

Nonthermal Processing Technologies for Food

Howard Q. Zhang, Gustavo V. Barbosa-Cánovas,
V.M. Balasubramaniam, C. Patrick Dunne,
Daniel F. Farkas, and James T.C. Yuan *EDITORS*



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Nonthermal Processing Technologies for Food



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Foreword

The consumer demand for fresh-like products generated gentle processing. Emerging technologies such as high hydrostatic pressure and pulsed electric field treatment did fit well into the hurdle concept and into the minimal processing scheme promising retention of freshness while providing safety and functionality of the product.

Today, we have industrial high-pressure and pulsed electric field treated products. Ozone, supercritical CO₂, ultrasound, and plasma treatment are either at pilot scale, industrially used, or on the verge of application. However, for me, after working for the first time with the first high-pressure unit at the University of Delaware, Newark, USA, exactly 25 years ago, there are still several issues to be addressed. Research work is still going on regarding inactivation, activation, or retention kinetics and mechanisms of microorganisms, nutrition, allergens, toxins, and viruses subjected to nonthermal processes.

Furthermore, it is my belief that many nonthermal processes described in the book also have the potential to do more than just mimicking existing conventional thermal processes. Our own approach to understand the potential of nonthermal processes and

then use them based on their unique mode of actions will lead to additional and unique applications. For example, high-pressure modification of proteins and polysaccharides, stress response induction by pulsed electric fields are examples of potential future applications of nonthermal processes for the generation of tailor-made foods.

Finally, it is essential for me to acknowledge the people who were the pioneers in the development of the “new” nonthermal processes. Amongst many, I want to mention about the pioneering work by Graham Gould, then Head of Microbiology, Colworth House, Unilever Research, UK, and Daniel Farkas, then the Chair of the Department Food Science, University of Delaware, USA. It is my firm belief that without those individuals the field of nonthermal processing would not be where it is today.

I wish this book all the success it deserves.

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Preface

Looking forward into the future of food science/technology/engineering, in the emerging area of non-thermal processing of foods, is definitely an adventure. It is open ended and full of uncertainties. Lessons learned from the past should always serve as a good basis for envisioning the future of this growing field, even though emerging and unexpected challenges in food processing are making the integration of “what is known” with “what is coming” difficult. This integration not only embraces the fascination with the new but also addresses the responsibility demanded of scientists for accuracy of research, and proper extrapolations from the laboratory bench to the production floor, and to the marketplace where the best predictions are made. We have the tools to visualize what is coming, but it is our dreams and vision, if not our ambitions, that inspire us to go beyond what can be viewed with mathematical models and complicated algorithms. The food industry, being one of the most conservative sectors in the food production chain, is experiencing the need for change and innovation, to a degree never encountered before. Consumers have become much more demanding, better educated in terms of food quality and nutritional aspects, forcing producers along with regulatory agencies to search for technologies that offer better products with greater safety. Scientists and avid researchers are incorporating knowledge acquired from very different and disconnected disciplines, in order to wisely blend this research pool of information with what is commonly known in food science/food engineering domains. The outcomes have been quite unexpected, though very much wel-

come in regard to food quality and safety, and it is envisioned that this trend will persist in the years to come.

Nonthermal processing of foods has essentially meant unprecedented opportunities for the industrial sector, in providing better health and wellness for the consumer, and unforeseen new food products of excellent quality without compromising safety. The challenges surrounding these emerging technologies are immense, but the long list of interested groups in support of their development is growing in an exponential fashion. Nonthermal processing technologies are being advanced and making a significant, positive impact in the food sector.

This handbook covers basic information and some of the recent developments in nonthermal processing of food, and the attempts, via predicted pathways, to identify future development in the field generated from the ingenuity and creative approach of a well-trained and resourceful community. The development of nonthermal processing techniques for processing of food has resulted in an excellent balance between safety and minimal processing, between cost and superior quality, and between novel approaches and use of existing process installations to optimize resources. Nonthermal processing could be perceived as an alternative to conventional thermal processing, but this is just a small piece of the role that nonthermal processing could play in the food factory of the future. Nonthermal processing can be effectively combined with thermal processing, and interesting synergistic effects have already been identified. Other significant synergisms could

be achieved by combining selected nonthermal technologies, as well as by combining these with other microbial stress factors, such as pH, water activity modifiers, and inclusion of antimicrobials and/or bacteriocins. At the same time, nonthermal processing facilitates the development of new products never envisioned before—a series of niche markets that will eventually receive wide attention in the years to come. The opportunities for such new products are countless, and most will have superb quality and very attractive prices.

Nonthermal technologies can be used for decontamination, pasteurization and, in some cases, sterilization, but in all examples of use, one of the key attributes of the processed product is excellent quality, wherein most products have “fresh” characteristics. There is no question that the quest for technologies capable of producing optimum-quality, safe-processed products has become a top priority in the world of food science and technology. Relevant factors to consider during exploration and application of these novel technologies include the following: the kind of microorganism inactivated; number of log cycles achieved; lethal doses required for inactivation; effect on enzyme activity as related to food quality factors; finding the most attractive process combinations to maximize synergy; how quality attributes are altered; how to scale up laboratory and pilot plant results to industrial applications; reliability of a given technology; adoption costs, such as engineering the process, initial investment, operation

of the process, maintenance, and depreciation; energy savings; environmental impact; and consumer perception of the technology and products of that technology. As a final point, the search for new approaches to processing foods should be driven, above all, to maximize safety, quality, convenience, costs, and consumer wellness; it cannot be used to force the utilization of a given technology. Any technology must fit the needs and desires of the consumer to be successfully implemented.

We have worked diligently to offer a thorough and objective overview of what nonthermal processing can offer today to the consumer and the industrial sector, what needs to be investigated further, and the expected developments. We have written some chapters in this handbook, but the contributions of other authors, who come from a wide array of backgrounds and prior experience in nonthermal processing, have been instrumental in presenting a well-balanced and self-provoking document that we hope will be useful to many in academia, industry, regulatory and other governmental agencies, and foremost to all of us, the consumers, and those who interpret the impacts of science on consumers.

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Introduction

Gustavo V. Barbosa-Cánovas and Daniela Bermúdez-Aguirre

The focus of food engineers and food scientists in the last 20 years has been on finding alternative process and preservation technologies that are environment friendly, low in cost, and able to preserve the quality attributes of the food product. A number of novel nonthermal technologies such as high pressure and irradiation are currently under commercialization and offer many of these advantages to the consumer. These new technologies have been extensively researched worldwide from a microbiological point of view, and study of composition factors and sensorial characteristics after processing has also been conducted. The interesting fact is that they are useful not only for inactivation of bacteria or enzymes but also for the development of ingredients and finished products with novel characteristics. Final quality of such products is outstanding compared with traditional thermal methods of preservation, while there are important savings in cost, energy, and processing times as well.

Here, we review some novel nonthermal technologies and their development in partnership by with industry, academia, and government, who together worked with regulatory agencies to satisfy the requirements for their use in the food industry in order to offer the consumer food products that are safe, nutritional, and tasty. The case is that some traditional regulations for pasteurization and sterilization have been modified to accommodate these emerging technologies where heat is not the main stress factor to inactivate microorganisms.

Why Nonthermal Technologies?

Thermal pasteurization and thermal sterilization are the two most common food unit operations used to process and preserve foods in the world. Heat is responsible for the microbial inactivation and reduction in enzyme activity that takes place in food products undergoing thermal treatment, and results in a safe product with longer storage life than its raw equivalent. The main purpose of thermal processing is the inactivation of pathogenic microorganisms and spores (depending on the treatment) to provide consumers with a microbiologically safe product. However, despite the benefits of thermal treatment, a number of changes take place in the product that alter its final quality, for example, flavor, color, texture, and general appearance.

In the last few decades, consumers have become more demanding about what they eat and the price they pay, including concern about the safety of their food; however, most products on the market have been overprocessed to ensure consumer safety and show significant damage in sensorial and nutritional characteristics. Now, consumers are looking for fresh-like characteristics in their food, along with high sensorial quality and nutrient content. Consumers are more aware of food content and the technologies used to process their food, showing a higher preference for natural products (Evans and Cox, 2006) free of chemicals and/or additives. Thus, the need for processing alternatives that can achieve microbial inactivation, preserve food's fresh-like

characteristics, and provide environment-friendly products, all at a reasonable cost, has become the present challenge of numerous food scientists/technologists around the world. Nonthermal technologies represent a novel area of food processing and are currently being explored on a global scale; research has grown rapidly in the last few years in particular. In some cases, it is very appropriate to combine preservation techniques looking for synergistic effects (hurdle technology approach). These novel technologies are very appealing to be utilized in combination, either among themselves or with traditional ones.

Food Spoilage

Food is an excellent vehicle for the transport of microorganisms. Because of the presence of water and the richness of the medium, a favorable environment exists for natural bacteria to grow; there are enough nutrients for bacteria to grow and multiply to a significant degree in a very short period of time. Under warm-temperature conditions, growth of microorganisms is even faster. Moreover, if unsanitary practices are followed during handling of food products, pathogenic microorganisms can be transferred to the food from surfaces, soil, water, or animals, generating a health risk for the consumer. Thus, processing operations that can inactivate pathogenic bacteria and reduce natural flora in vegetable and animal products are essential in the food industry.

The most common approach used to achieve these goals consists in thermal techniques, as applied in pasteurization and sterilization processes. Pasteurization is commonly used for high-acid food products ($\text{pH} < 4.6$) to inactivate target pathogenic bacteria and to extend product shelf life for a few weeks. It is also utilized for low-acid foods followed by refrigeration. The most common pasteurization method, high temperature short time, uses temperatures around 72°C for 15 seconds in the case of milk. Sterilization is a stronger thermal treatment used for low-acid food products ($\text{pH} > 4.6$); it inactivates spores, extends the shelf life of the product for months at a time, and uses temperatures around 121°C for several minutes (e.g., 15 minutes). Pasteurization

and sterilization have been used to inactivate cells of pathogenic microorganisms such as *Salmonella*, *Escherichia coli*, *Listeria monocytogenes*, *Staphylococcus aureus*, and spores of *Clostridium botulinum* in a significant number of food products, in addition to reducing spoilage microorganisms. Enzymatic activity can also be reduced when heat is applied to food and yields a product with better stability during storage.

Nevertheless, some microbial growth can be observed during food storage, for example, in pasteurized products (i.e., milk). The process does not inactivate all the microorganisms and heat-resistant spores can remain in the product after processing, and depending on storage conditions, growth of bacteria can accelerate. Some food products under specific conditions are processed to withstand long periods of storage. This is the case when manufacturing and shipping specific items from one country to another; the product must have enough shelf life to withstand being processed and consumed in two remote places. In more specific situations, such as food processed for military use or space missions, there are more strict requirements, one being product storage life in particular. For military use, the shelf life of food must be at least 3 years at room temperature. During this 3-year period, the growth of bacteria must be practically nil, or at an extremely slow rate, to avoid any spoilage. Military food is used under extreme temperature and moisture conditions, sometimes in desert environments, where food must be innocuous to soldiers and provide necessary energy and nutritive requirements. For space missions, shelf life must be longer than that required for commercial use. Food items must be microbiologically safe with adequate nutrients, taste good, and have a pleasing appearance. Although extreme temperature conditions are not considered a problem during space transportation and storage, the food must also be stable against high amounts of radiation.

At present, food scientists are studying the use of new preservation factors to preserve foods and extend product shelf life, which will be especially beneficial in research of military and space foods, as well as other foods. Some of these new preservation factors belong to the nonthermal technology

area of study, in which different physical hurdles are being explored to inactivate or delay the growth of bacteria. The main goal of these novel technologies is to process a safe product that retains the sensorial characteristics of the fresh product as closely as possible, in terms of nutrient content and sensorial properties.

Nutrient Content

The microbial safety of food remains an important aspect of food processing, but because of the required conditions to inactivate microorganisms, nutrient content in some food products is detrimentally affected after processing due to thermal sensitivity of some nutrients. For example, milk, eggs, fish, meat, and other important sources of protein; once they are subjected to thermal treatment (pasteurization or sterilization), the nutrient content is affected greatly. Thus, processing technologies that can maintain original nutrient content and do not change the structure and functionality of ingredients are highly desired in the food industry.

One nonthermal technology, high hydrostatic pressure (HHP), has shown a negligible effect on the nutrient content of food, for example, in processing of fruits and vegetables, where pressure has minimal effect on the anthocyanin content after processing (Tiwari et al., 2009). Anthocyanins are considered phytonutrients, and they not only are responsible for color but also have an important antioxidant effect on human health. However, anthocyanin content in juices after pulsed electric fields (PEF) treatment has shown contradictory results. Some researchers report a minimum effect on the pigment content after processing, while others show that there is degradation in anthocyanin content after pulsing (Tiwari et al., 2009). Other examples of nutrient retention in food products using nonthermal technologies are mentioned by Knorr et al. (2002), such as the minor loss of L(+) ascorbic acid in sonicated juice, better retention of ascorbic acid concentration in high pressure treated peas, complete retention of ascorbic acid in pressurized broccoli, and unchanged amino-acid content in PEF-treated grape juice (Garde-Cerdán et al., 2007), among others.

Sensorial Quality of Food

Changes in food's sensorial characteristics are commonly observed when thermal processing is used. Temperature works as a catalyzer in some chemical reactions occurring between the pigments, mineral salts, proteins, vitamins, amino acids, fats, and other chemical species in the food, promoting a number of physical changes. Browning, oxidation, protein denaturation, coagulation or precipitation, changes in microstructure and final texture, gelation, loss of color and flavor, loss of functionality, starch retrogradation, and related chemical processes occur in food components during thermal treatment.

High-pressure processing applied at room temperature yields a product with most of food's quality attributes intact; for example, pressurization does not affect covalent bonds, avoiding any development of strange flavors in the food (Knorr et al., 2002). Some studies of orange juice processed under pressure showed no important changes compared with the fresh squeezed product, retaining the same quality during storage for as long as 3 months at 5°C (Knorr et al., 2002). In consumer tests, when asked to compare fresh-squeezed, thermal pasteurized, and pressurized orange juices, consumers preferred the pressurized version (Evans and Cox, 2006).

In milk processing, high pressurization (>200 MPa) was found to increase casein solubility, protein and water content in curds, as well as curd yield (Knorr et al., 2002). This pressurized milk has been successfully used in cheese-making and yogurt production (Penna et al., 2007; San Martín-González et al., 2007). Another nonthermal technology applied to milk is PEF, which allows pasteurization of milk with only minor thermal damage to milk's properties and provides important energy and cost savings (Bolado-Rodríguez et al., 2000; Bendicho et al., 2002). Ultrasound has also been used in milk pasteurization, with important results; milk shows a higher degree of homogenization, whiter color, and better stability after processing. In this method, pasteurization and homogenization are completed in a one-step process (Bermúdez-Aguirre et al., 2009).

Better color in ultrasound-treated juice, better quality in pressurized strawberry jam, and better

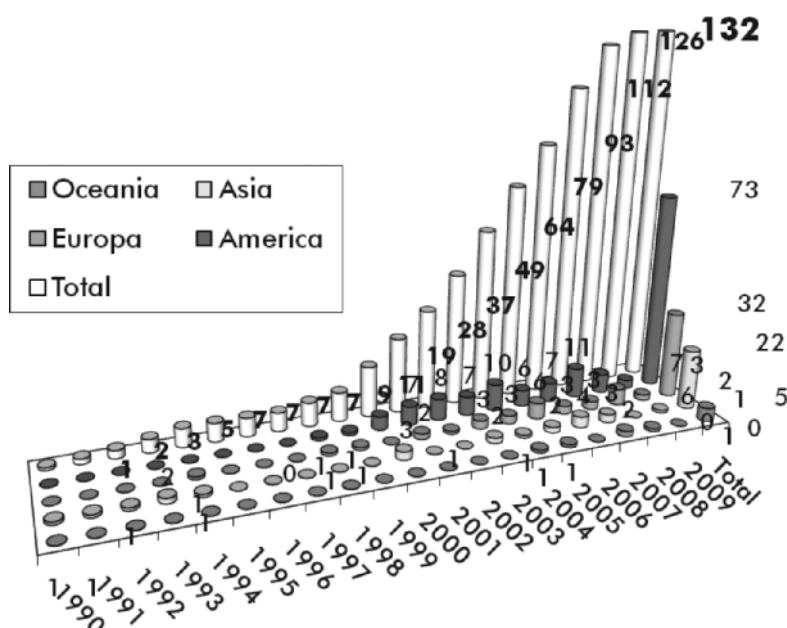


Figure 1. Number of high-pressure equipment units in use around the world as of 2009 (Tonello, 2009).

flavor and color in guava puree (Knorr et al., 2002) are other examples of quality enhancements experienced with nonthermal technologies. Minimal change in sensorial quality has been reported in fruit juices processed with ultrasound as well and in juices processed under dense phase carbon dioxide, showing important bacterial inactivation, which ensures microbial quality and product safety, but does not compromise the organoleptic properties (Tiwari et al., 2009).

Novel Nonthermal Technologies

These new technologies for food processing are normally applied under nonthermal conditions. Although temperature could be used in combination with some of these novel technologies to enhance effectiveness, most of the research conducted is at room temperature, and due to extremely short processing times, food remains fresh-like. Scientists are exploring the use of pressure, light, different types of electromagnetic radiation, sound, and other physical hurdles to inactivate bacteria. Consumers

are gradually becoming aware of novel technologies for food processing and sometimes refer to specific nonthermal methods, such as “cold pasteurization” (Cardello et al., 2007). A more detailed list includes the following: HHP, ultrasound, PEF, oscillating magnetic fields, irradiation, ultraviolet light, cold plasma, some chemicals (e.g., ozone, dense phase carbon dioxide, chlorine dioxide, electrolyzed water, and bacteriocins), and processing methods (e.g., intelligent packaging).

Some of the most explored technologies in this group are HHP and irradiation; both are currently used for commercial products and there are facilities for these technologies around the world. Figure 1 shows the growth in the number of HHP equipment in use around the world in the last 19 years. As a novel technology, development and improvement of such equipment have been based on specific requirements and needs of the food industry. Today, use of pressure (300–700 MPa) for commercial applications around the world in vessels ranging in size (35–420 L) has an annual production rate of higher than 150,000 tons (Wan et al., 2009).

Another nonthermal technology widely used to process food is irradiation; many toxicological tests have been conducted to show that this technology is safe for foods in specific cases, such as microbial inactivation, insect disinfestation, or improvement of quality. Since the 1990s more than 40 countries around the world have established safe and appropriate facilities for irradiation of food (Molins, 2001). These facilities have begun to show consumers that irradiation technology has more advantages than disadvantages. In some countries, the name of this technology has been changed to electronic pasteurization for better acceptance by consumers (Molins, 2001). The technique is regulated both nationally and internationally by IAEA, FAO, and WHO (Morehouse and Komolprasert, 2004). FDA considers irradiation to be more of an additive than a process for food.

PEF technology is probably the second most promising of the nonthermal technologies; it is already approaching industrial application. PEF application was successfully launched for fruit juices in the United States in 2006, achieving outstanding results in product quality. In the not too distant future, this technology will likely be launched by a number of European food companies for pasteurization of liquid foods, introducing all the advantages of this technology for the first time to consumers and food processors (Kempkes, 2008).

Processing Times

Food scientists are also looking for more convenient processing operations. Emerging nonthermal technologies have shown important reductions in processing times compared with traditional thermal processing operations. A short processing time is characteristic of most explored novel nonthermal technologies (Wan et al., 2009). For example, using PEF for pasteurization of liquid foods reduces the total processing time to less than a second; high pressure thermal sterilization is able to inactivate spores and produce a shelf-stable product in only a few minutes (around 5 minutes, depending on the characteristics of a product). This reduction in processing time is reflected in both energy and economic savings. Another key advantage of nonthermal

technologies is the environment-friendly aspect of such energy savings, which includes minimal waste after processing. PEF technology is a good example as this technology is a waste-free process (Knorr et al., 2002). In general, most of these technologies show a significant reduction in processing time compared with traditional thermal treatment; waste is minimal or nonexistent, and savings in energy is a common characteristic in the majority of those technologies already mentioned.

A Four-Member Team Approach

The development of nonthermal technologies has grown in the last several years because of the constant interaction between academia, industry, government, and the consumer, under the supervision of regulatory agencies. The first round of research was conducted by academia, but upon sharing the results with industry and government, and later on consumers, they too became interested and encouraged the continuation of this research, again under the supervision of regulatory agencies. HHP, for example, a technology that was probably looked at 20 years ago, began to be explored in labs; transfer and scale-up, from the pilot plant to industry, became a priority after viewing the encouraging results of these studies. Today, it is a commercial reality and has been adopted by industry for use in processing products with high quality and high added value, which includes processing already existing products and promoting the development of new products. Regulatory agencies such as the Food and Drug Administration have approved HHP use, first as a pasteurization alternative, and recently (February, 2009), in combination with heat, as an alternative for food sterilization known as pressure-assisted thermal sterilization (PATs) or pressure-assisted thermal processing (PATP) (NCFST, 2009).

Thermal Processing

There have been some innovations in this area, but conventional equipment is still used (Mermelstein, 2001). Sterilization and pasteurization have been extensively studied over the years, and in the case of

low-acid foods, sterilization must follow strict controls and, depending on the product, requires specific processing times to ensure that the spores of most resistant microorganisms are inactivated and beyond; this process results in loss of thermal-sensitive nutrients such as vitamins (Teixeira and Tucker, 1997). In past decades, food engineers/scientists followed a first-order kinetics or linear trend to interpret and describe thermal inactivation of bacteria. Thermal processing parameters such as D , z , and F_0 values have been used extensively to calculate lethality of the process. However, it has been shown that first-order kinetics is rarely followed by bacteria during inactivation and that safety in the canning industry is based more on overprocessing operations than on kinetics models (Corradini et al., 2005).

The nonisothermal conditions during thermal processing have been extensively reviewed and discussed by many authors in the last few decades (Corradini et al., 2006, 2007, 2008; Aragao et al., 2007; Smith-Simpson et al., 2007; Peleg et al., 2008). They have shown that most bacterial inactivation curves during thermal processing follow a nonlinear trend, and that alternative mathematical models can fit these curves (Aragao et al., 2007; Corradini and Peleg, 2004, 2007). Several efforts have been devoted to establishing better control during thermal processing using “intelligent systems” to monitor the process with on-line systems to optimize safety, quality, and process efficiency (Teixeira and Tucker, 1997). Local optimization algorithms (Miri et al., 2008) are used as well as comprehensive studies with time–temperature integrators (Mehauden et al., 2009), among other tools. Most of these approaches cannot be fully transferred to industry, because thermal processes (pasteurization or sterilization) must follow the established criteria, unless a variation has been successfully validated and approved by regulatory agencies.

In studies of nonthermal technologies, some concerns with thermal processes are also observed, such as nonlinearity during inactivation. These issues have been addressed together with increased knowledge of emerging nonthermal technologies, as cited by many authors (Raso et al., 2000; Rodrigo et al., 2003a,

2003b; Hassani et al., 2005); consequently, modifications resulting from these issues must be addressed in regulations and established standards by regulatory agencies.

New Definition of Pasteurization

The new alternatives for pasteurization of foods have resulted in various changes that must be addressed to meet the original standards for pasteurization. For one, the definition of pasteurization should be reviewed carefully for its applicability to other technologies with the same goal of pasteurization (as sought in years past). There are a number of important factors to consider when a new technology is thought to be the equivalent of thermal pasteurization, such as the most resistant pathogenic microorganisms in a given food, the efficacy of the novel technology, the characteristics of the food product, the conditions needed for food distribution and storage, and the intended use of the food (NACMCF, 2006). The new definition of pasteurization should meet all of the above-mentioned factors so that a safe product can be offered to the consumer; at the same time, it should describe the advantages of the novel technology to the consumer and the food processor. The National Advisory Committee on Microbiological Criteria for Foods (NACMCF) adopted a new definition for pasteurization in 2004: “Any process, treatment, or combination thereof, that is applied to food to reduce the most resistant microorganism (s) of public health significance to a level that is not likely to present a public health risk under normal conditions of distribution and storage” (NACMCF, 2006). In the same document, a list of novel technologies is included as a possible alternative for thermal pasteurization. Some novel thermal technologies such as microwave and ohmic heating are cited, and most of the nonthermal technologies such as HHP, PEF, ultraviolet, irradiation, chemical treatment, pulsed light, infrared, cold plasma, oscillating magnetic fields, ultrasound, and filtration are mentioned, including their criteria for use in pasteurization according to the type of microorganism, processing conditions, and research needs.

Additional Relevant Issues Regarding Novel Technologies

The search for other food processing alternatives not only provided the required tools for microbial inactivation but during the development of some novel technologies important discoveries were also made concerning specific foods that would help improve the quality of other products or the development of new ones. Some of the advantages of novel technologies are (or will be) their use in processing specific products such as military rations or space food items that require precise and very strict regulations. Novel technologies can be used to replace some of the current military rations as well, or to process some of the excess in agricultural and farm activities, avoiding important yearly economic losses around the world.

New Ingredients, New Products

The exploration of new preservation factors in food processing has not been limited to microbial and enzyme inactivation; during extensive research of nonthermal technologies, important discoveries regarding new properties in some food ingredients were observed. For example, HHP has the ability to modify the structure of proteins and polysaccharides, which allows changing the texture, functionality, and even appearance of food (Ross et al., 2003). These changes have been observed systematically according to the intensity of the applied pressure, and depending on the new use of the protein or the sugar, the changes can be modified accordingly. The possibility of having new ingredients for food processing has opened up a distinct and comprehensive world of opportunities in food research and development of new products. At the same time, new ingredients are helping to solve some quality issues in specific products. For example, during ultrasound treatment of milk, in addition to pasteurization, cavitation (which is the main effect of sonication) is responsible for breakdown of fat globules in milk and reorganization of microstructure in casein molecules and fat globules; the result is an homogenized product after processing (Bermúdez-Aguirre et al., 2008), avoiding one important step (homogenization) in the manufactur-

ing of milk by conventional methods. This new effect on milk can be “intelligently” used for some dairy products that present problems during storage, such as yogurt, in which syneresis is one of the main quality concerns; sonicating the milk prior to yogurt processing can minimize this problem.

Space Mission Food

Another big challenge in food science and technology is the processing of safe and nutritious foods for storage on long space missions, where the required long storage of these foods is not the only requirement. Other important factors determine whether they are acceptable, such as the requirement that space food be of reduced volume and mass with a minimum of waste. These food items must also satisfy all dietary requirements of astronauts for maintenance of health during a space mission, not only from the perspective of physical well-being and health aspects but also to address the possible psychological effects of such food on the astronaut (Chen and Perchonok, 2008). This last aspect is important for both space mission foods and military rations, because those consuming such food are away from home and under hard conditions for long periods of time; this food must be acceptable in appearance and content, and must evoke positive feelings and comfort, giving a definite sense of being closer to home. The NASA Advanced Food Technology Project at the Johnson Space Center is the agency currently in charge of feeding systems for astronauts. At this center, state-of-the-art technologies are evaluated to achieve various required goals, and one of them is to achieve a 5-year storage life for many space mission food items. The current food technologies being investigated are retort processing, freeze-drying, irradiation, and intermediate moisture processing; for instance, thermostabilized food products, a new trend in product design for space missions, are currently replacing some of the previous retorted items (Chen and Perchonok, 2008). The ongoing intensive study of some nonthermal technologies could very possibly deliver a viable option for future space mission meals. With the recent approval of PATP as a sterilization technology, this will open new frontiers for

food processing and the development of new food items for inclusion on space mission menus.

Military Combat Rations

One area of study receiving even more attention today is research related to military combat rations. In the last several years, important investments in terms of time and money have been made to achieve a world-class feeding system for US soldiers (Natick, 2008). Recent advances in food science and the development of state-of-the-art technologies have been applied to satisfy the dietary needs of soldiers from the nutritional, safety, and quality points of view. Soldiers often endure extreme, intense physical activity requiring high-energy meals, but in addition to satisfying their high-energy demands, these foods must be of the highest quality possible and at an affordable price to the military.

According to Chen and Perchonok (2008), one of the goals of the Department of Defense Combat Feeding Program by 2010 is to implement the use of nonthermal/combo technology for shelf-stable military rations. The novel technologies that have been investigated thus far (for Natick) are irradiation, PEF, high pressure, ohmic heating, microwave, and radio frequency (Cardello et al., 2007). HHP technology is the closest to being incorporated for military rations at this time. This technology has shown positive results in processing dairy products, fruits, and vegetables, potatoes, eggs, fish, and meats. According to RDECOM (2009), the product mashed potatoes in-a-pouch processed under PATS has been officially accepted for incorporation as an meals-ready-to-eat (MRE) product for military use. This is indeed a giant step ahead for use of one of the first nonthermal technologies researched. Investigated in food labs 20 years ago, today PATS is a reality in commercial pasteurization as well as commercial sterilization, and generates products that require strict and rigid quality control.

World Hunger Issues

Food scientists should not only focus on improving the quality and safety of foods marketed to demand-

ing consumers in the first-world countries but should also consider areas in the world experiencing hunger and poverty. Today, approximately 1.3 billion people live in extreme poverty, surviving on less than 1 dollar a day, and nearly 2 billion people live in poverty and marginal circumstances close to these conditions (ADA, 2003). However, this does not mean that food quality is not important. Even some consumers in the first-world countries agree that they should pay extra for high added-value food, such as freeze-dried products. Yet, most consumers in underdeveloped countries just want to pay the minimum amount in dollars necessary to satisfy their basic dietary needs. Access to food is another critical issue. According to the American Dietetic Association (ADA), food security is related to a person's access (at any given time) to adequate rations of safe, nutritious, and ethnically appropriate food (ADA, 2003). With the current level of agricultural production worldwide, food could be available to everyone on earth to feed the world's 850 million hungry people, but only if the biological, chemical, and physical factors that commonly generate loss of food around the world could be avoided or minimized (Marsh, 2008).

World hunger is by far another reason that study of novel technologies should focus on processing foods at a reasonable cost with adequate nutrient content and long shelf life; extending a technology's commercialization in faraway and hard-to-access locations could make a huge difference. Nonthermal technologies are not going to resolve the hunger problem on a day-to-day basis; the intelligent exploration of these technologies could, however, lead to processing highly nutritious food items with longer storage life, and possibly shipping these products to remote places; this in turn could satisfy basic food supply needs in underdeveloped countries. One example is the use of PATS, to be used for military rations in 2009 based on of its demonstrated advantages, as explained previously. Some military rations are used for humanitarian daily rations, which provide a full day's nutrition to the malnourished. These rations are designed to feed large populations of displaced people or refugees and contain ready-to-eat thermostabilized meals, similar to MRE products (Natick, 2008). Thus, the reality of feeding people

in poverty via novel technologies in the near future is a sound possibility, and foods would have better quality and most of the original nutrients intact.

Global Harmonization Initiative

Novel nonthermal technologies have been researched in different parts of the world with important results. Thousands of references can be found on the most developed nonthermal technologies, such as HHP. A wide variety of pressurized products are mentioned in these references, including a number of ethnic food products in specific countries. Researchers found that high pressure is an excellent technology for processing ethnic foods and extending product shelf life. However, due to inconsistencies in reporting processing conditions, today, there are problems in commercializing such products outside these ethnic niches. Added to this are the problems that arise with all novel nonthermal technologies in general when conditions used in a specific country to satisfy the sanitary regulations are not legally required in another country, making processing difficult and expensive, and commercialization of these products almost impossible. Furthermore, the time-consuming activity needed to demonstrate the safety of a specific product in another country is complex and expensive, causing delays in the food processing chain (Lelieveld and Keener, 2007; Sawyer et al., 2008).

Hence, a global regulation system that ensures the safety and quality of food regardless of country of origin is sorely needed. Lelieveld and Keener (2007) pointed out that, in addition to a global regulation system, there should be a governing body that regulates and monitors the enforcement of these food processing regulations. Although there are institutions that regulate food processing today, the resulting regulations often only apply to countries belonging to specific organizations (e.g., the *Codex Alimentarius*). Thus, the new regulations should apply to all countries and clearly show validity overseas. Meanwhile, with this goal in mind, a Global Harmonization Initiative (GHI) was launched 5 years ago by the International Division of the Institute of Food Technologists and the European Federation of

Food Science and Technology (EFFoST) to establish a world wide regulation system. Since then, GHI has made important advances in the last few years (Lelieveld, 2009).

Hurdle Technology

The use of novel technologies alone is often insufficient to achieve the desired processing goal, for instance, adequate microbial inactivation. Sometimes, the intelligent use of two or more preservation factors applied simultaneously, known as hurdle technology, can fulfill the requirements for a specific product. The concept of hurdle technology is not new. Processing factors have been combined in the past to extend the shelf life of food; examples include combining pH, acidity, heat, water activity, and/or antimicrobials. Today, a number of novel technologies are good candidates for use in combination with these past preservation factors, and preliminary results have shown important shelf life extension of products. Probably, the best example is the recent approval by the FDA (NCFST, 2009) of high pressure in combination with heat for commercial sterilization of food in the United States.

Other technologies combined with heat to enhance their effectiveness are PEF and ultrasound. In both cases, heat is used to weaken the cells during the process and to enhance the lethal effect against bacteria. Of particular note is the test of ultrasound in three different combinations: with heat (thermosonication), pressure (manosonication), and the combined factors sound, heat, and pressure (manothermosonication). These combinations have been effective in microbial and enzyme inactivation (Knorr et al., 2002).

Clearly, use of nonthermal processes in hurdle technology requires finding the right combinations of preservation factors. These factors should have a synergistic effect on cell inactivation in order to disrupt the vital functions of the microorganism; this is called a multitarget approach (Ross et al., 2003). Presently, the mechanisms of cell inactivation observed with nonthermal technologies are not all that clear. More knowledge about this topic will help determine the right combinations of preservation

factors needed to achieve higher inactivation and more lethal treatments against bacteria.

Final Remarks

Indeed, the area of nonthermal technologies is a vast world of opportunities for processing and preserving food with excellent quality. At present, there are many challenges facing food scientists, specifically those related to nonthermal technologies, although a number of these have been successfully tested in microbial inactivation. However, aspects related to mechanisms of cell inactivation and improvements in nonthermal processes and equipment are among the priorities that need to be addressed in the coming years. Currently, food scientists and engineers around the world are devoting much time to figuring out the majority of these issues. Still, other gaps in research remain, for example, the issue related to spore inactivation and the use of novel technologies to effectively inactivate vegetative cells. Results could be similar to that achieved with high pressure, which brought about the recent approval of high-pressure thermal sterilization—a result that could likewise be achieved with other technologies in the near future.

In addition, several features concerning the toxicological aspects of novel products should be evaluated to test if the applied energy generated by a nonthermal technology is strong enough to inactivate microorganisms and to preserve the nutritional content of food. At the same time, a nonthermal technology should not be responsible for creating undesirable compounds or toxic substances that could be harmful to consumers. Indeed, the issues now facing researchers in promoting a technology, from the lab to regulatory approval and commercialization, are the same issues that will provide consumers with better food and new food products exhibiting outstanding characteristics.

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Section I

Physical Processes

Chapter 1

Fundamentals of Food Processing Using High Pressure

Loc Thai Nguyen and V.M. Balasubramaniam

1. Introduction

Most processed foods are treated with heat to kill harmful bacteria, a process that often diminishes product quality. Considered one of the most important innovations in food processing in 50 years (Dunne, 2005), high-pressure processing (HPP) presents an alternative that retains food quality and natural freshness while extending microbiological shelf life (Farkas and Hoover, 2000). HPP, also commonly referred to as “high-hydrostatic pressure” processing or “ultra-high-pressure” processing, uses elevated pressures, with or without the addition of external heat, to achieve microbial inactivation or to alter food attributes. The pressures used in HPP are almost ten times greater than in the deepest oceans on earth. Common pressure units are listed in Table 1.1.

Long used in the material and process engineering industry for sheet metal formation and isostatic pressing of advanced materials such as turbine components and ceramics, HPP offers many advantages to food processors. Because HPP does not break covalent bonds, it preserves food freshness (Farkas and Hoover, 2000). The technology also provides food processors with an opportunity to process heat-sensitive, value-added foods with fewer additives and cleaner ingredient labels. Pressure can be applied at ambient temperature, thereby eliminating thermally induced cooked off-flavors. Finally, this technology

is efficient, as it can be used to process liquid foods in semicontinuous equipment and both liquid and solid foods in batch equipment. Table 1.2 summarizes some of the unique advantages of HPP.

The applications and limitations of high-pressure food processing have been reviewed extensively (Hayashi, 1991; Cheftel, 1995; Ledward et al., 1995; Ohlsson, 1996; Karin, 1998; Kunugi and Hayashi, 1998; Smelt, 1998; Thakur and Nelson, 1998; San Martin et al., 2002; Matser et al., 2004; Hogan et al., 2005; Torres and Velásquez, 2005; Rastogi et al., 2007). This chapter summarizes the basic process engineering principles related to HPP of food materials and emphasizes the importance of thermal effects during this preservation process.

2. Basic Principles Governing HPP

2.1. LeChatelier's Principle

LeChatelier's principle states that the application of pressure shifts the system equilibrium toward the state that occupies the smallest volume (Farkas and Hoover, 2000). Thus, any phenomenon (phase transition, change in molecular configuration, chemical reaction) that is accompanied by a decrease in volume is enhanced by pressure (and vice versa). This means that pressure stimulates reactions that result in a decrease in volume but opposes reactions that involve an increase in volume.

For a simple chemical reaction, the kinetics of transition from A to B with intermediate state A[‡] can be expressed as follows (Pfister et al., 2001):

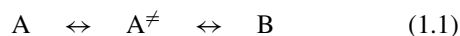


Table 1.1. Frequently used pressure units and conversion factors

	Atmosphere	Bars	Mega Pascal	Pounds/Inch ²
Atmosphere	1.000	0.987	9.901	0.068
Bars	1.013	1.000	10.000	0.069
Mega Pascal	0.101	0.100	1.000	0.00689
Pounds/inch ²	14.696	14.504	145.038	1.000

For this reaction, the process pressure (p), temperature (T), system's free enthalpy (ΔG), thermal energy (ΔE), volume (ΔV), and entropy (ΔS) can be related by the following:

$$\Delta G = \Delta E + p\Delta V - T\Delta S \quad (1.2)$$

Under isothermal condition, the kinetics of this reaction can be described by Equation (1.3):

$$\begin{aligned} \Delta V^\ddagger &= V^\ddagger - V_A = \left(\frac{\partial \Delta G^\ddagger}{\partial p} \right)_T \\ &= -RT \left(\frac{\partial \ln k}{\partial p} \right)_T \end{aligned} \quad (1.3)$$

where k is reaction rate constant and R is universal gas constant ($R = 8.314 \text{ J/mol}\cdot\text{K}$). $\Delta G^\ddagger$ and $\Delta V^\ddagger$

Table 1.2. Unique advantages of high-pressure processing

Description	Advantage
Pressure	Rapid and uniform distribution throughout the sample
Thermal distribution	Reduced impact of thermal gradient
Physical compression	Instant temperature increase and subsequent cooling on decompression
Product handling	Suitable for both particulate and pumpable foods
Process time	Less dependence on product shape and size
Functionality	Opportunities for new process/product development
Quality impact	Food may not undergo significant chemical changes
Reaction rate	Pressure accelerates traditional thermal inactivation kinetics

relate the changes in free activation enthalpy and activation volume. $V^\ddagger$ represents the volume of the activated system, while V_A represents the volume before activation (Pfister et al., 2001). A positive ΔV implies a shift toward the reactants at higher pressures. Depending on the mechanism, some reactions may be accelerated or retarded by pressure.

2.2. Isostatic Principle

It is generally believed that at the macroscopic level, pressure is transmitted in a quasi-instantaneous manner throughout the sample volume (Pascal principle). Thus, processing time during HPP is often thought to be independent of product size and geometry (Cheftel, 1995).

However, care must be taken to understand the interdependence of pressure and temperature during the HPP of food samples. Compression of the food sample results in a temperature increase (due to adiabatic heating). Water, carbohydrates, proteins, and fats are some of the basic building blocks of a complex food matrix, and each of these may respond uniquely under physical compression (Rasanayagam et al., 2003). The different rates of heating of each food matrix component under pressure may result in thermal gradients. Further, product near the vessel wall may lose heat to the environment.

Traditionally, the food industry has employed modest pressure treatment (3–30 MPa; 435–4,351 psi) for the homogenization of liquid foods. During homogenization, the liquid is forced to flow under high pressure through a narrow orifice. High product velocity and high shear characterize the homogenization process (Farkas and Hoover, 2000). Product heating can be expected. On the other hand, during HPP, the product is compressed isostatically (i.e., compressed in three dimensions), held, and then decompressed. Pressure reduces the volume of water by 10% at 300 MPa (43,500 psi) and by 17% at 600 MPa (87,000 psi) (Farkas and Hoover, 2000). Little product distortion occurs at the macroscopic level in food materials with high moisture. On the other hand, if the food material contains significant amounts of air (e.g., marshmallow, strawberry, and leafy vegetable), the air will escape from the product after

pressure treatment because of the difference in material compressibility. At HPP treatment pressures, gases in general are liquefied, if not dissolved in the liquid fraction of the food. On decompression, the gases expand and are released from the food matrix. Thus, products containing significant air may not be good candidates for pressure treatment. Similarly, dry solids form cake-like structures after pressure treatment. If food products do not contain sufficient moisture to maintain a water activity above 0.98, HPP may not provide effective microbial destruction.

3. Typical Process Description

HPP of solid foods starts with removing as much air as possible from the food and vacuum, packaging the products in flexible, high-barrier containers. Air removal is essential to ensure that a maximum number of containers can fill the pressure vessel during each cycle and that compression work will not be wasted on air in the system. The containers are loaded into a carrier basket or placed directly into the pressure vessel. Loading is similar in operation to a batch steam retort. Commercial batch vessel volumes range from 30 to 600 liters. A typical process cycle consists of loading the vessel with the prepackaged product and filling the remaining vessel void space with water, which acts as the pressure-transmitting fluid. The vessel is closed and the desired process pressure is achieved through addition of water delivered by an intensifier. After holding the product for the desired time at the target pressure, the vessel is decompressed by releasing the water (Balasubramanian et al., 2008).

Liquid foods can be processed in batch or semicontinuous mode. In the batch mode, the liquid product is prepackaged and pressure-treated as described for packaged foods. Semicontinuous pressure equipment employs two or more pressure vessels with free-floating pistons arranged to compress the liquid foods. A low-pressure transfer pump is used to fill the pressure vessel with the liquid food. After filling, the pressure vessel inlet valve is closed, and the pressure-transmitting fluid (usually water) is introduced behind the free piston to compress the liquid food. After the appropriate holding time, releasing

the pressure on the pressure-transmitting fluid decompresses the system. A pump is used to move the free piston toward the discharge port. The treated liquid food, which is held in a sterile tank, can then be filled aseptically into sterile containers. Three batch vessels in a semicontinuous system can be connected such that while one vessel discharges the product, the second vessel is being compressed, and the third vessel is being loaded. In this way, the output is maintained in a continuous fashion (Balasubramanian et al., 2008).

4. Packaging

The packaging requirement for the HPP process varies depending on the type of equipment (batch or semicontinuous) used. Semicontinuous systems are used in the case of pumpable liquid products, which are aseptically packaged after pressure treatment. On the other hand, flexible or semirigid packaging, with at least one flexible interface, is best suited for batch processing. A variety of existing flexible packaging structures may be used (Balasubramanian et al., 2004). Because high-moisture foods compress by 15–20% in the range of 600 MPa (87,000 psi) at ambient temperature, HPP packaging materials must be able to accommodate these reductions in volume and then return to their original volume without loss of seal integrity or barrier properties. For this reason, metal cans are generally not suited for the process.

Package size and shape will influence loading efficiency of the product within the pressure chamber. The package should be designed to achieve at least 75% loading for economical processing. Chapter 3 discusses the need for optimum package design in detail. Further, the mass ratio of product to void space water, and package size and shape, can influence the heat exchange between the pressure-treated product and the surroundings and may create thermal gradients within the food. As noted previously, the air present in the package headspace should be minimized to the extent possible to further improve the loading factor. High-barrier packaging materials with oxygen- and light-impermeable properties may be desired for extended refrigerated product storage.

This can also help preserve the fresh color and flavor attributes of many pressure-treated products (Hogan et al., 2005).

5. Pressure-Transmitting Fluids

During HPP, a pressure-transmitting fluid is used to transfer pressure to the prepackaged foods uniformly and instantaneously. The choice of pressure-transmitting fluid is based on the materials used to fabricate the pressure chamber. To prevent corrosion, commercial pressure vessels use a stainless steel liner. This enables the use of water as the fluid of choice for HPP treatment of foods. It is worth noting that the compression heating behavior of water is similar to that of most food materials. This can minimize thermal gradients between the food material and the compression fluid. Water has also emerged as a pressure-transmitting fluid of choice due to its availability, nontoxicity, and low cost. Chapter 2 covers additional details on equipment design construction and operation.

Castor oil, silicone oil, solutions of glycol–water mix, and sodium benzoate solutions are among the list of other pressure-transmitting fluids used in laboratory pressure equipment (Balasubramanian and Balasubramaniam, 2003). Depending on their thermal and physical properties (such as specific heat, viscosity, and compressibility), each solution may have a different rate of compression heating. For example, the heat of compression of water under pressure is 3.0°C per 100 MPa (14,500 psi), while that of silicone oil is about 20°C per 100 MPa. These differences can influence the magnitude of heat transfer among the pressure-transmitting fluid, food product, and the environment. The thermal gradient in the system subsequently could influence microbial inactivation and the quality of the processed foods (Balasubramanian and Balasubramaniam, 2003). If laboratory equipment (used for microbial or enzymatic kinetic studies) and commercial production equipment employ different pressure-transmitting fluids, the differences in respective heat transfer characteristics must be considered for reliable microbial challenge studies (Balasubramaniam et al., 2004).

6. Pressure–Temperature Response during Processing

During HPP, the temperature of food materials increases, as an unavoidable thermodynamic effect of compression (Ting et al., 2002). Figure 1.1 presents the typical pressure–temperature curve for a food sample subjected to high-pressure treatment. The temperature of the food sample increases because of physical compression (Figure 1.1, p_1 – p_2).

The magnitude of temperature change (Figure 1.1, T_1 – T_2) depends on the compressibility of the substance, thermal properties, initial temperature, and target pressure. For example, at 600 MPa (87,000 psi), the volume of a polar compound such as water is reduced by 17%. The maximum product temperature at the target process pressure is independent of the compression rate as long as heat transfer to the surroundings is negligible.

6.1. Pressure Come-Up Time

The time (Figure 1.1, t_1 – t_2) required to increase the pressure of the sample from atmospheric pressure to the target process pressure is often defined as “pressure come-up time” (Farkas and Hoover, 2000). The process come-up time is primarily a function of the desired target pressure, the volume of the pressure vessel, and the horsepower of the pump–intensifier

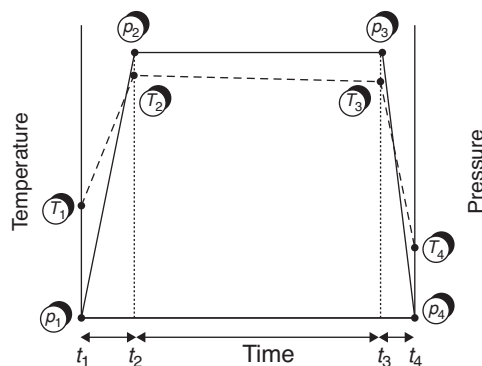


Figure 1.1. Typical pressure–temperature response of a water-based food material undergoing high-pressure processing. Come-up time, t_1 – t_2 ; holding time, t_2 – t_3 ; decompression, t_3 – t_4 .

employed. Typical commercial-scale high-pressure equipment is designed to have a come-up time in the range of 2–3 minutes to reach 600 MPa (87,000 psi) (see Chapters 2 and 3). Longer come-up times add to the total process time by reducing the hourly cycling rate. This affects product throughput. Variation in come-up time may also affect the inactivation kinetics of microorganisms. Therefore, consistency and awareness of these times are important in the process development of HPP (Farkas and Hoover, 2000; Ting et al., 2002; Balasubramaniam et al., 2004).

6.2. Pressure-Holding Time

Once the desired pressure is reached, and assuming that there is no significant pressure drop in the system as a result of heat exchange with the surroundings, no more additional energy is added to the process. Thus, pressure-holding time (Figure 1.1, t_2 – t_3) can be defined as the interval between the end of compression and the beginning of decompression. The products are held at the target pressure and temperature (if specified) for a predetermined holding time to achieve the desired microbial inactivation and/or quality.

The shortest processing time (<10 minutes) is often desired because process time has a significant effect on throughput (Balasubramaniam et al., 2004) (also see Chapter 3). Stability of product temperature during the holding time at pressure may depend on the insulation characteristics of the pressure vessel. If the equipment is not properly insulated, the temperature of the product decreases from T_2 to T_3 (Figure 1.1) during pressure-holding time (t_2 – t_3) due to thermal exchange through the pressure vessel walls.

6.3. Decompression Time

The time (Figure 1.1, t_3 – t_4) to bring a food sample from process pressure to near atmospheric pressure is often termed as “decompression time.” Most pressure equipments allow product to be decompressed in a few seconds. Certain food products may change their structure during decompression due to very rapid expansion of dissolved or occluded gas. If structural changes are undesirable, a slower rate of decompression

may be considered. The rate of decompression can be controlled by inserting a smaller venting line or by other throttling means; however, this will increase the cycle time.

During decompression, the product temperature drops toward T_4 , which may be lower than its initial temperature value (T_1). The difference between the sample initial temperature and final temperature after decompression (T_1 – T_4) can be indicative of the extent of heat loss from the product to the surroundings during processing (Ting et al., 2002).

6.4. Cycle Time

The total time for loading, closing the vessel, compression, holding, and decompression and unloading is commonly referred to as the “cycle time.” The cycle time and the volumetric efficiency (i.e., the percentage of the vessel volume occupied by the product) determine the system throughput and the cost of the HPP treatment (see Chapter 3).

6.5. Process Pressure

“Process pressure” (Figure 1.1, p_2 – p_3) refers to the holding pressure during the sample treatment. The accuracy of the pressure reading should be identified along with the pressure indicated. The recommended level of accuracy both to control and record pressure is $\pm 0.5\%$ (electronic) or $\pm 1.0\%$ (dial display) (Farkas and Hoover, 2000). Most mechanical Bourdon tube-type gauges lack good reliability under heavy use at elevated pressures. Strain gauges on the pressure vessel or displacement transducers on the external frame can be effective and reliable methods to measure pressure. It is recommended that at least two methods be used to measure pressure and an appropriate periodic calibration program should be in place (Balasubramaniam et al., 2004). A reference sensor or gauge should be available for periodic calibration of process instrumentation.

6.6. Product Initial Temperature

The initial temperatures (T_1) of the product, the pressure-transmitting fluid, and the process vessel

must be documented if the temperature is a specified condition for microbial inactivation during high-pressure treatment. For heterogeneous food samples, additional time may be needed to achieve temperature equilibrium within the sample. The high pressures used in food processing do not influence the type K thermocouple readings at temperatures below 500°C (Bundy, 1965). The reference thermocouple sensor should be located at a cold point or in an equivalent zone within the pressure vessel and calibrated to an accuracy of $\pm 0.5^\circ\text{C}$ (Farkas and Hoover, 2000). Standard methods and good laboratory practices regarding temperature measurement should be followed (e.g., Beckerath et al., 1998).

7. Treatment Effects during HPP

Depending on the pressure–temperature regime and duration of exposure, HPP can be used to deliver a variety of treatment effects on the food material. These include food pasteurization, sterilization, blanching, or freezing and thawing (Figure 1.2).

7.1. High-Pressure Pasteurization

Pasteurization treatment typically employs pressures in the range of 600 MPa (87,000 psi) at or near ambient temperatures for a specific holding time (Cheftel, 1995; Farkas and Hoover, 2000; Anon 2006). High-pressure pasteurization treatments inactivate pathogenic and spoilage bacteria, yeasts, and molds, but have limited effectiveness against spores and enzymes (Figure 1.2). The extent of bacterial inactivation also depends on the type of microorganism, food composition, pH, and water activity. Gram-positive organisms are more resistant than gram-negatives. Significant variations in pressure resistances can be seen among strains (Cheftel, 1995; Smelt, 1998). Water activity has a major influence on the rate of microbial inactivation. This is discussed in detail in Chapter 5.

Examples of high-pressure pasteurized products commercially available in the United States, Europe, and Japan include smoothies, guacamole, deli meat slices, ready-meal components, poultry products, oysters, ham, fruit juices, and salsa (Dunne, 2005).

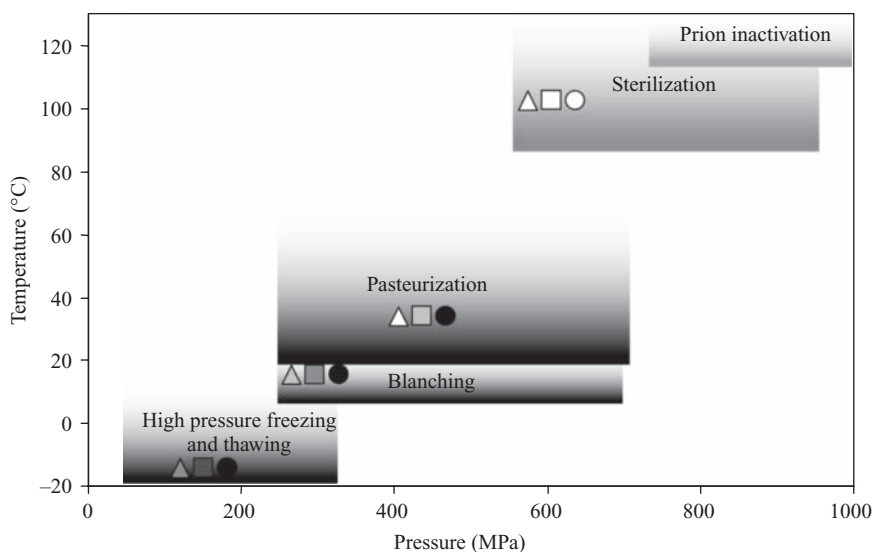


Figure 1.2. Different pressure–temperature regions yield different processing effects. Inactivation of vegetative bacteria, yeast, and mold (□), bacterial spores (○), and enzymes (Δ) are also shown. A filled symbol represents no effect, and an open symbol represents inactivation.

Chapter 4 presents a review of commercial products currently available in the market.

7.2. High-Pressure Sterilization

During typical pressure-assisted thermal processing (PATP) (also referred to as “pressure-assisted thermal sterilization” or “PATs”), the food is subjected to a combination of elevated pressures and moderate heat for 1–5 minutes. One of the unique advantages of PATP is its ability to provide a rapid and uniform increase in the temperature of treated food samples. Uniform compression heating and expansion cooling on decompression help to reduce the severity of thermal effects encountered with conventional processing techniques. Inactivation of various bacterial spores by the combined pressure–temperature treatment is a topic of ongoing research. Prions are even more resistant than spores under combined pressure–temperature treatment (see Chapter 5 for additional discussion). Limited studies evaluated PATP conditions under which prions can be inactivated. For example, Brown et al., (2003) reported that elevated temperatures (121–137°C) and pressures (690–1,200 MPa) are required.

7.3. Quality of Pressure-Sterilized Products

PATP technology reportedly reduces process time and preserves food quality, especially texture, color, and flavor as compared to retorted products (Hoogland et al., 2001; Krebbers et al., 2002, 2003; Juliano et al., 2006). Preheating and subsequent heat transfer during combined pressure–thermal treatment can influence the quality of PATP samples (Nguyen et al., 2007) (Figure 1.3). During 2009, the Food and Drug Administration approved a petition for pressure-assisted thermal sterilization of low-acid products (Food Processing, 2009). Shelf-stable, low-acid foods processed by this technology are not yet commercially available; however, the technology has the potential for sterilizing heat-sensitive products such as soups, egg products, coffee, tea, and mashed potatoes.

7.4. Pressure Pulsing

Application of two or more pressure pulses (referred to as “pressure pulsing” or “oscillatory pressure treatments”) has been shown to be more effective (Meyer et al., 2000) than single pulse treatments with an equivalent pressure-holding time. Pulse treatment can be utilized for both food pasteurization and sterilization. The measure of improved inactivation by pulsed pressurization must be weighed against the design capabilities of the pressure unit, the added compression costs, added wear on the pressure unit, possible detrimental effects on the sensory quality of the product, and the additional time required for cycling.

7.5. Pressure Applications during Freezing and Thawing

Conventional freezing at atmospheric pressure may cause structural damage due to the formation of larger ice crystals. Rapid freezing using cryogens can induce cracking, possibly due to the initial decrease of volume from cooling and the subsequent increase in volume from freezing (Kalichevsky et al., 1995). As per the LeChatelier’s principle, pressure opposes reactions associated with volume increase such as the state change that occurs during the transition from liquid water to ice. This provides opportunities for pressure-assisted freezing and thawing, pressure-shift freezing, and pressure-induced thawing so that food material can be preserved under subzero temperatures without ice crystal formation (Figure 1.4) (Benet et al., 2004). During pressure-assisted freezing and thawing, the phase transition occurs under constant pressure (Figure 1.4, a-b-e-f or f-e-b-a). During pressure-shift freezing, the sample is cooled under pressure to below 0°C, but is kept in the liquid state. Once the desired temperature is reached in the product, the pressure is released (Figure 1.4, a-c-d-f). This results in super cooling and rapid ice nucleation. Researchers demonstrated that this can reduce the freezing point and can promote rapid ice nucleation and growth throughout the sample, thus producing small ice crystals. The process can result in a better preserved microstructure and texture

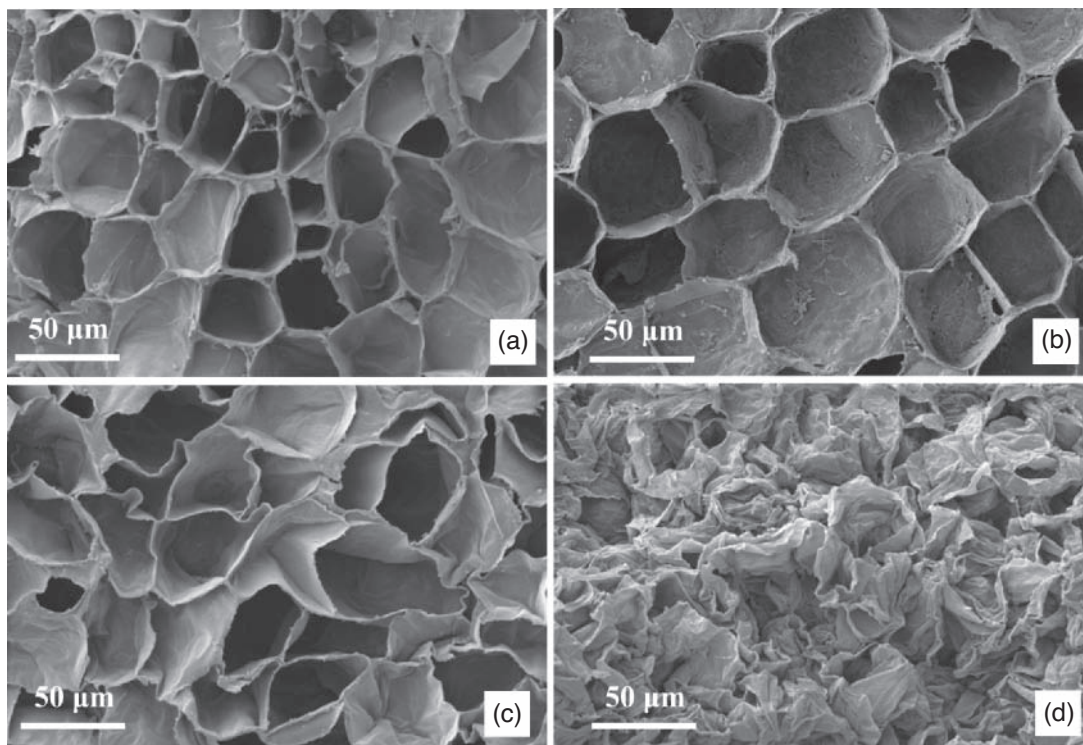


Figure 1.3. Microstructures of (a) control, (b) pressure treated (700 MPa, 25°C, 5 minutes), (c) pressure-assisted thermal processed (700 MPa, 105°C, 5 minutes), and (d) thermal processed (105°C, 0.1 MPa, 30 minutes) carrot samples (Nguyen et al., 2007).

and less drip losses than conventional frozen products (Otero et al., 2007). During pressure-induced thawing, a frozen product can be forced to the liquid state by applying pressure (Figure 1.4, pathway f-d-c-a). This facilitates faster thawing. Pressure-induced thawing is likely to have many applications in the food industry, especially for products in which significant sample deterioration occurs during thawing.

7.6. Pressure-Assisted Blanching

Eshtiaghi and Knorr (1993) reported that HPP at or near ambient temperatures can be effectively used to blanch food products. This process is similar to hot water or steam blanching, but with much reduced thermal degradation. This can help minimize problems associated with water disposal. For example,

the application of 400 MPa or 58,000 psi pressure at 20°C for 15 minutes blanched potato samples and provided a 4-log cycle reduction in microbial count while retaining 85% of the ascorbic acid. Complete inactivation of polyphenoloxidase was achieved when a 0.5% citric acid solution was used as the blanching medium. The addition of a 1% CaCl_2 solution to the medium also improved potato texture. The leaching of potassium from the high-pressure-treated sample was comparable with a 3-minute hot water blanching treatment (Eshtiaghi and Knorr, 1993).

8. Properties of Food Materials under High Pressure

HPP requires knowledge of the pressure dependency of various thermal and physical properties such as

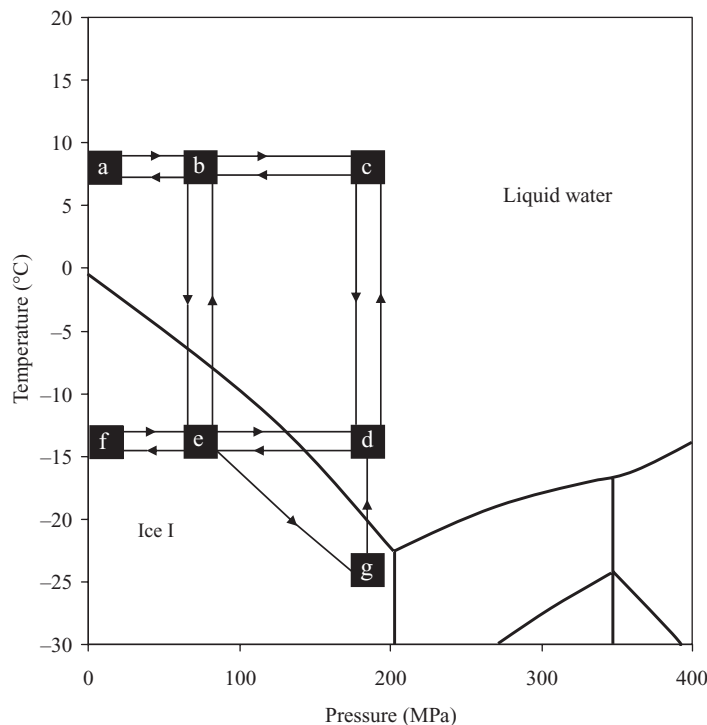


Figure 1.4. High-pressure application in freezing and thawing (Denys et al., 2001).

thermal conductivity, specific heat, density, and viscosity of food materials to evaluate heat transfer within the processed volume. While pressure primarily affects the volume of the system, heat transfer can cause both volume and energy changes within the system. Knowledge of combined pressure–thermal effects on food properties can facilitate the understanding of the uniformity of pressure treatment on microbial safety and the quality of food material.

After the pioneering work of Bridgman (Bridgman, 1931), the properties of water under pressure were well documented. Data are available from the International Association for the Properties of Water and Steam (IAPWS). A software implementation of IAPWS work can be obtained from the US National Institute of Standards and Technology (NIST) (Harvey et al., 1996). Very limited information is available on properties of food materials under pressure because of the practical challenges associated with the in situ measurement of these properties at

elevated pressures (Ramaswamy et al., 2005). The effect of pressure on the thermal conductivity, density, and viscosity of selected food materials is given in Figure 1.5. Thermal conductivity and density of material increase with an increase in pressure (Figure 1.5a and b). Water viscosity decreases from 0.1 to 200 MPa (14.5–29,000 psi), while the range of 300–600 MPa (43,500–87,000 psi) produces a slight increase of viscosity (Figure 1.5c). A further increase of pressure is associated with a drastic reduction in water viscosity.

8.1. Compressibility

During HPP of food materials, the gross structure of the food material is compressed. Compressibility is an intrinsic property of the material and is defined by the balance between attractive and repulsive potentials. Compression of a liquid decreases the average intermolecular distance and tends to

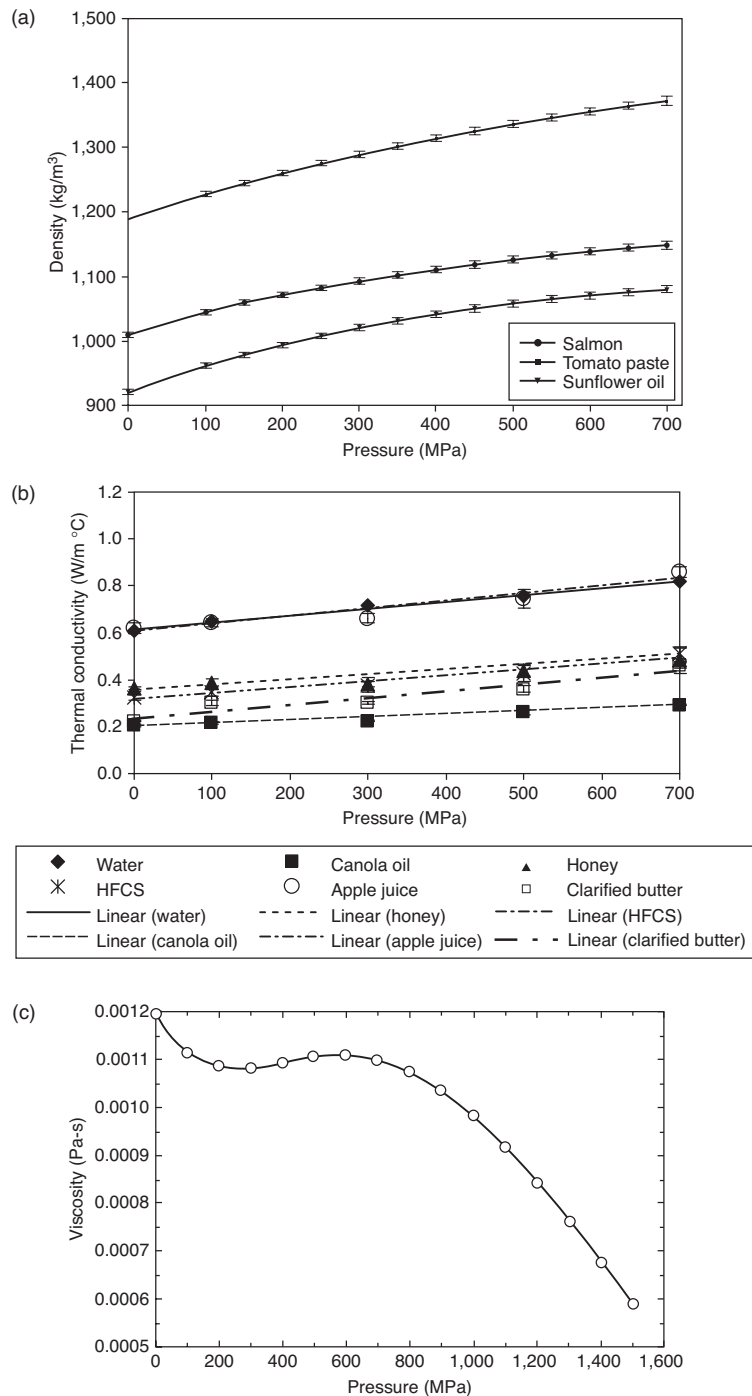


Figure 1.5. Selected properties of food materials under pressure: (a) density of salmon fillet, tomato paste, and sunflower oil as a function of pressure at 25°C (error bars represent uncertainty of density data) (Min et al., 2009); (b) thermal conductivity values of selected liquid foods under high pressure (data points with error bars indicate mean $\pm$ standard deviation) (Ramaswamy et al., 2007); (c) viscosity of water under elevated pressures at 25°C (Harvey et al., 1996).

reduce rotational and translational motion. Food material (e.g., orange juice) is considered to contain molecules that occupy space in excess of that needed for close packing. This excess is called “free volume,” and it is this volume that is reduced in initial compression (Rasanayagam et al., 2003). At elevated pressures, when the free volume has largely disappeared, a reduction in the van der Waals dimensions may occur and the compressibility is greatly diminished. Isothermal compressibility (β) is defined as the relative change in volume (V) with pressure (P):

$$\beta = -\frac{1}{V} \left(\frac{\partial V}{\partial P} \right)_T \quad (1.4)$$

Very limited information is available on the compressibility of food materials under pressure. The temperature of the food substances also changes during physical compression (Ting et al., 2002) (Figure 1.1). This temperature change causes thermal expansion of the material. The thermal expansion coefficient (α) is another thermodynamic property that provides a measure of the amount by which the density changes in response to a change in temperature at constant pressure:

$$\alpha = \frac{1}{V} \left(\frac{\partial V}{\partial T} \right)_p \quad (1.5)$$

Denys et al. (2000a) reported the thermal expansion coefficient of apple sauce and tomato paste at different combinations of temperature and pressure. The values were lower than that of pure water under these process conditions.

8.2. Heat of Compression

The instantaneous temperature change in materials during compression or decompression is often called the “heat of compression” (Otero et al., 2000; Rasanayagam et al., 2003; Ardia et al., 2004). The heat of compression can be estimated theoretically using the equation:

$$\delta = \frac{dT}{dP} = \frac{T\alpha}{C_p\rho} \quad (1.6)$$

where α , T , ρ , and C_p represent the thermal expansion coefficient, temperature, density, and heat

Table 1.3. Estimated compression heating factors (°C per 100 MPa) of water at various initial temperatures^a

Initial Sample Temperature (°C)	Heat of Compression Factor (°C per 100 MPa)
0	1.6
15	2.5
30	3.0
45	3.5
60	4.0
75	4.6
90	5.3

^aEstimated using NIST ASME software (Harvey et al., 1996).

capacity at constant pressure, respectively. Equation (1.6) is strictly applicable only to small pressure changes (Otero et al., 2000). An accurate estimation of volumes (Equation (1.3)) or thermal expansion (Equation (1.4)) under pressure is difficult to obtain due to challenges associated with developing reliable sensors and instrumentation that can withstand elevated pressure conditions. Alternatively, researchers often estimate the heat of compression values experimentally by directly monitoring temperature changes in the substance during compression or decompression (Otero et al., 2000; Rasanayagam et al., 2003; Patazca et al., 2007). Most foods exhibit a compression-heating behavior very similar to that of water, because water is usually their main ingredient. Among the food constituents, water, being a compact polar molecule, has the least heat of compression value under pressure (3°C per 100 MPa at 25°C) (Table 1.3). Nonpolar fats and oils with long-chain fatty acids have higher heat of compression values (up to 9°C per 100 MPa) (Table 1.4).

While for water, the heat of compression values increases with an increase in its initial temperature (Table 1.3); values of fats and oils are not much influenced by the initial temperature (Table 1.4). Proteins and carbohydrates have intermediate heat of compression values. The differences in the thermal response of water, fats, and oils can be attributed to their molecular structure and phase transition characteristics. If heats of compression values for various food constituents are known, the average temperature (T_2) of the test sample at the beginning of

Table 1.4. Heat of compression for various foods pressure treated at 25°C^a

Substance at 25°C	Temperature Change per 100 MPa ^a
Juice, tomato salsa, 2% fat milk, cream cheese, and other water-like substances	3.0
Tofu	3.1
Egg albumin	3.0
Mashed potato	3.0
Yogurt	3.1
Honey	3.2
Salmon	3.2
Chicken fat	4.5
Water/glycol (50/50)	From 4.8 to 3.7
Beef fat	6.3
Olive oil	From 8.7 to 6.3
Soy oil	From 9.1 to 6.2

Source: Otero et al. (2000), Rasanayagam et al. (2003), and Patazca et al. (2007).

^aWhile the initial temperature does not influence heat of compression values for fatty substances, the values increase with initial temperature for water-based foods (see also Table 1.3).

pressure-holding time can be estimated using simple mixture rule shown in the following equation:

$$T_2 = T_1 + \left(\frac{\sum_{i=1}^n \delta_i \times M_i}{M} \right) (\Delta P) + \Delta T_H \quad (1.7)$$

In this equation, T_1 is the sample initial temperature, M is the total mass, M_i is the mass of individual constituents, and P is the applied pressure. ΔT_H is temperature gain (or lost) between the test sample and the surrounding during product loading within the pressure chamber and pressurization. For example, if a product, consisting of several constituents at an initial temperature of 75°C, is compressed to 700 MPa (101,500 psi), it could reach a maximum process temperature of approximately 106°C as a result of compression heating. This temperature will be the average of the combined heats of compression of the several constituents present in the sample. The above

example only considers the temperature change in the product as a result of the heat of compression and assumes no heat exchange with the surroundings. However, in practice, heat exchange is likely to occur. Temperature changes resulting from heat transfer (ΔT_H) between the product and external factors such as compression fluid, pressure vessel, and the environment must be empirically determined by actual test. The time-dependent heat transfer between the test sample and the surrounding factors during the product loading, compression, and holding phase must be considered. It is worth noting that measured ΔT_H values are likely influenced by the insulation characteristics of the pressure equipment used, operator skill, and process conditions employed. Once ΔT_H values are determined, the initial sample temperature can then be suitably adjusted to achieve the desired final product temperature (Nguyen et al., 2007).

8.3. Thermal Conductivity

There are a limited number of studies reporting the thermal conductivity (k) of food materials under pressure. Denys and Hendrickx (1999) studied the k of tomato paste and apple pulp at pressures up to 400 MPa (58,000 psi). Zhu et al. (2007) studied k values of potato and cheddar cheese at pressures up to 350 MPa (50,750 psi) at 5°C. These foods showed a thermal conductivity increase with an increase in pressure. The increase in thermal conductivity was influenced by the amount of moisture present in the food material. Ramaswamy et al., (2007) reported on the thermal conductivity of selected liquid foods under pressure. Water and water-like substances (apple juice) were found to have the highest k values (up to 0.82 W/m°C at 700 MPa (101,500 psi) and 25°C), while fatty foods such as canola oil and clarified butter had the lowest values (0.29–0.40 W/m°C, respectively, at 700 MPa and 25°C). Honey and high-fructose corn syrup had intermediate values (Figure 1.5b). The estimated k values of all the food materials tested under pressure were higher than the corresponding k values of materials under atmospheric pressure (Ramaswamy et al., 2007).

8.4. Specific Heat

The specific heat values of foods at atmospheric pressure are measured using techniques such as the method of mixtures or differential scanning calorimetry. However, there is very limited literature using these techniques for estimating specific heat values of food materials under high-pressure process conditions. Data on the specific heat of pure water, as a function of elevated pressure and temperature, are readily available through the NIST database (Harvey et al., 1996). These values are approximately 10% lower than those estimated at ambient pressures. In the absence of experimental data, researchers often ignore the effect of pressure on specific heat in heat transfer calculations.

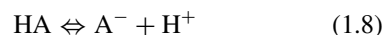
8.5. Density

When a food material is processed under pressure, there can be a significant decrease in the volume of the product. This decrease is due to a reduction in the “free volume” between molecules and the compacting of voids occupied by gases. These changes can, in turn, influence temperature process uniformity during HPP. The density of a material under pressure can be estimated by determining its change in volume under pressure and its mass. Density is then calculated as the ratio of mass to volume. The volume change of a material under pressure can be estimated by using a linear velocity differential transducer (Bridgman, 1931). Changes in sound velocity have also been used to measure density under pressure (Kovarskii, 1993). Denys et al. (2000a, 2000b) estimated the density of food materials using a bulk volume displacement method. Density of selected food materials was measured by a variable piezometer at 25°C up to 700 MPa (Min et al., 2009) (Figure 1.5a)

8.6. pH

During HPP, the pH of food materials, in general, shifts toward a lower pH value as a function of applied pressure. The direction of pH shift and its magnitude depends on the food composition. For a sim-

plified model, the dissociation of acid HA can be described by the equation:



The dissociation of HA is accompanied by a reduction in volume due to the more compact packing of solvent around the charged ions as compared to the uncharged HA molecule. As per LeChatelier’s principle, the dissociation of HA is favored by an increase of pressure, and as a result, the pH of a solution is reduced. El’Yanov and Hamann (1975) developed a theory on the dependency of a dissociation constant on pressure. This dependency is given by the following equation:

$$pK_a = pK_a^0 + \frac{\Delta V_m^0 p}{2.303RT(1 + bp)} \quad (1.9)$$

In this equation, pK_a is the dissociation constant, ΔV_m^0 is the molal volume change between associated and dissociated forms of buffering acid in solution, R is the universal gas constant (8.314 J/mol K), b is a universal constant (9.2 Pa⁻¹), p is pressure, and superscript 0 denotes values at atmospheric pressure. Heremans (1995) reported that apple pH decreased by 0.2 unit with 100 MPa increase in pressure. For a neutral pH phosphate buffer, a pressure of 68 MPa (9,860 psi) results in a decrease of 0.4 pH unit (Johnson et al., 1954). The prediction of pH change during HPP in various foods can be complicated by the composition and the unknown equilibrium constants. More research effort is needed in this area, and pH-measuring instruments that operate under pressure would aid these studies.

9. Process Uniformity during HPP

Combined pressure–heat treatment can provide either synergistic or antagonistic effects on the microbial safety and quality of the processed product. Thus, similar to traditional thermal processing, identification of the least processed volume (“cold-spot”) during HPP will help ensure safety of the processed foods. Knowledge of the least processed volume within a pressure chamber is especially critical for high-pressure sterilization of low-acid, shelf-stable foods. Although both pressure and temperature

can contribute to HPP process nonuniformity, for food processing calculations, pressure is assumed to be uniformly transmitted throughout the processed volume.

A number of factors can influence heat transfer-related process nonuniformity within a pressure chamber. These include the design of the pressure equipment as well as the geometry and insulation characteristics of the pressure chamber (Hartmann et al., 2004). The size of the pressure chamber will affect the rate of change of temperature gradients within the vessel. Larger size pressure vessels likely have slower temperature gradient changes.

The type of pressure-transmitting fluid used strongly influences temporal and spatial temperature distributions. During HPP, the temperature of the food material and the pressure-transmitting fluid increases as a result of physical compression. Subsequently, transient heat exchange takes place among the sample, the pressure-transmitting fluid, and the pressure chamber walls. The resulting temperature gradient in the system can also lead to density differences within the pressure-transmitting fluid and can, consequently, induce free convection (Hartmann, 2002; Otero et al., 2007). Redistribution of momentum and energy may then occur, and this fluid motion strongly influences the temporal and spatial distribution of temperature. Transient temperature and velocity fields also strongly influence each other. The viscosity of pressure-transmitting fluids is another important factor in process uniformity (Hartmann and Delgado, 2002). Fluid viscosity strongly affects the convective transport phenomenon, which contributes to the temporal and spatial distribution of temperature inside the pressure chamber.

The ratio of sample to vessel chamber volume, the size and shape of the package, and the insulation properties of the packaging material can influence the process uniformity due to temperature gradients during HPP (Otero et al., 2007). The packaging material can act as a heat barrier to maintain an “adiabatic” condition of the packed foods (Hartmann and Delgado, 2003). Heat of compression of food material and other relevant thermophysical properties can also influence the process uniformity (Ramaswamy et al., 2005).

10. Modeling Process Uniformity

The determination of temporal and spatial temperature distributions within a high-pressure chamber is dependent on the thermofluid dynamic effects. The process temperature and pressure gradient developed during HPP can be modeled by solving equations for conservation of mass, momentum, and energy. The treatment effect on the product can then be considered by including these gradient temperatures and pressures in relevant equations for microbial or enzymatic kinetics. Denys et al. (2000a) used residual enzyme activity and a numerical heat-transfer model for evaluating process uniformity in apple sauce and tomato paste. The residual enzyme activity distribution appeared to be dependent on the inactivation kinetics of the enzyme under consideration and the pressure–temperature combination considered. Hartmann et al. (2003) studied the influence of heat and mass transport effects on the uniformity of high-pressure-induced microbial inactivation. Their results showed that the effective inactivation rate increased with the increase in size of the high-pressure vessel. However, a more than one log variation in the residual surviving cell concentration could be observed, depending on the package material used, and the position and arrangement of the packages in the vessel. Hartmann et al. (2004) studied the thermofluid dynamics and process uniformity of HPP in a laboratory-scale autoclave using experimental and numerical simulation techniques. Ghani and Farid (2007) proposed a simulation study of heat transfer during HPP of food using computational fluid dynamics.

11. Approaches to Minimize Process Nonuniformity

Several approaches have been proposed to minimize thermal nonuniformity during pressure treatment. Temperature control of the product, package, pressure-transmitting fluid, and the pressure vessel for each cycle during HPP is critical (Farkas and Hoover, 2000). It is also important to consider the heat of compression of the various materials (e.g., food, pressure-transmitting fluid, and package) and

target pressure. The initial temperature of the materials can then be adjusted to minimize thermal gradients during pressure-holding time (Ting et al., 2002; Balasubramaniam et al., 2004). In addition, use of an external temperature control jacket for heating or cooling can help minimize temperature gradients within the system. Thermal insulation of the inner wall of the pressure chamber can also aid process uniformity (Hartmann et al., 2004). Finally, the selection of appropriate packaging materials can contribute to better thermal uniformity during HPP. For example, a high degree of uniformity can be achieved when packaging materials with good insulating characteristics are used (Hartmann and Delgado 2003, 2005).

12. Conclusion

HPP of foods offers a commercially viable alternative for food processors to preserve a variety of food materials with reduced thermal impact. Depending on the combination of pressure and temperature used, a variety of treatment effects, including freezing and thawing, pasteurization, sterilization, and blanching, are possible. The technology has been found to be effective for the control of a variety of pathogenic vegetative bacteria including *Salmonella*, *Escherichia coli*, and *Listeria* at room or modest temperatures. Combined pressure–thermal treatment demonstrated that spores could be inactivated. Although more research is needed to evaluate process uniformity and estimate in situ properties of food materials under pressure, HPP has demonstrated a significant advance in the quality of preserved foods.

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Chapter 2

High-Pressure Processing Equipment Fundamentals

Edmund Ting

1. Introduction

Early high-pressure researchers conducted their experiments in primitive steel and cast iron pressure vessels. These were designed without the benefits of modern engineering theory and materials. Danger was ever present because of equipment failures that could lead to the release of stored mechanical energy in flying objects as well as the release of potentially hazardous substances. During the late 1940s and early 1950s, theories of metal fatigue became well established. Advances in materials processing during this period resulted in advanced structural materials allowing for much safer high-pressure equipment. Today, the combination of advanced stress analysis, fracture mechanics, non-destructive inspection, high strength–high fracture toughness materials, and a century of experiences allow engineers to design and build higher pressure, larger volume, and safer pressure systems than ever before.

As commercial applications for high pressure increase, food processors interested in the use of high-pressure processing (HPP) need to understand the technology to be able to select and operate high-pressure systems in a cost-effective and safe manner. Every system consists of multiple subcomponents. This chapter describes the major components of modern HPP systems. It is not possible, nor is it intended, for any one to use this chapter to se-

lect or design high-pressure equipment. This chapter describes how pressure vessels are designed, constructed, and operated. The chapter also covers the necessary pump-intensifiers, closure systems, and controls so that persons interested in the commercial use of HPP can have a good basic understanding of the entire system.

2. Pressure Vessels and Their Materials and Methods of Construction

In every high-pressure system, there are components or parts of components which are loaded to high stress. Most of the time, these stresses are not sufficient to break the component outright on the first cycle. However, repeated loadings may result in the failure of the component. This “fatigue damage” is illustrated by repeatedly bending a steel paper clip. While most people cannot break the paper clip in one bend, after 10–50 bend reversals, the paper clip will break in half. This illustrates the principle of metal fatigue.

For any given stress, the number cycles a highly stressed part can endure is related to the strength of the materials of construction and the sensitivity of these materials to cracking. Unfortunately, most high-strength materials do not have good resistance to cracking. An example of a high-strength material with poor cracking resistance is the ceramic alumina. Alumina ceramic can tolerate very high stress, but once a crack forms, crack growth will be very fast and unpredictable. Thus, strong but brittle materials are not suited for pressure vessel construction. For all practical purposes, pressure vessels operating at

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pressure above 275 MPa (40,000 psi) are typically made from high-strength steel alloys with high fracture toughness.

Resistance to wear is another very important materials and design factor. Many highly stressed parts are also in relative motion with mating parts. The tribology (friction wear) between these parts can play a significant role in surface damage and subsequent crack initiation. The choice of materials that contact each other can play an important role in how each material will behave. For example, while there are many materials that have good tolerance to high stress, they might not be adequate under repeated loading and rubbing contact with certain other materials. Additionally, under the high stress of high-pressure equipment, the use of identical materials in rubbing contact is frequently undesirable as this can lead to cold welding of the parts.

Corrosion is also an important factor in components, which are subject to water contact. Many early pressure vessels required the use of nonrusting liquid compression media such as oils. The use of oil greatly affected the high-pressure process because of the greater compression heating characteristic of oils. During adiabatic compression, many oils can show compression heating of up to 10°C per 100 MPa (14,500 psi). Thus, compression to 1,000 MPa (145,000 psi) with an oil may cause a resulting temperature rise of up to 100°C. This makes the separation of pressure and thermal effects difficult when food is being treated. More data on heat of compression of various food materials is presented in Chapter 1.

Since food-processing equipment is subject to frequent washing and sanitizing, even water-compatible materials of construction, such as certain stainless steels, may be subjected to corrosion. Under corrosive conditions, certain otherwise very-high-strength materials are susceptible to becoming brittle and to easy cracking. Most importantly, in some situations, the pressure vessel material may come into direct contact with food acids or corrosive food products. In these situations, the suitability of materials of construction from the chemical and regulatory (Food and Drug Administration [FDA]) points of view needs to be considered.

3. Pressure Vessel Design

In the late 1800s, when Hite and his colleagues did their work (Hite et al., 1914), a pressure vessel was just a thick wall cylinder. Modern pressure vessels and closure systems can be a complex of parts. The construction of the vessel may use one of three common approaches depending on vessel operating pressure and diameter. The three cylindrical vessel construction methods are a single forged monolithic chamber, a series of concentric tubes shrunk fit on each other to form a multiwall chamber, and a stainless steel core tube compressed by a wire winding.

The monolithic or “monoblock” vessel is the simplest to make. At pressures less than 400 MPa (58,000 psi) and internal diameters less than 15 cm (5.9 inches), these are generally the least expensive to fabricate. The fatigue life of these vessels can be significantly increased by the use of autofrettage. Autofrettage is a fabrication method in which the pressure vessel is subjected to an enormous overpressure. This overpressure causes the region nearest the inside wall to undergo plastic deformation and the outer region of the vessel to shrink slightly. This puts the internal region of the vessel into compressive residual stress after the removal of the overpressure. The presence of a high compressive stress in the inner wall makes it difficult for cracks to grow, and fatigue life could be greatly enhanced. For vessels operating at very high pressures, it is difficult to create the high overpressures needed for autofrettage. Also, for large diameter chambers, thicker walls are needed. As the vessel wall thickness is increased, it becomes progressively more difficult to apply the right heat treatment to obtain the optimum mechanical properties. For all practical purposes, single wall, monolithic, vessels are not possible at pressures above 400 MPa (58,000 psi) and diameters above 15 cm (5.9 inches).

Multiwall pressure vessels can achieve the needed compressive residual stresses on the inside wall with an interference fit. This is achieved by shrink fitting one or more concentric cylinders, of increasing inside diameter, over the inside cylinder. The amount of interference, or residual compression force on the inner cylinder wall, is controlled mainly by the thermal or mechanical expansion of the materials of

construction. Multiwall chambers have an important advantage over autofrettagged monolithic chambers as in that crack propagation in multiwall chambers is physically limited to each layer of the chamber. Thus, an internal crack starting at the inner wall will not lead to a situation where the compressed contents can leak explosively from the vessel into the workspace. Thus, multilayer vessels are typically safer than single wall vessels and are generally designed to a “leak before break” code standard.

Wire wound pressure vessels generate their compressive residual stress on the inner cylinder wall by layering of high-strength wire under tension. The wire is wound onto a thin wall core of the pressure vessel. The advantage of wire wound pressure vessels is that the strength of the wire is independent of the size of the cylinder that is being wound. As a result, the physical properties of the wire can stay very high without concern for heat treating. The wire wound vessel shares, with the multiwall vessel, the added safety advantage of having a leak before break construction. A crack cannot grow continuously and uninterruptedly from the inside wall to the outside of the vessel. Additionally, wire wound vessels may weigh less than a multiwall vessel of equal capacity. Vessel weight is an important consideration during shipping and installation. Currently, the highest operating pressures and largest diameter pressure vessels are made using wire winding technology. While wire wound vessels may be considered to have cycle lives measured in the millions of cycles, there is a finite cycle life of the core cylinder. The core cylinder may require periodic replacement due to formation of small cracks. The need for periodic core cylinder replacement is an added operating cost for wire wound vessels used in food processing. Typically, a food-processing vessel may be subjected to 50,000 or more cycles per year (see Chapter 4 for discussion of operating cost).

4. Vessel Closures

HPP vessels used in food processing require rapid closing and opening systems that allow rapid loading and unloading of the vessel. Closure design is a major materials handling and safety concern. The challenge

of rapidly opening and closing a large pressure vessel closure is proportional to the diameter and operating pressure of the vessel. For example, a vessel with a 38 cm (15 inches) diameter closure has a cross sectional area of 1,136 cm² (176 sq inches). If the vessel is designed to operate at a pressure of 680 MPa (98,600 psi), the force acting on the closure is over 8,000 metric tons, roughly the weight of 20 fully loaded 747 aircraft.

Small diameter or lower pressure systems can use the vessel cylinder wall to carry the loads from the closure. These vessels typically use threaded- or breech-type closures or in some cases a pin closure. The pin passes through the vessel wall through the closure, and through the opposite side of the vessel. The closure loads are added to the pressure loads acting on the pressure vessel walls. Threaded closures are very reliable at lower pressures. However, if threaded vessels are abused or subjected to neglect, the threaded portion of the cylinder or closure could be subjected to cracking. Since a threaded closure crack typically will not result in leakage, these cracks can grow unnoticed to the point of failing, resulting in a rapid release of compressed energy into the workspace. This becomes a major catastrophe if the pressure vessel stores substantial energy.

At higher pressures and at larger diameters, closure loads become so large that a secondary structure is required to carry the closure loads. This secondary structure typically is an external frame or yoke made of high tensile strength steel or a wire wound frame in the shape of a yoke. For this reason, all high-pressure food-processing vessels use a secondary structure to hold the end closures in place (Figures 2.1 and 2.2).

Since the frame or yoke is subjected to high-stress cycling, some frames are constructed with prestressed steel to better resist cyclic loading. Prestressed frames are produced with pretensed tie-rods or wire wound yoke structures.

5. Operating Temperature Considerations

Most materials used in the construction of pressure vessels operating between 0 and 80°C perform

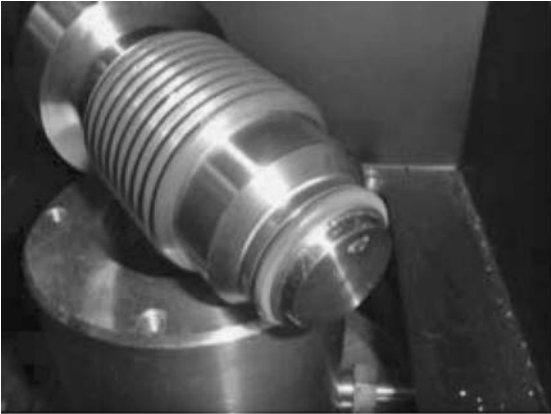


Figure 2.1. Typical small pressure vessels using a threaded closure. (Photo courtesy of Pressure Biosciences Inc.) (For color detail, see color plate section.)

without any problems. If pressure vessels are subject to very low temperatures, more than 20 degrees below 0°C, the fracture behavior of the vessel construction material can change and operating pressures must be reduced. At temperatures above 100°C, the beneficial residual compressive stresses added to the vessel during design or manufacturing can re-

lax and operating pressures must be reduced. It is for these reasons that all pressure vessels designed to American Society of Mechanical Engineers (ASME) Codes are stamped with a safe temperature range of operation in addition to their safe maximum operating pressure.

Food processors considering the use of high-pressure treatments must specify both the maximum operating working pressure needed and the lowest and highest operating temperatures needed (see Chapter 1 on typical process conditions employed in various food process operations). These specifications allow pressure vessels to be designed and constructed, using special steels, gaskets, seal materials, and modified construction methods, to accommodate the temperature and pressure range needed.

6. Pressure Vessel and Yoke Orientations: Vertical, Horizontal, and Tilting Systems

Pressure vessels, and their associated closure retaining yokes, can be operated at any desired angle of repose from horizontal to vertical. Units are available that tilt to receive product through the top opening, swing to a vertical position to engage a yoke during pressure treatment, and then tilt to release treated product from their bottom opening (see Figure 2.3).

Historically, large pressure vessels have been built to operate in the vertical position and are loaded and unloaded through their top opening Figure 2.4.

The vertical orientation enables a simple vertical hoist to be used for loading and unloading a carrier containing the product. Vertical orientation facilitates vessel placement inside a barrier to protect operators. Gas-filled pressure vessels operating at high pressures are usually placed in a pit to direct an explosive release of gas upward. Water-filled systems used in food processing operate with substantially less stored compression energy, and simple shielding against high-pressure leaks can provide the necessary worker safety.

In practice, the available ceiling height of the area, into which the pressure chamber is being installed, may dictate the orientation of the vessel (Figures 2.5



Figure 2.2. Wire winding can be used for both the pressure vessel and the yoke to support the end closure force. (Photo courtesy of Avure.) (For color detail, see color plate section.)



Figure 2.3. Pressure vessels, and their associated closure retaining yokes, can be operated at any desired angle of repose from horizontal to vertical. Units are available that tilt to receive product through the top opening, swing to a vertical position to engage a yoke during pressure treatment, then tilt to release treated product from their bottom opening. (Photo courtesy of Elmhurst.) (For color detail, see color plate section.)



Figure 2.4. Historically, large pressure vessels have been built to operate in the vertical position and are loaded and unloaded through their top opening. (Photo courtesy of Avure.) (For color detail, see color plate section.)



Figure 2.5. A second-generation yoke-based system with a 215 L volume which operates at a pressure of 600 MPa (87,000 psi). It is vertically orientated to minimize floor space. A high power pump-intensifier is located below the loading and unloading area. (Photo courtesy of Avure.) (For color detail, see color plate section.)

and 2.6). A horizontal orientation may make product movement in and out of the pressure vessel easier to integrate into a production line if ceiling height is a limitation and process floor space is available. From a pressure vessel point of view, while orientation



Figure 2.6. A third-generation 350 L volume, 600 MPa (87,000 psi) system that is capable of a 2,300 kg (5,000 pounds) per hour throughput. It is easily integrated into a horizontal product delivery subsystem. (Photo courtesy of Avure.) (For color detail, see color plate section.)

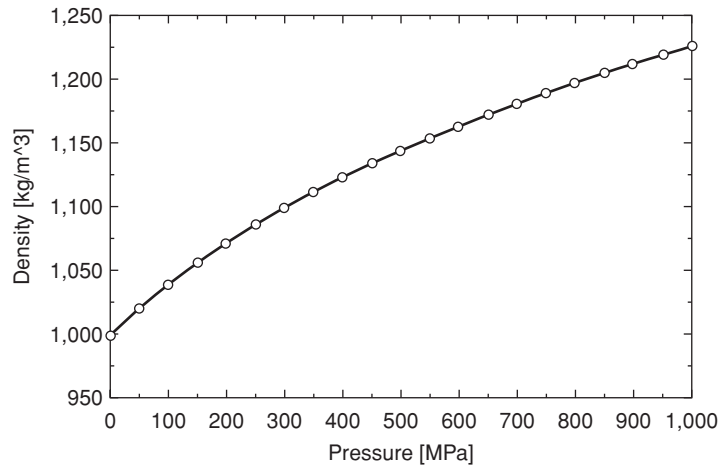


Figure 2.7. The density of water is plotted against pressure under adiabatic compression conditions. Starting condition is at 23°C.

has little effect on pressure performance, it has a major effect on product loading and unloading and compression water handling.

7. Pump–Intensifiers and Supporting High Pressure Components

All large pressure vessels require an external pump–intensifier system to achieve the designed operating pressure as quickly as possible. These pump–intensifier systems use an electric motor to drive a lower pressure pump to compress the hydraulic fluid that drives the intensifier used to compress the water entering the process vessel. The intensifier uses low-pressure hydraulic fluid to drive a large diameter piston. This piston is connected to a small diameter piston, which delivers the high pressure water to the vessel. For example, if the ratio of the area of the large piston to the small piston is 20:1, then 34 MPa (4,900 psi) on the large piston becomes 680 MPa (98,600 psi) on the small piston. With noncompressible liquids, the volume of liquid delivered to the large piston is 20 times the volume of the liquid delivered from the small piston. Since water at 680 MPa (98,600 psi) is compressed about 18%, the major energy consumption in operating an intensifier is in water compression at the higher operating pres-

ures. Figure 2.7 shows the compressibility of water with pressure. The shape of the compressibility curve suggests using a two-stage intensifier with the 2nd stage operating from 400 MPa (58,000 psi) to 680 MPa (98,600 psi).

The design of reliable and easy-to-maintain pump–intensifier systems is a complex task since an intensifier must cycle many times from atmospheric to operating pressure to deliver the necessary compression water to the vessel. Historically, the cost and time spent on system repair and maintenance mostly will be associated with pump–intensifier issues. Pump intensifiers are under continuous improvement to meet the needs of the food processing industry where pressure vessels are expected to operate in the range of 50,000–100,000 cycles each year.

8. Control Systems

Other than controlling the operation of the pump–intensifier and closures, a control system can be required to keep processing records and control-automated product movement. Depending on the requirements, the control system can be a complex and expensive part of an operating high-pressure installation. Electronic record keeping, as regulated by the

FDA, will need to meet strict software development standards. Control hardware must be designed to operate in a food plant environment, must be easily cleaned and sanitized, and be user friendly.

Processing conditions such as pressure and temperature may need to be monitored with certifiable certainty. National Institute of Standards (NIST) sensor traceability and sensor redundancy will likely be required for certain processes. For example, pressure transducer drift or failure modes must be anticipated and addressed when HPP is defined as a critical control point in a hazard analysis critical control point (HACCP) program. When product movement is automated, product bypass of the pressure treatment must be prevented. The use of batch or individual bar codes or Radio Frequency Identification (RFID) tags might be needed to track and certify product movement. In some situations, pressure-sensitive indicators may be attached to product carriers or individual products to give a positive indication of high-pressure exposure.

9. Other System Considerations

The effectiveness and uptime of a HPP system depends on the effectiveness of many subsystems ranging from the quality and temperature of the process water to the type, size, and shape of the packages being treated. Packaging must be designed to optimize loading volumetric efficiency to achieve cost-effective utilization of the pressure vessel. This is discussed in detail in Chapter 3. When the product is removed from the pressure vessel, it has to be dried and overwrapped for distribution. The extent of automation selected for loading and unloading the pressure vessel, drying the treated product, and overwrapping treated packages for distribution will reflect the production and marketing needs of the company producing the product.

10. Laws Regulating the Installation and Operation of High-Pressure Equipment

In many locations, the operation and safety of high-pressure food processing vessels come under state

and city regulations for boiler inspection and operation. These laws are passed by each state or local government and can vary significantly in coverage and requirements. While many states have adopted ASME's Section 8 Division 3, "Alternative Rules for the Construction of Pressure Vessels" as legal standards, many states also have exemptions from regulations for certain smaller pressure vessels (ASME, 2009). Some states, such as Texas, do not have pressure vessel laws. Technically, in these states, pressure vessels are not subject to inspection or regulation regardless of pressure or volume. For most states, meeting ASME Section 8 Division 3 requirements, as shown by an ASME Code stamp on the pressure vessel, is sufficient for compliance. ASME Section 8 Division 3 construction rules identify the allowable stress analysis methods for pressure vessel design, approved materials, testing methods, and other engineering practices. Additional Occupational Safety and Health Administration (OSHA) regulations need to be addressed in any industrial plant. For states without pressure vessel laws, OSHA general laws may cover pressure vessel safety. All pressure vessels used in commercial food processing should carry a stamp indicating that they have been designed, constructed, tested, and certified to operate at their designed pressure and temperature range.

In Europe, the safety of high-pressure equipment design and operation is subject to Pressure Equipment Directive (PED 97/23/EC). The PED allows a common standard to apply to all of the EU nations. Similar to the ASME Section 8 Division 3, the PED provides safety elements for design, manufacturing, materials of construction, testing, and other engineering practices. In the EU, certain other regulations, such as EMI, must be addressed in order to achieve the mandatory overall CE mark.

11. Conclusion

The use of HPP systems for the preservation of foods has taken its place along with other preservation technologies such as steam retorts, aseptic filling, and ammonia freezing systems. Design and operating codes ensure safety of HPP systems. Commercial pressure vessels and pump-intensifier systems

have shown their reliability and safety over millions of cycles of operation to yield millions of kilograms of pressure-treated products. Pressure vessel, closure technology, and pump–intensifier systems continue to benefit from ongoing research and development by equipment companies, suppliers of valves, tubing, and seals, and by university research programs.

The expansion of HPP has resulted in lower processing costs as pressure vessel and pump–intensifier costs are reduced due to increased demand for systems. It is expected that HPP will continue to capture an increasing share of the refrigerated foods market

due to the increased quality, safety, and shelf life of these foods.

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Chapter 3

High-Pressure Processing Pathways to Commercialization

Daniel F. Farkas

1. Introduction

Pressures from 200 to 700 MPa (29,000–101,500 psi) applied at or near room temperature can pasteurize low-acid foods and can commercially sterilize acid foods. Treatments do not depend on the size, shape, or composition of the food and the package as the effects of high pressure are transmitted through the package and food instantaneously and uniformly. High pressure does not break covalent bonds and, thus, flavors, colors, and nutritional values are conserved. High pressure can cause protein unfolding between 0 and 40°C. For example, pressure-treated meats appear denatured, but retain their raw flavor. Food spoilage enzymes are only partially affected by pressure treatments used for preservation. High pressure does not inactivate microbial spores and some viruses at or near room temperature.

During compression, high-moisture foods ($a_w \sim 1$) show a temperature increase of about 3°C for each 100 MPa (14,500 psi). Upon decompression, the temperature returns to the initial value, less any heat lost by the compression fluid to the vessel wall. At 600 MPa (87,000 psi), water volume is decreased by about 17%. Food packaging must be designed to accommodate this decrease in volume on compression, and should be able to expand to its original volume on decompression.

Commercial high-pressure food-processing equipment includes a pressure vessel, a vessel clo-

sure system, a high-pressure water pump–intensifier, controls, and instrumentation for process verification. Ideally, automated materials handling systems feed packages to the pressure vessel and remove treated packages upon decompression. Means must be provided to prevent untreated packages from bypassing pressure treatment.

The cost of high-pressure processing per unit of packaged product is determined by two factors. The first is the volumetric efficiency of the pressure vessel. Volumetric efficiency is determined by the volume of packages treated in each cycle divided by the available volume of the pressure vessel. Volumetric efficiency can be related to the diameter and the length of the pressure vessel, but must be determined experimentally with actual packages of product to be treated. The second factor is the number of compression cycles the high-pressure system can deliver per hour. The inherent high cost of high-pressure processing equipment requires careful planning to ensure maximum product throughput while minimizing operating costs for labor, utilities, repair, and maintenance. A volumetric efficiency of 75% or higher is desirable for commercial, high-pressure-treated, packaged food products. Cycle times of commercial high-pressure vessels can range from 6 to over 10 cycles per hour, assuming a hold time of 3 minutes at pressure. A higher number of cycles per hour can be achieved by rapid vessel loading and discharge, and by using higher horsepower pump–intensifiers to minimize the time needed to bring the vessel to the desired treatment pressure. Prepackage vacuum treatment to occluded gases and vacuum packaging of foods is essential to eliminate package headspace

gas. Occluded and headspace gas reduces volumetric efficiency and increases the compression time.

This chapter provides an analysis of cost and technology for the food companies considering the use of high-pressure food preservation.

2. Planning for High-Pressure Equipment Production Needs—Product Technical Plan

The decision to use high pressure as a commercial preservation method must be based on a technical and business plan. Cost data must be generated by a carefully planned and executed technical plan. Unit product treatment costs must meet identified financial, product, package, operating, and marketing criteria established in the product business plan.

The technical plan must supply accurate data for required high-pressure treatment conditions including product composition and bulk density, package shape and size, treatment pressure and hold time at pressure, product pH, and desired treatment temperature. Package shape and size must be specified so that an accurate pressure vessel volumetric efficiency can be estimated. However, accurate processing requirements can be determined using pilot plant equipment. Product prepared on pilot plant equipment can provide data on sensory qualities, shelf life, microbial safety, quality changes during storage, and product–package interactions. This information can be applied directly to commercial equipment.

Actual volumetric efficiencies must be determined by tests on full-scale equipment using production line packages of product. The product must be prepared under final production conditions to reflect the true bulk density of filled and sealed packages. Volumetric efficiency is a critical cost factor and every effort must be made to obtain a package–product–pressure vessel combination that will maximize volumetric efficiency. The bulk density of each package should be made as high as possible by removing occluded and headspace gases during the preparation, filling, and sealing operation. The bulk density of the filled packages should be optimized and the design size and shape of the package must maximize volumetric efficiency. Additionally,

if product temperature during compression, holding, and decompression is a critical part of process lethality, temperatures must be measured at selected locations in the vessel. These measurements must use actual product and a known controlled initial temperature (IT) for the product, compression fluid, and the vessel walls. Interior vessel walls can be insulated to reduce heat loss. Certain polymers used to insulate the interior walls of the vessel may achieve a higher temperature than the compression of water due to compression heating.

3. Product Business Plan

Marketing criteria can be developed through the pilot plant production of focus samples in sufficient quantities to determine consumer acceptance, price points, storage shelf life, handling requirements during distribution, package requirements, and labeling. The overall production cost of a unit of product will be determined by the commercial high-pressure processing system selected. Costs are determined by actual volumetric efficiency, equipment cycle rate per hour, uptime of the system, labor, repair, maintenance, floor space, and utility requirements. For example, compression water quality, temperature, and volume can be calculated and plans can be made for recycled water reconditioning.

High-pressure food treatment pilot plant facilities are available at universities, through equipment suppliers, and at tolling facilities. These pilot plant facilities are capable of processing a full range of plant- and animal-based foods. Food processors should budget for pilot plant testing in their technical, business, product development, and marketing plans. Detailed information should be developed on package handling and seal performance through actual product tests. Design work to integrate the high-pressure processing system into existing production lines should focus on minimizing labor cost.

4. Determining Commercial High-Pressure System Requirements

The development of realistic processing costs per package can begin when yearly production and peak

production requirements have been estimated and the final product–package design has been selected. The package selected will determine the number of packages that can be treated in available commercial pressure vessels of different diameters and lengths. The volumetric efficiency is the actual volume of the packages treated each cycle divided by the useable volume of the pressure vessel selected. Yearly manufacturing capacity of the pressure vessel is the yearly number of cycles of the vessel, times the volume of the vessel, times the volumetric efficiency of the vessel with the packaging selected. This figure is the volume of product to be processed per year and can be expressed in liters. The volume of packages treated each year is the basis for estimating the cost of processing.

As an example, a processor determines that there is a market for 5 million 1-pound packages of a vacuum-packed product per year. Tests show that the volume of the package selected, using a vacuum wrap, averages 0.475 L. The highest volumetric efficiency that can be obtained with this package, after testing several available commercial pressure vessels, is 0.8. The yearly volume that must be produced by this specific pressure vessel is:

$$\begin{aligned} &0.475 \text{ L/package} \times 5,000,000 \text{ packages/year} \\ &\quad \times 1/0.8(\text{volumetric efficiency}) \\ &= 3,000,000 \text{ L/year} \end{aligned}$$

The number of cycles the vessel must complete to achieve 3,000,000 L/year can be estimated on the basis of the volume of the pressure vessel selected and the number of hours the vessel is operated each year. In this case, the business plan indicated that the product and the package would be produced in a single 10-hour shift operating 250 days/year (2,500 hours/year). The packaged product would enter a refrigerated storage space and be pressure treated over 2, shifts each day to provide 5,000 hours/year of high-pressure system operation. It was determined that the system selected could operate at 10 cycles/hour (50,000 cycles/year). A 60-L vessel could provide 3,000,000 L/year. If a higher production rate were needed, a second 60-L vessel

could be installed. This second vessel could be operated in tandem with the existing vessel using the existing pump–intensifier. Alternatively, a single 100-L vessel could provide 3,000,000 L/year operating at 30,000 cycles/year. Operating cost of two 60-L vessels versus a single 100-L vessel can be compared.

5. Operating Costs of Commercial High-Pressure Systems

The actual system selected will reflect the available manufactured size of the pressure vessel closest to the design estimated size based on the vessel cycle rate per hour. The cost per package will depend on the total system operating costs and would include equipment lease payments for leased equipment if the equipment is not purchased.

The business plan must specify the number of hours the food preparation and packaging line will run each year and the peak hourly production needed to meet holidays and other special marketing opportunities. In the previous example, if the product were to be manufactured on a single line, operating ten hours per day, five days per week, for 50 weeks per year (2,500 hour/year), the nominal hourly production rate would be 2,000 packages per hour. This will yield 5 million 1-pound packages per year. If the packages must be treated directly after filling and sealing on a single shift, then the high-pressure system selected must be capable of treating:

$$\begin{aligned} &0.475 \text{ L/package} \times 2,000 \text{ packages/hour} \\ &\quad \times 1/0.8 (\text{volumetric efficiency}) \\ &= 1,200 \text{ L/hour} \end{aligned}$$

For ease of analysis it can be assumed that the high-pressure treatment system is leased on a yearly basis. The lease cost could include any auxiliary equipment and services such as a temperature-controlled water supply; installation; a service program; and an equipment-operating warrantee. The warrantee would be based on the planned number of cycles to be run each year. In this case, major repair costs would be covered in the lease cost per month, and maintenance costs would be paid by the

food processor. These costs would include regular replacement of items such as seals and tubing. Chapter 4 contains information on typical operating costs as a function of the cost of the high-pressure equipment. The cost per unit of the treated food is determined primarily by the cost of the high-pressure processing equipment.

In principle, there can be a number of combinations of vessel volume, cycle time, and volumetric efficiencies that can yield a suitable processing volume per hour. However, commercial high-pressure food-processing systems are sold with a limited number of pressure vessel volumes. Volumes range from 30 to 400 L. The size and shape of the food package will dictate the volumetric efficiency of any given vessel. Additionally, while it may be possible to cycle a fully automated 30-L vessel at 12 cycles per hour, a rate twice that of a 350 L vessel system, the smaller vessel may require specially designed packages to take full advantage of the potential production output. Six 30-L vessels each cycling at 12 cycles per hour would be needed to match a volume output of 2,100 L produced by a 350 L unit cycling at six cycles per hour. The larger number of cycles per year for the smaller vessels would increase operating costs due to replacement of wear parts such as seals, high-pressure tubing, and pump–intensifier wear. Thus, the trend has been toward the installation of one or more large volume vessels (also see Chapter 4 for processing cost).

6. Cycle Time Analysis

The cycle time of a high-pressure food-processing system can be optimized by minimizing the time required to accomplish each of the nine steps which make up a typical cycle. The nine steps are similar for pressure vessels oriented in the vertical (V) or horizontal (H) position, or in a tilting (T) mode. These steps are as follows:

1. Loading the vessel with the bottom (V, T) or discharge (H) closure in place. This includes the time to place the desired number of packages in the vessel and fill the remaining vessel void space with water. To speed loading, packages can be preloaded into a mesh carrier and the carrier placed into the vessel already filled with sufficient water to fill any void space (V, T).
2. Closing the vessel. The top (V, T) or the inlet (H) of the vessel is sealed with a closure designed to contain the water at the operating pressure of the system. Generally, the closure is designed to move slightly outward under pressure, from its zero pressure position, to engage the restraining yoke.
3. Moving the vessel or yoke to allow the top and bottom (V, T) or inlet and outlet (H) closures to engage the restraining yoke. Either the vessel (V, H, T) or the yoke (V, H) is moved so that the top and bottom (V, T) or inlet and outlet (H) closures of a horizontal or tilting system can press against the yoke as the vessel is brought to operating pressure.
4. Bringing the vessel to operating pressure. The vessel (V, H, T) is brought to operating pressure by activating the pump–intensifier(s) to deliver water into the vessel until the desired pressure is obtained. The compression time is a direct function of vessel volume, operating pressure, compressibility of the compression media, and the size of the motor driving the pump–intensifier system(s). A 100-horsepower pump–intensifier system, will, in principle, require half the time to bring a given vessel to pressure compared to a 50-horsepower pump–intensifier system. It can be seen that considerable cycle time can be saved using as large a pump–intensifier system as possible. The optimum size of the pump–intensifier system must be calculated based on pump–intensifier system costs and actual cycle time savings to determine the best economic combination. Water compression work is significant above 400 MPa (58,000 psi), and represents the major cost of operating the pump–intensifier system. This accounts for much of the compression time for operating pressures in the range of 600 MPa (87,000 psi). It may be useful to use two pump–intensifier systems to optimize water delivery. One system would operate from 0 to 400 MPa (58,000 psi). The second system would operate from 400 to 600 MPa (58,000–87,000 psi).

5. Holding at operating pressure (V, H, T). This time is fixed by the time needed to inactivate six log cycles of the pathogens or spoilage organisms to be controlled.
6. Decompressing the vessel (V, H, T). Decompression time is the time needed to allow the expanding water in the vessel to flow through the discharge line, so as to allow the vessel to return to atmospheric pressure. In some cases, it may be desirable to slow the rate of water flow to allow occluded and dissolved gasses to expand so as not to harm the structure of the material being treated. Additional 30 or more seconds may be required to allow for controlled expansion. A special throttling system is required to obtain the desired rate of decompression.
7. Moving the vessel or yoke to allow opening the top (V), bottom (T), discharge (H) closure.
8. Opening the vessel top (V), bottom (T), or discharge (H) closure to allow unloading of the product.
9. Unloading the vessel and closing the bottom (T), or discharge (H).

Vertical pressure vessels typically fill and empty product through the top vessel closure. The water used to fill voids may remain in the vessel unless there is a need for new water for each cycle. Horizontal pressure vessels receive product in carriers through the inlet end and discharge treated product through the outlet end in a through-flow manner. Water to fill the void spaces after loading must be added after the inlet closure is closed. Tilting pressure vessels can receive product through the top openings along with water and can discharge product through the bottom opening along with the water used for compression. The tilting system automatically closes the top closure as the pressure vessel moves from the tilted position to a vertical position inside the restraining yoke. After pressure treatment, the vessel is tilted to a 45-degree angle and the bottom closure is opened automatically to allow the gravity to discharge the product and water from the vessel onto a conveyor. The bottom closure is then automatically moved to the closed position to start the next cycle.

7. Packaging and Material Handling Factors

Vessels having a diameter of 38 cm (15 inches) may be needed to accommodate unique packaging requirements and deliver high volumetric efficiency. Also, larger diameter pressure vessels may be needed if a range of package sizes is to be processed.

High-pressure systems are manufactured upon the receipt of an order. Lead times for pressure vessels and yokes can be several months or longer. Once a decision has been made to use high pressure, negotiations should start with equipment suppliers to develop realistic delivery dates. Before the order is placed, it is essential that all test work be completed to determine volumetric efficiency, vessel cycle rate per hour, process pressure requirement, compression and decompression times, and product-hold times and temperatures. All this information is needed to allow realistic costing of the process and construction of the appropriate high-pressure processing system.

It is necessary to provide high-pressure systems manufacturers with detailed information on the equipment location in the production facility. Also needed are a product flow diagram and a proposed process line layout. Vertical units may require a ceiling height of up to 6–7 meters. Low ceiling heights may dictate the use of a horizontal or a tilting system.

The ability to automatically load and unload a system is an important cost consideration. For example, a tilting vessel system allows sealed packages to drop into the pressure vessel, positioned at a 45-degree angle, without the use of a carrier. The vessel is partially filled with water to cushion the fall of the packages. The elimination of a package carrier can increase the volumetric efficiency of the vessel.

Most batch systems use a carrier to accumulate a batch of packages prior to placing them in the pressure vessel. Carriers can be preloaded and placed automatically in a horizontal or vertical pressure vessel.

Multiple vessels may allow recovery of compression energy by directing the water from a decompressing vessel to a vessel ready for compression. Compression water may be recycled after filtering and temperature adjustment. Compression water will

be warmed slightly each cycle, if reused. While compression heating is dissipated on decompression. There is some heat transfer to the walls of the pressure vessels. This heat is retained and transferred to the colder compression water at the start of new cycle. If the recycled water is not cooled, this water can achieve an undesirably high temperature.

8. Product Manufacturing Specifications Affecting High-Pressure Food Preservation Equipment Selection

In addition to the information developed in the technical plan (pressure, hold time, temperature) and business plan (production rate, package design, number of shifts per year), food processors must determine how the high-pressure processing equipment will be integrated into a new or existing food-processing line. The equipment layout must reflect available space unless a new facility is to be built to house the high-pressure processing equipment.

A special application of high-pressure processing is pressure-assisted thermal sterilization (PATS) (Sizer et al., 2002; De Heij et al., 2003), which helps to obtain shelf-stable, low-acid, piece-form products (see Chapter 1). Products such as stews, mashed potatoes, pasta and cheese, which can only be heated by conduction, lend themselves to PATS. The integration of the preparation, filling, package closing, preheating, and IT adjustment require a dedicated processing facility.

The PATS process uses compression heating that takes place during the compression of package foods and water to raise the IT of the food and compression water from a value in the range of 90°C (194°F) to the value in the range of 121°C (250°F). A treatment pressure in the range of 600 MPa (87,000 psi) is used. Since compression heat is uniform throughout the food and compression water, the desired process F_0 can be delivered in a time, in minutes, close to the desired F_0 value.

At the end of the process hold time, the decompression of the food, packages, and compression water cools the system to a temperature in the range of the IT. The discharged packages then can be rapidly cooled to a desired storage temperature using chilled

water. The warm compression water can be recycled to a heated storage tank to be used to adjust the IT of incoming packages. The technology for IT adjustment, to ensure that each package is at or slightly above the design IT for the process, at the start of the compression cycle, must be developed for each PATS installation. Statistical quality control of IT is an absolute necessity. Also, technology must be developed for controlling the loss of heat, from the packaged food and compression water, to the pressure vessel walls, during holding at the process temperature and pressure. The use of plastic insulation inside the pressure vessel is possible (see Chapter 2). Appropriate temperature sensing instrumentation is required to verify the lethality of the process for each cycle.

Planning for PATS high-pressure processing requires specialists in both thermal sterilization and HPP. Packaging and package filling, vacuuming, and sealing must be considered. Any delays in the operation of the processing line prior to pressure vessel filling must be addressed by automatically recycling packages to ensure that IT values do not fall below the design values.

Since most high-pressure systems are free standing, the pressure vessel, yoke, pump-intensifier, water-conditioning equipment, and control equipment can be considered a single unit operation. Hazard Analysis of Critical Control Points (HACCP) can be developed around the system as a whole. The system will have the necessary pressure, time, temperature, and safety limits built into the control logic to provide printed histories of each treatment cycle. Undesirable variations from programmed pressure, temperature, and hold times will trigger alerts. Reprocessing of a batch, then, can be carried out if needed. An important advantage of high-pressure processing is the small effect that reprocessing has on product quality.

9. Some Guidelines for Selecting Products for Commercial High-Pressure Treatment

The cost of high-pressure processing may limit the use of this technology to certain types of products.

Chapter 4 provides the information on successfully processed products. The most successful products are those based on low-cost ingredients which can be combined to yield a safe, fresh-tasting, packaged product, of great convenience, and reasonable refrigerated shelf life. Products that do best are those that compete with local, fresh-made, convenience foods, which usually have a refrigerated shelf life of 7 days or less. High-pressure preserved foods with refrigerated shelf lives of over 21 days use no chemical preservatives and can be labeled as natural. The ability of high-pressure pasteurization to extend refrigerated shelf life from 7 days to over 30 days can provide a significant marketing benefit. Longer shelf life is possible if based on good manufacturing practices that control the initial population of spoilage microbes.

The quality of high-pressure preserved foods is equal to that of the freshly prepared refrigerated products. Because refrigerated foods have a limited shelf life, the most successful high-pressure preserved foods are those where high-pressure treatment can extend the useful refrigerated shelf life. Extended refrigerated shelf life combined with pasteurization to inactivate pathogens and spoilage molds, yeasts, and vegetative bacteria has proven to be a strong incentive for high-pressure processing.

The ability to extend shelf life and pasteurize refrigerated foods, alone, may not be a strong enough incentive to use high pressure due to process costs. Products must show other incentives such as the perception of high value equal to freshly prepared products. The treated product must command a price point that will meet the return on investment or other profit criteria set by the company. Potential products can be analyzed using the guidelines laid out in this chapter. For example, a series of market sales volumes may be assumed and processing costs may be estimated for several packaging sizes and shapes. The cost of ingredients can then be estimated to give a final process cost per unit of product. If ingredient costs are a significant part of the unit process cost, then the value-added requirement may be too high to allow successful use of high pressure without a substantial markup.

The corollary to this is to find raw ingredients that will be available year round at low cost. These ingredients can be converted to convenient, ready-to-eat, refrigerated products, with an extended shelf life using high-pressure processing. Guacamole is an excellent model. Avocados are produced year round in Mexico, converted to guacamole, and shipped under refrigeration to the United States. A refrigerated shelf life of over 30 days allows the product to compete with locally made products prepared from fresh avocados. Uniform quality, safety, uniform unit price, and convenience have allowed pressure-treated, refrigerated, guacamole to grow to a multimillion-dollar market. Additionally, the product can compete with frozen product based on a clean label since antibrowning agents are not needed.

High pressure can be used to treat food service packages since package size does not affect preservation conditions. Cook-chill products for food service may provide the margins needed to support the use of high-pressure preservation. Frozen food service products must be thawed while refrigerated vacuum-packed products may not have sufficient refrigerated shelf life to meet distribution needs. Acid and acidified bulk high-pressure-treated products can be made commercially sterile using high pressure.

High-pressure processing can produce unique conditions in certain foods. High pressure is used to shuck shellfish and to help in the removal of edible meat from crabs and lobsters. The yield and safety of these high-value raw materials can be enhanced by high-pressure treatment. Since high pressure can pasteurize fresh products with little or no change in their chemical composition, high pressure may be the method of choice for the preservation of products containing rich sources of heat labile nutrients, as an alternative to freezing preservation.

10. Conclusion

High-pressure processing of foods has found a place in commercial food processing. Process costs are decreasing as availability of the larger automated process systems increase. Successful foods are those yielding very high margins due to their fresh

qualities and extended refrigerated shelf lives resulting from high-pressure treatment. Equipment advances can be expected to further reduce the unit product costs of high-pressure-processed foods. As with all new food products, each product will need to be evaluated in the market place.

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Chapter 4

Case Studies on High-Pressure Processing of Foods

Carole Tonello

1. Introduction

This chapter illustrates how food-processing companies have commercialized high-pressure processing (HPP). Examples demonstrate how basic scientific findings and unique equipment designs have come together to yield successful commercial solid and liquid food products treated by high pressure.

2. Pioneers in Industrial High-Pressure Food Processing

2.1. Meidi-Ya: Cold Pasteurized Jams

The first commercial high-pressure processed foods were produced by the Meidi-Ya Company and were marketed in Japan in the early 1990s. Products included strawberry, apple, and kiwi jams, packaged in plastic cups (Hori et al., 1992; Dumoulin, 1998). Packaged jams were treated in a 50-L Mitsubishi Heavy Industry (Japan) cold isostatic press at 400 MPa (58,000 psi) for 20 minutes. This process was shown to provide commercial sterility in an acid product while maintaining fresh fruit sensory qualities.

This innovation was the result of an ambitious research and development program initiated by Hayashi from Kyoto University in the 1980s. He created the “Association of High Pressure Application” composed of food manufacturers, HPP equipment

suppliers, and scientists. The Association was supported by Japanese government funding from 1989 to 1993 (Hayashi, 1992).

2.2. Ulti: Freshly Squeezed Juices

The company Ulti in France, noting the development of HPP technology in Japan, was the first company in Europe to use HPP commercially. In 1994, Ulti launched pressure-pasteurized citrus juices to a local market. The juices marketed as “freshly squeezed” were processed in polyethylene bottles in a 15-L, 400 MPa (58,000 psi), production machine built by ACB Alstom in France. Ulti has progressively expanded its HPP production capacity to four machines using ACB-Alstom-designed equipment. Today, its HPP orange and grapefruit juices and line of smoothies, launched in 2008, are marketed across France.

2.3. Fresherized Foods: Revolutionizing the Fresh Guacamole Market

In 1997, a US company, Fresherized Foods (www.fresherizedfoods.com) (formerly Avomex), began the first industrial production of pressure-pasteurized avocado products at a plant in Mexico. Products were exported to the United States for food service use. The company started with a 50-L machine operating at 690 MPa (100,000 psi). This machine was supplied by ABB Pressure Systems, Sweden, now owned by Avure Technologies, USA (Ennen, 2001). Today, Fresherized Foods is the leading company in the high-pressure treatment of foods based on number of high-pressure machines (vessels

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from 215 to 350 L) and volume of production. Fresh-erized Foods uses machines built by Avure Technologies and NC Hyperbaric (Spain) with production facilities in the United States, Mexico, Peru, and Chile. The use of high pressure revolutionized the market for ready-to-eat (RTE) avocado products, and especially the guacamole market. HPP offers consumers much higher quality products than those preserved by heat or freezing. High-pressure pasteurized avocado products have a refrigerated shelf life of over 4 weeks. The strong sales of Fresh-erized Foods chilled avocado, RTE products have led several other avocado producers to invest in high-pressure equipment for processing guacamole or avocado paste.

2.4. España: High-Pressure Pasteurized RTE Meat Products

The Spanish company España (www.espuna.es) pioneered the use of HPP for the pasteurization of meat products. Sliced cooked ham with the label “High Pressure Pasteurized Product Remains Fresh Until Eaten” was launched in Spain in 1998 (Grèbol, 2003). Even now, it is being sold and distributed in several supermarket chains in Spain. The product’s ham slices are vacuum skin-packed with plastic film interleaves to facilitate the separation of slices by the consumer. It has a refrigerated shelf life of 60 days and it’s processed for 10 minutes at 400 MPa (58,000 psi) in a 320-L, horizontal, ACB-Alstom unit. In 2003, España launched a line of ready-to-microwave meat snacks consisting of small sausages, spicy diced chicken, turkey products, and bacon and cheese rolls. These have been successful in Spain, Great Britain, and France. In 2005, the company developed the first sliced cured ham stable for 40 days at nonrefrigerated temperatures (Astruc, 2006). This shelf-stable product is processed at 600 MPa (87,000 psi).

3. Worldwide High-Pressure Commercial Food Applications

All industrial uses of high pressure for food treatment may not be made public. Detailed data on commercial applications may be considered confidential

during the initial period of use while establishing a market. In most countries (except Japan), improvements in food-processing technologies may not directly translate into a stronger marketing position. Thus, few companies make the effort to use new process technologies as the basis for new product introductions. Rather marketers highlight improved convenience, flavor, and nutrition provided by high-pressure processing.

The following data summarize industrial information collected since 1992 from direct company inquiries, scientists, and equipment providers working on HPP food applications, and from food magazine publications. Data concerning the actual number of machines in production and associated vessel volumes have been corrected for the decommissioning of earlier equipment. Laboratory and pilot plant machines are not included except where they are used as tolling equipment for commercial food productions on a regular basis.

3.1. Equipment and Its Location

By mid-2008, about 125 industrial HPP machines were in production for food processing worldwide (Figure 4.1). Almost 85% of these machines were installed after 2000. The slow initial use of HPP in food processing can be attributed to the novelty of the process and a lack of knowledge of the marketing benefits of high-pressure processing. Another challenge was the limited capabilities of the HPP machines offered by equipment suppliers before 2000. Machines were not yet designed to meet food-processing conditions where stainless steel coverings, ease of cleaning, ease of maintenance, and high productivity are needed. High-pressure process equipment did not look like standard food process equipment.

About 60% of the world’s HPP equipment is located in the United States, Mexico, and Canada. Several units are in Peru and Chile. Europe has 22% of the installed HPP machine capacity located in Spain, Italy, Portugal, France, United Kingdom, Czech Republic, Germany, Belgium, and The Netherlands. The remaining 18% of the equipment is in Asia, especially in Japan and more recently in China and Korea. A few machines are located in Australia and New

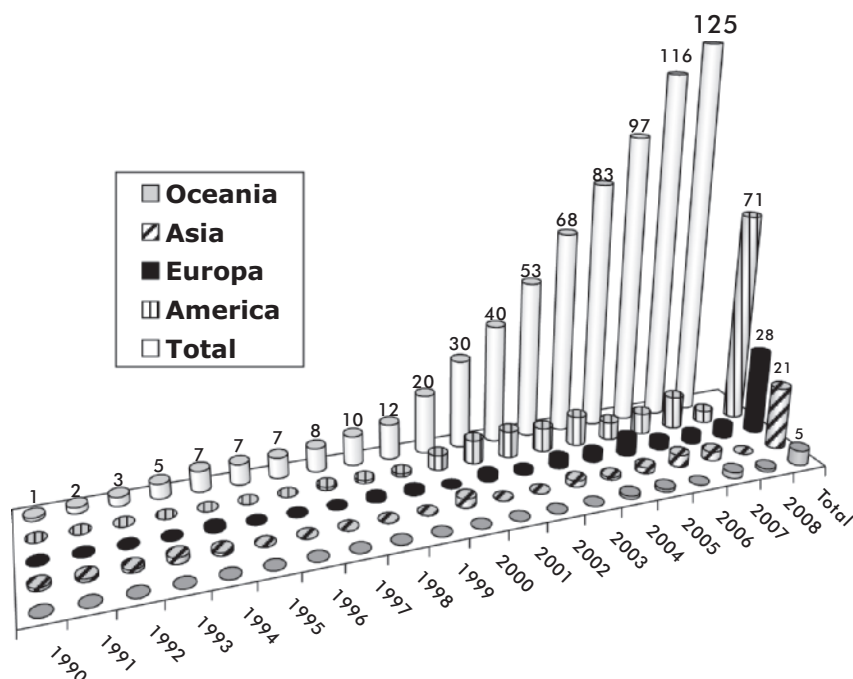


Figure 4.1. Evolution of the number of HPP industrial machines installed versus years and continents.

Zealand. To date, there are no machines in Africa. HPP appears to be advancing where consumers want premium, convenient RTE products. The safety and extended shelf life of HPP-treated RTE products is a bonus to the processors of these products.

HPP appears to have developed more rapidly in North America than in Europe possibly due to the need for extended safe, refrigerated shelf life and food preservation legislation more favorable to innovation. Food safety authorities in the United States have supported efficient techniques to kill microbial pathogens. United States Department of Agriculture (USDA)—Food Safety and Inspection Service—wrote in 2003, and updated in 2006, a guideline to control *Listeria monocytogenes* in RTE meat and poultry products (Anonymous, 2006). HPP was approved for its efficiency with minimum effect on organoleptic quality.

The European Union (EU), by contrast, considered HPP a novel technology and required food processors to comply with the “Novel Food Regulation

EC n°258/97.” Food processors intending to use high pressure as a preservation method needed to prove the safety of the process through a scientific study. The study needed to demonstrate not only the microbial safety of the product, but also toxicological and allergenic safety. Additionally, studies should demonstrate no detrimental effects to nutritional quality and the resulting product should not mislead consumers in perceived value. Eisenbrand indicated that HPP-treated food should not be considered novel. The lack of standardized requirements from one country to the other prevented compliance.

The Food Standards Agency (FSA) in the United Kingdom declared that “High-Pressure Processing per se is no longer considered a novel process” (Hattersley, 2001, 2002). FSA recommended that food processors need to only demonstrate adequate kill of pathogenic bacteria and have stringent measures in place to prevent the germination of *Clostridium botulinum* spores. The French position, in contrast, is illustrated by a novel food filing for

HPP treatment of smoked and dry cured duck ham (Briand, 2007). The file was considered unacceptable by the French food safety authorities because of a poor bibliography, lack of studies on the impact of HPP on nonpathogen microflora commonly present in the product, such as lactic acid bacteria, lack of information on possible pressure-induced spore germination, lack of information on the effect of HPP on physical and chemical changes of microorganisms growing or surviving post treatment, and also lack of data on toxicological aspects, such as comparative volatile compounds profiles, allergenic risks induced by the process, and packaging specific migrations. The conservative attitude of the EU countries toward new processes, and the uncertainty of costs and the time required to have a Novel Foods petition accepted, has slowed down the commercialization of HPP in Europe.

Japan has not embraced the commercial use of HPP in food processing. As far as known, only four companies produced pressure-treated foods in 2008. Earlier users, namely, Meidi-Ya, Wakayama, and Pokka, have abandoned the technology. Reliability problems with early HPP machinery may have discouraged these companies. Japanese machines suppliers who were the major players in HPP in the 1990s are large companies such as Mitsubishi Industries, Kobelco (Kobe Steel Group), and IHI Corporation. These companies may have decided to leave the market for high-pressure food processing machines due to its very small size in Japan and the high cost of developing reliable machines for the food industry.

3.2. High-Pressure Processing Companies

Worldwide, 60 companies are marketing more than 250 different HPP-treated products. The companies using HPP for food preservation appear to have selected this technology on the basis of the following advantages: ability to innovate new products, desire premium fresh qualities, want extended refrigerated shelf life without sacrificing microbial safety, or want to produce fresh-like products that cannot be heat-treated without losing nutritional or sensorial qualities. Companies owning one or several HPP machines represent a large range of sales volumes.

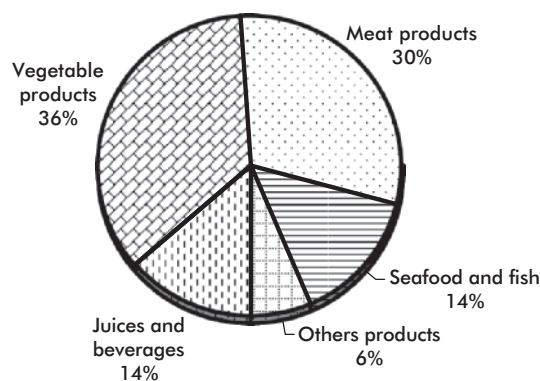


Figure 4.2. Distribution of high-pressure processing industrial machines versus food product type processed.

In 2008, 50% can be considered large organizations employing more than 500 workers and 17% of these companies are international groups with more than 5,000 employees. Small and medium enterprises represent the other half of the companies using HPP employing 10–250 people.

Roughly one-third of the HPP machines are in use for processing RTE vegetables, primarily avocado products (Figure 4.2). A third of the installed HPP machines are used to process meat products such as sliced or diced cooked pork, chicken, and turkey. The last third are used to process juices and beverages such as smoothies, seafood and fish, and other products such as dairy or for coprocessing or in tolling applications.

4. Commercial HPP Application by Food Sectors

4.1. Juices and Beverages

Juices, beverages, and vegetable products were the main commercial applications of HPP equipment, based on vessel volumes installed, during the 10-year period from 1998 to 2007 (Figure 4.3). Acid juices and beverages, with their low pH and high a_w , can be pasteurized using pressures in the range of 400–450 MPa (58,000–65,000 psi) and process times generally under 10 minutes. These process parameters matched the machines available at this period. Further investment in HPP equipment for beverage processing has been moderate. While semicontinuous

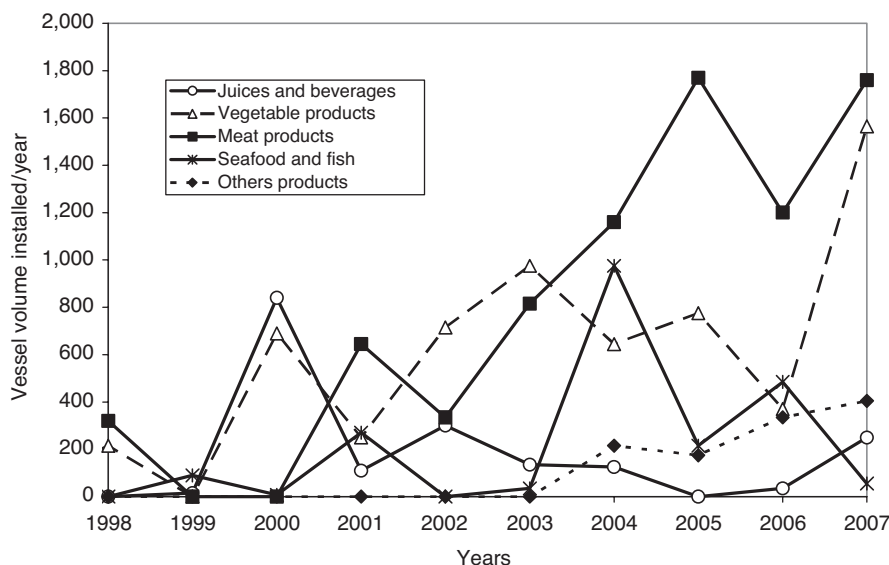


Figure 4.3. Evolution of high-pressure processing industrial machines vessel volume installed/year in the different food sectors versus year, during the last 10 years.

machines were available in 1990s, their capacity were too small for large companies. Juice and beverage companies using HPP are almost exclusively small enterprises with less than 50 employees processing bottled premium juices and smoothies for regional markets.

4.2. Vegetable Products

The HPP capacity devoted to vegetable products, primarily avocado processing, has been increasing over the last 10 years. Avocado processors have invested in equipment year after year to meet the expanding US market. Avocado processors found that pressures in the range of 600 MPa (87,000 psi) yielded a high-quality product with a hold time of less than 10 minutes. Guacamole packed in flexible bags allowed pressure vessels to be filled to a volumetric ratio at or near 70%. The relatively short hold time and volumetric efficiency made the process economically feasible.

4.3. Meat and Poultry Products

Pressure-treated meat has been the fastest growing sector in the last 5 years. Meat processors have

found that the uniformity of pressure treatment allows the pasteurization of any product independent of size, shape, composition, or package form. A benefit of high-pressure pasteurization of meat products is post-package treatment without the loss of fresh quality.

USDA regulations include HPP as acceptable processes to control *Listeria* in RTE refrigerated meat products. HPP processing provides a natural pasteurization technology to deliver RTE convenience meat products with a “clean” label.

The availability of larger diameter machines with increased volumetric efficiency and capacity per cycle has allowed meat processors to take advantage of HPP. Machines can be automated to further reduce processing costs.

4.4. Seafood

High pressure in the range of 200–300 MPa (30,000–43,500 psi) provides a simple and efficient method for the removal of edible meat from shell and carapace of shellfish and crustaceans. Industrial applications use high pressure to extract crustacean meat from crab and lobster, and open oysters and

other bivalves (shucking process). This last activity is generally run by small companies, which have invested in low-volume machines to meet the modest size of the market. By using a 1-minute hold at 300 MPa (43,500 psi), sufficient products can be treated to meet developing market needs. Seafood companies using HPP are located in the United States and Canada. There is one company in Japan. HPP of seafood has not been of interest to European seafood processors because the European consumer generally buys only fresh “live” oysters and lobsters.

The existence of several patents covering the use of HPP for opening bivalves (Miura and Hatsukade, 1992; Voisin, 2000) and extracting meat from crustaceans (Hognason and Jabbour, 2005; Raghubeer et al., 2006) has tended to restrict the development of the technology. The similarities among the patents make for a confusing intellectual property situation which is further confounded by claims covering the inactivation, by high pressure, of pathogens associated with seafood. Pathogen inactivation has been noted in the published research literature before the filing of these patents (Styles et al., 1991).

4.5. Other Products

Machines labeled for “other products” in Figure 4.3 are those used by coprocessors in the United States. Food companies use these machines on a per-cycle or per-kilogram processed. This avoids capital invest-

ment in this technology during product development, test marketing, early launching, or to meet a niche market opportunity.

4.6. Estimates of Worldwide High-Pressure Food Production

The total worldwide production of high-pressure treated food is showing steady growth. Estimates for production in 2008 are in the range of 200,000 metric tons/year (about 450 million lbs/year) and use the total vessel volume in production as indicated in Table 4.1. The 200,000-metric ton estimate is calculated using typical process data derived from food companies operating high-pressure food-processing lines. Process data include number of cycles per hour, operating hours per day, and working days per year. New machines are capable of higher hourly production rates so that actual production may be higher as new machines are installed. The estimate is corrected for machine downtime of about 10% for maintenance and repair, and with certain commodities, for seasonal downtime.

5. Incentives and Constraints to Be Considered in the Use of High Pressure for Food Processing

Capital costs, operating costs, and production rate per hour per high-pressure machine are decisive items

Table 4.1. Estimation of HPP food global production in 2008

Products	Vessel Volume in Liters	Number of Cycles/Hour	Vessel Filling Ratio	Working Hours/Day	Working Days/Year	Production in Metric Tons/ Year (lbs/year)
Vegetable products	6,412	5	0.65	12	300	75,020 (165,420)
Meat products	9,015	5	0.45	12	300	73,022 (161,012)
Seafood and fish	2,487	7	0.70	16	180	35,096 (77,387)
Juices and beverages	2,032	6	0.45	12	250	16,459 (36,293)
Other products	1,137	5	0.50	12	200	6,822 (15,043)
Total	20,962					206,420 (455,155)

for companies looking to establish an HPP facility. Additionally, realistic market demand, price points, range of product styles, and seasonal supply and demand issues must be known to a reasonable accuracy. High-pressure equipment suppliers can provide accurate data on capital and operating costs, including labor, utilities, repair, and maintenance, and up time. Production rates per hour must be determined from actual tests, with actual product, since the volumetric efficiency of any machine is directly related to the actual packaged product. Additionally, the planned number of hours per day the machine will be running must be known. For example, a food-processing line may produce product at twice the hourly capacity of the high-pressure process equipment using a single 8-hour shift. The high-pressure process equipment would be operated for two 8-hour shifts to treat the daily production. A refrigerated storage system would be needed to accumulate product to be pressure-treated during the second shift.

Food processors who are contemplating the use of high pressure to pasteurize or otherwise treat food products should start a dialog with equipment suppliers as soon as possible. This dialog will form the basis for both a business and technical plan and will highlight such needs as space for equipment, utilities, and process parameters such as hold time at pressure and needed process temperatures at pressure.

HPP machines continue to undergo improvements to increase the cycle rate per hour. Horizontal or tilting units can increase productivity over vertically mounted pressure vessels loaded and unloaded through the top closure (similar to a top loaded vertical retort). These systems use throughflow and automated loading and unloading. Throughflow design helps to prevent accidental mixing of treated and untreated product, can provide improved process temperature control, and, in some cases, eliminate the use of carriers.

The industry has found that an operating pressure of 600 MPa (87,000 psi) provides a satisfactory pasteurization pressure and hold time (3–5 minutes) for most vegetative microbes. Pressure vessels can be built to handle this pressure for up to hundreds thousands cycles before replacement. Wear parts and seals can perform satisfactorily at this pressure. This

pressure allows available intensifiers to compress water in 400-L vessels to 600 MPa (87,000 psi) in several minutes. The rate of compression directly influences cycle rate. Operating pressure and hold time at pressure may determine 75% of the cycle time. (See also Chapter 3)

6. Capital Costs and Production Rates

Capital costs for horizontal, throughflow, 600 MPa (87,000 psi), high-pressure food-processing units are primarily a function of pressure vessel volume. For example, in 2008, a unit with a 55-L vessel (Figure 4.4) costed in the range of US\$14,000 (€10,000)/L. A unit with a 420-L vessel (Figure 4.5) costed in the range of US\$7,500 (€5,500)/L. High-pressure intensifier systems for these machines are able to reach 600 MPa (87,000 psi) in about 2.5 minutes. A typical cycle time (without holding time) is in the range of 4 minutes for loading product, closing, filling the vessel with water, compressing to 600 MPa (87,000 psi), releasing the pressure (less than 3 seconds), opening the unit, and unloading. A 3-minute hold time at pressure gives a total cycle time of about 7 minutes or 8 cycles/hour. A 55-L vessel operating at 8 cycles/hour can treat 440 liters/hour. A 420-L vessel can treat 3,360 L/hour at 8 cycles/hour. The actual production rate per hour for any food product will depend on the volumetric efficiency of the packaged food in each pressure vessel. Volumetric efficiencies of 75% are possible with packages designed to optimize vessel loading. For packaged, RTE, sliced, meats, a volumetric efficiency may be 50% due to unique package designs. Thus a 55-L vessel can deliver about 220 1-L packages/hour. A 420-L machine can produce about 1,680 1-L packages/hour (Hernando Sáiz et al., 2008). The lower per liter capital costs of a larger vessel has provided a major incentive to purchase 300–400-L machines to obtain the lowest per package processing cost.

Figure 4.6 shows estimates of processing costs for juices, meats, and seafood products processed in (a) a 420-L machine and (b) a 55-L machine. Volumetric efficiency, operating hours per day, and operating days per year are those used in Table 4.1. The cycle rate per hour is based on actual production experience

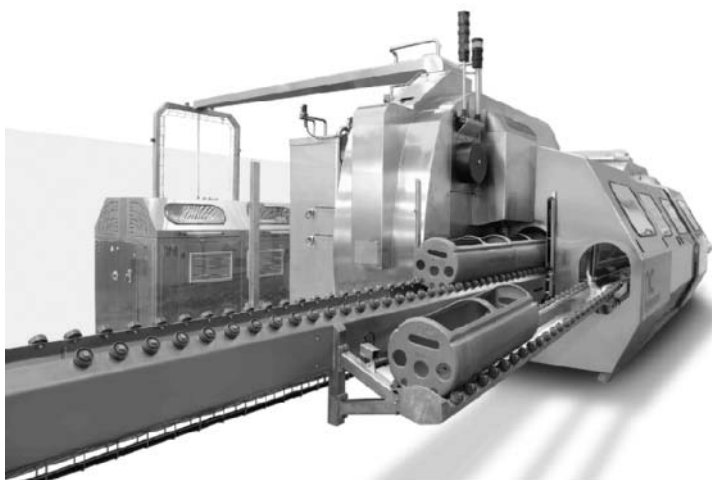


Figure 4.4. Wave 6000/420 NC Hyperbaric high-pressure processing equipment (420-L vessel volume—maximum working pressure: 600 MPa/87,000 psi).

with installed 55-L and 420-L machines. It should be noted that the production rates shown in Figure 4.6 are twice those shown in Table 4.1. This is due to the use of a 2-minute hold time for vegetable juice products allowing 10 cycles/hour at 600 MPa (87,000 psi) and 8.6 cycles/hour at 600 MPa (87,000 psi) with a 3-minute hold time for meat products. Seafood products processed to allow pressure-assisted separation of edible meat from shell can be treated at 14 cycles/hour using a 1-minute hold at 300 MPa

(43,500 psi). Fruit juices held at 450 MPa (65,250 psi) for 2 minutes allow 10–11 cycles/hour.

A depreciation period of 5 years is generally used for high-pressure food-processing systems; however, field experience indicates that properly maintained high-pressure systems can have a useful operating life in excess of 7 years. These operating cost figures yield processing costs between €0.044/kg and €0.104/kg using a 420-L machine. Processing costs are between €0.087/kg and €0.205/kg using a



Figure 4.5. Wave 6000/55 NC Hyperbaric high-pressure-processing equipment (55-L vessel volume—maximum working pressure: 600 MPa/87,000 psi).

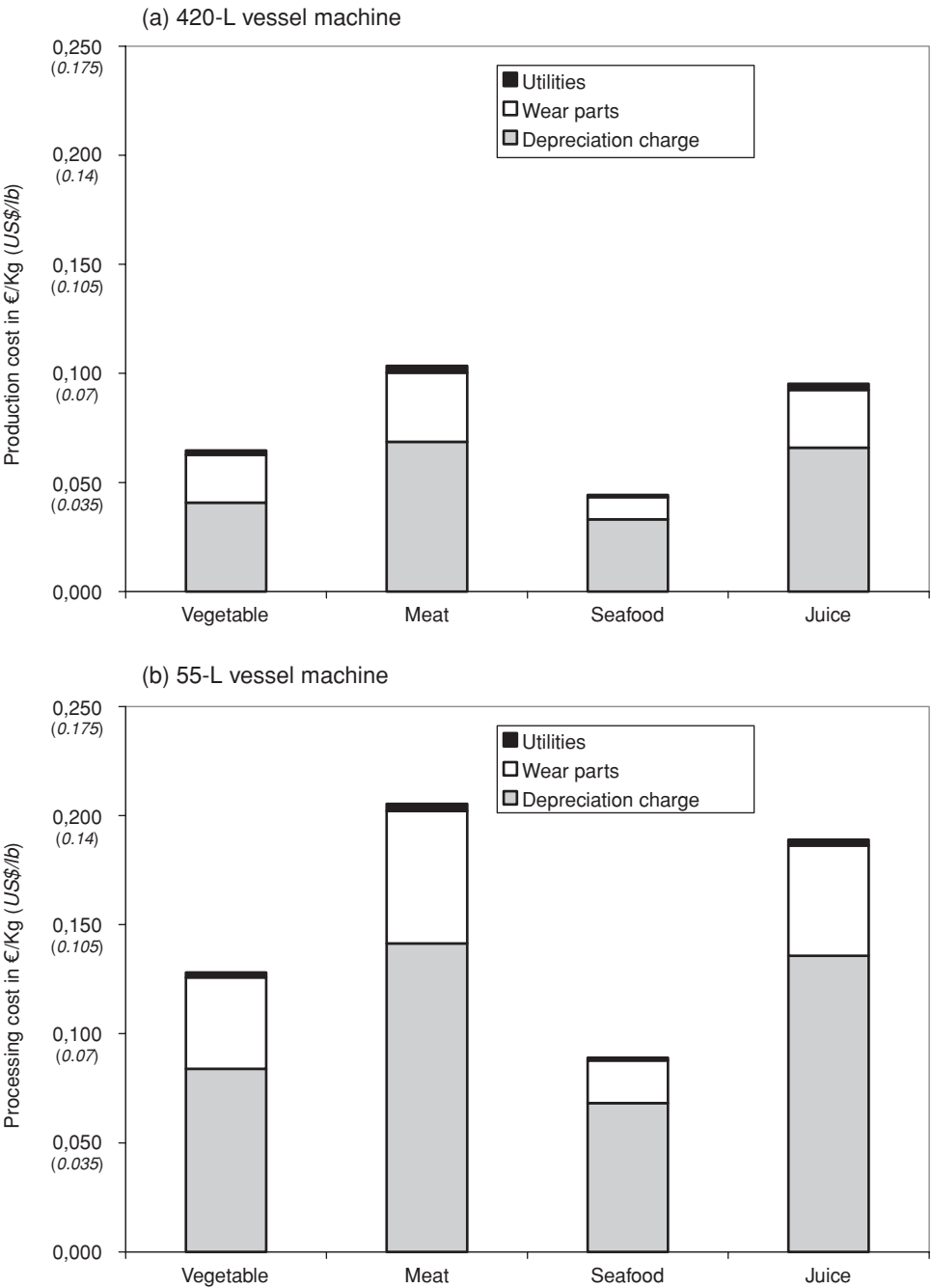


Figure 4.6. Estimated processing cost for vegetable products, meat products, seafood, and juice processed in (a) a 420-L machine and in (b) a 55-L machine.

55-L machine. Processing costs in the 420-L machine are about half to those of a 55-L machine due to the lower capital cost per liter of the pressure vessel and the associated operating costs of the 420-L machine for labor and maintenance. A 420-L machine can process almost 8 times more products each cycle than a 55-L machine with a cost per liter of the pressure vessel in the 420-L machine being only twice to that of a 55-L vessel.

Wear part costs for a 420-L unit are one half to those of the 55-L since the larger machine cycles less for a given yearly production rate. Mechanical parts wear out due to cycling, with the associated pressure increase and decrease, but not with hold time at pressure. Processing costs are 65–75% for depreciation, 22–33% for wear parts and maintenance, and 2–3% for utilities including process water and electricity. Labor costs must be added to the figures presented. Labor costs can be estimated from the number of workers required to operate the equipment each shift and can represent from 10 to 40% of the final processing cost. For automated systems, up to two additional workers may be required each shift including maintenance worker costs.

Seafood processing costs are lower because of high volumetric efficiencies and the modest pressures required. Vegetable product process costs, such as for guacamole packaged in flexible pouches at 600 MPa (87,000 psi), are lower than meat because of the higher volumetric efficiencies obtained by using flexible pouches for vegetables and trays for meat products. Juices packed in plastic bottles have a high-process cost because of the lower volumetric efficiency of the bottle.

7. Examples of Commercial High-Pressure Food-Processing Companies

7.1. AmeriQual-Contract Manufacturer for Kraft Foods and Tyson Foods

AmeriQual (www.ameriqua.com) installed an NC Hyperbaric 300 L (79 US gal)—600 MPa (87,000 psi) system at their facilities in Evansville (IN). This unit supported studies by Kraft Foods

(www.kraft.com) and AmeriQual in the Dual Use Science & Technology (DUST) Program, headed by Dr. C. Patrick Dunne at the US Army Natick Soldier Center, Natick, MA. Research focused on high-pressure sterilization of low-acid foods using high pressure to assist heat sterilization. AmeriQual, as a major supplier of military food rations for US Army, was interested in this technology to improve sensorial qualities of some of its shelf-stable products. It was found that high pressure combined with heat sterilization could greatly reduce process hold times.

In 2007, AmeriQual installed an additional 300-L horizontal system to be used by Tyson Foods (www.tyson.com) to pasteurize oven-roasted chicken products like breasts halves, thighs, and bone-in whole birds. These products were the first bone-in, packaged, products to be industrially processed under pressure. Whole chickens were oven-roasted to soften the bones so that they would not break or puncture the packaging during the pressure treatment. The 600 MPa (87,000 psi) process of several minutes duration, at room temperature, extended the refrigerated shelf life of these vacuum-packed preservative-free products from 14 to 45 days (Crews, 2007a). Two 300-L machines allowed a production rate of 45 metric tons (100,000 lbs)/day operating two 10-hour shifts.

7.2. Foster Farms: Preservative-Free RTE Poultry Products

Foster Farms (www.fosterfarms.com) started HPP RTE sliced turkey and chicken strips, free of nitrites and other preservatives, in 2007. Foster Farms wanted refrigerated, pasteurized, RTE, poultry products with a “clean” (preservative-free) label and extended refrigerated shelf life. The company invested in a pair of 300-L, horizontal, pressure vessels which were installed to operate in tandem using two intensifiers to shorten compression times by 50%. The 600 MPa (87,000 psi) treatment for several minutes at room temperature provided a refrigerated shelf-life of 55–60 days (Crews, 2007b). This is double the shelf life obtained without high-pressure treatment. HPP products are sold nationally. Foster Farms installed a third 300-L system in 2008.

7.3. SimplyFresco: Premium Tomato Sauces

SimplyFresco (www.simplyfresco.com) is a small firm based in San Antonio (TX) that produces high-quality tomato sauce preserved by high pressure. A 55-L, horizontal, HPP machine was obtained in 2006 to pasteurize a range of refrigerated, premium, pasta and salsa sauces for markets in Texas and southeastern states. The system was designed to operate at 8 cycles/hour with a volumetric efficiency of 80% using a flexible pouch packaging system. A hold time of a few minutes at 600 MPa (87,000 psi) at room temperature yields a refrigerated shelf life of 100 days and a throughput of more than 350 kg/hour (770 lbs/hour).

The sauces, all preservative-free, are made of fresh natural ingredients including peppers, onions, coriander, and garlic. As herbs and spices are not heat-treated, they keep their natural strong flavor. The sauces have a unique sensorial quality, very close to homemade sauces.

7.4. Abraham Traditional German Dry Cured Ham

Abraham Schinken GmbH & Co. KG (www.abraham.de) produces dry cured ham for the domestic German market, and starting in 1980, for export to the United States (Harms, 2006). Dry cured ham can be contaminated by *L. monocytogenes* at very low levels. These levels meet European food safety standards, but US legislation requires the absence of *L. monocytogenes* in 25 g of product. Abraham started to study the use of HPP in 2004, in collaboration with the Technical University Berlin and the Hygiene and Environment, Food Safety and Zoonoses Division, Hamburg. The process could eliminate *L. monocytogenes* to satisfy US standards while preserving sensorial qualities of this heat-sensitive product. Challenge tests showed a reduction of more than 5 log cycles in chilled dry cured ham inoculated with *L. monocytogenes*. Packaged hams were treated at 600 MPa (87,000 psi) with a 2-minute holding time at a temperature of 5°C (41°F). This process insures a high level of safety for a minimally contaminated

product where *L. monocytogenes* cannot grow during post-process storage. It was found that microbial reduction was less when the sliced ham was high-pressure-treated in the frozen state at −15°C to −12°C (5–10°F). This was the temperature of the product just after slicing. The current process includes a tempering step in a chill room to adjust the temperature of the ham slices to 5°C before HPP treatment.

A 150-L, horizontal, 600 MPa (87,000 psi) system was installed in the Abraham factory in 2005. The system can compress a full vessel of vacuum-packed ham products to 600 MPa (87,000 psi) in 3 minutes. If the ham is packaged in a modified atmosphere package (MAP), compression to 600 MPa (87,000 psi) will take 4 minutes. In the case of MAP products, compression is longer since the gas in the packages must be compressed to a supercritical fluid before the water in the system can be compressed. Cycle times are 7–8 cycles/hour.

Abraham was the first company in Europe to use MAP under pressure. It pioneered the use of semi-rigid, MAP, thermoformed, packaging. This packaging is flexible enough to recover its shape after processing under pressure. It was developed in collaboration with Wipak (www.wipak.de) and fulfills marketing requirements for this premium meat product. The package has good oxygen barrier properties and high flexibility. The volume of MAP gas is minimized to minimize compression time and maximize the volumetric efficiency of the package.

7.5. Rodilla Sandwich Market Expansion

Rodilla (www.rodilla.com) is a chain of sandwich shops located principally in Madrid (Spain). The company grew rapidly from 1997 to 2002 (Barciela, 2006), but subsequent growth was limited as long as the chain remained in the Madrid area. In 2003, the company evaluated the possibilities of nationwide and foreign expansion. A major obstacle to regional and national expansion was the distribution of fresh sandwich fillings made in a central location in Madrid. The fillings contained cheese or mayonnaise mixed with a wide range of ingredients including ham, cooked vegetables, shrimp, smoked salmon,

and nuts. The fillings had a refrigerated shelf life of 4–6 days depending on the ingredients. Rodilla wanted to keep their sandwich fillings preservative-free. Heat pasteurization was not feasible because it would destroy the fresh texture and flavor quality attributes. Rodilla spent 2 years looking for a technical solution to this problem. These studies led to experiments with high-pressure preservation of sandwich fillings in 2005. Studies showed that high pressure could pasteurize the fillings and provide a refrigerated shelf life of up to 21 days without changing the texture and flavor of the fillings.

Rodilla installed a 120-L, 600 MPa (87,000 psi), horizontal system. An immediate advantage was that the production costs could be reduced. The extended product shelf life allowed larger batches of filling to be prepared, and every type of filling did not need to be prepared every day. All fillings are processed at 500 MPa (72,500 psi) for several minutes in 1 or 2-kg flexible pouches for shipment to Rodilla shops all over Spain.

7.6. *Echigo Seika: High-Pressure Transformation of Rice and Cereals*

The Japanese company Echigo Seika (www.echigoseika.co.jp) pressure treats rice and cereal products. Since 1994 (Suzuki, 2002), the company has used high pressure to modify the structure of proteins and starches without heat, accelerate enzyme reactions, speed penetration of water into grains, accelerate the breakdown of cell walls, and eliminate air or other gases in grains. The process is called “high pressure-induced transformation” (Hi PiT) (Yamazaki, 2005a) and Echigo Seika has patented several of its processes. The patented processes require a combination of pressures from 200 to 400 MPa (29,000 to 58,000 psi) with moderate heat treatments at 50°C (122°F). The company operates two factories in Japan and one in China. Each factory has a processing line equipped with two 130-L, vertical, 400 MPa (58,000 psi) systems for processing rice or cereal grains in package or in bulk.

Echigo Seika produces four types of HPP products for the Japanese market and, in 2006, processed a

total of 2,400 metric tons (5,300,000 lbs) of these products in these three plants. High-pressure-treated, RTE, brown rice or white rice, is packaged in single serve trays. The products need only to be heated for 3 minutes in a microwave oven. Starch retrogradation is reduced and gelatinization is increased by the high-pressure treatment. This contributes to better sensory quality (Hayashi, 2005), and digestion in brown rice when compared to conventional cooked brown rice. These products have a shelf life of 1 year using a heat sterilization step after HPP.

Single portion, RTE, cereal mix is composed of eight kinds of grains including brown rice, black beans, soybean, azuki bean, oats, barley, millet, and red rice. High-pressure accelerates water hydration of the grains to improve the cooking step, which occurs during the final step of heat sterilization. A sweeter and more digestible product is obtained (Yamazaki, 2000).

Gamma aminobutyric acid (GABA)-enriched brown rice is known for its health benefits such as reducing blood pressure and improving menopausal disorders and liver functions. HPP is used for two purposes in the preparation of this product. First, it increases the contact between glutamic acid and glutamate decarboxylase, the enzyme catalyzing GABA synthesis. This is accomplished by rice cell wall structure modifications under pressure (Yamazaki, 2005b). Second, HPP acts as a precooking process to shorten the long cooking time normally required to prepare brown rice. Consumers have a more convenient product. The product is dried after HPP treatment, sold in traditional 0.5-kg pouches, and is stable during room temperature storage.

Hypoallergenic rice is prepared by HPP treatment of partially hydrated brown rice to enhance rice cell wall porosity. The increased cell diffusion facilitates salt-extraction of allergenic proteins (Yamazaki et al., 1998). This product is dried after extraction.

In 2002, the HPP processing cost of a 0.3-L, single-serve portion of rice was estimated by Echigo Seika to be 1.25 cents (US) on the basis of 100% utilization of the HPP equipment (2.50 cents at a 50% use rate). These costs included depreciation, energy, wear parts, operation, and administration (Sasagawa & Yamazaki, 2002).

7.7. Mitsunori RTE Clams

Mitsunori (www.mitunori.co.jp/html/kaisya-annai.html) is a Japanese company specializing in seafood processing. The company purchased a 55-L, horizontal system in 2007 for opening clams and whelks (scungilli). A pressure treatment in the range of 1 minute at 300 MPa (43,500 psi), with seawater as the compression medium, causes the shells to open. The meat can be removed manually. The shellfish meat is rinsed in seawater and packaged in flexible pouches or trays also filled with sea water. Refrigerated shelf life is from 3–6 days, depending on the type of seafood. HPP greatly reduces the cost of labor for opening small size clams and helps preserves freshness. This is very important for Japanese consumers who are used to eating raw seafood.

7.8. Donny Boy Premium Fruit Products

Donny Boy is an Australian fruit-processing company dedicated to the processing and marketing of high-pressure preserved fruit-based products including purees, sauces, and juices. These products are used in yogurt, ice cream, food service, and as beverages. HPP provides a pasteurized fresh fruit ingredient that can be used in products, such as yogurt and ice cream, which are not pasteurized after preparation. Fruit products are held at 600 MPa (87,000 psi) for a few minutes in a 55-L, horizontal system operating at room temperature. The company started the marketing of its first products, apricot, peach, and apple dice, for use in yogurt in 2007 under the trademark “Preshafruit” (www.preshafruit.com.au).

HPP fruit preparation for yogurt is not new. Danone applied for a Novel Food Authorization for it in France in 1998. The authorization was obtained in 2001 (Anonymous, 2001), but Danone did not commercialize the process. Thus, after two years of intensive studies and recipe development, Donny Boy is the first to market HPP fruit preparations. Products are packaged in flexible bags designed to give a volumetric efficiency more than 0.85. In 2008, their range of fruit preparations, including dice, has been extended to include strawberry, cherry, and mango. Donny Boy has also launched a line of exotic fruit

purees packaged in flexible, transparent, pouches, and lines of HPP fruit juices and smoothies. The juices and smoothies are packaged in bottles with a triangular cross section in order to maximize volumetric efficiency.

7.9. Fonterra Colostrum-Based Products

Fonterra (www.fonterra.com) is a New Zealand cooperatively owned processor of dairy products, and is a world-leading exporter of these products. Fonterra’s research on the high-pressure treatment of milk and milk fractions has resulted in the issue of several patents. These patents cover several applications including the preservation of starter cultures and probiotic strains of lactic acid bacteria. By optimizing pressure-processing conditions, spoilage microflora such as molds and yeasts are reduced (Carroll et al., 2004).

Fonterra has demonstrated the high-pressure preservation of heat-sensitive bioactive components such as lactoferrin, immunoglobulins, and growth factors. Unwanted microbial growth is prevented (Palmano et al., 2006; Carroll et al., 2006). The patent will form the basis of a commercial beverage using colostrum (Hembry, 2008). Fonterra has signed its first license in 2008 for industrial production for Asian market, where consumers associate colostrum with good health and better immunity. This beverage will be shelf stable up to 6 months at room temperature using an HPP treatment of 500 MPa (72,500 psi) and a pH below 4.5.

8. Conclusion

HPP has been successfully used in the commercial preservation of foods for more than 15 years and the number of installed high-pressure systems worldwide is constantly growing. The increasing use of HPP by food processors reflects industry needs for safe, refrigerated, convenient packaged foods that deliver just prepared freshness and have a reasonable shelf life. The technology has been adopted all over the world by large and small processors in all food sectors.

HPP is now mainly used as a nonthermal pasteurization technology. Exceptions are shellfish and crustacean meat extraction, commercial sterilization of acid foods, and to produce desirable changes in the structure of food and food ingredients. The high-pressure-induced transformation of Japanese rice and cereal products are good examples of this application.

The pressure-assisted thermal sterilization of low-acid foods, using a combination of pressure and high temperature, has shown that high-quality, shelf-stable products can be produced in a fraction of the time needed for conventional thermal processing. The technology takes advantage of compression heating as explained in Chapter 1. Industrial users of pressure-assisted thermal processing must demonstrate the successful application of the process to food safety authorities such as the US FDA or European Food Safety Agency (EFSA). The safety of the process must be demonstrated with commercial size process vessels as for any new thermal processing equipment. High-pressure equipment manufacturers must then build affordable and reliable large volume machines that can operate in the temperature range of 110–130°C (230–266°F) at 600 MPa (87,000 psi). Since the process depends on developing a uniform temperature throughout the pressure vessel by compression heating, insulation and vessel wall temperatures are important control factors.

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Chapter 5

Microbiological Aspects of High-Pressure Food Processing

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1. Introduction

High-pressure processing (HPP) can be used to pasteurize, sterilize, or develop novel high-pressure (HP)-treated products, however, any successful product must be microbiologically safe. For this reason, significant research worldwide has focused on the HPP inactivation of microorganisms in foods. This work has not only helped establish HPP technology as a useful tool for the production of safe and extended shelf-life foods, but has also helped microbiologists gain a better understanding of the mechanisms by which HPP inactivates microorganisms.

Inactivation of vegetative microorganisms by HPP requires pressure in the range of 300–700 MPa (43,500–101,500 psi) (Table 5.1). Bacterial endospores commonly require pressures applied at elevated treatment temperatures (Cheftel, 1995; Palou et al., 1999). gram-positive bacteria tend to be more pressure-resistant than gram-negative bacteria (Hoover et al., 1989; Patterson et al., 1995; Palou et al., 1999; Gervilla et al., 2000). In general, heat-resistant microorganisms are also generally resistant to HPs (Smelt, 1998). Cells in the exponential phase of growth have been found to be less resistant than those in the stationary phase (Issacs and Chilton, 1995; McClements et al., 2001). Some yeasts and molds can be very sensitive to pressure (Palou et al., 1998b; O'Reilly et al., 2000) and some viruses such as Aichi virus, a

picornavirus, can be very resistant (Kingsley et al., 2004).

2. The Effects of HPP on Vegetative Bacteria

HP can damage bacterial cell membranes, affect homeostasis, denature and inactivate proteins including enzymes involved in replication, and can alter the morphology of the cell. Some or all of these injuries can lead to cell death. Although not all of the mechanisms of HP-induced inactivation are fully understood, there is a great deal of valuable literature describing the studies that have attempted to characterize and comprehend the responses of microorganisms to HP (for review, see Hoover et al., 1989; Farkas and Hoover, 2000; Smelt et al., 2001; Patterson, 2005). HP-induced inactivation of microorganisms is largely dependent on species and, in some cases, there are significant differences in the pressure sensitivities of individual strains within the same species (Linton et al., 2001). Table 5.1 summarizes a selection of studies on the inactivation of vegetative bacteria in various substrates over a range of time, temperature, and pressure parameters.

As pressure increases, the death rate of microorganisms increases (Issacs and Chilton, 1995); however, increasing the duration of pressure treatment does not necessarily enhance the lethal effect (Patterson et al., 1995). Kinetic studies of HP-induced inactivation have usually reported a first-order kinetic reaction or a sigmoidal response, the latter indicating the possible presence of pressure-resistant subpopulations (Hoover et al., 1989;

Table 5.1. Inactivation of selected vegetative bacteria by high pressure

Microorganism	Substrate	Treatment Conditions			Log Reduction	Reference
		Pressure (MPa)	Time (minutes)	Process Temperature (°C)		
<i>Campylobacter</i>						
<i>C. jejuni</i>	Pork slurry	300	10	25	6	Shigehisa et al., 1991
<i>C. jejuni</i>	UHT milk	375	10	25	6	Solomon and Hoover, 2004
<i>C. jejuni</i>	UHT soy milk	375	10	25	6	Solomon and Hoover, 2004
<i>C. jejuni</i>	Chicken puree	375	10	25	6	Solomon and Hoover, 2004
<i>Citrobacter</i>						
<i>C. freundii</i>	Minced meat	280	20	20	5	Carlez et al., 1993
<i>Enterobacter</i>						
<i>E. sakazakii</i> A	Infant formula	600	1	25	6.8	Gonzalez et al., 2006
<i>E. sakazakii</i> B	Infant formula	600	11	25	3.1	Gonzalez et al., 2006
<i>Escherichia coli</i>						
<i>E. coli</i> 405 CECT	Goat’s fresh milk cheese	400	15	25	8	Capellas et al., 1996
<i>E. coli</i> MC 1061	Skim milk	500	15	20	6.5	Black et al., 2005
<i>E. coli</i> ATCC 29055	Apple juice	400	<1	25	8	Ramaswamy et al., 2003
<i>E. coli</i> O157:H7 NCTC 12079	UHT milk	600	15	20	<2	Patterson et al., 1995
<i>E. coli</i> O157:H7 NCTC 12079	UHT milk	200	15	60	8	Patterson and Kilpatrick, 1998
<i>E. coli</i> O157:H7 933	1% peptone	345	5	25	2.8	Alpas et al., 1999
<i>E. coli</i> O157:H7 932	1% peptone	345	5	25	5.6	Alpas et al., 1999
<i>E. coli</i> MG1655	UHT milk	700	15	20	2.3	Garcia-Graells et al., 1999
<i>Listeria</i>						
<i>L. monocytogenes</i> Scott A	1% peptone	345	5	25	<1	Alpas et al., 1999
<i>L. monocytogenes</i> SLR1	1% peptone	345	5	25	3.53	Alpas et al., 1999
<i>L. monocytogenes</i> Scott A	UHT milk	340	80	23	6	Styles et al., 1991
<i>L. monocytogenes</i> Scott A	UHT milk	600	10	20	7.5	Chen and Hoover, 2004
<i>L. monocytogenes</i>	Poultry meat	375	15	20	2	Patterson et al., 1995
<i>L. monocytogenes</i> NCTC 11994	UHT milk	400	24	8	6	McClements et al., 2001
<i>L. monocytogenes</i> NCTC 11994	UHT milk	375	30	20	1.5	Simpson and Gilmour, 1997
Lactic Acid Bacteria						
<i>Lactococcus lactis</i> ssp. <i>lactis</i>	Skim milk	400	20	20	3.2	Casal and Gomez, 1999
<i>Lactococcus casei</i> ssp. <i>casei</i>	Skim milk	400	20	20	3	Casal and Gomez, 1999

Table 5.1. (Continued)

Microorganism	Substrate	Treatment Conditions			Log Reduction	Reference
		Pressure (MPa)	Time (minutes)	Process Temperature (°C)		
<i>Lactobacillus viridescens</i>	Skim milk	500	5	20	6.1	Black et al., 2005
<i>L. viridescens</i>	Ham	500	5	25	4	Park et al., 2001
<i>Lactobacillus helveticus</i>	Ewe's milk	500	10	10	3	Gervilla et al., 1997
<i>Lactobacillus plantarum</i>	Model beer	600	5	15	8	Ulmer et al., 2000
<i>Lactobacillus sakei</i>	Cooked ham	500	10	40	4	Hugas et al., 2002
<i>Mycobacterium</i>						
<i>Mycobacterium avium</i> ssp. <i>paratuberculosis</i>	Milk	500	10	20	6.5	Donaghy et al., 2007
<i>Salmonella</i>						
<i>S. Senftenberg</i> 775W	Baby food	340	10	23	<2	Metrick et al., 1989
<i>S. Typhimurium</i>	Phosphate-buffered	350	15	20	5	Patterson et al., 1995
<i>S. enteritidis</i>	Phosphate-buffered	450	15	20	5	Patterson et al., 1995
<i>Shigella</i>						
<i>S. flexneri</i>	Buffer	350	15	25	6	Wuytack et al., 2002
<i>Staphylococcus</i>						
<i>S. aureus</i>	Poultry meat	600	15	20	3	Patterson et al., 1995
<i>S. aureus</i> 778	1% peptone	345	5	25	<1	Alpas et al., 1999
<i>S. aureus</i>	Cheese slurry	600	20	20	6	O'Reilly et al., 2000
<i>S. carnosus</i>	Cooked ham	500	10	40	1.29	Hugas et al., 2002
<i>Pseudomonas</i>						
<i>P. fluorescens</i>	Ewe's milk	450	10	10	4	Gervilla et al., 1997
<i>P. fluorescens</i>	Skim milk	300	5	20	8.2	Black et al., 2005
<i>P. fluorescens</i>	Minced beef	200	20	20	5	Carlez et al., 1993
<i>P. aeruginosa</i>	Buffer	400	10	20	6	Arroyo et al., 1997
<i>Vibrio</i>						
<i>V. cholera</i>	Artificial seawater	300	15	25	7	Berlin et al., 1999
<i>V. vulnificus</i>	Artificial seawater	300	15	25	7	Berlin et al., 1999
<i>V. parahaemolyticus</i>	Oysters	345	1.5	22	7	Calik et al., 2002
<i>V. parahaemolyticus</i>	Clam juice	170	10	23	6	Styles et al., 1991
<i>V. parahaemolyticus</i> O3:K6	Oysters	300	13	10	5	Cook, 2003
<i>Yersinia</i>						
<i>Y. enterocolitica</i>	Phosphate-buffered	275	15	20	5	Patterson et al., 1995
<i>Y. enterocolitica</i>	Pork slurry	300	10	25	6	Shigehisa et al., 1991
<i>Y. enterocolitica</i>	Cheese	400	10	20	7	De Lamo-Castellvi et al., 2005

UHT, ultrahigh temperature.

Earnshaw et al., 1995; Smelt, 1998). At low to moderate pressures, a so-called “tailing effect,” that is, a decrease in inactivation with time, has been found (Earnshaw et al., 1995; Patterson et al., 1995; Masschalck et al., 2000; Tay et al., 2003). The “tail-

ing” phenomenon may be caused by the inherent variation in pressure resistance of the population, the effects of experimental conditions, or cell-age distribution (Patterson et al., 1995; Earnshaw et al., 1995). Pronounced tailing has been observed by Masschalck

et al. (2000) in the pressure-resistant strain, *Escherichia coli* MG1655, limiting the level of inactivation under mild conditions (<400 MPa; 58,000 psi). In most cases, if higher pressures are applied, this tailing effect is less dramatic.

3. Mechanisms of Pressure-Induced Inactivation

Pressure-induced damage to microorganisms can include cell membrane disruption (Hauben et al., 1996; Pagán and Mackey, 2000), ribosomal destruction (Niven et al., 1999), inactivation of enzymes (Degraeve et al., 1996; Simpson and Gilmour, 1997), inactivation of membrane-bound transport systems (Ulmer et al., 2002), and damage to the proton efflux system (Wouters et al., 1998). Dramatic changes to the morphology of bacteria following pressure treatment have been observed with the aid of scanning electron microscopy (Ritz et al., 2001; Kaletunc et al., 2004). The effects of HP on cell structure include the collapse of intracellular vacuoles at relatively mild pressures (0.6 MPa), separation between the cell wall and cytoplasmic membrane, and ribosomal destruction (Earnshaw et al., 1995). These effects lead to the impairment of cell functions, slowing growth rate or causing cell death.

The cell membrane appears to be the primary site of pressure-induced damage in microorganisms, and can result in leakage of intracellular components and loss of homeostasis (Farkas and Hoover, 2000). HP treatment causes an increase in extracellular levels of adenosine triphosphate (ATP) (Smelt et al., 1994) and an increase in the uptake of propidium iodide (Ulmer et al., 2000) and ethidium bromide (Benito et al., 1999), indicating loss of membrane permeability and function. Mañas and Mackey (2004) described the effects of HP on the membranes of stationary-phase cells versus exponential-phase cells of *E. coli*. The increased sensitivity to pressure of exponential-phase cell membranes was linked to physical perturbations of the cell envelope, loss of osmotic responsiveness, and exclusion of intracellular proteins and RNA. Wouters et al. (1998) observed reduced activity of the integral membrane protein

F₀F₁ ATPase in *Lactobacillus plantarum* treated at 250 MPa in addition to impairment of acid reflux and maintenance of intracellular pH.

E. coli in the exponential phase subjected to HP displayed a loss of viability which was related to a permanent loss of membrane integrity. In stationary phase, the cell membranes became leaky during pressure treatment, but resealed partially or wholly after decompression (Pagán and Mackey, 2000). Hauben et al. (1996) proposed that a transient permeabilization of the outer membrane of *E. coli* is caused by HP. *E. coli* becomes sensitive to nisin and lysozyme during compression; however, if these peptides are added to the cells posttreatment, *E. coli* remains resistant. It appears that structural damage to the outer membrane (pore/lesion formation) is rapidly repaired after pressurization. Similar results were found by Black et al. (2005) on examining membrane integrity using fluorescent dyes during pressure treatment. It was found that cells of *Pseudomonas fluorescens* were permeabilized to propidium iodide by a sublethal pressure treatment and most cell membranes resealed after treatment. According to MacDonald (1992), cell membranes with increased fluidity are less resistant to HP. Work by Ulmer et al. (2002) supports this view, as cells of *L. plantarum* with membranes in the liquid-crystalline (fluid) phase were shown to be less resistant to HP than those with membranes in the gel (rigid) phase.

In addition to membrane damage, enzyme inactivation and protein denaturation are other important factors in the HP inactivation of microorganisms. In *E. coli*, the activity of isocitrate dehydrogenase, a vital enzyme in the respiratory cycle, was reduced by 90% when cells were held at 400 MPa (58,000 psi) for 2 minutes. This loss of activity was reflected in the loss of viability of the organism (Isacs and Chilton, 1995). Ritz et al. (2000) analyzed the action of HP on the membrane proteins of the important food pathogen, *Salmonella* Typhimurium. The outer membrane showed greater damage than the cytoplasmic membrane when pressure was applied. Some proteins of the outer membrane appeared to be completely denatured, while a few were resistant; for example, the protein LamB was more resistant to HP

in acidic conditions. Ulmer et al. (2000) showed that a moderate treatment, 200 MPa (29,000 psi), caused inactivation of the membrane-bound hop-resistance protein, HorA, in *L. plantarum*, resulting in reduced resistance to hops during beer storage. Pressure damage to F₁F₀ ATPase in *L. plantarum* caused by treatment at 250 MPa (36,250 psi) resulted in loss of the ability of the cell to maintain intracellular pH. Prolonged pressure treatment eventually led to cell death (Wouters et al., 1998).

In a study by Niven et al. (1999), it was shown that ribosomal destruction occurred in parallel with loss of cell viability in *E. coli*. After treatment, ribosomes with reduced stability were found to be capable of recovery under optimal conditions; however, cell viability often continued to decline, indicating that other factors are involved in the death of pressure-damaged cells. It was suggested that ribosomal destabilization was caused by leakage of Mg²⁺ from pressure-permeabilized membranes. Alpas et al. (2003) used differential scanning calorimetry to compare the pressure sensitivities of *E. coli* O157:H7 and *Staphylococcus aureus* and found that ribosomal denaturation coincided with cell death. HP-induced inactivation of microorganisms can occur as a result of one destructive event to the membrane or protein moieties, or more likely, as a result of a combination of pressure-induced effects.

4. The Effects of Suspending Medium on Pressure-Induced Inactivation

The pressure-induced inactivation of microorganisms is extremely dependent on the suspending medium (Table 5.1). Foods, for example, dairy products, are generally physicochemically complex and many foods or their ingredients have been shown to give a baroprotective effect to harbored microorganisms. For example, according to Garcia-Graells et al. (1999), milk has a strong protective effect on *E. coli* MG1655, with a 7-log reduction following treatment at 400 MPa (58,000 psi) in phosphate buffer, and only a 3-log reduction at 700 MPa (101,500 psi) in milk, both in 15 minutes at 20°C. This baroprotective

effect has also been shown in bovine milk for a range of organisms (Gervilla et al., 1997, 2000). *Enterobacter sakazakii* can be protected from HPP in infant formula at moderate pressures of 200 and 400 MPa (29,000–58,000 psi). This protection is less evident at higher pressures (Gonzalez et al., 2006). Calcium and other minerals associated with casein micelles of milk have been found to protect *E. coli* and *Listeria* against the lethal effects of HPP (Hauben et al., 1998; Black et al., 2007a).

Lowering the water activity (a_w) of a food product generally confers a baroprotective effect on microorganisms. The protective effect of low a_w , however, is very dependent on the type of solute used (Molina-Höppner et al., 2004). At equal a_w values, a sucrose solution gives greater baroprotection to *Listeria monocytogenes* than a sodium chloride solution (Koseki and Yamamoto, 2007). Van Opstal et al. (2003) demonstrated the protective effect of high sucrose concentrations (10–50% w/v) on *E. coli* in phosphate buffer. Solutes can act by preventing the inactivation of enzymes and in maintenance of the fluidity of the cytoplasmic membrane (Molina-Höppner et al., 2004).

Over a range of 480–600 MPa (69,600–87,000 psi), the differences in barotolerances of *Listeria innocua*, *L. monocytogenes*, *E. coli* O157:H45, and *Vibrio mimicus* in oysters compared to phosphate-buffered saline (PBS) was approximately 5 log CFU/mL (Smiddy et al., 2005). The protective effect was thought to be related to the high salt content of the oysters as bacteria examined in tryptone soy broth with yeast extract (TSBYE) containing added sodium chloride were also found to be more resistant to pressure than bacteria pressure-treated in TSBYE without added salt. Smiddy et al. (2005) demonstrated that *L. monocytogenes* with the ability to accumulate compatible solutes (betaine and L-carnitine) display greater pressure tolerance than mutants incapable of transporting compatible solutes. They hypothesized that the protective role of these osmolytes was maintenance of membrane fluidity and prevention of protein unfolding.

In general, meat products do not confer significant protection to pressure-treated bacteria, especially when compared to other foods such as milk.

Patterson et al. (1995) found that ultrahigh-temperature (UHT) milk gave more baroprotection than poultry meat to *L. monocytogenes*, *E. coli* O157:H7, and *S. aureus*. Carballo et al. (1997) showed that similar reductions of total aerobic bacteria and psychrotrophic bacteria in beef patties were achieved at 300 MPa (43,500 psi) regardless of the fat content, either 9.2 or 20.3%, with the exception of *S. aureus* which was protected in the higher fat-content patties. In a study of HPP-cooked ham and minced pork patties, microbial reductions were found to be higher in pork patties (Lopez-Caballero et al., 2002). This effect may be due to the salts and sugars of the cooked ham having a protective effect, or possibly that the nature of the contaminating microbiota was more pressure-resistant. The microbiota of the patties was dominated by pressure-sensitive *Pseudomonas* species while the ham was dominated by less sensitive lactic acid bacteria.

Also, acid foods can have the opposite effect on microorganisms subjected to HP. That is, the added hurdle of low pH enhances pressure inactivation and may prevent recovery of injured cells (Linton et al., 1999; Jordan et al., 2001; Dogan and Erkmen, 2004). Both Garcia-Graells et al. (1998) and Pagán et al. (2001) found that HPP sensitizes *E. coli* to acidic conditions posttreatment.

5. Injury and Repair

HPP may leave a proportion of a microbial population injured, depending on the pressure applied, suspending medium, and duration of treatment (Patterson et al., 1995). This can lead to either underestimation or overestimation of the inactivation achieved by HPP (Cheftel, 1995). Depending on posttreatment conditions, these cells may revive in the nutrient-rich environment that many foods provide, or die due to increased sensitivity to acidic conditions or the presence of antimicrobial additives such as beer hops (Ulmer et al., 2000; Ganzle et al., 2001). Dogan and Erkmen (2004) reported that *L. monocytogenes* was more sensitive to HPP in fresh orange and peach juices than in brain heart infusion broth, most likely due to the low pH of the juices. Chilton et al. (2001) showed that different types of damage

were inflicted on the outer and inner membranes of *E. coli* by sublethal pressures. Repair of the cytoplasmic membrane requires RNA and protein synthesis. Repair of the outer membrane was shown not to be energy-dependent or requiring synthesis of RNA or protein.

The recovery from pressure-induced injury of four foodborne pathogens (*L. monocytogenes*, *S. aureus*, *E. coli* O157:H7, and *Salmonella* Enteritidis) in milk stored at 4, 22, and 30°C was described by Bozoglu et al. (2004). The authors suggested that two types of injury, I1 and I2, occurred in all four bacteria. I1 injury is most likely a structural damage that will prevent colonies growing on selective agar, but will allow growth on a nonselective medium. The second type of injury, I2, is metabolic injury, which if given suitable conditions can recover to an I1 state. The inability of I2 cells to grow on either selective or nonselective media and the potential of I2 cells to recover raises concern that their presence will not be detected unless proper storage or shelf-life studies are conducted. Bull et al. (2005) also highlighted the pitfalls of *L. monocytogenes* detection following pressure treatment. In this study, it was suggested that some selective agents in enrichment broths might be toxic and would impair recovery of injured cells. Optimized Penn State University (oPSU) enrichment broth was found to significantly increase the likelihood of detecting the pathogen in HP-treated milk.

Depending on the nature and extent of injury, microorganisms may be more susceptible to further treatments or hurdles, such as mild heat or bacteriocins, resulting in improved product stability (Cheftel, 1995; Chilton et al., 2001). It is of the utmost importance to consider the fate of pressure-injured cells, as some recent studies have reported recovery and regrowth of injured cells even after 3–5 days of storage (Black et al., 2005; De Lamo-Castellvi et al., 2005). Black et al. (2005) observed recovery of *P. fluorescens* during storage in milk at 4°C following treatment at 250 MPa (36,250 psi). Increasing the pressure treatment to 300 MPa (43,500 psi) or the addition of another hurdle, that is, the addition of nisin, prevented recovery in milk. See Section 4 on combination processes for more examples.

6. Pressure-Inactivation Kinetics

Many survival curves have been generated in the study of inactivation kinetics from application of pressure to pure cultures of microbial cells and spores. From the survival curves has followed modeling of the inactivation kinetics to predict the end point of processes incorporating HPP. Comparisons have commonly been made to thermal inactivation. Processing using heat has been traditionally followed using first-order kinetics based on the assumption that all cells or spores in a population possess equivalent resistances to the lethal treatment; however, for the most part, pressure inactivation curves generally follow nonlinear inactivation kinetics. Instead of straight lines that can easily be used to determine D values (decimal reduction times) to characterize heat resistance of a microorganism, HPP survival curves often show shoulders (concave-down) and tails (concave-up) or a sigmoidal shape (Cerf, 1977; Peleg and Cole, 1998).

Several mechanistic or empirical models originally based on growth curves have been proposed for use in describing nonlinear survival curves. These include among others, the modified Gompertz (Bhaduri et al., 1991), log-logistic (Cole et al., 1993), Baranyi (Baranyi and Roberts, 1994), and Weibull models (Peleg and Cole, 1998). A fundamental assumption of most of these models is that cells or spores of a population do not have identical resistances to the lethal treatment and these differences are permanent (Chen and Hoover, 2003a).

The Weibull model, which has advantages in simplicity and flexibility, has worked reasonably well to predict pressure inactivation. Chen and Hoover (2003a) found both the Weibull distribution and log-logistic model providing good fits of data derived from inactivation of *Yersinia enterocolitica* in phosphate buffer and UHT whole milk over a pressure range of 300–500 MPa (43,500–72,500 psi) at ambient temperature. The modified Gompertz model was unable to fit the data curves as well as the Weibull and log-logistic models. Tailing was seen in all survival curves.

For the case of *L. monocytogenes* Scott A in whole milk, Chen and Hoover (2003b) produced survival

curves at four different treatment temperatures (22, 40, 45, and 50°C) and two pressure levels (400 and 500 MPa; 58,000 and 72,500 psi). Again, tailing was seen in all survival curves, and the modified Gompertz produced a poorer fit of data in modeling. The Weibull and log-logistic models were comparable in producing very good fits of data. Similar trends were seen with *S. Typhimurium* DT 104 in UHT milk (Guan et al., 2005a); however, in the case of pressure inactivation of *S. aureus* and *E. coli* O157:H7 in UHT milk, the modified Gompertz and Weibull models gave the better fits depending on the process temperature (Guan et al., 2005b).

For coliphage λ cI 857, pressure-inactivation curves also demonstrated tailing in all cases. The Weibull and log-logistic models consistently produced best fits for data in all survival curves. A simplified Weibull model was proposed in which the number of parameters were reduced from two to one by setting the shape factor, n , at the mean value because there was no significant differences in the values of n for the buffered medium used. The simplified model gave a fit comparable to the full Weibull model.

Bacterial spores normally require an elevated treatment temperature, above ambient, to demonstrate any significant degree of inactivation by HPP. The primary circumstance for pressure inactivation of bacterial spores is one in which exposure to the combination of pressure and elevated temperature induces germination so that spores lose their impressive resistance to lethal effects and are subsequently inactivated by treatment conditions (Black et al., 2007b). Thus, the process temperature is a critical parameter in optimizing spore inactivation even if pressure is not incorporated with the higher temperatures used in pressure-assisted thermal sterilization (PATS) types of treatments. For food-processing applications employing pressure in the pasteurization of low-acid foods, pressure inactivation of spores normally is conducted above 40°C to induce germination. The survival curves for HPP inactivation of spores have commonly been nonlinear (Black et al., 2007b).

Spores of *Geobacillus* (nee *Bacillus*) *stearothermophilus* have been examined in the modeling

of HPP-inactivation kinetics for bacterial spores. Anata et al. (2001) successfully adapted a nonlinear regression method based on n th-order kinetics to inactivation data obtained from spores inoculated into mashed broccoli and cocoa mass. The model worked well for both model foods in which temperatures between 60 and 120°C and pressures between 50 and 600 MPa (7,250–87,000 psi) were examined. It was found that increasing the moisture content of the cocoa mass reduced the protective effect of the fat. Both Patazca et al. (2006) and Rajan et al. (2006) selected *G. stearothermophilus* for their work as well. Patazca et al. (2006) identified the log-linear regression model for best-fit data from combination treatments of 500–700 MPa (72,500–101,500 psi) and 92–111°C for 0.01–360 seconds in distilled water. Rajan et al. (2006) inoculated their spores into egg patties and found the Weibull model to best describe data at 700 MPa (101,500 psi) and 105°C. It was noted that the Weibull model had advantages in mathematical simplicity and accuracy in predicting tailing.

de Heij et al. (2003) suggested a predictive model incorporating parameters, pressure equipment material and dimensions, product characteristics, and target microorganisms with the final product temperature a critical processing parameter. Their proposed two-step approach involves calculation of temperature distribution by the heat of conduction in the vessel using an axisymmetric one-dimensional finite element model followed by use of the Eyring–Arrhenius equation to calculate the inactivation of spores as a function of time, product temperature, and pressure level.

7. The Effects of HPP on Bacterial Spores

Bacterial spores are extremely resistant to the effects of HPP. It is generally accepted that high levels of inactivation of spores of *Bacillus* or *Clostridium* species cannot be achieved by applying commercially used pressure treatments (200–600 MPa; 29,600–87,000 psi) alone (Black et al., 2007b). HP can be used as a part of a hurdle approach to killing spores in food. See Chapter 28 on combined processes. HPP has been combined with various an-

timicrobial substances such as citric acid (Roberts and Hoover, 1996), sucrose laurate (Shearer et al., 2000), and bacteriocins (Lopez-Pedemonte et al., 2003; Kalchayanand et al., 2003) to inactivate spores.

Pressure-induced germination of spores was identified as early as 1969 by Clouston and Wills. This loss of dormancy by application of HP has been shown by Paidhungat et al. (2002) to involve the germinant receptors of spores. HPP can cause germination of spores of *Bacillus subtilis* even in the absence of nutrient germinants. Pressure-induced germination sensitizes spores to other processing hurdles or to subsequent cycles of HP. A strategy of cycled HP has been investigated by some authors. The first cycle is to germinate spores and is followed by further cycles to inactivate the cells (Gola et al., 1996; Mills et al., 1998; Lopez-Pedemonte et al., 2003). The problems of superdormancy and an inability to achieve 100% germination, however, hamper these methods (Black et al., 2007b). To date, the most efficient hurdle-based approach to achieve sterilization using HPP is a combination of HP and high temperature. The process of PATS or pressure-assisted thermal processing (PATP) is discussed in more detail below.

8. Pressure-Assisted Thermal Sterilization

HPP is often referred to as a nonthermal processing method, but this reference can be questioned given the natural generation of compression heat (for additional discussion on adiabatic heat, see Chapter 1) during pressure applications and the not uncommon use of elevated temperature during pressure treatment as a means to enhance the effectiveness for inactivating microorganisms in a processed product. Incorporating heat at a higher level is called PATS, and is the combination of pressure and high temperatures to realize moist-heat sterilization. In PATS, initial pressure treatment temperatures in the approximate range of 70–90°C are used. Such an approach represents a viable means to inactivate spores and other recalcitrant organisms or enzymes in low-acid foods. The advantage offered by PATS is a reduced or better controlled exposure to heat, due to the uniformity of compression heating, that can enhance

product quality while eliminating viable spores (Wilson and Baker, 1997; Meyer et al., 2000; Matser et al., 2004). PATS can be directed against other pressure-resistant forms of microorganisms, such as human infectious viruses (e.g., Aichi virus), parasites (e.g., oocysts of *Cryptosporidium* and *Cyclospora*), and fungal ascospores (e.g., *Byssoschlamys fulva*, *Byssoschlamys nivea*, and *Neosartorya fischeri*). Brown et al. (2003) found that infectious doses of scrapie prions could be effectively inactivated by short pressure pulses of 690–1,200 MPa (100,050–174,000 psi) at treatment temperatures of 121–137°C.

PATS takes advantage of the instantaneous and spatially homogeneous adiabatic heating of compression to better control temperature application. All compressible substances increase in temperature during physical compression (Ting et al., 2002). There is an increase of approximately 3°C for every 100-MPa increase in pressure in foods high in water content (Balasubramaniam et al., 2004), while foods high in fats and alcohol may exhibit compression heating approaching 9°C per 100 MPa (Rasanayagam et al., 2003). In a PATS application, the initial treatment temperature (IT), of the product is raised to the desired level, followed by pressure treatment of 500–700 MPa (72,500–101,500 psi). For example, if the temperature of the food is 80°C and a pressure of 700 MPa (101,500 psi) is then applied, the temperature uniformly increases to approximately 115°C as a result of the heat of compression added to the initial product temperature (Balasubramaniam et al., 2004; see Chapter 1 for additional discussion). When pressure is released, the reversible adiabatic heat is immediately dissipated so there is instantaneous cooling back to the initial product treatment temperature with no dependency on product size. Meyer et al. (2000) found a twin pulse of pressure to work best in the elimination of spores to insure moist-heat sterilization. Spore lethality from PATS can be considered a combination of protein denaturation and enzyme inactivation by heat with the aggregation of proteins under pressure (Rodriguez et al., 2004).

The process temperature is not only dependent on the initial temperature of the product and magnitude of pressure applied, but also on the uniformity of the product temperature at HP. Heat transfer effects

affecting spore inactivation can be caused by temperature differences in the food product, the compression fluid, and the walls of the pressure vessel (Balasubramanian and Balasubramaniam, 2003). Heat loss into reduced-temperature surroundings can be a critical factor affecting spore elimination by PATS (de Heij et al., 2003). Ting et al. (2002) stated that maintenance of proper temperature levels was necessary over the entire length of PATS treatment to avoid tailing seen in spore inactivation curves.

Spores of the dominant pathogen to low-acid and shelf-stable products, *Clostridium botulinum*, are very resistant to inactivation by combinations of heat and HP, often requiring exposures to 121°C and 800 MPa (116,000 psi) to generate significant reductions (Hendrickx and Knorr, 2002). As one might anticipate, variations in resistances to PATS treatment by spores of *C. botulinum* exist. For example, exposure to 75°C and 827 MPa (119,915 psi) for 15 minutes in buffer only reduces spores of *C. botulinum* type A by 3.2 log₁₀ CFU/mL (Reddy et al., 2003), but spores from four different strains of nonproteolytic type B are reduced by 6 log₁₀ CFU/mL in buffer or crabmeat treated at 827 MPa (119,915 psi)/75°C/30 minutes (Reddy et al. 2006). Reductions of spores of *C. botulinum* by PATS at 600 MPa (87,000 psi) and 80°C ranged from 0 to 5.5 log₁₀ CFU/mL. Margosch et al. (2004) and Rodriguez et al. (2004) demonstrated a 4-log₁₀ CFU/mL inactivation of *C. botulinum* spores at 80°C/100 MPa (14,500 psi)/15 minutes. Thus, while PATS treatment parameters do vary from laboratory to laboratory, it is also relevant that spores of *C. botulinum* demonstrate an inherent range of inactivation differences based on strain variations.

Other spores of clostridia have been examined for inactivation by PATS. The nonpathogenic types of *Clostridium* spores appear more sensitive to inactivation than spores of *C. botulinum*. Ahn et al. (2007) used 121°C and 700 MPa (101,500 psi) for 1 minute to inactivate 7–8 log₁₀ CFU/mL spores of *Clostridium sporogenes* and *Clostridium tyrobutyricum* suspended in water. Kouchma et al. (2005) inactivated spore strips of *C. sporogenes* embedded in egg patties using 110°C and 690 MPa (100,050 psi) for 4 minutes. Ahn et al. (2007) evaluated spore suspensions of *Thermoanaerobacterium thermosaccharolyticum*

(formerly *Clostridium thermosaccharolyticum*) and *Bacillus amyloliquefaciens* in water and found 121°C and 700 MPa (101,500 psi) for 5 minutes inactivated 4.5 log₁₀ CFU/mL.

Scurrah et al. (2006) surveyed spores of *Bacillus* species from dairy sources. *Bacillus sphaericus* proved most resistant to PATS inactivation in skim milk at 600 MPa (87,000 psi) and 75, 85, and 95°C. When pressure-treated for 1 minute at 72°C and 600 MPa (87,000 psi), the range of responses of the 40 isolates of bacilli ranged from no inactivation to 6-log₁₀ spores/mL. Gao et al. (2006a) found optimal process parameters to eliminate *B. subtilis* spore populations of 6 log₁₀ to be 87°C and 579 MPa (83,955 psi) for 13 minutes in milk buffer. For spores of *G. stearothermophilus* (formerly *B. stearothermophilus*), Gao et al. (2006b) reported that 86°C and 625 MPa (90,625 psi) for 14 minutes gave 6-log₁₀ reductions in a milk buffer. In water, Patazca et al. (2006) found thermal resistances of spores (expressed as *D* values) of *G. stearothermophilus* varied according to the pressures used.

The observation that pressure can protect or stabilize spores to inactivation from heat has been reported. For example, Rajan et al. (2006) found application of 700 MPa (101,500 psi) reduced the *D* values for PATS treatments from 95 to 110°C, but treatment at 121°C at this pressure resulted in a *D*-value increase. Margosch et al. (2006) examined spores of *C. botulinum* and *B. amyloliquefaciens* and found that a heat exposure to 100°C alone inactivated more spores than treatment at 600 or 800 MPa (87,000 or 116,000 psi) and 100°C (and above).

The phenomenon of tailing has been reported in PATS studies (Margosch et al., 2006; Ahn et al., 2007). Tailing could be the result of pressure stabilization of spores; however, this could also be due, all or partly, to spore superdormancy or nonlinear inactivation kinetics naturally inherent in these studies involving large populations of spores or cells.

9. The Effects of HPP on Yeasts and Molds

The spoilage of fruit and vegetable products from the growth of yeasts and molds makes them an im-

portant target of HPP research. Many yeasts and molds can spoil fruit juices and fruit purees even under refrigeration temperatures (Splittstoesser, 1987; Pitt and Hocking, 1997). The success of several pressure-treated fruit and vegetable products available in Japan, the United States, and Europe proves the efficacy of HPP against agents of food spoilage. The synergy of naturally acidic foods (fruit juices and purees) and HP is an effective barrier against spoilage by most yeasts and molds found in these products (Stewart and Cole, 2001).

Yeasts and molds of importance in the food industry for causing spoilage include the yeasts, *Saccharomyces cerevisiae* and *Zygosaccharomyces bailii*, molds such as *Penicillium*, *Aspergillus*, and heat-resistant molds, such as *Byssoschlamys*, *Neosartorya*, and *Talaromyces*. Although *S. cerevisiae* is most notable for its beneficial role in the brewing and baking industries, it is also a powerful agent in food spoilage from growth and release of ethanol, carbon dioxide, hydrogen sulfide, and other off-odors (Thomas, 1993). Ascospore formation in *S. cerevisiae* can be induced on fruit surfaces where low concentrations of sugars and ethanol are present (Miller, 1989). *Byssoschlamys* species, such as *B. nivea* and *B. fulva*, are able to grow fermentatively at low oxygen levels and produce powerful pectinases that result in disintegration of the fruit structure. These species may also produce mycotoxin, such as patulin (Hocking and Pitt, 2001).

In general, vegetative cells of molds (e.g., conidia) are regarded as sensitive to HPP, while the ascospores of yeasts and molds are analogous to bacterial spores and are generally found to be the most pressure-resistant form of yeasts and molds (see below for ascospore inactivation). For example, Ogawa et al. (1990) observed a >5-log₁₀ reduction of each of nine species of yeasts and molds in fruit juice when treated at 350 MPa (50,750 psi) for 30 minutes or 400 MPa (58,000 psi) for 5 minutes. These authors demonstrated higher pressure resistances for ascospores than vegetative cells. They observed that heat-resistant fungi are also commonly more resistant to pressure than heat-sensitive fungi. Raso et al. (1998) compared the resistance of *Z. bailii* ascospores and vegetative cells suspended in apple,

orange, pineapple, cranberry, and grape juices to heat and HP. Ascospores were found to be 5–8 times more heat-resistant than the vegetative cells. The population of vegetative cells treated at 300 MPa (43,500 psi) for 5 minutes decreased by almost 5 log₁₀ cycles while a 0.5–1-log₁₀ reduction in the numbers of ascospores was observed for the same treatment. Decimal reduction times (*D* values) for *S. cerevisiae* suspended in pasteurized orange juice at 350 MPa (50,750 psi) were determined to be 4–76 seconds for ascospores and 1–38 seconds for vegetative cells at pressures between 350 and 500 MPa (50,750 and 72,500 psi) (Parish, 1998).

Some studies have identified processing conditions that will eliminate yeasts and molds in both model and real foods. Palou et al. (1998b) demonstrated that *Z. bailii* suspended in a model system with an *a_w* of 0.98 and pH 3.5 could be inactivated at sublethal pressures of 207 (30,015), 241 (34,945) and 276 MPa (40,020 psi) if cyclically applied over 20 minutes. A range of yeasts and molds, including *Penicillium*, *Aspergillus*, and *S. cerevisiae* in cauliflower, onion, spinach, lettuce, and asparagus, were eliminated at pressures between 300 and 400 MPa (43,500 and 58,000 psi) (Arroyo et al., 1999). Inactivation was found to be the most effective at 5°C as compared to 10 or 20°C. Inactivation of spores of *Penicillium roqueforti* in cheese slurry was not evident until a pressure of 300 MPa (43,500 psi) at 20°C was reached, after which cell numbers decreased rapidly (O'Reilly et al., 2000).

In contrast to the sensitive species discussed above, there are a number of fungal species that display notable resistances to HP. Pressures greater than 600 MPa (87,000 psi), often in conjunction with temperatures higher than 60°C, are necessary to inactivate ascospores of heat-resistant molds (Butz et al., 1996; Palou et al., 1998a). Treatment at 689 MPa (99,905 psi) and 60°C for 25 minutes was insufficient to inactivate ascospores of *B. nivea* suspended in apple and cranberry juice concentrates. This may reflect the reduced *a_w* of these concentrates. HP is progressively less effective as *a_w* is reduced. Oscillatory pressure (five 1-second cycles at 689 MPa (99,905 psi) and 60°C) reduced the ascospore count by 1 log₁₀ (Palou et al., 1998a).

Chapman et al. (2007) examined the barotolerance of the heat-resistant molds, *B. fulva*, *B. nivea*, *N. fischeri*, and *Neosartorya spinosa*. They found that older spores were generally more resistant to HP. In the case of *B. nivea*, a 2.5-log₁₀ reduction was achieved for 3-week-old ascospores, whereas 9-week-old ascospores were reduced by only 0.5 log₁₀ using similar treatment conditions. Similar results were found by Dijksterhuis and Teunissen (2004) for ascospores of *Talaromyces macrosporus* with older spores typically showing increased pressure resistance. The age-related changes in pressure resistance of ascospores may be due to ongoing changes in the ultrastructural state of the multilayered ascospore wall (Dijksterhuis and Teunissen, 2004; Chapman et al., 2007). Younger ascospores may succumb to HP because of a weaker underdeveloped cell wall, whereas mature ascospores have a denser cell wall which may protect them from HP.

Effects of HPP on yeasts and molds, which lead to cell death, include membrane damage and release of intracellular components (Perrier-Cornet et al., 1999), loss of homeostasis (Abe and Horikoshi, 1995), and disruption of the reproductive cycle (Kawarai et al., 2006). HPP promotes the acidification of the vacuoles of yeast (Abe and Horikoshi, 1995); pressures as low as 40–60 MPa (5,800–8,700 psi) can reduce the vacuolar pH of *S. cerevisiae* from 6.05 to 5.88 (defined using 6-carboxyfluorescein). Acidification disturbs the cellular ion homeostasis so that degradation of proteins takes place in the vacuole. Kawarai et al. (2006) treated *S. cerevisiae* at 50–150 MPa (7,250–21,750 psi) for 30 minutes at 30°C and found that action filaments disappeared which hampered cellular budding. The authors also demonstrated that in vitro polymerization of actin monomers was inhibited by exposure to 100 MPa (14,500 psi).

10. Activation and Germination of Yeast and Mold Ascospores by HP

HPP can induce the activation and germination of dormant ascospores (Reyns et al., 2003; Dijksterhuis and Teunissen, 2004); however, compared to HPP-induced germination of bacterial endospores, little

is known of the mechanisms controlling the break of spore dormancy. Reyns et al. (2003) found that pressures of ≥ 200 MPa at 20°C induced the activation and germination of *T. macrosporus* ascospores. Raising the pressure above 400 MPa (58,000 psi) sensitized the ascospores to subsequent heat treatment so that at 500 MPa (72,500 psi), activation was followed by 3 log₁₀ of HPP-induced inactivation if holding times were increased. Optimum temperatures for HPP-induced ascospore activation were 10–20°C and 60°C, and activation was not particularly pH-dependent over a pH range of 3.0–6.0. In a recent study by Chapman et al. (2007), HPP-induced germination was also observed for *N. spinosa*, *B. fulva*, and *B. nivea*. Ascospores of *N. spinosa* were the most sensitive to HPP-induced activation. Dijksterhuis and Teunissen (2004) suggested that influx of water through the HPP-damaged envelope of the ascospore caused activation.

11. The Effect of Suspending Medium on Pressure-Induced Inactivation of Yeasts and Molds

Many of the yeasts and molds of concern in the food industry are the spoilage agents of fruit beverages, jams, jellies, and vegetable preserves (Splittstoesser, 1987; Thomas, 1993). These products often have high levels of sugar or other solutes, while the reduced a_w provided by these solutes have a preservative effect (Pitt and Hocking, 1997). They also provide protection to fungal contaminants against the lethal effects of HPP.

Rhodotorula rubra suspended in solutions of sucrose, glucose, fructose, or sodium chloride, were treated at 200–400 MPa (29,000–58,000 psi) and shown to have an a_w -dependent barotolerance (Oxen and Knorr, 1993). The protective effect started at values below a_w 0.92–0.88 and was independent of pH between pH 3.0 and 8.0. Palou et al. (1997) reported a linear relationship between survival of *Z. bailii* and a_w (controlled by sucrose) after HPP at 354 MPa (51,330 psi) for 5 minutes.

Goh et al. (2007) pressure-treated (600 MPa, 87,000 psi) suspensions of yeast cells (*S. cerevisiae*, *Pichia anomala*, and *Hanseniaspora uvarum*)

and fungal spores (*Penicillium expansum*, *Fusarium oxysporum*, and *Rhizopus stolonifer*) in citrate phosphate buffer formulated with sucrose or glycerol and NaCl at equivalent a_w values of 0.925, 0.903, and 0.866. Sucrose was found to have a stronger baroprotective effect for *S. cerevisiae* than the other solutes at two of the three investigated water activities. For *P. expansum* at 0.903 a_w , NaCl gave the best protective effect. Palou et al. (1999) proposed that enhanced survival at reduced a_w could be due to cell shrinkage which causes thickening of the cell membrane, reducing the membrane permeability, and protecting the cells from destruction by HP.

12. Process Implications for Controlling Yeast and Molds

Key process parameters for effectiveness of HPP with regard to inactivation of fungi include ascospore age, suspending medium, and the presence of resistant species. As often suggested for many different food types, Chapman et al. (2007) emphasized that HPP needs to be combined with other hurdles to ensure that the quality of shelf-stable fruit products is superior to currently available heat-processed products.

13. The Effects of HPP on Viruses

The first virus to be pressure-treated was the plant pathogen, tobacco mosaic virus by Giddings et al. in 1929. Unfortunately, few studies were conducted on the pressure inactivation of animal and human viruses. The advent of more sophisticated cell culture techniques and molecular methods has greatly aided study in this field of research. Research has yielded a considerable amount of data on the responses of viruses to HPP (some of which is summarized in Tables 5.2 and 5.3). The field is still an emerging one. Research in the area of viral inactivation by HPP has taken two main routes, the first being the inactivation of food-borne viruses such as hepatitis A virus (HAV) and noroviruses, the second is medical applications such as inactivation of viruses in blood plasma (Bradley et al., 2000) and other biomedical products, as well as vaccine development (Silva et al., 1992).

Table 5.2. Inactivation of human and animal viruses by high-pressure processing

Virus	Substrate	Treatment Conditions			Loss in Infectivity (log ₁₀)	References
		Pressure (MPa)	Time (minutes)	Process Temperature (°C)		
Adenovirus	CCM	400	15	20	5	Wilkinson et al., 2001
Aichivirus A846/88	CCM	600	5	21	No reduction	Kingsley et al., 2004
Avian influenza A H7N7	CCM	500	0.4	15	5	Isbarn et al., 2007
Bovine enterovirus	Oysters	450	5	20	5	Murchie et al., 2007
Coxsackievirus A9	CCM	500	5	21	7	Kingsley et al., 2004
Coxsackievirus B5	CCM	600	5	21	No reduction	Kingsley et al., 2004
Feline calicivirus	CCM	275	5	22	7	Kingsley et al., 2002
Foot-and-mouth disease virus	Buffer	240	120	-15	6	Oliveira et al., 1999
Hepatitis A virus	CCM	450	5	22	7	Kingsley et al., 2002
Herpes simplex virus type 1	CCM	400	10	25	7	Nakagami et al., 1992
Human cytomegalovirus	CCM	300	10	25	4	Nakagami et al., 1992
Human immunodeficiency virus (KK-1 clinical strain)	CCM	450	10	25	>3	Otake et al., 1997
Human parechovirus-1	CCM	500	5	21	4	Kingsley et al., 2004
Infectious bursal disease virus	CCM	230	120	0	5	Tian et al., 2000
Poliovirus	CCM	600	60	20	<1	Wilkinson et al., 2001
Rotavirus (human)	CCM	300	2	25	8	Khadre and Yousef, 2002
Simian immunodeficiency virus		250	60	22	5	Jurkiewicz et al., 1995
Sindbis virus	Buffer	250	480	Not specified	5	Gaspar et al., 2002
Vesicular stomatitis virus	Buffer	260	720	20	4	Silva et al., 1992

CCM, Cell culture medium.

Studies on viral inactivation have revealed the heterogeneous nature of virus responses to HPP. Some viruses are extremely pressure-resistant; for example, poliovirus is only inactivated by less than 1 log when subjected to 600 MPa (87,000 psi) for 1 hour (Wilkinson et al., 2001). Others have been found to be very sensitive, such as feline calicivirus (FCV) which is completely inactivated by pressures as low as 275 MPa (40,000 psi) for 5 minutes (Kingsley et al., 2004).

14. Mechanisms of Pressure Inactivation of Viruses

Viral inactivation has been shown to be more dependent on the magnitude of pressure applied than the duration of the treatment. Jurkiewicz et al. (1995) showed that the infectivity of simian immunodeficiency virus (SIV) was reduced by 5-log units after treatment at 250 MPa (36,250 psi) for 1 hour at 21.5°C. Lower pressure treatments of 200 and

Table 5.3. Inactivation of bacteriophage by high-pressure processing

Bacteriophage	Substrate	Treatment Conditions			Loss in Infectivity (log ₁₀)	References
		Pressure (MPa)	Time (minutes)	Process Temperature (°C)		
Q-beta coliphage	Oysters	600	10	20	6	Smiddy et al., 2006
Phage C2	Oysters	700	10	20	6	Smiddy et al., 2006
Lactococcal phage P001	M17 broth + Calcium	600	30	25	6	Muller-Merbach et al., 2005
Lactococcal phage P008	M17 broth + Calcium	600	60	25	3	Muller-Merbach et al., 2005
MS2	Buffer	600	5	21	3.3	Guan et al., 2006
Bacteriophage ΦX	Buffer	300	120	40	4	Brauch et al., 1990
Bacteriophage T4	Buffer	400	10	4	8	Groß and Ludwig, 1992

150 MPa (29,000–21,750 psi) required 3 and 10 hours, respectively, to achieve similar reductions. Muller-Merbach et al. (2005) suggested that the size and shape of the virus can have an effect on its resistance to HPP. It was found that both P001 and P008 bacteriophage were more resistant than their lactococcal hosts and that P008 was the more resistant of the two. The resilience of P008 may be attributed to its morphology; P008 is an isometrically headed phage whereas P001 has a prolate or elongated head (Muller-Merbach et al., 2005).

The mechanisms of viral inactivation by HPP are thought to involve the dissociation and/or denaturation of the proteins of the virus coat (Silva et al., 1992), or in the case of enveloped viruses, damage to the envelope (Nakagami et al., 1992) rather than damage to viral nucleic acids (Kingsley et al., 2002). The pressure-induced changes to the coat can be subtle alterations to capsid proteins (Kingsley et al., 2002) or receptor recognition proteins (Pontes et al., 2001), which can lead to loss of infectivity.

Herpes simplex virus and human cytomegalovirus were reduced by more than 7 and 4 logs, respectively, following 10-minute treatments at 400 MPa (58,000 psi) and 25°C. Electron microscopic examination revealed damage to the envelopes which prevented the viruses from binding to cells and initiating infection (Nakagami et al., 1992). Kingsley et al. (2002) found that the capsid of HAV remains intact following HPP suggesting that pressure may denature attachment

proteins within the capsid rendering the virus unable to initiate infection. Similarly Pontes et al. (2001) attributed pressure-induced inactivation of rotavirus to alteration of the viral spike protein, VP4.

Treatment of some viruses at low temperature has been found to enhance pressure-induced inactivation. Murine norovirus treated at 350 MPa (50,750 psi) for 5 minutes at 30°C were inactivated by 1.15 log₁₀ PFU/mL compared to 5.56-log₁₀ reduction when the same treatment was performed at 5°C (Kingsley et al., 2007). Oliveira et al. (1999) combined HP (240 MPa; 34,800 psi for 2 hours) and low temperature (−15°C) to inactivate 6 logs of the foot-and-mouth disease virus compared to 4 logs of inactivation at room temperature. The synergy of pressure and low temperature may be explained by the low-temperature-induced exposure of nonpolar sidechains to water resulting in the virus proteins being more compressible and more likely to be denatured under pressure.

15. Effect of Suspending Medium on Pressure Inactivation of Viruses

Similar to pressure-induced inactivation of bacteria, the nature of the suspending medium or food is an important factor to consider in viral inactivation by HPP. A protective effect of substrate on viruses has been reported by Kingsley et al. (2002) and Murchie et al. (2007). Bovine enterovirus (BEV)

and FCV studied as surrogates of HAV and norovirus, respectively, were pressure-treated in shellfish, seawater, and culture medium and found to be most resistant when treated in oysters and mussels (Murchie et al., 2007). HAV treated in seawater of 27.4-kppt salinity was protected from the effects of HPP compared to an isotonic tissue culture medium (Kingsley et al., 2002).

Most of our current knowledge on pressure-induced inactivation of virus comes from studies performed in tissue culture media (Table 5.2). As more challenge studies of viruses in food are carried out, the interactions of viral particles and substrate components under pressure will become clearer.

16. Virus Surrogates

The difficulties of working with certain virus cultures have made the use of surrogates a vital aspect of virus research. Viruses, such as noroviruses, are difficult to culture in culture medium and, therefore, surrogates, such as FCV, are used to predict their response to pressure and other treatments. The use of bacteriophage as surrogates for enteric viruses has numerous advantages; they are easy to work with in the laboratory and are not hazardous to human health. Some recent studies have attempted to find suitable surrogates for use in the validation of HPP of food (Guan et al., 2006, 2007; Smiddy et al., 2006; Murchie et al., 2007).

Virus surrogates are generally chosen because of their structural similarity and genetic relatedness to a target virus. Murchie et al. (2007) found a 5-minute exposure to 350 MPa (50,750 psi) at 20°C sufficient to inactivate BEV in seawater or “naturally” infected mussels or oysters. BEV is structurally similar to HAV and FCV, a norovirus surrogate. This study and a study by Kingsley et al. (2007) on murine norovirus inactivation by HPP indicate that noroviruses are highly susceptible to pressure.

A range of bacteriophages has been screened for use as potential surrogates (Guan et al., 2006, 2007; Smiddy et al., 2006), some of which are listed in (Table 5.3). Pressures of up to 800 MPa (116,000 psi) were required to inactivate high levels of Q β and c2 phage in oysters (Smiddy et al., 2006). Such pres-

ures are not required for inactivation of the enteric viruses that have been studied thus far and, therefore, cannot be used as surrogates for HPP validation. Guan et al. (2007) examined the pressure sensitivities of the coliphages φ X 174 (ssDNA virus), MS2 (ssRNA virus), λ imm434 (dsDNA virus), and T₄ (dsDNA virus), and suggested that despite their morphological differences, MS2 and T₄ may be potential surrogates for human enteric viruses.

17. Other Applications of HPP and Viruses

HPP is a technology that has many potential uses not directly associated with food safety. For example, Muller-Merbach et al. (2005) demonstrated that HPP could be utilized for the treatment of milk used in cheesemaking in order to inactivate bacteriophage of the cheese starter cultures (Table 5.3). While not harmful to humans, bacteriophage can cause huge economic losses in the dairy industry by attacking the starter cultures of dairy fermentations. HPP could also be used in the pasteurization of blood and blood products (Nakagami et al., 1992; Bradley et al., 2000) without damaging plasma proteins such as immunoglobulins.

Some viruses do not disassociate during pressure treatment or can reassemble post-pressure forming nonviable viral particles. Pressure-inactivated viral particles can retain many of the physical and chemical characteristics of the native virus, and remain highly immunogenic (Silva et al., 1992; Oliveira et al., 1999; Pontes et al., 2001), thus opening up the possibility of producing vaccines.

18. Future Research Needs

The area of microbial inactivation by processes incorporating HP has matured dramatically. Nonetheless, there is still a need to reliably predict the effective end point of pressure processes for preservation of food products. Variations in microbial susceptibility will continue to occur on the basis of the food type, pressure equipment used, processing conditions, and quality specifications of the treated product. Thus, it is safe to assume that work will continue on pressure

inactivation with attention paid to process resistance mechanisms.

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Chapter 6

Biochemical Aspects of High-Pressure Food Processing

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1. Introduction

Pressures greater than 100 MPa (14,500 psi), when applied to foods at or near a water activity of 1.0, result in a reduction in the volume of the food. The chemical and biochemical response of the food is governed by the principle of Le Chatelier and Braun (Pfister et al., 2001). The principle states, "If a constraint is imposed on a system that is in a state of equilibrium by modifying external conditions, the point of the equilibrium will be shifted to such a degree that the external constraint becomes reduced." Le Chatelier's principle applies to equilibrium conditions and means that all materials exhibit compressibility (Balny and Masson, 1993). Heremans (1982) concludes that the principle of microscopic ordering governs the behavior of materials under compression. The principle of microscopic ordering states that at constant temperature, an increase in pressure increases the degree of ordering of molecules of a given substance. Therefore, pressure and temperature exert antagonistic forces on molecular structure and chemical reactions (Balny and Masson, 1993). The application of high pressures to foods results in molecular compression accompanied by a decrease in volume that promotes macromolecular interactions, structural changes, and phase transitions (Palou et al., 1999).

The application of high pressure influences biochemical reactions in foods as well as the chemical

structure of cells, biopolymers, and molecular constituents since many of these reactions and structural changes involve a reduction in volume (Palou et al., 1999). Hoover et al. (1989) reported that pressure influences reaction systems by reducing the available molecular space and increasing the interaction among biopolymers. Kauzmann (1959) associated the formation of hydrogen bonds with a decrease in volume. Thus, reactions involving the formation of hydrogen bonds are favored by the application of high pressures (Palou et al., 1999). Masson and Reybaud (1988), however, concluded that hydrogen bonds may be insensitive to pressure. van Doorn (2008) reports that hydrogen and covalent bonds are not affected by high pressures.

In contrast to fundamental chemical reactions, it is difficult to obtain precise physical meaning to volume changes associated with high-pressure treatment of biochemical solutions or foods (Balny and Masson, 1993). Experimentation with reaction volumes for ionic interactions demonstrates that the evolution of charges is accompanied by a decrease in volume (Heremans, 1982). The decrease in volume associated with ionic interactions is attributed to increased electrostriction of the solvent around the free charges. Therefore, the choice of buffers for high-pressure research is largely determined by electrostatic considerations (Heremans, 1982).

Heremans (1982) divided hydrophobic effects of high-pressure treatment into two categories: hydrophobic solvation or solubility of apolar species in water; and solvent-induced hydrophobic interaction among apolar species. The model for hydrophobic solvation is biopolymer folding; the model

for hydrophobic interaction is biopolymer–ligand or biopolymer–biopolymer interaction. Kauzmann (1959) suggested that volume increases enhance the association of hydrophobic molecules, while Ben-Naim (1980) reported that volume increases enhance the association of aliphatic molecules in water. Weber et al. (1974) reported that volume decreases enhance the association of aromatic molecules in water. A simplified qualitative model proposed by Heremans (1982) indicated that the association of aliphatic chains is enhanced by volume increases and association of aromatic rings is enhanced by the volume decreases resulting from high-pressure treatment.

Cheftel (1995) cited biochemical studies indicating that pressures greater than 200 MPa (29,000 psi) often result in the dissociation of oligomeric structures into their subunits, partial unfolding and denaturation of monomeric structures, biopolymer aggregation attributed to structural unfolding, and formation of an insoluble network, gelation, in solutions with adequate biopolymer concentrations. High-pressure treatment of foods and food formulations and the resulting structural changes and chemical reactions may potentially introduce unique textures and selected functionality to improve the quality of food systems. This benefit is in addition to providing safe and stable foods by inactivating microorganisms.

2. Water's Role in High-Pressure Processing of Foods

The concentration of water in foods processed by high pressure as determined by water activity (a_w), must be close to 1.0. Thus, water should be the most predominant constituent in these foods. Adiabatic compression of water increases the temperature by approximately 3°C per 100 MPa (Tauscher, 1995). The freezing characteristics of water in foods are altered under high pressures (also see Chapter 1). This provides opportunities for storage of foods at subzero temperatures without ice crystallization, rapid thawing of frozen foods, and increase in the rate of freezing by decompression of compressed foods (Palou et al., 1999). Ionization of water is promoted by high pressure. In an aqueous system, water molecules sur-

rounding ionized molecules align according to the influence of the ion charges. This results in more compact molecular arrangement. Ionization of the acidic or basic constituents of biomolecules involves decreases in volume and is enhanced by increased pressure (Hoover et al., 1989; Johnston et al., 1992).

The partial molar volume of organic molecules in water is smaller than the partial molar volume of organic molecules in nonpolar solvents (Kauzmann, 1959). The decrease in molar volume in nonpolar solvents is smaller for aromatic than for aliphatic hydrocarbons. The molecular flexibility that increases from aromatic to saturated rings to aliphatic hydrocarbons results in small increases in the molecular volumes as alcohols are mixed with water (Brower et al., 1969). Pressures may exhibit a greater effect on flexibility and stability of aliphatic molecules than on aromatic molecules in foods.

3. Effects of Pressure on Food Proteins

The effects of high pressure on proteins and other biopolymers is reviewed by Heremans (1982), Balny and Masson (1993), and Palou et al. (1999). The three-dimensional structure of native proteins results from the compact folding of the polypeptide chain (Balny and Masson, 1993). The volume of native proteins includes the sum of the compositional volume of constituent atoms, the porous volume of internal cavities, and volume changes attributed to solvation dependent on the solvent. Thus, the native structure that governs the biological activity and functionality of proteins is a delicate balance of stabilizing and destabilizing interactions in a selected environment within the polypeptide chains and with the solvent (Balny and Masson, 1993). Pressure acts as a physicochemical parameter disturbing the balance of intramolecular and solvent–protein interactions (Jaenicke, 1981).

The quaternary structure of proteins is mainly retained by hydrophobic interactions that are susceptible to pressure (Weber et al., 1974). Moderate pressures, less than 150 MPa (21,750 psi), favor the dissociation of oligomeric proteins accompanied by a decrease in molecular volume. For example, the dissociation of lactic dehydrogenase results in a volume

decrease of 500 mL/mol (Schade et al., 1980). Dissociation may be followed by subunit aggregation or precipitation (Masson et al., 1990). Pressures greater than 200 MPa (29,000 psi) induce unfolding of proteins and reassociation of subunits from dissociated oligomers. For example, at pressures less than 150 MPa (21,750 psi), β -casein is reversibly depolymerized whereas at greater pressures a temperature-dependent reversible reassociation is observed (Balny and Masson, 1993).

Substantial changes in tertiary structure are observed at pressures greater than 200 MPa (29,000 psi) (Li et al., 1976). Reversible unfolding of small proteins such as ribonuclease A occurs at greater pressures, 400–800 MPa (58,000–116,000 psi), demonstrating that the volume and compressibility changes during denaturation are not completely dominated by hydrophobic effects (Li et al., 1976). Denaturation is a complex process involving intermediate forms leading to multiple unfolded forms (Li et al., 1976). The presence of stable intermediates during denaturant-induced equilibrium unfolding reactions is well established (Ku wajima, 1989). The intermediate states of selected proteins exhibit common characteristics, the compact globularity of the molecule with native-like secondary structure, but unfolded tertiary structure (Ku wajima, 1989). The term “molten globule” was introduced as a third thermodynamic state of protein molecules in addition to the previously established thermodynamic native (unfolded) and denatured states (Ku wajima, 1989; Semisotnov et al., 1991; Ptitsyn and Uversky, 1994; Ptitsyn, 1995). High-pressure-induced (600 MPa or 87,000 psi) stable intermediate molten globule states of β -lactoglobulin (Yang et al., 2001; Yang et al., 2002) are potentially relevant to protein functionality in foods.

Alterations of the secondary structure of proteins may occur at pressures greater than 700 MPa (101,500 psi), dependent on the rate of compression and the extent of the secondary structure rearrangement, leading to irreversible denaturation (Balny and Masson, 1993).

The primary alterations of protein structure occur at the sites of electrostatic and hydrophobic affinity along the protein molecules. High pressures result

in deprotonation of ionic groups and disruption of salt bridges and hydrophobic affinity, resulting in conformational and structural changes in proteins. High pressures are not observed to alter or break covalent bonds or disulfide bridges of proteins in the pressure ranges 100–700 MPa (101,500 psi) (Balny and Masson, 1993). Structural transitions of proteins are accompanied by large changes in hydration and functionality (Palou et al., 1999). Hydration changes are the major source of volume decreases attributed to dissociation and unfolding of proteins (Balny and Masson, 1993). Hydrophobic affinity in proteins may be either disrupted or stabilized dependent on the magnitude of the pressure applied (Johnston et al., 1992).

The effects of pressures on proteins may be reversible or irreversible, similar to the effects of increasing temperatures on the denaturation of proteins (Balny and Masson, 1993). Reversible effects are generally observed at pressures less than 200 MPa (29,000 psi), whereas irreversible effects occur at pressures greater than 300 MPa (43,500 psi). Unlike thermal denaturation and unfolding induced by denaturing agents, volume determinations and limited changes in heat capacity indicate that pressure-induced denaturation corresponds to partial unfolding of the protein (Balny and Masson, 1993). Combined pressure and thermal treatments (also see Chapters 1 and 5) may contribute to commercial sterilization of foods (Ting et al., 2002; de-Heij et al., 2003). Combination of pressure and temperatures can result in protein structures with new properties. These properties may have use in new foods (Frauenfelder et al., 1990). As pressures increase to approximately 100 MPa (14,500 psi), the unfolding temperatures of proteins increase, whereas at greater pressures the temperatures of unfolding generally decrease. The practical consequence is that the pressure at which proteins unfold is generally lower at ambient temperatures than at higher temperatures (Balny and Masson, 1993).

Structural changes in proteins resulting from high pressure also affect the functionality of the proteins in food systems. A frequent change in functionality of proteins is the inactivation or activation of enzymes resulting from volume decreases in the reaction,

conformational changes of the enzyme, or dissociation of the enzyme (Morild, 1981). Inactivation of selected enzymes in model buffers with treatment pressures ranging from 0.1 to 900 MPa (14.5–130,500 psi) was ranked in order of ease of inactivation from easiest to the most difficult as follows: lipoxygenase, lactoperoxidase, pectinesterase, lipase, phosphatase, catalase, polyphenol oxidase, and peroxidase (Seyderhelm et al., 1996). Combining pressure with a moderate temperature increased enzyme inactivation rate. Pressure treatment of foods demonstrated a protective effect of food constituents on the pressure inactivation of most enzymes (Seyderhelm et al., 1996). Protein functionality such as solubility, precipitation, agglomeration, stabilization, emulsification, gelation, hydration, and hydrophobic affinity are closely related to the structural conformation of the protein. The solution–gel transition of protein solutions is affected by pressure. Although selected pressure-induced protein gels may exhibit great hydrophobicity and gel strength, pressure-induced gels are often unstable to heat (Balny and Masson, 1993).

High pressures dramatically alter the structure and functionality of proteins. The potential for microbial inactivation with only small increases in temperature and the development of improved protein functionality, leading to unique and innovative foods, is great. The volumetrically uniform and instantaneous application of pressure throughout foods is an advantage of high pressure over thermal processing. High-pressure acceleration or inhibition of enzyme activity and chemical reactions introduces a new concept to food technology (Balny and Masson, 1993). Cheftel (1992) and Messens et al. (1997) provide a provisional classification of potential applications of pressure on food proteins including restructuring and texturization through unfolding and aggregation, low temperature gelation, gelation of muscle proteins with little or no salt, and rheological changes in emulsified foods. Other effects of pressure are meat tenderization through unfolding, dissociation, and/or proteolysis, enzyme inactivation (blanching) through unfolding or structural alteration, discoloration of hemoglobin, increased sensitivity of protein foods to proteases with improved digestibility, reduced allergenicity through dramatic structural alterations,

increased binding of specific ligands such as flavors, dyes, vitamins, inorganic compounds to proteins, and increased surface hydrophobicity and accompanying insolubility.

The high-pressure treatment of proteins will often define the role of high-pressure treatment of foods. High-pressure equipment available today allows the comprehensive characterization of the behavior of proteins under pressure (Gross and Jaenicke, 1993). The structural changes in proteins resulting from exposure to high pressures will define protein functionality, bioactivity, and chemical reactivity. All these are of great importance to the chemistry of food safety, quality, successful production, and consumer acceptance of foods.

4. Effects of Pressures on Food Carbohydrates

Carbohydrates are basic nutrients that serve as sweeteners, gelling agents, stabilizers, preservatives, and precursors for the formation of flavors and pigments in food processing (Pfister et al., 2001). The functional properties of carbohydrates such as gelling characteristics, retrogradation of starches, and water binding may be influenced by high pressure (Pfister et al., 2001).

Starches can be gelatinized using pressure or heat. The extent to which starch can be gelatinized depends on the source of the starch, treatment pressure, treatment time, and temperature of pressurization (Palou et al., 1999). Pressure-induced gelatinization is a two-step process. In the first step, hydration of amorphous parts of the starch granules occurs. This leads to swelling of the granules and distortion of the crystalline regions. In the second step, the crystalline regions become more accessible to water (Oh et al., 2001). The effect of high pressure on gelatinization is due to the stabilization of hydrogen bonds, which maintain the starch granule in the native state. Pressure-treated starches are unique in the sense that the granular structure remains intact. Heat treatments destroy starch granules by promoting granular dissolution and forming transparent solutions (Palou et al., 1999). The starch granule

surfaces were most resistant to high-pressure treatment in a 10% suspension of potato starch in water subjected to pressure treatment at 600 MPa (87,000 psi) for 2 or 3 minutes. The inner part of the starch granule was almost completely filled with a gel-like network, with empty spaces growing in diameter toward the center of the granule (Błaszczaka et al., 2005). The pressure induced gelatinization of selected waxy and normal rice, corn, tapioca, and potato starches provided starch solutions characterized by pasting behavior, degree of swelling, and changes in birefringence. Potato starch was less affected by pressure treatment than the other starches, as it retained birefringence after a pressure treatment of 600 MPa (87,000 psi) for 30 minutes. Waxy rice, waxy corn, and tapioca starches were completely gelatinized after an equivalent high-pressure treatment, whereas normal rice and corn starches were only partially gelatinized. The pasting curves of normal starches exhibited an increase in the initial viscosity after pressure treatment, whereas the initial viscosities of the waxy starches after treatment at 600 MPa (87,000 psi) for 30 minutes were equal to their respective peak viscosities (Oh et al., 2001).

High-pressure treatment of carbohydrates may result in minor chemical changes in foods; however, modifications of molecular structures are minimal because covalent bonds are not affected by pressure treatment in the range of 700 MPa (101,500 psi). High-pressure energy input is too small and a volume increase is associated with the splitting of covalent bonds. Pressure treatments greater than 1,000 MPa (145,000 psi) resulted in confirmed splitting of glycosidic bonds (Pfister et al., 2001).

The influence of high pressure on deesterification of pectins and saccharide degradation is observed. However, deesterification is attributed to enzymatic reactions rather than high-pressure treatment itself. Total pectin in pressure-treated carrots is equivalent to total pectin in carrots cooked for 3 minutes. However, with increasing pressure, from 100 to 700 MPa (101,500 psi), the amount of high methoxyl pectin in the carrots decreased while low methoxyl pectin increased. Thus, the effects of pressure on pectin degradation and texture of raw carrots were different than

the effects of heating on pectin degradation and carrot texture. The degradation of pectin during high-pressure treatment was attributed to pectin methyl esterase activity in the carrot. The degree of esterification decreased during the course of pressure treatment of pectins in carrots (Kato et al., 1997). Strawberry jams treated by high-pressure processing (HPP) and conventional processing were examined for quality changes during storage for 1–3 months at 5 and 25°C. The fresh flavor and natural color of strawberry jam immediately after HPP were superior to the quality of thermally processed jam. The superior quality was maintained during storage at 5°C for 2–3 months. However, storage at 25°C resulted in rapid deterioration of quality including off-flavors, discoloration, browning, and degradation of sucrose and vitamin C. The commercial value of high-pressure-processed strawberry jam deteriorated quickly at 25°C. On the other hand, thermally processed strawberry jam did not change in quality at 25°C storage for 3 months. The rapid deterioration in pressure-processed strawberry jam is attributed to dissolved oxygen and active enzyme systems (Kimura et al., 1994).

Many food constituents such as sucrose, glucose, and fructose provide a baroprotective effect on the inactivation of bacteria and yeasts by high pressure. Cell shrinkage at reduced water activities or increased soluble solids concentrations result in thickening of the cell membrane, thereby reducing membrane permeability and protecting the cells from high-pressure inactivation (Palou et al., 1999). *D*-values were observed for high-pressure inactivation of *Saccharomyces cerevisiae* and *Listeria innocua* in commercial applesauce with concentrations of soluble solids ranging from 13 to 60%. Applesauce at room temperature inoculated with *S. cerevisiae* was pressure treated at 300 MPa (43,500 psi) for hold times between 0 and 150 seconds. Applesauce inoculated with *L. innocua* was treated at 375 MPa (54,375 psi) at hold times between 0 and 300 seconds at room temperature. Results demonstrated that soluble solids concentrations greater than 30% provide a baroprotective effect on inactivation by pressure treatment. The use of high-pressure technology by the food industry to inactivate microorganisms

may be limited in foods with high soluble solids concentrations (Chauvin et al., 2006) (see also Chapter 1.5.4).

The application of high pressure in combination with moderate or elevated temperatures may influence Maillard reactions as shown by the extent of development of brown colors in foods. Maillard reactions involving xylose-lysine, xylose- β -alanine, or glutaraldehyde- β -alanine are inhibited by high-pressure treatments. Browning was inhibited at 200–400 MPa (29,000–58,000 psi) in xylose-lysine systems at pH 8.2 and temperature 50°C (Tamaoka et al., 1991). Browning of glucose-lysine systems with or without a high-pressure treatment of 600 MPa (87,000 psi) at 50°C, with a range of pH values between 5.1 and 10.1 was studied. Hill et al. (1996) observed that pH values near 8.0 and 10.0 enhanced browning, while pH values near 5.1 and 6.5 inhibited browning. Inhibition of browning during high-pressure treatments was attributed to a decrease in pH values (Hill et al., 1996).

5. Effects of Pressure on Food Lipids

Lipids constitute one of the three major components of food products along with carbohydrates and proteins. Lipids are composed of fats and oils. Fats are solid or semisolid at room temperature and oils are liquid at room temperature. Lipids are essential nutrients of human diets and are concentrated sources of energy. Triacylglycerols (TAGs), three fatty acid esters of glycerols, constitute the greatest portion of fats and oils in foods. The physical characteristics of fats and oils are strongly dependent on the molecular structure of TAGs (Oh, 2002). Lipids are of great importance for the preparation and production of foods because of their melting behavior, pleasant cream-like or fatty taste, mouthfeel, stabilization and formation of flavor components, prolongation of shelf life, and emulsifying effect of fatty acids (Pfister et al., 2001). The functional properties of lipids are such that the rate of oxidative changes and crystallization may be influenced by high-pressure treatments.

The influence of high-pressure treatment on oxidative changes of lipids during storage depends on environmental conditions such as a_w , pH, metal

ions, oxygen, degree of saturation of fatty acids, and content of antioxidants. The rates of oxidation of lipids in foods due to HPP are contradictory. A protective effect of high-pressure treatment is observed. However, peroxidative effects were demonstrated in storage trials involving oils treated with high pressure (Pfister et al., 2001). Pressure effects on lipid oxidation of linolenic acid were evaluated. Lipids treated at 350 MPa (50,750 psi) for 21 hours at 40°C were compared with lipids treated for an equivalent time and temperature at atmospheric pressure. During the initiation step of the oxidation, there was an increase in oxidation products. However, subsequent lipid oxidation steps were inhibited by high-pressure treatment (Kowalski et al., 1993). Cod muscle pressure treated in the range of 202–608 MPa (29,220–88,160 psi) for 15 and 30 minutes exhibited an increase in the peroxide value of the extracted oils with increasing pressure and processing time. The presence of fish muscle accelerated lipid oxidation during high-pressure treatments. Isolated fish oils were relatively stable against autoxidation after pressure treatments as great as 608 MPa (88,160 psi) (Ohshima et al., 1993). A pressure treatment of 700 MPa (101,500 psi) for 10 minutes at room temperature was applied to extra virgin olive oils and to seed oils including grape-stone, sunflower, soybean, peanut, and corn oils to document any changes in the lipid fraction of the oils. The application of high pressure produced changes in the *p*-anisidine values, but not in the peroxide values and volatile hydrocarbons of the selected oils. Olive oil is more resistant to oxidation than seed oils. The effect of high pressure on oils is dependent on the origin, composition, initial quality, and age of the oils (Severini et al., 1997). The effects of high pressure on lipids must be evaluated directly on individual foods. Also, changes occurring in fats and oils during storage of the foods must be distinguished from changes occurring during the high-pressure treatment (Pfister et al., 2001).

High pressure increases the crystallization rate of TAGs (Larsson, 1994). The crystallization behavior of edible fats and oils is not only dependent on chemical composition, temperature, and treatment time, but also on factors such as thermal history, dispersion of

fat (o/w emulsion), or presence as a continuous or discontinuous phase. High pressure may accelerate the crystallization of fats by shifting the solid/liquid transition point to higher temperatures (Buchheim et al., 1996). Pressure treatment of milkfat in an emulsion accelerated the rate of crystallization by increasing the melting point by about 0.2°C per MPa of pressure (Buchheim and El-Nour, 1992). Furthermore, the effects of high pressure on crystallization of milkfat emulsions are dependent on the magnitude of pressure and length of application time (Buchheim et al., 1996). Buchheim et al. (1996) reported that a pressure treatment of 200 MPa (29,000 psi) resulted in maximum acceleration of milkfat crystallization in the range of 100–400 MPa (14,500–58,000 psi). Slower crystallization rates of milkfat at pressures greater than 350 MPa (50,750 psi) were observed.

Form V to Form VI transitions of cocoa butter were investigated at storage temperatures between 21 and 28°C. Pressure treatments of cocoa butter melt at 21°C did not affect the rate of Form V crystallized cocoa butter transition to Form VI during temperature fluctuations. Pressure treatments of 100, 300, or 600 MPa (87,000 psi) on Form V crystallized cocoa butter did not alter the rate of Form V to Form VI transitions during storage. High-pressure treatments can be applied to foods containing high fats with little effect on potential polymorphic transitions (Oh and Swanson, 2006). High pressure may accelerate or retard crystallization of fats. This may be useful for aging ice cream mixes and physical ripening of dairy cream during butter making (Dumay et al., 1996).

6. Effects of High Pressure on Dairy Products

Several review manuscripts describe the significant and unique effects that result from high-pressure treatment of milk (Cheftel, 1995; Huppertz et al., 2002; Trujillo et al., 2002; López-Fandiño, 2006; Considine et al., 2007). Huppertz et al. (2002) describe the effects of high pressure on milk as:

- disruptive to casein micelles and the structure of whey proteins;
- shifting the mineral balance in milk;

- inducing crystallization of milkfat;
- increasing the pH of milk;
- reducing the turbidity of milk; and
- reducing the rennet or acid coagulation time of milk, increasing cheese yield.

6.1. Casein Micelles

The effects of high pressure on casein micelles were studied using skim milk (Harte et al., 2002). The effects were noted by observing absorbance and transmittance (Buchheim et al., 1996; Gaucheron et al., 1997). Direct observation of micelles, under transmission electron microscopy, were performed by Gaucheron et al., 1997; Keenan et al., 2001; Needs et al., 2000; Harte et al., 2002. The reduction in micelle size induced by high pressure was observed by light scattering (Kelly et al., 2002) and analytical centrifugation (Harte et al., 2002). The effect of high-pressure treatment of milk on casein micelle size is temperature-dependent (Huppertz et al., 2002). High-pressure treatment markedly increases the transfer of individual caseins from the colloidal to the soluble phase of milk (López-Fandiño et al., 1998). Dissociation of individual caseins in milk by high pressure is dependent on pH (Arias et al., 2000; Huppertz et al., 2002).

6.2. Milkfat and Milk Enzymes

Crystallization of milkfat is induced by high-pressure treatment of cream (Buchheim and El-Nour, 1992; Buchheim et al., 1996). Buchheim et al. (1996) observed that high-pressure treatment of milkfat emulsions of equivalent fat composition and concentration, but different mean fat droplet sizes, reduced globule size and delayed crystallization of milkfat at ambient and high pressures. No increase in the products of lipolysis was observed following treatment of milk with pressures ranging from 400 to 800 MPa (58,000–116,000 psi) (Buchheim et al., 1996).

Enzymes in milk are resistant to high-pressure treatment (Huppertz et al., 2002). Alkaline phosphatase (López-Fandiño, 1996; Rademacher et al., 1998), lactoperoxidase (López-Fandiño, 1996; Seyderhelm et al., 1996), phosphohexose-isomerase

(Rademacher et al., 1998), and γ -glutamyl-transferase (Rademacher et al., 1998) are resistant to pressures lower than 400 MPa (58,000 psi) at 25°C. The resistance to inactivation of these enzymes in milk makes them unsuitable for selection as indicators of the magnitude of high-pressure treatment of milk (Huppertz et al., 2002). The proteolytic activity of pressure-treated (100–400 MPa) (58,000 psi) milk after 48 hours at 37°C was equivalent to the proteolytic activity of raw milk. This was consistent with large residual plasmin activity following high-pressure treatment of milk (López-Fandiño, 1996). Pressure treatments of milk as low as 206 MPa (29,870 psi) induced a reduction in lactate dehydrogenase activity. Activity decreased with pressure treatment until complete inactivation was obtained at 620 MPa (89,900 psi) (Kouassiet al., 2007).

6.3. Cheese and Yogurt from High-Pressure-Treated Milk

The potential of pressure treatment from 200 (29,000 psi) to 600 MPa (87,000 psi) to shorten the coagulation time of milk, increase curd yield, and provide acceptable cheese quality is described by several researchers (Desobry-Banon et al., 1994; López-Fandiño, 1996; Drake et al., 1997; Huppertz et al., 2002; Trujillo et al., 2002). Desobry-Banon et al. (1994) suggest that decreased coagulation time is related to reduction in casein micelle size. This leads to increased surface area and increased probability of interparticle collisions. Further observations suggest that high-pressure treatment of milk decreases the enzymatic hydrolysis of κ -casein and promotes aggregation and gel formation of milk (López-Fandiño et al., 1997; Huppertz et al., 2002). High-pressure treatment of milk may result in opposing mechanisms affecting the aggregation of milk. The direct effect of pressure on micelles increases hydrolysis of κ -casein and promotes aggregation, while the alteration of the structure of β -lactoglobulin reduces the rate of aggregation (Needs et al., 2000). Increased curd yield is probably a result of both the incorporation of denatured whey proteins and enhanced moisture retention in the curd (Huppertz et al., 2004a). The quality of the resulting cheese is probably the

major factor in determining the potential application of high-pressure treatment of milk in cheese making (Huppertz et al., 2002). Drake et al. (1997) observed no differences in flavor among Cheddar cheeses made from pasteurized or high-pressure-treated milk, but the high-pressure-treated milk produced a Cheddar cheese with a pasty, weak texture attributed to the presence of whey proteins and greater moisture content.

High pressure increases the rigidity and force required at the breaking point of acid-induced gels in milk. The results are dependent on the pressure magnitude and treatment time (Johnston et al., 1992). The volume of whey released after cutting the gel decreased with increasing pressure, indicating greater resistance to syneresis. This is consistent with microscopic observations of a greater density of network strands in gels from milk treated at 600 MPa (87,000 psi) (Johnston et al., 1992; Huppertz et al., 2002).

Pressure treatment of milk at 250 MPa (36,250 psi) increases casein micelle size by ~30%, whereas pressure treatment of milk at 400 or 600 MPa (58,000–87,000 psi) reduced casein micelle size by ~50% (Huppertz et al., 2004b). Casein micelles in yogurts made from milk treated at 600 MPa or 675 MPa (87,000 or 97,875 psi) are described as individually distinguishable smooth-surfaced particles and densely packed strands by Needs et al. (2000) and as clusters of coalesced micelles by Harte et al. (2002). Yogurts made from high-pressure-treated milk coagulated at a higher pH and were similar in firmness and water-holding capacity to yogurts made from heated milk (Ferragut et al., 2000; Harte et al., 2002).

The firmness of yogurt made from high-pressure-treated milk increases with increasing treatment pressure (Needs et al., 2000), and is greater for yogurt made from milk treated with high pressure at 55°C than milk treated at 10°C or 25°C (Ferragut et al., 2000). Schwertfeger and Buchheim (1998) suggested that disintegration of casein micelles, loss of electrostatic stabilization of casein micelles near a pH of 5.3 (Banon and Hardy, 1992), destruction of the hydration sphere around micelles at pressures greater than 150 MPa (21,750 psi), and increased frequency

of particle collisions influence the quality of the acid coagulum formed (Huppertz et al., 2002).

The primary effects of high-pressure treatment of milk include alterations in casein micelles, salts, and whey proteins. These alterations result in increased pH and reduced lightness and turbidity. High-pressure treatment of milk may reduce coagulation time, increase cheese yield, and increase the firmness and water-holding capacity of yogurt (Huppertz et al., 2002).

6.4. Whey Proteins

β -Lactoglobulin, the major whey protein of ruminant species (Kontopidis et al., 2004), is the subject of many high-pressure treatment experiments which examine structural changes. Proteolysis of β -lactoglobulin is enhanced during a high-pressure treatment of 200 MPa (29,000 psi) (Hayashi et al., 1987). Pittia et al. (1996) report that pressure treatments of 300–900 MPa (43,500–130,500 psi) reduce the foamability of β -lactoglobulin compared to native β -lactoglobulin. These workers observed a greater capacity for protein–protein interactions, and attributed the modifications in functional properties to pressure-induced structural changes. Irreversible molecular modifications in tertiary structure, surface hydrophobicity, sulfhydryl group interaction, and association state of β -lactoglobulin at pressures of 200–900 MPa were described by Tanaka et al., 1996; Iametti et al., 1997; Belloque et al., 2000.

Yang et al. (2001) noted the potential for increasing the hydrophobicity and functionality of β -lactoglobulin through high-pressure formation of a thermodynamically stable intermediate molten globule state. The tertiary structural alterations of β -lactoglobulin by high pressures greater than 500 MPa (72,500 psi) increased hydrophobicity and binding affinity of fluorescent hydrophobic probes *cis*-parinaric acid (CPA) and 1-anilino-naphthalene-8-sulfonate (ANS). Pressures above 500 MPa (72,500 psi) did not improve affinity for ligands like palmitic acid, capsaicin, carvacrol, α -ionone, β -ionone, cinnamaldehyde, or vanillin (Yang et al., 2003). Hoang (2006), using fluorescence spec-

troscopy and headspace-solid phase microextraction (HS-SPME) gas chromatography, demonstrated that at a pressure of 600 MPa (87,000 psi), β -lactoglobulin increased surface hydrophobicity. No linear relationships were observed between high-pressure treatment and the binding of diacetyl, 2-methyl butyraldehyde, δ -decalactone, or ethyl lactate with β -lactoglobulin. Headspace analysis of pressure-treated β -lactoglobulin resulted in significant increases in retention of selected flavor compounds when compared to native β -lactoglobulin (Hoang, 2006). High-pressure-induced denaturation of α -lactalbumin and β -lactoglobulin in milk and whey demonstrated that β -lactoglobulin was less baroresistant than α -lactalbumin. Both proteins were considerably more resistant to high-pressure-induced denaturation in whey than in milk (Huppertz et al., 2004b). Reversible antioxidant properties (Moller et al., 1998) and increased antigenicity of β -lactoglobulin (Kleber et al., 2006) were also observed following high-pressure treatment of whey proteins.

A pressure of 586 MPa (84,970 psi) was selected to alter whey protein structure and functionality in order to increase the commercial use of whey protein concentrate (WPC). Cantonese noodles formulated with 10% of the pressure-treated WPC exhibited increased water absorption, increased lightness, and reduced or comparable cooking loss when compared to a control formula containing untreated WPC (Kadharmestan et al., 1998). In addition, the handling properties of wheat flour doughs containing 10% pressure-treated WPC were improved. Resulting bread volumes increased when compared to control doughs containing 10% untreated WPC. Kadharmestan et al. (1998), using differential scanning calorimetry, demonstrated that 586 MPa (84,970 psi) pressure-treated WPC was comparable to WPC resulting from high-temperature treatment (90°C). Scanning electron micrographs of spray-dried WPC were described as globular in nature and pressure- or heat-treated WPC were described as glass-like, porous, or spongy in nature. Incorporation of 10% pressure-treated WPC into wheat flours significantly increased mixograph water absorption and extended mixing time compared to control wheat

flours containing no WPC. Pressure-treated WPC decreased mixing time performance compared to wheat flour doughs fortified with 10% untreated WPC (Kadharmestan et al., 1998).

Liu et al. (2005) reported an increase in the hydrophobic binding affinity and apparent dissociation constants by WPC for selected aromatic flavor compounds including benzaldehyde, heptanone, and octanone. However, significant increases in the retention of benzaldehyde, heptanone, or octanone in high-pressure-treated WPC solutions were not observed. Lee et al. (2006a) observed an increase in hydrophobicity of whey proteins following 690 MPa (100,050 psi) pressure treatment at ambient temperatures. High-pressure-treated whey proteins exhibited decreased solubility, which correlated with increased surface hydrophobicity ($r = -0.95$). The hardness of high-pressure-induced 20, 25, or 30% whey protein gels increased with increasing whey protein concentrations. Hardness also increased with high-pressure treatment time between 5 and 30 minutes (Lee et al., 2006a). Whey proteins treated in phosphate buffer at pH 5.8 and 690 MPa for 5 minutes exhibited increased emulsifying activity and emulsion stability in model oil-in-water emulsions. Lee et al. (2006b) demonstrated that the addition of high-pressure-treated whey protein to low fat processed cheese foods yielded a product with acceptable firmness and meltability, but the product had an undesirable sandy or grainy texture. The sandy, grainy texture and the rough protein matrices observed with scanning electron microscopy were attributed to the unfolding of whey proteins during the treatment (Lee et al., 2006b).

7. Pressure Effects on Beef, Pork, Poultry, and Seafood

Consumers demand high-quality meat and seafood products with natural flavor, fresh appearance, and pleasant texture. High pressure represents an appealing nonthermal process for meat products to preserve quality and avoid postprocessing contamination. High pressure exhibits potential for the preservation of meats and seafood, and is promising for the development of new products (Hugas et al., 2002).

Meats and seafood are composed of water, protein, fat, and minerals. From a physical point of view, an increase in pressure leads to a phase transition of meat molecules, which may be reversible after decompression.

From a chemical point of view, high pressure is less destructive of molecular structure than the thermal treatment of meats. Covalent bonds are not broken, but weak energy bonds such as hydrogen bonds and hydrophobic affinity can be irreversibly disrupted (Cheftel, 1995). Compression of water results in a decrease in its melting point and an increase in ionization leading to a decrease in the pH. High pressures may lead to protein denaturation, enzyme inactivation, or enzyme activation. High pressure may result in a reversible transition of lipids from liquid to a solid state including gelation. When there is a mixture of lipids, as in a cell wall, high pressure may lead to a disruption of cell membranes. The vitamins in meats are modified very little by high pressure. The effects of high pressure on meat and seafood products depend on the product and processing conditions (Hugas et al., 2002).

The effectiveness of high-pressure technology on meat or seafood matrices is highly dependent on the pressure magnitude, temperature, and treatment time. In general, high pressure at low or moderate temperatures results in the inactivation of microbial vegetative cells and enzymes, without changing the organoleptic characteristics of meats. High-pressure treatment can induce changes in meat structure and texture, and can increase the functionality of some ingredients. High pressure offers the opportunity to develop new meat products based on cold gelation of starches, nonthermal coagulation of proteins, and selective enzyme inactivation (Hugas et al., 2002).

Pressures ranging from 300 to 800 MPa were applied for 20 minutes at 20–70°C on postrigor beef *longissimus dorsi*. Texture profile analysis demonstrated an increase in firmness with increasing pressure at ambient temperature. A similar increase in firmness is observed with increasing temperatures at ambient pressure. At temperatures of 60 and 70°C, pressures of 200 MPa resulted in large and significant decreases in meat firmness. The results observed for meat firmness were mirrored by results observed for

gumminess and chewiness in high-pressure-treated meats (Ma and Ledward, 2004). Microbiological, physicochemical, and sensory quality of a Spanish dried beef product “Cecina de León” was evaluated after a pressure treatment of 500 MPa (72,500 psi) for 5 minutes and stored at 6°C for 210 days. Pressure treatment at 500 MPa (72,500 psi) for 5 minutes inhibited the growth of enterobacteria, enterococci, and pseudomonas, and delayed the growth of lactic acid bacteria, Micrococcaceae, yeasts, and molds. Also, no noticeable changes in physicochemical and sensory parameters were observed after pressure treatment or during refrigerated storage. High-pressure treatment is an efficient method for preserving Cecina de León without decreasing sensory quality (Rubio et al., 2007).

Duck foie gras pressure-treated at 400 MPa (58,000 psi) for times between 10 and 30 minutes at 50°C or at 300 MPa (43,500 psi) for 30 minutes at 50°C resulted in characteristics described as less fat melting, softer texture, and less cooked flavor. The microbiological quality was similar to that obtained with heat treatments (El-Moueffak et al., 1995). The application of pressures of 200 and 400 MPa for 30 minutes favors water- and fat-binding properties of chicken and pork batters. Textural properties of meat batters were influenced by cooking temperature. High pressures influence the texture of batters, so that pressure-treated batters are firmer, more cohesive, springy, or chewy than nonpressure-treated batters (Jimenez-Colmenero et al., 1998).

The extent of gelling of washed sardine mince (2.5% NaCl), treated at pressures between 150 and 500 MPa for 10–30 minutes at 0–75°C, was observed and compared with gelling using conventional thermal processing. The best conditions for achieving acceptable rheological parameters were pressure treatments around 300 MPa (43,500 psi) for 10 minutes at temperatures less than 10°C. High-pressure treatment of sardines considerably improved the rheological values of a mince which exhibits little gel-forming ability under conventional heat treatment (Perez-Mateos and Montero, 1997). Pressure treatment of fish skins at 250 and 400 MPa (36,250–58,000 psi) for 10 or 20 minutes was applied during pretreatment in acid at 10°C to facilitate

destabilization of acid labile crosslinks, or during extraction in water at 45°C to accelerate collagen hydrolysis. The use of high pressure to help extract gelatin from fish skins is a useful alternative to heat treatment. The HPP drastically reduces the longest phase of the extraction. This makes it possible to produce a gelatin of high gelling quality in only a few minutes (Gómez-Guillén et al., 2005).

Physical and biochemical changes in oysters following pressure treatment at 260 MPa (37,700 psi) for 3 minutes, or heat treatment for 10 minutes at 50°C, or 8 minutes at 75°C were compared to physical and biochemical changes in untreated oysters. High pressure and heat treatment both modified the proximate composition of oyster tissues. The protein content of high-pressure-treated oysters (6.9%) was significantly smaller than the protein content of heat-treated oysters or the control (7.9–9.1%). Pressure treatment improved tissue color of oysters more than thermal treatments. One significant advantage of high-pressure treatment over thermal treatment of oysters is that the pressure facilitates opening of the oysters by separating the muscle of the oyster from the shell (Cruz-Romero et al., 2006).

8. Effects of High Pressure on Vegetable and Fruit Quality

Quality and functionality of vegetables and fruits are heavily influenced by stress reactions of plant cells prior to and during food processing (Dornenburg and Knorr, 1998). Vegetables and fruit products such as purees, pie fillings, preserves, and juices are widely consumed in the United States. Cooling, freezing, drying, pasteurization, and commercial sterilization are some of the chemical-free processes that can be used for vegetable and fruit product preservation (Silva and Silva, 1997).

HPP of vegetables and fruits at 300–700 MPa inactivates microbes to produce a safe food that is free of additives and retains its taste and texture for an extended period of time under refrigeration. High-pressure treatment of vegetables and fruits can reduce enzyme activity and inactivate microorganisms. Pressure has little effect on molecular interactions among small molecules such as volatile carbonyls,

pigments, vitamins, phenols, sensory compounds, nutritional, and health-related qualities of vegetables and fruits (Palou et al., 1999).

Blanching is a thermal treatment used to inactivate enzymes such as peroxidases. Peroxidases can induce undesirable changes in the color and flavor of vegetables during storage. However, thermal blanching results in sensory changes and loss of nutrients. High-pressure treatment of vegetables was introduced by the food industry as an alternative to thermal processing (Quaglia et al., 1996). Pressures of 400–900 MPa (58,000–130,500 psi) for 5 and 10 minutes in combination with a mild heat treatment at 60°C were applied to green peas to evaluate the use of high pressure for blanching. Increasing the pressure from 400 to 900 MPa resulted in greater inactivation of peroxidase and with retention of ascorbic acid, while longer treatment times had less effect on changes in enzyme activity, ascorbic acid content, or texture. Pressure-treated vegetables and fruits exhibited acceptable textures, although there was a significant reduction in the firmness of fresh peas. The best results were achieved using 900 MPa (130,500 psi) for 5 minutes. High pressure can be used as an alternative to the thermal blanching of peas (Quaglia et al., 1996).

Pressures between 100 and 400 MPa were applied to inoculated tomatoes and lettuce. Treatment times and temperatures were 10 minutes at 20°C and 20 minutes at 10°C. Tomatoes and lettuce were inoculated with selected strains of gram-positive and gram-negative bacteria, molds and yeasts, as well as spores of gram-positive bacteria. Populations of gram-positive and gram-negative bacteria, molds and yeasts on tomatoes, and lettuce decreased 1 log cycle at treatment pressures of 300 MPa (43,500 psi) for both time and temperature treatment conditions. Treatment at 300 MPa (43,500 psi) altered sensory properties of the tomatoes and lettuce. The skins of the tomatoes loosened and peeled away, while the flesh remained firm. The color and flavor were slightly changed. The high-pressure-treated lettuce remained firm with an acceptable flavor, but underwent browning. Moderate pressure treatments of vegetables in combination with other treatment conditions such as temperature may be required to re-

duce the populations of pathogens and spoilage microbes while avoiding undesirable sensory qualities (Arroyo et al., 1997). Cryofracture scanning electron microscopy revealed pressure-induced cellular changes in cauliflower and spinach leaves. Pressure increased cell wall permeability and enabled movement of water and metabolites from the inside to the outside of the cell. The structural parenchyma cell organization disappeared in spinach leaves and cavity formation was observed after high-pressure treatment. Cauliflower exhibited a firm structure with a translucent appearance. Cauliflower is more acceptable than spinach for high-pressure treatment (Prestamo and Arroyo, 1998).

The effect of several pressure–temperature–treatment combinations on protein digestibility, texture, and microstructure changes of black beans were evaluated. Pressure, time, and temperatures combinations included 275, 410, 550, or 690 MPa for 5, 10, and 15 minutes at 25, 45, 65, and 85°C. Protein digestibility was dependent on pressure, whereas texture was most affected by temperature. Swelling of cell walls and minimal cell separation was observed in black beans treated at 690 MPa (100,050 psi) for 10 minutes at 25°C. Cell separation increased when beans were pressure-treated at 690 MPa (100,050 psi) using a temperature of 85°C. High pressure in combination with elevated temperature improves the protein digestibility and softens black beans. High pressure is an alternative process for bean preparation (Sangronis, 1999).

Shelf-stable fruit preserves and pie fillings are traditionally heat processed to ensure food safety and prolong shelf life. Fruit products exposed to high temperatures can present a cooked flavor, and lose desirable texture, color, and nutrient quality. HPP can achieve the “fresh-like” quality desired by consumers, while inactivating common pathogenic and spoilage microorganisms. High pressure is also capable of inactivating enzymes, such as polyphenoloxidase, pectin methylesterase, and polygalacturonase, which result in the browning or softening of fruit (Meyer et al., 2003).

In 1990, Japan was the first market to introduce fruit-based foods preserved by high pressure (see also Chapter 4). The Japanese market included several

fruit preserves, orange and grapefruit juices, salad dressings, fruit yogurts, and fruit sauces (Aleman et al., 1998). HPP reached a commercial reality in the United States in 1993. The first high-pressure processed food marketed in the United States was Classic Guacamole[®], a refrigerated fresh guacamole manufactured by Avomex (Mermelstein, 1998).

The quality and shelf life of pressure-treated guava puree was compared to thermally pasteurized puree. At 600 MPa (87,000 psi), a 6 log inactivation of microorganisms was observed with minimal color changes, degradation of pectin, cloud formation, and better retention of ascorbic acid when compared with fresh guava puree. There was complete inactivation of polyphenoloxidase, pectin methylesterase, and polygalacturonase inactivation in heat-treated guava puree. The pressure-treated guava puree (600 MPa or 87,000 psi for 15 minutes at 25°C) maintained quality similar to freshly extracted guava puree for 40 days when stored at 4°C (Gow and Hsin, 1996).

High-pressure treatment led to higher overall antioxidant activity of orange juice during storage at 0–15°C compared with conventional pasteurization (Polydera et al., 2004). The effects of pressure treatment of 350 MPa on orange juice carotenoids, β -carotene, α -carotene, zeaxanthin, lutein, and β -cryptoxanthin, associated with pro-vitamin A and radical-scavenging capacity values, resulted in significant increases of 20–43% in the carotenoid content of fresh orange juice (De-Ancos et al., 2000).

High-pressure technology may result in food products with novel functionality and characteristics. Process optimization for high-pressure technology is needed to achieve and maintain the superior sensory quality of high-pressure-treated food products (Oey et al., 2008).

9. Conclusions

HPP at pressures ranging from 300 to 700 MPa, offers unique opportunities to develop new food products with fresh-like and unique textures, flavors and appearance, excellent nutritional and health-promoting qualities, convenient preparation, and increased shelf stability (Palou et al., 1999; Torrey 2007) at refrigerated temperatures. High-pressure

treatment of food systems and food constituents can modify the functional properties of proteins, carbohydrates, and lipids. High pressure can provide safe and stable foods while minimally altering the sensory qualities (Knorr, 1993). Thus, HPP can better meet expectations of consumers for “fresh-like” and “health-promoting” foods (San-Martín et al., 2002).

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Chapter 7

Sensory Quality of Pressure-Treated Foods

Alan O. Wright

1. What “Quality” Means

This chapter is a guide for obtaining sensory data on the quality of food products treated by high-pressure processing (HPP). For the purposes of manufacturing a product, the definition of quality is based on specific measurable, sensory, and analytical criteria (Nielsen, 1998). Data should reflect a balanced focus on both consumers’ and producers’ interests and objectives. Sensory data adds value by presenting human sensory perceptions in quantifiable terms that can be correlated with other measures and used in the decision-making process. When asking questions and reporting responses, the use of proven sensory methods and techniques will strengthen the data and will help guide product development, maintain consumer satisfaction, and sustain profit margins for the company. Useful tools such as descriptive profiling are available to obtain this data. When consistently used, these tools provide a data history from which to base current and future decisions.

These tools and related principles will be discussed as they relate to the evaluation of consumer perceptions of quality in HPP treated products. They can be applied to other products and processes as well. When defining “quality” as it pertains to the consumer, it is sometimes viewed in terms of the mass market and how much a product is liked by the “average” consumer. More recently, manufacturers are realizing that multiple segments (defining

marketable niches within consumer groups) can more profitably replace what was once a single segment—“the average consumer.” Even though the product might not appeal “on average” in a broader category, the degree of liking of a tailored product can be very strong for a given segment. Economies of scale have to be considered, but by defining quality in terms of a more limited and clearly defined market segment, combined sales and consumer satisfaction can actually increase. A manufacturer may be poised to produce several closely related but slightly different products. Having HPP capabilities in house is an advantage in prototyping a variety of related products for multitargeted customers.

Survey tools (such as “select all that apply” scales) and analysis techniques (such as “principle component analysis”) exist to assess the diversity and depth of consumer market segments. Trained panelists can be used to identify critical characteristics of products within each segment. These attributes can be associated with consumer evaluations to confirm the degree of liking for various attributes in a product category. A clear definition of salient attributes enables product developers to develop by design rather than by chance. Designed products may be evaluated by sensory panels to assure they carry the highest-weighted attributes into a targeted market segment. Very slight differences in formerly singular product categories have been marketed successfully to appeal to multiple segments (e.g., Prego tomato sauces, Vlasic® pickles, Hershey’s® chocolates, Coca-Cola’s® cola beverages, and so forth). Some segmentation examples include: by personality type, economic status, and

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This chapter remains with the U.S. Government.

hedonic preferences. Some degree of knowledge about category strength and manufacturing costs are required; however, some companies have overextended themselves by producing for unwarranted and unsustainable market segments. The key is knowledge. Many books are available on the measurement of consumer satisfaction (O'Mahony, 1986; Lawless and Klien, 1991; Lawless and Heymann, 1999; Meilgaard et al., 2006; Frewer and Trijp, 2007). Manufacturers would do well to understand and articulate within their organizations who their targeted consumers are and then, in a unified manner, appeal to the distinguishable and primary elements of that consumer.

Segmenting is important as it relates to HPP-treated foods because of the unique contributions HPP offers to a variety of products. One needs to identify, understand, and measure fundamental characteristics of a consumer segment in order to predict, build, and verify the product's desirability to the targeted consumer. Surveys, focus groups, ethnography (trained observations), sensory evaluation practices, adaptive experimentation (AE) and other similar methods such as Rule Developing Experimentation (RDE—a type of combinational experimentation) (Moskowitz and Gofman, 2007), are being used and refined to help identify segments. Many segments remain unidentified and unexplored because of the novel nature and the unique properties imparted by emerging technologies. Our knowledge base needs further expanding. Only a limited number of products have been created or evaluated with HPP; thus, the field is somewhat embryonic. A key concept to keep in mind is, we've become a knowledge economy, and only by investing in knowledge can we expect economic improvement (Kouzes and Posner, 1995).

HPP has unique physical effects on food products (see Chapter 1.6). Measurable freshness factors—such as turgidity retainment in plant tissue, as well as a lack of thermal damage—are important effects derived from HPP. Protein conformational changes, color preservation and changes, de-aeration, and component infusion are notable examples of emerging HPP effects that consumers may find desirable in various applications. (Ramirez-Suarez and Morrissey, 2006; Considine et al., 2008).

Thus the potential that many new consumer marketing segments remain to be defined. Principles of sensory science and analytical measures can help to define and retain products that meet consumer's wants and needs.

2. Creating Quality

Quality is created by identifying and articulating important attribute specifications and retaining those specifications within the product. Quality products are value-added products that consistently address three key variables: targeted and defined consumers; consistency of the key product attributes; and sensitivity to the voice of the consumer with an ability to respond and adapt.

Targeted consumers are identified by primary characteristics. For example, a segment of consumers who like BBQ sauce is identified. Focus groups, PCA (principle component analysis), RDE, RSM (response surface methodology), and/or conjoint analysis are some of the methods used to provide data which identify segments and highlight consumer-valued attributes in a product (O'Mahony, 1986; Lawless and Klien, 1991; Lawless and Heymann, 1999; Meilgaard et al., 2006; Frewer and Trijp, 2007; Moskowitz and Gofman, 2007). Assume the following traits are identified as valuable for a defined consumer segment: "All Natural" and "Locally Grown" product labels; a flavor profile with a slightly sweet BBQ sauce, fresh-like chipotle notes; and a lingering hickory smoke aroma and flavor on the palate. A survey might reveal that 83% of this segment of consumers shop frequently at Trader Joe's® markets. With these value-yielding traits of the primary consumer defined, measuring the product's "quality" is much easier. Specifications, sampling, and testing are then aligned to support these components or values.

For example, after further sensory and analytical research it is determined that the quality specifications should include the following: all raw ingredients sourced from verified "all natural" vendors in the area and processed at a local plant; a finished Brix of 18.2–18.4°, and the hickory note derived from flavored beef drippings. These drippings require a 2-hour natural flame cook of 12% lean beef cooked

over hickory wood. All drippings are collected and added at a 3.7–3.8% level. Trained sensory panelists check the flavor of the drippings before adding to the sauce. Time–intensity ratings (0 to 15+ scale) are performed to confirm a “hickory smoke” intensity of 3 at 0 seconds, 11 at 45 seconds, and 5 at 90 seconds. These ratings are determined to be optimal by comparing Hedonic data of targeted consumers with time–intensity data from trained panelists.

The product preparation, packaging, and processing steps are as follows: liquid components are cooked, cooled, and filled into containers. Clean chipotle is freshly cut and added to the containers to constitute 6.3% of the formulation. Containers are sealed and the product is HPP treated at 550 MPa (79,750 psi) for 3 minutes.

Sales and marketing personnel would focus efforts to offer the product exclusively through Trader Joe’s[®] and use their consumer research data to demonstrate the quality and value of this exclusive product.

3. Validation of Quality (Subjective and Objective)

Subjective measures (see Figures 7.1 and 7.2) are those which are “subject to opinion,” while objective measures (see Figures 7.3–7.7) stand alone as an

Please rate the OVERALL ACCEPTABILITY of this sample.

- ☐ (9) Like extremely
- ☐ (8) Like very much
- ☐ (7) Like moderately
- ☐ (6) Like slightly
- ☐ (5) Neither like nor dislike
- ☐ (4) Dislike slightly
- ☐ (3) Dislike moderately
- ☐ (2) Dislike very much
- ☐ (1) Dislike extremely

Figure 7.1. Consumer-liking measurement scale. This type of scale was made popular from Peryam and Pilgrim’s work (1957). Here is an example of a standard 9-point Hedonic scale. This type of scale has been around a long time. Most sensory people know what the results mean in context of other products. It is a reliable type of scale that is easy to use on paper ballots (i.e., it fills out and scans easily), and there is a lot of historical data gathered with this type scale.

How much do you like or dislike this sample?

- Greatest imaginable like
- Like extremely
- Like very much
- Like moderately
- Like slightly
- Neither like/dislike
- Dislike slightly
- Dislike moderately
- Dislike very much
- Dislike extremely
- Greatest imaginable dislike

Figure 7.2. Labeled affective magnitude (LAM) (Schutz and Cardello, 2001), a consumer scale which uses validated spacing of word anchors (newer and tells more, ratio statements can be made—sample xyz is liked 3x more than sample abc).

“object” independent of opinion—they are what they are. Human sensory measures are individually subjective but can become objective (see Table 7.1) with an appropriate number of subjects or after attribute, reference, and sample training (see Figure 7.8). Two main groups of panelists are used: naive consumers who tell how much they like something; and trained or partially trained judges/panelists who assess intensity and describe or discriminate attributes of products. Quality measures such as intensity, presence, or absence of clearly defined characteristics such as sweetness, cohesiveness, moistness, and hickory aroma are often viewed as those measured by appropriately calibrated instruments. Trained panelists are human subjects who have become “calibrated” to measure known sensory properties. Thus trained, the panelist’s responses become standardized and consistent. Training reduces variance and enables the sensory specialist to objectively analyze the data for significance (see Table 7.1).

In sensory analysis, various tools, such as scales, references, training, and statistics, are used to “objectify” the human perception. Other methods not

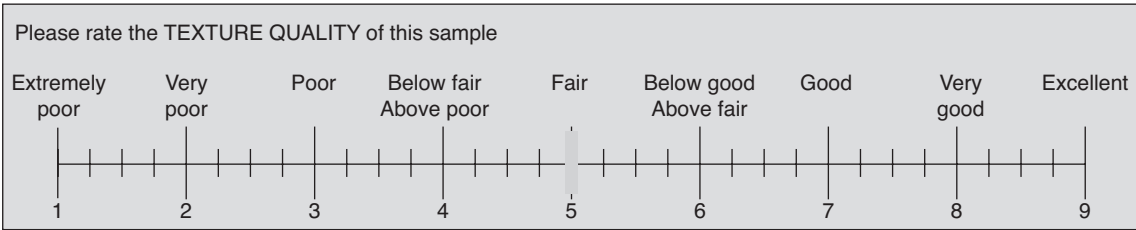


Figure 7.3. Quality scale used for specific quality attributes (texture in this example) by trained panelists.

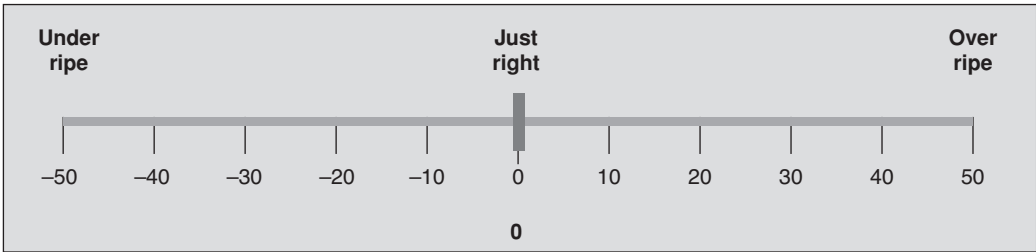


Figure 7.4. “Just Right” scale and results used for tomato texture perception study of 12 treatments. This scale is balanced along one attribute—ripeness.

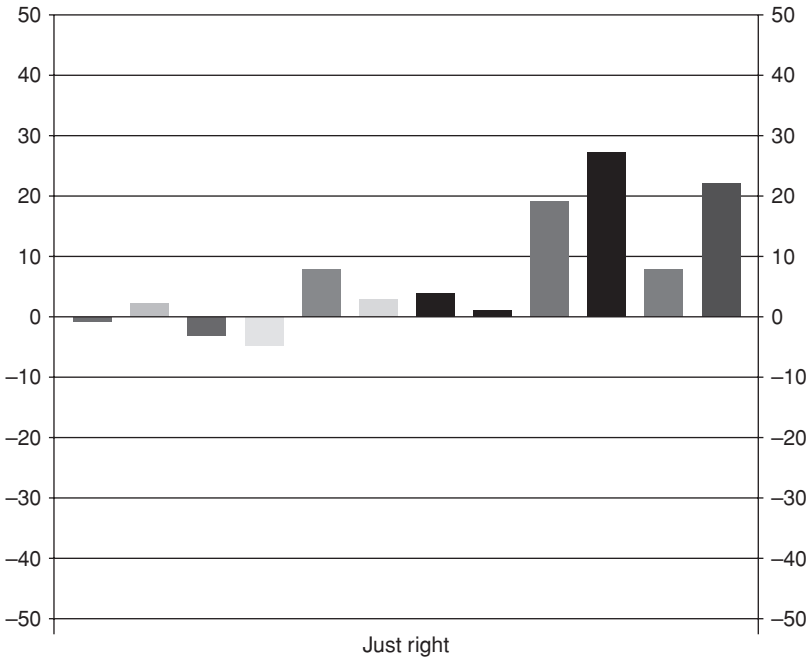


Figure 7.5. This graph displays the results of the “ripeness” from data acquired from the “Just Right” scale. This could have been generated from naive consumers who were asked to rate their perception of how optimal the color of the fruit was or the data could have been generated from trained panelists who are rating the “ripeness” from specific criterion. The 12 treatments are seen graphically in a way that is intuitive and easy to see relative comparisons. (For color detail, see color plate section.)

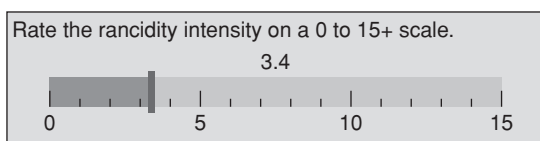


Figure 7.6. Intensity scale for the attribute “Rancidity” using a 0 to 15+ scale (the “0” indicates no rancidity, the “15” indicates the limits of perceptibility, and the “+” indicates that there are continued levels of rancidity possible but theoretically those levels exceed the threshold level of human perceptibility). Often, references will be used with an intensity measure like this (e.g., a 0 reference, a 7 reference, and a 12 reference would be suitable).

already mentioned include quantitative descriptive analysis (QDA), texture profile method (TPM), free choice profiling (FCP), and flavor profile method (FPM) (Keane, 1992). Scales can be used in ratings and when enough people have rated a product, the results become objective measures. Instrumentation can also be used and correlated with sensory findings. Acid profiles, texture analysis (e.g., TA.XT2 machine, Bostwick consistometer), Hunter colorimeter, gas chromatograph, mass spectrometry, moisture analysis, and water activity are some of the instrumentation methods that can be correlated

with sensory analysis. Arguments can be made for and against the use of instrumentation in lieu of human subjects. Consideration should be given to the type of measure one desires to obtain and the relationship of cost:benefits and frequency of use of equipment and personnel. The availability of human panelists, the urgency and quality of the result to be obtained, cost risk factors, and the business model of the manufacturer also play a role. Instrumentation is unlikely to ever fully replicate the total integrated and contextual experiences of a human being’s sensory experience and perception. Thus, sensory evaluation holds a valuable place in the decision-making processes of product development and product life cycle factors.

4. Process and Product Improvements for HPP Foods

From time to time, process and product changes and improvements necessitate changes to products. Some of the reasons include: consumer attitude shifts; raw ingredient supplier changes; government regulation changes; raw ingredient quality/availability changes due to supply chain disruptions; new technological

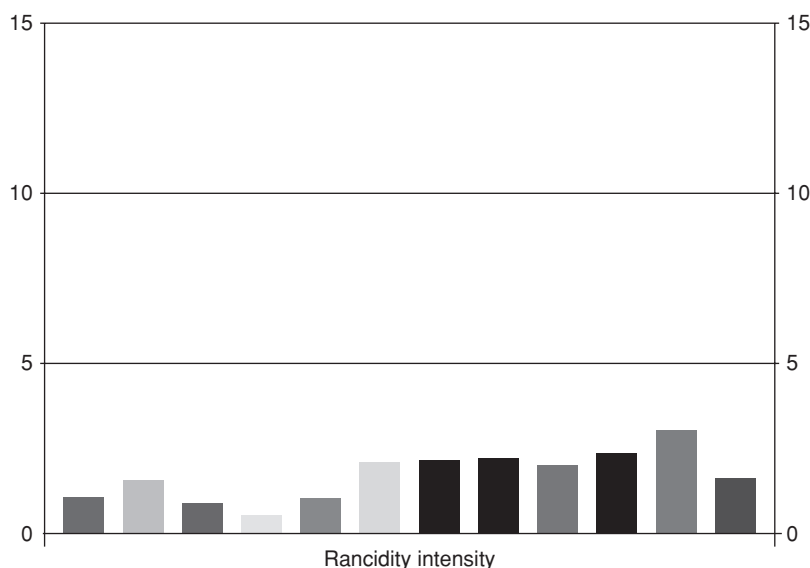


Figure 7.7. Bar graph results of “Rancidity intensity” for 12 different samples. (For color detail, see color plate section.)

Table 7.1. Comparison of means for significance (different letters within a row indicate significance between the mean of that cell with the means of other cells in the same row)

Attribute	No Wrapper, Bar Sample Only						With a Wrapper on the Bar						P Value	Sig
	119	228	337	446	553	661	119	228	337	446	553	661		
Overall accept-ability rating	ab	ab	d	cd	d	ab	ab	a	d	cb	cd	a		
Perceived product benefit	6.19	6.00	4.60	4.95	4.52	6.17	5.92	6.62	4.63	5.56	5.13	6.62	0.0001	0.001
	cab	cb	e	e	e	cb	cd	a	e	cb	de	ab		
	4.78	4.51	3.58	3.64	3.60	4.45	4.33	5.37	3.54	4.54	3.83	5.06	0.0001	0.001

Duncan's analysis (comparison of means) determined using SAS, October 15, 2007, by Alan O. Wright.

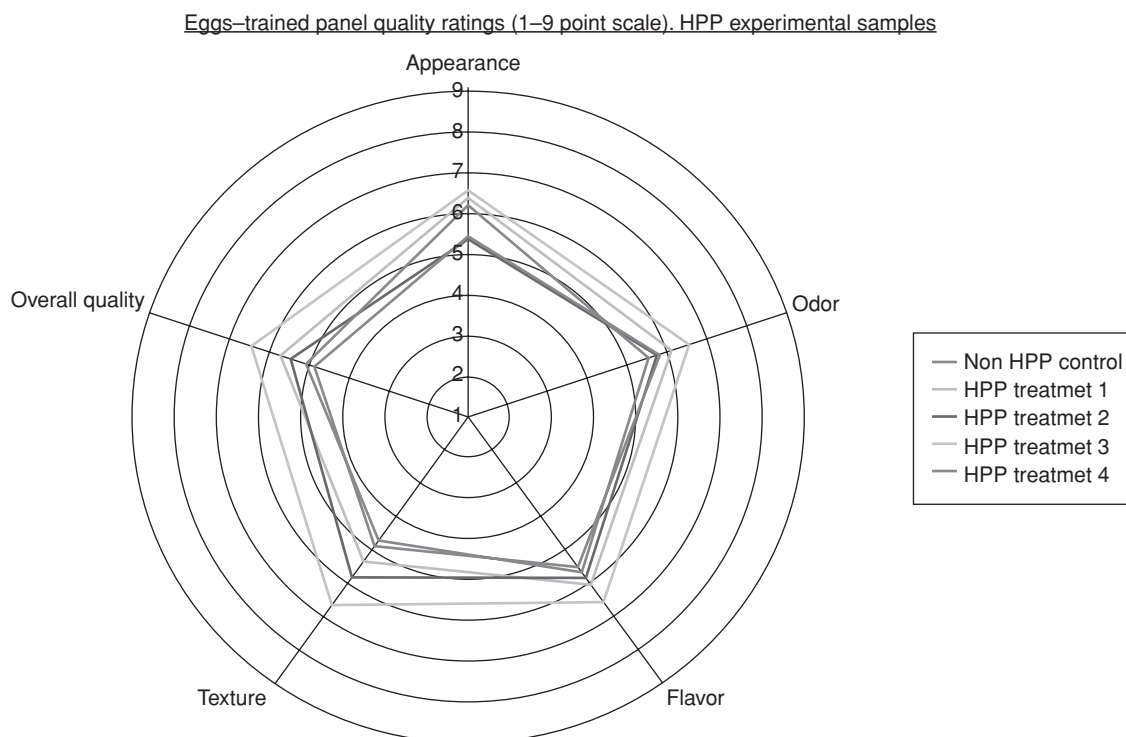


Figure 7.8. Spider graph display of HPP data from several treatments. The spider graph provides a visual comparison/fingerprint of products overlaying each other. In this graphic, the HPP treatment 3 is clearly the highest rated in all categories. (Note: this graph was derived from a 1- to 9-point quality scale (see Figure 7.3), *not* a 1- to 9-point Hedonic scale (see Figure 7.1)). (For color detail, see color plate section.)

developments; and the need to enhance products in order to be more competitive. Sensory measures consistently employed offer several benefits: they may expose supplier raw ingredient changes or defects; they may confirm consumer acceptability to known or unknown changes; they may assure consistency of product; and they may reveal product flaws and safety concerns. In short, sensory practices assure the relevance of objective measures and confirm that the product is in line with the targeted consumer's expectations.

5. Examples of Quality Measurements Appropriate to HPP Treated Foods

The scales and instruments should be regularly used in a consistent manner in order to assure that data of the past can be effectively measured against the

data of the present. When changes are needed, the sensory specialist should assure that sufficient note taking is included to describe changes to methods in order to accommodate as much analysis as possible of "old" data with "new" data. The Hedonic scale (see Figure 7.1) is commonly used to measure consumer liking. It typically uses a 1- to 9-point Likert scale (see Figure 7.1). Point 9 indicates "extremely liked," while 1 indicates "extremely disliked." The Hedonic is an affective scale and should not be confused with descriptive (see Figure 7.3) or discriminative scales. These scales are commonly used to determine if a difference exists (e.g., triangle test) or to describe characteristics of a product (e.g., Figure 7.6, 0–15+ intensity of attribute scale and Figure 7.9, Degree of Difference scale).

Various methods are employed to collect consumer data. It may be collected electronically (e.g.,

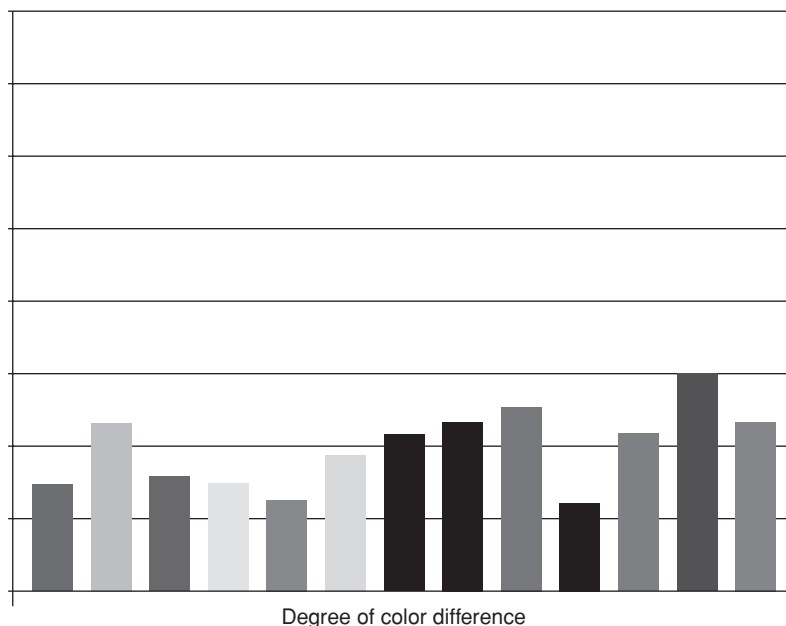


Figure 7.9. Degree of difference scale and results (treatments vs. control) When scales are different, it is sometimes useful to standardize the rating so that other attributes or characteristics can be viewed on the same graph. Caution should be used when multiple scales are used. Work to assure the scaling of a graph is the same as the scaling that was used to get the rating and to not create misleading information to those viewing graphs. Errors are commonly made in data acquisition and data presentation that can be misleading or confusing when multiple scales are used together to collect data and to present the data. Data Standardized to a 9-point scale—data appropriately presented (number removal simplifies understanding to an intuitive level). (For color detail, see color plate section.)

SIMS 2000, Sensory Computer Systems, LLC[©], www.sensorySIMS.com). Data may be collected by pencil and paper surveys. It may be acquired verbally in one-on-one interview surveys or even through observation alone (ethnography). Data may be collected in central location testing (CLT) facilities through contractors or at one's own CLT facility. Data may be collected over the internet, in round table discussions, in labs, at the bench top, in malls, in churches, in schools, and in other places where people gather. Data may be collected through home-use trials. It may be collected in restaurants, grocery stores, dining halls, and sporting arenas. Though the places and methods are extensive, the key to good data collection is to have a good experimental design that can allow results to be validated by appropriate statistical analysis. There must be a solid understanding of how to collect the data within the context of interest

for the product and consumer without creating bias. When carefully employed, sensory evaluations provide insightful information that serves to maintain consumer satisfaction and quality control.

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Chapter 8

Hydrodynamic Pressure Processing of Meat Products[§]

M.B. Solomon, M. Sharma, and J.R. Patel

1. Introduction

Food safety and quality, and tenderness in particular, are two of the top priorities facing the meat industry. Thus, a means for controlling and assuring both a meat product's safety and tenderness level is essential. Tenderness is the major criterion driving consumers' decisions to purchase or repurchase meat. Unfortunately, tenderness has proven to be the most difficult quality factor for meat producers and meat packers to manage. Development of commercial methods to ensure consistently tender meat products is of primary importance for enhanced consumer acceptance of meat. A variety of techniques has been utilized for tenderizing meat, but none have been completely successful. Techniques applied individually or in combination include: mechanical, chemical, temperature conditioning, aging, electrical stimulation, high-pressure-heat treatments, and alternative carcass positioning. A number of these techniques require additional holding periods, space, and labor. Furthermore, several of these methods have been criticized for their lack of consistency in tenderizing meat.

[§] Mention of brand or firm names does not constitute an endorsement by the US Department of Agriculture over others of a similar nature not mentioned.

2. History and Origin of Hydrodynamic Pressure Processing

The concept for tenderizing meat using shock waves from underwater detonation of explosives, called hydrodynamic pressure processing (HDP), was first introduced and patented by Godfrey (1970). Hydrodynamics refers to the motion of fluids and the forces acting on solid bodies immersed in these fluids. The HDP process should not be confused with research using high-pressure processing or hydrostatic-pressure processing (HPP). Hydrostatic pressure refers to the characteristics of liquids at rest and the pressure in a liquid or exerted by a liquid on an immersed object. In HPP technology, meat products are submerged in a fluid in a pressure vessel, and pressure is generated by pumping fluid into the closed vessel (see Chapter 1). In contrast to the instantaneous shock wave generated using HDP, HPP technology tenderizes meat with constant pressure exerted over an extended duration (minutes or hours) and at temperatures as high as 40°C.

The Hydrodyne[®] process and equipment patented by Long (1993, 1994) was designed to overcome deficiencies of the original patent by Godfrey (1970) for tenderizing meat using an explosive charge in a tank filled with water. Long explained in his patent that Godfrey's principle was sound, but the tank and position of the meat in relation to the tank and explosive charge would present serious difficulties for the commercial tenderization of meat. Long's (1994) modifications included a shock-absorbing support system for a suspended vessel with a baffled locking cover to recover water expelled during the detonation.

Nonthermal Processing Technologies for Food, Edited by Zhang, Barbosa-Cánovas, Balasubramaniam, Dunne, Farkas, and Yuan. This chapter remains with the U.S. Government.

3. Principles of HDP for Meat Tenderization

In 1992, the USDA's Food Technology and Safety Laboratory (FTSL), formerly named the Meat Science Research Laboratory, began testing the HDP concept and developing HDP technology. Solomon and Long (1995) and Solomon et al. (1995) were the first to report on the successful use of HDP to tenderize beef and lamb, respectively. Improvement in meat tenderness for a variety of muscle foods (beef, lamb, pork, and poultry) has been demonstrated using HDP technology. Zuckerman and Solomon (1998) and Zuckerman et al. (1999) were the first to identify a cause of tenderization. They showed that tenderization resulted from the disruption of the myofibrillar structure and the structural integrity of the muscle and connective tissue complex. The HDP-treated meat displayed no outward visual signs of change, but upon cooking was found to be significantly more tender (by 25%) than non-treated control samples. Furthermore, a direct relationship between the tenderness of HDP-treated meat and the redistribution of proteins in the homogenate fraction of the HDP-treated samples was reported by Spanier and Romanowski (2000). They observed that the response to HDP was similar to that seen when the myofibrillar fragmentation index was used to correlate levels of meat tenderness during the aging of meat.

HDP involves underwater detonation of a high-energy explosive in a containment vessel to generate a shock wave pressure front at velocities exceeding the speed of sound. The shock wave passes through the liquid medium and vacuum packaged meat. Food products that contain a considerable amount of water and little dissolved gas have compressibility properties similar to that of water (also see Chapter 1). To be an acoustical match with water, the object, in this case, meat, must possess an E/D ratio similar to water, where E is the bulk modulus of elasticity and D is the density of the object (Kolsky, 1980). Because boneless meat contains approximately 75% water, it is a good acoustical (mechanical impedance) match with water (Kolsky, 1980) and is quite suitable for this process. The shock wave in water reflects off any object that is not an acoustical match (Kolsky, 1980).

Shock waves generated and transmitted through water move equidistant from an explosive source depending on the shape of the explosive (Batsanov, 1994). The shock wave, with targeted pressure fronts of 70 to 100 MPa (10,100–14,500 psi) in the HDP process, occurs in fractions of milliseconds. Adiabatic compression of water (or of aqueous solutions) causes a temperature increase of only 2–3°C per 100 MPa, depending on the initial temperature and the rate of pressure increase (Batsanov, 1994). Pressure release causes a decrease in temperature of the same order of magnitude. Since the dynamics of the shock wave pressure front in the HDP process occur in fractions of milliseconds and at pressure levels less than 100 MPa (14,500 psi), there is virtually no increase in the temperature of the meat or water.

Detonation of an explosive under water produces both a shock wave and a gas bubble. When a shock wave is generated, the wave front raises the surrounding materials to a high pressure, then induces a flow velocity behind the wave and quickly subsides (Glass, 1994). A flow velocity is similar to an aftershock or reverberation of subsiding pressure waves. Cole (1948) defined the shock wave as the largest single source of energy. The first wave front to reach the meat surface in the HDP process is a compression wave (Craig and Rye, personal communications). Secondary pressure pulses from the resultant gas sphere contractions are apparent in a longer duration than the shock wave, but the energy associated with the gas sphere is typically one-third or less of the shock wave energy (Cole, 1948). It is dependent on the type/composition of the explosive used. The effects of the gas-sphere-generated energy (secondary wave) on the submerged object (meat) depend on the geometry and composition of the explosive charge, target, and shape of the nearby surfaces. As the wave front traveling at hypervelocity initially passes through the meat, a compression force is generated (Hyde, 2005). The shock wave generated from the detonation of an explosive decays quickly while expanding. It has a curved front, normally not a perfect sphere, and therefore the pressure jump across its front changes with time as function of location. As the primary wave front reaches the free unsupported acoustic mismatch surface at the bottom of the

container, the wave front is reflected off the bottom of the container by a steel platform. As a result, a near doubling in pressure occurs when the transient pressure wave changes to a tensile wave (Craig and Rye, personal communications; Gustavson et al., 2001; Hyde, 2005). The relationship between the wave velocity and the wave period of the ensuing shock wave appear to be critical components of the HDP process for successful meat tenderization.

The explosives used in HDP develop a very rapid and shattering action when detonated. This shattering power is referred to as the “brisance” of the explosive. By varying the composition and the shape (cylindrical, rectangular) of the explosive, brisance properties can be modulated. The various explosives that can be used in the HDP process differ in: the amount of gas that is produced per quantity of explosive; amount of heat liberated; detonation velocity; pressure that gases exert upon their immediate surroundings; and shattering power developed by the explosive. Thus, a number of these parameters influence the shape and strength of the resultant shock wave.

4. HDP Technology and Meat Tenderization Process Enhancements

Research has suggested that although HDP does improve meat tenderness, the magnitude of tenderness improvement is dependent on several factors. These include: configuration and composition of the shock wave container; type of shock absorbers used (Solomon and Eastridge, 1999; Solomon et al., 1999a; Solomon and Berry, 2000); quantity of explosive (Solomon, 1998a; Solomon, 1999); placement of the explosive (Solomon, 1998a; Solomon and Eastridge, 1999); shape of the explosive (Eastridge et al., 2002; Solomon et al., 2004); boundary conditions of the shock wave container (Eastridge et al., 2000; Solomon and Berry, 2002); and geometry of the steel reflecting surface of the container (Callahan et al., 2006). Other variables that affect the response of the muscle to achieve the maximum tenderization need to be evaluated. These variables include (1) whether the muscle is treated fresh or frozen/thawed (Berry et al., 1997; Solomon et al., 2004); (2) conditions after treatment (Callahan et al., 2002; Solomon

et al., 2002); and (3) postmortem time of treatment and storage times (Paroczay et al., 2002; Solomon et al., 2002). HDP can be combined with other postharvest intervention strategies (Solomon et al., 1998; Liu et al., 2006). The effectiveness of combined treatments has yet to be determined.

Three types of shock wave containers have been used in the HDP process. They are: plastic explosive containers (PEC) of various sizes (208 L, 115 L, and 98 L) fitted with a flat, steel reflector plate; a stationary 1,060-L commercial prototype steel hydrodynamic unit (CHU); and a stationary small-scale 54-L laboratory model hydrodynamic unit (LHU). The HDP process (Solomon et al., 1999a) generated in a 115-L PEC resulted in a 43% tenderness improvement for boneless beef strip loins. Shear force was reduced from 8.3 to 4.7 kg, compared to samples treated with HDP in the CHU (original commercial prototype Hydrodyne[®] unit with four shock absorbers). Shear force values were reduced by 33% (8.2–5.5 kg). As shock absorbing devices were added (and their composition changed from rubber to steel) to support the CHU vessel, meat tenderization was significantly decreased (Solomon and Eastridge, 1999). Shear force values generated in PECs with no shock absorbing supports decreased to 3.7 kg (47%). Shear values in the CHU with four shock absorbing supports decreased to 4.2 kg (40%). Shear values in the CHU with eight shock absorbing supports decreased to 5.9 kg (16%) and with 16 shock absorbing supports was 6.2 kg (11%). This last value was not significantly different from the control shear values of 7.0 kg. Results of this study suggested that type of container and shock absorbing system play an important role in the performance of the HDP process. Solomon and Berry (2000), comparing the plastic containers with the LHU model, reported that HDP generated in 115-L PECs resulted in a 40% decrease in shear force (6.8–4.1 kg) compared to samples treated with HDP in the 54-L LHU, which improved 28% (6.8–4.9 kg).

In developing parameters for tenderizing meat, the majority of HDP experiments have used a binary explosive composed of a liquid and a solid. Additional trials evaluated the effectiveness of molecular explosives (Eastridge et al., 1998; Solomon, 1998b;

Marriott et al., 2001). Results suggest that at lower pressure fronts (<100 MPa; <14,500 psi), binary explosives are more effective at improving tenderness than molecular explosives, but at higher pressure fronts (>125 MPa (>18,125 psi)) molecular explosives may be more effective than binary ones. The magnitude of tenderness improvement from HDP is also dependent on additional factors. For example, increasing the quantity of explosive used (Solomon et al., 1997; Solomon, 1998a; Meek et al., 2000) does not always increase tenderness. The shape of the explosive charge (Eastridge et al., 2002; Solomon et al., 2004) affects the dynamics of HDP. A cylindrical shaped charge placed horizontally, parallel, to the meat results in more uniform tenderizing effect than a rectangular (folded) shaped charge (Eastridge et al., 2002). The rectangular shaped charge resulted in 10% reduction in shear force, while changing to a cylindrical shape reduced shear force by 16%. With the rectangular charge, a 38% shear force reduction was achieved. However, the magnitude of improvement among all samples was more variable. The cylindrical shaped charge had higher mean shear force reduction than the rectangular shaped charge, but among samples, shear force was less variable. Solomon et al. (2004) found that a larger proportion of samples treated by HDP using the rectangular shaped charge (81%) were successful in shear force reduction compared to the cylindrical shape (56%). Successful shear force reduction (tenderization) was defined as $\geq 10\%$ reduction in shear force value.

The position (distance) of the charge from the meat source (Eastridge et al., 1998; Solomon, 1998b; Meek et al., 2000) showed that although peak pressure fronts increase as the explosive charge is moved closer to the meat, the results indicate that shear force reductions are inconsistent. Different contact boundary conditions (e.g., air, water, and surface support material) for the PEC containers have been evaluated (Eastridge et al., 2000; Spanier et al., 2000; Solomon and Berry, 2002). Eastridge et al. (2000) reported that the deeper the air boundary for suspended PEC containers (suspended in air by plastic ropes), the more effective the HDP treatment. Spanier et al. (2000) found that HDP treatment of beef strip loins resulted in greater shear force reduction using PEC contain-

ers suspended in air by plastic ropes (40% reduction in shear force) vs. PECs seated on a styrofoam® base (21% reduction). Solomon and Berry (2002) reported that type of support surface (soil vs. concrete) and surrounding boundary environment (air vs. water) on soil for placement of the plastic containers influenced the performance of the HDP process for tenderizing meat. Beef strip loin shear force was reduced to 24% (11.7–8.9 kg) using a concrete support surface with the container surrounded by air. A 39% shear force reduction (11.9–7.3 kg) was found for a soil support surface with the container surrounded by air and a 24% shear force reduction (12.1–9.2 kg) was found with a soil support surface with the container surrounded by water.

Beef eye of rounds were used in a study (Callahan et al., 2006) designed to test the theory that maximum tenderizing effects would occur when the reflector plate had a curved (bowl) shape similar to the shape of the emanating shock wave. A 41-cm diameter weld cap was used as the curved reflector bowl on the bottom of a PEC in place of the standard 1.3 cm thick 41 cm diameter flat steel plate. A 150 g folded, rectangular shaped charge was detonated at 31 cm above the surface of the meat. Immediately after HDP treatment, 3.2 cm thick steaks were removed from control and HDP samples for shear force evaluation. Cooking time and yield were similar for control and HDP. The resulting 5% reduction in shear force, although statistically significant, was highly variable (–9 to 25%) among individual samples. From these results, the curved reflector bowl did not appear to improve the tenderization effects of HDP treatment.

Berry et al. (1997) conducted two studies to evaluate the sensory and cooking properties from HDP treated US Select grade strip loins using PECs. The first experiment involved frozen/thawed loins that were treated with HDP. Steaks were cooked to 65, 71, or 77°C. Steaks were evaluated for sensory properties by a 10-member trained panel. With previously frozen loins, HDP produced significantly higher tenderness ratings and shorter cooking times than controls. Increasing end point temperature produced lower cooking yields, longer cooking times, more well-done cooked color scores, but had no effect on sensory properties. In the second experiment, fresh

(never frozen) loins were subjected to HDP. HDP significantly improved tenderness scores compared to controls. In both studies, juiciness and flavor scores were not affected by HDP treatment. Furthermore, Solomon et al. (2004) found that HDP reduced shear force values in both fresh and frozen/thawed meat samples. Variability in instantaneous tenderization using HDP is related to postmortem time of application of the treatment (Paroczay et al., 2002).

The magnitude of instantaneous shear force value reduction was higher in fresh meat samples, most likely due to the freeze/thaw cycle. After 6 days of refrigerated storage following HDP treatment, shear force reduction resulting from HDP treatment was sustained for both fresh and frozen/thawed meat samples (shear force values were ≤ 3.9 kg) compared to 4.5 kg for control samples. Solomon et al. (2001) reported that the HDP treatment yielded instantaneous improvements in tenderness and these improvements were maintained even after a period of frozen storage. To further evaluate the ability of HDP to tenderize tough meat, intentionally cold shortened loins with initial shear values of 8.3 kg were treated with HDP and the percentage reduction in shear force value ranged from 37 to 47% for HDP samples (Solomon et al., 1997).

Fresh beef strip loins were HDP-treated either on day 2, 4, or 7 postmortem. Shear force reduction were detected (11% for day 2; 25% for day 4; and 28% for day 7). Shear force values of non-treated control samples dropped from 7.1 to 6.2 kg due to aging from 2 to 7 days; however, these differences were not significant. HDP-treated samples showed no additional improvement in tenderness with aging. Solomon et al. (2002) found that HDP-treated beef ages faster and to a greater degree than non-treated controls. Shear force values of control steaks dropped from day 0 to day 12 but no additional reductions were detected beyond day 12. HDP treatment instantaneously reduced shear force values (28% reduction compared to controls) on day 0 and samples continued to show reductions in shear force through day 5 (3.9 kg) and through day 16 (3.1 kg). Control samples never reached a 4-kg shear force value although they were aged for 29 days. Similar improvements in tenderness with aging were found with pork

loins (Callahan et al., 2002). Pork loins that were treated with HDP at 2 days postmortem were evaluated for shear force value on the day of the HDP treatment and then after aging for 3, 5, 11, and 18 days. No improvements in tenderness were found on day 0; however, HDP-treated steaks reached minimum shear force values by day 11 (3.8 kg), whereas the non-treated controls required an additional 7 days of aging (18 days total) to reach a similar shear force value (3.8 kg).

Two studies (O'Rourke et al., 1999; Solomon et al., 1999b) were conducted to determine whether the orientation of muscle fibers during the HDP process has any effect on tenderization. Horizontally (based on muscle fiber orientation) positioned semitendinosus muscle sections resulted in greater shear force reduction and increased improvements in tenderization than vertically positioned muscle samples.

5. Microbial Safety Resulting from HDP Treatment of Meat

The microbial safety and inactivation mechanism of HPP are discussed in Chapter 5. During HPP, several morphological changes in the microbial cell occur, which may lead to cell death. Some of the changes include: compression of gas vacuoles; cell lengthening; separation of the cell membrane from the cell wall; contraction of the cell wall with the formation of pores; modifications of the cytoskeleton and strand formation; modifications of the nucleus and intracellular organelles; coagulation of cytoplasmic protein; and release of intracellular constituents out of the cell (Shimada et al., 1993). Further, various microbial enzymatic systems are inhibited or inactivated by pressure. Pressure damage to the cell membrane denatures or displaces membrane-bound ATPase. The lack of ATP hydrolysis leads to cell death because protons will not be actively transported outside of the cell, leading to acidification of the cytosol. Kheadr et al. (2002) reported changes in cell morphology as well as dissociation of the cytoplasmic membrane during high-pressure processing, resulting in bacterial death. The cytoplasmic membrane is also believed to be the most damaged cellular target of high-pressure treatments (Earnshaw, 1992).

The exact mechanisms of the bactericidal effect of HDP are still unknown. One possible mechanism could be a shock-wave-cavitation interaction during HDP processing (see also Chapters 9 and 10). The shock waves initially produce a high positive pressure, which is rapidly transformed into tensile stress within microseconds, resulting in the formation of microscopic-vapor-filled cavities. These cavities expand momentarily and then violently collapse after a very short time, creating small, high-speed (up to 400 m/second) jets of fluid (cavitation) generating very high energy densities. Cavitation causes high localized temperature and pressure gradients that could be bactericidal. Cavitation depends on the pressure of the medium, the presence of microscopic bubbles in the samples, and the existence of a liquid–air interface (Loske et al., 2002). Along with cavitation, micro jets, acceleration, and shearing forces may be associated with bactericidal inactivation.

6. Inactivation of Food-borne Pathogens in Meat by HDP

HDP treatment can reduce parasites in pork loins. When treated by HDP with pressure fronts of 46 MPa (6670 psi), larvae per gram (LPG) of *Trichinella spiralis* in pork loins were significantly lower than LPG in untreated controls (Gamble et al., 1998). However, larvae of *T. spiralis* in pork loins treated by HDP using larger pressure fronts of 55–60 MPa (7,975–8,700 psi) were not significantly reduced when compared to untreated loins. Neither HDP treatment eliminated the infectivity of *T. spiralis* larvae.

Ground beef inoculated with *E. coli* O157:H7 and treated with HDP had significantly lower populations than untreated inoculated beef, but reductions were marginal (Podolak et al., 2006). *Salmonella* Typhimurium in minced chicken treated with HDP were not reduced significantly (0.2–0.3 log CFU/g) compared to populations on chicken not treated with HDP (Patel et al., 2006).

Interestingly, the number of cells of *E. coli* O157:H7, *S. Typhimurium*, and *L. monocytogenes* that were strongly attached to beef cubes were significantly reduced by HDP treatment compared to

counts of loosely attached cells (Patel and Solomon, 2005). Reductions in populations of *E. coli* O157:H7, *Salmonella*, and *L. monocytogenes* were 0.44 log CFU/g, 0.35 log CFU/g, and 0.26 log CFU/g, respectively, after HDP treatment. Although these reductions are not large, these results may indicate that gram-negative bacterial cells may be more sensitive to HDP than gram-positive cells (see Chapter 5). The most prevalent genera identified from ground beef treated with HDP, and then stored for 14 days at 4°C, were gram-positive streptococci. The most prevalent bacteria in non-HDP treated ground beef stored under the same conditions were the gram-negative bacteria *Serratia* sp., *Pseudomonas* sp., *Hafnia* sp., and *Yersinia* sp., followed by streptococci (Williams-Campbell and Solomon, 2002). Gram-negative cells may have been inactivated or injured by HDP treatment and subsequent storage at 4°C. On sausages immersed in water, HDP significantly ($P < 0.05$) reduced titers of Feline Calicivirus (FCV), a surrogate for norovirus, by 2.70 log₁₀ TCID₅₀/mL, and Hepatitis A by log₁₀ by 1.10, respectively, when compared to nonpressure-treated controls (Sharma et al., 2008).

7. Combination of HDP with Antimicrobial Interventions (see also Chapter 28)

HDP has been most effective in killing bacterial food-borne pathogens when combined with other antimicrobials or thermal treatments. Populations of *Listeria monocytogenes* on inoculated beef frankfurters were reduced from 5.6 to 4.6 log CFU after HDP treatment (Patel et al., 2009). Populations of *L. monocytogenes* inoculated on hot dogs treated with a combination of the bacteriocin nisin and HDP were reduced from 5.6 to 3.9 CFU/g, whereas hot dogs solely treated with HDP were reduced to 4.6 log CFU/g. The addition of sodium lactate and sodium diacetate to hot dogs treated with HDP did not reduce counts of *L. monocytogenes* significantly more than controls (Patel et al., 2009). The combination of HDP and heat also reduced numbers of pathogenic bacteria in beef steaks. On blade-tenderized steaks which were treated with HDP and then cooked at

71.1°C, *E. coli* O157:H7 counts were significantly lower (<1.08 CFU/g) than populations on steaks which were only blade-tenderized (2.20 log CFU/g), and slightly lower than populations on steaks that received HDP treatments only (1.84 CFU/g) (Patel et al., 2005). Populations of *E. coli* O157:H7 on blade-tenderized steaks treated with HDP were reduced by 0.3 log CFU/g compared to populations on steaks that did not receive HDP treatments (Patel et al., 2005).

8. Shelf Life Extension of Meat Treated by HDP

HDP can extend the shelf life of ground beef and beef stew pieces. Total aerobic bacterial populations in two ground beef samples which were stored at 5°C for 20 hours and then treated with HDP were significantly lower, 3.15 and 5.49 log CFU/g, than those in ground beef that did not receive HDP treatment, 5.45 and 6.88 log CFU/g (Williams-Campbell and Solomon, 2002). Similarly, total aerobic counts on beef stew pieces stored under the same conditions as the ground beef and treated with HDP had significantly lower populations, 2.48 and 4.95 log CFU/g, compared to populations on untreated beef stew pieces, 4.30 and 6.29 log CFU/g. Ground beef treated with HDP and stored at 4°C had populations of spoilage bacteria of 3.72, 4.01, and 4.58 log CFU/g compared to populations of 5.22, 7.56, and 9.11 log CFU/g in untreated ground beef on days 0, 7, and 14, respectively (Williams-Campbell and Solomon, 2002). Boneless pork loin muscle treated with HDP did not contain significantly lower populations of aerobic bacteria or coliforms than untreated controls (Moeller et al., 1999). Extended refrigerated transport of pork loins may have affected bacterial counts in this study. It is unclear why HDP extends the shelf life of ground beef but had a lesser effect in studies conducted on food-borne pathogens. Differences between inactivation of total aerobic populations and pathogen populations may be due to the internalization of bacteria during HDP treatment. Cells of *E. coli* labeled with green fluorescent protein (GFP) penetrated to a depth of up to 300 µm from the exterior of a treated beefsteak, while cells on the untreated

controls penetrated to a depth of up to 50 µm (Lorca et al., 2002). It is thought that pathogenic cells that penetrate up to 300 µm do not pose a health risk to consumers prior to cooking, because normal cooking temperatures (55–82°C) are thought to kill cells penetrating at this depth.

9. Future Research Opportunities to Improve HDP

Although the effectiveness of the HDP process has been demonstrated in beef, pork, lamb, and poultry, commercialization has been hampered by four technical problems: safety, throughput, packaging, and performance. Aside from the obvious safety concerns when using explosives, the use of explosives necessitates a type of batch processing system that is incompatible with the high throughput of most modern meat processing plants. Meat has to be vacuum packaged to remove all the occluded and headspace gas. This is necessary to prevent rupturing or tearing of the package during the HDP process. The vacuum packaging material for the HDP process must be robust enough to withstand the brisance or shattering power of the explosive. Before these issues can be resolved to develop a successful commercialized system, further understanding of how to maximize the shock wave properties and the effects of the shock waves on the muscle system must be determined.

As previous experiments have shown, HDP can be an effective process in increasing tenderness of cuts of meat and may have potential to serve as a food safety intervention. However, working with explosives in a food processing setting presents issues with worker safety and implementation on a large processing scale. Nonexplosive methods for generating hydrodynamic pressure may be required for use of HDP in food processing.

Electrohydraulic shock wave (ESW) treatment has also been effective in reducing populations of bacterial suspensions. The extracorporeal shock wave lithotripsy used in renal infections has led to generating underwater shock waves using a high voltage discharge (Figure 8.1) (Loske et al., 2002). Unlike HDP, ESW treatments can generate multiple shock waves while simultaneously emitting

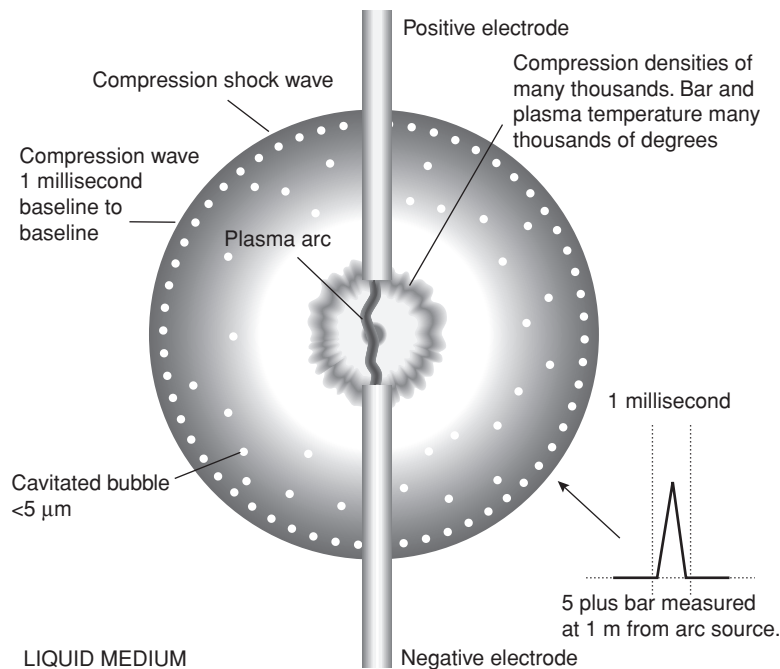


Figure 8.1. Illustration of underwater high voltage discharge for generating shock waves.

ultraviolet (UV) light, unlike HDP (see Chapter 26, Electrolyzed water). The use of UV light, coupled with the application of repeated shock waves, may enhance the bactericidal effect of ESW over HDP. A high voltage discharge (15–25 kV) across a pair of electrodes immersed in water creates a high-pressure plasma bubble that expands supersonically against the surrounding fluid, producing a spherically radiated shock wave. This shock wave is reflected off an ellipsoidal reflector. Since the electrodes are located at the focus point closest to the reflector, shock waves are concentrated at the second focus. Pressure amplitude and shock wave energy depend on the discharge voltage. Further, several hundred shock waves can be delivered in few minutes. This is not possible using explosives, as it requires additional time to replace the explosive after each shock wave generation. The underwater high voltage discharge used to generate shock waves in electrohydraulic generators has a continuum in the ultraviolet (UV) spectrum between approximately 55 and 150 nm. This radiation, along with acoustic cavi-

tion, may contribute to microbial death. The number of shock waves also influences its bactericidal effect. Populations of *E. coli* in exponential growth phase cells were reduced by 4.7 log CFU/mL, while those in stationary phase were reduced by 4.1 log CFU/mL when treated with 350 shock waves (Loske et al., 2002) of ESW. However, treatments of exponential growth and stationary phase *E. coli* cells treated with 150 shock waves did not result in significantly lower populations when compared to cells not receiving ESW treatments (Loske et al., 2002). Treatments of 350 shock waves killed more cells of *L. monocytogenes* (3.2 log CFU/mL) than of *S. Typhimurium* (1.7 log CFU/mL) and *E. coli* O157:H7 (0.56 log CFU/mL), indicating ESW treatment may be more effective against gram-positive food-borne pathogens than gram-negative ones (Alvarez et al., 2004). Currently, there are no reported studies evaluating the effectiveness of ESW treatments on meats.

The pulsed shock wave (PSW) is a patented food preservation technology based on powerful electrical discharge in liquids (Zuckerman et al., 2001). PSW

is generated due to the formation of extremely high pressures in the discharge plasma channel (see also Chapter 20 plasma). Fast plasma expansion due to large plasma density and temperature gradients, together with non-elastic properties of the liquid, lead to the formation of a pulsed shock wave. By changing parameters of the electrical system one can control the parameters of generated PSW and ultimately its bactericidal effect (Zuckerman et al., 2002). Pressure fronts between 80 and 100 MPa have been recorded in the sub-microsecond period when the wave is generated (Zuckerman et al., 2002). Treatment with four pulses reduced populations of *Lactococcus* and *Lactobacillus* sp. by 3 log CFU/mL, which was less than the 5 log CFU/mL reduction observed in non-pathogenic *E. coli* and *Staphylococcus aureus*. PSW treatments were least effective against the yeast *Saccharomyces cerevisiae*. Populations of *S. cerevisiae* were only reduced by 2 log CFU/mL after four pulses (Zuckerman et al., 2002). PSW treatment decreased the activity of alkaline phosphatase, an enzyme that is used as an indicator of dairy product microbial quality and safety (Zuckerman et al., 2004).

Hydrodynamic pressure can be generated through a “water hammer” process resulting from the sudden acceleration or deceleration of a column of liquid. A high pressure air gun can be used to fire a piston into a pressure vessel containing liquid, and vacuum packaged meat resting at the bottom of the vessel. As the piston impacts the liquid, a shock wave is generated with pressure fronts similar to those generated through HDP. Electrically based pulsed plasma technology also provides an alternative method to generate shock waves. This method is similar to technologies used to disrupt kidney stones in human patients. Multiple shock waves can be applied at a rate of one cycle per second and can generate pressure fronts ranging from 40 to 100 MPa (5,800–14,500 psi). These shock waves are focused on one location. This technology has been shown to reduce shear force values of beef loins by 18–24%, postrigor chicken by 22%, and turkey by 12% (Claus et al., 2001; Claus et al., 2002). These pressure-generating technologies represent opportunities to apply hydrodynamic pressure technologies to improve the quality and safety of meat products.

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Chapter 9

Physicochemical Effects of High-Intensity Ultrasonication on Food Proteins and Carbohydrates

Jochen Weiss, Ibrahim Gulseren, and Gunnar Kjartansson

1. Introduction

Application of high-frequency sound waves to liquids at sufficiently high intensities leads to mechanical and chemical effects that may alter the structure and composition of the material (Suslick and Price, 1999). The discovery of the chemical effects of high-intensity ultrasound dates back to 1927 when Richards and Loomis published their first paper on sonochemistry entitled “The chemical effects of high frequency sound waves” (Richards and Loomis, 1927). They described, for the first time, that application of “power” or “high-intensity” ultrasound could accelerate the rate of a number of conventional chemical reactions or could induce redox processes similar to reactions induced by ionizing radiation (Heusinger, 1990b). However, the origin of these effects remained unclear until 1935. The spontaneous emission of light from water was observed when it was exposed to intense ultrasound. This light was attributed to the collapse of the ultrasonic-induced cavities (Mason and Lorimer, 2002; Mason and Peters, 2002). Brohult, in 1937, using a higher power ultrasonic energy, described a third type of sonochemical reactions. These reactions appeared to lead to the degradation of synthetic or biological polymers (Brohult, 1937). Since then, the field of sonochemistry and power ultrasound has steadily moved for-

ward. It has been driven by the development of better analytical techniques that allow accurate recording of the spatially and temporally distributed physical and chemical effects that are the results of the application of high-intensity ultrasound (Bernstein et al., 1996; Kanthale et al., 2003). Development of a new generation of ultrasonic transducers has allowed an increase in the volume-specific energies making the development of scaled-up reactor systems feasible (Povey and Mason, 1998).

2. The Physics of High-Intensity Ultrasound

Ultrasound can be divided into two categories: (1) high frequency, low energy, diagnostic ultrasound in the mega hertz (MHz) range; and (2) low frequency, high energy power ultrasound in the kilo Hertz (kHz) range. Application of ultrasound generates a number of forces that can act on molecules and particles dispersed in the solvent. These forces are radiative in nature and include acoustic streaming (or steady ultrasonic) forces; the average Stokes force; the Oseen pressure; and the Bernoulli attraction forces. These radiative forces occur because of the propagation of acoustic pressure waves through the medium. The wave energy is observed by the solvent, dispersed molecules, and by particles (Mason, 1999; Mason and Lorimer, 2002; Mason and Peters, 2002). The energy density of the ultrasonic field is less than $\sim 10^{-9}$ J per atom. This is not high enough to break chemical bonds. The chemical and most of the mechanical

effects are the results of an indirect phenomenon known as cavitation. Cavitation is the generation of bubbles in the solvent as the radiative wave energy propagates (Šponer, 1991; Crum, 1995a; Leighton, 1995). The bubbles either pulsate rapidly or expand to a critical size and collapse violently. Rapidly pulsating bubbles are referred to as stable cavitation. The violently collapsing bubbles are known as transient cavitation. During pulsating or collapsing cavitation, shrinking or collapsing bubbles focus the energy of the sound field to amplify it by more than 11 orders of magnitude. This level of energy is sufficient not only to break chemical bonds but also to induce luminescence (Bernstein et al., 1996).

When ultrasound propagates through a liquid medium, the medium is compressed and sheared (Price et al., 1995; Price and Clifton, 1999). At low intensities, the pressure and shear waves are not large enough to cause significant changes in the fundamental physical properties of the medium that govern the propagation. Thus, the waves continue to propagate sinusoidally. However, the propagation of compression and shear waves at large intensities can result in the deformation of the wave as it travels from the transducer through the medium (Figure 9.1). This deformation can be explained in terms of the velocity of an ultrasonic wave, which depends on the elas-

tic modulus and the density of the medium (Mason, 1999):

$$v_{\text{ult}} \propto \sqrt{\frac{\text{Elastic modulus}}{\text{Density}}} \quad (9.1)$$

The transmission of a high-intensity ultrasonic wave causes a change in the local pressure. This change in pressure can change the elastic modulus and density of materials (see Chapter 1). The increase in elastic modulus is generally larger than the increase in density. This causes the velocity of the wave at the pressure maxima to be greater than at the pressure minima. The peaks of the initially sinusoidal ultrasonic wave will, therefore, tend to “catch up” with the troughs causing waveform distortion. The resulting wave now has a saw-tooth-like appearance associated with an abrupt decrease in pressure. Bubbles are generated if this pressure change is large enough and is above the so-called “cavitation threshold” (Leighton, 1995). Formation occurs under acoustic pressures that are significantly lower than those theoretically required to initiate homogeneous nucleation. This suggests that a heterogeneous nucleation process is involved. Nuclei must be present in the liquid to aid the creation of the cavities. This phenomenon is important in food processing. It indicates that the composition of the system, as measured by the presence of suspended particles, dissolved ions, surface-active agents, and other compounds, will have an important influence on the generation of cavities. The rate of formation and equilibrium concentration of cavities are the source of the mechanical and chemical effects that are utilized during the food process operation (Povey, 1998; Povey and Mason, 1998).

The formation and collapse of bubbles at 20 kHz occurs over extremely short periods of time. The bubbles grow and collapse in a few microseconds (Hardcastle et al., 2000). A number of theoretical models have been proposed to describe the observed cavitation events under the influence of high-intensity ultrasound. For example, Blake analyzed the condition of a small bubble in a solution when the pressure in the liquid suddenly increases or decreases (Blake et al., 1997; Blake et al., 1999). The

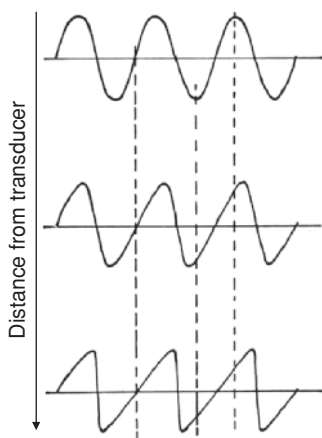


Figure 9.1. Distortion of initially sinusoidal high amplitude ultrasonic waves as they propagate through an elastic medium away from the ultrasonic transducer.

analysis revealed that a critical bubble size R_{crit} must exist at which a pressure balance across the phase boundary can no longer be maintained and explosive growth must follow. He calculated this critical size as:

$$R_{\text{crit}} = \left(\frac{3R_0^3}{2\sigma} \left(p_0 + \frac{2\sigma}{R_0} - p_v \right) \right)^{\frac{1}{2}} \quad (9.2)$$

where R_0 is the size of the bubble nuclei, p_0 is the hydrostatic pressure, p_v is the vapor pressure of the liquid and σ the surface tension. Explosive growth can be triggered if $p_0 \geq p_v - 4\sigma/3R_{\text{crit}}$. Minnaert (1933) in a different approach tried to establish a relationship between the bubble radius and the applied frequency of the applied sound wave (Suslick et al., 1987; Suslick and Price, 1999):

$$f_0 = \frac{1}{2\pi R_0} \sqrt{\frac{3\gamma p_0}{\rho}} \quad (9.3)$$

where R_0 is the resonant cavity size, f_0 the wave frequency, γ the specific heat ratio of the gas, p_0 the total external pressure, and ρ the density of the media. Equation (9.3) explains why high-intensity ultrasound equipment usually operates at low frequencies such as 20 kHz since cavitation bubbles grow larger as the frequency of the ultrasonic wave decreases. The collapse of a larger bubble can be expected to be more violent and thus can generate highly turbulent flow conditions and extremely high, localized temperatures and pressures (Povey, 1998; Povey and Mason, 1998). A widely accepted theory known as the hot-spot theory predicts local temperatures to be approximately 2,000–5,000 K and pressures to range from 10 to 100 MPa. The microsecond collapse of the cavities is so rapid, that the heat released cannot be conducted away by these high temperatures to the solvent phase. Calculated cooling rates within the bubble, using heat transfer theory, are in the order of 10^{10} K s^{-1} . Thus, adiabatic conditions within the bubble can be assumed and the temperature inside the bubble at the end of the collapse can thus be estimated as:

$$T_{\text{max}} = T_0 \left(\frac{V_0}{V_{\text{max}}} \right)^{\frac{c_p}{c_v} - 1} \quad (9.4)$$

where c_p/c_v is the polytropic index K of the cavity medium. This index may range from 1.6 to and 1.7 depending on the type of gas dissolved. For example, the polytropic index is 1.66 for Argon and 1.63 for Helium (Hoffmann et al., 1996). Considering the frequency dependence of the bubble size in Equation (9.3), the temperature should therefore decrease as the frequency increases since the size of the bubble decreases. In reality though, thermal conductivity effects begin to play a role for smaller bubbles and the collapse is no longer adiabatic at higher frequencies. Thus, collapse temperatures reach a maximum at approximately 300 kHz. This indicates that the chemical effects of high-intensity ultrasound are closely related to the conditions inside the bubble. From a food processors' point of view, if the goal is to predominantly utilize the chemical effects of high-intensity ultrasound, higher frequency transducers are preferable.

It is important to note that the collapse of bubbles may not happen uniformly. For example, in the vicinity of solid surfaces a bubble can collapse nonspherically and the liquid rushing into the cavity can generate a liquid jet with speeds as high as 110 ms^{-1} (Mason, 1992). Bubbles are not likely to collapse individually. Instead, multiple bubbles can collapse close to each other and exert very high shear forces. For example, for 5- μm bubbles generated at 1 MHz, a shear stress of 500 Nm^{-2} has been reported (Hoffmann et al., 1996). For larger bubbles at lower frequencies, these stresses can be substantially higher.

3. General Physicochemical Effects of Cavitation

The collapsing cavities can be viewed as tiny microreactors in which complex hot-gas-phase reactions occur (Luche, 1998). For example, the solvent or other volatile compounds that are present within the gas phase of the cavity can be thermolytically cleaved to yield radical species that can readily react with each other. A simplified reaction scheme assuming only water vapor in the gas phase is shown in Figure 9.2. The reaction not only proceeds within the gas phase but molecules in the liquid shell in the

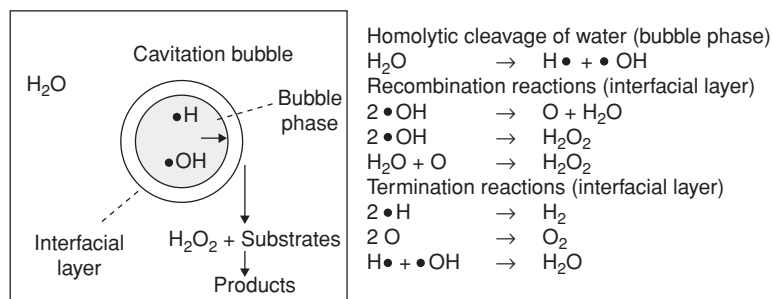


Figure 9.2. Simplified reaction scheme illustrating the chemical processes that may occur inside cavitational bubbles, in the interfacial layer, and in the bulk layer with water as a solvent.

immediate vicinity of the bubble are affected as well. The energies may be high enough to cause sonolysis of nonvolatile compounds with active radicals being expelled into the solvent phase. Follow-up reactions then proceed in the solvent phase due to the presence of these newly generated, highly reactive species. For example, radicals may react with each other to form such prominent reactants as hydrogen peroxide. Alternatively, the radicals can react with any other dissolved reactants. Simultaneously, intense mixing of gas and liquid occurs, especially if liquid jets are generated which can accelerate heat and mass transfer and fluid flow. Researchers have also reported that reactions that involve single electron transfer are accelerated by ultrasound (Hardcastle et al., 2000). Research is still ongoing to find an explanation for this phenomenon. An entire field of research, sono-electrochemistry, has emerged to investigate the effect of ultrasound on electrochemical reactions.

The mechanical effects associated with the bubble collapse depend on the nature of the system in which the cavitation occurs (Raso et al., 1999). For example, in a homogeneous solution (solutions or simple molecular dispersions), degradation of large molecules, such as polymer chains, may be observed (Kruus et al., 1988; Price, 1990a). The solvation layer around neutral and charged molecules is disturbed. This may lead to premature precipitation of molecules from solution (Cains et al., 1998). If dissolved gases are present, bubble collapse may cause the degassing of the solvent. In heterogeneous systems in which multiphase boundaries are present, solid surfaces may be subjected to microstreaming.

This allows the removal of surface layers and can improve penetration of reactants (Price and Clifton, 1999; Suslick and Price, 1999). An example is the ultrasonic cleaning of metal parts. For dispersed particles, the particles may actually be broken up into smaller fragments resulting in a particle size reduction. If the particles are liquid such as in an emulsion premix, a fine emulsion may be produced (Behrend et al., 2000). However, the highly turbulent conditions, due to the collapse of the cavities, may also lead to collisions of the particles and can lead to coalescence (De Sarabia et al., 2000).

It should be mentioned that in biological systems, at lower ultrasonic intensities, improvements in microbiological reactions and environmental remediation have been reported. While at high ultrasonic intensities, cells may be disrupted and the cell content may be released into the solvent phase. This may lead to the inactivation of bacteria, mold, and yeasts (Povey and Mason, 1998).

4. High-Intensity Ultrasound Processing Parameters

For food processors, it is important to understand the parameters that influence the application of ultrasound to achieve a desired process. A fairly large number of physicochemical properties can affect the process. A brief review will be presented of the key process and environmental parameters that are of importance to ultrasonic processing (Hunnicke, 1990; Raso et al., 1999; Mason and Peters, 2002; Gogate et al., 2003).

4.1. *Intensity*

The intensity of the ultrasonic wave is one of the key processing parameters that influence the sonochemical effects of high-intensity ultrasound. Minimum intensity is required to induce cavitation. This minimum is dependent on the frequency and the physicochemical properties of the medium that is treated. As the intensity increases, larger numbers of cavitation bubbles are generated, thus increasing the observed sonochemical effects. However, if the number of cavities is very high, the bubbles coalesce to form larger, longer living bubbles. Eventually, this effect will dampen the amount of energy that can be transferred into the system. However, if the amplitude of the transducer is too high, the liquid is no longer able to maintain contact with the transducer surface. This effect is known as decoupling and it leads to an immediate decrease in transmitted energy. Very large amplitudes decrease operating lifetime of the transducer. Eventually, cracks and fractures may destroy the transducer.

4.2. *Frequency*

The frequency of the high-intensity ultrasound waves has a major influence on size of the cavities as has been discussed. Because the time available for expansion and collapse of bubbles shortens with increasing frequencies, it becomes more difficult to maintain the extent of the cavitation at higher frequencies. At frequencies in the megahertz region, cavitation completely ceases because a finite amount of time is required for the molecules to physically separate and form the cavity. Production of transducers with high-power output at high frequencies has proven to be difficult. Most power ultrasound transducers, available for commercial application, are in the range of 20–100 kHz.

4.3. *Presence of Gases*

The presence of dissolved or occluded gases has a positive effect on the efficiency of ultrasound due to improved generation and collapse of cavities. The introduction of gas cavities (bubbles) into a system

increases the number of nucleation sites. This results in a more uniform energy distribution throughout the system. Monoatomic gases with high specific heat capacities, such as argon, have yielded improved cavitation effects (Mason, 1992). The energy released upon collapse of the bubbles is greatest for gases with the largest ratio of specific heats (see Equation (9.4)) such as helium, neon, and argon. In contrast, carbon dioxide yields very low cavitation energies.

4.4. *Temperature*

Mason and coauthors investigated the effect of temperature on the efficiency of particle size reduction using power ultrasonication (Mason, 1992). They observed a decrease in the delivered intensity of ultrasonic power to the solution from 79 to 23 Wcm⁻² as the temperature increased from 0 to 90°C (decoupling). This inverse relation between temperature and ultrasonic power has been explained by the increase in vapor pressure of the solvent resulting in a delayed time of collapse of gas bubbles and decoupling. Simultaneously, both viscosity and surface tension, properties that influence generation of cavities, decrease with increasing temperature. As the solvent reaches the boiling point, vapor bubbles interfere with the cavitation bubbles effectively dampening all sonochemical effects. In some processes though, the increase in temperature can lead to synergistic effects possibly due to temperature-induced structural changes that may increase the susceptibility of the system to ultrasound.

4.5. *Solvent Properties*

Vapor pressure, viscosity, and surface tension of the solvent have an important influence on the formation and collapse of bubbles. The cavitation bubbles are filled with vapor generated from the surrounding liquid. More volatile solvents will enhance cavitation at lower ultrasonic intensities while solvents with low vapor pressures make it difficult to induce cavitation. However, if the solvent is too volatile and the bubble is filled vaporized solvent molecules, then the collapse will be cushioned, thus reducing the intensity of the sonication. Because solvent molecules have

to be separated and interfaces need to be formed, a lower surface tension typically enhances cavitation. The addition of surfactants can enhance the sonochemical effects of ultrasonication. Solvent viscosity ideally should be low to provide the least possible resistance to generation and collapse of the bubbles. It becomes increasingly difficult to induce cavitation as solvent viscosity increases.

4.6. Pressure

Application of an external pressure during sonication, so-called manosonication, will influence the extent of the cavitation. The intensity of the collapse of the bubbles increases with increasing external pressure and thus greater sonochemical effects have been observed with manosonication.

5. Ultrasonic Power in Processing Applications

High-intensity ultrasonic transducers are typically built to resonate a single fixed frequency. Generally, the frequency of low-intensity ultrasound equipment can be readily adjusted (Hunnicke, 1990; Gogate et al., 2003). For scale-up purposes, it is critical to understand the energy distribution of the ultrasonic waves that are radiated to the material being treated. This quantity is not readily available since the ultrasonic generator may only specify the maximum amount of power input (e.g., 600 W) delivered to the transducer. Processors should consider measuring the energy actually transmitted into the system using available dosimetry on the basis of physical or chemical methods (Mason and Lorimer, 2002; Mason and Peters, 2002). Unfortunately, to date, no consensus exists on how to standardize the reported ultrasonic energy input in published studies. The sensitivity of methods varies and researchers working close to the cavitation threshold may prefer to use chemical dosimeter methods rather than calorimetric or power consumption methods (see below). Food processors must pay special attention to the method of dosimetry used in order to interpret the reported power input and to compare results with other studies.

One of the difficulties associated with quantifying an ultrasonic process is the fact that the ultrasonic energies are spatially distributed within the treatment vessel (Gogate et al., 2003). This is one of the biggest problems when it comes to scale-up of high-intensity ultrasonic processes. The ultrasonic energy decreases with increasing distance from the transducer because the ultrasonic wave gradually loses energy through absorption and scattering. Figure 9.3 shows a spatial map of the energy densities in the vicinity of an ultrasonic probe (Sáez et al., 2005). The active reaction zone exists close to the transducer where cavitation events are maximal. Results of an ultrasonic process are always a function of the residence time distribution within this active reaction zone. Rapid mixing is not only a by-product of the sonication process, but in fact a requirement to ensure that molecules in solution are uniformly treated. It is difficult to achieve a uniform process outcome in highly viscous samples or in reactor vessels with dead zones.

5.1. Direct Determination of the Amplitude of the Ultrasonic Wave

Observation of the mechanical motion of the transducer probe may yield an estimate of the ultrasonic power delivered to a solution as long as the probe is directly coupled with the surrounding liquid. Transducer vibration amplitudes are in the range of ten microns and can be directly observed with a metallurgical microscopy. This is achieved by marking a spot on the surface of the transducer and focusing the microscope onto the marked point while the transducer is turned off.

When the power is turned on, the marked point will be transformed into a line, with the length of the line being proportional to the amplitude. Alternatively, the acoustic pressure can be detected using acoustic balances as direct acoustical dosimetry. Unfortunately, very precise, acoustical balances only yield a mean acoustic pressure and are difficult to set up outside of the laboratory. Hydrophones, another direct measure of acoustic pressure, are capable of resolving spatial differences in the acoustic pressure. Thus, they allow the mapping of the distribution of the ultrasonic energies within a reactor. Hydrophones

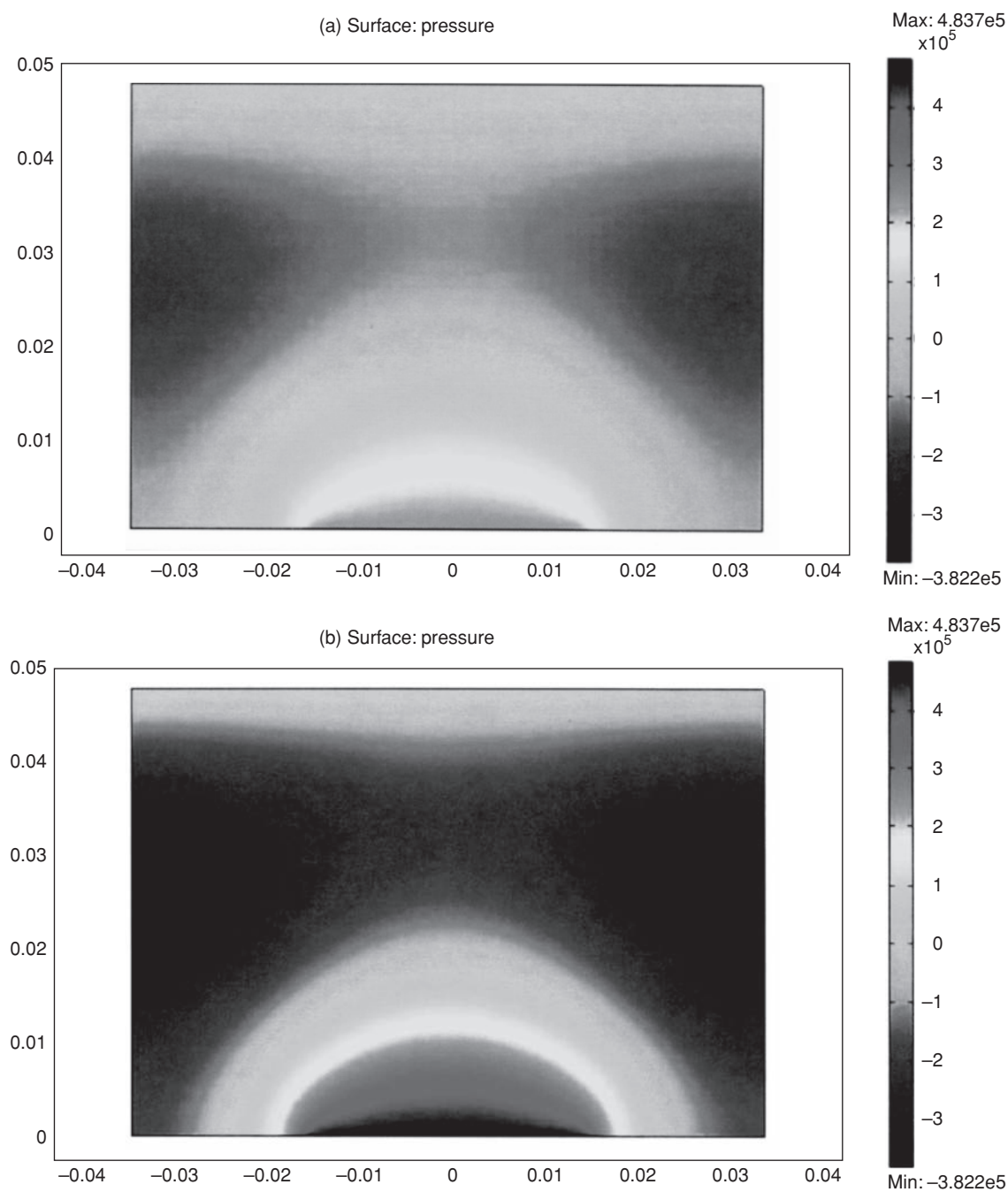


Figure 9.3. Distribution of energy densities as a function of the distance from the ultrasonic transducer (Sáez et al., 2005). (For color detail, see color plate section.)

are not resistant to many organic or corrosive solvents and are thus not suitable for use in many food process operations. A third method to measure the energy transmitted into the system is direct measurement of the electrical power that is consumed. The base power consumption of the transducer is taken while running in air without a load. The base power consumption is then deducted from the power consumption of the sonicator in the sample to obtain the actual net ultrasonic power input.

5.2. Calorimetric Determination of the Ultrasonic Power

One of the most common methods used to determine the ultrasonic energy absorbed by a solution during treatment is by calorimetry. The heat produced by the transducer is measured when the system is operating under adiabatic conditions, where no heat is lost to the environment. In practice, it may be difficult to completely insulate the reactor vessel. In this case, the heat loss has to be taken into consideration when calculating the ultrasonic intensity.

Bobber showed the ultrasonic intensity can be determined by measuring the time-dependent increase in temperature of a sample irradiated in a reactor vessel (Bober, 1998). The heat loss of the reactor after shutdown of the ultrasonic transducer is shown in Figure 9.4. The ultrasonic intensity I can be calculated according to Equation (9.4) from, the slope of the initial rise in temperature dT/dt_a ; the slope of heat loss after the shutdown of the sonicator dT/dt_b ; the sample mass m , the heat capacity of the solvent c_p ; and the radius of the ultrasonic probe r

$$I = \frac{mc_p}{\pi r^2} \left(\left(\frac{dT}{dt} \right)_a - \left(\frac{dT}{dt} \right)_b \right) \quad (9.5)$$

This method has the advantage of only using temperature measurements. Very little information about the physicochemical properties of the system are required. Since the method accounts for the total mass of the irradiated system, it represents a specific quantity (W/m^2) that makes comparison between ultrasonic processes using different ultrasonic probes

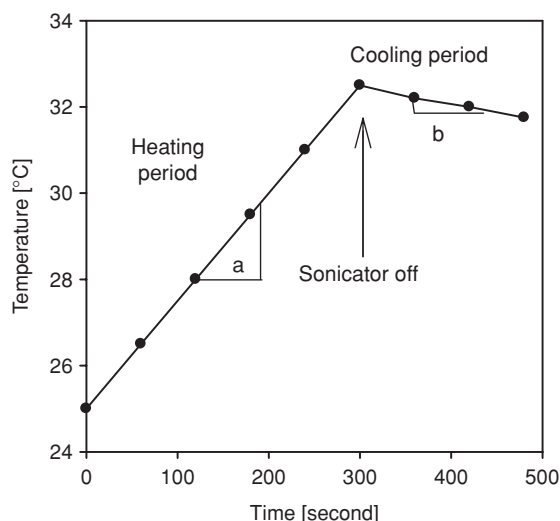


Figure 9.4. Principle of calorimetric determination of ultrasonic intensity. Gains and losses in heat can be determined from the slopes of the temperature increase while the sonicator is turned on and the slope of the temperature decrease after the sonicator has been turned off.

feasible. Once the system is calibrated, a number of products can be evaluated for energy absorption.

5.3. Indirect Measurements of Ultrasonic Energies Using Chemical Probes

A large number of chemical dosimeters exists that are based on the production of a specific chemical species in proportion to the ultrasonic energy absorbed (Heusinger, 1990b; Mason and Cordmas, 1996; Povey and Mason, 1998). Since not all chemical events inside the cavitation bubble give rise to the production of chemical products in the bulk solution, chemical dosimetry may not reflect the total energy entering the system. Chemical dosimeters may be more sensitive than, for example, the calorimetric measurements and may be better indicators of the sonochemical effects of ultrasonic irradiation. Iodine dosimetry is an accepted method for absorbed chemical energy measurement in aqueous solution. It is based on the rapid oxidation of iodide ions by sonic-energy-produced hydrogen peroxide to produce molecular iodine. A spectrophotometric

analysis of the sonicated solution containing potassium iodine is used to calculate the amount of hydrogen peroxide produced. Concentrations of generated H_2O_2 are typically low and the equipment has to be completely free of interfering reagents that could cross-react with H_2O_2 . Another method is the terephthalate dosimeter. Unlike the iodine dosimetry method, it is very sensitive and often used to detect the cavitation threshold. Terephthalic acid that is dissolved under alkaline conditions produces strongly fluorescent radicals under the influence of ultrasonication. The concentration of the fluorescing radicals increases linearly with increasing sonication time at constant ultrasonic energy input conditions. Moreover, for specific treatment times, the concentration of radicals linearly reflects the power input at different frequencies. The Fricke dosimeter can be used to measure absorbed sonic energy. It is based on the oxidation of an air saturated solution of Fe^{2+} to Fe^{3+} . The reaction is observed at 304 nm. In practice, a solution of ferrous ammonium sulfate reacts with hydroxyl radicals produced as a result of the ultrasonic irradiation as long as oxygen is present.

6. Effect of High-Intensity Ultrasound on Functionality of Proteins

In addition to being an essential part of the human diet, proteins are functional food components that can stabilize foams and emulsions, form gels, and can exercise biological activity *in vivo* and *in vitro*. They are a key part of the toolbox of components used by food scientists to create foods with desirable appearance, texture, flavor, and aroma. The many functions carried out by proteins are a manifestation of their structure. For example, the surface activity of proteins, which allows them to be excellent emulsifiers, is derived from the amphiphilicity of proteins. The specific structures of proteins also give rise to the high biological specificity that is characteristic of enzyme activity. Since high-intensity ultrasound treatment can lead to a modification of the structures of macromolecules, changes in protein functionality can be expected after application of high-intensity ultrasound. Recent literature on the power ultrasound treatment of proteins in solution has documented

protein functionality with respect to: emulsion and foam stabilization; gel formation; and enzyme activity. Depending on the experimental conditions (solvent, temperature, pressure, ionic strength, etc.) and the type of proteins treated, changes could be beneficial or could reduce their functional effectiveness. Power ultrasound is increasingly used to improve the efficiency of the extraction of proteins from plant and animal raw materials. However, the extracted proteins may undergo physicochemical changes during the ultrasound treatment. A comprehensive understanding of the specific effects of high-intensity ultrasound on structure–function relationships of proteins is thus required to use the technology in the most beneficial and efficient manner. For example, the extent of recovery of an enzyme by ultrasonic disruption of microbial cells can be determined by measurements of enzymatic activity after recovery. This must be compared with the initial activity of the enzymes in the cells to be extracted.

Published investigations on the effect of high-intensity ultrasound on proteins report a wide range of applied ultrasonic intensities. This is because transducer capacities range anywhere from 50 to 2,000 W. A clear delineation of the volume-specific ultrasonic energy to which the samples were subjected is critical because physical effects of high-intensity ultrasound often do not scale linearly with intensity. Findings with respect to the effect of high-intensity ultrasound on enzyme activity may range from complete inactivation enzyme activity to substantial increases in enzyme activity. Sonication may be combined with heat and pressure treatments, to modify the fundamental physical effects of high-intensity ultrasound (e.g., number and extent of cavitation events).

7. Modification of Surface Activity by High-Intensity Ultrasound

A number of studies have reported that the ultrasonic treatment of purified protein solutions, for a wide variety of experimental conditions, may increase the surface activity of proteins at the solution–air interface (Weiss and Seshadri, 2001; Güzey, 2002; Guzey et al., 2004; Gülseren et al., 2007; Güzey

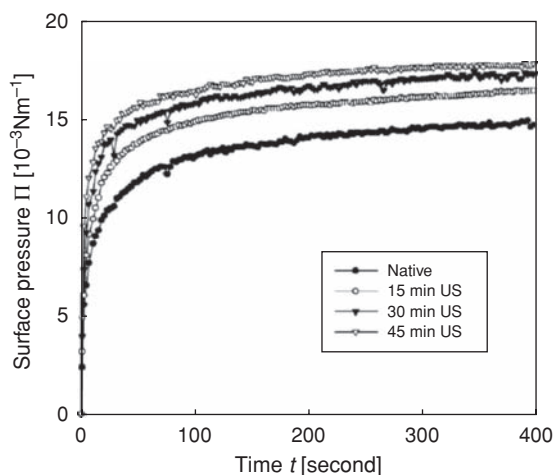


Figure 9.5. Change in surface pressure as a function of time for aqueous native and sonicated (15, 30, and 45 minutes) BSA solutions at a concentration of 3×10^{-6} M. Solutions were prepared in $0.1 \times$ PBS buffer. Sonication intensity was 20 W cm^{-2} (Güzey et al., 2006).

et al., 2006) (Figure 9.5). Longer ultrasonic processing times were found to increase the surface activity of bovine serum albumin (BSA) solutions, whereas the operational mode (i.e., pulsed or continuous sonication) did not have a significant effect. While thermal processing is known to enhance surface activity, thermosonication increased the surface activity beyond that of a comparable thermal treatment. This fact suggests the formation of a nonnative, molten-globular state that is profoundly different from a thermally denatured state. The investigations suggested an increased mobility and a slight increase in the amount of α -helix content (Güzey et al., 2006). This is contrary to the loss of structure that is generally reported by heat unfolding. Sonication treatments increased both short- and long-term surface activities, equilibrium surface pressures, and diffusion coefficients of BSA (Güzey et al., 2006).

The enhancement of surface activity by sonication was found to be independent of solution pH (Figure 9.6). The highest surface activity was observed around the isoelectric point, pH 5, for both native and sonicated BSA samples (Gülseren, 2004). This is in agreement with previously published studies

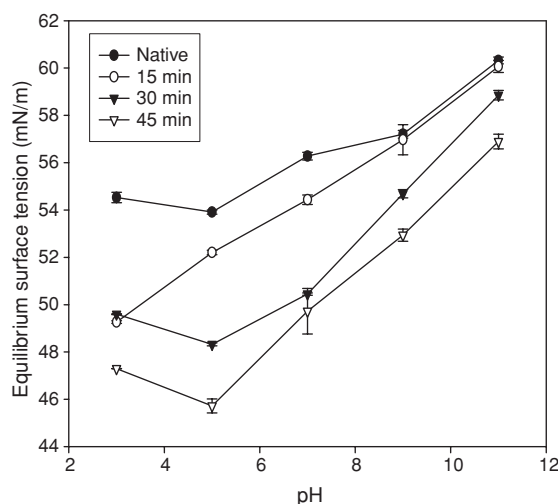


Figure 9.6. Equilibrium surface pressure of native and 15, 30, and 45 minutes sonicated (20 W cm^{-2}) BSA solutions as a function of solution pH. All solutions were prepared at a BSA concentration of 1.10^{-6} M in $0.1 \times$ PBS buffer (Gülseren, 2004).

where the surface excess concentration of BSA at the air–water interface (Lu and Su, 1999); the initial adsorption rate at a solid–solution interface (Elgersma et al., 1992); and foaming activity (i.e., surface coverage at the air–water interface) (Yu and Damodaran, 1991) were shown to be maximal around the isoelectric point of the proteins under investigation. For short-term adsorption kinetics, samples adjusted to pH 3, adsorbed to the interface; reduced the surface tension very rapidly; and reached equilibrium shortly thereafter. Basic solutions had slightly higher diffusion coefficients calculated from long-term adsorption kinetics, but were accompanied by higher equilibrium surface tension values (Gülseren, 2004).

8. Structural Investigations of the “Ultrasonically Induced” State of Proteins

A series of structural investigations on BSA was carried out by Gülseren et al., 2007, in an effort to elucidate the possible nature of an ultrasonically

induced state. The thermal unfolding temperature of BSA was not affected by the application of high-intensity ultrasound. However, a slight reduction in thermal denaturation enthalpy was observed. Similar to thermosonication, this finding showed that a high-intensity ultrasound-induced state appears to be distinctly different to a thermally unfolded state. Minimal changes were observed in the global structure of BSA, but surface charge increased particularly at basic pH values (e.g., $\text{pH} > 9$). The surface charge of proteins increased on the either sides of the isoelectric point, but the extent of the increase in sonicated proteins was higher compared to the native protein value.

Dynamic light scattering measurements indicated that the particle size of BSA increased up to 3.4 times after 90 minutes of sonication. No significant change in the oligomeric state of BSA was observed when analyzed by blue native PAGE analysis. Similarly, the amount of free sulfhydryl groups in BSA decreased after sonication where the extent of the reduction was proportional to the sonication time. The increased particle size and decreased number of free sulfhydryl groups suggests that protein aggregates may have been formed.

Circular dichroism (CD) spectroscopy and FTIR analysis indicated changes in the secondary structure of BSA. In addition, surface hydrophobicity increased. The authors concluded that mechanical, thermal, and chemical effects of ultrasonication resulted in structural changes in BSA that altered the functional properties of the macromolecule due to formation of an ultrasonically induced state that differed from a thermally, mechanically, or solvent-induced state (Gülseren et al., 2007).

Independent of pH, the influence of sonication treatment was accompanied by subtle changes in the molecular structure of BSA, such as increases in surface charge and reduction in free sulfhydryl content. Isomerization states of BSA were proposed as the basis of the differences in surface activity of acidic and basic BSA solutions. BSA molecules tend to aggregate around the isoelectric point, whereas at acidic and basic pH values, a loosened structure and a lower free sulfhydryl content would be typical. Reduction of free sulfhydryl groups (i.e., dissociation)

at high pH values may decrease the surface activity of BSA (Gülseren, 2004).

It is important to note that the results obtained for BSA may not be typical for other proteins. Investigations carried out at the University of Tennessee, showed the effect of ultrasound on the surface activity and structure of a number of different proteins (Güzey, 2002). A pronounced difference in susceptibility of proteins was noted. For example, lysozyme, which is a compact rigid protein, was most sensitive protein to ultrasound followed by BSA, fatty acid free BSA, and β -lactoglobulin (Güzey, 2002) (Figure 9.7).

In addition to the structural modifications outlined above, other researchers have reported a number of other findings in terms of functional changes in proteins treated with ultrasound. In a comprehensive review on the sonication of meat; protein fragmentation; proteolytic degradation; tenderization of meat; and modified muscle microstructure were mentioned among the benefits of ultrasonication (Jayasooriya et al., 2004). A sonication-induced redox reaction

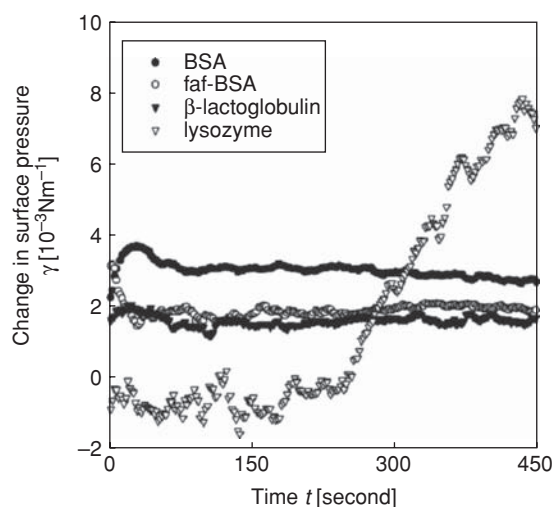


Figure 9.7. Comparison of change of surface pressure for four different proteins (3×10^{-4} M) upon sonication with 20 W cm^{-2} at 20°C for 45 minutes. Data were obtained by subtracting surface pressure values of ultrasound treated solutions from the surface pressure values of the corresponding native protein solution over an adsorption period of 450 seconds. (Güzey, 2002).

was observed for reduced horse heart cytochrome *c*. Interestingly, instead of losing secondary and tertiary structure as did myoglobin and hemoglobin, this protein was oxidized to ferric-cytochrome *c*. Its active site conformation was not affected (Barteri et al., 1996).

9. Influence of High-Intensity Ultrasonication on Gelation Behavior

In addition to the studies on protein structure and adsorption kinetics, the gelation behavior of ultrasonically pretreated whey protein isolates (WPI) was investigated (Weiss et al., 2000). Native or sonicated WPI solution and lactose were fermented using a standardized lactic acid bacteria culture mix. The strength of the acid-induced protein gel formed was found to depend primarily on the thermal pretreatment temperature that the proteins were subjected to prior to the fermentation. Gel strength increased with increasing extent of protein denaturation. However, an additional increase in gel strength of thermosonicated proteins was observed compared to thermally treated proteins. The gelation performance increased with increasing power level and sonication time (Weiss et al., 2000). In another study, the fermentation of manothermosonicated milk formed firmer yoghurt samples with high viscosity and consistency (Vercet et al., 2002a). Similar results were presented by Wu et al. (2001), but unlike Vercet et al., a shorter fermentation time was suggested for sonicated samples.

10. Ability of Ultrasonicated Proteins to Stabilize Emulsions

A great number of studies have been published on the ultrasonic preparation of protein and surfactant stabilized emulsions, including multiple-layered emulsions; o/w and w/o emulsions; and polymerized emulsions. For example, ultrasound reduced the particle diameters of primary and secondary emulsions and stabilized the system against creaming (Güzey, 2002). Production of proteinaceous microcapsules was facilitated by high-intensity ultrasound and the oxidative environment generated by sonication

(Suslick and Grinstaff, 1990). The high-intensity ultrasound treatment of milk, with or without heat, reduced the particle size of fat globules (Villamiel and De Jong, 2000). The production of emulsions by high-intensity ultrasound is well established and is increasingly being used in industry in through-flow, high-efficiency, emulsification systems.

Little is known as to how the pretreatment of proteins by high-intensity ultrasound alters their ability to stabilize emulsions produced by standard homogenization. The amount of information is relatively limited on structural changes of protein molecules in solution or adsorbed to the surface of emulsion droplets during sonication. The ability of native and sonicated WPI to stabilize a corn oil in water emulsion was investigated (Gülseren, 2004). The emulsification activity of WPI was assessed by determining the particle size of the emulsion during homogenization. Results showed significant changes in the particle-size distribution of emulsion droplets stabilized by sonicated WPI solutions compared to native WPI solutions. High-intensity ultrasound was not found to improve the emulsification ability of WPI (Gülseren, 2004). This finding was attributed to the fluid dynamic processes occurring during homogenization. The time-scale for the droplet surface disruption in a high-pressure homogenizer is within the range of a few milliseconds. Thus, the increase in short-term surface activity may not be high enough to cause any significant differences in the emulsification capacity. According to Krause et al. (2000), under certain circumstances, resistance of proteins to denaturation can be as important as surface hydrophobicity when used for emulsification.

11. Influence of High-Intensity Ultrasound on the Foam Stabilization of Proteins

Investigations on the effect of ultrasonic treatment of purified protein solutions have reported increased surface activity at the solution–air interface (Weiss and Seshadri, 2001; Güzey, 2002; Gülseren, 2004; Güzey et al., 2004; Gülseren et al., 2007; Güzey et al., 2006). The increased flexibility and surface activity might yield proteins that are better able to

stabilize foams. A research group in the United Kingdom demonstrated that foam stability and bubble uniformity increased and bubble diameter decreased in whey protein concentrate (WPC) foams upon sonication (Lim and Barigou, 2005a; Lim and Barigou, 2005b). This suggests that high-intensity ultrasound might be used to improve the performance and quality of food foams. This area is subject to intense investigations. The group also reported that an increase in protein concentration allowed for production of foams with a better texture, but that the increased strength of protein films at the interface required the use of higher ultrasonic wave amplitudes.

High-intensity ultrasound can produce and also destroy foams. Nonprotein materials such as polymeric liquids and surfactants in combination with high-intensity ultrasound were used to destabilize foams (Sandor and Stein, 1993; Morey et al., 1999; Lim and Barigou, 2005a). Foam controlled by sonic energy has a successful industrial application.

12. Effect of Ultrasonic Energy on Enzymatic Activity (also see Section 9.6)

There is a wide range of effects of high-intensity ultrasound on enzyme activity. Studies have reported increased enzyme activities while others have reported no effect or decreased or complete inactivation of enzymes. These differences can be attributed to the structure of the targeted enzymes; the environmental conditions; and the intensity of the ultrasonic waves. For example, at low ultrasonic energy levels ($0.27\text{--}0.85\text{ Wcm}^{-2}$), the activity of invertase increased with sonication intensity, regardless of pH, possibly due to substrate activation by ultrasonication (Sakakiabra et al., 1996). The release rate and activity of invertase from *Aspergillus niger* were shown to increase under ultrasonication using at moderate intensities (Vargas et al., 2004).

Manothermosonication of pectic materials decreased the activity of pectinmethylesterase (PME) whereas polygalacturonase (PG) remained fully active. Authors concluded the observed decreases in activity were not due to pressure but to thermal, chemical, and mechanical effects of the sonic en-

ergy cavitation. The ultrasonic treatment of tomato products was found to inactivate enzymes, and improve rheological properties (Vercet et al., 2002b). Similarly, β -subunits of tomato endopolygalacturonase were inactivated by manothermosonication. These subunits have been shown to be problematic in tomato processing because they may protect other degradative enzymes from thermal denaturation (Vercet et al., 2002a). Increasing treatment time and cavitation intensity increased the loss of pectinmethylesterase (PME) activity. A sonication temperature below the thermal inactivation temperature increased the enzyme inactivation rate of sonication. This was possibly due to decreased vapor pressure in the bubbles generated in the solvent. At higher thermostonication temperatures, the rate of enzyme inactivation increased significantly suggesting that ultrasound and heat exhibited synergistic effects (Raviyan et al., 2005).

Increasing ultrasonic energy levels decreased the lactose hydrolysis rate of β -galactosidase due to a decreased enzyme activity (Şener et al., 2006). Sonication applied to the fermentation of milk revealed that both β -galactosidase and lactic acid bacteria were vulnerable to high-intensity ultrasound. Since lactic acid bacteria are generally considered to be beneficial to human health, a balance between the release of β -galactosidase and the number of viable cells in the final product has to be established. β -galactosidase is an endoenzyme and its release positively affects the subsequent hydrolysis. Prolonged sonication could cause substrate inhibition as a consequence of low cell viability and low enzyme concentrations in the cells (Wang and Sakakibara, 1997). A case study on the optimization of β -galactosidase release conditions from *E. coli* cells has been published (Feliu et al., 1998).

Ultrasound accelerated starch hydrolysis for a variety of glycosidases (Barton et al., 1996). The authors suggested that the disruption of molecular clusters and the homogeneity generated by ultrasonic mixing were basis for the improved hydrolysis.

Reduction in trypsin activity due to sonication was related to the unfolding of the enzyme leading to irreversible structural changes. A variety of peptide fragments were detected in the sonicated solutions (Tian

et al., 2004). Tryptic digestion and fragment identification of BSA could benefit from high-intensity ultrasound, since less than one minute sonication led to significant increases in the digestion rate of BSA by trypsin (López-Ferrer et al., 2005).

Using manothermosonication, Vercet et al. (2002a) have shown that *Pseudomonas fluorescens* lipase inactivation was mainly due to mechanical damage. For proteases from the same organism, the damage seemed to be mainly induced by the generation of free radicals. The authors hypothesized that in some cases, synergism among heat, sonication, and pressure might be nonexistent.

The recovery efficiency of *E. coli* penicillin acylase increased upon 10 minutes of sonication. The authors commented that longer sonication durations could increase the efficiency, but that the structural integrity of the enzymes could be damaged (Bansal-Mutalik and Gaikar, 2003). Under temperature-controlled conditions, penicillin acylase released from *E. coli* cells was optimized for both ultrasonic and high-pressure cell disruption techniques. The enzyme remained fully active with both techniques (Fonseca and Cabral, 2002).

Ultrasonic treatment of a variety of purified and buffered enzyme solutions, resulted in only alkaline phosphatase remaining fully stable and active. All other microbial and mammalian enzymes underwent some inactivation depending on the ultrasonic processing conditions (Özbek and Ülgen, 2000).

Mason et al. (Mason and Cordmas, 1996; Mason et al., 1996) briefly reviewed the increased activity of immobilized enzymes, chymotrypsin, and amylase during sonication. Immobilization of enzymes is a natural phenomenon observed in soil. Sonication was shown to be useful in mobilizing and dispersing acid phosphatase in soil samples. This treatment increased the reproducibility of enzyme assays in soil samples (De Cesare et al., 2000).

Manothermosonication of peroxidases showed that both heat and sonication were found to act synergistically toward the inactivation of the enzymes almost independent of pH (Lopez and Burgos, 1995). The authors hypothesized that the inactivation mechanism could be due to splitting the heme group from the apoenzyme.

13. The Effect of High-Intensity Ultrasound on Functionality of Carbohydrates

Carbohydrates are fundamental building blocks that food scientists utilize to create a desired food product. They can be found in both plants and animals. Contrary to proteins though, their molecular weight and precise molecular structure can vary widely even within a single type of carbohydrate. Their structure is generally linear although random coil structures or ordered helices may be assumed under certain solvent conditions. Nevertheless, the molecules are significantly stiffer and more rigid than proteins, a property that has important implications for their interaction with high-intensity ultrasound waves. In plants, microbes, and fungi, a great portion of the biomass exists as cellulose. The self-association of cellulose gives rise to the characteristically high mechanical strength of plant cells. Many linear carbohydrates, in the absence of branching, have the ability to form crystalline structures that are difficult to dissolve and extract. The development of new technologies to improve extraction remains a high priority. High-intensity ultrasound has been shown to be useful not only in the extraction of carbohydrates, but in postextraction chemical modifications to tailor their functionalities.

14. Classical and Ultrasound-Assisted Extraction of Carbohydrates

Classical techniques for solvent extraction of carbohydrates from organic materials are based upon appropriate choices of solvents. Initial extraction with alcohol, isolates polar and apolar compounds, while solvents with lower polarity isolate lipophilic materials (Silva et al., 1998). Specific classes of carbohydrates may be extracted with water adjusted to various pH values. Prior to the extraction, materials can be cut or milled to increase the surface area. Stirring or mechanical agitation are used to increase the rate of extraction. Techniques used to improve extraction yields and rates may be used in combination with high-intensity ultrasound.

14.1. Maceration

Maceration is a batch process where a solid containing desired solute is placed in a sealed container in constant contact with a solvent. This allows the solvent to penetrate into the cellular structure in order to dissolve the desired solutes. Extraction efficiencies can be increased by shaking or by using a mechanical stirring to create a uniform mixer of solids and solvent. An ultrasonic bath can improve extraction efficiency. Maceration is a batch-extraction process. Renewal of the solvent may be necessary during the extraction process to reduce the solute to a minimum (Silva et al., 1998).

14.2. Decoction

Decoction is an extension of the maceration process. Typically, boiling water is adjusted to a suitable pH and added to the milled sample. In decoction, the material is actually brought to a rolling boil. This type of extraction can be more efficient than simple maceration, but it is not suitable for solvents with low boiling points such as methanol–water or ethanol–water mixtures (Silva et al., 1998). Application of high-intensity ultrasound during decoction is problematic because of the interference of the steam bubbles with the cavitationally generated bubbles (see Section 9.4). The ultrasonic waves are greatly dampened by steam bubbles and the process becomes relatively inefficient.

14.3. Percolation

One of the more efficient and popular methods for the extraction of solutes from plant materials is percolation. Percolation requires little pretreatment of raw materials apart from grinding the material to a suitable particle size. The solvent is repeatedly passed through the raw material in the form of a porous filter cake on a filter medium. The solute may be continually phase separated from the solvent in a second step and the regenerated solvent added back to the percolation process. The process is nevertheless a batch process. The material being extracted remains in the percolator until the desired level of extraction

is reached. This may take more than 24 hours (Silva et al., 1998). High-intensity ultrasound is used primarily as a pretreatment step. Ground raw materials may be dispersed in water and briefly sonicated to perforate the plant cell walls. This increases the mass transport of solvent and improves the extraction kinetics and yield (Li et al., 2004a, 2004b).

14.4. Nonconventional Methods

Presently, there are also a number of nonconventional extraction methods in use that may improve extraction of solutes from raw materials. Additional energy can be introduced into the extraction process in order to increase the transfer of solutes from sample to solvent. These include microwave-assisted extraction, pressurized liquid extraction (Huie, 2002), as well as vortex (turbo) extraction (Vinatoru, 2001a). Apart from the pressurized liquid extraction, where high-intensity ultrasound can be applied simultaneously, sonication is mostly used to pretreat the raw material.

15. Mechanical Effects of High-Intensity Ultrasonication on Carbohydrates

Ultrasonication can enhance the extraction efficiency of carbohydrates. With regards to polysaccharides, ultrasound appears to be particularly useful for the extraction of low molecular weight polymers such as simple sugars or oligomers (Mason et al., 1996). A number of studies have focused on the extraction of complex carbohydrates, and reports of successful extraction of dextran, xylan, pectin, chitin, and cellulose have been published (Hromadkova et al., 1999; Hromadkova et al., 2002; Hromadkova and Ebringerova, 2003).

Many researchers have suggested that it is the mechanical effects of ultrasound on the solid matrix being extracted that are responsible for the improved efficiencies of the extraction. It appears that high-intensity ultrasound provides a greater penetration of solvent into the cellular materials. This can lead to an improved solute yield. The generation of impinging jets near solid surfaces, such as sugar beet cell walls,

seemed to be particularly useful in the extraction of sugar. If energies are high enough, the cell walls may actually be broken down. This facilitates the release of intracellular content. In studies that involve the sonication-assisted extraction of a variety of high molecular weight carbohydrates, little or no change in their molecular structure, functional properties, and biological activities were reported. This situation can dramatically change once ultrasound is applied to dispersions of the extracted carbohydrates in the absence of other interfering plant compounds. This is discussed in Section 9.16.

Ultrasound-assisted extraction has been used for sample preparation for total mineral content analysis. Ultrasonic sample preparation appears to prevent losses due to decomposition prior to analysis. This may sometimes occur if the samples are subjected to wet-digestion or dry-ashing procedures (Capelo et al., 2005). Upon application of high-intensity ultrasound to plant matter, two different physical effects are observed (Vinatoru, 2001a). First, sonication may destroy the integrity of cell storage structures that serve as reservoirs for lipids or starches. Second, ultrasound can facilitate the swelling and hydration of plant cells and cause an increase in the number of pores in the cell wall. The mechanical effect of ultrasound during extraction has been verified for marigold leaves that were sonicated at two different ultrasonic frequencies (20 and 500 kHz). It was observed that the lower frequency destroyed all the storage structures in the leaves while the higher frequency left them virtually unaffected (Toma et al., 2001). Lower ultrasonic frequencies allow power levels to be reduced while maintaining sufficient cavitation to improve the extraction process.

16. Chemical Changes in Carbohydrates caused by High-Intensity Ultrasonication

While the mechanical effects of high-intensity ultrasound are important for the extraction process, sonochemical processes may lead to a number of unexpected chemical reaction products. While these products may not necessarily form during the extraction, they clearly have been shown to form if a disper-

sion of carbohydrates is sonicated in the absence of other interfering plant matter. Section 9.4 discussed that conditions, in the cavitation bubbles, that may lead to the production of $H^\cdot$, $OH^\cdot$, and nitrogen oxides (NO_x) (Lohse and Hilgenfeldt, 1997). $OH^\cdot$ can react readily with organic compounds such as carbohydrates or two $OH^\cdot$ radicals may recombine to form H_2O_2 (Mead et al., 1976). H and OH radicals in aqueous solution react with carbohydrates by abstracting an H-atom attached to the carbon atoms in the sugar molecules (Heusinger, 1990a). In D-glucose, the attack occurs almost at random at various positions, although a slight preference for attack at the C_1 -H and the C_6 -H has been noted (Heusinger, 1991). In the presence of oxygen, polymers in the molecular weight range of 4,000 Da were produced from glucose (Snell, 1965).

Hydrolysis and cleavage has been demonstrated for a variety of polysaccharides (Kardos, 2001) and the use of high-intensity ultrasound to degrade polymers has been widely recognized (Price, 1990b). For example, sonication of starch led to formation of shorter chain molecules and reducing sugars (Tomasik and Zaranyika, 1995). Similar results have been observed for rayon and hydroxyalkyl cellulose derivatives (Malhotra, 1982). A variety of other reactions that involve carbohydrate oligomers from sonolytically produced fragments have been reported (Heusinger, 1990b; Fuchs and Heusinger, 1994). Unique reactions include the formation of carbon–deuterium bonds for labeling; formation of carbon–heteroatom bonds to produce, for example, bromide-containing sugar derivatives (Kudo and Heusinger, 1983); glycosylation (Brochette-Lemoine et al., 2000); and formation of carbon–carbon bonds (Brochette-Lemoine et al., 2000; Kardos, 2001).

During cavitation, the exerted shear stresses in the vicinity of the collapsing bubbles are large enough to cause uncoiling and stretching of polymer molecules. Linear, stiff molecules such as carbohydrates appear to be much more susceptible to the ultrasonic degradation process than proteins. Not surprisingly, larger carbohydrates are more susceptible to degradation by high-intensity ultrasound. There appears to be a minimal molecular weight below which the

molecules at a specific applied ultrasonic intensity are no longer susceptible to the chain scission. For example, measurements of the intrinsic viscosity of sonicated chitosan solutions, as a function of ultrasonic power level and treatment time, showed a gradual decrease in the molecular weight of the chitosan polymer treatment time until a constant molecular weight was reached (Baxter et al., 2005) (Figure 9.8). There is still uncertainty about the mechanism involved in the primary chitosan polymer degradation. Some authors have argued that carbohydrates cleave around the midpoint (Hoff and Glynn, 1972) while others have argued that cleavage may occur at various locations within the chain (Schmid, 1939; Baxter et al., 2005). Whether the applied mechanical forces are solely responsible for cleaving, the degradation of the primary polymer is still a matter of debate (Crum, 1995b; Mason et al., 1996; Mason, 1997; Stephanis et al., 1997). It is possible that the

chain scission is a secondary effect of chemical reactions initiated by free radicals that have been generated in the process of cavitation (Ramsden and McKay, 1986). Polymer degradation rates decrease with increasing frequency. This is an indication that chemical reactions are less responsible than mechanical effects for depolymerization.

A number of factors influence the mechanical degradation of carbohydrates. For example, carbohydrates are more readily degraded as the share rate (a function of the extent of cavitation) increases (Moore and Parts, 1968). Thus, increasing ultrasonic power levels lead to decreased average molecular weights of carbohydrates. Similarly, the degradation efficiency decreases with increasing frequency since cavities collapse less violently. Increased temperature appears to reduce molecular entanglements and improve the degradation process (Bestul, 1954). However, if temperatures approach

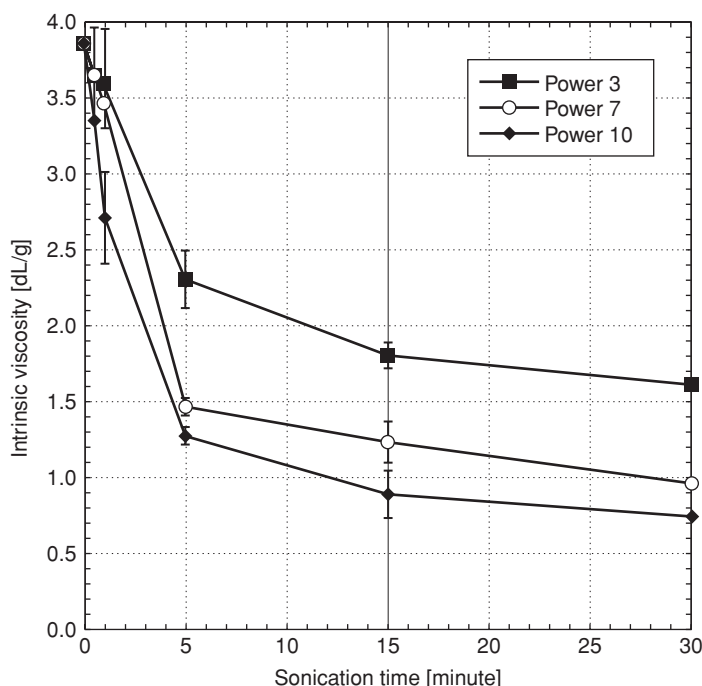


Figure 9.8. Scanning electron micrographs of lyophilized FWP shell powder (a–c) and NAS shell powder (d–f) after (a, d) traditional extraction, (b, e) 1-hour-sonication-assisted extraction, and (c, f) 4-hour-sonication-assisted extraction. (Reprinted with permission from Kjartansson et al., (2006a) American Chemical Society.)

the boiling point of the solvent, vapor bubbles can weaken the ultrasonic field and decrease the efficiency of the polysaccharide degradation. The nature of the solvent–carbohydrate interactions can influence the degradation process. Solutions of carbohydrates in “poor” solvents assume a different configuration than carbohydrates in “good” solvents. The decrease of intrinsic viscosities in poor solvents indicates that polymer coils may have a tendency to aggregate and not fully expand. This decreases their susceptibility to high-intensity ultrasound (Schmid, 1939). Increasing solvent viscosity decreases the extent of cavitation. Carbohydrates are less degraded in high-viscosity solvents (Tanford, 1961). Finally, it should be noted that the molecular structure, flexibility, and strength of the internal bonds of carbohydrate polymers have the greatest influence on their susceptibility to ultrasonic degradation (Ram, 1970).

Ultrasonically cleaved fragments may actually recombine under certain conditions (Brochette-Lemoine et al., 2000; Kardos, 2001). This may lead to polysaccharide dispersions with a significantly narrower molecular weight distribution. This phenomenon may help in the production of high-quality carbohydrate extracts.

17. Effect of High-Intensity Ultrasonication on Specific Carbohydrates

17.1. Dextran

Dextran is a glucan which can be enzymatically synthesized from sucrose. Dextran has contiguous α -1 $\rightarrow$ 6-linked D-glucopyranose units as part of its main chain. Dextran may yield relatively high molecular weight polymers (Robyt, 1995). Low molecular weight dextrans are of great importance to the medical industry (Alsop, 1993; Debelder, 1993; Goto et al., 2004). The traditional way of producing lower molecular weight dextrans is by depolymerizing high molecular weight dextrans followed by fractionation. Depolymerization can be achieved by partial acid hydrolysis (Alsop, 1993), enzymolysis (Corman and Tsuchiya, 1957), ultrasonication (Lorimer et al., 1995), and shear degradation (Carrasco et al., 1987).

17.2. Xyloglucan and Pectin

Xyloglucan and pectins can be extracted from apple or citrus peels. Both by-products of fruit juice production (May, 1990). Traditionally, xyloglucan is extracted from apple pomace by concentrated (1–4 M) KOH or NaOH (Renard et al., 1995). Caili et al. (2006) studied the effect of ultrasound on extracting xyloglucan from apple pomace. The authors reported that application of ultrasound increased the efficiency of the traditional extraction process and accelerated the extraction threefold. Panchev et al., (1988; 1994) studied the extraction of pectin from apple pomace and citrus peel. The conventional extraction process involves the hydrolysis and extraction of pectin from plant cell walls and their solubilization at a low pH and high temperature for prolonged periods of time (Kertesz, 1951; El-Nawawi and Shehata, 1987). The use of ultrasonication increased yield but did not affect the quality of pectin (Panchev et al., 1994). Panchev and coworkers reported that intermittent sonication of apple pomace at 1–1.2 Wcm⁻² increased the yield of pectin by 28% when compared to the standard extraction procedure (Panchev et al., 1994).

17.3. Chitin

Chitin is the second most abundant polymer in nature and a by-product from marine food production, mainly crustacean waste (Zakaria et al., 1998). Chitin is an essential part of supporting tissues and exoskeletons of arthropods (crustaceans and arachnids), insects and cell membranes of microorganisms. Chitin exists in the form of composites in crustacean shells. In the exoskeleton it is bound to polypeptides (proteins) and calcium carbonate, which function as inorganic fillers. Chemically, chitin, (1 $\rightarrow$ 4)-2-acetamido-2-deoxy- β -D-glucan, is a nontoxic and biodegradable polymer of *N*-acetylglucosamine and glucosamine residues. The monosaccharide unit to which the acetyl groups are attached in chitin, are linked together by β (1-4)-glycosidic bonds (Simpson et al., 1994). Chitin is the basic raw material used in the production of chitosan, a biologically highly active molecule that

has exhibited antimicrobial, lipid, and metal-binding properties. Hence, chitin use involves either conversion to chitosan or direct utilization as a self-standing product (Knorr, 1991).

The extraction of chitin from crustacean shells is a time-consuming process that involves extensive demineralization and deproteinization treatments. These require high concentrations of strong acids and strong alkali applied at high temperatures (Muzzarelli, 1997; Wiley, 1998). Kjartansson and coauthors (Kjartansson et al., 2006a, 2006b) studied the effect of ultrasound assisted extraction of chitin from both fresh water prawns (FWPs) and North Atlantic shrimp (NAS). They discovered that for FWP, the yield of extracted chitin decreased significantly compared to untreated controls when ultrasound was applied as a pretreatment. Scanning electron micrographs revealed the effects of sonication on the morphology of the FWP shells (Figure 9.9a–c). Removal

of minerals and protein left the chitin fibers in the form of stacked layers. However, an application of 1 hour of sonication during demineralization and deproteinization fractured chitin sheets, while 4 hours of sonication resulted in extensive perforation of the shell fragments (Figure 9.9c). The crystallinity indices of chitin decreased as the time of sonication increased. The degree of acetylation of chitin was unaffected by sonication, but the degree of acetylation of chitosan produced from sonicated chitin decreased (Kjartansson et al., 2006b).

Similar results were reported by Cardoso and coauthors (Cardoso et al., 2001). The authors reported that sonication of chitin seemed to improve its homogeneity. Increasing the surface area of fragments enhanced their accessibility to reagents. It was concluded that presonicated chitin could be deacetylated up to 95%. Commercially available chitosan is about 88% deacetylated.

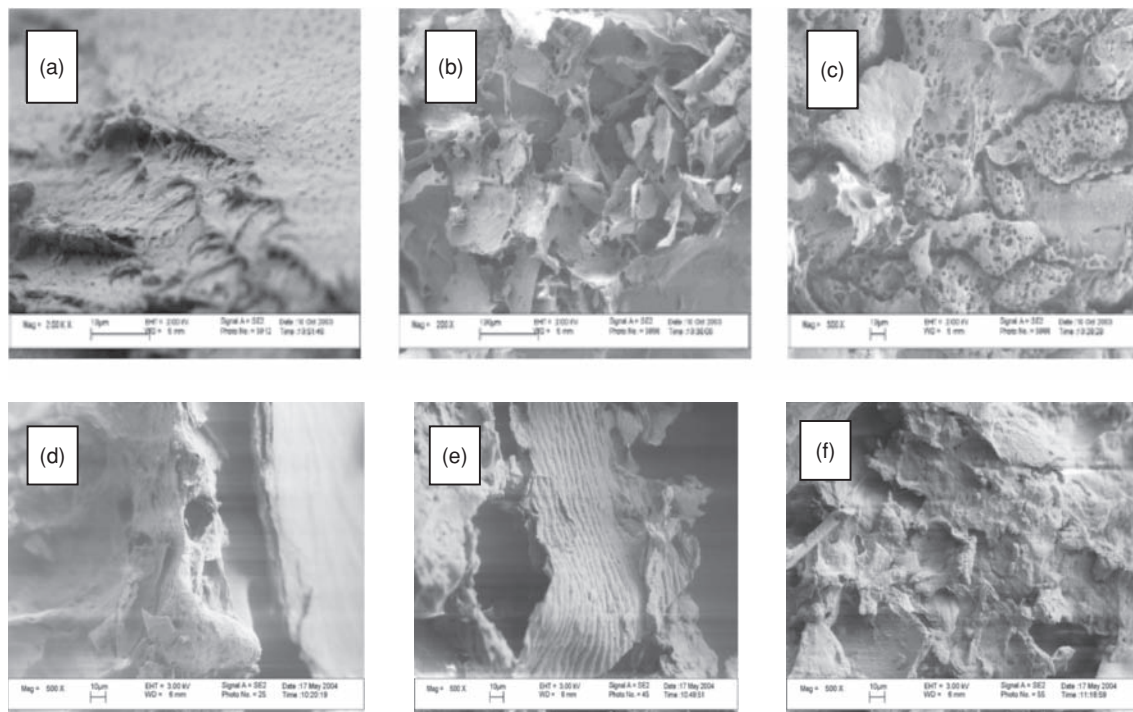


Figure 9.9. Intrinsic viscosity of sonicated chitosan dispersions as a function of sonication time for ultrasonic intensities of 16.5 (Power 3), 28.0 (Power 7), and 35.2 (Power 10) Wcm^{-2} (Baxter et al., 2005).

A comparison of the yields obtained from freshwater prawn (FWP) shells and NAS showed significant differences between the two species in terms of relative and true yields. Removal of minerals was not affected by sonication.

The crystallinity index of chitin from NAF shells decreased from 87.6 to 79.1% and 78.5% after 1 and 4 hours of sonication. Chitosans from these shells had crystallinity indices of 76.7, 79.5, and 74.8% after deacetylation. FTIR scans indicated that the degree of acetylation of chitins was unaffected by sonication. Comparison of the ultrasonic extraction results of NAS with that from FWPs indicated that more impurities were left in the NAS-chitin (Kjartansson et al., 2006a). Scanning electron micrographs (Figure 9.9d–f) show the effect of sonication on the morphology of freeze-dried NAS shells. Removal of minerals and proteins by traditional extraction left the chitin particles in the form of stacked layers. Application of 1-hour sonication during demineralization and deproteinization did not fracture the chitin sheets. After application of 4 hours of sonication (Figure 9.9f), the material showed signs of perforation. FWP shells were much more susceptible to ultrasonically induced forces than NAS.

17.4. Lignin

Sun and Tomkinson used ultrasound to improve alkali extraction of lignins from wheat straw (Sun and Tomkinson, 2002). Dissolution of lignin increased from 43 to 50% as the treatment time was increased from 5 to 35 minutes. They demonstrated that the purity of lignins was higher with ultrasound extraction than without. The molecular weight and thermal stability of lignin preparations produced by ultrasound-assisted alkali extraction was higher than that of lignin fractions isolated without the use of ultrasound. No differences in structural features between alkali and ultrasound-alkali-extracted lignin were found (Sun and Tomkinson, 2002).

17.5. Starch

The unique chemical and physical characteristics and nutritional quality of starch distinguish it from other

carbohydrates. Choi investigated the effect of ultrasound on dilute-sulfuric-acid-catalyzed hydrolysis of starch at moderate temperatures in the range of 90–100°C (Choi, 1994). An increase in the hydrolysis rate occurred during sonication. This was attributed to the extreme rise of local temperature and pressure created by the collapse of cavitation bubbles (Choi, 1994). The ultrasound-mediated enzymatic depolymerization of starch was studied (Sinisterra, 1992). At low power intensity, some enzymes were not deactivated and sonication, using a cleaning bath, increased the hydrolysis rate by a factor of 1.2–2 possibly due to better homogenization of the slurry.

18. Influence of High-Intensity Ultrasound on Gelation of Carbohydrates

Influence of high-intensity ultrasound has a variable effect on the ability of select carbohydrates to form a gel. Here, it is important to distinguish whether the application of ultrasound is for the extraction or a postextraction treatment. The results of Panchev and coauthors on the extraction of pectin with high-intensity ultrasound were discussed in Section 9.17. In those studies, the use of ultrasonication increased yield but did not affect the strength of gels formed with the ultrasonically extracted pectins (Panchev et al., 1994). Similarly, Hromadkova (Hromadkova et al., 1999) reported that high-intensity ultrasound in combination with a hot dilute alkali solution significantly enhanced the extractability of polysaccharides. In this work, insoluble residues of sage were extracted after ethanol extraction of lipid compounds. There was no effect on polysaccharide gelling ability.

In contrast, Seshadri et al. found that the effect of ultrasound on a pretreated pectin dispersion lead to weaker gels with increased sonication power and time. When a power law model was fitted to the viscosities of the treated pectin solutions, it became apparent that the flow behavior of the dispersion changed from viscoelastic to Newtonian (Seshadri et al., 2002). The turbidity of ultrasonically pretreated pectin decreased by 50% resulting in a more

transparent gel. Cote and Willet (Cote and Willet, 1999) studied the influence of ultrasonication at 20 kHz and 1.5 MHz, jet cooking with high-pressure steam, and twin-screw extrusion, on dextran gelation under various condition. Results were determined by size exclusion chromatography and viscometry. Although all three methods reduced the molecular weight and viscosity of dextran, the greatest degree of depolymerization was observed using 20-kHz ultrasonication.

19. Immunology of Ultrasonically Treated Carbohydrates

Polysaccharide fractions of plant cells of herbal origin are of a great medicinal interest. These compounds provide an inexhaustible source of raw materials for the pharmaceutical industry (Rice, 1995). Ebringerova and coworkers (Ebringerova et al., 2002a) studied the structure and functional relationship of two acidic heteroxylan types; the 4-O-methylglucuronoxylans (GXs) from beechwood; three medicinal herbs *Rudbeckia*, *Altheae*, and *Mahonia*; and the arabino-(glucurono) xylan from corn cobs. Depolymerization of arabino-(glucurono) xylan by ultrasonication resulted in a decrease in the immune activity. In the case of the GX samples, neither the uronic acid content nor the distribution pattern of the uronic acid was found to play a role in the expression of immunomodulatory activity (Ebringerova et al., 2002a).

20. Conclusions

High-intensity ultrasound can be used to improve extraction and functionality of proteins and carbohydrates. Because of the complex nature of the cavitation process that drives the physical and chemical effects of high-intensity ultrasound, food processors have to understand the fundamental physical processes and parameters to achieve a desired process outcome. Susceptibility of food biopolymers to high-intensity ultrasound depends on their molecular structure. Linear stiff molecules such as carbohydrates are more susceptible to the mechanical effects of high-intensity ultrasound than, for example,

globular proteins. Some of the discrepancies reported in literature are due to the fact that sonication is currently predominantly applied to a complex food or agricultural system rather than a well-defined solution of food polymers.

In the case of proteins, the degree of the changes in protein functionality depends on the ultrasonic intensity, duration of the sonication treatment, and the environmental conditions (temperature, pressure, solvent pH, and ionic strength). The observed changes are due to intense modifications of the fundamental protein structure as well as changes in folding of the proteins (secondary and tertiary structures) (Figure 9.10). It is thus important for food processors to understand the extent of the structural changes caused by high-intensity ultrasound. In some cases extensive damage to protein structures may be desirable. The inactivation of enzymes or the debittering of proteins are examples. Enzyme activity, adsorption kinetics, gelation kinetics, and foam stabilization can be influenced by sonic energy. In all cases, the effect of high-intensity ultrasound is protein specific and processors need to evaluate the specific effects of high-intensity ultrasound treatment on a protein-by-protein basis.

In the case of carbohydrates, high-intensity ultrasound has improved the extraction of carbohydrates from plant materials. The physicochemical properties of the extracted carbohydrates appear to be similar to their classically extracted counterparts. On the contrary, treatment of carbohydrates dispersed in appropriate solvents at sufficient ultrasonic intensities can lead to significant changes in the structure of carbohydrates. This occurs through cleaving of the stiff primary carbohydrate polymer. Smaller polymer fragments were obtained. Moreover, high-intensity ultrasonication at higher frequencies can induce and accelerate chemical reactions. These reactions can lead to better solubility, increased thickening and gelling activity, and biological activities (e.g., antimicrobial and immunological activities).

The use of high-intensity ultrasound to produce sonochemical effects is growing in the food and agricultural industries. The shifting of heterogeneous reaction pathways to yield more uniform products is an example. Ability of high-intensity ultrasound to

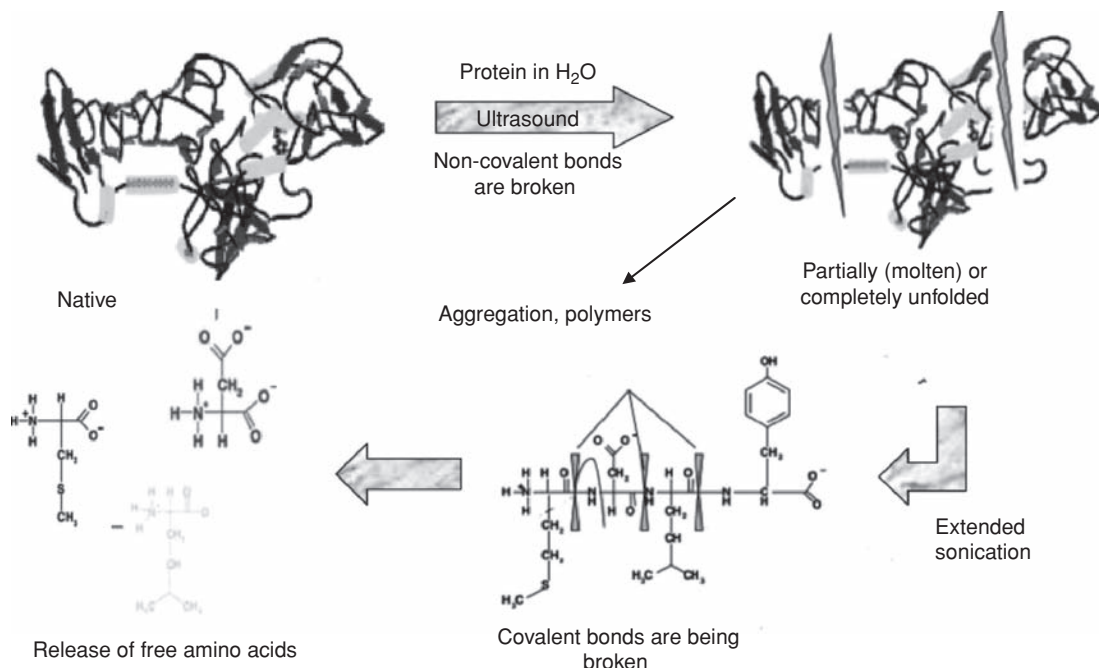


Figure 9.10. Schematic of ultrasonically induced structural rearrangement, aggregation and breakdown processes upon treatment of proteins with increasing ultrasonic wave amplitudes.

accelerate kinetics of a wide range of radical driven reactions could lead to the generation of a wide variety of modified food proteins and carbohydrates. Using high-intensity ultrasound may also lower solvent requirements, and allow the use of less aggressive and more environmentally benign reagents.

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Chapter 10

Ultrasonic Processing

Hao Feng and Wade Yang

1. Introduction

The use of acoustic energy as a processing aid has been explored in various industrial sectors for over 50 years. Its application in food processing is relatively new. This article summarizes current research and development activities on food processing and preservation applications. Ultrasound refers to sonic waves that are at frequencies higher than sound audible to the human ear. Ultrasonic waves for food processing can be divided into two categories based on the difference in frequency and sound intensity. High-frequency ultrasound (also called diagnostic ultrasound) operates at frequencies of 2–20 MHz with sound intensities in the range of 0.1–1 W/cm². High-frequency ultrasound is used in food quality analysis, medical imaging, and non-destructive inspection. Power ultrasound (also known as high-intensity ultrasound) operates at lower frequencies, typically 20–100 kilo Hertz (kHz), with a sound intensity ranging from 10 to 1,000 W/cm² (Feng and Yang, 2005). The high energy level available in power ultrasound makes it suitable for use in the food industry to enhance processes such as oil extraction (Li et al., 2004a; Sharma and Gupta, 2004); surface decontamination (Seymour et al., 2002; Zhou et al., 2007); microbial inactivation (Baumann et al., 2009; Baumann et al., 2005a; Ugarte et al., 2006); enzyme inactivation (Raviyan et al., 2005); and

starch–protein separation (Zhang et al., 2005a). Most power ultrasound applications are performed in a liquid and can be referred to as sonication. This chapter focuses on the applications of power ultrasound in food processing. Review articles on the use of high-frequency ultrasound in the food industry are available (McClements, 1997, 1999; Coupland, 2004). See Chapter 9 for additional references.

2. Generation of Ultrasound and Ultrasound Systems

2.1. Generation of Ultrasound

In 1880, the brothers Pierre and Jacques Curie discovered that a plate cut in a particular plane from a Rochelle salt crystal (sodium potassium tartrate tetrahydrate, KNaC₄H₄O₆·4H₂O) had the property of generating an electrical potential when subjected to mechanical pressure. This is called the piezoelectric effect. The inverse effect occurs when an alternating voltage is applied across a certain plane of the crystal. The alternating voltage causes a change in linear dimension and thus mechanical vibration. In 1881, researchers started utilizing these crystals to generate and detect sound waves. Some ceramic materials, such as barium titanate, lead zirconate, and lead titanate, also exhibit piezoelectricity and are used in ultrasonic transducers. Other means to produce mechanical vibrations to generate ultrasound include whistles, magnetostrictive methods, and spark discharges in water (Mackersie et al., 2005).

2.2. Mechanical Methods

The most widely used mechanical means to generate ultrasound are whistles and sirens. Whistles and sirens convert mechanical energy into vibration to generate sound. Sirens use a gas to facilitate ultrasound generation, while whistles can use either a gas or a liquid to generate ultrasound. Air-borne ultrasound, generated by sirens or whistles, has low power intensity and finds applications in de-foaming, agglomeration of fine dusts, and augmentation of air drying. Liquid whistles can be used to effectively improve mixing and homogenization efficiency.

2.3. Magnetostrictive Transducers

A magnetostrictive transducer utilizes the property of ferromagnetic materials, such as nickel, that change their shape when subjected to a magnetic field. In magnetostrictive transducers, a large number of nickel plates or laminations are arranged in parallel. The edge of each laminate is attached to the bottom of a process tank or other surface to be vibrated. The magnetostrictive material is wrapped with a coil of wire, which, when supplied with an oscillating electric current, will generate an alternating magnetic field. This magnetic field will cause the magnetostrictive material to contract or elongate, producing a sound wave that travels through a sonicating fluid.

2.4. Piezoelectric Transducers

Piezoelectric transducers consist of a single or double thick piezoelectric ceramic disc made of materials such as barium titanate, lithium sulfate, lead metaniobate, or lead zirconate titanate (PZT). The ceramic disc can be considered a composite of many tiny piezoelectric crystals. The ceramic-disc-electrode is compressed to a desired pressure between a block of aluminum and a block of steel, to a preset degree of compression prior to bolting. When an oscillating voltage is applied to the electrodes, the ceramic assembly will expand or contract, with the change of polarity of the applied voltage. As sound waves are generated due to the physical displacement, they propagate into the material to be treated.

PZT is the most effective piezoelectric generator of sound waves but has poor silver-to-PZT adhesive qualities. This makes it more suitable for immersion probes. Lithium sulfate is the best receiver of sound waves, but its solubility in water makes it an expensive material to use. In normal conditions, barium titanate is the most commonly used piezoelectric material. Lead metaniobate has a Curie temperature of 550°C which allows it to be used in high-temperature environments.

2.5. Probe System

The probe system delivers power ultrasonic energy directly to the food being treated.

A probe system consists of a generator for turning electrical energy into high-frequency alternating current, a transducer (or converter) for converting the alternating current into mechanical vibrations, and a delivery probe for conveying the sonic vibrations into a loading medium to couple ultrasonic vibrations to the treated material (Figure 10.1). The probe is usually made from titanium, aluminum, or steel, and may take the shape of a rod, plate, bar, or sphere, depending on the shape of the load and the required gain.

The ultrasonic probe is in direct contact with foods, or can be inserted into a treatment chamber or flow cell of specified geometry to transmit energy into a food system with better energy efficiency. A high sound intensity (W/m^2) system or a high volumetric acoustic power density (APD) (W/m^3) system can be designed by varying the volume of treatment chamber.

There are some potential drawbacks associated with the use of a probe system. The pitting and erosion of the probe, especially at the tip and of a metal treatment chamber can bring metal ions and powders into the food. These contaminants can degrade food quality. The temperature control of the loading medium in a probe system may need special attention. The strong cavitation near the tip of a probe may generate free radicals which can be detrimental to foods. The intense-reaction zone near the tip of a probe can result in a non-uniform energy distribution inside a continuous, through-flow reactor. For optimal energy coupling to the food, it is necessary

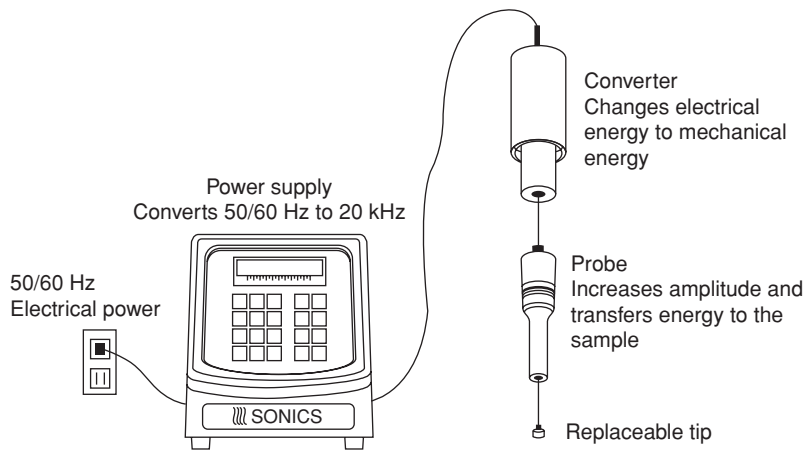


Figure 10.1. A typical ultrasonic probe system. (Courtesy of Sonics & Materials, Inc.)

to establish an impedance matching between the ultrasonic generator, the ultrasonic transducer, and the loading medium.

2.6. Tank System

An ultrasonic tank or bath system consists of a generator, a number of transducers, and a sonication bath. In this system, the ultrasound generating elements do not contact the food directly. Instead, they are usually mounted beneath the bottom a water bath. Ultrasound has to be transferred through water to reach the food placed in the sonication bath. Both the sound intensity (W/m^2) and the volumetric APD (W/cm^3) of a tank system are low compared to a power ultrasound probe system. The ultrasonic tank has the disadvantages of poor temperature control and difficulty in quantifying power delivered to the food during sonication. Acoustic energy distribution inside the tank is not uniform due to the standing wave formation in the tank. Repeatable results may be difficult to obtain. The degassing prior to a treatment is often needed to allow better transmission of acoustic energy into the food being sonicated. Despite these limitations, tank systems have been widely used due to their simplicity in design and ease of operation. The most notable application of the tank system is surface cleaning of mechanical parts, electronic circuit boards, jewelry, and glassware. In recent years, the ultrasonic tank system has been tested

for cleaning or decontamination of raw-food surfaces (Seymour et al., 2002; Yang et al., 2005; Kim et al., 2006; Zhou et al., 2009).

2.7. New Reactors

Due to the ultrasound generation mechanism, traditional ultrasound systems are inherently fixed-frequency units whether used in probe or bath system. A problem associated with a fixed-frequency system is the non-uniform acoustic field established inside a treatment chamber due to standing wave formation. The spatial variation in APD will create problems for certain ultrasonic applications. For instance, in the direct probe power ultrasonication of a liquid food for pasteurization, bacteria tend to concentrate on nodal planes where APD is zero. Thus, the bacteria would not be inactivated.

Since most power ultrasound treatments can be enhanced by increasing cavitation activity, different methods have been explored for maximizing the cavitation. One approach is to use more than one transducer, each with a different frequency. Researchers have demonstrated severalfold increases in cavitation activities in laboratory testing of dual or multifrequency ultrasound systems (Feng et al., 2002). Power ultrasound systems using this concept have to use two or more transducers, and associated signal generator. This complicates the design and operation of the system and increases the cost. A different concept,

the multifrequency, multimode, modulated (MMM) system has been developed to facilitate variable frequency power sonication applications. In the MMM unit, the ultrasonic power supply produces two types of oscillations. The first is a variable-frequency-sweeping oscillation around a central operating frequency. The second is an amplitude-modulated output signal. The frequency of amplitude modulation follows subharmonic low frequency vibrating modes of the mechanical system. The MMM technology can utilize the coupled vibrating modes in a mechanical system by applying advanced digital signal processing to create driving wave forms that synchronously excite many vibrating modes (harmonics and subharmonics) in an acoustic load. MMM will help to produce a uniform distribution of high-intensity acoustical activity to make the entire available vibrating domain acoustically active while eliminating the creation of standing waves (Prokic, 2001).

New concepts have also been proposed to address ablation of probes and metal powder formation from metal treatment tanks. Hielscher Ultrasonics has developed a reactor that sonicates a medium in a closed system indirectly. The medium is led through a glass tube, having no contact with the probe (Freitas et al., 2006) and thus free of contamination. Sonertec, Inc. uses a cylindrical duct made of a nonresonant material (e.g., polymeric/PTFE) that is surrounded by several transducers in the form of longitudinal bars. The prismatic transducers are acoustically coupled to the outside wall of the tube by a thin layer of lubricant, thereby increasing the power intensity at the face. This design produces converging cylindrical acoustic waves, generating a confined cylindrical cavitation zone concentric to the tube in the flowing liquid. This design maintains the cavitation away from the inner wall of the tube so as to avoid surface erosion due to cavitation (Dion and Agbossou, 2002).

3. Ultrasound Measurement

The characterization of a sound field in a liquid can be achieved by measuring sound pressure distribution with a device called a hydrophone (see Section 9.5). A hydrophone is a sound-to-electricity transducer

that takes real-time measurements of the acoustic pressure in a liquid. It is difficult to characterize the sound field of most industrial power ultrasound applications using direct hydrophone measurement. Thus, other methods have been used to measure acoustic power radiated into a liquid during sonication. It has been found that power consumption of an ultrasonic unit at different locations in the system can have different values. The output power of the generator, the power consumed by the transducer (by measuring the applied voltage), and the acoustic power dissipated in the liquid are linearly related, but all different. Power consumption of the generator is important as it will help determine the energy utilization of an ultrasonic processing system. It can also be used as a parameter for process design and scale-up considerations. In ultrasound research, a good understanding of power input and dissipation in an ultrasound treatment will help resolve the poor reproducibility problem; build a platform for comparing work conducted by different research groups; and help establish a controllable variable. Power consumptions then can be related to the output parameters such as microbial inactivation rate or yield of a sonochemical reaction.

Food processors using sonication as a processing aid should be interested in three power values for any ultrasonic processing system. The first value is P_{in} , the actual power consumed by the generator that can be determined using a high-precision wattmeter (W) on the electrical input line. The second important power value is P_{diss} . P_{diss} is the power dissipated in the treatment vessel. The value of P_{diss} can be measured by calorimetry as the ultrasonic waves in the process vessel dissipate as heat. The rate of temperature rise in a batch treatment vessel can provide an estimate of the power delivered to the vessel (P_{diss}). P_{diss} can be estimated using Equation (10.1).

$$P_{diss} = mc_p \left(\frac{dT}{dt} \right) \quad (10.1)$$

where m is mass of liquid (kg), c_p is the specific heat capacity of the liquid (J/(kg K)), and (dT/dt) is the initial slope (K/s) of the temperature versus time curve measured for the first 30 seconds of sonication.

The third power value of interest to the food processor is the fraction of P_{diss} used to form

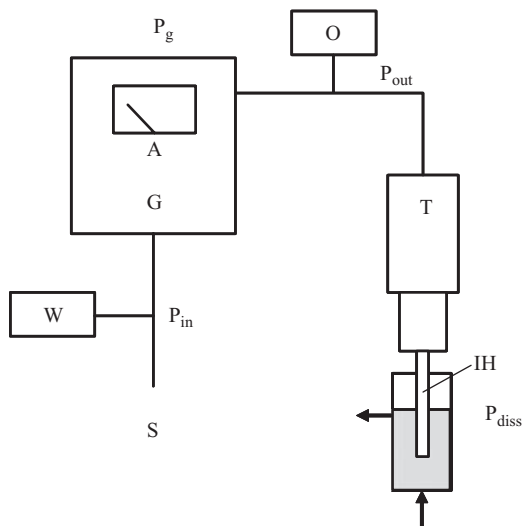


Figure 10.2. Ultrasonic apparatus: A, power reading on the generator; G, generator; IH, immersion horn; O, voltage and intensity probes; S, electrical source; T, transducer; W, wattmeter. (Adapted from Contamine et al., 1995.)

cavitation-induced free radicals, P_{rad} . While the value of P_{rad} may be small, it is a key factor in the use of power ultrasonication for microbial inactivation and for the breakdown of protein and starch polymers (also see Sections 9.6, and 9.16). P_{rad} must be determined using chemical dosimetry (Contamine et al., 1995; Kimura et al., 1996).

Commercial sonic energy systems of the type shown in Figure 10.2 draw power P_{in} , and convert the line voltage and frequency to ultrasonic energy of the desired frequency and power. This power, P_{out} , is used to drive the sonic energy transducer. The relative level of the output power may be measured by a voltmeter display on the power supply. The efficiency of conversion of P_{in} to P_{out} will depend on the circuitry used in the power supply and the power factor associated with P_{out} . The voltage wave and current wave must be synchronized to a power factor of one. The power factor for P_{out} can be determined by measuring the separate values of the voltage (V) and current (I) arriving at the sonic energy transducer using appropriate instrumentation. $P_{\text{out}} = VI \cos \Phi$, where Φ is the phase shift between V and I .

4. Acoustic Cavitation

4.1. Transient Cavitations

Useful food processing application of power ultrasound often depends on a phenomenon called acoustic cavitation. During cavitation, gas- or vapor-filled cavities in a liquid go through rapid formation, growth, and implosion. As the sound waves travel through a liquid, a series of compression and rarefaction activities take place. A cavity forms when the negative pressure at the rarefaction portion of the sound wave exceeds the local tensile strength of the liquid. Nucleation by select additives may facilitate the formation and the increase in number of cavitation bubbles at sites where the tensile strength of the liquid is minimal. The formation-growth-implosion behavior of the cavitating bubbles will depend upon the physical and chemical properties of the media.

Transient cavitation bubbles can be generated if the sound intensity exceeds 10 W/cm^2 . Upon creation, the bubbles normally have an effective residence time of less than 100 ns. They will quickly expand their sizes in a few acoustic cycles and terminate in a violent collapse or implosion when the size reaches a critical value. The implosion of transient bubbles is accompanied by localized extreme physical conditions, such as very high temperatures (e.g., 5,000 K); pressures (e.g., 1,000 atm); or the formation of shock waves and water jets that may have an inflow velocity of up to 156 km/hour (Leighton, 1994). It is believed that the localized high-temperature, high-pressure, and high-velocity jets are responsible for most of the physical, sonochemical, and bactericidal effects of power ultrasound treatments on foods. Shock waves due to the ultrahigh pressure resulting from the implosion of cavitation bubbles can create mechanical cleavage of large biopolymers such as proteins, starches, and chitin (see Section 9.17) or joint surfaces of different materials.

4.2. Stable (static) Cavitation

Stable (static) cavitation can be generated at a sound intensity of $1\text{--}3 \text{ W/cm}^2$. A stably oscillating bubble possesses a longer residence time, during which

it undergoes an increase in equilibrium size as gas dissolved in the liquid is pumped into the bubble through rectified diffusion (Leighton, 1994). As the acoustic energy increases, stable bubbles can turn into transient bubbles due to increasing bubble size. When the bubble size reaches a critical level, the bubbles will implode. Bubble can also grow to a size big enough to allow the bubble to float to the surface. This process is called ultrasonic degassing. When the oscillating frequencies of stable bubbles reach their resonance frequencies, intense localized shearing can occur adjacent to the bubbles. These shear forces can cause disruptive mechanical effects in the medium or the surfaces contacted by the bubbles (Mason and Lorimer, 2002).

4.3. Approaches to Enhance Cavitation Activity

There are two ways to increase acoustic cavitation activity. The first is to increase the number of cavitating bubbles. This can be achieved by lowering the cavitation threshold or using multifrequency techniques. Another method is to increase the power of bubble implosion. This can be achieved by applying an external static pressure during sonication or raising the level of APD (W/m^3) (Feng, 2005).

Sonication of a liquid can create bubbles with a wide range of sizes, although not all the bubbles generated can cavitate. According to Mason and Lorimer (1999), the cavitation activity can be maximized when the natural resonance frequency of the bubble is equal to the frequency of ultrasound. A single-frequency sonication unit may generate cavitation effects in bubbles of a limited size range. The natural resonance frequency (f_r) of the bubble is given by (Mason and Lorimer, 2002)

$$f_r = \frac{1}{2\pi R_r} \left[\frac{3\gamma P_h}{\rho} \right] \quad (10.2)$$

where R_r is the radius of the bubble, ρ is the density of the liquid, P_h is the hydrostatic pressure, and γ is the ratio of the specific heats.

Increasing attention has been paid to the use of multifrequency techniques to improve the efficacy of a sonication treatment. The rationale behind this approach is to create a sonication environment un-

der multiple frequencies where bubbles with a wide range of sizes can go through implosion for strengthening the activity of cavitation. For example, Ciuti et al. (2003) reported that in a dual frequency system, the enhancement on ultrasonic cavitation, as measured by iodine release, is more than the addition of cavitation effects at the individual frequencies.

The effect of external static pressure on cavitation can be described by Equations (10.3) and (10.4) (Mason and Lorimer, 2002):

$$\tau = 0.915 R_m \left(\frac{\rho}{P_h + P_A} \right)^{0.5} \left(1 + \frac{P_{vg}}{P_h + P_A} \right) \quad (10.3)$$

$$P_{\max} = P_g \left\{ \frac{(P_h + P_A)}{P_g} \right\}^{K/(K-1)} \quad (10.4)$$

where τ is collapse time, R_m is the radius of the cavity at the start of collapse, P_A is the applied acoustic pressure, $P_{\max}$ is the maximum pressure at the collapse, P_{vg} is the pressure in the bubble at the start of bubble collapse, P_g is the pressure of gas, and K is the polytropic index of the gas mixture. An increase in external pressure (P_h) will result in a more rapid implosion (shorter τ) at a higher pressure $P_{\max}$. In recent years, the combination of elevated external pressure with ultrasound and mild heat has been used to inactivate microorganisms and food enzymes in what is termed in literature as manothermosonication (MTS) (Pagán et al., 1999). A significant increase in inactivation rate has been widely reported in laboratory MTS testing (Lee et al., 2009).

5. Power Ultrasound Applications

5.1. Emulsification

Power ultrasound has been used to emulsify foods, cosmetics, and pharmaceuticals. Emulsions obtained by sonication are reported to be stable—even without the addition of surfactants, and to have a uniform droplet-size distribution (Mongenot et al., 2000; Freitas et al., 2006). During ultrasound-assisted emulsification, the localized shock waves from bubble collapse create high temperature and high pressure. This can emulsify two immiscible fluids into a homogenized mixture (Mason et al., 1996;

Behrend and Schubert, 2001). Ultrasound-enhanced emulsions of maltodextrin in milk were reported to have retained cheese aroma better (Mongenot et al., 2000). Milk homogenized by ultrasound was found to reduce the total fermentation time of yogurt by 0.5 hour (Wu et al., 2001). The application of ultrasound did not inactivate yogurt bacteria in an 8-minute treatment and the viscosity of the sonicated yogurt was significantly higher than the control.

An increase in APD and treatment time will decrease the mean droplet diameter in an emulsion (Abismail et al., 1999; Behrend et al., 2000). The effect of continuous phase viscosity on droplet size in an oil-in-water system was reported to be negligible when a low fraction of the oil was used (Behrend et al., 2000). Changes in hydrostatic pressure and gas content affect droplet sizes mainly through changes in the APD (Behrend and Schubert, 2001). The treatment at a higher hydrostatic pressure was accompanied by a higher APD and hence smaller mean droplet sizes. Gas saturation or partial degassing prior to emulsification resulted in a shift in maximum energy density. At constant APD, no effect was found on droplet size (Behrend and Schubert, 2001). The generation of metal ions and powders from ultrasonic probes and reactor walls is an inherent problem associated with traditional direct contact power ultrasound units. This is a concern in potential food applications. Freitas et al. (2006) used a contact-free, through-flow cell, suitable for aseptic production, and produced an oil-in-water emulsion with a mean droplet diameter of 0.5 μm that was free of contamination.

Ultrasound can be used to break an emulsion at low APD values (Sun et al., 2001). It has been reported that oil or liquid droplets in an emulsion tend to concentrate on nodes of an ultrasonic standing wave field. There they separate from the emulsion by aggregating into larger droplets (Haake and Dial, 2002).

5.2. Size Reduction

Ultrasonic-size reduction, including cutting, slicing, slitting, and chopping, has found commercial applications in the food industry (Cardoni and Lucas, 2005). Ultrasonic equipment for size reduction uses

a titanium horn designed to function as a knife. Ultrasonic cutting equipment can cut sticky and brittle foods such as nuts, raisins, peanuts cereal bars, and other hard, dried nuts or fruits cleanly with minimum waste.

Ultrasonic cutting occurs when local tensile stresses in food increase to critical values that exceed what can be dissipated by sliding processes (Schneider et al., 2002). As a result, transversal cracks and abrasion of particles caused by shear deformation are avoided. The fast reciprocating vibration of ultrasonic blades also considerably reduces friction between knife and food and results in straight and clean cuts. Four major types of food products, that is homogenous and compact solids, porous solids, vegetable tissues, and animal tissue, were subject to ultrasonic cutting to demonstrate the advantages (Schneider et al., 2002). Ultrasonic cutting of homogenous and porous solids avoided the problems of large deformation, rupture by shearing, cracking, and crumbling associated with conventional cutting. The cut-surface roughness was also reduced. However, acoustic energy coupling between the knife and the food allowed secondary effects such as melting and cavitation to take place. Off-flavors were reported in high-moisture and fat-rich extruded cheese (Schneider et al., 2006). Enzymatic reactions were noticed in vegetable tissues (Schneider et al., 2002). These reactions have been attributed to cavitation at the blade and moist food contact surface. The blade profile design was found to be important since a poor design will cause blade failure due to high stress (Cardoni and Lucas, 2005). The ultrasound frequency and amplitude was reported to affect the maximum vibration speed of the knife. A higher maximum vibration speed led to a reduction in cutting force and an improvement in cut quality (Zahn et al., 2005). It was also found that increasing vertical cutting velocity would increase the cutting work. However, at a particular cutting velocity, the cutting work would decrease with increasing amplitude (Zahn et al., 2006).

5.3. Crystallization

Crystallization comprises two principal physical processes, nucleation and crystal growth. Ultrasound

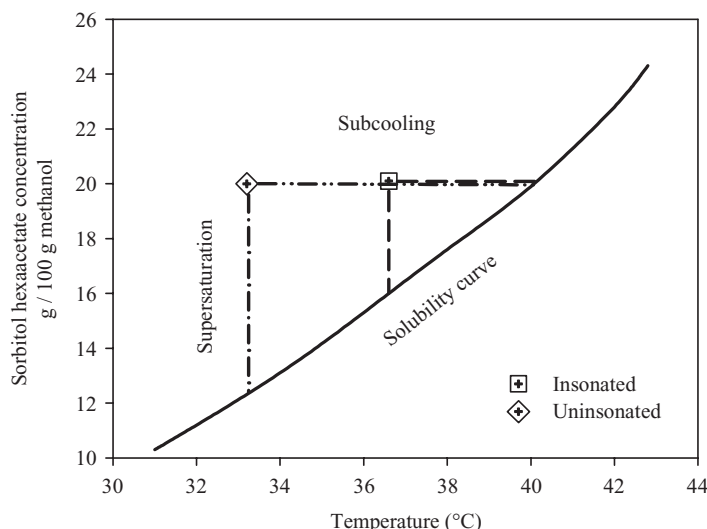


Figure 10.3. Cooling crystallization of sorbitol hexa-acetate from methanol with and without ultrasound treatment. (Adapted from McCausland, 2003.)

has been used to enhance crystallization since 1927 in a process called sonocrystallization. This is a non-invasive method of using ultrasound to control the point of nucleation and the number of nuclei formed (Richards and Loomis, 1927). Benefits of sonocrystallization include controlled initiation of nucleation, enhanced yield, improved filtration characteristics, improved product properties, and increased process reproducibility. Factors affecting crystallization include APD, frequency, exposure time, and pulsation.

Ultrasound can induce nucleation in particle-free solutions and replace the need for seed crystals. The crystallization equipment can be operated remotely for contained and sterile food applications. Ultrasound helps to nucleate a solution at lower levels of supersaturation as shown by a reduction in metastable zone width (MZW). This is seen in Figure 10.3. A 30-second burst of ultrasound reduced the MZW of sorbitol hexa-acetate from 6.8 to 3.2°C and, hence, also decreased the corresponding supersaturation level.

The collapse of sonic formed bubbles in a solution is commonly considered as the trigger for nucleation. Several theories have been proposed to ex-

plain this phenomenon. One model postulates that a positive pressure change, during the final stage of the collapse of a cavitation bubble, reduces the crystallization temperature. Another theory relates the subsequent rapid local cooling rates (10^7 – 10^{10} K second⁻¹) to an increased localized supersaturation. A third hypothesis states that the cavitation events overcome the excitation energy barriers associated with nucleation (Ruecroft et al., 2005). Once primary nucleation sites have been formed, ultrasound can help increase the rate of nucleation and growth of the crystals. This can be facilitated by disruption of seeds or nuclei that already existed in the solution. The use of ultrasound can also help to control the crystal size distribution. A short burst of ultrasound allows the growth of large crystals while continuous sonic exposure, throughout the duration of the process results in small crystals. An added advantage of using ultrasound in crystallization is the cleaning effect of ultrasound. This effect helps stop encrustation of crystals on heat transfer surfaces and hence helps to improve heat transfer efficiency (Mason, 1998).

Ultrasound has been investigated for hastening the conversion of type III polymorphic triglycerides to type V in cocoa butter. Type V has the desirable

Table 10.1. Examples of ultrasound-assisted extraction of food components, food ingredients, and phytochemicals

Source	Solute	Solvent	Reference
Sugar beet	Sugar	Water	Chendke and Fogler, 1975
Defatted flakes	Soybean protein	Water	Wang, 1981
Chinese plants	Helicid	Ethanol	Zhao et al., 1991
Abomasa	Rennin	HCl	Kim and Zayas, 1991
Wheat germ	Germ oil	Supercritical CO ₂	Chen et al., 1997
Grape seeds and whole grape	Tartaric acid or malic acid	Water or methanol	Palma et al., 2002
Corn cob	Xylan	NaOH	Hromadkova et al., 1999a
Sage (<i>Salvia officinalis</i> L.)	Polysaccharides	Ethanol	Hromadkova et al., 1999b
Caraway seeds	Carvone or limonene	Hexane	Chemat et al., 2004
Soybean	Soybean oil	Hexane, isopropanol, or mixed solvent	Li et al., 2004a, 2004b
Kabkab date	Date syrup	Water	Entezari et al., 2004
Chicken liver	Lutein	Hexane	Sun et al., 2006
Prawn shell	Chitin	HCl	Kjartansson et al., 2006
Egg yolk	Lutein	Hexane	Yue et al., 2006
Garlic	Essential oil	Diethyl ether, hexane, or ethyl acetate	Kimbaris et al., 2006
<i>Chlorella pyrenoidosa</i>	Polysaccharides	Ethanol	Shi et al., 2006
Grape stem	Resveratrol	Ethanol	Cho et al., 2006
Pomegranate peel	Phenols	Acetone, methanol, ethanol, water, and ethyl acetate	Yasoubi et al., 2007

texture and mouth feel (Frederic and John, 1997a, 1997b). Ultrasonic treated cocoa butter may retard fat bloom development in chocolate (Baxter et al., 1997). Ultrasound treatment of anhydrous milk fat at 65 kHz was used to control crystal size in the final crystallization slurry and was found to improve the separation efficiency by about 20% (Jo et al., 2002). Sonocrystallization was also found to increase lactose recovery by a factor of 6.3 compared to the conventional crystallization method (Bund and Pandit, 2007).

5.4. Solvent Extraction

Solvent extraction is a separation process of removing specific components from solid or liquid foods by means of an immiscible solvent. It can be classified into liquid–liquid, solid–liquid, and supercritical fluid extraction. The ultrasound-assisted extraction uses the mechanical effect of cavitation: increase the interface mass transfer rate; alter the permeability of biological cell membranes; increase the activ-

ity of substances that are bound in cell structures; and disrupt cell walls to facilitate release of content (Mason et al., 1996). Table 10.1 shows some examples of ultrasound-assisted extraction of food or bioactive components. The use of ultrasound results in increased solute extraction in a shorter time and at lower temperatures. It helps to reduce thermal degradation of sensitive aroma compounds from garlic (Kimbaris et al., 2006) and increase the extractability of polysaccharides from sage (Hromadkova et al., 1999b). Ultrasound-assisted extraction can achieve a significantly higher soybean oil yield compared to conventional methods without the need for time-consuming preparation steps (Li et al., 2004b). Analyses of ultrasound-treated samples indicated no significant changes in the composition of fatty acids in soybean oil and much less degradation of lutein from chicken liver (Sun et al., 2006). However, digestive enzyme extract from stomach tissue exhibited darker and less green color and was more turbid than controls when ultrasound was used (Kim and Zayas, 1991).

6. Inactivation of Microorganisms

Inactivation of microorganisms with power ultrasound has been used to reduce the food safety concerns of human pathogens in foods. Studies are commonly conducted in liquid media with ultrasound alone or in combination with other hurdles such as temperature (thermosonication), pressure (manosonication), or combinations of heat and pressure (manothermosonication). Although surface decontamination of solid objects with air-borne ultrasound has been proven effective in the inactivation of a virus (Hoover et al., 2002), more studies are needed before a general conclusion can be drawn. The following discussion hence focused on the inactivation of microorganisms by power ultrasound in liquid foods.

Power ultrasound treatment for microbial inactivation at sublethal temperatures generally results in low kill rates especially at a low APD (Ugarte et al., 2007). When sonication is conducted at a lethal temperature (thermosonication), the microbial kill rate appears to be greatly enhanced by the combined action of heat and ultrasonic energy (Earnshaw et al., 1995). One example is the thermosonication inactivation of *Listeria monocytogenes* in milk at 60°C. The *D* value of the combined treatment at 60°C was 0.3 minutes, which represented a seven-fold increase in the rate over heat inactivation at the same temperature. It was found that an upper temperature limit existed for microorganisms treated by thermosonification (Ugarte et al., 2006). Beyond this temperature, thermosonication did not cause additional inactivation (Figure 10.4). A further increase in the efficacy of inactivation has been reported when a low pressure (200–500 kPa) is introduced into a thermosonication system to achieve a manothermosonication (MTS) treatment. Lee et al. (2009) reported that the rate of MTS inactivation of *Escherichia coli* K12 in a 0.01 M phosphate buffer (pH 7.0) was increased by 22% and 1,105% compared to thermosonication and heat alone treatment, respectively.

The inactivation mechanism of ultrasound can be explained through the effect of cavitation on microbial cell walls. The water jets of liquid generated, due

to the asymmetric implosion of transient cavitating bubbles, may cause severe cell envelope damage and cleavage of the texture of the polymeric materials of the cell walls. In terms of the chemical effects, transient cavitation can create OH^- and H^+ radicals and hydrogen peroxide. These have bactericidal capabilities (see also Section 9.16). Also, stable cavitating bubbles can generate microstreaming alongside the bubble and create high hydrodynamic shear stresses, which cause cell membrane damages (Wu, 2002).

It has been found that spores, gram-positive and cocci cells are more resistant to inactivation by power sonication than vegetative, gram-negative, and rod-shaped bacteria. For the same species of microorganisms, the resistance to ultrasonic energy varies among different strains (Baumann et al., 2005a; Rodriguez-Calleja et al., 2006). According to the literature, ultrasound inactivation can be characterized by log-linear kinetic parameters at sublethal temperatures. At lethal temperatures the inactivation kinetics does not appear to follow a log-linear relationship (D'amico et al., 2006; Lee et al., 2009). Shoulders may relate to cell disaggregation, while tailing has been attributed to a progressive loss in cavitation intensity during sonication. This may be the case for an open system where degassing can occur, with the subsequent loss of cavitation intensity.

Pagán et al. (1999) examined the effect of sonication in conjunction with external pressure on the recovery of gram-positive and gram-negative cells. Recovery media had added sodium chloride opposed to nonselective media. They observed that the recovery in both cases was virtually identical. Mañas and Pagán (2005) attributed the absence of sublethal injured cells to irreversible physical damage to the outer cell membrane. This has also been confirmed in a recent study conducted by Ugarte et al. (2006). Ugarte and co-workers present scanning electron microscopy images of ultrasound treated *Escherichia coli* K12 cells. Cells showed extensive damage including cell perforation (Figure 10.5). Perforation caused by sonication is a unique phenomenon which had not been reported in previous studies. The extensive and marked changes in cell

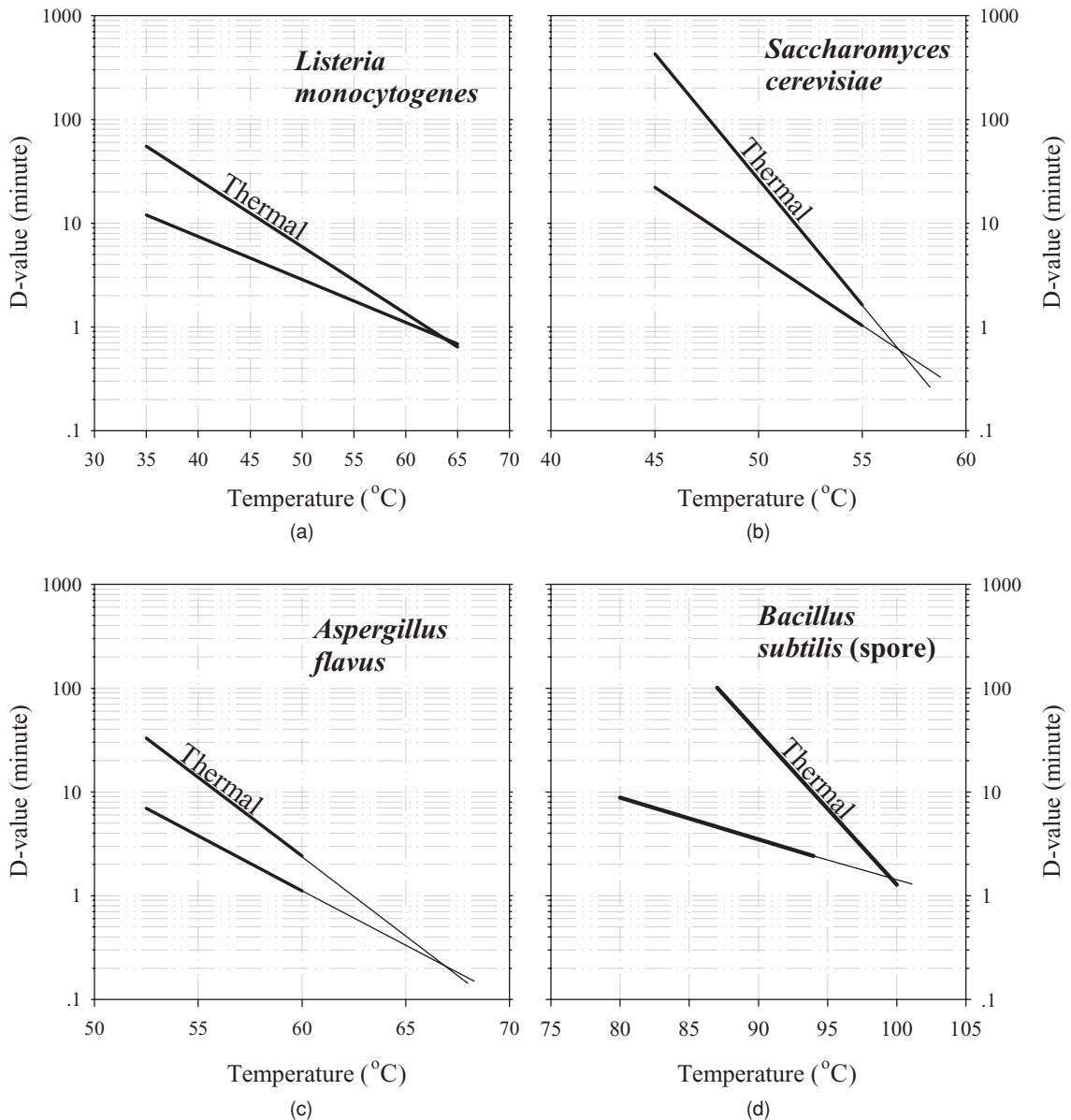


Figure 10.4. Resistance of *L. monocytogenes* (a: Ugarte et al., 2006), *S. cerevisiae* (b: López-Malo et al., 1999), *Aspergillus flavus* (c: López-Malo et al., 2005), and *Bacillus subtilis* spores (d: Sala et al., 1999) to thermosonication treatment in comparison with thermal alone treatment. There are two lines for each microorganism, with the upper one denoting thermal treatment and the lower one denoting thermosonication treatment. Thick solid lines represent the temperature range reported in the literature while the thin solid lines are an extrapolation from the data reported.

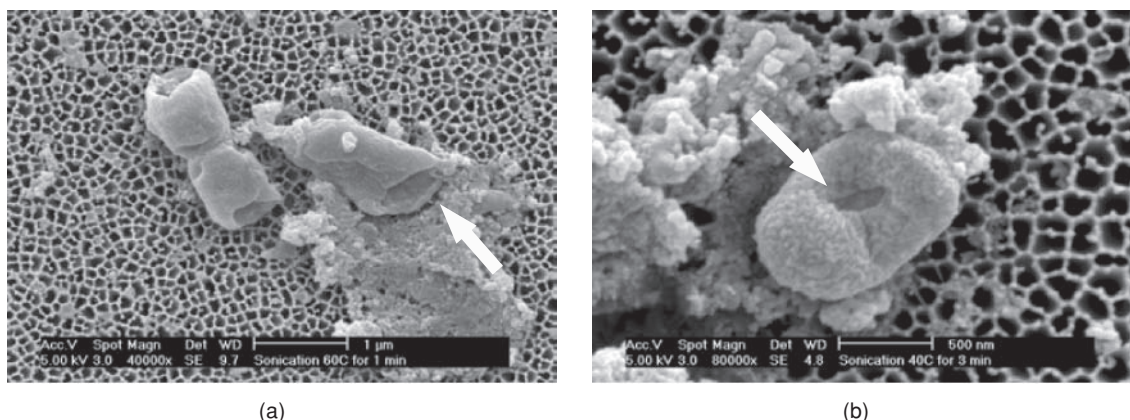


Figure 10.5. *E. coli* K12 cells observed with ESEM. (a) Sonication at 60°C in apple cider for 1 minute (40,000 magnification) and (b) sonication at 40°C in apple cider for 3 minutes (80,000 magnification). (Adapted from Ugarte et al., 2006.)

morphology demonstrate that power ultrasound inactivates microbial cells irreversibly through physical impacts.

More data are needed on the resistance of vegetative cells, yeast, molds, spores, virus, and food toxins to power ultrasonic treatment in both model and food systems. Studies on the effect of ultrasound on quality attributes of foods are crucial for any power sonication applications. A good understanding of the microbial destruction kinetics and the food product quality degradation kinetics is indispensable. This is needed for the substantiation and optimization of a practical ultrasound food preservation operation. The control parameters, sonication protocols, and ultrasonic equipment used must be well defined and reported, so that data from different research groups can be compared, whereas inactivation data are often difficult to compare because of the diverse inactivation conditions, testing protocols, and ultrasound systems used. To facilitate a more effective comparison of inactivation data among different researchers, it is recommended that APD be reported. APD is the dissipated acoustic power measured with the calorimetric method as given in Equation (10.1) divided by volume of the treatment sample. Further information on microbial inactivation with power ultrasound can be found in comprehensive review articles by Miller et al. (1996), Mason et al. (2003a), and Condón et al. (2005).

7. Enzyme Inactivation and Activity Control

Power ultrasound can reduce the activity of enzymes in foods. Inactivation studies have been carried out on citrus, tomato, and dairy products. For example, the effect of MTS on tomato (Vercet et al., 2002) and orange (Lee et al., 2005) pectic enzymes has been examined. According to existing literature, the inactivation of enzymes by power sonication at low temperatures is not very effective. Most experiments have been conducted at temperatures high enough to cause enzyme inactivation. Power ultrasound enhanced thermal inactivation of pectin-methylesterase (PME) at 61°C, and increased the inactivation rate by 288-fold. At 72°C, the increase was 70.5-fold (Raviyan et al., 2005). These results are similar to ultrasound enhancement of thermal microbial inactivation. The most effective inactivation is achieved by MTS. Lopez et al. (1998) reported tomato polygalacturonase (PG) MTS inactivation tests (200 KPa). A 52.9- and 26.3-fold increase was found over that of heat inactivation for thermoresistant PG I and PG II, respectively. More ultrasonic food enzyme inactivation data can be found in the references of Sala et al. (1999) and Mason et al. (2003b).

While power ultrasound enhanced enzyme inactivation has been attributed to different mechanisms,

it is generally agreed that sonication depolymerized macromolecules. The shear stress generated by stable cavitation is considered to be an important factor in causing the degradation of high molecular weight polymers. Free radical formation and resultant scavenging of amino acid residues are also important in enzyme inactivation.

Besides inactivating food enzyme activity, ultrasound can also increase the activity of enzymes if proper treatment conditions are maintained. It has been observed that thermosonication of pectinmethylesterase (PME) in orange juice increased the activity of PME when the treatment temperature was 30°C (Zhang et al., 2004). An increase of up to 200% in the activities of α -amylase and glucoamylase bound to porous polystyrene was reported when a sound intensity of 5 kW/m² was used (Schmidt et al., 1987).

8. Cleaning and Surface Decontamination

Ultrasonic cleaning is one of the most successful and most widely used ultrasound applications (Povey and Mason, 1997; Quartly-Watson, 1998; Maisonhaute et al., 2002a; Seymour et al., 2002). Ultrasonic cleaners have been employed for cleaning jewelry, lenses, coins, watches, dental and surgical instruments, fountain pens, industrial parts, and electronic equipment. However, the fundamental aspects of the cleaning process await more thorough explanations. Maisonhaute et al. (2001, 2002a, 2002b) used electrochemical approaches to explain the mechanism of cleaning by acoustic cavitation. They reported that acoustic bubbles were oscillating at a distance of only a few tens of nanometers above the surface to be cleaned. The flow resulting from the bubble collapse can lead to important drag and shear forces on the surface, causing surface cleaning or erosion. The cavitation effects of ultrasound are stronger than the adhesion force holding a particle to the surface (possibly Van der Waals attraction) (Maisonhaute et al., 2002b). It has been reported that cavitation related phenomena, such as microstreaming and macrostreaming, are important in detaching particles from surfaces (Lamminen et al., 2004).

Surface treatment of food products with power ultrasound has been tested for poultry disinfection (Sams and Ferial, 1991; Lillard, 1994); fresh produce decontamination (Seymour et al., 2002; Ajlouni et al., 2006; Zhou et al., 2009), removal of biofilms (Rediske et al., 1999; Baumann et al., 2005b), and vegetable seed decontamination (Scouten and Beuchat, 2002; Kim et al., 2006). More studies are needed to further improve the efficacy of ultrasonic surface decontamination treatments. A key technical issue for such a decontamination operation will be the generation of a strong and uniform cavitation field inside the cleaning tank.

9. Ultrasonically Enhanced Heat and Mass Transfer

The physical actions of ultrasonic waves at fluid and solid interfaces can be used to enhance heat and/or mass transfer in many food processing operations. The enhancement is attributed to a reduction in heat and mass transfer resistance at the interface and is often related to the activity of cavitation. The microstreaming caused by stable cavitation helps reduce the thickness of the boundary layer, thus reducing the resistance to convective heat and mass transfer. The high speed in-flow of liquid generated by water jets disrupts the boundary layer and also helps enhance heat and mass transfer. In ultrasound-assisted drying and other operations, an increase in mass transfer diffusivity in solids has been observed. The increase in diffusion may be caused by a series of rapid and successive compressions and rarefactions when sound waves propagate through the food product (Simal et al., 1998). The use of ultrasound to increase heat or mass transfer rate has been reported for both liquid and air applications. These include hot air drying, osmotic dehydration, meat brining, extraction, and membrane separation.

Sastry and co-workers (Sastry et al., 1989; Lima and Sastry, 1990) explored ways to enhance fluid-to-particle convective heat transfer with power ultrasound. They reported an increase in the convective heat transfer coefficient from 500 to 1,200 W/m² K when ultrasound was applied and found that the extent of sonic enhancement was strongly dependent

on fluid rheological properties. The enhanced fluid-to-particle heat transfer was also reported in a test conducted at the Campden & Chorleywood Food Research Association when heating potato cubes with 4% starch slurry. Without ultrasound, it took the cubes 205–540 seconds to reach 60°C, while with sonication, the heating time was reduced to 170–420 seconds (Leadley and Williams, 2001).

In ultrasound-assisted hot air drying, the moisture diffusivity in apple was increased 4.6 times, compared to hot air drying at the same temperature (31°C) when apple samples were directly exposed to ultrasonic waves (de la Fuente et al., 2004). Acoustic energy was also found to increase drying rate when it was applied in the drying of rice (Da-Mota and Palau, 1999) and onions (Muralidhara and Ensminger, 1986). In liquid–solid mass transfer processes, ultrasound accelerated water loss and sugar gain in osmotic dehydration of apples (Simal et al., 1998). A solution temperature of 70°C must be maintained to achieve the same solute gain in apples as that obtained at 40°C with sonication. As a result, a lower temperature can be used in ultrasound-assisted osmotic dehydration to preserve the natural flavor, color, and heat-sensitive nutritive components. In the brining of meat, however, it was found that an increase in salt gain was achieved only when an APD threshold of about 8.8 W/cm³ was reached (Benedito et al., 1999). This intensity threshold was also observed in other mass transfer processes. It was postulated that the effect of acoustic streaming becomes greater than the influence of natural convection when the APD is greater than the threshold (Mulet et al., 1999).

Power ultrasound was also used to increase the rate of moisture transfer during the rice parboiling process by combining the soaking and gelatinization steps into one, so as to shorten the lengthy process of conventional parboiling of rough rice (Wambura et al., 2008). A significantly higher moisture absorption was observed in ultrasound-enhanced soaking than conventional soaking. It was found that the moisture uptake of rough rice after 3 hours of sonication-enhanced soaking (i.e., 49% wet basis) was equivalent to 10 hours of conventional soaking (i.e., 44% w.b.), indicating that ultrasound-assisted soaking reduced 70% of soaking time.

10. Bioseparation

Power ultrasound has been used for the purpose of bioseparation. In starch–protein separation experiments, Zhang et al. (2005a) has found that sonication helped recover 97.3–99.5% of the total starch from degermed corn flour (67.5% total starch) and hominy feed (46.4% total starch)—two low-value dry-milling by-products. The quality of the resulting starch is comparable to regular commercial cornstarch. In a quick germ/quick fiber process, one in which no SO₂ is added during steeping to enhance starch separation, ultrasonication in the fine fiber stream was found to result in a starch yield (66.9–68.7%) almost as high as that of a traditional wet-milling operation (68.9%). The quality of starch from the ultrasound treatment was comparable to or better than conventional wet-milling starch, as evidenced by lower protein content, comparable color, and similar pasting properties measured with a Rapid Visco Analyzer (RVA) (Zhang et al., 2005b). In a corn wet-milling process, power ultrasound was used to rapidly remove corn pericarp prior to steeping. This resulted in a reduction in steeping time, and improved the isolated starch gelatinization and pasting properties (Liu, 2002; Yang and Liu, 2004; Wang et al., 2006). Wang and Wang (2003) used ultrasonication in rice starch isolation tests (without addition of chemicals) and isolated up to 76.2% starch without changing the surface morphology of starch granules. In an ultrasound-assisted tomato peeling test with a 2% lye solution, peel loss was reduced by 3%, compared to peeling under commercial conditions where a lye solution of 10% was used (Lee and Feng, 2004). Power ultrasound treatment was also used to extend the shelf life of roasted peanuts by removing oils on peanut kernel surfaces. A 10-minute power ultrasound treatment removed, as can be discerned from microscopic examinations, nearly all the surface oil and increased the shelf life by up to 17% (Yang et al., 2005).

11. Power Ultrasound Applications

Power ultrasound is currently not an off-the-shelf technology and commercialization requires careful development and attention must be paid to scale-up

Table 10.2. Selected commercial applications of power ultrasound in the food industry (Adapted from Patist and Bates, 2010)

Application	Description	Production Rate (m ³ /hour)	Power (kW)	Benefit (k\$/year)	Payback Time
Defoaming	Increased production capacity	>6	0.3	1,000	6 weeks
Emulsification	Reformulation & improved shelf life	>7	32	500	1 year
Extraction	Yield increase	>36	32	2,000	4 months

issues. Numbers of laboratory scale ultrasound prototypes have been successfully scaled up and commercialized in Europe and in the United States (Patist and Bates, 2010). For example, Cavitus Pty Ltd (2007), among others, has developed commercial high-intensity ultrasound extraction systems for the food and beverage industry. Ultrasound application in wine industry uses a 32-kW unit to treat 50 m³/hour of must for the extraction of grape color and anthocyanin during the fermentation process. For applications such as mayonnaise, an excellent white color is achieved which reflects the small particle size and narrow size distribution. In another commercial application, the traditional homogenizer was replaced by ultrasound, which enabled 50% reduction in the use of expensive emulsifier. In oilseed processing industry, ultrasound was used for defoaming that led to 5–10% throughput increase. This translated to a return on the investment of less than 2 months. The energy consumption of the ultrasonic defoaming system is in the order of 300–600 Watts. An automatic oak wine barrel cleaning system using a 4-kW ultrasonic transducer can be used to effectively remove tartrates and Brett organisms (Clark, 2008). An ultrasound-assisted seasoning process developed by Procter & Gamble increases the coating adhesion rate from 66.7% for traditional method to 98.6% (Quan, 2010). Ultrasonic cutting is another application that has been commercialized in the food industry. Selected power ultrasound applications in the food industry are tabulated in Table 10.2.

Manufacturers of high-power ultrasound equipment have been focusing on the design of large flow-continuous treatment chambers (flow cells).

**Figure 10.6.** 16 kW continuous power ultrasound treatment system. (Courtesy of Hielscher USA, Inc.) (For color detail, see color plate section.)

This facilitated the reduction of cost per volume material treated. A typical large flow chamber provides 8–16 kW of power for flows ranging from 5 to 500 L/minute, depending on the application (Figure 10.6). Larger flow rates would require multiple systems in series or parallel. Current power ultrasound systems have an energy efficiency of around 85% which indicates that most of the power sent to the transducer is transferred into the medium.

Glossary

Acoustic power density (APD): It is given by the dissipated power into a liquid measured with the calorimetric method divided by the volume of the liquid.

Cavitation: The formation, growth, and implosion of tiny gas bubbles or cavities in a liquid when ultrasound travels through it.

Magnetostrictive transducer: Transducers driven by the effect of certain metals, especially nickel, and certain other materials such as ytterbium compounds, which expand and contract in an alternating magnetic field.

Manothermosonication: An ultrasound treatment in a liquid at lethal temperature and elevated pressure.

Piezoelectric transducer: Transducers driven by the effect of certain crystals, such as lead-zirconate-titanate, and other materials, which expand and contract in an alternating (charged) electrical field.

Power density: Ultrasound power from the generator or that dissipated in a treatment medium divided by the area of insonating surface of the probe.

Power ultrasound: Ultrasound waves with frequencies in the range of 20–100 kHz and power intensity in the range of 10–1,000 W/cm².

Sonication: An ultrasound treatment in a liquid.

Thermosonication: An ultrasound treatment in a liquid at lethal temperature.

Water jet (also liquid jet and microjet): Inflow of high-speed liquid at a solid–liquid interface caused by asymmetric implosion of a cavitating bubble in the vicinity the solid.

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Section II

Electromagnetic Processes

Chapter 11

Pulsed Electric Fields Processing Basics

Olga Martín-Belloso and Robert Soliva-Fortuny

1. Introduction

The utility of electric fields to stabilize food products is known from the beginning of the twentieth century. The lethal effects of nonpulsed alternating currents against microorganisms were studied to design processes that, however, had a strong thermal effect. Thus, this technology was used to treat grape must (Tracy, 1932) or to pasteurize milk (Moses, 1938). However, the technology was replaced by others during the 1940s due to the high energy costs (Palaniappan and Sastry, 1991). A new process called electrohydraulic treatment was investigated in the 1950s. This treatment consisted of the application of high-intensity field discharges provoking the formation of electric arches, and in turn the generation of pressure shock waves in a liquid medium placed within a pressure vessel, thus leading to the inactivation of microorganisms and enzymes (Palaniappan and Sastry, 1991). In the 1960s, several studies demonstrated that the effect of electric fields against microorganisms was mostly due to the effects of electricity on cell disruption, thus leaving electrochemical reactions and temperature increase as secondary effects. These effects can be substantially minimized when energy is applied in the form of homogeneous nonarcing pulses (Doevenspeck, 1961; Sale and Hamilton, 1967).

During the 1990s and up to our days, the number of research groups studying the effectiveness of

pulsed electric field (PEF) treatments on several food products has continuously increased. Most of the work has concentrated on pasteurization of liquid food products, but enzyme inactivation, as well as electroporabilization of plant cells, or induction of stress reactions and secondary metabolite production are still under investigation (Toepfl et al., 2005).

The increasing consumer interest for highly nutritious fresh-like food products, together with the search for environmentally friendly processing technologies, has aided in the development of emerging nonthermal technologies. PEFs, understood as the use of external electrical fields of moderate or high intensity of short duration—a few microseconds—can induce the formation of pores in the cell membranes, leading to a rapid breakdown and the induction of structural or functional changes in the food matrix with reduced energy costs. However, commercial exploitation of PEF systems has been inhibited up to the moment, since efforts still need to be undertaken to develop energy-efficient industrial equipment and to study potential applications on several food products.

This chapter will review the principles of action of this emerging technology as well as the main key advantages. The design of PEF systems as well as the influence of the main critical parameters will also be discussed including their main limitations and challenges for future research. Eventually, some potential applications for the pasteurization of liquid foods, induction of stress reactions, or permeabilization of biological tissues applied to food matrices will be presented.

2. Mechanisms of Action

2.1. Mechanism of Cell Electroporation

The application of electrical fields to biological cells causes buildup of electrical charges at the cell membrane (Schoenbach et al., 1997). Membrane disruption occurs when the induced membrane potential exceeds a critical value of 1 V in many cellular systems, which, for example, corresponds to an external electric field of about 10 kV/cm for *Escherichia coli* (Castro et al., 1993) or to fields in the range of 1–2 kV/cm for plant cells.

Several theories have been proposed to explain the effects of PEF on cells. Among them, the most studied are electrical breakdown and electroporation or disruption of cell membranes (Zimmermann, 1986; Castro et al., 1993).

The electrical breakdown theory considers the cell membrane as a capacitor filled with dielectric material. The accumulation of charges with opposite polarity on both sides of the membrane causes the building-up of a naturally occurring 10-mV transmembrane potential (Zimmermann, 1986). The application of an external electrical field induces a stronger polarization of the membrane, thus resulting in an increase in the transmembrane potential, which in turn leads to the reduction in the cell membrane thickness. When a critical value—on the order of 1 V—is exceeded, membrane breakdown occurs, depending on the initial thickness of the cell membrane as well as on its barrier functional properties (Zimmermann, 1996). It is assumed that breakdown causes the formation of transmembrane pores that are filled with conductive solution, thus facilitating the electrical discharge at the membrane and speeding up membrane decomposition. Hence, if the amount and size of pores is large enough in relation to the total membrane surface, irreversible membrane breakdown is attained. The electrical breakdown of cellular membranes has been explored based on model systems such as phospholipid vesicles, planar bilayers, or even microorganisms but, until now, there is no clear evidence on this mechanism at the cellular level (Zimmermann et al., 1974; Chernomordik et al., 1987; Ho and Mittal, 2000).

The mechanism that explains the formation of pores and their temporal stabilization to allow transport phenomena between the intra- and extracellular media is not yet very well defined. Kinoshita and Tsong (1977) proposed a two-step mechanism for pore formation in which the initial perforation is a response to an electrical suprathreshold potential followed by a time-dependent expansion of the pore size. When the voltage applied is high enough, application of the voltage field causes immediate widening of these pores. According to this, larger pores will be obtained by increasing the intensity of the electric field as well as the pulse duration or by reducing the ionic strength of the medium. If the voltage exceeds a threshold value, the pore diameter may increase to a point where normal attractive forces cannot reseal the breach when the field force is removed. The value for the field to cause rupture depends on several factors. For instance, an electric field of 2.2 kV/cm is necessary to induce pores of about 1 nm in diameter in human erythrocytes (Kinoshita and Tsong, 1979). Exposure to fields of duration between 10^{-7} and 10^{-4} seconds did not result into permanent effects. However, at times greater than 10^{-4} seconds, the induced ruptures did not reseal and permanent channels were created (Sale and Hamilton, 1967).

Electroporation is based on the premise that proteins and lipid bilayer in cell membranes are temporarily destabilized by a high voltage electric field pulse (Castro et al., 1993). Then, the biological role of the cell membrane, acting as a semipermeable barrier responsible for mass transfer, can be seriously affected. After being exposed to an electric field, cell plasma membranes become permeable to small size molecules, which can eventually cause swelling of the cell and even rupture of the membrane (Vega-Mercado et al., 1996a). The process that is thought to occur initiates with the formation of spontaneous hydrophobic pores and the movement of ions and water dipoles through them. After this stage, membrane lipid molecules would rearrange to form more stable hydrophilic pores. Molecular reorientation theories are supported by experimental evidence, which indicates pore formation on the surface of cells. Protein channels, pores, and pumps in cell membranes are

likely to host this spontaneous pore formation in cell membrane due to their high sensitivity to changes in the transmembrane potential (Tsong, 1990).

Most microscopic studies indicate physical evidence of the effect of PEF on cell membranes. Microscopy observations have revealed perforated cells (Márquez et al., 1997; Elez-Martínez et al., 2005), increased roughness of cell walls (Calderón-Miranda et al., 1999), shrinkage of cytoplasm and thinning of cell walls (Pothakamury et al., 1995), and leakage of cellular contents (Simpson et al., 1999; Elez-Martínez et al., 2005). In addition, Picart et al. (2002) reported some morphological changes in *Listeria innocua* by means of atomic force microscopy. However, cytoplasm shrinkage and destruction of cell organelles when applying high-voltage treatments has been shown to affect only a relatively low number of cells, which suggests that inactivation pathways are more complex than the previously proposed.

2.2. Mechanism of Action on Food Components

The mechanisms that mediate the effects of PEF on food constituents are still unclear. However, evidence on the effect of intense PEF treatments on the functionality of some bioactive molecules, such as enzymes and proteins, has been reported during the past years. Nonetheless, the reported changes have been shown to be irreversible in some cases, which makes the difference with thermal treatments. The amino acid groups present in proteins create highly asymmetric spatial distributions of charge that lead to strongly polarized regions in their molecular structure (Laberge, 1998). Because of a complex network of noncovalent (electrostatic forces, ion pairing, van der Waals forces, hydrogen bonding, and interaction hydrophobic effect) as well as covalent interactions (disulfide bonds), the structural stability and functionality are maintained (Wong, 1995). This equilibrium could be altered by strong electric fields, which could cause protein unfolding and denaturation, breakdown of covalent bonds and oxidation–reduction reactions, like those between sulfide groups and disulfide bonds, because of

the charge polarization (Barsotti and Cheftel, 1999). Several authors have suggested that PEF treatments induce changes at the level of the secondary structure of proteins. Yang et al. (2004) found that inactivation of pepsin by PEF was correlated to the loss of β -sheet secondary structure of pepsin molecules. Consistently, Zhong et al. (2007) reported dramatic changes in the secondary structure of PPO and peroxidase (POD) enzymes treated with intense treatments of 25 kV/cm for 1,740 μ s and 744 μ s, respectively.

It is known that electric fields can influence the conformational state of charged molecules by interacting with charges, dipoles, or induced dipole chemical reactions. Charged groups and structures are highly susceptible to various types of electric field perturbations and these changes cause modifications in the structure and, consequently, in the functionality of the food constituent. According to Chen and Wu (2006), the possible mechanisms involved in the loss of functionality of cell membrane proteins include Joule heating damages, electroconformational changes, or both. Barsotti et al. (2002) reported that series of 20 exponential decay pulses of 31.5 kV/cm at 1 Hz applied to a buffer solution containing ovalbumin induced at least a partial unfolding of the protein structure or enhanced the ionization of SH groups into a more reactive S^- form. The number of pulses and the energy applied per pulse significantly influenced SH group reactivity. However, these modifications appeared to be transient, provided that when the pulse-processed ovalbumin solution was kept at 4°C for more than 30 minutes, the extent of the observed changes decreased and were completely reversed after 24 hours. Consistently, Perez and Pilosof (2004) reported the formation of aggregates involving covalent bonds in β -lactoglobulin exposed to PEF treatments of 12.5 kV/cm, thus resulting in a 40% modification of the native structure of the protein. It was also shown that the thermal stability of β -lactoglobulin was greatly reduced when applying PEF treatments. Consistently, the consequences of the changes in the conformational structure of proteins can be often macroscopically assessed. Li et al. (2006) reported a significant increase in the solubility, emulsibility, foaming capacity, hydrophobicity,

and degree of denaturation of a soybean protein isolate with increasing electric field and treatment time. Nevertheless, these effects greatly differ between treatments, food components, and food matrices. Most studies conclude that field strength, pulse width, pulse number, and pulse shape are the main critical parameters affecting protein inactivation.

The effect of PEF treatment on other food constituents needs to be further investigated. Pulsed electrical treatments have been found to degrade some small organic compounds, including phenols, in water solutions (Sun et al., 2000). However, no significant changes in the content of phenolics or carotenoids are reported when contained within the food matrix (Min et al., 2003a; Sánchez-Moreno et al., 2005; Cortés et al., 2006). Little influence has been reported on food lipidic constituents. No changes in the fatty acid content have been attributed to the PEFs (Odriozola-Serrano et al., 2006; Garde-Cerdán et al., 2007; Zulueta et al., 2007). On the contrary, it has also been shown that PEF treatments induce fewer changes in vitamin content than conventional thermal processing treatments. Elez-Martínez and Martín-Belloso (2007a) reported that pulses applied in monopolar mode, as well as a higher electric field strength, treatment time, pulse frequency and width, led to higher reductions in the vitamin C content of both orange juice and cold, vegetable-based “gazpacho” soup, although the PEF-treated products always showed higher vitamin C retention than those of the heat-pasteurized products. However, it is unlikely that vitamin depletion can be attributed to conformational changes induced by electric fields.

3. Process Engineering of Pulsed Electric Field Treatments

3.1. Electrical Properties of Food Products

When an electric field is applied through a treatment chamber containing a food product, the polarization of dipolar molecules, as well as the displacement of ions within the food product, account for the induction of capacitive and resistive electrical currents (Riley and Watson, 1987; Zhang et al., 1995). A food product contained between two electrodes dis-

sipates energy as resistance, but also stores energy as a condenser. Thus, the performance of PEF treatments greatly depends on the electrical properties of the medium to be treated. Resistance, conductivity, and dielectric properties of a food affect the interactions between the product and the electric field (Lewis, 1993).

Each material has a specific resistance that is called resistivity. Depending on its resistivity, a product will offer more resistance to the flow of electricity. The inverse parameter is conductivity and represents the ability of the medium to conduct and electric current. Conductivity increases as ionic strength of the medium and temperature increase. Hence, media rich in ionic species, such as tomato juice, may pose problems in achieving a significant voltage for a supercritical field strength. The choice of electrode configuration and geometry with high load resistivity may help to reduce this effect and to improve voltage division in the discharge circuit (Toepfl et al., 2006).

The relative dielectric constant of a food is a measure of the extent to which it concentrates electrostatic lines of flux. It is the ratio of the amount of stored electrical energy when a potential is applied, relative to the permittivity of air or vacuum. The higher the dielectric constant, the more energy the medium is able to store.

3.2. Considerations Concerning Pulsed Electric Field Treatments

PEF generation systems transform low voltage energy into pulsed fields of high intensity. Generally, the time between pulses is much longer than the duration of the pulse, since slow charging and fast energy discharge are required. The generation of an electric field, E , between two electrodes is directly proportional to the charging voltage, U , and inversely proportional to the distance between them, d (Equation (11.1)).

$$E = \frac{U}{d} \quad (11.1)$$

The electric power is usually stored in a bank of capacitors that are discharged into the treatment chamber across a high-voltage switch and protective

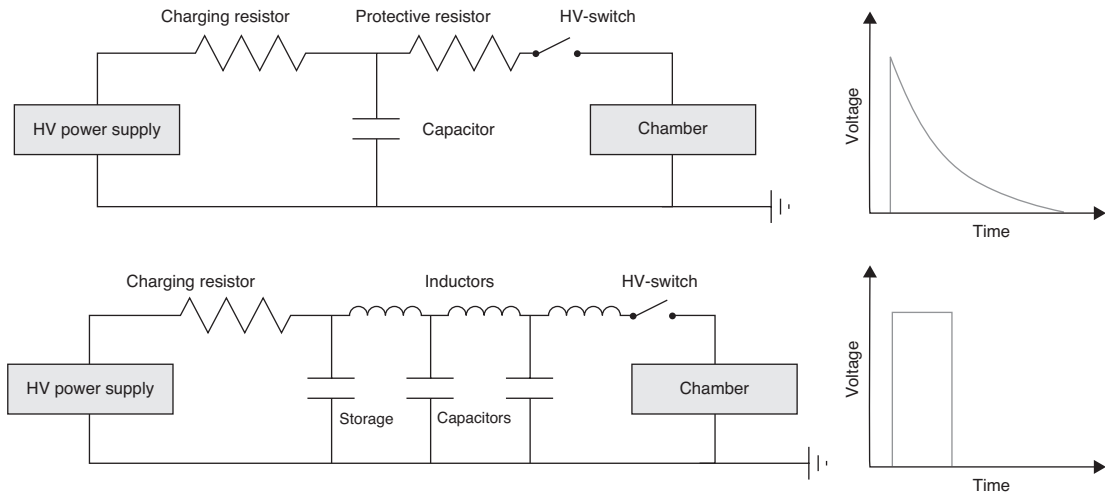


Figure 11.1. Simplified circuitry for the generation of exponential decay and square wave pulses.

resistors within microseconds. Pulse duration (τ) directly depends on the medium resistance (R) and the capacity of the condensers bank (C_0) as given by Equation (11.2).

$$\tau = R C_0 \quad (11.2)$$

Hence, total treatment time will be the product of the number of pulses by pulse duration. The discharge process that generates the pulsed field in the treatment chamber containing the medium to be treated can be performed using different circuit conformations that result in exponential decay, square, or oscillating pulsed waves. Oscillatory decay pulses are the least efficient because they do not achieve continuous exposure to high-intensity fields, which prevents the cell membrane from irreversible breakdown over a large area (Jeyamkondan et al., 1999). Thus, the most commonly used pulse waves are exponential decay and square (Figure 11.1). Exponential decay waves rise rapidly to a maximum value and decay slowly to zero. A simplified circuit for the generation of exponential waves consists of DC power supply that charges a capacitor bank connected in series with a charging resistor (R_s). When a trigger signal is applied, the charge stored in the capacitor flows through the food in the treatment chamber. Increasing the gap between electrodes to obtain high flow

rate capacity implies increasing the charging voltage and, therefore, significant stressing of the switching system. On the contrary, a square waveform can be obtained by using a pulse-forming network consisting of an array of capacitors and inductors and solid state switching devices (Figure 11.1).

Several switching systems have been applied in PEF equipment, including vacuum or gas spark gaps, thyatron switches, semiconductor, or high-power transistor switches. Each type of switch allows different repetition rates and maximum withstandable voltage. A spark gap consists of an arrangement of two conducting electrodes separated by a gap usually filled with gas such as air. When a voltage exceeding a breakdown value is supplied, a spark is formed, thus ionizing the gas and drastically reducing its electrical resistance. An electric current then flows until the path of ionized gas is broken or the current reduces below the breakdown value. However, maximum repetition rates around 100 Hz and a maximum lifetime of approximately 10^6 shots are strong limitations that must be taken into account for spark-gap switches. Thyatron switches have been widely used for pulse applications. A typical hot-cathode thyatron uses a heated filament cathode, completely contained within a shield assembly with a control grid on one open side, which faces a plate-shaped anode.

As long as a slight positive voltage is applied to the control electrode, gas between the anode and cathode ionizes and conducts current. Thyatron switches provide high repetition rates within the kHz range. Thyristor switches are replacing thyatron switches. The thyristor is a four-layer semiconducting device, with each layer consisting of an alternately N-type or P-type semiconductor. Thyristor configurations such as silicone controlled rectifier (SCR) or gate turn-off (GTO) require less complex circuitry and have a better performance for pulse applications. Their main limitations are the reliability in long-term use, treatment chamber blocking, or electromagnetic interference (Toepfl et al., 2005).

Depending on the chamber configuration, the field strength and pulse duration will not provide enough information about the intensity of the treatment. Chamber geometry can substantially condition the area that exceeds the critical electric field strength necessary to achieve desired results. In that case, the amount of energy that is supplied to the food product takes relevance to understand the overall effects of PEF treatments and, in turn, it helps to compare the effectiveness of different treatments. For exponential and square wave pulses, the energy density (Q , J/m³) supplied to the medium can be computed by Equation (11.3), whereas Equation (11.4) accounts for the energy density supplied when square pulses are applied.

$$Q = N \frac{U_0^2 C}{2v} \quad (11.3)$$

$$Q = N \frac{U_0^2 C}{v} \quad (11.4)$$

where N is the number of pulses delivered to the medium, U_0 is the peak voltage (V), C is the capacitance of the capacitor (F), and v is the volume of the treatment chamber (m³).

Energy density stands out as an advantageous parameter when quantifying operational costs of PEF treatments. However, it does not adequately describe processing parameters such as pulse duration or number of pulses per volume unit, which are required to provide complete information about the treatment.

The polarity of the pulsed field can be reversed by using a system with two capacitor banks placed in series. A DC power supply is used to charge the first capacitor bank and a series switch is used to discharge the stored energy through the second capacitor bank and the treatment chamber. The pulse has positive polarity when the output of the DC power supply is positive with respect to the ground. When the voltage in the treatment chamber is near zero, a shunt switch is turned on so that energy stored in the second capacitor bank is discharged through the chamber as a negative pulse. Hence, a pair of bipolar pulses is produced each time the main capacitor is charged. Another possibility is to use instant-charge-reversal pulses, which are considerably different from standard bipolar pulses. Standard bipolar systems require a relaxation time between pulses, and this time can be too long even when using high-frequency pulsers. Instant-charge-reversal pulses can drastically reduce energy requirements of standard bipolar pulse generation systems.

4. Processing Critical Parameters

PEF processing involves several factors that are critical to attain pasteurization effects. Some of them are intrinsic to the food product and the others are extrinsic, depending on the system parameters as well as on the environmental conditions that are held during treatments.

The principal parameters intrinsic to the food product that determine the effectiveness of PEF treatments are the electrical conductivity, pH, food composition, and structure and characteristics of the agent to be inactivated.

The main processing parameters and environmental conditions affecting the inactivation of microorganisms and enzymes by PEF are electric field strength, treatment time, pulse number, pulse width, pulse shape, pulse polarity, and treatment temperature.

4.1. Food Intrinsic Parameters

The effectiveness of PEF treatments greatly depends on the composition and physical properties of the

food product. Concerning the composition of the medium, the presence of ions in the food matrix may substantially change the result of a PEF treatment. In this regard, a large amount of cations or anions would account for a high ionic strength, which is associated with high conductivity or low resistivity. In general, PEF pasteurization is most lethal under conditions of low ionic strength, low conductivity, and high resistivity. Apart from the influence on electric strength, conductivity plays a role in determining the difference between ionic strength in the medium and the cytoplasm (Jayaram et al., 1992). The presence of ions generally increases conduction through the material, but large concentrations may have an opposite effect. In foods with low ionic strength, the membrane will be weakened, thus becoming more susceptible to PEF treatments. For instance, Vega-Mercado et al. (1997a) achieved 3, 2, and 1 log reductions of *E. coli* in simulated milk with ionic strengths of 28, 56, and 156 mM when applying 16 pulses of 40 kV/cm at 10°C. Bivalent ions such as Mg^{2+} or Ca^{2+} have a protective effect on membranes, thus leading to a lower effect of PEF treatments on microorganisms. Regarding fruit juices, the order of juice conductance from highest to lowest is tomato, orange, pineapple, grape, apple, and cranberry (Raso et al., 1998). Furthermore, lipids may act as an insulating barrier against the applied voltage and exert a protective effect on the cells (Grahl and Markl, 1996).

PEF treatments show an increased antimicrobial effect at low medium pH. Liu et al. (1997) reported a synergistic killing effect against *E. coli* between the PEF treatment and the addition of organic acids at pH 3.4, but not at pH 6.4. Inactivation of the pathogen by combinations of electric pulse and organic acids was enhanced by an increase in temperature, field strength, and number of electric pulses. In addition, inactivation was greater when cells were suspended in ionic media than in nonionic media. Consistently, Wouters and Smelt (1997, 1999) reported an increased effectivity of PEF treatments at low pH in inactivating *E. coli*, *Saccharomyces cerevisiae*, *Lactobacillus plantarum*, and *L. innocua*. The synergism between organic acids and PEF can be attributed to the fact that both treatments target the cell membrane.

In this way, PEF would permeabilize the cell wall and membrane, enhancing the entry of undissociated acids into the bacterial cell. However, lower pH may also act antagonistically with PEF. Evrendilek and Zhang (2003) found that a pH of 3.6 and temperatures of 4 and 40°C caused significant decreases in the inactivation of *E. coli* O157:H7 treated by PEF. In contrast, pre-PEF treatments, pHs of 5.2 and 7.0, at 35°C did not result in any resistance of microbial cells to inactivation by PEF treatment.

4.2. Processing System and Environmental Parameters

Inactivation of microorganisms and enzymes by PEF increases when the electric field increases. The electric field (E) is defined as the quotient of the electric potential difference between electrodes (U) by the separation distance (d). Field intensity, together with treatment time, is the most critical parameter to achieve food pasteurization, which is consistent with the electroporation theory, in which the induced potential difference across the cell membrane is proportional to the applied electric field. It has been shown that the critical external field strength (E_c) is highly dependent on cell size as well as cell orientation in the field (Heinz et al., 2002). Thus, with decreasing cell size the required field strength sharply increases and variations in cell shape can cause considerable increase of the field strength necessary to achieve inactivation. In this way, electroporation in yeast cells such as *S. cerevisiae* occurs at field strengths above 2 kV/cm, whereas smaller bacterial cells such as *L. innocua* require a minimum of 15 kV/cm.

Treatment time is defined as the product of the pulse width and the average number of pulses. Generally, an increase in treatment time results in increased microbial or enzyme inactivation. Moreover, an increase in the treatment time necessarily means an increase in either the pulse duration or the number of pulses. The pulse width (τ), also called pulse duration, is defined as the time where the peak field is maintained for square wave pulses or the time until decay to 37% of the peak intensity for exponential pulses (Toepfl et al., 2005). For treatments

with similar field strength and treatment time, microbial inactivation decreases with pulse width (Jayaram et al., 1992; Elez-Martínez et al., 2004, 2005). Thus, for the same amount of energy supplied to the product, short pulses of a few microseconds usually give greater microbial inactivation than a few pulses of long duration. In addition, an increase in pulse duration may entail an unacceptable increase in temperature, so the energy density that is supplied to the system must be taken into consideration. Indeed, since water electrolysis may occur when the pulse width is greater than 10 μ s, extreme pulse widths may cause microbial death by the generated electrolytic by-products rather than to solely by PEF treatment (Ho and Mittal, 2000). Pulse width also influences the critical electric field. Namely, for pulse widths greater than 50 μ s, E_c is 4.9 kV/cm, whereas with pulse widths less than 2 μ s, E_c is 40 kV/cm (Schoenbach et al., 1997). However most systems do not allow modifying pulse duration and this makes treatment time to depend proportionally on the number of pulses (n). All studies show an increase in inactivation of microorganisms and enzymes with greater n , though the marginal inactivation attained at a large number of pulses is usually much smaller than those achieved during the first few pulses. Ho et al. (1995) reported that when $n > 10$ there was a statistically insignificant effect on the inactivation of *Pseudomonas fluorescens*. Nevertheless, treatment times achieving several microbial reductions do not suffice to attain significant inactivation of most enzymes.

Pulses are performed either in monopolar or bipolar and usually exhibit either square wave or exponential decay shape. In an exponential pulse, the voltage increases to a selected peak field intensity and decreases exponentially. There is major agreement on the fact that square wave pulses are more effective than exponential wave pulses. Zhang et al. (1994) reported that for 20 pulses of the same energy applied to apple juice, square wave pulses resulted in a 0.5 greater log colony reduction than exponential decay pulses. Moreover, pulse polarization also plays a significant role. Bipolar pulses are usually more lethal against microorganisms than monopolar pulses (Qin et al., 1994; Ho et al., 1995; Elez-Martínez et al., 2004, 2005). However, there are some controversial

results about the effect of polarity on the inactivation of enzymes. Bipolar pulses achieved higher inactivation of polyphenoloxidase (PPO) and pectin methylesterase (PME) than monopolar fields (Giner et al., 2002; Elez-Martínez et al., 2007b), whereas either the inverse behaviour or no significant differences between polarities have been reported for POD in orange juice and tomato PME, respectively (Giner et al., 2000; Elez-Martínez et al., 2007b).

Processing temperature plays a determinant role in the effectivity of PEF treatments. There is an overwhelming agreement on the fact that temperature has a synergistic effect on treatment efficacy (Jayaram et al., 1992; Zhang et al., 1995; Pothakamury et al., 1996; Vega-Mercado et al., 1997b; Reina et al., 1998; Shin and Pyun, 1999). Temperatures in the range of 45–55°C appear to be the most effective in attaining microbial reduction, which is thought to be due to the phase transition of the cell membrane phospholipids, from a rigid gel to a liquid-crystalline structure. For instance, an increase from 1 to 4 log cycles inactivation of *Salmonella dublin* has been reported in milk when increasing treatment temperature from 40 to 50°C (Dunn and Pearlman, 1987). In this same way, Heinz et al. (2003) observed a clear synergistic effect of elevated treatment temperature of 35–65°C with PEF treatments on microbial inactivation in apple juice. The need to preheat the juice before treatment provided the possibility to recover the dissipated electrical energy after treatment, thus resulting in a strong reduction of the energy required to achieve a 6-log inactivation from above 100 to less than 40 kJ/kg.

5. Pulsed Electric Field Processing Systems

The generation of high field strengths and the design of adequate treatment chambers that allow uniform treatment with minimal temperature increase are among the most determinant aspects influencing the effectiveness of PEF systems. Most available systems, specially those applying high intensities, incorporate a cooling unit in order to prevent the product from an excessive rise in temperature during treatment.

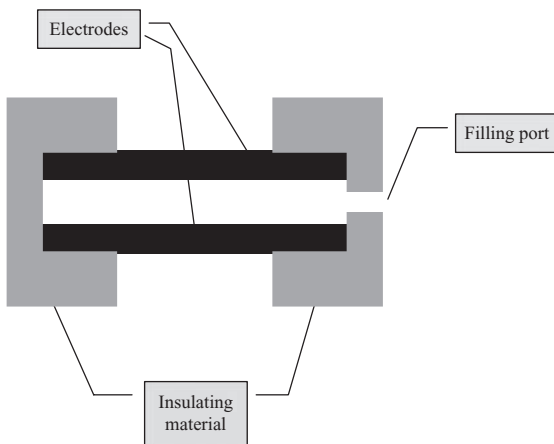


Figure 11.2. Schematic of a batch treatment chamber with parallel electrodes.

Treatment systems can be classified as batch or continuous, which substantially conditions the design of treatment chamber. The first batch or static chambers that were used for food processing were designed by Sale and Hamilton (1967) and Dunn and Pearlman (1987) and allowed maximum field strengths of 25 and 30 kV/cm, respectively. These models consisted of two parallel electrodes supported by an insulating material (Figure 11.2). Stainless steel electrodes have been traditionally used in most systems, although other materials such as gold, platinum, carbon, and metal oxides have been proposed as alternatives to the currently used electrodes (Bushnell et al., 1996). Among the different electrode configurations, parallel plates provide the most uniform electric field in a large usable area between the plates, but treatment intensity is reduced in boundary regions (Toepfl et al., 2005). Thus, a considerable part of the volume may remain undertreated in batch chambers, especially when a homogenizing system is not incorporated. This can be prevented by using continuous treatment chambers with multiple treatment zones in series or baffled flow channels (Zhang et al., 1995). Another chamber configuration, developed by Lubicki and Jayaram (1997), uses a glass coil surrounding the anode that avoids product exposure to the electrode surface. The volume of the

chamber was 20 cm³ and requires a filling liquid with high conductivity and similar permittivity to the sample used because there is no inactivation with a nonconductive medium.

Continuous systems have essentially the same high voltage pulse generator devices but incorporate a pumping continuous system that allows achieving continuous processing, which is necessary for industrial application. High flow rates require pulses to be applied with high repetition rates, which can lead to undesirable heating of the treated media. A cooling unit must be then incorporated in order to maintain a constant temperature. Different chamber configurations have been proposed for continuous treatment systems. A parallel plate continuous chamber, designed as an evolution of batch chambers, was proposed by Qin et al. (1994). The system was essentially a horizontal chamber containing a series of U-shaped channels and allowed the circulation of water to cool down the product. However, the baffled tube made difficult to monitor the intensity of the treatment applied to the food product. Therefore, a coaxial design was developed by the same research group using a finite element electric field computation (Qin et al., 1995a). Coaxial chambers are basically composed of an inner cylinder surrounded by an outer annular cylindrical electrode that allows food to flow between them. A protruded outer electrode surface enhances the electric field within the treatment zones and reduces the field intensity in the remaining portion of the chamber. A co-linear chamber is another possible configuration, consisting of a set of hollow, cylindrical electrodes separated by insulators. The product is pumped through the drilling and the flow is not disturbed by any impediments (Toepfl et al., 2005).

Geometry of the treatment chamber has a decisive impact on its total resistance. The ratio of the chamber resistance and that of the protective resistor is very important, as the applied charging voltage is divided between them. The protective resistor is necessary to prevent breakdown of the switching system in case of short circuit. A great amount of energy might be lost if the resistance of the protective resistor is in the same range as that of the treatment chamber.

6. Limitations and Challenges when Designing Pulsed Electric Field Processing Systems

Several factors limit the exploitation of PEF technology on an industrial scale. On the one hand, there is a need for the development of generators that provide sufficient electrical field strength, power, and repetition rate at reasonable costs. Once experimental systems can be scaled up, more reliable data will be available to estimate the specific requirements and costs for different applications. Adequate cooling systems that allow energy recovery need to be developed to attain systems that can be industrially exploited. In addition, special attention needs to be devoted to treatment uniformity. Thus, chambers that achieve homogeneous fields with appropriate degassing systems need to be implemented. In this regard, the presence of bubbles is of outstanding importance. Góngora-Nieto et al. (2003a) reported the perturbing effect of air bubbles present within treatment chamber, indicating that a significant drop in field strength occurs in boundary regions of bubbles, thus causing food-safety problems. On the other hand, suitable materials for processing systems need to be developed. Problems due to electrochemical reactions between the electrode and the food product have been discussed in the past (Morren et al., 2003; Toepfl et al., 2004). Apart from the reduction in electrode life time, release of particles and heavy metals from the electrode may account for toxicity problems. There is a challenge to replace the commonly used stainless steel electrodes by other materials or to modify the pulse generator systems to reduce the amount of electrochemical reactions. The application of carbon electrodes may overcome this problem (Toepfl et al., 2004). Also, the application of shorter pulses or switching systems without leak current could help to avoid electrochemical reactions (Mastwijk, 2004).

7. Potential Food Processing Applications of Pulsed Electric Fields

7.1. Applications on Liquid Foods

Most applications of PEF technology on liquid foods are devoted to assure safety of the treated product by

destroying hazardous microorganisms that could potentially grow in the food. Microbial inactivation can be successfully achieved by using PEF technology under adequate processing conditions. Table 11.1 provides a summary of the results achieved by several researchers on food-borne pathogenic microorganisms in different food products. Most studies that have been undertaken in the last years have studied the utility of PEF to get stable fruit juices. Namely, the effect of PEF on the shelf life of orange juice has been studied by many researchers because of its economic importance. Jia et al. (1999) reported that a treatment of 30 kV/cm during 480 μ s was effective in inactivating the native microflora of orange juice without significantly altering the sensory character of the fresh-squeezed juice. An effect similar to that obtained with a thermal pasteurization treatment (94.6°C, 30 seconds) was achieved when applying 35 kV/cm during 59 μ s (Yeom et al., 2000a, 2000b; Ayhan et al., 2002). Under these conditions, microbial counts below 1 log CFU/mL were reported during 112 days of storage both at refrigeration and at ambient temperatures. Similar results were reported by Min et al. (2003a), who obtained microbiologically stable juices during 112 days at 4°C after processing at 40 kV/cm during 97 μ s. More intense treatments of 35 kV/cm during 1,000 μ s were required by Elez-Martínez et al. (2006a) to achieve both microbial and enzymatic stability in orange juice during at least 56 days of refrigerated storage at 4°C. Qin et al. (1995b) applied a treatment of 36 kV/cm and 250 μ s, thus achieving microbial stability during more than 3 weeks. Evrendilek et al. (2000) reported a shelf life of at least 67 days when processing at 35 kV/cm and 94 μ s. Similar results were found by these authors for apple cider processed under same conditions. Consistently, Min et al. (2003a) reported a microbiological shelf life of at least 112 days for tomato juice processed at 40 kV/cm during 57 ms, similar to that achieved with a pasteurization treatment applied at 92°C for 90 seconds. On the other hand, the effects of PEF on most enzymes can be at least as irreversible as those achieved through thermal processing (Table 11.2). Yeom et al. (2000b) reported a 88% reduction in PME activity of orange juice, instead of a 98% reduction for thermally processed juices. No increase in PME activity was reported during

Table 11.1. Effect of PEF treatments on food-borne pathogens in some selected food matrices.

Medium	Microorganism	Treatment Conditions	Log Reduction	Reference
0.1% w/v peptone	<i>Pseudomonas fluorescens</i>	Batch: 10 kV/cm, 10 pulses, 2 μ s, 20°C	6	Ho et al., 1995
0.1M NaCl (pH = 7.2)	<i>Yersinia enterocolitica</i>	Batch: 75 kV, 250 pulses	7	Lubicki and Jayaram, 1997
Whole milk	<i>Staphylococcus aureus</i>	Continuous: 35 kV/cm, 150 pulses, 8 μ s, <40°C	4.5	Sobrinho-López et al., 2006
Skimmed milk	<i>Escherichia coli</i>	Batch: 45 kV/cm, 64 pulses, 1.8-6 μ s, 15°C	3	Martín et al., 1997
Skimmed milk	<i>Salmonella dublin</i>	Continuous: 25 kV/cm, 100 pulses, 1 μ s, 50°C	4	Sensoy et al., 1997
Liquid egg	<i>Listeria innocua</i>	Continuous: 50 kV/cm, 32 pulses, 2 μ s, 36°C	3.5	Calderón-Miranda et al., 1999
Liquid egg yolk	<i>E. coli</i> O157:H7, Salmonella Enteritidis	Continuous: 30 kV/cm, 105 pulses, 2 μ s, 40°C	5	Amiali et al., 2007
Liquid egg white	<i>E. coli</i> O157:H7, Salmonella Enteritidis	Continuous: 30 kV/cm, 60 pulses, 2 μ s, 40°C	2.9 3.7	Amiali et al., 2006
Peas soup	<i>Bacillus subtilis</i>	Continuous, 33 kV/cm, 30 pulses, 2 μ s, 55°C	5.3 6.5	Vega-Mercado et al., 1996b
Apple juice	<i>E. coli</i> O157:H7	Continuous: 30 kV/cm, 43 pulses, 4 μ s, 25°C	5	Evrendilek et al., 1999
Melon juice	<i>E. coli</i>	Continuous: 35 kV, 400 pulses, 4 μ s, <39°C	3.8 4.3	Mosqueda-Melgar et al., 2007
	<i>Salmonella</i> Enteritidis		4.3	
	<i>Listeria monocytogenes</i>		3.9	

subsequent storage. Consistently, Elez-Martínez et al. (2006a) did not observe changes in the residual PME and POD activities in PEF-treated orange juice during 56 days of storage. On the other hand, the sensory and nutritional properties of juices are substantially preserved. In this regard, the color, pH, acidity, soluble solids content, viscosity, flavor, or vitamin C content of juices such as orange, apple, and tomato were better preserved when processing by PEF than when using heat treatments (Evrendilek et al., 2000; Yeom et al., 2000b; Min and Zhang, 2003; Min et al., 2003a, 2003b; Elez-Martínez et al., 2006a). A similar trend was reported by Min et al. (2003b) in the lycopene content of PEF-treated tomato juice. Plaza et al. (2006) and Elez-Martínez et al. (2006a) reported a slight decrease in the antioxidant capacity of PEF-treated orange juice throughout storage. Nevertheless, these changes were not significantly different to those observed in the fresh-juice.

Results on milk and milk products are often less successful because of the composition and electrical properties of milk. Skimmed raw milk processed at

40 kV/cm for 40 μ s and stored at 4–6°C had a shelf life of 14 days (Qin et al., 1995b). Odriozola-Serrano et al. (2006) required much higher treatment intensities (35.5 kV/cm, 1,000 μ s) to attain microbial stability of whole raw milk during 5 days under refrigeration. Combination with mild heat treatments can significantly improve the performance of PEF treatments. Fernández-Molina et al. (2000) reported a shelf life of 30 days for PEF-treated milk by applying a mild heat treatment (73°C, 6s) before processing at 50 kV/cm during a total treatment time of 60 μ s. Substantial inactivation of some milk enzymes can be achieved provided that enough energy is supplied to the medium, which greatly limits application on these products (Soliva-Fortuny et al., 2006).

Hermawan et al. (2004) observed an increase in shelf life of PEF processed whole liquid egg, without significant changes in viscosity, electric conductivity, color, pH, or soluble solids during storage at 4°C. However, several authors have pointed out the need of combining PEF treatments with other techniques such as addition of nisin or mild thermal processing

Table 11.2. Effect of PEF treatments on enzyme inactivation in some selected food matrices

Medium	Enzyme	Treatment Conditions	% Inactivation	Reference
8.8% NaCl	Pectin methylesterase (tomato)	Batch: 24 kV/cm, 8,000 μ s, 15°C	93.8	Giner et al., 2000
Distilled water	Pectin methylesterase (tomato)	Batch: 30 kV/cm, 40,000 μ s	<10	Van Loey et al., 2002
Orange juice	Pectin methylesterase	Continuous: 35 kV/cm, 59 μ s, 60.1°C	88	Yeom et al., 2000a
Orange juice	Pectin methylesterase	Continuous: 35 kV/cm, 1,500 μ s, 40°C	80	Elez-Martínez et al., 2007b
McIlvaine buffer (pH = 6.5)	Polyphenoloxidase (apple)	Batch: 24.6 kV/cm, 6,000 μ s, 15°C	96	Giner et al., 2001
McIlvaine buffer (pH = 6.5)	Polyphenoloxidase (apple)	Batch: 31 kV/cm, 1,000 μ s	<10	Van Loey et al., 2002
McIlvaine buffer (pH = 6.5)	Polyphenoloxidase (peach)	Batch: 24.3 kV/cm, 5,000 μ s, 25°C	70	Giner et al., 2002
McIlvaine buffer (pH = 6.5)	Polyphenoloxidase (pear)	Batch: 22.3 kV/cm, 6,000 μ s, 15°C	62	Giner et al., 2001
Tomato juice	Lypoxigenase	Continuous: 35 kV/cm, 50 μ s, 30°C	80	Min et al., 2003b
Orange juice	Peroxidase	Continuous: 35 kV/cm, 1,500 μ s, 35°C	100	Elez-Martínez et al., 2006b
Buffer solution	Peroxidase (leek)	Continuous: 22 kV/cm, 1,863 μ s, 40°C	34.7	Zhong et al., 2005
Caseine solution	Protease (<i>P. fluorescens</i>)	Continuous: 14–15 kV/cm, 196 μ s	0	Vega-Mercado et al., 2001
Skimmed milk	Protease (<i>B. subtilis</i>)	Continuous: 35.5 kV/cm, 866 μ s, 46°C	81.1	Bendicho et al., 2003
Whole milk	Protease (<i>B. subtilis</i>)	Continuous: 35.5 kV/cm, 866 μ s, 46°C	57.1	Bendicho et al., 2003
Skimmed milk	Protease (<i>P. fluorescens</i>)	Continuous: 14–15 kV/cm, 196 μ s	60	Vega-Mercado et al., 2001
Buffer (pH = 2,1)	Pepsine	Continuous: 41.8 kV/cm, 126 μ s	83.8	Yang et al., 2004
Buffer (pH = 6,5)	Lysozyme (egg white)	Continuous: 38.2 kV/cm, 126 μ s	0	Yang et al., 2004
Deionized water	α -amilase (<i>B. licheniformis</i>)	Batch: 80 kV/cm, 60 μ s, 20°C	90	Ho et al., 1997
Buffer (pH = 5,1)	Glucosaoxidase (<i>A. Niger</i>)	Batch: 63 kV/cm, 60 μ s, 20°C	75	Ho et al., 1997
1 mM EDTA	Papaine (papaya)	Continuous: 50 kV/cm, 2,000 μ s, 35°C	90	Yeom et al., 1999
Buffer (pH = 7,2)	Lactate dehydrogenase (bovine heart)	Batch: 31.6 kV/cm, 192 μ s, 30°C	0	Barsotti et al., 2002
Buffer (pH = 2,9)	Quimotripsine	Continuous: 34.2 kV/cm, 126 μ s	4	Yang et al., 2004

to achieve microbial stability in egg products (Calderón-Miranda et al., 1999; Bazhal et al., 2006; Sampedro et al., 2006)

7.2. Applications on Solid Foods

The use of PEF treatment for the preservation of meat, fish, and other solid food products does not seem realistic as application of low-intensity electric field pulses usually has a detrimental effect on the microstructure. Other uses of PEF treatments on solid foods are more realistic. For instance, the application of moderate intensity pulses as a pretreatment for the extraction of valuable materials, mostly from plant sources, as well as for dehydration of organic materials from plant and animal origin, have good prospects for future research. Eshtiaghi and Knorr (1999) report a yield of 87%, similar to that attained after enzymatic maceration, together with an increased content of soluble solids and pigments. In the same way, increase in extractability of fermented black tea leaves and fresh or dry mint leaves under moderate electric field processing was reported by Sensoy and Sastry (2004). In a similar way, Jemai and Vorobiev (2006) have evaluated the use of PEFs as an intermediate treatment for the cold juice extraction from sugar beet. It was reported that the purity of pressed juices after a PEF treatment was systematically higher compared to that of untreated juices (96–98% and 90–93%, respectively). Tissue softening as a consequence of cell membrane permeabilization and loss of turgor in vegetable products such as apple, potato, and carrot treated by moderate PEF provides an opportunity to improve plant cutting and pressing processes (Fincan and Dejmek, 2003; Lebovka et al., 2004). Drying times and/or drying temperatures can be also significantly reduced with moderate PEF treatments. Ade-Omowaye et al. (2001, 2003) reported substantial reductions in drying and osmotic dehydration times of coconut and bell peppers, respectively, using pulse intensities around 2 kV/cm. Lebovka et al. (2007) pointed out an essential influence of PEF treatment at moderate electric field strengths (0.3–0.4 kV/cm) on drying of potato disks.

PEF can also provide a potential to induce stress reactions in plant systems or cell cultures. A mild sublethal treatment (0.6 kV/cm) of maize germs has been shown to increase oil yield and phytosterol productions, resulting in plant oil with a higher phytosterol concentration. Indeed, PEF treatments have also increased oil yield and isoflavonoid content in soy beans and olives (Guderjan et al., 2005).

8. Economical and Environmental Considerations of Pulsed Electric Field Treatments

PEF, as most emerging non-thermal techniques, can also help to improve environmental performance of food processing. In the last few years, the consumers' demand for "fresher" food products produced in an environmentally sustainable way, that is organic produce, has promoted the development of alternative processing techniques that require lower energy inputs or fewer resources than conventional techniques and, in addition, can improve utilization of raw materials and generated by-products. The energy requirements optimization of unit operations in the food industry have been extensively studied in the past decades for conventional processes, but their replacement by emerging non-thermal techniques may still potentially reduce energy consumption and operation costs (Toepfl et al., 2006). As most studies on PEF technology have been performed in a laboratory-scale, the results obtained are often difficult to scale-up.

PEF application for liquid pasteurization requires a higher input of energy than a thermal treatment, as heat recovery rates in thermal pasteurization are in the range of 90–95%. As already outlined, treatment intensities required to destroy microorganisms in liquid food products using PEF are much higher than the intensities applied to induce changes in plant or animal cells. Estimated investment costs for liquid pasteurization systems at industrial scale (5 t/hour) would be in the range of 2–3 million US dollars (Toepfl et al., 2006). However, the feasibility of applying this technology to processing of liquid foods greatly depends on both the electric properties of the product to be treated and the susceptibility

to PEF treatment of pathogenic and spoilage microorganisms that can be potentially found in each product. Pulse energy dissipation depends on the product and the energy density input, thus resulting in significant differences in temperature increase. Specifications on the energy input applied to the product are not provided in all studies but are very important in terms of energetic efficiency of the process. Hence, if the energy density is too high, the product needs to be cooled with the subsequent loss of efficiency. Heinz et al. (2003) evaluated the impact of temperature on the PEF inactivation of *E. coli* in apple juice, indicating the potential of a combined treatment of PEF and mild heat. It was found that an increase in treatment temperature from ambient to a range of 35–55°C can reduce the energy required for achieving 6-log reductions of *E. coli* from far above 100–40 kJ/L when operating at an initial treatment temperature of 55°C. Aronsson et al. (2005) reported substantially higher energy requirements of 384 and 160 kJ/L when operating at initial temperatures of 30°C to attain 5-log reductions of *E. coli* and *S. cerevisiae* suspended in a phosphate buffer. When the product does not allow processing under high temperatures, considerations regarding energy cost due to cooling gain importance. Góngora-Nieto et al. (2003b) reported that the energy applied in PEF pasteurization of liquid whole egg at temperatures below 40°C showed an optimum level of 357 kJ/L, 714 kJ/L including cooling requirements, which yielded a shelf life of around 26 days. The cooling energy was found to be a direct function of the energy delivered by the pulses. Thus, prolonged treatments are undesirable because they imply large energy expenditures. To minimize cooling energy, a sound approach would be the use of regenerative heat exchange between the product inlet cold stream and the heated stream following PEF. Optimization of processing conditions based on the minimum amount of energy needed to achieve stability of the product should be considered in future studies. The overall energy requirement of PEF treatments should take into account the efficiency of the heat exchanging units in addition to the energy consumed by the electrical system.

PEF alone can inactivate enzymes but energy inputs required are too high to allow the use of this tech-

nology at a reasonable cost. Energy inputs necessary to attain a 90% enzyme activity reduction have been reported to be far above 34, 25, 12 MJ/L for PME, PPO, or polygalacturanase, respectively (Giner et al., 2000, 2001, and 2003). A combination of mild heat and PEF may also help to attain sufficient levels of enzyme inactivation. Thus, when operating at elevated treatment temperatures and making use of synergetic heat effects, the PEF energy input might be reduced close to the amount of 20 kJ/L required for conventional thermal pasteurization, assuming 95% of heat recovery (Toepfl et al., 2006). This would make PEF technology feasible for obtaining high-quality products at a reasonable energy cost.

The application of PEF on processes involving changes in plant or animal cells, such as extraction, pressing, or drying, has a greater impact on the final energy costs, since these operations may require large amounts of mechanical or thermal energy for prolonged holding periods. Thus, the energy required for electroporating tissue cells is much lower than that required for inactivating microbial cells. Permeabilization rates for apple and potato tissue can be substantially decreased from 100 kJ/kg (thermal treatment), 60–100 kJ/kg (enzymatic treatment) or 20–40 kJ/kg (mechanical pressing) to 1–5 kJ/kg when applying low-intensity PEF treatments (Toepfl et al., 2006). Moderate-intensity PEF treatments have been shown to substantially reduce the amount of energy necessary to perform press extraction of several fruit and vegetable juices (Knorr et al., 1994; Bazhal and Vorobiev, 2000; Shilling et al., 2007). Jemai and Vorobiev (2006) reported that a PEF treatment with an energy input of approximately 3 MJ/t was optimal in assessing sugar beet juice press extraction, thus resulting in a significant reduction of the amount of energy that is usually required for thermal extraction.

Furthermore, oil extraction can be substantially increased with an additional PEF energy input, resulting in a reduction of specific energy consumption. Results by Guderjan et al. (2005, 2007) on maize, olive, soybean, and rapeseed oils suggest that PEF treatments can be used as a pretreatment before oil separation, increasing oil yield, and the content of functional food ingredients such as phytochemicals, isoflavonoids, or tocopherols under gentle

conditions. For instance, an increase of 7.4% in olive oil yield was found after applying an energy input as low as 18 kJ/kg oil output.

The use of PEF to improve drying processes is also advantageous from the point of view of energy savings. Taking into consideration that PEF treatment of vegetable or animal tissues is between the range of 2–20 kJ/kg (Toepfl et al., 2006) and that energy inputs required for conventional drying oscillate between 4 and 6 MJ/kg, it is evident that there is a potential to reduce the total energy input for product drying by increasing mass transfer rates and the subsequent reduction of drying times.

9. Final Considerations and Future Needs

During the last years, PEF has been investigated as a potential emerging nonthermal technology for food preservation. Moderate microbial reductions, up to pasteurization levels, have been achieved in most studies on food products at low temperatures and short residence times, thus allowing the retention of the fresh-like and nutritional characteristics. Higher energy levels than those required to destroy microorganisms are needed to inactivate enzymes. The efficacy of the treatments has been shown to greatly depend on several process parameters, medium properties, and treatment conditions. Results published to date indicate that PEF is most effective when applied to stressed cells, especially if the stress has an effect on cell membrane integrity. Thus, PEF technology can achieve outstanding results when combined with others such as addition of antimicrobials, acidification of the food medium, or mild heating as a hurdle approach.

Unfortunately, some work is still needed before this technology can be effectively transferred to the industry. Research carried out by numerous groups has been conducted with several equipments under heterogeneous conditions, which greatly hinders experimental results from being compared. Future works will need to focus on understanding the kinetic mechanisms of inactivation as well as the effects of PEF on minor constituents of foods. In addition, the development of industrial-scale equipment as well

as an exhaustive evaluation of the operational costs precludes the commercial exploitation of PEF. Furthermore, the application of moderate intensity PEF treatments in order to permeabilize cellular tissues has a great potential to improve several conventional processes in food industry such as drying or extraction processes from the point of view of energy efficiency and suggests in-depth studies in the future.

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Chapter 12

Engineering Aspects of Pulsed Electric Fields

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1. Introduction

Pulsed electric field (PEF) is one of the most appealing nonthermal technologies for preservation of liquid foods due to reduced heating effects compared to traditional pasteurization methods (Barbosa-Cánovas et al., 1999). The common interest of academia and food industry on optimizing the PEF system to assure food safety and product quality have led to improvement in the technology in recent years and successful results were obtained to be utilized for industrial implementation. From an engineering point of view, as in the case of most novel technologies, PEF technology is still in the early stages of commercialization and scaling up to cost-effective industrial operations is highly dependent on further research on the engineering principles behind this technology to fully understand the mechanisms at play (Góngora-Nieto et al., 2002).

A typical PEF system is based on a high voltage pulse generator with a treatment chamber with a suitable fluid handling system and necessary monitoring and controlling devices. Food product is pumped through the treatment chamber, either in a static or in a continuous design, where two electrodes are connected together with a nonconductive material to avoid electrical flow from one to the other. Generated high voltage electrical pulses are applied to the electrodes and high intensity electrical pulses are conducted to the product placed between the two

electrodes. The food product experiences a force per unit charge, the electric field, while the dose of the application is adjusted by means of electric field intensity (peak voltage and the gap between electrodes) and the number of pulses (treatment time). Treated product is then removed or subjected to subsequent pulses with recirculation until the treatment dose is complete. The main process parameters that determine PEF treatments are electric field strength, shape and width of the pulse, treatment time, frequency, specific energy density, and temperature. Among all, electric field intensity and treatment time (number of pulses) are the basic control parameters affecting energy density applied during PEF process. The intensity of these parameters determines the final lethal effect on the microbial population while width and frequency of the pulses contribute to define the process time (Wouters et al., 2001).

There is substantial variety in PEF equipments operating in different laboratories around the world and the need for unification of the process parameters to assess equivalency among different system as well as reducing the difficulty in comparing experimental results is certain (Wouters et al., 2001). Currently, the majority of research reported on PEF defines process conditions in terms of two common variables: intensity of electric fields calculated from voltage and treatment time, which does not help to assess the feasibility or the efficiency of the system. Instead, if the energy density or energy per pulse together with the process conditions were provided, comparable and reproducible data could be obtained. Moreover, unless the voltage value measured with a probe in the treatment chamber, most of the studies assume

perfectly efficient operating systems, that is, the electrical energy at the input to flow is equal to calorimetric heat out of the system. A proper energy balance in order to accurately calculate the treatment intensity requires evaluation of the system efficiency and behavior of the system components during PEF since several factors including the change of the electrical properties during the process, system configuration, treatment chamber, or pulse shape may introduce inefficiency to the system. This inefficiency results in effective electric field intensity and treatment time being different from the reported values. This chapter will review the basic engineering aspects as well as the mathematical relationships utilized to design an optimum PEF process by verifying the effect of electrical and flow properties of the system components on energy consumption during PEF process.

2. Electrical Components

The high-intensity PEF processing system is a simple electrical system consisting of a high-voltage source, capacitor bank, switch, and treatment chamber. Generation of PEFs requires a fast discharge of electrical energy within a short period of time. This is accomplished by the pulse-forming network (PFN), an electrical circuit consisting of one or more power supplies with the ability to charge voltages (up to 60 kV), switches (ignitron, thyatron, tetrode, spark gap, semiconductors), capacitors (0.1–10 μF), inductors (30 μH), resistors (2 Ω –10 M Ω), and treatment chambers (Góngora-Nieto et al., 2002). High-voltage pulses are supplied to the system via a high voltage pulse generator at required intensity, shape, and duration. The simplest PFN is a resistance–capacitance (RC) circuit in which a power supply charges a capacitor that can deliver its stored energy to a resistive load (treatment chamber) in a couple of microseconds, by activation of a switch (Góngora-Nieto et al., 2002).

2.1. Power Supply

High-voltage pulses are supplied to the system via a high-voltage pulse generator at required intensity, shape, and duration. The high-voltage power supply

for the system can either be an ordinary source of direct current (DC) or a capacitor charging power supply with high frequency AC inputs that provide a command charge with higher repetitive rates than the DC power supply (Zhang et al., 1996). The simplest PFN is an RC circuit in which a power supply charges a capacitor that can deliver its stored energy to a resistive load (treatment chamber) in a couple of microseconds, by activation of a switch (Góngora-Nieto et al., 2002). Total power of the system is limited by the number of times a capacitor can be charged and discharged in a given time. The electrical resistance of the charging resistor and the number and size of the capacitors determine the power required to charge the capacitor, wherein a smaller capacitor will require less time and power to be charged than a larger one. The capacitance C_0 (F) of the energy storage capacitor is given by Equation (12.1):

$$C_0 = \frac{\tau}{R} = \frac{\tau \sigma A}{d} \quad (12.1)$$

where τ (second) is the pulse duration, R (Ω) is the resistance, σ (S/m) is the conductivity of the food, d (m) is the treatment gap between electrodes, and A (m^2) is the area of the electrode surface. The energy stored in a capacitor is defined by the mathematical expression:

$$Q = 0.5 C_0 V^2 \quad (12.2)$$

where Q is the stored energy, C_0 is the capacitance, and V is the charge voltage.

2.2. Pulse Shape

The relative electric value of each component in a PFN determines the shape of the pulse. The simplest PFN is an RC circuit as mentioned above in which a power supply charges a capacitor and the energy is delivered to a resistive load (treatment chamber) in a few seconds by activation of a switch. The pulse generated in these types of systems is exponentially decaying, where the voltage across the resistive load (PEF chamber) as a function time is described by:

$$V(t) = V_0 e^{-\frac{t}{\tau}} \quad (12.3)$$

where V_0 is the voltage charged in the capacitor of the PFN, t is the pulse duration, and $\tau = RC$ is the time constant. In an RC circuit the pulse duration is approximately five time constants (Cogdell, 1999). Application of exponential decaying pulses on food implies that the food is exposed to a spectrum of electric fields with intensity proportional to the voltage potential ($V(t)$) received by the load. The research for ideal pulse shape for a PEF system still continues; however, it is generally accepted that pulse-PFNs generating square pulses can apply a relatively constant electric field to the food. With a rectangular or square pulse, the electric field strength reaches the threshold level, within a short time, while the excess electric field strength is low. This would yield a high level of inactivation per unit of power, assuming that below the threshold level there is no inactivation (de Haan and Willcock, 2002).

2.3. Switch

The switches are the most critical components in a PEF system that functions to discharge the stored energy through the PFN circuit instantaneously. The switch plays an important role in the efficiency of the PEF system, and it is selected based on its ability to operate at a high voltage and repetition rate. There are two main groups of switches currently available: ON switches and ON/OFF switches. ON switches provide full discharging of the capacitor but can only be turned off when discharging is completed. ON switches can handle high voltages with relatively lower cost compared to ON/OFF switches, however, the short life and low repetition rate are some disadvantages to be considered for selection. The Ignitron, Gas Spark Gap, Trigratron, and Thyatron are some of the examples from this group. ON/OFF type switches have been developed in recent years that provide control over the pulse generation process with partial or complete discharge of the capacitors. Improvements on switches, mainly on semiconductor solid-state switches, have resulted in longer life spans and better performance. The Gate turn off (GTO) thyristor, the insulated gate bipolar transistor (IGBT), and the symmetrical gate commutated thyristor (SGCT) are some examples from this group (EPRI and

Army, 1997; Barbosa-Cánovas et al., 1999; Barsotti and Cheftel, 1999; Góngora Nieto et al., 2002; Sepulveda and Barbosa-Cánovas, 2005).

2.4. Treatment Chamber

One of the most important and complicated components in the PEF processing system is the treatment chamber. Several different designs have been developed through the years for this key component, wherein high voltage delivered by the power supply is applied to the product located between a pair of electrodes. The basic idea of the treatment chamber is to keep the treated product inside during pulsing, although the uniformity of the process is highly dependent on the characteristic design of the treatment chamber. When the strength of applied electric fields exceeds the electric field strength of the food product treated in the chamber, breakdown of food occurs as a spark. Known as the dielectric breakdown of food, this is one of the most important concepts to be considered in treatment chamber design. Dielectric breakdown of the food is generally characterized as causing damage on the electrode surfaces in the form of pits, a result of arching and increased pressure, leading to treatment chamber explosions and evolution of gas bubbles. Intrinsic electrical resistance, electric field homogeneity, and reduction and generation of enhanced field areas are some other important design criteria for a successful design in terms of energy consumption and low product heating (Sepulveda and Barbosa-Cánovas, 2005).

Treatment chambers are mainly grouped based on their operating manner as either a batch or continuous; batch systems are generally found in early designs for handling of static volumes of solid or semi-solid foods. Hamilton and Sale (1967), Dunn and Pearlmann (1987), and Mizuno and Hori (1991) are some examples of static chamber designs, a basis for the evolution of continuous chambers that would provide advantageous pumping for efficient use in industrial applications. The concentric cylinder, concentric cone, and co-field treatment chambers are some examples of successful continuous flow chambers.

The evolution of treatment chambers began with static chambers consisting of U-shaped polyethylene spacers composed of carbon electrodes supported on brass blocks (Hamilton and Sale, 1967). The electrode area and amount of food that could be treated were regulated by using different spacers. The maximum electric field that the chamber could handle was limited to 30 kV/cm due to the electrical breakdown of air above the food. Dunn and Pearlmann (1987) continued to improve designs with a chamber consisting of two stainless steel electrodes separated by a cylindrical nylon spacer. This chamber was one of the earliest designs incorporating parallel plate geometry using flat electrode surfaces separated by an insulated spacer. Liquid foods were introduced through a small aperture located in one of the electrodes, which could also be used for temperature measurement during processing. The limitations experienced with this chamber geometry were mainly due to surface tracking on the fluid, resulting in arcing. Thus, conical geometry naturally evolved offering the advantage of ease in eliminating bubbles (Zhang et al., 1995b). Two round-edged, disc-shaped stainless-steel electrodes were polished to mirror surfaces, and polysulfone or Plexiglas was used as insulation material. Cooling was maintained by circulating water at pre-selected temperatures through jackets located in the electrodes. This chamber was completely sealed and thus different from other geometries, so to prevent possible sparking or high pressure development, a pressure release device was included within the treatment chamber.

The earliest attempts at continuous treatment chamber design were by Dunn and Pearlmann (1987) in their use of different geometries based on the same principle of circulating food through a closed system. The electrodes were separated from the food by ion conductive membranes made of sulfonated polystyrene, while an electrolyte was used to facilitate electrical conduction between electrodes and ion permeable membranes. New geometry designed for static operation was modified by adding baffled flow channels inside for operation as a continuous chamber (Zhang et al., 1995b; Qin et al., 1996).

The concept of enhanced electric fields in the treatment zone was a milestone for treatment cham-

ber designs. The design was composed of a continuous treatment chamber with coaxial conical electrodes and introduced by Bushnell et al. (1993), later followed by the continuous treatment chamber with parallel electrodes coaxial cylindrical electrodes (Qin et al., 1997). Among all designs to date, coaxial and co-field arrangements are currently favored over most designs. The coaxial arrangement has inner and outer cylindrical electrodes, shaped to minimize the electrical enhancements with uniform fluid flow. In a simple coaxial chamber design, the electric field intensity is not uniform and changes with change in location, as shown in Equation (12.4) (Zhang et al., 1995b):

$$E = \frac{V}{r \ln(R_2/R_1)} \quad (12.4)$$

where r is the radius of electric field measurements and R_2 and R_1 are the radii of the outer and inner electrodes, respectively. The advantageousness of coaxial configurations in providing a well-defined electric field distribution is based on the idea of predicting and controlling nonuniformity of electrical field distribution. Co-field designs introduced by Yin et al. (1997) were composed of two hollow electrodes separated by an insulator providing a tube for product flow. Based on the same principles for coaxial arrangement, co-field designs enabled handling a higher load resistance, allowing the pulser to operate at lower currents in the treatment chamber when compared with coaxial design (Dunn, 2001). Several research teams have proposed a number of different geometries for chambers, such as the glass coil static (Lubicki and Jarayam, 1997), needle-plate, and ring-cylinder continuous treatment chambers (Sato et al., 2001). Limitations and the applicability of each arrangement have shaped new routes toward innovative designs.

Electrically pulsing devices generally have an electrode gap filled with a more or less conductive liquid acting as resistors. In the case of PEFs, the treatment chamber represents the electrical load consisting of two or more electrodes filled with the liquid food product treated. The chamber has to be designed in such a way that electrical field acting on the liquid is homogeneous across the entire active region.

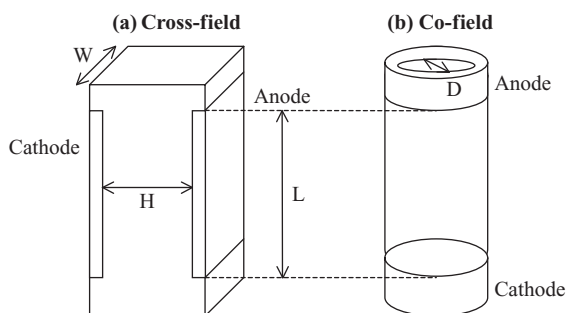


Figure 12.1. (a) The cross-field treatment chamber design with inner dimensions W, H , and electrode length L . (b) The co-field treatment chamber design with inner diameter D and electrode length of L .

This can be adjusted in principle with planar, coaxial, and axial geometries. Zhang et al. (1995a) discussed several possible treatment chamber geometries that have been used in experiments so far and grouped the geometries on the basis of the direction of flow and field. According to this grouping, field and flow directions are either normal or parallel to one another.

Cross-field geometry where the field direction is normal to the flow direction is the simplest geometry for treatment chambers. A simplified scheme of co-field treatment chamber geometry is given in Figure 12.1a where the direction of flow and electric field are perpendicular to each other within a rectangular duct of insulating material and two limited electrodes on opposite sides (Yin et al., 1997). For this type of design, the length (L) and distance (gap) of the electrodes determine the electric field distribution where the treatment volume can be extended in the direction along the width (W). Coaxial tubes, flat circular tubes, conical surfaces, or combination can be used in this geometry. Based on this principle, coaxial treatment chamber design was composed of a continuous treatment chamber with coaxial conical electrodes and introduced by Bushnell et al. (1993), later followed by the continuous treatment chamber with parallel electrodes coaxial cylindrical electrodes (Qin et al., 1997). The coaxial arrangement has inner and outer cylindrical electrodes, shaped to minimize the electrical enhancements with uniform flow.

On the other hand, co-field or co-flow treatment chamber geometries where field and flow are parallel to each other are the most commonly used geometries used for PEF operations as shown in Figure 12.1b. The central part is an electrically insulating tube through which liquid flows. On either side of this chamber the liquid contacts an electrode, causing an electric field in the chamber. In the co-field geometry, the electrodes are two metal pipes, which also serves as the entrance and exit for the liquid. Colinear geometry for which the metal pipes have a slightly larger inner diameter than the chamber is a successful modification based on this principle (Zhang et al., 1995a).

It is also possible to use multitreatment chambers in a PEF processing system. The option of two or more chambers in series has been applied by several researchers. For the co-field geometry, either the inlet or the outlet is at high electric potential and a long insulating tube is required to obtain a low-field fluid path from this high electric potential to zero potential. For two co-field chambers in series, the high potential is applied between both chambers. Treatment in chamber is limited, depending on temperature and pressure. Two chambers will allow approximately twice the treatment possible in one chamber. This option might be interesting if the optimal choice for PEF treatment is not the one sufficient pulse, but multiple pulses that are applied some time apart (Lelieveld and de Haan, 2007).

2.5. Electric Field Intensity

Electric field intensity has been identified as the most relevant factor affecting microbial inactivation by PEF treatment. Electric field intensity in combination with total treatment time during PEF is mainly effective on the extent of membrane disruption in bacterial cells (Hamilton and Sale, 1967). Understanding the electrical principles behind PEF technology is essential for a comprehensive analysis of the PEF system. The electrical field concept, introduced by Faraday, explains the electrical field force acting between two charges. When unit positive charge q located at a certain point within the electric field is generated in the treatment gap (E_r), it experiences force F identified

by position vector r (Blatt, 1989). The electrical field per unit charge is then defined as:

$$E_r = \frac{F_{qr}}{q} = \frac{\text{Newton}}{\text{Coulomb}} \quad (12.5)$$

The electrical potential difference (V) between voltage across two points, separated by a nonconductive material, results in generation of an electric field between these points, with an electrical intensity (E) directly proportional to the magnitude of potential difference (V) and inversely proportional to the distance (d) between points as:

$$E = \frac{V}{d} = \frac{\text{Volt}}{\text{Meter}} \quad (12.6)$$

Transferring Equation (12.5) into the form in Equation (12.6):

$$V = E \cdot d = \frac{\text{Newton} \cdot \text{Meter}}{\text{Coulomb}} \quad (12.7)$$

The Laplace equation can be used as a general expression to describe the general electric field, depending on the voltage under different conditions within boundary conditions, where ϕ represents the electrical potential:

$$\nabla \phi^2 = 0 \quad (12.8)$$

In the case of coaxial treatment chamber arrangement, the inner and outer cylindrical electrodes are shaped to minimize the electrical enhancements with uniform fluid flow. In a simple coaxial chamber design, the intensity of the electric field varies with respect to the radial distance from the inner electrode as shown in the following equation (Zhang et al., 1995b):

$$E = \frac{V}{r \ln \left(\frac{R_2}{R_1} \right)} \quad (12.9)$$

where r is the radius of electric field measurements and R_2 and R_1 are the radii of the outer and inner electrodes, respectively.

2.6. Treatment Time and Dose

PEF treatments are applied in the form of short pulses to avoid excessive heating or undesirable electrolytic

reactions (Barbosa-Cánovas and Sepúlveda, 2005). Liquid foods are electrical conductors for carrying electrical charges. High-voltage PEFs are created when a large flux of electrical current flows through the treatment chamber in a very short period of time (microseconds). Time elapsed between each pulse is much larger than the pulse width since charging of the capacitor is slower than instant discharging (Zhang et al., 1995a).

The simplest configuration of a PFN is the direct discharge of the capacitor to a treatment chamber with purely resistive load and no other associated loads, which produces exponentially decaying pulses. In the case of a charged capacitor (C_0) discharging through a resistor (R), the voltage across the food in the treatment chamber decreases exponentially with a pulse width (Góngora-Nieto et al., 2002):

$$\tau = RC_0 \quad (12.10)$$

In RC circuits, the total pulse duration equals approximately (5τ) five time constants (Cogdell, 1999). Considering the exponential decaying behavior of the delivered energy, τ can be adopted as the effective pulse width, calculated as the time required for the input voltage to decay to $1/e$ (37%) of its maximum value (Zhang et al., 1995b; Grahl and Märkl, 1996; Barsotti and Cheftel, 1999; Góngora-Nieto et al., 2002). Treatment time (t) for a PEF application is defined as a function of pulse width (τ) and number of pulses (n):

$$t = n\tau \quad (12.11)$$

Pulse repetition rate, maximum voltage, and peak current are the key parameters in specifying a PEF generator. In order to establish the desired treatment dose in terms of number of pulses per treatment volume of the treated of fluid (n), treatment chamber volume (v), volumetric flow rate (F), and pulse repetition rate (f) can be adjusted as (Zhang et al., 1995a):

$$f = \frac{nF}{v} \quad (12.12)$$

The electrical components of the PEF system and related mathematical expressions explaining their relationship were reviewed. Understanding the

electrical properties (conductivity, resistivity) of the liquid food product affecting electrical components of the system is also essentially required.

3. Conductivity and Intrinsic Electrical Resistance

Among physical properties of biological materials, electric properties are the least known and described; however, one can find the examples of their application in the food industry. The most important characteristics that alter the biological changes produced during PEF are conductivity, ionic strength, pH, the presence of particles, presence of gas bubbles, dielectric properties, sugar content (for juices), and temperature (Barbosa-Cánovas et al., 1999).

Electrical conductivity is the reciprocal of resistance through a unit cross-sectional area A over a unit distance L , or the reciprocal of resistivity. Intrinsic electrical resistance is one of the most important design criteria for PEF treatment chamber for defining the pulse width, peak electric field, and power per pulse delivered to the treated product. The resistance of a treatment chamber can be analytically determined, provided the effective electrode area (A), the distance between the electrodes (d), and the electrical conductivity of the treated product (σ) are known, by:

$$R_{Ch} = \frac{d}{\sigma A} \quad (12.13)$$

When we simplify the PEF system into a basic electrical discharge circuit (Figure 12.2) we can see that the total resistance of the circuit (R_T) is a sum of individual resistances including treatment chamber resistance (R_{Ch}), transmission line resistance (R_t), switch resistance (R_s), and any other resistance present in the series circuit (Barbosa-Cánovas and Sepúlveda, 1995).

$$R_T = R_s + R_t + R_{Ch} \quad (12.14)$$

The circuit is basically a voltage divider; therefore, the larger the chamber resistance in comparison with the total resistance, the higher the peak voltage reached at the chamber electrodes. If all resistance in the system except the treatment chamber resis-

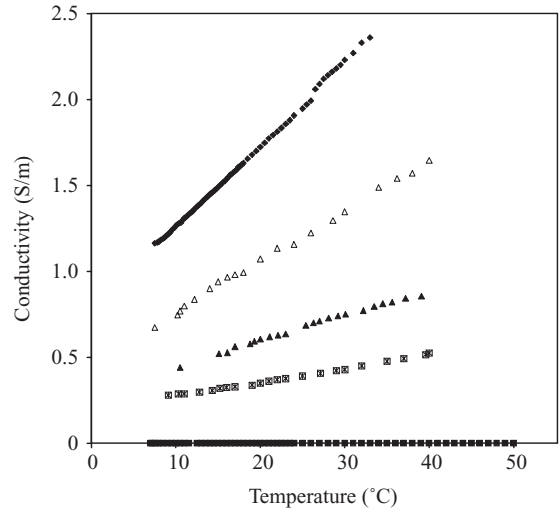


Figure 12.2. The effect of temperature on electrical conductivity of McIlvaine buffer for different ratios of dilutions with distilled water. Buffer dilution ratio (buffer:water): (◆) 1:0 (buffer only), (Δ) 1:1, (▲) 1:3, (□) 1:15 (apple cider), (■) 0:1 (water).

tance (R_{Ch}) kept constant, then the performance of the system is governed by the resistance of the treatment chamber according to the Ohm's law (voltage is equal to current times resistance) (Blatt, 1989).

The increasing trend of electrical conductivity for ionic solutions as a function of temperature is of major concern affecting the electrical properties of the PEF system. In an experimental setup to evaluate the impact of temperature and concentration on the electrical conductivity of McIlvaine buffer solutions (Figure 12.2), the conductivity of buffer solution increased in a range of 1–2.5 S/m when the temperature rise is from 10 to 40°C. The higher the conductivity of the food product treated in the treatment chamber, the lower the electrical resistance to the flowing current. This in turn, reduces the proportion of applied charging voltage in the form of electric field strength between the electrodes of the treatment chamber. The conductivity of the media, which is related to the material's capacity to conduct electric current, will influence the maximum electric field achievable for a given input power and the maximum temperature rise during processing. Lowering the conductivity (high resistivity) reduces the temperature rise and applied

power, thus increasing the electric field intensity and overall microbial effectiveness as suggested by Vega-Mercado et al. (1996a).

The rate of increase for conductivity as a function of temperature was higher for concentrated buffers where strong temperature dependency of ionic solutions is generally explained by their kinetic state and an increase in temperature increases the mobility of ions throughout the solution causing an increase in the conductivity (Heinz et al., 2002).

4. Controlling and Monitoring

Operation and performance of the PEF system are generally controlled by a central computer connected to the high-voltage pulse generator. The computer controls the voltage and pulsing frequency in addition to operation of pumps. Data logs of temperature at different points, including flow rate, voltage, current, and power curves of applied pulses, are also recorded using appropriate probes and an oscilloscope card fed into the central computer (Sepúlveda and Barbosa-Cánovas, 2005).

5. Specific Energy and Temperature

From an engineering point of view, in order to estimate energy requirements and temperature rise during the PEF process, complex energy balance calculations are required. Therefore, estimation of energy density or energy per pulse is infrequently reported; in a few cases it is only qualitatively correlated (Evrendilek et al., 2000; Giner et al., 2000). For a continuous single pass process without recirculation the energy Q (J) dissipated during the discharge of the capacitor C (μF) at a charging voltage V (kV) is given by:

$$Q = \frac{1}{2} C V^2 \quad (12.15)$$

Only under ideal conditions where the ratio of the chamber resistance to the total resistance is 1, all the energy discharged from the capacitor can be delivered to the treatment chamber. Generally, a fraction of the total energy is delivered to the food product which defines the peak voltage received by the treat-

ment chamber (Barbosa-Cánovas and Sepúlveda, 1995):

$$V_{\text{chamber}} = V_{\text{charging}} \frac{R_{\text{chamber}}}{R_{\text{total}}} \quad (12.16)$$

Therefore, the energy per pulse delivered to the treatment chamber can be described as:

$$Q_{\text{pulse}} = \frac{1}{2} \frac{R_{\text{Ch}}}{R_{\text{T}}} C V^2 \quad (12.17)$$

where C is the capacitance of the charging capacitor (μF) and V (kV) is the charging voltage.

Upon delivery of the energy to the food product, during PEF operation, the bulk temperature of the fluid increases due to ohmic heating (Mastwijk, 2007). The temperature increment is related to the input power and is the result of conversion of electrical energy into heat.

Heating of the product is defined as:

$$\Delta T = \frac{Q}{m C_p} \quad (12.18)$$

where ΔT is the temperature rise, Q is the total energy delivered to the treated product, m is the mass of the product being treated, and C_p is the specific heat capacity of the product. For a continuous PEF process, considering the energy balance completed above:

$$f Q_{\text{pulse}} = \rho F C_p \Delta T \quad (12.19)$$

where mass (m) is replaced by density (ρ) times volumetric flow rate (F) of the product and pulsing frequency (f) is taken into account. The ratio between pulsing frequency f (1/s) and flow rate F (mL/s) defines the number of applied pulses (n), when multiplied by the treatment volume v (mL) can be set by the processor to reach the desired degree of inactivation (Zhang et al., 1995a).

As mentioned above, with a pilot plant PEF system and a cylindrical concentric treatment chamber, under variable charging voltage and pulse frequency conditions, the microbial inactivation kinetics of *Escherichia coli* with respect to energy density as a function of fluid flow are given in Figure 12.3. Inactivation kinetics with respect to energy input at 35 kV/cm, where the energy density (J/mL) is

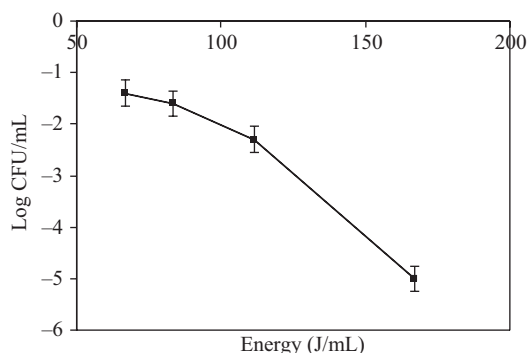


Figure 12.3. Inactivation of *E. coli* (ATCC 11775) in apple cider as a function of input energy at 35 kV/cm (■) for 50 microseconds of PEF with a coaxial treatment chamber.

calculated by adjusting the energy per milliliters for different flow rates of the medium inoculated with *E. coli* (ATCC 11775) are listed in Table 12.1. As the flow rate of the medium is decreased, residence time of the treatment volume inside the treatment chamber increased; therefore, energy density (per milliliter) increased. Table 12.1 indicates that a decrease in flow rate from 2.0–0.8 L/minute resulted in temperature rise from 32.3 to 57.9°C in the outlet of the treatment chamber. It is possible to achieve several log cycle reductions with the use of higher flow rates; however, in order to design a PEF process with the optimum process conditions, the energy density and temperature rise should also be considered.

Inactivation results at 35 kV/cm and 12 pulses (30 μ s) indicate that at energy inputs lower than approximately 150 J/mL, the microbial response is almost linear. However, at higher energy inputs, an abrupt increase in the slope of the survival curve is ob-

served. The observed shape of the survival curve is a typical PEF inactivation curve possessed exponential decay pulses due to negligible microbial inactivation at electric fields lower than the critical electric field. Representing inactivation studies with the use of specific energy consumed during the process would certainly allow better quantification of the process feasibility as well as efficiency.

6. Fluid Flow in Coaxial Treatment Chamber Design

The flow regime within the chamber can be determined by the ratio of inertial forces to viscous forces, defined as the Reynolds' number:

$$Re = \frac{\rho v L}{\mu} \quad (12.20)$$

where, ρ is the density of the fluid, v is the average fluid velocity, L is the characteristic treatment length, and μ is the dynamic (absolute) fluid viscosity. The velocity profile in the coaxial treatment chamber can be determined by applying momentum balance equations in cylindrical coordinates for incompressible fluid flow through an annulus (Bird et al., 1960). Figure 12.4 shows an incompressible fluid flowing in steady state in the annular region between two coaxial circular cylinders of radius r and R .

The steady state laminar flow (Re is less than 2,100) of an incompressible (density ρ is constant) Newtonian [$\tau_{rz} = -\mu(dv_z/dr)$] fluid inside the treatment chamber while neglecting end effects and slip conditions at the wall (pure liquid) can be modeled by

Table 12.1. Treatment temperatures, inactivation, and input energy density of PEF-treated apple cider inoculated with *E. coli* and pumped with selected flow rates at 35 kV/cm electric field strength and 30 microseconds

Flow rate (L/minute)	T_{in}	T_{out}	Inactivation log (N/N_0)	Input Energy (J/mL)
2.0	13.3	32.3	-1.4	67
1.6	13.5	36.4	-1.6	84
1.2	13.3	43.5	-2.3	111
0.8	13.6	57.9	-5.0	167

T_{in} , inlet temperature of the product; T_{out} , outlet temperature of the product.

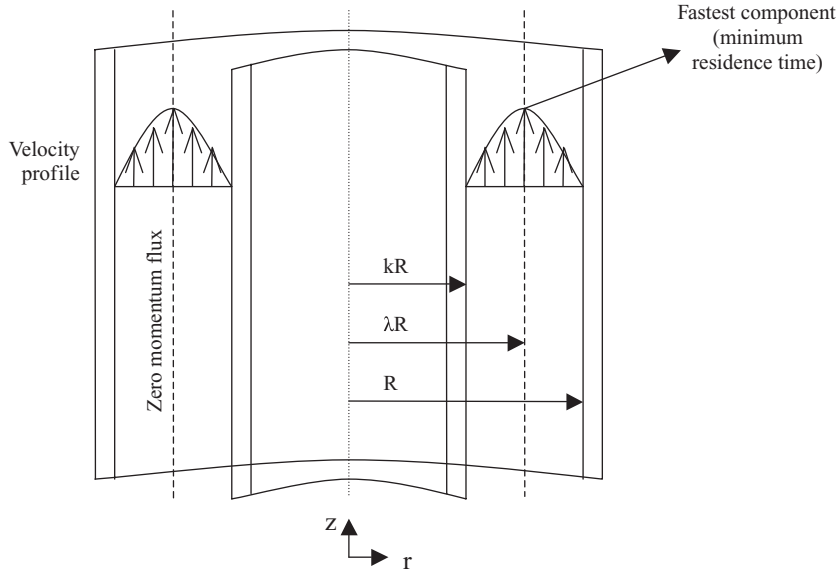


Figure 12.4. Cross-sectional view of the upward flow through coaxial cylinder treatment chamber with cylindrical annulus (adapted from Bird et al., 1960).

setting up a momentum balance over a thin cylindrical shell and arriving at the differential equation (Bird et al., 1960):

$$\frac{d}{dr}(r\tau_{rz}) = \frac{(P_0 - P_L)r}{L} \quad (12.21)$$

where $(P_0 - P_L)$ is the ΔP , which is the pressure drop through the effective length. Then, $\tau_{rz} = -\mu(dv_z/dr)$ can be substituted and the equation becomes:

$$\frac{d}{dr} \left[r\mu \left(\frac{dv_z}{dr} \right) \right] = \frac{-(P_0 - P_L)r}{L} \quad (12.22)$$

Taking out the constant viscosity and integrating the above equation:

$$-r \frac{dv_z}{dr} = \frac{-\Delta P r^2}{2\mu L} + C_1 \quad (12.23)$$

The constant C_1 cannot be determined immediately, due to the lack of information on the momentum flux at either of the fixed surfaces $r = kR$ or $r = R$. But there will be a maximum in the velocity curve at some plane $r = \lambda R$ at which the momentum flux will

be zero. Solving the equation for C_1 :

$$\frac{dv_z}{dr} = \frac{-\Delta P R}{2\mu L} \left[\left(\frac{r}{R} \right) - \lambda^2 \left(\frac{r}{R} \right) \right] \quad (12.24)$$

Integration with respect to r :

$$v_z = \frac{-\Delta P R^2}{4\mu L} \left[\left(\frac{r}{R} \right)^2 - 2\lambda^2 \ln \left(\frac{r}{R} \right) + C_2 \right] \quad (12.25)$$

Solving the equation for C_2 by using the boundary conditions:

- Boundary condition 1: at $r = kR$ $v_z = 0$
- Boundary condition 2: at $r = R$ $v_z = 0$

Substituting the boundary conditions and solving for C_2 and λ , respectively, the velocity distribution for steady incompressible flow in an annulus is:

$$v_z = \frac{-\Delta P R^2}{4\mu L} \left[1 - \left(\frac{r}{R} \right)^2 + \left[(1 - k^2) / \ln \left(\frac{1}{k} \right) \right] \times \ln \left(\frac{r}{R} \right) \right] \quad (12.26)$$

Therefore, by using the above equation for velocity distribution we can calculate:

The maximum velocity (the center line of the annulus $r = \lambda R$):

$$v_{z,\max} = -\frac{\Delta PR^2}{4\pi L} \left(1 - \frac{(1-k^2)}{2\ln(\frac{1}{k})} \right) \quad (12.27)$$

The average velocity:

$$\bar{v}_z = \frac{-\Delta PR^2}{8\mu L} \left[\frac{(1-k^4)}{(1-k^2)} - \frac{(1-k^2)}{\ln \frac{1}{k}} \right] \quad (12.28)$$

The volumetric flow rate:

$$Q = \pi R^2(1-k^2)\bar{v}_z \quad (12.29)$$

For designing a PEF process with optimum conditions, it is important to determine the residence time of the treated fluid within the effective electrode length inside the coaxial cylindrical treatment chamber where the electric fields are delivered to the flowing product. Based on the derived velocity profile, the velocity of the fastest moving particle should be taken into account due to its minimum residence time inside the treatment chamber.

7. PEF System Efficiency

There is very limited data published regarding to the energy consumption of PEF systems and lethality with respect to energy received by the food (Heinz et al., 1999). The lack of studies is possibly due to the fact that even if the energy delivered to the treatment chamber is accurately measured, the energy received by the food may not be entirely used to inactivate the microbial flora. However, before going into the efficiency on cellular basis, unknowns related to the optimum PEF conditions should be explored.

A simplified energy balance over the treatment chamber based on the calculated input energy and output energy in terms of measured heat output are represented in Table 12.2. Based on the results, for a single pass continuous PEF operation where a fluid product with density (ρ) of 1013.7 kg/m³ and specific heat capacity (C_p) of 3.8 kJ/kg°C flowing through the system with a flow rate of 1.2 L/minute, the theoretical energy balance equation is given in Equation (12.18). This equation holds when the input energy is completely utilized by the treatment chamber to convert electrical energy to heat as output. However, in the actual case, only one part of this energy will heat the fluid food that passes through the PEF chamber. This ratio (φ) is less than 1 and

Table 12.2. Maximum input energy and output energy in terms of measured heat dissipation

V_{Ch} (kV)	Q_{pulse} (J)	Number of pulses	E_{in}		ΔT	E_{out}	
			Q_{in} (J/s)	J/mL		Q_{out} (J/s)	J/mL
20	100	5	410	21	4	264	13
25	156		852	43	12	641	32
30	225		1,022	51	14.4	923	46
35	306		1,256	63	15	1,064	53
20	100	12	990	50	8.9	632	32
25	156		1,675	84	23.6	1,547	72
30	225		2,228	111	30.2	2,143	107
35	306		3,032	152	35.4	2,512	126
20	100	20	1,650	83	13.6	965	48
25	156		2,725	136	38.4	2,578	129
30	225		3,713	186	49.5	3,512	176
35	306		5,053	253	53.6	3,803	190

V_{Ch} , charging voltage; Q_{pulse} , energy per pulse; E_{in} , input energy as the charging voltage; E_{out} , measured heat dissipation from the PEF system; ΔT , temperature difference ($T_{in} - T_{out}$).

depends strongly on electrical conductivity of the food Equation (12.19).

It was mentioned earlier in this chapter that the PEF system resembles a basic electrical circuit and serves as a voltage divider and according to Ohm's law, the performance of the system is governed by the resistance of the treatment chamber. Therefore, if the resistance of the treatment chamber decreases as a result of increase in the conductivity of the food being treated, the ratio of chamber resistance to the constant total resistance is lower resulting in more discrepancy between the input charging voltage and the actual voltage in the treatment chamber.

The influence of the intensity of the charging voltage and number of pulses (treatment time) on the efficiency of the system can be better observed in Figure 12.5a and 12.5b. The theoretical values of temperature rise for an ideal system ($\varphi = 1$) are shown in Figure 12.5a. The exponential curves for increasing pulse frequencies were expected due to the direct proportionality of temperature difference (ΔT) with the square power of charging voltage (V_{Ch}^2). On the other hand, for the actual case, in Figure 12.5b, where the observed temperature rise was plotted with respect to the same input charging voltage a different pattern was followed. Keeping in mind that φ is the ratio of voltage in the treatment chamber to the charging voltage, the concavity of the curves in Figure 12.5b

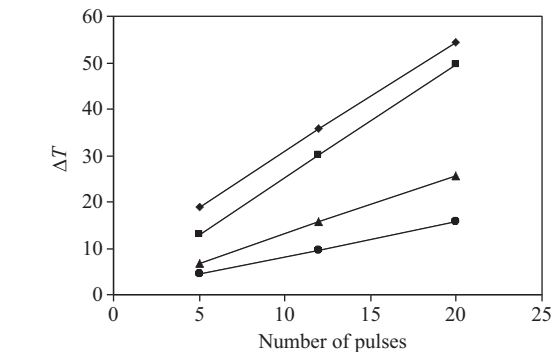
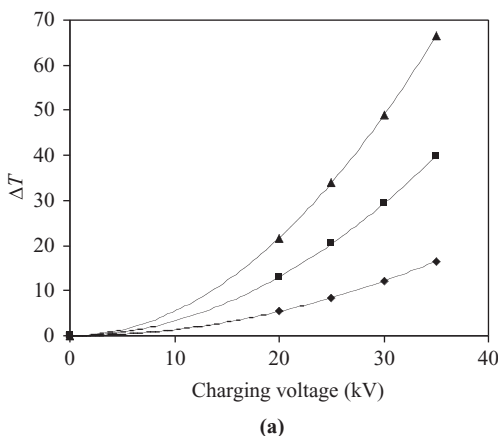


Figure 12.6. Temperature increase (observed) as a function of applied pulses for (◆) 35 kV, (■) 30 kV, (▲) 25 kV, and (●) 20 kV.

indicates that, as the charging voltage increases, the value of φ gets smaller, possibly due to the increase in conductivity, leading to more deviation from ideality.

The effect of treatment time on temperature rise to be compared with the effect of charging voltage (Figure 12.6) reveals that the effect of pulse frequency (treatment time) on temperature rise is stronger especially for higher voltages (30–35 kV). This approves previously explained change in voltage proportions with respect to fluid conductivity and treatment chamber resistance as result of temperature development.

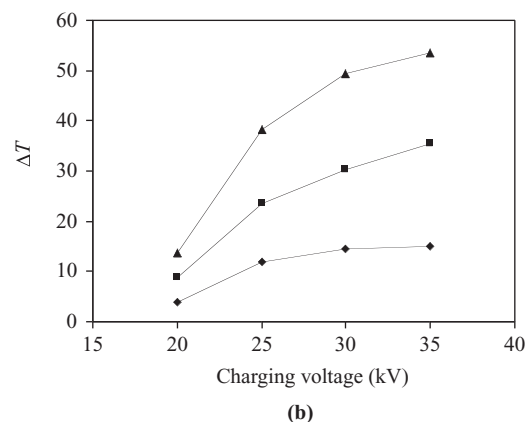


Figure 12.5. Temperature increase as a function of charging voltage for (▲) 20, (■) 12, (◆) 5 pulses. (a) Theoretical temperature rise when $\varphi = 1$, (b) observed temperature rise.

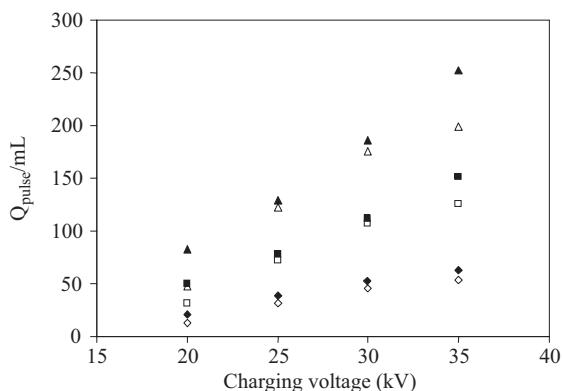


Figure 12.7. Energy densities (energy per pulse) as a function of charging voltage for input at (▲) 20 pulses, (■) 12 pulses, (◇) 5 pulses; and output at (△) 20 pulses, (□) 12 pulses, (◇) 5 pulses.

Based on the data given in Table 12.2, the plot of energy per pulse versus charging voltage (Figure 12.7) for both theoretical and observed values provides evidence for the increasing inefficiency of the system for higher voltages. The ratio φ changes as a function of capacitor charging voltages and approaches to 1 only for smaller charging voltages at all three pulsing frequencies where the effect of temperature is negligible on the effectiveness of the PEF system. As the number of pulses applied increases and reaches to its maximum value (20 pulses), the difference between input and the output increases possibly due to changing electrical properties of the system with increased temperature.

Therefore, by using data in Table 12.2, the energy consumption by PEF can be estimated, compared to thermal pasteurization and the difference between the actual and the theoretical values can be determined. With the use of Equation (12.18), the energy requirement for bringing 50 mL of water from room temperature (20°C) to pasteurization temperature (71.7°C) is calculated as 10.7 kJ. For the same amount of water, the actual and theoretical energy requirement for a PEF system with 35 kV and 20 pulses (50 μ s) assuring sufficient log cycles reduction of *E. coli* are 9.5 kJ and 12.65 kJ, respectively.

8. Final Remarks

The focus of this chapter was to investigate the effects of the possible factors contributing to the variation in PEF system efficiency. The impact of electrical properties as a function of temperature and concentration, the effect of energy density and fluid flow rate on microbial inactivation and the efficiency of the system as a function of charging voltage, number of pulses were discussed by comparing the actual and theoretical energy densities in a PEF system. After a brief review of the engineering principles behind the PEF technology, the impact of temperature and concentration on electrical conductivity was discussed first followed by the effect of product flow rate on the temperature development inside the chamber as a function of energy density. A simplified energy balance over the treatment chamber enables us to verify that PEF system performance deviated from ideality for intense PEF conditions whereas an increase in the charging voltage decreases the ratio of voltage in the treatment chamber to the charging voltage (φ) leading greater deviation of PEF system performance from ideality. In this chapter, the obtained results enabled us to take a step forward by suggesting the potential factors that influence the system performance in terms of the discrepancy between input and output energy values. However, further analysis is required to evaluate the main source of inefficiency by analyzing the electrical resistance, current, and voltage proportion of each component of the system by using electrical engineering principles.

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Chapter 13

Pulsed Electric Field Assisted Extraction—A Case Study

Stefan Toepfl and Volker Heinz

1. Introduction

Disintegration of cellular material, a key step prior to operations such as juice winning, extraction, or pressing is often performed by enzymatic maceration, thermal treatment, or mechanical grinding. These techniques may require a significant amount of thermal or mechanical energy as well as holding times and storage tanks. Side activities of enzymes (added or natural) and thermal degradation during holding time can cause losses of nutritionally and physiologically valuable compounds and lower product quality. When applying pulsed electric fields (PEFs) to cellular tissue, an increase in mass transfer coefficients was observed because of cell membrane permeabilization (Knorr et al., 1994; Bazhal and Vorobiev, 2000; Fincan et al., 2004). Based on this effect, a PEF application can replace or substitute conventional techniques in fruit and vegetable juice processing. First applications of PEF for disintegration of biological material have been described by Doevedspeck (Doevedspeck, 1960) and Flaumenbaum (Flaumenbaum, 1968), reporting a 10–12% increase of juice yield when applying electroporation to apple tissue. Systematic studies on microbial inactivation were conducted by Sale and Hamilton (Sale and Hamilton, 1967). First attempts to commercialize PEF technology for disintegration of cells have been made by Krupp, Germany, in the 1980s, with the development of the ELCRACK[®] process (Sitzmann

and Münch, 1988) based on experiments conducted by Doevedspeck (Doevedspeck, 1960; Doevedspeck, 1961). During the 1990s, the interest in PEF application in universities and research centers increased and until 2006 about 450 research papers are cited in the *Food Science and Technology* (FSTA) abstracts. The applicability of PEF to enhance, modify, or replace a variety of operations during food processing has been shown in an unprecedented amount of publications from approximately 25 groups worldwide (Barbosa-Cánovas et al., 1999). Bazhal et al. (Bazhal et al., 2001) investigated juice expression of apple under simultaneous mechanical pressing and PEF treatment, a significant increase in juice yield was reported after a treatment at 520 V/cm and 100 μ s treatment time. For carrot juice, an increase of juice yield from 60.1 to 66.4% was found in comparison to an untreated sample; in the same way, the dry matter of the sample was increased from 13 to 15% (Knorr et al., 2001). The beneficial impact of a PEF treatment for wine production was reported (Sigler et al., 2004). The impact of an electroporation on mass transport coefficients in red beetroot was investigated by Fincan (Fincan et al., 2004), suggesting a bimodal Fickian diffusion model. Viscoelastic properties of the tissue changed due to loss of turgor and release of intracellular compounds. Lebovka et al. (Lebovka et al., 2004b; Lebovka et al., 2005) investigated the stress deformation and relaxation of carrots, potatoes, and apples. It was shown that through formation of pores and loss of turgor a PEF treatment alone does not completely eliminate the textural strength of the tissue similar than a thermal treatment. Jemai and Vorobiev (Jemai and Vorobiev,

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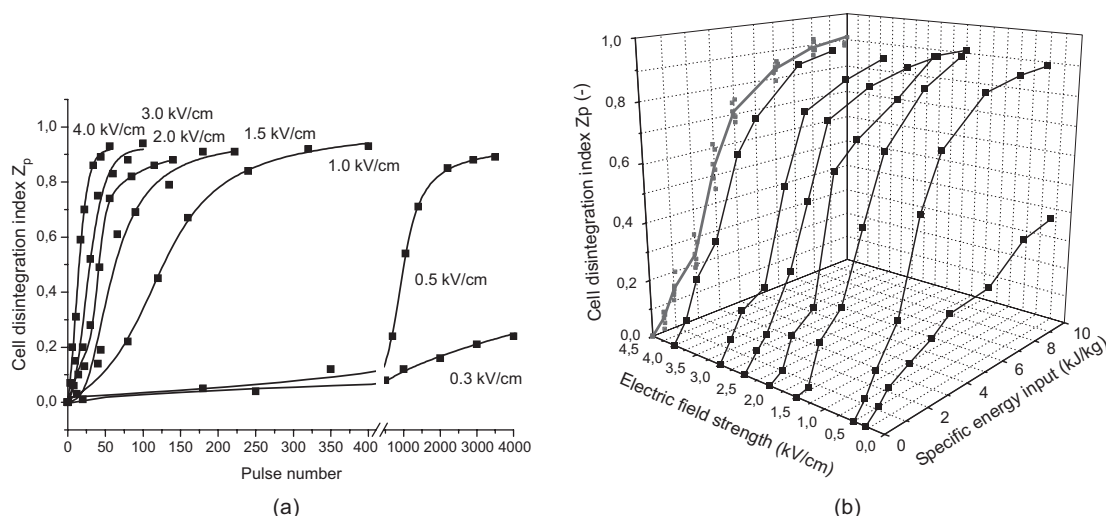


Figure 13.1. (a) Impact of PEF treatment at different electric field strengths on tissue integrity of apple (Royal Gala) samples dependent on pulse number. (b) Scatter diagram of cell disintegration index Z_p of apple (Royal Gala) samples after PEF treatment at a field strength of 0.3–4 kV/cm.

2002) showed the enhancing effect of PEF treatment on the diffusion coefficients of soluble substances in apple slices. These results clearly indicate that PEF can successfully be applied to disintegrate biological tissue and to improve the release of intracellular compounds. Though providing a large potential to achieve a nonthermal, low energy disintegration of plant or animal matrices several industrial applications are still limited at present, as mainly a lack of industrial scale treatment systems inhibited a broad application so far. In this study, the impact of a PEF treatment on fruit juice winning and selected examples of PEF enhanced mass transport will be presented.

2. Application Examples

2.1. PEF Impact on Tissue Integrity

To evaluate PEF-induced tissue permeabilization, the cell disintegration index after a method developed by Angersbach et al. (Angersbach et al., 1997; Angersbach et al., 1999) can be used. This method is based on interfacial polarization effects of the Maxwell-Wagner type at the intact membrane interfaces. Measurement of the frequency-dependent

passive electrical properties of biological cell systems (for the vegetable and muscle tissues characterized in frequency ranges from 10^3 to 10^7 Hz) allows the simple quantification of the degree of membrane disintegration as disintegration index Z_p , ranging from 0 for intact to 1 for fully disintegrated tissue. The impact of a PEF treatment at different electric field strength and pulse number on Royal Gala apples is shown in Figure 13.1a. Typical sigmoid curves have been obtained dependent on pulse number, similar than reported for potato tissue (Knorr and Angersbach, 1998). Increase of electric field intensity showed higher PEF efficiency for tissue disintegration at same pulse number, a disintegration index of 0.6 can be obtained after 20 pulses at 4 kV/cm or after approximately 150 pulses at 1 kV/cm. It is noteworthy that also a very low electric field strength of 0.3 kV/cm resulted in a permeabilization after a sufficient number of pulses. The electrical energy delivered per pulse has an exponential dependency on peak voltage (and therefore field strength) applied. In Figure 13.1b, the cell disintegration index after treatment at different field strength has been related to the total specific energy input. The scatter diagram shows the impact of electric field strength and energy input on tissue integration. It can be seen that

exceeding a sufficient level of electric field strength no further improvement of energy efficiency is found. An YZ-projection of data points and average for all samples treated at 1 kV/cm or higher is shown by the red squares and line. The cell disintegration level obtained is then dependent on total energy input only. For Royal Gala apples this minimum level of electric field strength was in a range of 0.5 kV/cm, an operation at 0.3 kV/cm resulted in cell permeabilization, but a higher total energy input was required to achieve a similar level of permeabilization. Up to an energy input of a range of 10 kJ/kg, an increase in cell disintegration was found.

2.2. PEF-enhanced Apple Juice Yield in Laboratory-Scale

To evaluate the impact of PEF application on enhancement of juice winning, laboratory scale tests have been performed using an electric field strength between 1 and 5 kV/cm and an energy input between 1 and 30 kJ/kg. Yield of PEF-treated variants ranged from 67.9 to 71.3%, but significant differences in juice yield were observed also for the control samples (64.5–68.5%). For this reason, the effects of mash treatment on juice yield shown are expressed relative to the respective control of the same mash. Application of pulsed electric fields increased juice yields in the range of 1.7–7.7%, whereas enzymatic mash treatment resulted in a 4.2% increase.

Exemplarily the increase in juice yield after treatment of Royal Gala is shown in Figure 13.2. The average juice yield for control samples was 69.2%. A treatment with an energy input of 4.32 kJ/kg showed the highest efficiency for this variety, whereas increasing energy input resulted in a decrease of juice yield. Considering the impact of PEF on tissue disintegration (see Figure 13.1), this treatment is related to a Z_p of approx. 0.75. A further increase in disintegration appeared to result in a mash structure with adverse effect on liquid–solid separation. Similar results were found for other varieties in laboratory-scale experiments.

Accelerated release of the juice, as reported by (McLellan et al., 1991), was not observed, but the

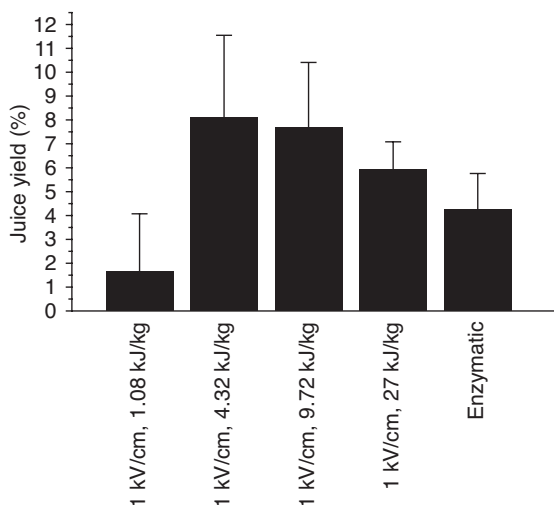


Figure 13.2. Increase in juice yield from Royal Gala apples by PEF application and enzymatic maceration in comparison to untreated control samples. Each treatment has been repeated three times.

mash became squashier as a result of the PEF treatment. Textural characteristics were in accordance with previous observations (Lebovka et al., 2004), describing changes of the force relaxation curves of apples due to the PEF-induced damage of the apple cells after PEF treatment. Treatment temperature did not show an effect on PEF efficiency during juice winning in a range of 10–30°C (data not shown).

However, it should be taken into consideration that juice yield is only one aspect, even though the most important in determining the profitability of juice production. In contrast to the pomace obtained after enzymatic mash treatment, press residues resulting from PEF treatment can still be exploited for pectin extraction, which is not depolymerized by enzyme application (Schilling et al., 2007; Schilling et al., 2008).

A comparison of juice yield for Granny Smith, Braeburn, and Royal Gala is shown in Figure 13.3, as PEF-treatment parameters 30 pulses at 2 kV/cm have been selected. The PEF impact was most pronounced for Royal Gala, whereas Braeburn showed a minor increase in juice yield only. Differences in juice extractability after a PEF treatment may be related to structural properties of different varieties.

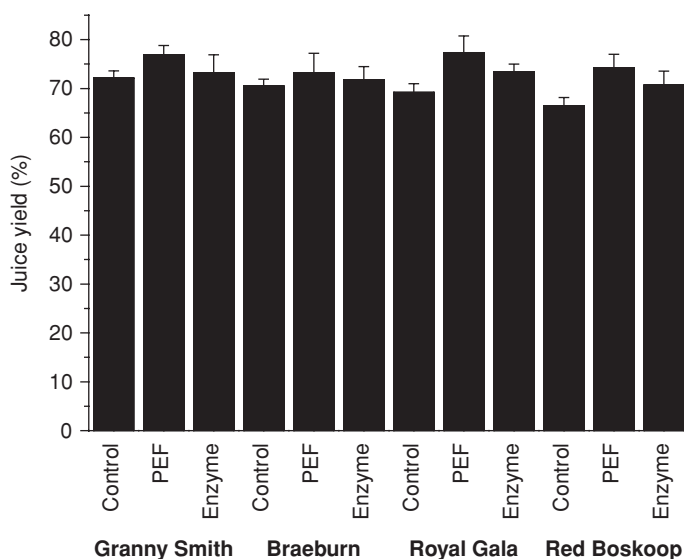


Figure 13.3. Comparison of juice yield after PEF or enzymatic treatment for three different apple varieties (pressing in laboratory scale, three replications per treatment, PEF treatment: 2 kV/cm, 30 pulses, 4,32 kJ/kg).

Red Boskoop was used to investigate the impact of a PEF treatment on polyphenol extractability in laboratory-scale in cooperation with University of Hohenheim, Germany (Schilling et al., 2007), differences in the phenolic composition between the control juices and PEF-treated samples a/b for field intensities of 1 kV/cm and 3 kV/cm were insignificant. Considering pH, TSS (total soluble solids), TA (total acids) and the sugar–acid ratio, the juices obtained after PEF treatment of apple mash did not differ from the respective controls. Uniformly, total soluble solids amounted to 13.0 ± 0.2 Brix, and densities were 1.052 ± 0.001 g/cm³. TA, calculated as malic acid, was approximately 10.1 ± 0.4 g/L. Sugar contents of the juices were not affected by different mash treatments. Analogous results were found for malic acid (10.8 ± 0.4 g/L). Also, the pectin contents of the juices were not diminished by PEF treatment amounting 1.1 ± 0.1 g GA/L. Due to the pectolytic activity, the pectin content of the macerated samples was only 0.08 ± 0.01 g GA/L. In accordance to studies of PEF application for preservation (Zárate-Rodríguez and Ortega-Rivas, 2000; Ayhan et al., 2002; Lechner and Cserhalmi, 2004) it

was confirmed that also a treatment of apple mash does not lead to substantial changes in the chemical composition of the resulting juices. All parameters are in agreement with specifications of EEC food regulations; the samples were equivalent to control samples.

2.3. Impact on Juice Yield in Technical Scale

To scale-up laboratory-scale experiments on PEF applicability for fruit juice production, a pilot system with a production capacity of up to 5 t/hour was realized. The system was used for technical scale tests during autumn 2005 and in spring 2006 to investigate PEF effect on freshly harvested and stored apples in cooperation with the University of Hohenheim, Germany, and the University of Applied Sciences Geisenheim, Germany. A typical mixture of apple varieties used for industrial juice production has been obtained by Eckes-Granini, Nieder-Olm, Germany. Liquid–solid separation has been performed using a Z23–3 (Flottweg, Vilsbiburg, Germany) decanter centrifuge

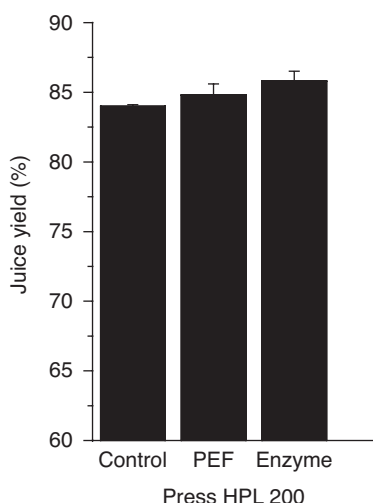


Figure 13.4. Comparison of juice yield in technical scale from typical industrial mixture of fresh apples after different pretreatments and liquid–solid separation using a horizontal filter press. PEF: 2 kV/cm, 10 kJ/kg.

or a horizontal filter press HPL 200 (Bucher, Niederweningen, Switzerland) after a mash treatment with PEF or MA-Xpress (Erbslöh, Geisenheim, Germany) pectolytic enzyme. The yield obtained after different pretreatments is shown in Figure 13.4.

Using the press for liquid–solid separation a slight trend toward an increase of juice yield after a PEF application can be observed. Liquid–solid separation was performed with a press filling of 150 kg. No significant differences were found between control, enzymatic, and PEF-treated mash after Tukey ($P > 0.05$). In addition to the final yield after pressing, the impact of PEF treatment on pressing curves and juice flow was investigated. A pressing curve for control, enzyme, and PEF-treated sample is shown in Figure 13.5. It is obvious that a PEF treatment caused a decrease in juice flow in particular during beginning of the pressing. Whereas an enzymatic maceration allowed a juice yield of 75% after 19 minutes, this yield was obtained after 25 and 34 minutes for control and PEF sample, respectively. After 50 minutes, a yield of 85% was obtained for the enzyme treated fruit mash, indicating the superior impact in comparison to control and PEF-treated mash. Though structural changes and PEF-induced cell disintegration of the apple mash were clearly visible by fast enzymatic browning, the impact on liquid–solid separability showed to be adverse. Structure disintegration and loss of mash compressibility might have caused a blockage of drainage channels as well as a loss of structural support necessary for liquid flow within the mash. Additionally, the press design might play a role; a HPL 120 horizontal hydraulic filter press with

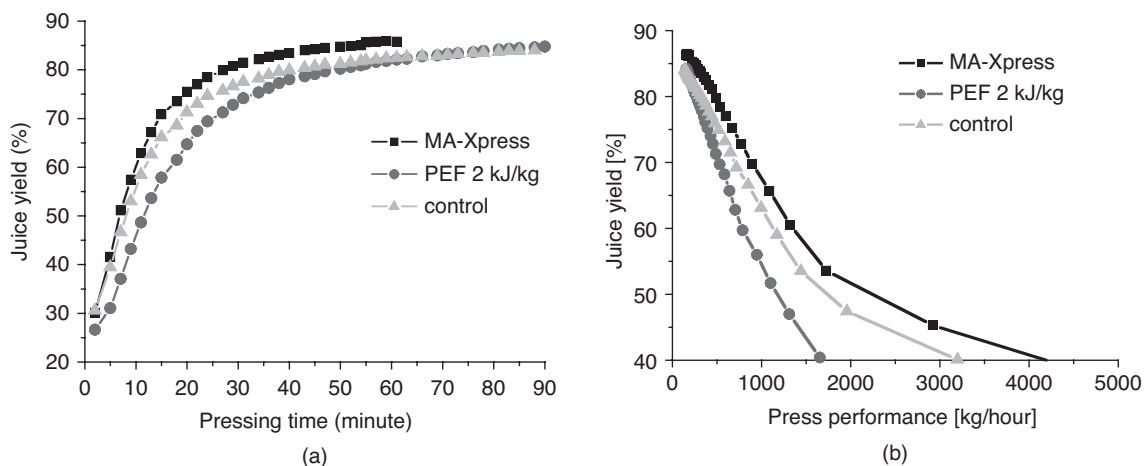


Figure 13.5. (a) Juice yield from industrial apple mixture dependent on press time and pretreatment. (b) Performance–yield diagram with fresh apples (industrial mixture) after different mash treatments. PEF parameters: 3 kV/cm, 10 kJ/kg; enzyme: MA-Xpress, HPL 200 press.

drainage elements implemented was used, the surface volume ratio of this press type is lower in comparison to other press types. The effect of decreased juice flow will cause a drastic reduction in production capacity in comparison to an enzyme treated mash.

Application of a decanter centrifuge for juice winning from stored apples (harvest in autumn 2005, processing March 2006) resulted in a yield increase dependent on treatment intensity (see Figure 13.6). Above a treatment intensity of 10 kJ/kg, the juice contained too many solid particles. An adjustment of decanter settings and/or modification of screw and weir might help to improve separability of apple mash. It was reported by Guenther (Guenther, 2006, personal communication) that during pilot-scale tests using a PEF equipment of KEA Tec, Karlsruhe, Germany, an adaptation of parameter settings was required but subsequently a plain juice was obtained.

As the effect of mushy juice has not been found during laboratory-scale experiments using a basket press the press design appears to have an impact on liquid–solid separability. Further experiments to evaluate separability of PEF-treated apple mash were performed using fresh apples and a wrapped cloth

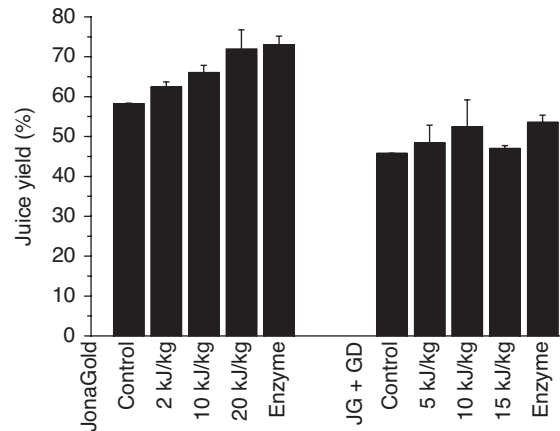


Figure 13.6. Impact of PEF treatment at different intensities on juice yield using a decanter centrifuge. PEF treatment at 2 kV/cm, Jona Gold (JG), and Golden Delicious (GD) were used, juice yield determined gravimetrically, including an eventual transition of solids to juice.

press with a lot size of 50 kg. The impact of different PEF-treatment intensities on juice yield from Royal Gala and Jona Gold in comparison to untreated and enzyme treated samples is shown in Figure 13.7. Press curves have been monitored, every 2 minutes

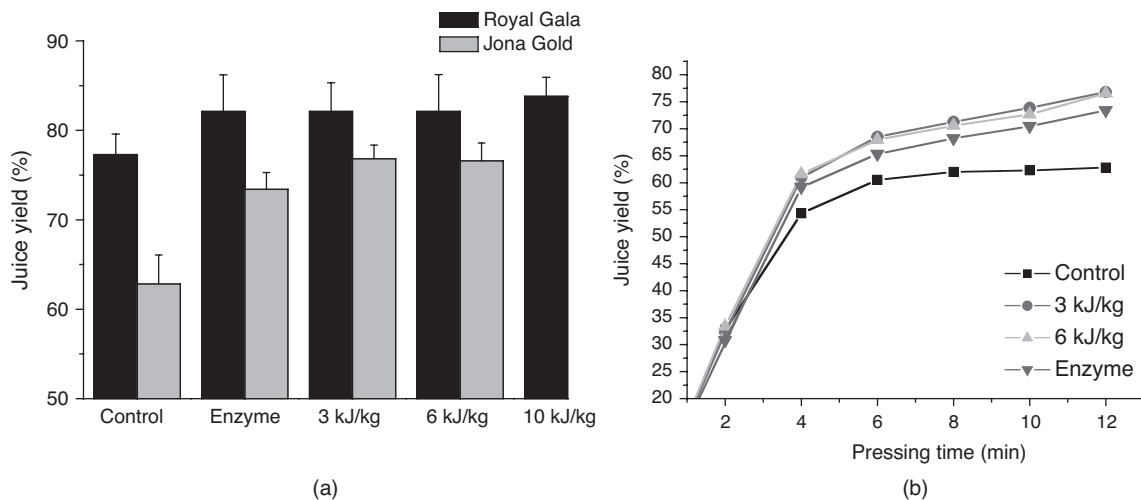


Figure 13.7. (a) Juice yield obtained in wrapped cloth press from two apple varieties after different PEF treatments at 2 kV/cm and different specific energy input in comparison to enzyme treatment and untreated sample. (b) Press curve of Jona Gold mash after different pretreatments using a wrapped cloth press. Pressure was increased from 0 to 20 bar in increments of 4 bar.

the pressure was increased by an increment of 4 bar. After 12 minutes the pressing was stopped, the yield of each pressing step was determined gravimetrically. The press curve for Jona Gold is shown in Figure 13.7b, indicating that using other press types might provide a potential to utilize mash structural changes after a PEF treatment in a beneficial way. A wrapped cloth press has been selected as the mash is divided into several lots, during this study, layers of mash wrapped in pressing cloth have been used. In comparison to a filter, press a higher surface volume ratio is obtained. Even if such press type is operable in batch mode, only these findings are relevant for selection of appropriate liquid–solid separation techniques after a PEF treatment. The application of belt presses could provide a highly promising and continuously operated alternative to application of filter presses. The potential of influencing tissue structure and integrity has been shown by this study; further experiments should concentrate on improvement and adaptation of separation techniques on structural changes of fruit mashes after a PEF treatment. Contrary to previous experiments in laboratory scale, some juice-quality parameters showed an impact of a PEF treatment during technical-scale tests. Total acids of decanter (4.1 g/L for control, 4.2 and 4.6 for PEF and enzyme treatment) and press juices (4.6, 4.9, and 5.1 g/L, respectively) were increased, along with a slight decrease in pH value and an increase of glucose, fructose, and saccharose content for decanter juices. Phenolic compounds showed an increase after PEF treatment, which might be related to improved release in comparison to control sample and shorter processing time in comparison to enzyme treatment. In general, all juice-quality parameters were in accordance within the European Code of Practice for Fruit Juices (AIJN, 1996), allowing the production of juices substantially equivalent to commonly processed ones (Toepfl, 2006; Schilling et al., 2008). No degradation of apple pectin occurred during or after a PEF treatment, indicating the potential to improve fruit juice production sustainability. Whereas by an enzymatic treatment, a pectin depolymerization and deesterification reduces the quality and applicability of apple pectin as a gelatinization agent, no detrimental effect of a PEF treat-

ment was found. These findings allow utilization of pomace for pectin extraction while achieving a similar juice yield than after an enzymatic treatment. For production of cloudy apple juices, an enzyme application can not be performed, as turbidity stability would be degraded by pectin hydrolysis. During processing of cloudy apple juice an increase of yield can be expected while maintaining pectin and turbidity properties.

2.4. Carrot Juice Recovery

A treatment of carrot mash was performed to evaluate the separability using tissue with different structural properties. A comparison between an untreated sample, different PEF-treatment intensities, and a Suprator[®]-homogenizer-treated mash (Ludwig et al., 2003) is shown in Figure 13.8. A temperature increase up to 80°C was required to allow pumping of carrot mash and to improve juice separation. After a PEF treatment at 10 kJ/kg specific energy input and a temperature increase to 80°C, a similar juice yield than after a Suprator[®]-treatment was obtained. The energy input required for a mechanical homogenization is in a range of 15–30 kJ/kg, whereas a

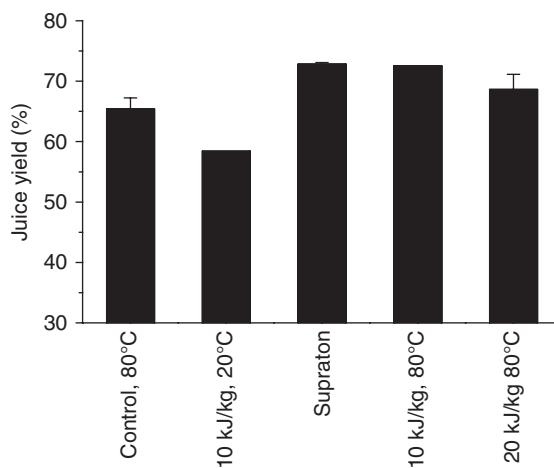


Figure 13.8. Carrot juice yield using a decanter centrifuge after different pretreatments in comparison to untreated control and Suprator[®]-homogenizer. PEF treatment at 2 kV/cm. To increase mash and juice viscosity to allow pumping and separation, a temperature increase up to 80°C was necessary.

PEF treatment with an energy input of 10 kJ/kg provided good results. In comparison to an untreated control (also heated to 80°C), the juice yield was increased by approximately 7%. For carrot tissue, the solids transition found for treatment of apple mash was not observed, indicating that separability after a PEF treatment is dependent on material structural properties.

2.5. PEF-Impact on Anthocyanin Extraction

In addition to juice yield enhancement, the extraction of valuable intracellular contents can be improved by PEF application (Lebovka et al., 2001; Chalermchat et al., 2004; Fincan et al., 2004; Lebovka et al., 2004). Exemplarily, the impact of a PEF pretreatment on anthocyanin extraction from purple fleshed potatoes is shown in Figure 13.9. Blanching at 70°C was required for all samples to retain typical pigment color by preventing enzymatic browning. If no blanching was applied, browning after a PEF treatment was faster than for control samples. After PEF treatment, the extractability of anthocyanins from purple fleshed potatoes Garden Huckleberry (*Solanum scabrum*) (Lehmann et al., 2007) was enhanced. Four different varieties have been screened regarding their anthocyanin content and the potential

of PEF application to increase extraction yield, for all varieties an increase was found after PEF treatment (data not shown).

3. Cost Estimation

A PEF treatment system consists of a pulse modulator and a treatment chamber where the foodstuff is exposed to the pulsed electric field. The generation of pulsed power requires a fast, repetitive discharge of energy storage into an electrical load matched to the pulse modulator. The principle of pulse modulation and key requirements regarding modulator design are discussed in Chapter 2.1. PEF application to enhance extraction of fruit or vegetable juices, plant oil, or other valuable substances can be performed batch-wise or continuous on whole tubers or fruits or mashes. Exemplarily, a chamber for continuous treatment of fruit mashes or potato tubers are shown in Figure 13.10.

Considering the processing parameters reported, an electric field strength in the range of 1–2 kV/cm, exponential decay or rectangular pulses with a pulse width in range of μs and a total energy input of 5 to 10 kJ/kg the design parameters for an industrial scale system with a production capacity of 10 t/hour of fruit mash can be determined. Based on a treatment-chamber diameter of 50 mm, a peak pulse voltage of 20 kV will be sufficient. To limit the impact of product conductivity, use of a co-linear treatment chamber is suggested, which provides a high load resistance. Two subsequent treatment zones are formed by a setup of three electrodes, consisting of a central high-voltage electrode and two grounded counterparts. The average residence time within a treatment zone with a diameter of 50 mm and a gap of 50 mm will be 2.8×10^{-2} s. To subject every volume element to an average number of 20 pulses (10 in each zone), a minimum repetition rate of 350 Hz will be required. At a flow rate of 10 t/hour and an energy input of 10 kJ/kg, an energy supply with an average output power of 30 kW is required. For commercially available high-voltage power supplies, costs in a range of 1 k€/kW are commonly assumed. The overhead for pulse modulation, capacitors, and control equipment is dependent on modulator

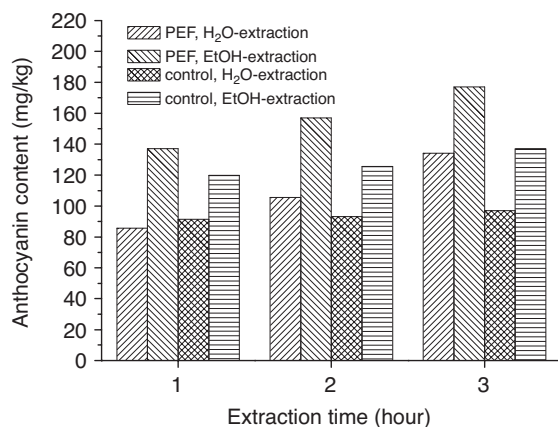


Figure 13.9. Impact of PEF treatment on aqueous and ethanol extraction of anthocyanins from purple fleshed potatoes. PEF parameters: 1.5 kV/cm, 150 pulses.

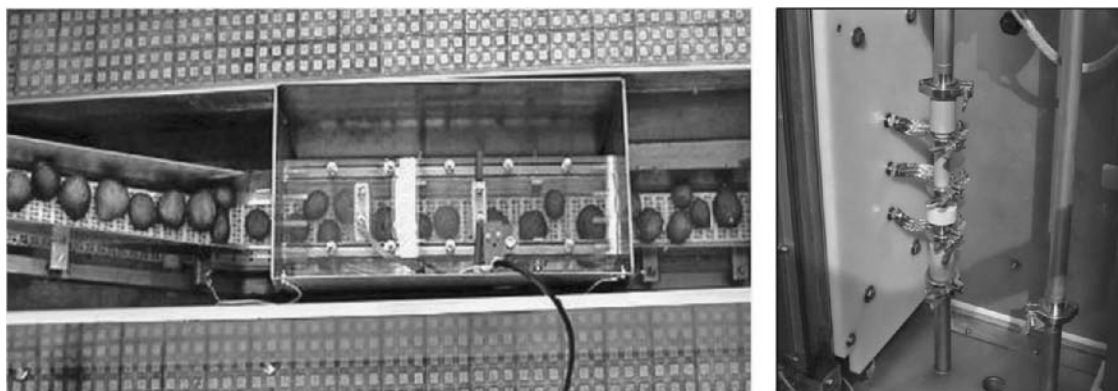


Figure 13.10. Treatment chamber examples. (For color details, please see color plate section.)

typology, the ratio between peak and average power, type of components used, and the number of units realized up to four times the power supply costs. The total pulse modulator costs can range from 2 to 6 € per kW average power installed.

An energy input of 10 kJ/kg corresponds to an electric power consumption of approximately 3 kWh/t of product. Based on a price of 10 ct/kWh the pure electric energy costs for the PEF treatment can be estimated as 0.30 €/t, adding 10% overhead a total power price of 0.33 €/t is assumed. For a conventional enzymatic maceration, the treatment costs can be estimated at 7.50 €/t, where a significant amount is contributed by enzyme costs. A calculation of profitability is shown in Table 13.1, taking into account the costs for investment as well as variable and maintenance costs. It is obvious that the high initial costs for installation of a PEF system can amortize within a very short period of time, as the treatment costs per ton are in a range of 2–3 €/per ton of mash. This estimation is based on the assumption that at least the same juice yield is obtained after PEF or enzymatic pretreatment, which is confirmed by literature data available as well as first reports from the first industrial installation in Germany (Guenther, 2006, personal communication). During production of cloudy apple juice, an enzyme addition is not applicable due to limited turbid stability—in this case a significant increase in juice yield can be obtained in comparison to conventional, untreated processing. Additional consumer benefit due to less detrimental

impact on product quality is not included into this balance, same as the drastic reduction of processing time and the potential to extract native structure pectin from the pomace. For the application of

Table 13.1. Estimation of total costs of a PEF cell disintegration in comparison to an enzymatic maceration for a production capacity of 10 t/hour and an operation time of 1,875 hour/a. Load voltage, 20 kV; average power 30 kW; investment cost 150,000 €

Cost Type	Unit	Enzymatic Maceration	PEF
Production per a	t	18,750	18,750
Investment	EUR	37,500	150,000
Residual value.	EUR	—	—
Replacement value	EUR	45,000	175,000
Expenditure	EUR	45,000	175,000
Depreciation range	Years	7	7
Interest	%	7	7
Depreciation	EUR/a	6,000	22,000
Interest	EUR/a	3,150	12,250
Maintenance	EUR/a	9,150	10,000
Fixed costs p.a.	EUR/a	18,300	44,250
Variable costs p.a.	EUR/a	140,625	6,188
Total costs p.a.	EUR/a	158,925	50,438
Variable costs p.t	EUR/t	7.5	0.33
Total costs p.t	EUR/t	8.48	2.69
Δ Total costs p.a.	EUR/a	108,487	
Δ Total costs p.t	EUR/t	5.79	
Reflux time	Years	1.38	
Profitability	%	119	

Table 13.2. Parameters of PEF equipment available from DIL

	ELCRACK® 5 kW	ELCRACK® 30 kW
Average power	5 kW	30 kW
Peak voltage	25 kV	30 kV
Pulse shape	Rectangular	Rectangular
Pulse polarity	Mono- or bipolar	Mono- or bipolar
Pulse repetition	1–1,000 Hz	1–1,000 Hz
Pulse width	3–30 μ s	3–30 μ s
Dimensions	60 cm $\times$ 130 cm $\times$ 50 cm	160 cm $\times$ 230 cm $\times$ 60 cm
Weight	250 kg	1,000 kg
Housing	IP 54, air cooled	IP 54, water cooled
Treatment capacity	2 t/hour	10 t/hour

PEF for disintegration of other cellular tissue or to improve drying or extraction, similar processing parameters (1–3 kV/cm, 10 kJ/kg) have been reported (Toepfl, 2006), the total costs for the PEF treatment in the range of 2.69 € per ton of product, therefore, can be utilized to characterize the expenses to expect for other applications with similar production capacity.

4. Industrial Scale Equipment Availability

Pulse modulators and treatment chambers for technical and industrial scale treatment are realized at DIL, since 2006 DIL is owner of the ELCRACK® brand. Based on a solid state typology using a pulse transformer modular 5 and 30 kW average power systems are available. Design parameters are shown in Table 13.2. Treatment capacities range up to 10 t/hour for disintegration of fruit or vegetable tissue; parallel operation is possible to obtain higher flow rates. Exemplarily, a 5 kW ELCRACK® pulse modulator is shown in Figure 13.11 (For color details, please see color plate section). The turn key design and low space requirements allow a versatile use and an easy implementation into existing processing lines.

**Figure 13.11.** 5 kW ELCRACK® technical scale system. (For color details, please see color plate section.)

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Chapter 14

Improving Electrode Durability of PEF Chamber by Selecting Suitable Material

Minjung Kim and Howard Q. Zhang

1. Introduction

Pulsed electric field (PEF) technology is a nonthermal food preservation method using high-voltage PEF. It is based on a pulse power delivered to the product between two electrodes in the PEF chamber (Gngora-Nieto et al., 2002). Many researches have proved that PEF treatment in liquid foods is effective in microbial inactivation, and PEF treatment causes less degradation of ascorbic acid or flavor compounds in high-acid liquid products, thus resulting in better quality compared to thermal process (Qin et al., 1995–Evrendilek et al., 2000). While the microbial inactivation and engineering aspect of PEF have been profoundly investigated, electrochemical reaction or electrode corrosion in PEF process had little attention.

Electrode placed in PEF chamber is contacted with the food and electrical interface forms between the electrode and the liquid food. In the electrical interface, electrode is influenced by the charge of electrolyte held at the electrode and an electric field forms between the electrode and the layer of ions. If no voltage is applied, the electronation and de-electronation reactions reach equilibrium and net current across the interface is zero (Bockris and Reddy, 1970). When a voltage is applied to the electrode, thickness of the layer of ions at the interface increases and they be-

have as a capacitor. If the applied voltage is below a certain threshold voltage, capacitive current flows across the interface. The capacitive current does not involve any chemical reactions or charge transfer; it only causes accumulation or removal of electrical charges on the electrode and in the electrolyte near the interface. If the applied voltage is above the threshold voltage, Faradaic current flows and the electrochemical reactions may occur at the interface. The electrochemical reactions in PEF process can result in chemical changes in food products, electrode fouling including electrode corrosion and deposit of particles on the electrode surface, and release of electrode materials. There have been several measures to reduce the electrode fouling, such as changing chamber design (Dunn and Pearlman, 1987; Jayaram and Lubicki, 1997), adjusting pulse repetition (Morren et al., 2003), and lowering energy absorption (Mittal et al., 2000; Robbins, 2001).

Electrode corrosion in PEF process carries migration of electrode material. The released electrode material may cause color alteration or catalyze undesirable reactions in food products (Samaranayake, 2003) and eventually shorten the life time of electrode. Selecting proper electrode materials can be an important factor to prevent corrosion and food contamination. Some researchers recommended food grade, chemically inert materials, such as carbon, gold, platinum, and metal oxide, as electrodes in PEF system (Bushnell et al., 1996). Other researchers suggested the use of electrically conductive polymers as electrode materials (Qin et al., 1997). Samaranayake (Samaranayake, 2003) compared the

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electrochemical behaviors of different electrode materials in ohmic heating process. However, there is limited literature on the effect of electrode materials on the electrode corrosion or the electrochemical behavior of different electrode materials in PEF processing at this time.

Titanium, platinized titanium, stainless steel 316, and boron carbide were selected as electrode materials in this study due to their corrosion resistance. Titanium is well known for its corrosion resistance and is usually used in areas where austenitic stainless steels cannot provide sufficient corrosion resistance (Peters and Leyens, 2003). Commercially pure titanium or low-alloy titanium grades are used when corrosion resistance is main requirement rather than strength. Platinum is known as a noble material and does not oxidize in the air at any temperature. Due to its inertness, platinum is used for electrical contacts or corrosion-resistant apparatus. Since pure platinum is expensive, platinization of titanium has been used in many cases, especially when electrochemical process is involved (Kamachi et al., 2000). Grade 316 stainless steel, belonging to the austenitic stainless steel group, contains chromium (16%), nickel (10%), and molybdenum (2%) (Schweitzer, 2003). The molybdenum gives 316 stainless steel better corrosion resistances than other stainless steels; thus, wide applications, such as food processing, architecture, and transportation, are possible. Boron carbide is one of the nonmetallic and refractory compounds. Refractories are generally considered to be inert and corrosion resistant compared to metallic alloys (Rigaud, 2000).

The objective of this study was to reduce electrode corrosion and electrode material migration into the food by selecting corrosion-resistant material as electrode in PEF chamber.

2. Materials and Methods

2.1. Materials

Titanium (Grade 2) (All Metal Sales, Inc., Cleveland, OH), platinized-titanium (All Metal Sales, Inc., Cleveland, OH; US Filter, Union, NJ), stainless steel 316 (All Metal Sales, Inc., Cleveland, OH), and boron carbide (Boride Products, Traverse

City, MI) were used as electrodes. All electrodes were made as the same geometry, which was tubular shape with outer diameter 0.75 inch, inner diameter 0.25 inch, and length 2 inch. The ACS grade citric acid monohydrate (Sigma, St. Louis, MO), disodium phosphate (Sigma, St. Louis, MO), and trace metal grade double distilled nitric acid (GFS Chemicals, Powell, OH) were purchased from the suppliers.

2.2. Media

Citric acid buffer solution was prepared by adding 2 L of 0.1 N citric acid solution and 0.9 L of 0.2 N disodium phosphate solution into 11.6 L of distilled water to make 14.5 L of buffer solution. Citric acid was added into each buffer solution separately to adjust pH 3.5 and electrical conductivity 0.22 S/m at room temperature.

2.3. PEF Treatment System

The PEF system consisted of four co-field continuous flow tubular treatment chambers, heat exchanger, I.D. 0.5 inch stainless steel tubing, a gear pump (Micropump, Inc., Vancouver, WA), and fluid reservoir (Figure 14.1). The flow of fluid was monitored and controlled by ABB control panel. Each PEF treatment chamber consisted of tubular electrodes and an insulator. The chamber diameter was 0.635 cm

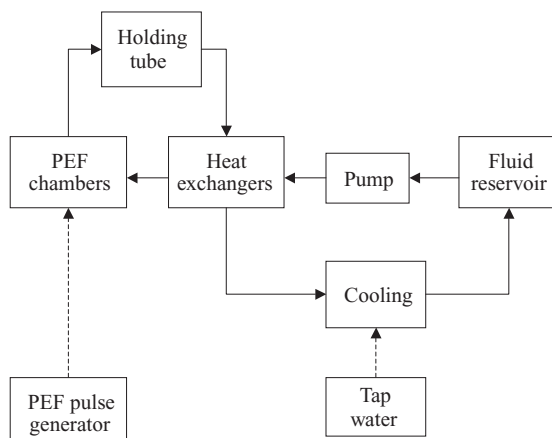


Figure 14.1. Flow chart of the pilot plant PEF system.

and the gap distance between the electrodes was 1.0 cm. Bipolar square waveform of pulses was used. Recirculating media system was used, since the trace metal in sample might not be detectable after one circulation of PEF process.

The PEF processing conditions were 28 kV/cm electric field strength, 600 pps pulse repetition rate, and 2 μ s pulse duration. Total treatment time was 44 μ s per one circulation and flow rate was 125 L/hour. The temperature of media during process was monitored at the points of inlet and outlet of the fluid reservoir, PEF chamber, and heat exchanger.

2.4. Experimental Procedure

A volume of 6.25 L was subjected to PEF processing in each experimental run. One circulation of media, which means media running from the fluid reservoir through the PEF processing to the fluid reservoir again, took 3 minutes, and total 80 circulations conducted in each experimental run. Temperature and electrical conductivity of media were monitored and controlled to be constant during the recirculating process. Prior to each experimental run, the PEF system, including fluid reservoir, stainless steel tubing, heat exchanger, and the PEF chamber was cleaned with water. After flow rate of citric acid buffer solution was stabilized at 125 L/hour, two control samples were taken from the fluid reservoir before the PEF process. The PEF-treated samples for electrode corrosion analysis were taken from the fluid reservoir after the 1st, 4th, 8th, 16th, 32nd, 64th, and 80th circulations. Three replicates were collected at a time, and the whole experimental run was duplicated. Replicates were collected at three different locations in the fluid reservoir and these sampling locations were kept same for all experimental runs. The same experimental procedure was performed for four different electrode materials.

2.5. Analysis of Electrode Corrosion

2.5.1. ICP-MS Concentrations of Ti from the titanium electrodes, Pt from the platinized titanium electrodes, Fe from the stainless steel electrode, and B from the boron carbide electrodes in the media

were considered as migration of electrode materials into the media and taken as measures of electrode corrosion. Perkin Elmer Elan 6000 inductively coupled plasma mass spectrometer (ICP-MS) for B and Pt analyses and Thermo Finnigan Element 2 ICP-MS for Ti and Fe analyses were used in quantitative analysis (Official methods of analysis of AOAC International, 2000). High-density polyethylene (HDPE) bottles (Fisher Scientific, NJ) were used for ICP-MS analysis sampling. HDPE bottles were cleaned using adapted method from EPA methods. After cleaning with deionized water, bottles were leached with 30% (v/v) nitric acid for 3 days and then leached with 2% (v/v) nitric acid for another 3 days. After leaching, the bottles were rinsed with deionized water and dried in a laminar hood and maintained capped until used. A 10 mL of sample was pipetted into a HDPE bottle and stabilized by adding concentrated nitric acid (2%, v/v) in the minimized condition of contamination. ICP-MS system was standardized with untreated buffer solution (stabilized with 2% (v/v) nitric acid). ICP-MS conditions were 1,300 W power, 15 L/minute plasma gas flow rate, and 0.5 L/minute auxiliary gas flow rate.

2.5.2. Scanning Electron Microscope The effect of corrosion on electrode surfaces was examined by a JEOL JSM-820 scanning electron microscope (SEM) at 15 kV. Each electrode was numbered: number 1, 3, 4, and 6 electrodes were served as ground electrodes and number 2 and 5 electrodes were served as high electric field applying electrodes in PEF chambers. Since only one side of the ground electrodes was exposed to the electric field and the other side was grounded, grounded sides of electrodes were used as unused control surfaces in SEM analysis. Unused control surface, used surface on ground electrode, and used surface on high electric field applying electrode were analyzed and compared by SEM.

2.6. Statistical Analysis

A linear regression model was used to determine if the type of electrode material had significant effects on corrosion. A statistical model was constructed

using the following equation:

$$Y_i = \beta \times t_i + \varepsilon$$

where Y_i = concentration of migrated metal elements in media; β = the slope; t_i = PEF running time; ε = random error.

Significance level was 0.05 in this analysis. All statistical analyses were conducted using Minitab 14 (Minitab Inc., State College, PA).

3. Results and Discussion

3.1. Electrode Corrosion

One of the important electrode corrosion measurements is the concentration of electrode materials migrated into the food in PEF processing. The comparison of migrated element concentration (in ppb) among four electrode materials is shown in Table 14.1. The migrated concentration of Ti, Pt, Fe, and B from titanium, platinized titanium, stainless steel, and boron carbide electrodes, respectively, were significantly different with one another ($p \leq 0.05$). While the migrated concentrations of titanium and platinized titanium electrodes were not significantly different at pH 3.5 in the ohmic heating process (Samaranayake, 2003), the migrated concentration of titanium electrode in PEF processing shows sig-

nificant difference from that of platinized titanium electrode at the same pH.

The migrated concentrations of all elements in the media increase proportionally as the number of circulations increase. The migrated boron concentrations were as high as 200 times compared to the migrated titanium concentrations. Since the migrated concentration represents the corrosion resistance, titanium is considered the most corrosion-resistant material and boron carbide is the least corrosion-resistant material in PEF processing. The order of corrosion resistance among four materials in this study is titanium, platinized titanium, stainless steel, and boron carbide.

3.2. Electrode Surface Morphologies

Changes of electrode surface morphologies before and after PEF processing were examined by scanning electron microscopy (SEM). Since electric field is applied to the inner edge of the electrode, images of the inner edge within 1 mm region were taken for comparison. Figures 14.2–14.7 show the changes of four different electrode surfaces. As it can be seen in Figure 14.2 (a), surface of titanium electrode is smooth, and the edge forms irregular shape caused by the manufacturing before the PEF processing. However, the surface changes to be rough

Table 14.1. Concentrations of migrated electrode elements into the media with respect to recirculation time

Number of Circulation	Run Time (minute)	Migrated Element Concentration (ppb ¹)			
		Ti ^{a, 2}	Pt ^{b, 3}	Fe ^{c, 4}	B ^{d, 5}
1	3	0.06 (0.06)	0.23 (0.04)	3.00 (1.73)	5.83 (5.49)
4	12	0.15 (0.07)	0.78 (0.05)	8.50 (2.12)	53.33 (2.88)
8	24	0.38 (0.09)	1.52 (0.04)	13.67 (2.89)	129.83 (4.88)
16	48	1.09 (0.20)	3.12 (0.16)	24.00 (0.00)	283.17 (7.05)
32	96	2.50 (0.35)	6.10 (1.19)	43.67 (2.08)	617.00 (28.49)
64	192	5.79 (0.35)	11.72 (2.63)	78.67 (0.58)	1216.00 (23.14)
80	240	7.46 (0.61)	14.35 (2.89)	95.00 (1.41)	1510.33 (35.06)

The values are means of six replicates ($n = 6$) with respective standard deviation in parentheses.

Different superscript letters (a–d) with the means denote significant differences ($\alpha = 0.05$).

¹ppb: parts per billion.

²Ti: titanium.

³Pt: platinum.

⁴Fe: iron.

⁵B: Boron.

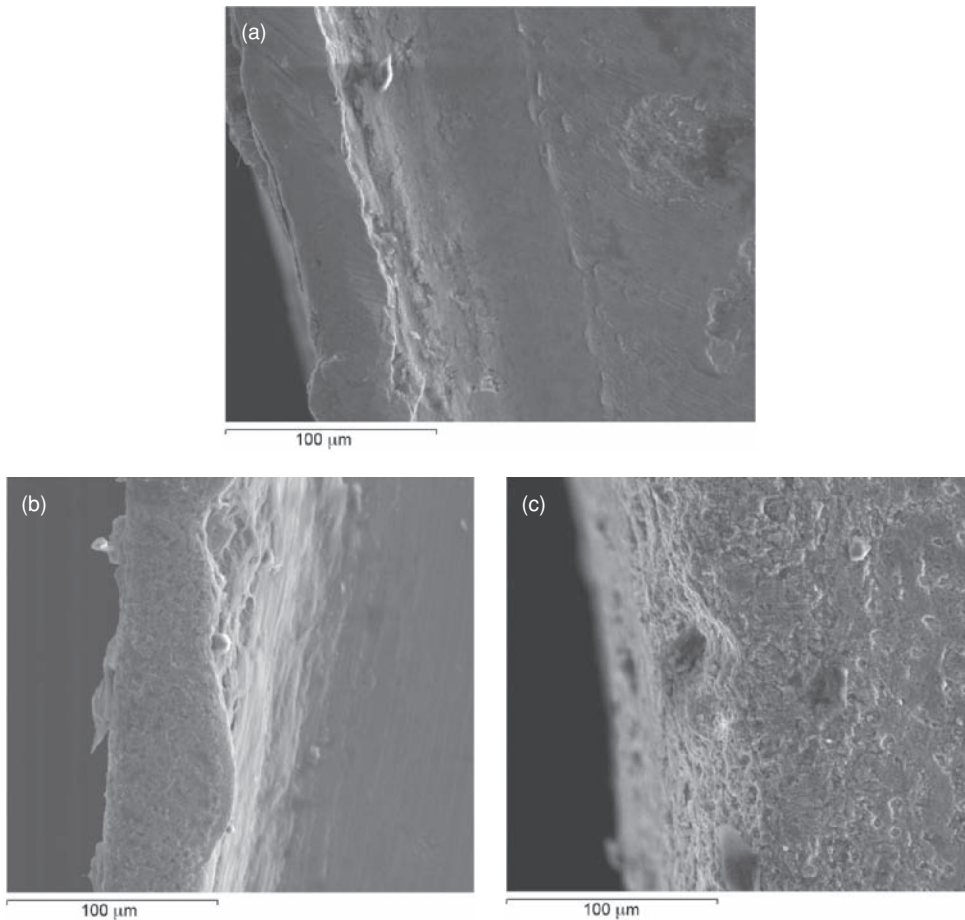


Figure 14.2. SEM micrographs of titanium electrodes (magnification: $\times 500$). (a) The surface of titanium electrode before PEF processing; (b) the surface of ground electrode after 35-hour PEF processing; and (c) the surface of high electric field electrode after 35-hour PEF processing.

and ragged on the edge (Figure 14.2 (b), (c)) after 35-hour of PEF processing. High electric field electrode, which is placed in the middle connecting to the other two ground electrodes in the PEF chamber, exhibits rounded inner edge indicating more corrosion compared to the ground electrodes. The roughness of high electric field electrode surface is, also, more severe than ground electrodes.

Platinized titanium shows a globular surface before PEF processing (Figure 14.3 (a)). Ground electrode surface becomes smooth from erosion, yet retains globular characteristics on the edge after

14-hour of PEF processing (Figure 14.3 (b)). On the other hand, the edge of high electric field electrode becomes smooth and the region next to the edge shows raggedness (Figure 14.3 (c)). The comparison of surface morphologies between ground and high electric field electrodes after PEF processing is shown in Figure 14.4. While the ground electrode does not show much difference between the edge and middle region, the high electric field electrode shows a clear difference. It is distinguished by a smooth region, ragged region, and globular region as a result of erosion and/or corrosion.

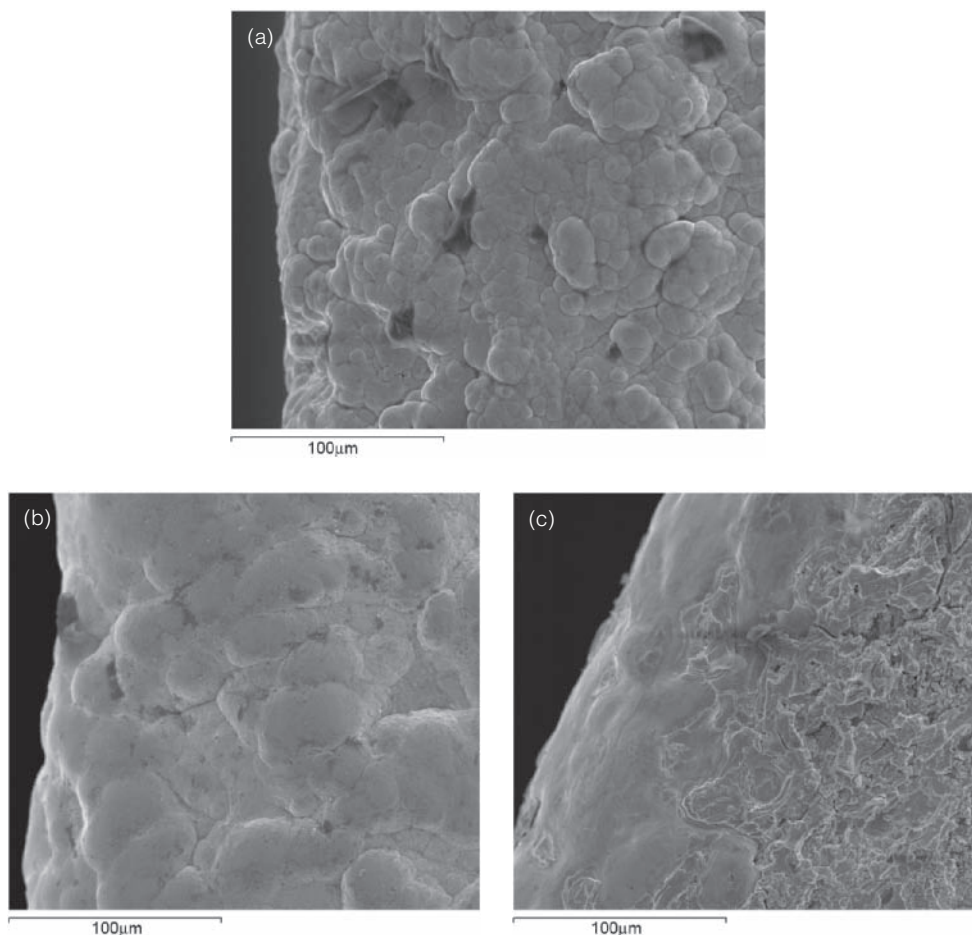


Figure 14.3. SEM micrographs of platinized titanium electrodes (magnification: $\times 500$). (a) the surface of platinized titanium electrode before PEF processing; (b) the surface of ground electrode after 14-hour PEF processing; and (c) the surface of high electric field electrode after 14-hour PEF processing.

Stainless steel shows clear evidence of corrosion in both ground and high electric field electrodes after 12-hour PEF processing. Unused stainless steel surface is relatively smooth, although some cracks and layers are observed (Figure 14.5 (a)). After PEF processing, the edges of stainless steel electrodes do not appear to be eroded much compared to other materials (Figure 14.5 (b), (c)). However, a lot of corrosion pits are observed on the surface (Figure 14.6). The corrosion pits of high electric field electrode are clear and severe compared to those of ground electrodes.

The areas where the corrosion pits appear are, also, different between the two. While the corrosion pits are observed in the region within $150\text{ }\mu\text{m}$ from the edge on the ground electrodes, they are observed in $400\text{ }\mu\text{m}$ from the edge on the high electric field electrodes.

The erosion of inner edge on boron carbide electrodes was severe enough to be observed by the eyes and it is consistent with the results by ICP-MS analysis. Unused boron carbide electrode surface is even with machine marks and has tiny holes resulted

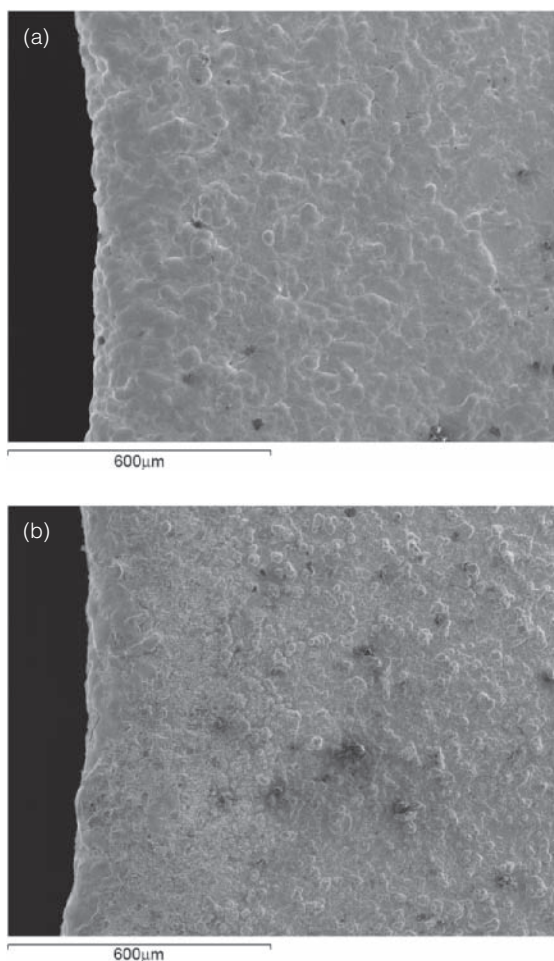
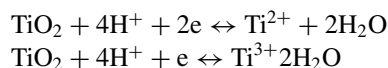


Figure 14.4. Comparison of platinized titanium electrode surfaces between ground electrode and high electric field electrode after 14-hour PEF processing (magnification: $\times 100$). (a) The surface of ground electrode and (b) the surface of high electric field electrode.

from air bubbles during manufacturing (Figure 14.7 (a)). The sharp inner edges of unused boron carbide change to be round by erosion and/or corrosion and the surface becomes to be very smooth after 13-hour PEF processing (Figure 14.7 (b), (c)). The region where the erosion occurs does not have any machine marks and the borderline between eroded and non-eroded region is clear in both ground and high electric field electrodes.

3.3. Titanium Electrodes

The inner edges of titanium electrodes colored with white to yellow and bluish color after PEF processing, indicating the formation of titanium oxide film. The corrosion resistance of titanium comes from the formation of protective titanium oxide film on the surface. Because titanium metal is highly reactive and has high affinity for oxygen, the protective oxide films form spontaneously and instantly when fresh metal surfaces are exposed to air and/or moisture. These oxide films can even reheal the damaged oxide film in the presence of oxygen or water (Donachie, 2000). Titanium forms different oxides depending on environmental conditions, such as TiO_2 (rutile, yellowish color), Ti_2O_3 (anatase, white color), $\text{TiO}_{1.9}$ (oxygen deficit, bluish color), and Ti_3O_5 (violet color) (James and Straumanis, 1976; Donachie, 2000). The titanium oxide films on surface contribute to the least migrated concentration in the media, furthermore, the least corrosion of titanium electrode among four materials in PEF processing. When the oxide is sufficiently thick and stable, electron exchange occurs between the electrolyte and the oxide film and the semiconductive properties of the oxide determine the current/potential behavior of the system. When the oxide is sufficiently thin (0.4–3 nm), electron exchange occurs between the electrolyte and the underlying metal by direct tunneling or resonance tunneling and the current/potential characteristics are similar to those of the bulk metal (Been and Grauman, 2000). The migrated concentration of Ti in the media can be explained by deposition of underlying metal with thin oxide films or the following cathodic reactions of titanium oxide under acidic condition (James and Straumanis, 1976).



3.4. Platinized Titanium Electrodes

Platinized titanium electrodes in this study exhibit significantly lower concentration of migrated Pt element from the electrode in the media compared

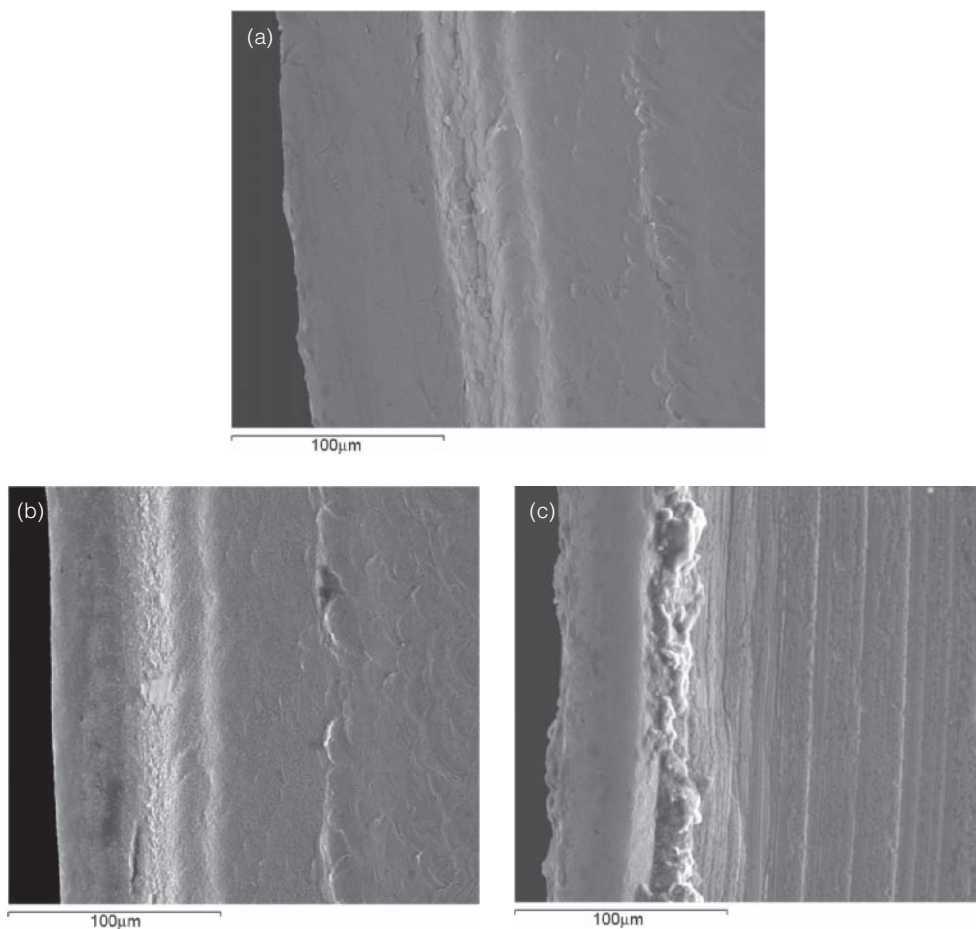


Figure 14.5. SEM micrographs of stainless steel electrodes (magnification: $\times 500$). (a) The surface of stainless steel electrode before PEF processing; (b) the surface of ground electrode after 12-hour PEF processing; and (c) the surface of high electric field electrode after 12-hour PEF processing.

to stainless steel and boron carbide electrodes, but higher concentration than titanium electrode. Even though platinum is considered as more stable cathodic metal than titanium, platinized titanium electrodes show less corrosion resistance than titanium electrodes in PEF processing. It is considered that platinum coating on the titanium electrode might have effect on this result. In many cases, electrodeposited coating of platinum group metals has pores with increasing thickness and spontaneous cracking or even mechanical detachment from anodes can oc-

cur due to attack of the substrate through pores in the coating (Shreir, 1976). Therefore, pure platinum electrode may show different results from this study. In the SEM images of platinized titanium surfaces, platinum coated on the titanium shows some degree of porosity, which can be subject to corrosion. In addition to the porosity, the surfaces of electrodes are globular with bumps and valleys and these bumps can be worn out through the PEF processing. Figure 14.3 (b) exhibits wearing out of the top on globular bumps after PEF processing.

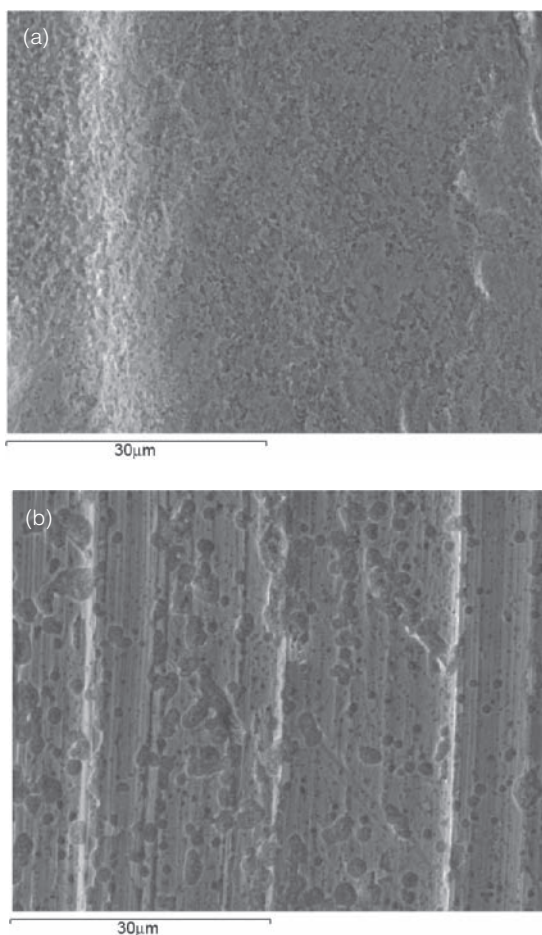


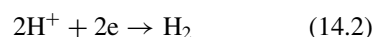
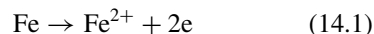
Figure 14.6. SEM micrographs of corrosion pits on stainless steel electrodes after 12-hour PEF processing (magnification: $\times 2000$). (a) The surface of ground electrode and (b) the surface of high electric field electrode.

3.5. Stainless Steel Electrodes

Adherent surface films were observed inside and outside of stainless steel electrode edges after PEF processing. The observed films were transparent, with a light golden color. The corrosion resistance of stainless steel 316 depends on formation and stability of a passive film on the surface, and chromium and molybdenum act in synergy to enhance the stability of the film (Qiu, 2002). Passive film, which consists of an inner layer of chromium oxides and an

outer layer of iron oxide and hydroxides, undergoes variation with time and sufficient time is needed to achieve equilibrium with the surrounding medium (Proverbio and Bonaccorsi, 2002). The corrosion attack occurs when the surrounding environment does not allow the formation of the protective layer. It is considered that electrical pulses of PEF inhibit the stability of passive film, thus, the corrosion occurred on the surface in spite of the presence of passive film.

The concentration of migrated Fe from stainless steel electrodes into the media is lower than that of B from boron carbide in this study. However, the surface images by SEM analysis indicate that pitting corrosion attacked the electrode surface. Since the pits on the electrode are not deep enough in spite of their abundance, it is considered that the pitting corrosion just started and it will continue and get worse on the electrode surface. Pitting corrosion is one of the most dangerous forms of local attack for steels. The corrosion can happen by the following reaction at pH 3.5.



While the amounts of weight loss of titanium, platinized titanium, and boron carbide electrodes after PEF processing showed consistence with the corrosion-resistant order of ICP-MS results, the weight loss of stainless steel was smaller than expected level, which was based on ICP-MS result. The deposits of particles or adhering substances in corrosion pits on the surface are possible explanation for the inconsistency between ICP-MS result and weight loss of electrode in stainless steel.

3.6. Boron Carbide Electrodes

Boron carbide electrodes are currently used in OSU PEF systems and the lifetime is estimated as about 100 hours. They were selected as electrodes due to their excellent resistance to erosion. In addition to that, boron that can be migrated from the boron carbide electrodes into food is considered as mineral source. Boron has been known essential mineral in plants for a long time, and evidence has been

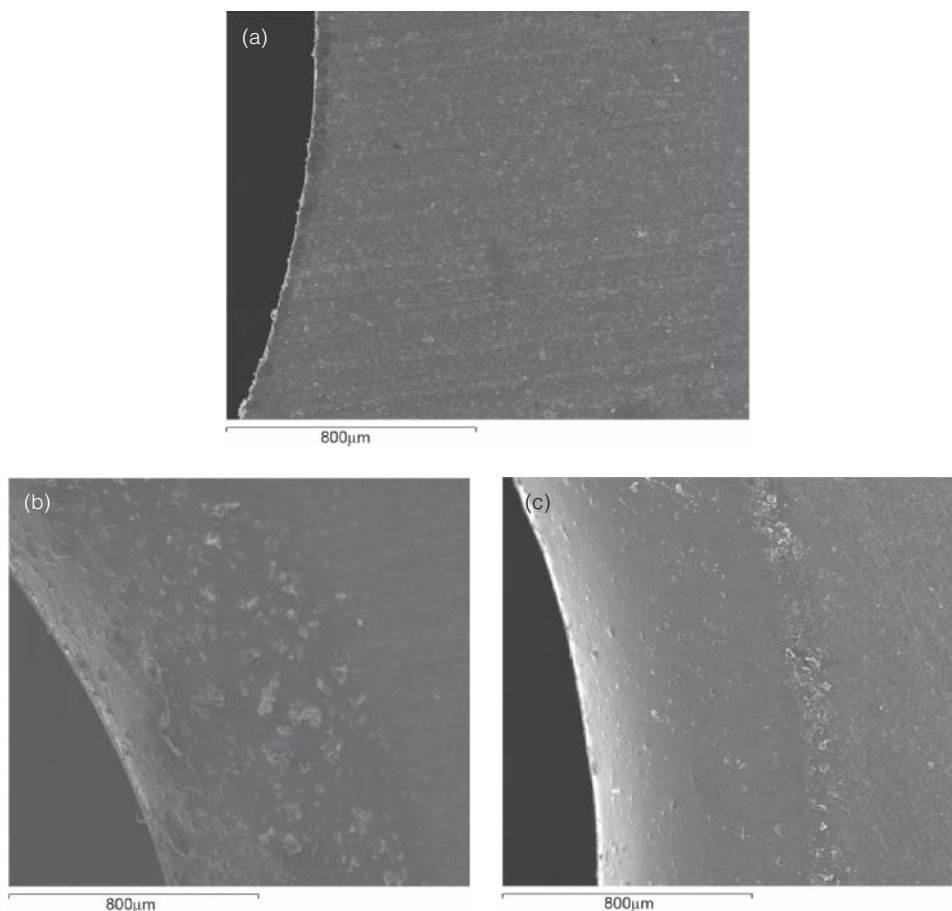


Figure 14.7. SEM micrographs of boron carbide electrodes (magnification: $\times 75$). (a) The surface of boron carbide electrode before PEF processing; (b) the surface of ground electrode after 13-hour PEF processing; and (c) the surface of high electric field electrode after 13-hour PEF processing.

accumulating which suggests that boron has an essential biochemical role in higher animals including humans (Nielsen, 1997).

In spite of known resistance to erosion, boron carbide was the most corroded material in this study. After 12-hour of PEF processing, corrosion of boron carbide electrodes was noticeable by the eyes while other materials were not, after the same processing time. The concentration of migrated boron is also conspicuously higher than those of other materials. Boron carbide is relatively corrosion-resistant at room temperature under dry atmosphere; how-

ever, the propensity to degradation increases rapidly with increasing temperature and specific chemical, mechanical, and physical gradients (Rigaud, 2000). Since oxidation shortens the life of ceramic material when carbon is used as a matrix interphase (Viricelle et al., 2001), mass loss in the boron carbide might occur by erosion and oxidation. Formation of boric oxide (B_2O_3) can retard the oxidation, however, the temperature that boric oxide forms is as high as $450^\circ C$. Since the maximum temperature of media in this study was adjusted below $60^\circ C$, boric oxide formation against oxidation did not occur. Boron

Table 14.2. Comparison of estimated element intakes through consumption of an 8 fL oz PEF-treated meal with the upper limit of daily dietary intakes for adult consumers

Element	Estimated Intake Through 8 fL oz. Meal ($\mu\text{g}/8 \text{ fL oz.}$) ^a	Upper Limit of Daily Dietary Intakes ($\mu\text{g}/\text{day}$)
Ti	0.015	600 ^b
Pt	0.047	0.3 ^b
Fe	0.409	45,000 ^c
B	3.726	20,000 ^c

^aThe estimation is based on unit conversions: 1 ppb = 1 ng/mL; 8 fL oz = 236.59 mL; 1 ng = 10 μg .

^bReilly (Reilly, 2002).

^cFood and Nutrition Board, Institute of Medicine (Food and Nutrition Board, Institute of Medicine, 2001).

carbide electrodes in the PEF system are corroded under electrochemical gradient without self-protection.

3.7. Toxicity of Migrated Element Amounts

Intakes of the migrated elements were evaluated with respect to a typical 8 fL oz meal using averages of migrated concentrations after one circulation. The comparison of the estimated intakes of PEF-treated products with upper limit of daily dietary intakes is shown in Table 14.2. The amounts of estimated contaminant intakes are way below the upper limit of daily intakes for all four materials.

4. Conclusion

Four different electrode materials, titanium, platinized titanium, stainless steel 316, and boron carbide, show different corrosion resistance in PEF process. Titanium was the most corrosion-resistant material as electrode in PEF system. After 35-hour of PEF process, titanium electrodes were not changed much on the edge visibly, while boron carbide electrodes were worn out after 12 hours of PEF process. The amount of migrated Ti element in the media was significantly different from those of migrated Pt, Fe, and B in the media. Platinized titanium electrodes showed better corrosion resistance compared to stainless steel 316 and boron carbide electrodes;

however, it was not as good as titanium. Stainless steel 316 electrodes exhibit severe pitting corrosion in SEM analysis. Boron carbide, which is currently used in OSU PEF system, was the least corrosion-resistant material. The estimated intakes of Ti, Pt, Fe, and B through consumption of 8 fL oz PEF-treated products were far below the upper limit of dietary intakes of each material. Titanium can be recommended as electrode material in PEF system due to its high corrosion resistance, long life time, and toxicological safety.

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Chapter 15

Radio Frequency Electric Fields as a Nonthermal Process[§]

David J. Geveke

1. Introduction

Radio frequency electric field (RFEF) processing of liquid foods using high field strengths at relatively low temperatures has only recently been investigated. It is similar in many respects to pulsed electric field (PEF) technology processing, which has been extensively studied during the last two decades. Genesis Juice (Eugene, OR) recently introduced the first PEF-treated products in the United States. The juice produced using PEF tastes better than that produced using heat pasteurization, although at a slightly greater cost. RFEF processing uses relatively simple equipment to generate high electric fields and may offer potential cost benefits to the food industry. Several research groups have demonstrated the inactivation of a range of microorganisms both in model systems and in food products using a diversity of RFEF systems (Uemura & Isobe, 2002; Geveke & Brunkhorst, 2003). RFEF, also referred to as high electric field alternating current, is the application of radio frequency waves of very high field strength for a very short time to foods placed between two electrodes. Although RFEF is a nonthermal processing

method, an increase in temperature can occur during treatment depending on sample composition and processing conditions. While much has been discovered regarding RFEF processing, much more needs to be determined.

The present chapter gives an overview of the current state of art in microbial inactivation in food products by RFEF. Critical process parameters determining inactivation are discussed. Some issues are presented that need further investigation in order to commercialize RFEF technology to preserve liquid foods.

2. Historical Background

The bactericidal effect of an electric current has been known for over a century, but the lethality of applying direct or alternating current resulted from thermal effects. The effects of quick high electric field pulses on microbes were first systematically studied in the 1960s (Sale & Hamilton, 1967). It was determined that passing an elevated electric field (10–25 kV/cm) through a liquid held between two electrodes, in very short pulses (2–20 μ s), did not significantly raise the temperature, but did cause a lethal effects on microbes. Since then, numerous research groups around the world have investigated PEF processing of liquid foods. During the past decade, researchers began exploring the use of radio frequency power supplies to generate high electric fields of short duration to liquid foods (Geveke et al., 2002; Uemura & Isobe, 2002). The remainder of this chapter relates to RFEF processing.

[§]Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the US Department of Agriculture.

3. Mechanisms of Action

Nonthermal inactivation of microorganisms by RFEF is thought to occur by electroporation (Chang et al., 1991; Geveke, 2003). In an electric field, a voltage is formed across the cell membrane. The opposite charges on either side of the membrane are attracted to each other and the membrane becomes thinner. At a sufficiently high voltage, pores are formed in the membrane and the cell ruptures (Zimmermann, 1986). In order to investigate this phenomenon, scanning electron microscope (SEM) images were produced for untreated, thermally treated, and RFEF nonthermally treated *Escherichia coli* K12 (Geveke et al., 2006). Untreated cells were typically individual, rod-shaped with smooth surfaces. The shapes of thermally treated cells were distorted by large, irregular depressions and evaginations of their surfaces. The shapes of nonthermally treated cells were less distorted than those thermally treated, indicating that the mechanisms for thermal and RFEF inactivation are dissimilar. In addition to using SEM to detect visible damage to the cytoplasmic membrane, flow cytometry measurement may be used to study possible changes in cell wall and membrane morphology (Ukuku & Shelef, 1996). Ruptures in the membrane structure of bacterial cells can lead to leakage of internal substances of bacteria, thus causing them to be inactivated. ATP plays a key role in the energy status of the cell and in regulating enzyme activity. Non-thermal RFEF processing of *E. coli* K-12 in apple juice led to the efflux of intracellular ATP and UV-absorbing materials suggesting that mechanism of inactivation of RFEF is by disruption of the bacterial surface structure leading to the damage and leakage of intracellular biological active compounds (Ukuku et al., 2008). The suspected membrane damage as a result of RFEF treatment needs to be further investigated by measuring the leakage of intracellular proteins, nucleic acids, and enzymes and by examining the morphological changes described above.

4. RFEF Treatment Systems

Currently, RFEF treatment systems are not commercially available. This situation is similar to PEF re-

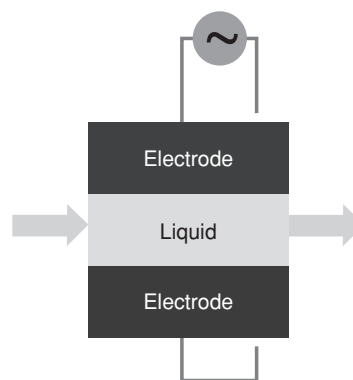


Figure 15.1. Diagram of simplified RFEF system showing a treatment chamber and a power supply. Liquid flows between two parallel plate electrodes with an alternating current across them.

search in that all of the treatment systems are custom built. As the field of RFEF research is a new one, there are only a few groups around the world who have designed and assembled treatment systems (Uemura & Isobe, 2002; Geveke & Brunkhorst, 2003). All of the systems include a liquid feed pump, a treatment chamber where the liquid comes in contact with high voltage electrodes, and a radio frequency generator that supplies the high voltage to the electrodes. A simplified diagram of the basic components of a RFEF system is presented in Figure 15.1.

In addition to the basic RFEF components, the system generally includes a feed tank, a heat exchanger between the pump and the treatment chamber to regulate the inlet temperature, a heat exchanger after the treatment chamber to remove any ohmic heat generated by the treatment, controllers, and monitoring equipment (Geveke & Brunkhorst, 2003). As has been mentioned, RFEF processing is a new research area and, consequently, the size of the equipment is still rather limited. The flow rates of liquid that these novel systems can treat varies from 150 mL/minute (Uemura & Isobe, 2002) to 1,400 mL/minute (Geveke & Brunkhorst, 2008). The most powerful RFEF system for nonthermal processing of liquids is located at the US Department of Agriculture Eastern Regional Research Center (Figure 15.2).



Figure 15.2. RFEF pilot plant system including fluid handling equipment, 80 kW power supply, and monitoring equipment (photograph by Peggy Greb, USDA-ARS).

Additional information regarding the two key components of RFEF systems, generators and treatment chambers, are presented in the following sections.

5. Generation of RFEF Fields

High electric fields are produced by placing a liquid between two electrodes that are in close proximity to each other and applying a high voltage to the electrodes. In the case of RFEF, an alternating current generator is used to supply the power. This potentially simpler method of generating high electric fields may have lower capital costs than those associated with PEF processing (Geveke, 2003).

Several types of AC power supplies for nonthermal RFEF processing have been reported in the literature. Geveke & Brunkhorst (2003) designed and constructed a power supply that consisted of four 1 kW RF amplifiers (Industrial Test Products, Port Washington, NY, model 1000 A) and four step-up transformers (Industrial Test Products). These were connected in series and produced a voltage of 4.0 kV_{peak} over a frequency range of 20–100 kHz. A function generator (Tektronix, Beaverton, OR;

model AFG 310) drove the amplifiers. Geveke & Brunkhorst (2004b) proposed and fabricated a larger RFEF power supply to achieve greater treatment capacity. It consisted of an 80 kW RF power supply (Ameritherm, Scottsville, NY, model L-80) and a custom-designed matching network (Ameritherm) that enabled the RF energy to be applied to a resistive load. This system produced a voltage of 6.9 kV_{peak} over a frequency range of 21–40 kHz. Uemura and Isobe (2002) used a power supply consisting of a PC-controlled signal generator (Hewlett-Packard, model HP8904 A) and an amplifier (NF Circuit Block, model 4510). These produced a voltage of 0.3 kV_{peak} at a frequency of 20 kHz.

There are numerous companies that sell AC power supplies throughout the world. The three criteria for selecting a power supply for nonthermal microbial inactivation applications are voltage, frequency, and cost. According to the electric field strength model (Hulshager et al., 1981), there is a critical electric field strength that is the minimum field required to irreversibly rupture the cell membrane. Therefore, the voltage delivered to the treatment chamber should be great enough to achieve an electric field strength of approximately 10 kV/cm or greater (Sale &

Hamilton, 1967; Geveke & Brunkhorst, 2003). The frequency should range between approximately 1 and 250 kHz. In designing RFEF systems, it is necessary to have a minimum of one half-cycle in each chamber. It is safer to design for a minimum of two half-cycles to ensure against non-uniform particle velocities. Based on reasonable flow rates and chamber dimensions, the frequency cannot be much below 1 kHz. Regarding an upper frequency limit, there is a lag between the applied voltage and the induced voltage across the membrane due to the capacitance of the membrane (Kotnik et al., 1998; Geveke et al., 2002). Although it is very difficult to predict the reduction in the induced transmembrane voltage due to the lag, it is clear that the voltage is significantly reduced as the frequency is increased from 100 kHz to 1 MHz. In PEF processing, typically, pulse widths of 2 μ s are used. Pulse widths of less than 1 μ s are rarely used. If PEF pulse widths are considered equivalent to RFEF half-cycles, then a pulse width of 2 μ s would equate to a frequency of 250 kHz. Therefore, based on the theoretical modeling of Kotnik et al. (1998) and the experience with PEF processing, it seems reasonable to limit the frequency to ≤ 250 kHz. Finally, the quality of the power does not appear to be important (Geveke & Brunkhorst, 2003, 2004b); therefore, equipment should be selected based on the cost to power ratio (\$/kW).

6. Treatment Chamber Design

Many designs of treatment chambers have been used in PEF and RFEF processing. One of the simplest designs is shown in Figure 15.1. In this case, the electric flux lines are perpendicular to the direction of the liquid flow. Uemura and Isobe (2002) used this type of treatment chamber. The electrodes were made of titanium strips separated by a 0.2 mm gap.

An alternative design is to orient the electric flux lines parallel to the direction of the liquid flow (Sensoy et al., 1997; Geveke & Brunkhorst, 2004a). These are sometimes referred to as cofield flow chambers. An example is shown in Figure 15.3. The chamber is made of Rexolite, a transparent cross-linked polystyrene copolymer (C Lec Plastics, Philadelphia, PA). The treatment chamber is

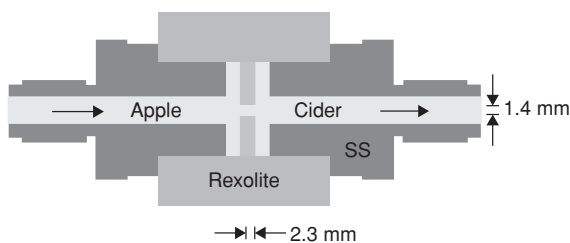


Figure 15.3. Cross-section of a converged cofield flow treatment chamber including Rexolite insulation and stainless steel (SS) electrodes. The diameter and length of the cylindrical channel through the Rexolite, the high electric field zone, were 0.14 cm and 0.23 cm, respectively (Geveke & Brunkhorst, 2008).

designed to converge flowing foods, in this case, apple cider, into a narrow area in order to reduce the power requirement (Matsumoto et al., 1991; Sensoy et al., 1997; Geveke & Brunkhorst, 2004a). Liquid food enters and exits the Rexolite chamber through the annuli of cylindrical stainless steel electrodes (Swagelok, Solon, OH, part no. SS-400-1-OR). Between the electrodes in the treatment chamber, there is a thin partition with a channel of circular cross section through the center. The diameter and length of the channel are 0.14 cm and 0.23 cm, respectively. The chamber design includes a 0.20 cm space between the end of each of the electrodes and the central channel to reduce the potential for arcing.

In order to increase the RFEF treatment time and microbial inactivation, the liquid food is often passed through a series of treatment chambers. Uemura and Isobe (2002) used four treatment chambers in series. Four pairs of electrodes were sandwiched between Teflon plates that contained a hole for the liquid to flow through. AC voltage was applied to each of the pair of electrodes in parallel. In the case of cofield flow treatment chambers, there are two ways to connect the chambers in series. One configuration has the liquid flowing in series through treatment chambers in which the first electrode on each of the treatment chambers is grounded as shown in Figure 15.4a. The remaining electrode on each of the treatment chambers is connected to the RFEF power supply in parallel. Upon exiting the treatment chamber, the liquid

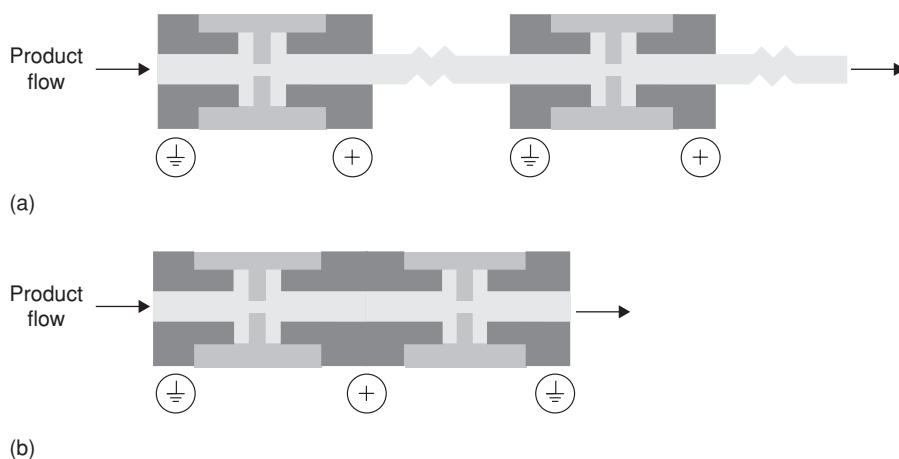


Figure 15.4. Configurations of two cofield flow treatment chambers in series: (a) separated, by plastic tubing to electrically isolate the chamber, and a heat exchanger to control the processing temperature, and (b) connected.

flows through a sufficient length of plastic tubing to electrically isolate the treatment chamber from the surrounding equipment and ensure that the maximum field is achieved within the chamber. The temperature of the liquid food rises during RFEF processing due to ohmic (resistance) heating. Therefore, the liquid food flows through heat exchangers after each treatment to control the processing temperature. Another way of connecting two cofield flow treatment chambers in series is presented in Figure 15.4b. The chambers are joined by stainless steel tubing. The inner electrodes between the chambers are connected to the RFEF power supply. The outer electrodes are grounded. The advantage of this setup is that there is no concern about isolating the chambers from the surroundings. The disadvantage is that, for a given field, the temperature rise is twice that for a single-treatment chamber.

QuickField™ (Tera Analysis Ltd, Svendborg, Denmark) finite element analysis software has been used to model the anisotropic electric field strength within converged cofield flow treatment chambers having diameters and lengths of 0.10 cm and 0.20 cm, respectively (Geveke & Brunkhorst, 2004a), and 0.12 cm and 0.20 cm, respectively (Geveke et al., 2006). In scaling up the process, larger treatment chambers have been used. The electric field strength has been modeled for a larger treatment chamber hav-

ing diameter and length of 0.14 cm and 0.23 cm using QuickField™ (version 4.3). The results of the model indicate that the food flows from a field-free region to a high field strength region within the channel (Figure 15.5). The field is nearly uniform which promotes evenly processed foods. If the diameter were to be much larger relative to the length, the field would become nonuniform. There would be a region near the center of the treatment chamber where the electric field is too low to inactivate bacteria and only heats the food. This would reduce the energy efficiency of the process.

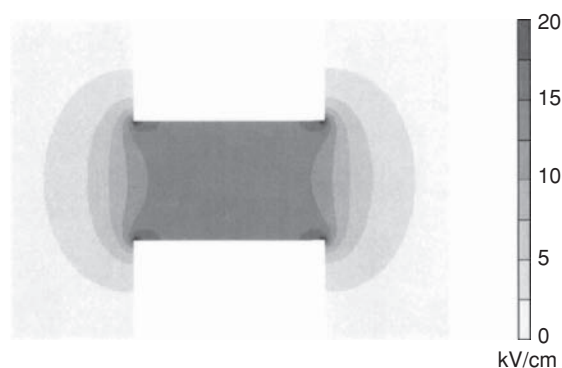


Figure 15.5. Modeled anisotropic RFEF strength within the converged section of the treatment chamber shown in Figure 15.3. The diameter and length of converged section is 0.14 cm and 0.23 cm, respectively.

7. Main Processing Parameters

There are a number of operating variables involved in RFEF nonthermal processing which make this technology somewhat complex. Several research groups have tested various parameters; however, the equipment, medium, and microorganisms used by the different groups imply that comparisons of results may not always be valid. Information regarding several key parameters of RFEF processing is presented in the following sections.

7.1. Electric Field Strength

As the electric field strength increases, the effect on bacterial cells increases. For instance, Figure 15.6 presents data on RFEF processing of apple cider at varying field strengths. Increasing field strength has also been shown to increase inactivation of *Saccharomyces cerevisiae* in water (Geveke & Brunkhorst, 2003) and of *E. coli* in apple juice (Geveke & Brunkhorst, 2004b). In one study, increasing the field up to 16 kV/cm increased inactivation; however, above this intensity, inactivation remained constant (Geveke & Brunkhorst, 2004a). There are reports in the PEF literature of similar behavior. Inactivation of *Lactobacillus brevis* greatly increased with field strength up to 15 kV/cm, whereas, at higher fields, inactivation remained constant (Jayaram et al., 1992). Likewise, inactivation of *Listeria in-*

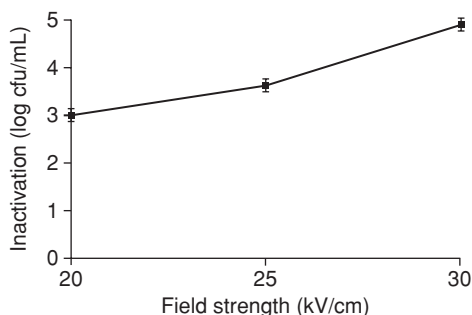


Figure 15.6. Effect of electric field strength on the inactivation of *E. coli* in apple cider at 220 μ s RFEF treatment time at an outlet temperature of 60°C. Controls (<1 kV/cm field strength) at the identical temperature and time established that there was no thermal inactivation.

Table 15.1. Effect of RFEF treatment chamber outlet temperature on the inactivation of *E. coli* in apple cider at a total treatment time of 420 μ s and hold time of 5.4 seconds

Temperature (°C)	Electric Field (kV/cm)	Inactivation (log CFU/mL)
50	<<1 (control)	0.0 $\pm$ 0.1
55	<<1 (control)	0.0 $\pm$ 0.1
60	<<1 (control)	0.2 $\pm$ 0.1
50	20	1.3 $\pm$ 0.2
55	20	2.4 $\pm$ 0.7
60	20	5.0 $\pm$ 0.1

nocua increased with field strength up to 30 kV/cm, but remained constant at higher fields (Wouters et al., 1999).

There is an upper limit to the electric field strength. At field strengths exceeding 30–40 kV/cm, arcing across the electrodes occurs. This is detrimental to the liquid food and to the treatment chamber.

7.2. Treatment Temperature

The effects on microbial inactivation of temperature and electric field strength are synergistic. For example, Table 15.1 presents data on RFEF processing of apple cider at varying temperatures. At an outlet temperature of 50°C, all of the inactivation, 1.3 $\pm$ 0.2 log, is attributable to nonthermal effects. At 60°C, the thermal inactivation component is 0.2 $\pm$ 0.1 log and the nonthermal inactivation component is 4.8 $\pm$ 0.2 log. This synergism of temperature and RFEF was observed earlier during RFEF processing of apple juice (Geveke & Brunkhorst, 2004b). The same phenomenon has been reported in PEF processing (Wouters et al., 1999; Bazhal et al., 2006). Using synergistic effects of elevated treatment temperature, 65°C, and PEF, the processing energy was reduced by more than 60% for a microbial inactivation of 6 log (Heinz et al., 2003).

7.3. Treatment Time

As the treatment time increases, the effect on bacterial cells increases. For instance, Figure 15.7 presents

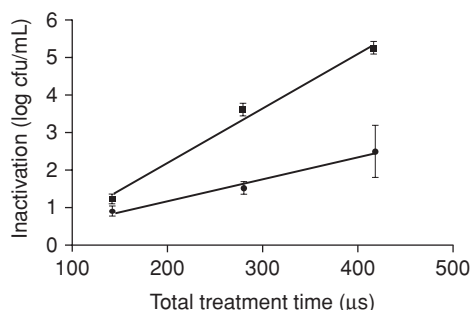


Figure 15.7. Effect of RFEF total treatment time on the inactivation of *E. coli* at an electric field strength of 20 kV/cm; (●) 55°C, (■) 60°C. A control (<1 kV/cm field strength) established that the thermal inactivation was 0.2 ± 0.1 log at 60°C and the longest time. At all other times and temperatures, there was no thermal inactivation (Geveke & Brunkhorst, 2008.)

data on RFEF processing of apple cider at varying treatment times and at two outlet temperatures, 55 and 60°C. The data follow first-order kinetics with a multiple regression correlation coefficient (r^2) of 0.972 and 0.986 for 55 and 60°C, respectively. The calculated D values for 55 and 60°C are 194 and 74 μ s, respectively. When the cider was ohmically heated (<1 kV/cm) to 55°C, the bacteria was unaffected. Likewise, when the cider was ohmically heated to 60°C, the bacteria was unaffected except for at the longest time, 420 μ s, which resulted in a thermal inactivation of only 0.2 ± 0.1 log.

Increasing RFEF treatment time has also been shown to increase inactivation of *S. cerevisiae* in water (Geveke & Brunkhorst, 2003) and of *E. coli* in apple juice (Geveke & Brunkhorst, 2004a, 2004b). Treatment time can easily be increased by adding treatment chambers in series. This is routinely done in PEF processing (Wouters et al., 1999; Evrendilek et al., 2004). There is no limit to the amount of treatment chambers that may be added in series.

8. Applications

Nonthermal processing of liquids with high-intensity RFEF has only been studied for the past several years. Microbial inactivation using low intensity RFEF, with field strengths less than 5 kV/cm, was shown to be due to heat alone (Geveke et al., 2002). Us-

ing a high-intensity RFEF power supply and treatment chamber to achieve a 30 kV/cm electric field, the population of *S. cerevisiae* in water was reduced by 3.1 log at a treatment chamber inlet temperature of 26°C and outlet temperature of 45°C (Geveke & Brunkhorst, 2003). As a control experiment, when the electric field was eliminated and the inlet temperature was raised to match the outlet temperature of 45°C, the reduction was only 0.3 log. Application of a 14 kV/cm RFEF reduced *E. coli* in saline water on the order of 2 log at approximately 60°C with a hold time of less than 1 second (Uemura & Isobe, 2002).

RFEF processing inactivated *E. coli* in apple juice at nonthermal conditions. The population of *E. coli* was reduced by 1.4 log after being exposed to a 24 kV/cm peak electric field (Geveke & Brunkhorst, 2004a). When the field was eliminated and the inlet temperature was raised to match the outlet temperature of 45°C, the reduction was <0.1 log. Using a scaled-up RFEF system, the population of *E. coli* in apple juice was reduced by 2.7 log after being exposed to a 20 kV/cm electric field at a treatment chamber outlet temperature of 60°C, and hold time of 3 seconds (Geveke & Brunkhorst, 2004b). When the juice was ohmically heated to the same outlet temperature of 60°C, and held for the same time of 3 seconds, the population of *E. coli* was unaffected.

Orange juice containing *Bacillus subtilis* spores was RFEF processed at 121°C under pressurized conditions to elevate the boiling point (Uemura and Isobe, 2003). Applying a 16.3 kV/cm field reduced the viable *B. subtilis* spores by 4 log in <1 second; however, no control was performed at the identical time and temperature to determine whether the spore reduction was due to thermal or nonthermal effects. RFEF processing orange juice at an outlet temperature of 65°C reduced the population of *E. coli* by 3.3 log relative to the control (Geveke et al., 2007). No loss in ascorbic acid or enzymatic browning was observed due to RFEF processing.

9. Challenges

There are many challenges to having a new technology adopted by industry. In addition to proving

that a new process can sufficiently inactivate microorganisms, the technology developer must demonstrate that the process provides a better product and is economically interesting in comparison to existing processes, in terms of investment and operating costs. There are also government regulations and consumer acceptance issues with which to contend. Several of these subjects are discussed in the following sections.

10. Operating Costs

The energy required for a 5-log reduction of *E. coli* in apple cider using RFEF at a processing temperature of 60°C is approximately 300 J/mL. By comparison, the estimated energy of PEF processing ranges from 100 to 400 J/mL (Barsotti and Cheftel, 1999; Schoenbach et al., 2002). The estimated energy cost for RFEF pasteurization is \$0.0060/L of apple cider using the US Department of Energy's data for the average industrial electric price for the first 9 months of 2008 of \$0.0699/kWh. By contrast, the energy costs for conventional thermal pasteurization with heat regeneration is approximately \$0.00068/L (Kozempel et al., 1998). The additional cost of RFEF pasteurization is about \$0.0053/L, which is minor compared to the overcall cost of producing apple cider.

11. Regulations

Currently, there is no specific legislation applicable to RFEF processing. Such is also the case for PEF processing. In the United States, PEF-treated juices have been introduced to the market without explicit regulation. As the RFEF and PEF processes are very similar, it is a good assumption that RFEF-treated products will likewise be brought to the market.

12. Conclusions

The development of RFEF processing of liquid foods using high field strengths at relatively low temperatures has made great strides over the past few years. RFEF processing uses moderately simple equipment to generate high electric fields. The high fields are applied for a very short time, that is less than 500 μ s, to foods. The RFEF power supply should deliver a field

greater than 10 kV/cm to the food in order to inactivate microorganisms. The power supply frequency should range between approximately 1 and 250 kHz. Because the treatment times are so brief, some of the food may not receive any treatment if the frequency were too low. On the other hand, if the frequency is too high, the induced transmembrane voltage may be significantly decreased, resulting in a loss of inactivation. The quality of the power does not appear to be important; therefore, equipment should be selected based on the cost to power ratio.

The main processing parameters are field strength, temperature, and treatment time. In general, microbial inactivation increases as the electric field strength increases. There are some cases where inactivation remains constant above certain field strengths. In addition, the probability of arcing within the food increases as field strengths exceed 30–40 kV/cm. The effects on microbial inactivation of temperature and electric field strength are synergistic. At operating temperatures of 60°C, the synergism results in reduced processing costs. Inactivation increases as the treatment time increases. There is no limit to the amount of treatment chambers that may be added in series to raise treatment times.

Several research groups have demonstrated the inactivation of a range of microorganisms both in model systems and in food products using a variety of RFEF systems. Nonthermal RFEF processing has been shown effective at inactivating *S. cerevisiae* and *E. coli*. The population of *E. coli* in apple cider was reduced by 5 log at an electric field strength of 20 kV/cm, a treatment time of 420 μ s, and a flow rate of 84 L/hour.

The energy required for a 5-log reduction of *E. coli* in apple cider using RFEF processing is approximately 300 J/mL. The estimated energy cost for RFEF pasteurization is \$0.0060/L of apple cider. By contrast, the energy costs for conventional thermal pasteurization with heat regeneration is approximately \$0.00068/L. The additional cost of RFEF pasteurization is minor compared to the overcall cost of producing apple cider.

RFEF processing is similar in many respects to PEF processing which has been extensively studied during the last two decades. The first PEF processed

commercial products became available in the United States in 2005. It is anticipated that RFEF processed commercial products will follow shortly.

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Chapter 16

Use of Oscillating Magnetic Fields in Food Preservation

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1. Introduction

Traditionally, in the past, food preservation methods were used to prevent spoilage in products when fresh food was not available. Nowadays, preservation methods are used to preserve food quality in addition to producing safe products. Although many foods today are thermally pasteurized to extend shelf life, the food industry is currently looking for alternative nonthermal pasteurization methods to produce minimally processed and fresh-like foods, and they have shown increasing interest in some techniques, such as high hydrostatic pressure (HHP), pulsed electric fields (PEFs), oscillating magnetic fields (OMFs), and irradiation.

In many applications, it would be desirable to only preserve the food, without causing any changes, except to destroy the microorganisms that could cause its eventual spoilage. In this case, high-intensity magnetic fields applied at moderate frequency could be used to destroy or inactivate the microorganisms in a mainly nonelectrically conductive environment.

Destruction of microorganisms in food subjected to an OMF, applied in the form of pulses, can be achieved in a very short treatment time, resulting in no significant temperature rise in the food and no plasma production.

The objective of this chapter is to present a general overview of the history and definition of OMFs,

as well as to review the effects of magnetic fields on microorganisms and their application in food preservation.

2. Magnetic Fields

A magnetic field is a force field that surrounds electric current circuits, but the phenomenon can also be found in the vicinity of ferromagnetic materials. The first documented observations of magnetic phenomena were recorded by the ancient Greeks, and also by the early Chinese who described the affinity of iron toward lodestone. However, it was not until the eighteenth century, as demonstrated by the Danish scientist Hans Christian Oersted, that the flow of an electric current in a wire was associated with a magnetic field. Further investigations by Andre-Marie Ampere concluded that magnetic forces arise from the movement of electrical charges.

The existence of a magnetic field is ascertained by the effects on particles found within it. The most relevant effects are: (i) force on a moving electrically charged particle, (ii) force on a stationary charged particle when the magnetic field is changing, and (iii) mutual force acting between two objects or electric current circuits that are surrounded by a magnetic field (Du Trémolet de Lacheisserie et al., 2005). Generally, the interaction of a magnetic field with a food will be of the second kind.

The magnetic field produced around a magnetic source can be defined by measuring the force the field exerts on a moving charged particle, such as

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an electron or ion. A charged particle (q) entering a magnetic field (B) at a certain relative speed (v) experiences a magnetic force (F), calculated as:

$$\vec{F} = q(\vec{v} \times \vec{B}) \quad (16.1)$$

The unit of magnetic field is Newton-second per Coulomb-meter (or Newton per Ampere-meter), and is called Tesla (T).

Magnetic fields can be static (SMF) or oscillating (OMF). Field intensity is constant with time for SMF, whereas in the case of OMF, it is applied in the form of constant amplitude or decaying amplitude sinusoidal waves. In addition, a magnetic field may be homogeneous or heterogeneous. Homogeneous magnetic fields have a uniform field intensity (B) throughout the entire area enclosed by the magnetic field coil, while in a heterogeneous field, B is nonuniform, with the intensities decreasing as distance from the center of the coil increases (Pothakamury et al., 1993).

In addition, the composition of a food will substantially define its susceptibility to a magnetic field. Carbon atoms exhibit isotropic susceptibility to magnetization, which means that the degree of magnetization is equal along the three orthogonal axes. However, bonded carbon atoms and, consequently, most complex organic molecules present anisotropic susceptibility, which implies different susceptibility to magnetization depending on the direction the field affects these compounds. Another interesting property is related to the way that some compounds are affected by magnetic fields in vacuum. Most organic and inorganic molecules are diamagnetic; thus, they are more susceptible to magnetization under vacuum conditions. Contrarily, free radicals and compounds containing transition elements are paramagnetic, meaning they are less affected by a magnetic field in vacuum than under standard conditions (Mulay, 1964).

2.1. Generation of Magnetic Fields

A magnetic field is generated when an electric current flows through a coiled wire. The magnetic field is concentrated near the coil. The more loops of wire in existence, the greater the cross-section of each loop;

and the greater the current passing through the wire, the stronger the field. The magnetic field intensities that are able to inactivate microorganisms range from 5 to 50 T. OMFs of this density can be generated by using (Gersdorf et al., 1983):

1. superconducting coils;
2. coils that produce DC fields; and
3. coils energized by the discharge of energy stored in a capacitor.

When the core of paramagnetic or ferromagnetic material (commonly, soft iron) is placed inside the coil, magnetic fields can be produced that are much more intense. Magnetic field intensities of up to 3 T can be generated by inserting iron in the coil. The core concentrates the magnetic field, which makes it much stronger than that of the coil itself. As a current is passed through the coil, small magnetic regions within the material, also called magnetic domains, align with the applied field, thus resulting in an increase in the magnetic field strength. However, as the current is increased, all the magnetic domains eventually become aligned, a condition called saturation. Once the core becomes saturated, a further increase in current will only cause a relatively minor increase in the magnetic field. Therefore, iron cores reach magnetic saturation above 3 T, but air-core coils can be used to attain higher intensities.

Magnets with superconducting coils will then need to be used to generate magnetic fields up to 20 T (Figure 16.1). The coils themselves are made of tiny superconductive filaments placed in a copper matrix. Copper is needed for mechanical stability and thermal stability; in case the temperature or intensity reaches values above a critical point, superconductivity is lost. The superconducting portions of most such magnets are composed of niobium–titanium. This material has a critical temperature of 10 K and remains stable until about 15 T. More expensive magnets can be made of niobium–tin (Nb3Sn), having critical temperatures of around 18 K. When operating at 4.2 K, the magnets are able to withstand much higher magnetic field intensity, ranging from 25 to 30 T. In order to keep the superconductor filaments below the critical temperature, liquid

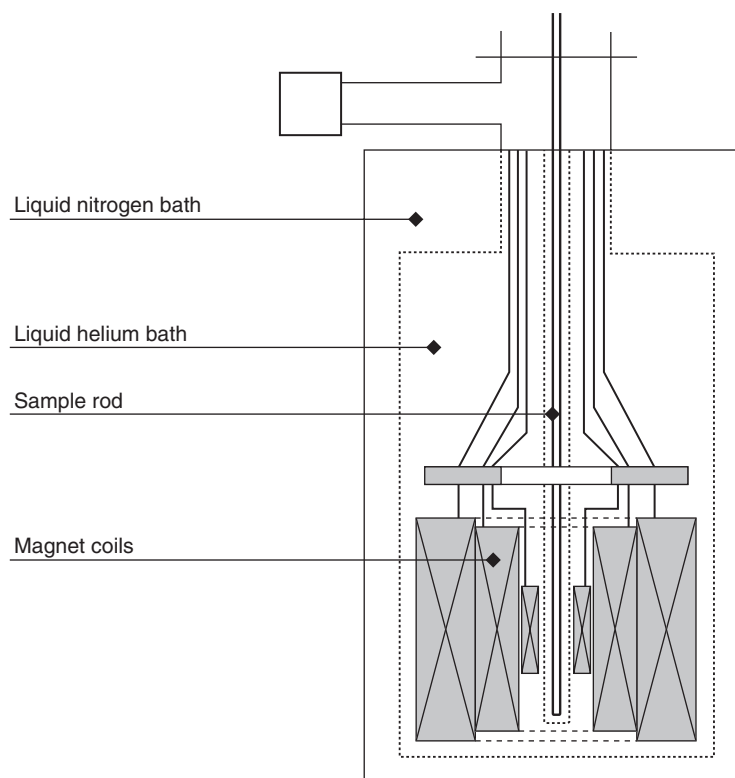


Figure 16.1. Schematic of a superconducting magnet cross-section.

helium is used as a coolant for superconducting materials with critical temperatures around 4.2 K, whereas liquid nitrogen is used for higher temperatures. Superconducting magnets have several advantages over resistive electromagnets. The field is generally more stable, leading to less noisy measurements; they can be smaller, allowing more freedom when configuring the rest of the device; they consume much less power, since consumption is negligible in the steady state (Ašner, 1999). Higher fields, however, can be obtained with cooled resistive and hybrid magnets, because the superconducting coils enter the normal, nonsuperconducting state at high fields, resulting in Joule heating phenomena.

Hybrid magnets are obtained by combining a superconducting magnetic coil with a water-cooled resistive coil. The resulting combination generates high magnetic fields of more than 30 T, while using just one-third the power of traditional resistive magnets

(Iwasa, 1996). The energy is then released in form of pulses of short duration, and the current is supplied to the coil from a condenser bank. Similarly, some systems use the energy stored in a capacitor bank for later release by generating an oscillating electrical current that, in turn, generates an OMF. OMF applied in the form of pulses reverses the charge of each pulse, and the intensity of each pulse decreases with time to about 10% of the initial intensity. The frequency and intensity of the magnetic field are determined by the capacitance of the capacitor and the resistance and inductance of the coil (Pothakamury et al., 1993).

3. Effects of Magnetic Fields on Living Cells

Static and OMFs have been studied for their potential use as microbial inactivation methods. The influence of magnetic fields on living cells became evident in

the early twentieth century with the observation of protoplasmic streaming in cells. The velocity of the protoplasmic stream in *Chara braunii* seaweed was increased up to 15% when a magnetic field was applied in parallel to the major axis of the cell, whereas the perpendicular magnetic field did not affect the protoplasmic velocity (Kameda and Nakabayashi, 2007).

The discovery of magnetotactic behavior in bacteria was based on the fact that certain motile, aquatic bacteria orient and migrate along magnetic field lines when subjected to a magnetic field on the order of a geomagnetic field, or greater. Thus, for magnetotactic bacteria from Northern Hemisphere collection sites, the predominant direction of migration in drops of water and sediment on a microscope slide is parallel to the magnetic field corresponding to northward migration in the geomagnetic field. If the direction of the local magnetic field is reversed when the magnetotactic bacteria are moving, they execute a “U-turn” and continue migrating in the same direction relative to the local magnetic field. The predominant migration direction of magnetotactic bacteria in the magnetic field can be reversed by subjecting the cells to a strong magnetic field pulse, oriented oppositely to the ambient field.

All magnetotactic bacteria contain magnetosomes, which are intracellular structures comprising magnetic iron mineral crystals enveloped by a phospholipid membrane (Gorby et al., 1988). The magnetosome membrane is a structure that anchors the mineral particles at particular locations in the cell, and it is the locus of biological control over the nucleation and growth of the mineral crystal.

Recent experiments on cell division suggest the application of intense static magnetic fields as a tool for the manipulation of biological systems (Valles et al., 2004), in which the magnetic field appeared to couple with the intrinsic anisotropies in the diamagnetic components of the cells. Hence, different intensities of between 2.5 and 8 T were applied to immobilize (nonswimming) *Paramecium caudatum* suspended in a density-matched medium. The organisms aligned with their long axis parallel to the applied magnetic field, which is in accordance with the observations described for algae. It has also been ob-

served that the organism alters its swimming behavior when exposed to magnetic fields. Since biological materials are weakly diamagnetic, intense inhomogeneous magnetic fields are necessary to induce the desired changes. The reduction in the swimming velocity of paramecia exposed to magnetic fields is explained by the partial alignment of the diamagnetically anisotropic molecules in the cell membrane with the applied magnetic field.

In a similar study, a strong dependence of the accumulation of a bacterial polysaccharide with respect to the orientation of the magnetic field was reported (Adamkiewicz and Pilon, 1983). Microscope slides were dipped vertically into cultures of *Streptococcus mutans* for 24 hours, resulting in over 100% more bacterial polysaccharide accumulation on the side facing north, as determined chemically. Inversion of the magnetic field by 180 degrees caused a similar inversion of the preferential accumulation. The preferential accumulation occurred on dia-, para-, and ferromagnetic slides, and was related to a physiologic response linked to adherence on vertical surfaces against gravity.

Magnetic fields can have different effects on microbial growth and reproduction. Yoshimura (1989) classified the effects produced on microbial cells as: i) inhibitory, ii) stimulatory, and iii) none observable. The effect of magnetic fields on microorganisms is summarized in Table 16.1.

Results concerning the effects of magnetic fields on microbial growth are often inconsistent. The magnetic fields either stimulated or inhibited microbial growth, or in some cases had no effect at all. The results presented in Table 16.1 show that the effect of magnetic fields on the microbial population of foods may depend on the magnetic field intensity, number of pulses, frequency, and product characteristics (e.g., resistivity, electrical conductivity, and thickness), although these results are not well understood. Before considering this technology for food preservation purposes, consistent results concerning the efficacy of the method are still needed.

The possibility that strong static (nongradient) magnetic fields might have an influence on biological materials and processes has been discussed for many years, including in reports that implicate high

Table 16.1. Effect of magnetic fields on microorganisms

Microorganism	Type of Magnetic Field ^a	Field Strength (T)	Frequency of Pulse (Hz)	Effect	Reference
Wine yeast cells	Heterogeneous SMF	0.04	0	Growth inhibited, exposed for 5, 20, 25, 60, 120, or 150 minutes; no inhibition for 10, 15, 17 minutes of exposure.	Kimball, 1937
<i>Serratia marcescens</i>	Heterogeneous SMF	1.5	—	Growth rate remains same as controls up to 6 hours; growth rate decreases between 6 and 7 hours and again increases between 8 and 10 hours; at 10 hours cell population same as controls.	Gerencser et al., 1962
<i>Staphylococcus aureus</i>	Heterogeneous SMF	1.5	0	Growth rate increases between 3 and 6 hours; then decreases between 6 and 7 hours, cell population at 7 hours is same as controls.	Gerencser et al., 1962
<i>Saccharomyces cerevisiae</i>	Heterogeneous SMF	0.465	0	Rate of reproduction reduced, incubated for 24, 48, or 72 hours.	Van Nostran et al., 1967
<i>S. cerevisiae</i>	SMF	0.56	0	Decreased growth rate; interaction between temperature and magnetic field only during the logarithmic phase.	Van Nostran et al., 1967
<i>Escherichia coli</i>	SMF	0.3	0	Growth simulated.	Moore, 1979
<i>Halobacterium halobium</i> , <i>Bacillus subtilis</i>	SMF	0.015 0.03 0.06	0	Growth inhibited.	Moore, 1979
<i>Pseudomonas aeruginosa</i> , <i>Candida albicans</i>	OMF	0.015 0.03 0.06	0.1–0.3	Growth simulated; stimulation increases with increase in frequency.	Moore, 1979
<i>E. coli</i>	OMF	0.15	0.05	Inactivation of cells at concentration of 100 cells/mL.	Moore, 1979
<i>Streptococcus thermophilus</i> in milk	OMF	12.0	6,000 (1 pulse)	Cell population reduced from 25,000 cells/mL to 970.	Hofmann, 1985
<i>Saccharomyces</i> in yogurt	OMF	40.0	416,000 (10 pulses)	Cell population reduced from 3,500 cells/mL to 25.	Hofmann, 1985
<i>Saccharomyces</i> in orange juice	OMF	40.0	416,000 (1 pulse)	Cell population reduced from 25,000 cells/mL to 6.	Hofmann, 1985

Table 16.1. (Continued)

Microorganism	Type of Magnetic Field ^a	Field Strength (T)	Frequency of Pulse (Hz)	Effect	Reference
Mold spores	OMF	7.5	8,500 (1 pulse)	Population reduced from 3,000 spores/mL to 1.	Hofmann, 1985
Yeast cells	SMF	0.57	—	No changes observed in growth.	Yoshimura, 1989
Yeast cells	OMF	—	—	Inactivation increase.	Yoshimura, 1989
<i>E. coli</i>	Homogeneous SMF	7.0	—	Adversely affected on growth of bacterium in early logarithmic growth phase. However, in stationary phase, cell number under high magnetic field was about 2–3 times higher than control, indicating that magnitude of decrease in cell number was reduced by high magnetic field.	Tsuchiya et al., 1996
	Heterogeneous SMF	5.2–6.1	—		
	Heterogeneous SMF	3.2–6.7	—		
<i>Bacillus subtilis</i> MI1 13	Homogeneous SMF	7.0	—	In stationary phase, cell number in inhomogeneous magnetic field was about twofold higher than reference, indicating that magnitude of decrease in cell number was reduced by high magnetic field.	Nakamura et al., 1997
	Heterogeneous SMF	5.2–6.1	—		Nakamura et al., 1997
<i>S. marcescens</i>	SMF	8.0 mT	—	Growth inhibited when exposed for 24 and 48 hours.	Piatti et al., 2002
<i>Shewanella oneidensis</i>	Homogeneous SMF	14.1 T	—	No detectable effects on bacterial counts. Transcriptional expression levels of 65 genes were altered: 21 genes were upregulated and others were downregulated.	Gao et al., 2005

^aSMF, static magnetic field; OMF, oscillating magnetic field.

magnetic fields in the alteration of the cleavage plane during cell division and other cellular disorders (Maret and Dransfeld, 1985; Maret, 1990; Iwasaka et al., 2002). Nevertheless, the common viewpoint today is that achievable static magnetic fields do not have a lasting effect on biological systems.

Van Nostran et al. (1967) used a culture of *Saccharomyces cerevisiae* to study the influence of

a magnetic field of 0.46 T on the microbial growth pattern. Cell populations were determined at 24, 48, and 72 hours intervals, and possible interactions between the magnetic field and other environmental parameters, such as time, temperature, and osmotic pressure, were considered statistically. The main effect of the high magnetic field was a significant reduction in cell multiplication during each time

interval. Significant interactions were found to occur between temperature and the magnetic field at 24 hours, whereas synergies between the magnetic field and osmotic pressure were not found.

Ikehata et al. (2003) evaluated the response of the budding yeast, *S. cerevisiae*, when exposed to static magnetic field treatments. After exposure to 5 T vertical magnetic fields, differences were found in the sedimentation pattern of cells that depended on the location of the dish in the magnet bore. Sedimented cells were localized in the center of the dish when placed in the lower part of the magnet bore, whereas cell sedimentation was uniform in dishes placed in the upper part of the bore because of the diamagnetic force. A genome wide gene expression profile of the yeast cells after exposure to 5 T magnetic fields for 2 hours suggested that the expression level of any gene in yeast cells was not affected, although the sedimentation pattern was altered. In addition, exposure to 10 T for 1 hour and 5 T for 24 hours also did not affect the gene expression. On the other hand, a slight change in expressions of several genes, which are related to respiration, was observed by exposure to a field of 14 T for 24 hours.

Gao et al. (2005) evaluated the effect of a static magnetic field of 14.1 T on log phase cells of the bacterial strain *Shewanella oneidensis* MR-1 by using a whole genome microarray of this bacterium. Although differences were not observed between the treatment and control after measuring the optical density, colony-forming unit (CFU), as well as post-exposure growth of cells, transcriptional expression levels of 65 genes were altered according to microarray data. Among these genes, 21 were upregulated while other 44 were downregulated, compared with untreated cultures. Consistently, Paul et al. (2006) evaluated the biological impact of static magnetic field strengths up to 30 T through the use of transgenic Arabidopsis plants engineered with a stress-response gene. Field strengths in excess of about 15 T caused the widespread induction of stress-related genes and transcription factors, as well as a depression of genes associated with cell wall metabolism.

Nonetheless, it has been observed that when magnetic fields become heterogeneous, the effects on microorganisms can be significantly boosted even with

low field intensities. Fojt et al. (2007) reported a 21% reduction in the CFU counts of a *Paracoccus denitrificans* culture exposed to 10 mT for 24 minutes at 50 Hz with respect to the control samples. Furthermore, the enzymatic reduction of nitrate and/or nitrite to nitrogen gas, catalyzed by the microorganism, was significantly decreased after exposure to the magnetic field. However, the extent of these effects can significantly vary depending on the conditions applied and the microorganisms exposed to these conditions. Gerencser et al. (1962) observed that the growth of *Serratia marcescens* and *Staphylococcus aureus* can be slightly modified when exposed to heterogeneous magnetic fields under certain conditions. The growth of *S. marcescens* was retarded between 6 and 10 hours of exposure to a 1.5-T magnetic field at 27°C, but after 10 hours the number of cells in the culture was similar to a nonexposed control culture. On the other hand, the growth of *S. aureus* at 37°C when exposed to the same magnetic field conditions was not significantly altered, but when the gradient of heterogeneity of the magnetic field was increased, significant changes in the growth pattern were observed.

Inhibition or stimulation of the growth of microorganisms exposed to magnetic fields may be a result of the magnetic fields themselves or the induced electric fields. The latter are measured in terms of induced electric field strength and induced current density. To differentiate between magnetic-field and electric-field effects, a cylindrical enclosure containing cells and a medium that can be adapted to *in vitro* studies employing uniform, single-phase, extremely low frequency (ELF) magnetic fields is recommended (Barbosa-Cánovas et al., 1998a).

4. Mechanisms of Microbial Inactivation

Static or OMFs could potentially inactivate food microorganisms. Different theories have attempted to explain the influence of magnetic fields on biological systems. Bacterial cell surfaces possess net negative electrostatic charges by virtue of ionized phosphoryl and carboxylate substituents on outer cell envelope macromolecules, which are exposed to the

extracellular environment. Most protozoan and bacterial cells are negatively charged to varying degrees. For example, gram-negative bacteria have an outer layer of lipopolysaccharides and proteins, which forms a highly charged surface that is stabilized by cation binding. Variations in the structure and chemical composition of these compounds have been shown to affect bacterial surface charge. The force generated by a magnetic field in a food system may be able to create an ion current from one location to another. In turn, the transport of ions may also occur between both sides of cell membranes, which could open and close voltage sensitive membrane channels and would initiate electroporation processes.

The first theory to explain the effect of magnetic fields on biological systems supposes that the bonds between ions and proteins can be loosened by a relatively weak magnetic field. In the presence of a steady background magnetic field, such as that of the earth, the biological effects of magnetic fields are more pronounced around particular frequencies, coincident with the cyclotron resonance frequency of ions (Coughlan and Hall, 1990). To understand this phenomenon, the reason for what happens when a charged particle, namely, an ion, enters a static and uniform magnetic field needs to be observed. The particle will move in a circle due to the Lorentz force. The circular motion may be superimposed with a uniform axial motion, resulting in a helix, or with a uniform motion perpendicular to the field, for example in the presence of an electrical or gravitational field, resulting in a cycloid. The angular frequency ($\omega = 2\pi f$) of this cyclotron motion, also known as gyrofrequency, for a given magnetic field strength B depends on the charge/mass ratio of the particle and is given by Equation 16.2:

$$\omega = \frac{eB}{m} \quad (16.2)$$

where e is the elementary charge and m is the mass of the charged particle.

The movement of a charged particle in a magnetic field is shown in Figure 16.2. When v and B are parallel, F is zero (see Equation (16.1)). When v is normal to B , then the ion moves in a uniform circular

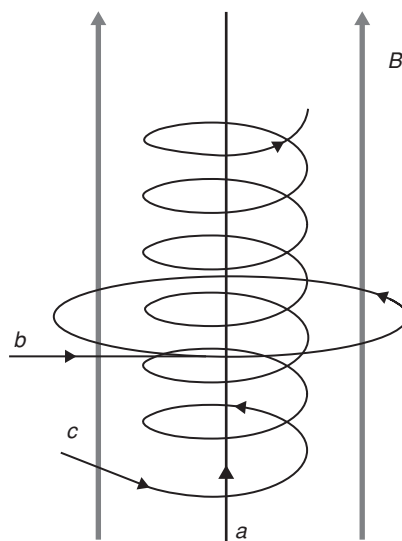


Figure 16.2. Charged particle in a magnetic field: a when v is parallel to B ; b when v is normal to B ; c when v makes an arbitrary angle with B .

path. For other orientations, the ions move in a helical path (Figure 16.2).

The ion cyclotron resonance (ICR) model has been suggested as an explanation for many of the weak, ELF magnetic field interactions observed in living systems. There is abundant evidence indicating altered biological response following magnetic field exposures that bear the unique signature in which integral ratios of ionic charge-to-mass equal the ratio of magnetic frequency to magnetic intensity (Liboff, 1997). Thus, cyclotron resonance occurs when f , the linear frequency, equals the frequency of the magnetic field, which allows the transfer of energy from the magnetic field to the ions with f equivalent to frequency of the magnetic field. Calculation of the corresponding ICR frequency f gives 2–100 Hz for most ions of biological relevance. This range covers the majority of technical (e.g., 16.33, 50, 60 Hz) and natural (e.g., Schumann Resonance 7.8 Hz) low frequency alternating fields. The geomagnetic field is subjected to temporal variability as well as to static local magnetic anomalies. Therefore, the natural ICR frequency of Ca^{2+} shifts worldwide and time dependently by up to 7 Hz (Pazur, 2004). The interaction

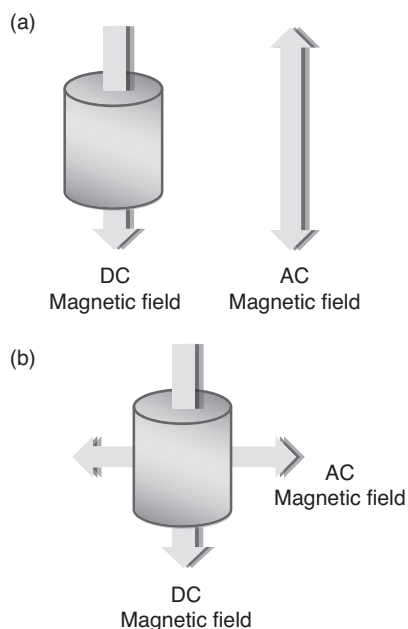


Figure 16.3. Two equivalent ways to obtain ICR: (a) DC magnetic field parallel to AC magnetic field; (b) DC magnetic field perpendicular to AC magnetic field. For a given charge-to-mass ratio, the resonance frequency is the same in both cases.

site of the magnetic field would be the ions in the cell, and they transmit the effects of magnetic fields from the interaction site to other cells, tissues, and organs.

When an OMF at frequencies close to ICR is externally applied to a matrix containing ions in parallel to an SMF (Figure 16.3a), it may couple with the corresponding ionic species in such a way as to selectively transfer energy to these ions and thus indirectly to the metabolic activities in which they are involved (San Martín et al., 1999). Furthermore, it should be appreciated that there are two equivalent conditions that give rise to ICR phenomenon. In the first case (Figure 16.3a), a charged particle executes cyclotron resonance in a DC magnetic field if an AC magnetic field is simultaneously applied in a direction parallel to the DC field. In the second case (Figure 16.3b), ICR occurs in the presence of an AC magnetic field perpendicularly oriented to the DC field (Liboff, 1997). The earth's total field, cor-

responding to the DC field, ranges from 25 to 70 μT . As already outlined, most of the slightly and double charged ions of biological interest have corresponding gyrofrequencies in the ELF range of 10–100 Hz for these field strengths.

A second model that can explain the effect of magnetic fields on ions is based on the ion parametric resonance (IPR) model. The IPR model considers the movement of a charged ion, such as a calcium ion, moving in a spherically symmetric constraint, with no direct interaction of other molecules or viscous forces. When exposed to a weak external DC magnetic field, modulated by an additional low frequency field, parallel to the static field (Figure 16.3a), the ion motions can be described as coherent combinations of circular orbits. Essentially, the IPR model considers how an ion cofactor in a key molecular complex, for example an enzyme binding site, alters the conformation states of that molecular complex, resulting in an observable change in the biological system in which it is contained (Blanchard and Blackman, 1994). In an ion–enzyme complex, the result could be a change in reaction kinetics. The ionic influence is controlled by the restructuring of internal energy states resulting from the externally applied magnetic fields. The effectiveness of this restructuring in creating observable biological changes is in turn influenced by the resonance relationship between the frequency of the applied AC magnetic field, the flux density of the applied DC field, and the particular ion's charge-to-mass ratio, as well as by special features of candidate ion-molecular interactions that allow resonance response to occur for a sufficient time to affect a change. Mathematically, there is an important distinction in the definition of resonance between the ICR and IPR models. The IPR model describes the detailed relationships between the intensity of the magnetic field (BAC), its frequency (f_{AC}), and the static magnetic field (BDC), leading to predictions of transition probabilities of ions at resonance. The influence of the electric field, which is decoupled from the magnetic field at power frequencies, is not considered by the model. The IPR model also considers possible effects when several ions are at or near resonance simultaneously, assuming an additive role for each ion's influence. This

makes a distinction with the previously launched IPR model, with application limited to calcium, and perhaps magnesium ions (Lednev, 1991).

The observations made by Coughlan and Hall (1990) support the theory of magnetic fields loosening the ions bound to proteins. Applying an SMF to a calcium-binding protein causes the plane of vibration to rotate, or precess, in the direction of the magnetic field at one-half the cyclotron frequency of the bound calcium. An OMF applied at calcium cyclotron frequency disturbs the movement and results in loosening of the bond between the calcium and protein.

An alternative theory proposed by Hofmann (1985) suggests that the inactivation of microorganisms may be due to the energy coupling from OMF to the magnetically active parts of the DNA molecules. Within 5–50 T range, the amount of energy per oscillation coupled to 1 dipole in the DNA is 10^{-2} – 10^{-3} eV. Several oscillations and a collective assembly of enough local activation may result in the breakdown of covalent bonds in the DNA molecules and inhibition of the growth of microorganisms. Lai and Singh (2004) reported that the DNA of rat brain cells acutely exposed to a 60-Hz sinusoidal magnetic field for 2 hours showed significant increases in single- and double-strand breaks. These effects could be blocked by applying free radical scavengers melatonin and N-tert-butyl- α -phenylnitron, suggesting that magnetic fields are responsible in some way for the production of free radicals. The use of Trolox (a vitamin E analog) or 7-nitroindazole (a nitric oxide synthase inhibitor) blocked magnetic-field-induced DNA strand breaks, further supporting the role of free radicals on the effects of magnetic fields. Treatment with an iron chelator also blocked the effects of the magnetic field treatments on the cell DNA. Authors hypothesized that exposure to low frequency magnetic fields initiated an iron-mediated process, for example the Fenton reaction, thus increasing the formation of free radicals in brain cells, leading to DNA strand breaks and cell death.

Inhibition or stimulation of the growth of microorganisms exposed to magnetic fields may be a result of the magnetic fields themselves or the induced electric fields. The latter are measured in terms of induced

electric field strength and induced current density. To differentiate between an electric field and magnetic field effects, a cylindrical enclosure containing cells and a medium that can be adapted to *in vitro* studies employing uniform, single-phase, ELF magnetic fields is recommended.

5. Critical Process Factors

The critical process factors influencing the effectiveness of magnetic field treatments on microbial populations have not been completely identified. The main studied factors include magnetic field characteristics, electrical resistivity, microbial type, microbial growth stage, treatment time, and treatment temperature.

5.1. Magnetic Field Characteristics

The growth and reproduction of microorganisms may be stimulated or inhibited by exposure to magnetic fields. Pulsed treatments of 5–50 T and frequencies of 5–500 kHz generally reduce the number of microorganisms by at least 2 log cycles (Hofmann, 1985). High-intensity magnetic fields can affect membrane fluidity and other properties of cells (Frankel and Liburdy, 1995). Inactivation studies often show some inconsistent results (see Table 16.1), making it impossible to clearly assess the microbial inactivation efficiency of magnetic fields or to predict its effectiveness on microbial populations.

The heterogeneity of the magnetic field can also strongly determine its effectiveness on microbial inactivation. Tsuchiya et al. (1996) found that cell survival of *E. coli* was greater under heterogeneous (5.2 to 6.1 T and 3.2 to 6.7 T) magnetic fields compared with homogeneous (7 T) fields.

5.2. Electrical Resistivity

As in all electromagnetic nonthermal technologies, the electrical properties of a food product strongly determine its ability to undergo processing. It is widely known that each electric field has an associated magnetic field, and vice versa. In the specific case of OMF, an electrical current will be induced in the

exposed product when the magnetic flux changes, starting from the product's surface. The electrical resistivity, also known as specific electrical resistance, is a measure of how strongly a material opposes the movement of electrically charged particles. Thus, the magnetic field intensity applied will depend on the electrical resistivity and thickness of the food product magnetized. The electrical resistivity of a food needs to be greater than 10–25 Ohms-cm in order to allow OMF microbial inactivation. Most foods have electrical resistivity within this range; for example, orange juice resistivity is 30 Ohms-cm.

5.3. Microbial Growth Stage

Tsuchiya et al. (1996) reported a growth stage/exposure time-dependent response for *E. coli* bacterial cultures. Assuming that magnetic fields could act as a stress factor, cells collected after 30 minutes of incubation under magnetic field treatment (lag or early lag growth phase), or in the stationary phase after long-term magnetic field treatment, were subjected to mild heat treatments. However, no differences were observed between the treated and control samples.

5.4. Temperature

Little is known about the dependence of microorganism inactivation upon temperature. Berk et al. (1997) evaluated the effect of 71 and 106 mT static magnetic fields on three potentially pathogenic amoebae. They found that the number of amoebae in the three species was significantly reduced after a 72-hour exposure to magnetic fields at temperature 20°C or above. Final counts of magnetic-field-exposed amoebae ranged from 9 to 72% lower than counts in unexposed samples. In one test where temperature was kept at 20°C for 48 hours, exposure to the magnetic field was not inhibitory.

6. Magnetic Fields Applications in Food Preservation

OMFs can be applied to stabilize and preserve either solid or liquid foods. Preservation of solid foods

with OMF involves sealing the food in a plastic bag, whereas for liquid foods, the product is pumped through a pipe in continuous flow. OMF treatments consist of subjecting the product to 1 to 100 pulses with frequencies between 5 and 500 kHz at temperatures ranging from 0 to 50°C for a total exposure time of 25 to 100 ms (milliseconds). Frequencies higher than 500 kHz are less effective for microbial inactivation and tend to heat the food material (Barbosa-Cánovas et al., 1998b). Magnetic field treatments are carried out at atmospheric pressure and moderate temperatures; the product is slightly heated to temperatures ranging from 2 to 5°C. According to Hofmann (1985), exposure to magnetic fields above 2 T will cause inhibition of growth and reproduction of microorganisms. Field intensities of 5–50 T and frequencies of 5–500 kHz were applied to liquid media, reducing the number of microorganisms by at least 2 log cycles. Further, OMF technology may be used to improve the quality or increase the shelf life of food products in combination with traditional processing techniques, for example low pasteurization treatments. Within the magnetic field range of 5–50 T, the amount of energy per oscillation coupled to 1 dipole in the DNA is 10^{-2} – 10^{-3} eV (Hofmann, 1985). OMF of this intensity can be generated using superconducting coils, coils that produce DC fields, or coils energized by the discharge of energy stored in a capacitor (Gersdorf et al., 1983).

Lipiec et al. (2004) studied the effects of high-density OMF pulses on the survival of certain selected pathogenic microorganisms in potatoes. They reported log reductions for bacteria of over three orders of magnitude with OMF treatments, whereas fungi appeared to be more resistant to treatments, with less than 2 log reductions. The least resistant of the tested microorganisms were *Ervinia carotovora* and *Streptomyces scabies*. On the other hand, the tested fungus *Alternaria solani* proved to be the most resistant. However, the results achieved could substantially vary depending on the applied conditions and food matrix, namely, on its electrical properties. Harte et al. (2001) exposed *E. coli* and *S. cerevisiae* to 18 T static and pulsed magnetic fields at different temperatures, in either phosphate and McIlvaine buffer or peptonated water, as a prior experiment

for later application to real food products. No inactivation or cell injury was detected due to magnetic fields, whether static or pulsed. In a parallel work, the inactivation effects of 18 T pulsed OMF on *E. coli* ATCC 11775 in combination with ultrasound, hydrostatic pressure, pulsed electric fields, or bacteriocin treatments were studied (San Martín et al., 2001). No additional inactivation or cell damage due to pulsed magnetic field exposure at 42°C was observed. Controversial results like these concerning the efficacy of treatment conditions on different food products should be investigated further before considering this technology for food preservation purposes.

No special preparative steps need to be followed before treating food products with OMF. Frequencies higher than 500 KHz are less effective for microbial inactivation because the temperature of food products will increase due to Joule heating processes. Magnetic field treatment is considered safe because the intensity of the field only persists within a very short distance from the coil and drops drastically upon moving away from the treatment zone.

7. Research Needs

Each nonthermal technology has specific applications in terms of the types of foods processed. OMF technology is potentially useful for processing both liquid and solid foods. However, the main limitation of most nonthermal technologies, OMF included, is the inadequate inactivation of resistant microbial forms such as spores. Taking a more combined methods approach is necessary to study the possible synergies between treatments.

Although preliminary studies indicate promising results, it will be a while before most nonthermal technologies can be used on a commercial scale. The majority of research today is focused on the application of nonthermal technologies, but little on understanding the mechanisms of inactivation of microorganisms, such as spores or enzymes.

Research has demonstrated that bacteria, yeasts, and molds are not equally susceptible to nonthermal methods. The question that now needs to be addressed is whether the processing time of nonthermally processed foods is based on the most re-

sistant organism present in the food. Another question would be: do the microorganisms follow a linear and predictable inactivation during nonthermal processing? For example, is a specific organism that is known to be resistant to electric fields also resistant to another nonthermal technology? Additional studies are thus necessary to arrive at the actual inactivation mechanism at play. With proper understanding of the inactivation mechanisms present in nonthermal technologies, some of the limitations can potentially be overcome, and where one technology fails, others can be used. It is also necessary to compare the quality and shelf life of foods treated by different nonthermal methods to determine whether one method is more suitable than another for specific foods.

The most important issue involved in the commercialization of nonthermal technologies is regulatory approval. Foods processed thermally or nonthermally must comply with the safety regulations set forth by the Food and Drug Administration prior to being marketed or consumed. Therefore, it is not enough to develop a food processing method to satisfy only pasteurization or sterilization requirements; it is also necessary to ensure that the process is safe for equipment operators and consumers.

There is a significant lack of information on the ability of OMF treatment to inactivate pathogenic microorganisms and surrogates. A main area that needs to be elucidated is the confirmation that magnetic field treatment is an effective process for inactivation of microbes. Once established, significant data gaps will still need to be closed before this technology can be safely and practically applied to food preservation. Some of the more significant research needs are:

- to establish the influence of magnetic fields on the inactivation of microorganisms;
- to elucidate the kinetics of microbial inactivation by magnetic fields;
- to identify key resistant pathogens;
- to determine the mechanisms involved in microbial inactivation by magnetic fields;
- to identify critical process factors and effects of microbial inactivation;

- to validate the process and evaluate indicator organisms and appropriate surrogates; and
- to identify process deviations and ways to address them.

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Chapter 17

Irradiation of Ground Beef and Fresh Produce

Christopher Sommers and Xueting Fan

1. The Problem—Why Irradiate Beef

The US Department of Agriculture's (USDA) Food Safety and Inspection Service (FSIS) has determined that the contamination level for the human pathogen *Escherichia coli* O157:H7 in ground beef was 0.17% in 2004 compared to 0.30% in 2003, 0.78% in 2002, 0.84% in 2001, and 0.86% in 2000 (USDA FSIS, 2005). From 2003 to 2006, there have been 22 recalls of beef and ground beef due to contamination with *E. coli* O157:H7 (Table 17.1). Surveillance and intervention efforts including the implementation of hazard analysis and critical control point plans have reduced, but not eliminated, microbial contamination of meat and poultry carcasses (CDC, 2000; USDA FSIS, 2003).

While substantial progress has been made in decreasing the incidence of *E. coli* O157:H7 in ground beef, approximately 2 out of every 1,000 ground beef patties (GBPs) sold in the United States still contains *E. coli* O157:H7. Because of the low infectious dose associated with *E. coli* O157:H7 and the severity of associated infections, even a low incidence and contamination level represent a significant risk to public health.

Between 1993 and 2003, the ten largest beef processing companies spent an estimated \$400 million on new equipment and an added \$250 million to their operating costs to fight *E. coli* O157:H7. The overall cost of *E. coli* O157:H7 to the beef industry

since 1993 was approximately \$2.8 billion (Eustice and Bruhn, 2006). Frenzen et al. (2005) estimated that Shiga-toxin-producing *E. coli* O157:H7 (O157 STEC) caused 73,000 illnesses, 2,000 hospitalizations, and 60 deaths in the United States annually. The annual estimated cost (\$2,003) due to *E. coli* O157:H7 was an estimated \$370 million for premature deaths, \$30 million for medical care, and \$5 million for lost productivity.

The US Centers for Disease Control and Prevention estimates that if 50% of poultry, ground beef, pork, and processed meats in the United States were irradiated, the potential benefit of the irradiation would be a 25% reduction in the morbidity and mortality rate caused by these infections. This estimated net benefit is substantial, as the measure could prevent nearly 900,000 cases of infection, 8,500 hospitalizations, more than 6,000 catastrophic illnesses, and 350 deaths each year (Tauxe, 2001).

2. Irradiation and How It Works

Food irradiation is the process of using ionizing radiation, in the form of x-rays, gamma rays, or accelerated electrons (electron beam or e-beam), to improve the microbial safety, disinfest, delay the maturation of, and extend the shelf life of food products.

Ionizing radiation kills microorganisms by damaging their chromosomes. Irradiation induces single- and double-stranded DNA strand-breaks, resulting in transition mutations, transversion mutations, frameshift mutations, and deletion mutations (Glickman et al., 1980; Raha and Hutchison, 1991; Sargentini and Smith, 1994; Wijker et al., 1996;

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This chapter remains with the U.S. Government.

Table 17.1. Recalls of ground beef due to contamination with *E. coli* O157:H7

Recall Number	Date	Pounds Recalled
FSIS-RC-027-2006	August 18, 2006	909
FSIS-RC-026-2006	August 5, 2006	4,337
FSIS-RC-024-2006	July 31, 2006	120
FSIS-RC-021-2006	July 17, 2006	315
FSIS-RC-015-2006	May 5, 2006	150,000
FSIS-RC-046-2005	November 1, 2005	94,400
FSIS-RC-047-2005	November 3, 2005	6,200
FSIS-RC-035-2004	September 17, 2004	59,000
FSIS-RC-030-2004	August 3, 2004	497,000
FSIS-RC-019-2004	June 22, 2004	101,600
FSIS-RC-014-2004	April 29, 2004	45,000
FSIS-RC-007-2004	February 24, 2004	90,000
FSIS-RC-064-2003	December 15, 2003	5,620
FSIS-RC-057-2003	October 31, 2003	500
FSIS-RC-056-2003	October 31, 2003	102,200
FSIS-RC-044-2003	August 29, 2003	220
FSIS-RC-041-2003	August 23, 2003	76,000
FSIS-RC-034-2003	August 8, 2003	659,000
FSIS-RC-032-2003	August 5, 2003	194,700
FSIS-RC-021-2003	April 28, 2003	180
FSIS-RC-011-2003	March 11, 2003	106,000
FSIS-RC-010-2003	March 8, 2003	1,126

Ground beef recall information obtained from the USDA FSIS website at: http://www.fsis.usda.gov/fsis_recalls/Recall_Case_Archive_2003/index.asp.

Alper, 1998) in the chromosomes of pathogenic bacteria such as *E. coli* O157:H7 and *Salmonella*, either killing them or rendering them unable to reproduce.

Ionizing radiation damages DNA via two mechanisms: (1) direct action against the bacterial chromosome by photon-induced breakage of the DNA phosphodiester backbone or (2) indirect damage to the DNA by the radiolysis products of water, primarily hydroxyl radicals. Indirect damage accounts for >70% of the DNA damage induced by ionizing radiation (Sommers et al., 2002a, 2002b).

3. Irradiation of Beef in the United States

Irradiation of raw meat was approved by the US Food and Drug Administration (FDA) in 1997 and the US Department of Agriculture's Food Safety Inspection Service in 1999 (USDA FSIS, 1999). Red meats, in-

cluding beef, can be irradiated to doses of 4.5 kGy for refrigerated meat or 7.0 kGy for frozen meats. The reasons for different upper dose limits for refrigerated versus frozen meat will be discussed later in the chapter. In 2004, the USDA's Agricultural Marketing Service (USDA-AMS) made irradiated frozen ground beef available to schools as part of the National School Lunch Program (USDA AMS, 2003). However, the higher cost of irradiated versus nonirradiated product has not made irradiated ground beef a viable option to school districts that participate in the program.

Irradiated ground beef and beef cuts is available commercially in the United States and is sold in a variety of forms including case-ready ground beef and steaks, frozen GBPs, and chubs. Irradiated ground beef must be labeled with the radura symbol as required in 21CFR179 in the US Code of Federal Regulations.

4. Toxicological Safety of Irradiated Meat

The toxicological safety of irradiated meat has been studied for approximately 45 years. Long-term feeding studies conducted in multiple animal species that consumed large quantities of radiation pasteurized and sterilized meat and poultry (6–74 kGy) have found no increased risk of cancer or birth defects associated with consumption of irradiated meat (WHO, 1994; FDA, 2005). These feeding studies have focused primarily on meat and poultry because of the high fat content of those products, which is subject to both thermolytic and radiolytic chemical changes (WHO, 1994).

More recent studies on the safety of irradiated meat have focused on the mutagenicity or clastogenicity of unique radiolytic products that are derived from irradiation of fatty acids, the 2-alkylcyclobutanones (2-ACBs). The most abundant 2-ACB in ground beef is 2-dodecylcyclobutanone (2-DCB), which is derived from palmitic acid, the most abundant saturated fatty acid in beef. A person consuming a 100-g cooked GBP would be expected to consume 3–6 µg of 2-DCB (Public Citizen, 2003; Knoll et al., 2006; Sommers, 2006).

To date, no 2-ACB has been found to cause mutations in DNA in various in vitro systems including the *Salmonella* mutagenicity test, the *E. coli* TRP Test, 5-FU-induced forward mutations in *E. coli*, and the generation of 6-thioguanine-resistant mutants in the human TK6 lymphoblasts (Burnouf et al., 2002; Sommers, 2003; Gadgil and Smith, 2004; Sommers and Mackay, 2005; Sommers and Schiest, 2005; Sommers, 2006). Claims that 2-ACBs are mutagenic remain unsubstantiated and should be regarded as inaccurate.

Saturated fatty acids, such as palmitic acid and oxidized unsaturated fatty acids, are potent inducers of oxidative stress, mitochondrial toxicity, cell membrane damage, DNA strand and chromosome breakage (clastogenesis), necrosis, and apoptosis of human and rodent cells in vitro at concentrations of 50–200 μM (Beeharry et al., 2003; Udilova et al., 2003; Uloth et al., 2003; Ji et al., 2005; Nogueirra de Sousa Andrade et al., 2005). Excessive consumption of fatty acids has been linked to an increased risk of tumor promotion and colon cancer in rodents and humans (Coquhoun and Guri, 1998; Zock, 2001; Bartsch et al., 2003).

In contrast to saturated fatty acids and oxidized polyunsaturated fatty acids, 2-ACBs (50–200 μM) are either noninducers, or very weak inducers, of DNA strand or chromosome breakage in human and rodent cells in vitro depending on the cell line used and exposure conditions (Burnouf et al., 2002; Knoll et al., 2006; Sommers, 2006).

Because 2-ACBs are nonmutagenic and display weaker clastogenicity in comparison to their fatty acid parents, and are present in μg (2-DCB) versus gram (palmitic acid) quantities in irradiated meat, it is highly unlikely that 2-ACBs would have a significant impact on human health as part of a healthy well-balanced diet (Smith and Pillai, 2004; Gadgil and Smith, 2006; Sommers, 2006). Research on potential 2-ACB toxicity has been extensively reviewed by both Health Canada and the US FDA (Health Canada, 2002; FDA, 2005).

Claims of cancer-inducing potential associated with consumption of irradiated meats remain unsubstantiated.

5. Inactivation of *E. coli* O157:H7 by Irradiation

There have been a number of studies on the irradiation of beef and ground beef for the inactivation of *E. coli* O157:H7. Inactivation kinetics is typically listed as D_{10} value, or the radiation dose needed to inactivate 90%, or 1 $\log_{10}$, of pathogen. The USDA FSIS, in its final rule that allows irradiation of red meat lists the D_{10} value of *E. coli* O157:H7 to be 0.25 kGy for refrigerated product versus 0.45 kGy for frozen meat products (FDA, 1999). Therefore, a minimum radiation dose of 1.25 kGy would be needed to inactivate 5 $\log_{10}$ of *E. coli* O157:H7 in refrigerated meat versus 2.25 kGy for frozen meat. Because dose uniformity ratios (the ratio of the minimum and maximum absorbed radiation doses) of 2:1 to 3:1 are typical in commercial irradiation facilities, irradiation would be expected to inactivate 5–9 $\log_{10}$ of *E. coli* O157:H7 in refrigerated and frozen ground beef.

Irradiation is not a substitute for lack of sanitation and poor hygienic practices during slaughter. Additional $\log_{10}$ reductions of *E. coli* O157:H7 in beef are obtained during slaughter due to carcass rinses and other procedures as outlined in hazard analysis and critical control point plans.

A list of *E. coli* O157:H7 radiation D_{10} values is presented in Table 17.2. Fat level of ground beef has relatively little effect on the radiation resistance of *E. coli* O157:H7. The D_{10} values which were obtained in these studies vary depending on inoculation method, microbiological media used for recovery and enumeration of the pathogen, the method of freezing, vacuum or aerobic packaging, and maintenance of product temperature.

The maximum dose allowed by the US FDA is currently 4.5 kGy for refrigerated red meat versus 7.0 kGy for frozen red meat. The difference in upper radiation dose limits for refrigerated versus frozen meats is based on the differences in radiation resistance of pathogens in the refrigerated versus the frozen states. Many studies have shown that the radiation resistance of food-borne pathogens increases with decreasing temperature (Sommers and Niemira

Table 17.2. Radiation D_{10} -values for *E. coli* 0157:H7 suspended in ground beef

Reference	Radiation Source	Temperature	D_{10} -Value
Thayer and Boyd, 2001	cesium-137	4°C	0.34 kGy
		−12°C	0.61 kGy
		−20°C	0.98 kGy
Black and Jaczynski, 2006	e-beam	4°C	0.24 kGy
		−20°C	0.33 kGy
Rocelle et al., 1994	cobalt-60	3–5°C	0.24–0.25 kGy
		−17°C	0.31 kGy
Lopez-Gonzalez et al., 1999	e-beam	−15°C	0.34–0.63 kGy
	cobalt-60	−15°C	0.59–0.62 kGy
	cobalt-60	4°C	0.13 kGy
Chiasson et al., 2004	cesium-137	4°C	0.28 kGy
Thayer and Boyd, 1993	cesium-137	4°C	0.30 kGy
Ito and Harsojo, 1998	cobalt-60	Frozen ^a	0.46 kGy
Oh-Jin-Kwon et al., 1997	e-beam	Frozen ^a	0.41 kGy

^aTemperature not reported.

In Press; Thayer et al., 1995; Lopez-Gonzalez et al., 1999; Sommers et al., 2001; Thayer and Boyd, 2001; Black and Jaczynski, 2006). Greater than 70% of the damage to macromolecules such as DNA is indirect through radicals, primarily hydroxyl radicals, produced by the radiolysis of water (Sommers et al., 2002a; Sommers et al., 2002b). The increased radiation resistance of microorganisms at subfreezing temperatures has been attributed to the lower water activity (a_w) of meat at subfreezing temperatures and to decreased hydroxyl radical mobility following the radiolysis of water when in the frozen state (Bruns and Maxcy, 1979; Taub et al., 1979). Therefore, the physical state of water, product temperature, and the initial freezing point of the food matrix are important factors that determine the efficacy of the radiation processing of foods.

6. Organoleptic Quality of Irradiated Ground Beef

There have been several studies that have used trained and untrained panels to evaluate the effect of irradiation on the organoleptic quality of irradiated ground beef. Luchsinger et al. (1997) irradiated frozen ground beef (2.0 and 3.5 kGy, 10 and 22% fat) and stored the samples for 14 days at −19°C.

A trained panel found that irradiation had minimal effects on flavor, texture, or aroma of GBPs. In another study using a trained panel it was found that irradiated beef (19% fat, 3 and 4.5 kGy) had less beef flavor and aroma and more off-flavor than the nonirradiated samples after 27–29 days of storage at −28°C (Wheeler et al., 1999). In a study conducted by Lopez-Gonzalez et al. (2000), a trained panel found that irradiated fresh ground beef (2 kGy, 20% fat) had less cooked beef/brothy flavor than the nonirradiated ones. There were no differences in other sensory attributes between irradiated and nonirradiated samples.

In studies using untrained panels, which are closer to how consumers would react to irradiated ground beef, Murano et al. (1998) irradiated and stored frozen GBPs (20% fat) in different packaging schemes for up to 7 days at −25°C. and found that packaging type and storage affected texture and aftertaste of irradiated ground beef, but no undesirable change in flavor, texture, juiciness, or aftertaste was caused by irradiation. GBPs irradiated under vacuum and stored in air were more tender than the nonirradiated samples. In a study by Wheeler et al. (1999), a consumer panel found that irradiated GBPs (4.5 kGy, 62–104 days of storage at −28°C) had lower score on taste than the nonirradiated

GBPs while no difference was found at doses lower than 3 kGy.

Giroux et al. (2001), using an untrained panel, found no significant difference in odor and taste between irradiated (23% fat, up to 4 kGy) and nonirradiated GBPs during 7 days of storage at 4°C. Vickers and Wang (2002), using an untrained panel, found that ratings of overall liking, flavor liking, and texture liking for irradiated fresh GBPs (1.5 kGy, fat content not defined) did not differ from those of the nonirradiated GBPs. The rating for juiciness was higher in irradiated patties than the nonirradiated ones. Lorenzen and Heymann (2003), using a consumer panel, found that irradiation of frozen GBPs (1.0 kGy, fat content not defined) had little effect on overall liking, tenderness, juiciness, and flavor of cooked patties. In a study by Zienkewicz and Penner (2004), a consumer panel could not differentiate between irradiated (25% fat, 1.5 kGy, 3-month storage) and nonirradiated ground beef. In a study by Fan et al. (2004), untrained panelists from the USDA Agricultural Research Service's Eastern Regional Research Center could not differentiate between nonirradiated and irradiated frozen GBPs (3 kGy, 15% fat, -20°C, 12-month storage) that would be distributed by the USDA's Agricultural Marketing Service, on a voluntary basis, as part of the National School Lunch Program.

From the scientific literature we can conclude that trained panels, in some cases, could detect differences in the organoleptic qualities of irradiated (≤ 1.5 kGy refrigerated, ≤ 3 kGy frozen) and nonirradiated ground beef. Untrained panels generally found minimal differences in sensory attributes between irradiated and nonirradiated ground beef. Based on these scientific studies, and food irradiation industry sponsored testing, commercially available refrigerated ground beef is typically irradiated to a maximum radiation dose of 2.3 kGy, while frozen ground beef is irradiated to a maximum dose of 3.0 kGy.

7. Consumer Acceptance and Sales of Irradiated Foods

Approximately 18 million pounds of ground beef are irradiated annually in the United States.

Irradiated ground beef is currently available in 2,500–3,000 grocery and convenience stores (Eustice, 2006). This is an encouraging development because irradiated ground beef has only been on the market since the 2000–2001 fiscal year. Since its introduction to the market, there have been no recalls of irradiated ground beef, or food-borne illness outbreaks associated with irradiated ground beef, due to contamination with *E. coli* O157:H7.

Following the FDA approval for irradiation, consumer attitudes toward irradiated meat and poultry have changed in the last 15 years. In 1993 only 29% of consumers viewed irradiation of meat favorably, versus 50% in 1999, and a 69% in 2003 (Frenzen et al., 2001; Johnson et al., 2004).

Key factors in successful acceptance of irradiated ground beef by consumers and introduction of irradiated ground beef into the market place include (1) coordinated education efforts by retailers, public health officials, and trade associations (Eustice and Bruhn, 2006; Hoefer et al., 2006); and (2) actively and aggressively challenging misleading and inaccurate information about the safety of irradiated foods (Eustice and Bruhn, 2006).

Factors that negatively impact the purchase of irradiated ground beef include (1) the cost of irradiation and the cost of transport, which may increase in coming years, to and from the three facilities currently irradiating ground beef in the United States. Irradiated beef costs 13–30 cents per pound more than nonirradiated ground beef (Brown, 2002; Melgares, 2002; Burros, 2003; Roos, 2003); (2) low availability, that is if it is not available, consumers cannot purchase it (Crowley et al., 2002; Hoefer et al., 2006); and (3) the difficulty in overcoming inaccurate information about the safety and wholesomeness of irradiated ground beef (Eustice and Bruhn, 2006).

Positive information, when presented alone, has been demonstrated to increase product acceptance. In a study on consumer's willingness to purchase irradiated ground beef (Nayga et al., 2004), only about half of the respondents indicated willingness to buy irradiated ground beef before the presentation of information on irradiation. After the presentation of information on the nature and benefits of food irradiation, about 90% of the respondents indicated

a willingness to buy irradiated ground beef. Consumers are willing to pay a premium of about \$0.77 for a pound of irradiated ground beef (Nayga et al., 2006).

However, negative media coverage and activities by groups opposing products or technologies will have a stronger influence on consumers' judgments than positive attributes (Fiske, 1980; Ahluwalia et al., 2000). By highlighting the importance of educating consumers with accurate, scientific information about irradiated foods, a more favorable attitude toward irradiated products will develop.

8. Cooking Temperature, Meat Thermometers, and Risk

The risk associated with consumption of undercooked ground beef becomes more serious when consumer preferences and cooking habits are considered.

Current guidelines for the cooking of beef call for reaching an internal temperature of 160°F in order to inactivate pathogenic bacteria such as *E. coli* O157:H7 (USDA FSIS, 2004). However, research by US FDA and USDA FSIS show that only 60% of households have a meat thermometer, and only 6% of consumers use it on a regular basis (Cates, 2002). Anderson et al. (2004) showed that less than 5% of participants used a thermometer to determine doneness of meat. Almost 45% percent of the study participants reported not knowing the recommended cooking temperature for ground beef. In addition to the lack of consumer knowledge is the preference of many consumers for beef cooked to a medium-rare temperature.

9. Heat Sensitivity Following Irradiation

As reviewed earlier in this chapter, ionizing radiation inactivates pathogenic bacteria through breakage and oxidative DNA damage to the bacterial chromosome. Such damage increases the sensitivity of microorganisms to heat through thermolability of the bacterial DNA (Kim and Thayer, 1996; Sommers and Schiestl, 2004; Alvarez et al., 2006). This concept is impor-

tant, given the preference of some consumers for ground beef cooked to a 145°F medium-rare (65°C) temperature.

While irradiation at the doses specified would result in 5–9 log reduction of *E. coli* O157:H7, it is one of the radiation sensitive food-borne pathogens. The order of radiation resistances of food-borne pathogens in raw and ready-to-eat meats are *Salmonella* spp. > *Listeria monocytogenes* ≥ *Staphylococcus aureus* > *E. coli* O157:H7 (Thayer et al., 1995). While less frequent, food-borne illness outbreaks associated with ground beef can be associated with contamination by *Salmonellae* (CDC, 2002).

In original research presented in this chapter, we evaluated the ability of a medium-rare cooking temperature (internal temperature of 145°F) to inactivate *Salmonella senftenberg* ATCC 8400 suspended in ground beef following irradiation (1.5 kGy) and 3 weeks of refrigerated (4°C) storage.

Extra-lean ground beef was used in this study due to consumer preference for low-fat ground beef for hamburgers. Ground beef (93% lean) was purchased at a local supermarket. The ground beef was subdivided into 200-g aliquots, placed in No. 400 Stomacher bags, and vacuum sealed using a Multivac Model A300 vacuum packager (Multivac Inc., Kansas City, MO). The ground beef was then radiation sterilized to a dose of 42 kGy (–30°C).

S. senftenberg ATCC 8400 (American Type Culture Collection, Manassas, VA) was maintained frozen at –70°C in 15% glycerol contained in sterile polypropylene cryogenic vials. Culture identity was confirmed by Gram staining and on the basis of reactions on the Gram-Negative Identification Cards of the Vitek AMS Automicrobic System (bioMérieux Vitek, Inc., Hazelwood, MO). Following identification and cryopreservation *S. senftenberg* was propagated and maintained in Trptic Soy Agar (TSA) (BD-Difco, Sparks, MD).

S. senftenberg was propagated in multiple cultures of 100-mL Tryptic Soy Broth (TSB) (BD-Difco, Sparks, MD) in baffled 500-mL Erlenmeyer culture flasks at 37°C (150 rpm) for 18 hours. The bacteria were then sedimented by centrifugation and resuspended in a ten fold reduced volume of Butterfield's phosphate buffer (BPB) (Applied Research

Institute, Newtown, CT). The ground beef was inoculated, 1/10th volume of *S. senftenberg*, into the sterile ground beef and mixed for 90 seconds in a Stomacher Mixer (Tekmar, Co., Cincinnati, OH). The inoculum levels for *S. senftenberg* in the ground beef were $> 10^8$ CFU/g. The inoculated meat was then aliquoted (200 g) into No. 400 Stomacher bags, vacuum-packaged, and refrigerated until ready for irradiation or storage (4°C).

The inoculated ground beef was irradiated to a dose of 1.5 kGy. A Lockheed Georgia Company self-contained ^{137}Cs irradiator was used for all exposures. The radiation source consisted of 23 individually sealed source pencils placed in an annular array. The 22.9 cm $\times$ 63.5 cm cylindrical sample chamber was located central to the array when placed in the operating position. The inoculated samples were placed vertically in the sample chamber to insure uniformity of dose. The dose rate was 0.10 kGy/minute. The temperature during irradiation was maintained at $4(\pm 1.0)^\circ\text{C}$ by the gas phase of a liquid nitrogen source, which was introduced directly into the top of the sample chamber. The temperature was monitored using two thermocouples placed in close proximity to the samples. The dose delivered was verified using 5 mm alanine pellet dosimeters, which were measured using a Bruker EMS 104 EPR Analyzer.

Following irradiation, the nonirradiated and irradiated ground beef was stored at 0–4°C for 3 weeks. At Weeks 0, 1, 2, and 3 samples were removed from refrigerated storage and formed into 200 g (2.5 cm thick $\times$ 10 cm wide) patties or “burgers.” The burgers were then cooked to an internal temperature of 145°F using a double-sided electric grill, and 10 g of the meat from the center of the cooked burgers removed to enumerate the surviving *S. senftenberg*.

After cooking, the samples were assayed for colony-forming units (CFU) by standard pour plate method (Sommers and Thayer, 2000). Approximately 90 mL of BPB was added to the sample bags containing 10 g ground beef. The samples were then mixed by stomaching for 2 minutes and 1/10 serial dilutions performed in BPB. One milliliter of the diluted samples was then placed in sterile petri plates and 20–25 mL of TSA added. The petri plates (three per dilution) were then incubated for 4 hours at 25°C

to allow for recovery of injured bacteria and then for 2 days at 37°C prior enumeration of CFUs. Extended incubation to 3 days did not change the number of CFU/plate.

Each experiment was conducted independently three times. The mean plate counts of the treated samples (N) were divided by the average control plate counts (N_0) to give a $\log_{10} (N/N_0)$ survivor ratio. Descriptive statistics and graphical representation was completed using the statistics package of Microsoft Excel (Redmond, WA) and Sigma Plot Version 8.0 (SPSS, Inc., Chicago, IL).

Results of this original research (Figure 17.1) indicated that cooking nonirradiated burgers to an internal temperature of 145°F resulted in a $\sim 2 \log_{10}$ reduction of *S. senftenberg* inside the GBPs, which persisted during the 3-week storage period. Irradiation (1.5 kGy) resulted in a $\sim 2 \log_{10}$ reduction of *S. senftenberg* that again persisted during the 3-week storage period. Cooking the irradiated GBPs to an internal temperature of 145°C for 3 weeks following irradiation resulted in a $> 6 \log_{10}$ reduction of *S. senftenberg*.

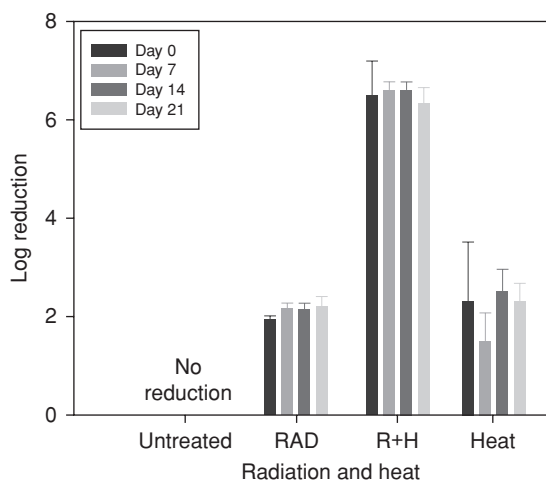


Figure 17.1. Inactivation of *S. senftenberg* in 200 g extra-lean GBPs by irradiation (RAD) (1.5 kGy), cooking to an internal temperature of 65.5°C (heat) and combination of irradiation and heat (R + H). After irradiation, both irradiated and nonirradiated patties were stored for 21 days at 4°C. At 0, 7, 14, and 21 days of storage, the samples (both irradiated and nonirradiated) were cooked and surviving population of *Salmonella* determined ($n = 3$).

Thus irradiation, in addition to inactivating 99% of *S. senftenberg*, sufficiently damaged the pathogen such that its thermosensitivity at a medium-rare cooking temperature persisted during 3 weeks of refrigerated storage, resulting in inactivation of $>6 \log_{10}$ (or 99.9999%) of the most radiation-resistant common vegetative food-borne pathogen.

10. Irradiation of Fresh and Fresh-cut Fruits and Vegetables

Fruits and vegetables are low in fat and rich in fiber and an excellent source of vitamins, micronutrients and contain phytochemicals that play a key role as antioxidants. Therefore, diets with at least five servings of fruits and vegetables are protective against many types of cancers, diabetes, and possibly other chronic diseases. As a result, consumption of fresh and fresh-cut fruits and vegetables in the United States has increased every year in the last decade. Unfortunately, the increasing consumption of fresh produce is accompanied with an increase in the number of outbreaks and recalls due to contamination with human pathogens. Centralized and widely distributed processing plants, an increase in global trade, likely being consumed raw, a longer food chain, an increase in consumption, and an aging population that is susceptible to food-borne illness may play a role in the increased number of food-borne illnesses that implicate fresh produce. Recent outbreaks of *E. coli* O157:H7 in spinach and lettuce and *Salmonella* linked to hot peppers and tomatoes have attracted intense media coverage and increased public awareness of food safety. The number of illnesses associated with fresh produce is a continuing concern and there is a growing need to reduce the presence of pathogens. The fresh produce industry is in need of a kill step to ensure the safety of produce. Ionizing radiation is known to effectively eliminate human pathogens such as *E. coli* O157:H7 on/in fresh produce. However, the commercial application of irradiation on fresh produce is still limited.

10.1. Pathogen Reduction

Earlier studies on irradiation of fresh produce are mostly about inhibition of ripening/maturation, ex-

tension of shelf life and phytosanitary application. Recent studies have been focusing on irradiation inactivation of human pathogens on fresh-cut produce. The radiation resistance of a pathogen is often represented by *D* values, which are the amounts of radiation energy required to inactivate 90% of reduction of specific pathogens. *D* values of food-borne pathogens inoculated on the surface of fresh and fresh-cut produce vary. The reported *D* values on fresh produce ranged from 0.12 to 0.47 kGy for *E. coli* O157:H7 and from 0.16 to 0.46 kGy for *Salmonella* spp. It has been recently found that internalized pathogens are more resistant to irradiation compared with those which are surface inoculated (Niemira, 2008). *D* values of *E. coli* O157:H7 inside of fresh produce were 0.30–0.45 kGy while those inoculated on the surface of corresponding vegetables were 0.12–0.28 kGy (Fan et al., 2008). It is unclear why the *D* values were higher for internalized pathogens. Perhaps the environments in the plant tissues surrounding the pathogens were different from the surface. Higher antioxidants inside the plant tissue may protect pathogen from irradiation injury. Fan et al. (2004) have shown irradiation resistance of *L. monocytogenes* was higher in solution of calcium ascorbate (an antioxidant) compared to those present in buffer. Additional research is warranted to develop a complete understanding of this phenomenon.

Nthenge et al. (2007) found that irradiation eliminate pathogenic bacteria internalized within hydroponically grown lettuce plants. Aqueous chlorine at 200 ppm failed to eliminate *E. coli* O157:H7 in lettuce tissue; however, the bacteria were not detected in 0.75-kGy-gamma-ray-treated plants. The study suggested that irradiation was more efficacious than was aqueous chlorine to control internal contamination in hydroponically grown lettuce. Therefore, irradiation can be used to inactivate *E. coli* O157:H7 and consumers benefit from a safer food product.

10.2. Quality of Irradiated Fresh Produce

Studies have demonstrated that most of fresh-cut fruits and vegetables can tolerate 1 kGy radiation (Fan et al., 2008; Fan and Sokorai, 2008a). Some vegetables such as fresh-cut cilantro can tolerate

3.85 kGy radiation (Foley et al., 2004). Shelf life of some fruits and vegetables can be extended by low dose irradiation due to the reduction of spoilage microorganisms. For example, Koorapati et al. (2004) showed irradiation at doses above 0.5 kGy prevented microbial-induced browning and blotches of sliced mushroom. It appears that deterioration in quality of irradiated fresh-cut produce is mainly due to softening, loss of ascorbic acid, and change in flavors. Effects on most of quality attributes (such as vitamin C) are often small compared to variation among cultivars and the changes in storage. Studies have shown that irradiated fresh produce may have higher antioxidant content as irradiation increase synthesis of phenolic compounds (Fan, 2005). The losses in quality can be minimized by combining the produce with other sanitizers or techniques such as modified atmosphere packaging, heat treatment, calcium infiltration, and antibrowning agents (Prakash and Foley, 2004; Niemira and Fan, 2006). For example, Boynton et al. (2006) showed that fresh-cut cantaloupes packaged in modified atmosphere (4% O₂, 10% CO₂) and irradiated at 1 kGy had the highest rating in sweetness and cantaloupe flavor intensity and lowest in off-flavor after 17 days storage compared to the control and 0.5-kG samples. Foley et al. (2004) combined chlorine (200 ppm) with low dose radiation to eliminate population of *E. coli* O157:H7 on cilantro and found the combined treatment significantly reduced level of the pathogen on fresh cilantro while maintained product quality. The combination of sanitization of whole produce has also been applied to lower the microbial load of fresh-cut produce prior to irradiation. Fan et al. (2006) surface pasteurized whole cantaloupes with 76°C water for 3 minutes. Fresh-cut cantaloupes, prepared from the pasteurized fruit were then packaged in clamshell containers and were exposed to 0.5-kGy radiation. They found that samples treated with combined heat and low dose radiation had lower microflora populations than either treatment alone and maintained the quality of the product. Therefore, combination of hot water pasteurization of whole cantaloupe and low dose irradiation of packaged fresh-cut melon can reduce the population of native microflora while maintaining quality of this product. Overall, studies conducted in

last decade demonstrated that most of fresh-cut fruits and vegetable can tolerate 1 kGy radiation. At 1 kGy, *E. coli* O157:H7 and *Salmonella* could be reduced by 3–6 logs.

10.3. Regulatory Approval

In 2008, the FDA approved the use of irradiation up to 4.0 kGy on fresh lettuce and fresh spinach, to improve microbial food safety and to extend shelf life (FDA, 2008). Irradiation of other fruits and vegetables are approved only for insect control and shelf-life extension with a maximum allowable dose of 1 kGy.

10.4. Safety of Irradiated Fresh Produce

Irradiation of fat-containing food generates minute quantities of a family of compounds derived from fat called 2-alkylcyclobutanones (2-DCB). 2-ACBs are unique radiolytic compounds. Some studies found that 2-ACBs are slightly genotoxic and may promote tumor growth in mice (Delincée and Pool-Zobel, 1998; Marchioni et al., 2004) even though animal feeding studies showed no adverse effects attributed to irradiation treatment. Because the eatable parts of all fresh fruits and vegetables have little fat, the formation of 2-ACBs is not a concern for fresh produce.

Furan (C₄H₄O) is regarded as a possible carcinogen according to the Department of Health and Human Services and the International Agency for Research on Cancer, because it causes cancer in animals in studies where the animals are exposed to furan (IARC, 1995; NTP, 2004). This compound is commonly found in foods that have been treated with traditional heating techniques, such as cooking, jarring, and canning (FDA, 2004). Fan and Sokorai (2008b) irradiated 19 fruits and vegetables and measured furan formation in those irradiated produce. Overall, they found that irradiation produced low ppb levels of furan only in a few fruits. No detectable levels of furan were found in any of vegetables tested. It appears that the presence of high amounts of sugars and low pH are prerequisites for furan formation in fresh-cut produce. Considering the low ng/g of

furan detected in the limited number of fruits as well as the volatility of furan, irradiation-induced furan is unlikely to be a concern for fresh-cut produce.

10.5. Packaging Materials

Most of polymeric packaging materials that are used by the produce industry have been approved by FDA. However, these polyolefins may contain additives that have not been approved for use during irradiation. Therefore, packaging materials intended for the irradiation of prepackaged fresh-cut produce in the presence of oxygen may still need a premarket approval. In addition, packaging materials are very complex and emerging new packaging materials presents a challenge to FDA. New materials such as degradable and antimicrobial packages, adjuvants (antioxidants, stabilizers, etc.), plasticizers, colorants, and adsorbent pads may need more research before being evaluated and approved by FDA (Kamolprasert, 2007).

11. Conclusions

Irradiation is a safe FDA-approved process for improving the microbiological safety of meats such as ground beef, Iceberg lettuce and spinach. Irradiation, when performed properly, does not affect the taste, aroma, texture, and overall liking of ground beef. Consumer attitudes toward irradiation of meat and poultry have improved over the last 15 years. Irradiation may provide additional protection to consumers through maintenance of increased heat sensitivity of bacterial food-borne pathogens during refrigerated storage of ground beef and fresh produce.

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Chapter 18

Pulsed Ultraviolet Light

Ali Demirci and Kathiravan Krishnamurthy

1. Introduction

Microorganisms can exist in water, food, and environment in a typical farm and food processing plant. Contamination of these microorganisms can be avoided by choosing an appropriate method of decontamination and by avoiding cross-contamination and post-contamination. A major public health concern of today is foods contaminated with pathogenic microorganisms, such as *Salmonella* spp., *Clostridium perfringens*, *Staphylococcus aureus*, *Campylobacter jejuni*, *Escherichia coli* O157:H7, and *Listeria monocytogenes*, which have significant harmful effects on the health of people throughout the world.

Food preservation techniques have been developed to ensure the safety of the food. Therefore, preservation is critical for modern mass food production and distribution. As such, various preservation technologies have been developed and adopted successfully. Though, several technologies, such as heat treatment, cold temperature treatment, irradiation, microwave radiation, pulsed electric field, magnetic field, high pressure, and ohmic heating treatments, are available for inactivation of these microorganisms (Juneja and Sofos, 2002), there is always a need to investigate novel technologies as an alternative to existing preservation methods, to improve efficiency, minimize cost, and yield minimal quality changes. Ultraviolet (UV) light processing is getting more and more attention from food

industry as an alternative technology for inactivation of pathogenic and spoilage microorganisms during food processing.

2. Ultraviolet Light

UV light has been used as a bactericidal agent from as early as 1928 (Anonymous, 1989). UV light is divided into four regions (Table 18.1), namely, UV-A (315–400 nm), UV-B (280–315 nm), UV-C (200–280 nm), and vacuum UV (100–200 nm) according to their wavelength (Perchonok, 2003).

Light consists of discrete fundamental packets of energy known as photons which have zero mass, no electric charge, and an indefinitely long lifetime. These photons contain vast amount of energy, which is determined by the wavelength of light Equation (18.1). For instance, UV-light photon at 254 nm has energy of 470 kJ/mol based on Equation (18.1):

$$E = h\nu = \frac{hc}{\lambda} \quad (18.1)$$

where E is the energy of photon, h is the Planck's constant (6.626×10^{-34} J/second), ν is the frequency of light, c is the speed of light in vacuum, and λ is the wavelength of light. Comparison of energies of photon at different electromagnetic spectrum in UV light calculated from the above equation is also given in Table 18.1. Photons in ultraviolet region have more energy than visible or infrared region and hence it may account for predominant inactivation of pathogens. For instance, the energy of the photon in UV-C range and far infrared range are 12.40–4.43 eV and 0.41–0.12 eV, respectively. Increase in

Table 18.1. Characteristics of UV, visible, and infrared regions of electromagnetic spectrum

Region	Wavelength (nm)	Frequency (Hz)	Photon Energy (eV)	Molar Photon Energy (kJ/mol)
Vacuum UV	100–200	3.00×10^{16} to 3.00×10^{15}	124–12.4	11,975–1,197
UV-C	200–280	3.00×10^{15} to 1.07×10^{15}	12.40–4.43	1,197–427
UV-B	280–315	1.07×10^{15} to 9.52×10^{14}	4.43–3.94	427–380
UV-A	315–400	9.52×10^{14} to 7.49×10^{14}	3.94–3.10	380–299
Visible light	400–700	7.49×10^{14} to 4.28×10^{14}	3.10–1.77	299–171
Near Infrared	700–1,400	4.28×10^{14} to 2.14×10^{14}	1.77–0.89	171–85.5
Mid infrared	1,400–3,000	2.14×10^{14} to 9.99×10^{13}	0.89–0.41	85.5–39.9
Far infrared	3,000–10,000	9.99×10^{13} to 3.00×10^{13}	0.41–0.12	39.9–12.0

wavelength results in decrease in the energy level. For instance, the molar photon energy at 100 and 10,000 nm are 11,975 and 12 kJ/mol, respectively. The infrared portion of the pulsed UV light induces rapid temperature increase upon absorption.

When a light of initial intensity (I_0) falls on food surface, portion of the light is transmitted through the food, while the rest is reflected back and/or scattered. As the UV light penetrates through the food material, its intensity decays along a distance of x beneath the food surface (Palmieri et al., 1999) as given in Equation (18.2):

$$I = TI_0 e^{-x} \quad (18.2)$$

where T is the transparency coefficient of the food material, I is the intensity of light at a distance x from the surface, I_0 is the initial intensity of UV light, and x is the distance below the food surface. The residual amount of light is dissipated as heat and transferred to the inner layers through conduction (Palmieri et al., 1999). Therefore, the intensity of UV light exponentially decays within the food material. UV light is more effective for surface sterilization and sterilization of highly transparent liquids such as water.

As the energy of photons in UV-light range is high they can even cause ionization of molecules, whereas visible light and infrared region causes vibration and rotation of molecules, respectively. When molecules absorb the energy, it is elevated to an excited state. The excited molecule can either (i) relax back to the ground state by releasing the energy as heat, (ii) relax back to the ground state by releasing energy as photons, or (iii) can induce some chemical changes. Ab-

sorption of UV light by microorganisms can undergo these changes and hence inactivate microorganisms due to chemical changes and temperature buildup.

3. Pulsed UV Light

UV light can be applied in two modes, namely, continuous mode and pulsed mode. In continuous mode, constant energy UV light is released continuously in a monochromatic or polychromatic wavelengths. Conventional UV-light systems, such as low-, medium-, or high-pressure mercury lamps, emit radiation in continuous mode. In pulsed mode, the electrical energy is stored in a capacitor over a short period of time (few milliseconds) and released as very short period pulses (several nanoseconds). The electrical energy is transferred through a lamp filled with inert gas (xenon or krypton), which causes ionization of gas and produces a broad spectrum of light in the wavelength region of ultraviolet to near infrared (Figure 18.1).

The intensity of pulsed light is at least 20,000 times more than that of sun light (Dunn et al., 1995). Typically, the pulse rate is 1–20 pulses per second and the pulse width is 300 ns–1 ms. Therefore, light pulses with several megawatts of instantaneous energy is produced though the total energy is comparable to a continuous UV-light system. Therefore, pulsed UV-light treatment is a more effective and rapid way of inactivating the microorganisms than continuous UV-light sources because the energy is multiplied manifold (Dunn et al., 1995). Comparison of continuous and pulsed UV light is listed in Table 18.2.

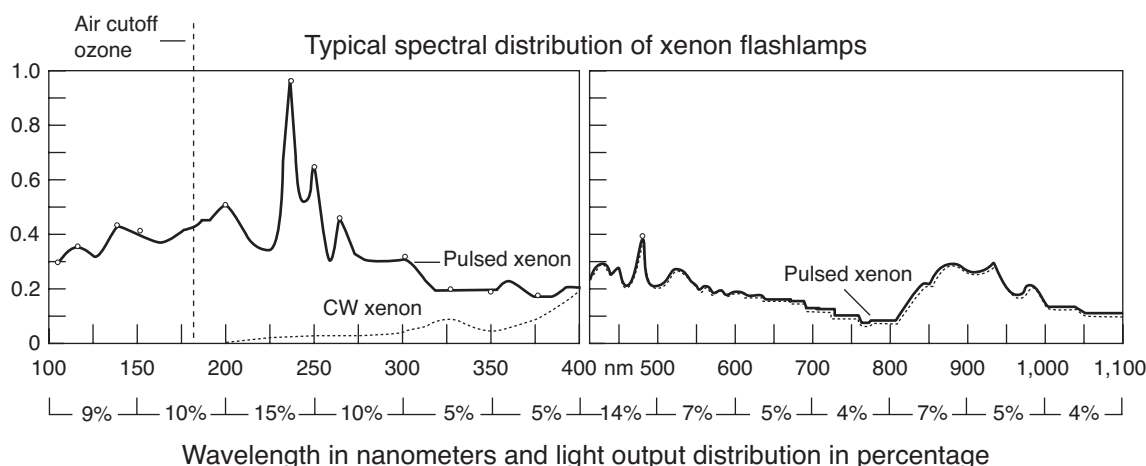


Figure 18.1. Spectral distribution of pulsed UV light (Xenon, 2003).

Pulsed light is a broad-spectrum radiation from UV light to infrared radiation. Pulsed light is also referred as pulsed UV light, high-intensity light, broad-spectrum white light, and pulsed white light (Green et al., 2003). To maintain consistency, the term *pulsed UV light* will be used throughout this chapter. Also, the UV portion of the pulsed UV light has higher energy level followed by visible light and infrared region. A typical lamp produces a polychromatic radiation in the wavelength range of 100–1,100 nm, with 54, 26, and 20% of the energy at UV light, visible, and infrared regions, respectively (Figure 18.1). UV region plays the most important role in activation by creating thymine dimers, but infrared region

also contributes to inactivation by creating localized heating.

4. Inactivation Mechanism of UV Light

UV light exhibits germicidal properties in the wavelength range of 100–280 nm (UV-C region). The inactivation efficiency of UV light follows a bell-shaped curve, where maximum inactivation occurs from 254 to 264 nm (Masschelein, 2002). Conventional mercury lamp produces UV light at 254 nm, and hence this wavelength is often used for comparison of inactivation efficiencies of continuous

Table 18.2. Comparison of continuous and pulsed UV light

Parameter	Continuous UV Light	Pulsed UV Light
Wavelength	254 nm	100–1,100 nm (typical)
Instantaneous energy	Less	Magnified several thousand folds
Inactivation mechanism	Photochemical DNA damage by thymine dimer formation	Damage to cells by photochemical changes and by localized heating
Natural cooling of lamp	No	Enables lamp to cool between pulses
Mercury	Commonly used as source	Provides mercury free alternative
Inactivation efficiency	Normal	Up to four times increased inactivation efficiency as compared to continuous UV light
Temperature increase during UV treatment	No significant temperature increase	Significant temperature increase due to infrared

UV-light systems. Therefore, it is crucial to design a disinfection system which emits higher intensities of wavelengths in the absorption region of interest.

As only the absorbed UV-light energy induces photophysical, photochemical, and/or photothermal effect necessary for inactivation of pathogenic microorganisms, it is crucial to have a proper lamp design. Base pairs of DNA absorb UV light (Masschelein, 2002) effectively due to their aromatic ring structure. In general, pyrimidines “thymine (DNA), cytosine (DNA and RNA), and Uracil (RNA)” are strong absorbers of photons in the ultraviolet range and thus produce photoproducts which results in bacterial inactivation.

UV -light damages the DNA of bacteria by primarily forming thymine dimers and thus bacterial DNA cannot be unzipped for replication. Therefore, bacteria cannot reproduce (Bank et al., 1990; Jay, 2000). Though cyclobutyl pyrimidine dimer formation is the main inactivation mechanism, there are other photoproducts formed during UV-light exposure including pyrimidine pyrimidinone [6–4]-photoproduct, Dewar pyrimidinone, adenine–thymine heterodimer, cytosine photohydrate, thymine photohydrates, single-strand break, and DNA–protein cross-link. Setlow and Setlow (1962) reported that DNA chain breakage, cross-linking of strands, hydration of pyrimidines, and formation of dimers between adjacent residues in the polynucleotide chain are the major photochemical changes that occur in DNA upon UV-light exposure. Formation of photoproducts depends on wavelength, DNA sequence, and protein–DNA interactions (Mitchell, 1995). Aromatic amino acids, such as phenylalanine and tryptophane, also absorb ultraviolet light (Henis, 1987) and thus inactivate these amino acids present in microorganisms.

Photoreactivation occurs in the wavelength range of 330–480 nm due to activation of DNA photolyase, which splits thymine dimers (Walker, 1984). It is also hypothesized that thermal stress induced on bacteria due to UV-light exposure leads to bacterial rupture especially at higher flux densities (0.5 J/cm^2). Overheating of bacteria is caused due to differences in UV-light absorption by bacteria and surrounding medium and thus bacteria becomes a local vaporization cen-

ter and may generate a small steam flow performing membrane destruction (Takeshita et al., 2003).

UV-A (315–400 nm) has better penetration capacity than UV-C and affects bacterial cells by causing membrane damages and/or generate active oxygen species or H_2O_2 (Kramer and Ames, 1987). However, UV-A has very little impact on microbial cells unless exogenous photosensitizers are used in the process and absorbed by the bacterial cell (Mitchell, 1978). Antimicrobial effect of pulsed UV light is predominately caused by the UV-light portion of the broad spectrum.

The inactivation mechanism for spores is different from that of vegetative cells mainly because of the structural differences. Riesenman and Nicholson (2000) reported that the thick protein coat induces resistance in spores of *Bacillus subtilis*. The DNA of bacterial spore has a different conformation than the DNA of the vegetative cell. Setlow and Setlow (1987) reported that *Bacillus* spores did not produce any detectable amount of thymine-containing dimers. The predominant photoproduct was 5-thyminyl-5,6 dihydrothymine adduct; later, it was termed as “spore photo-product.”

Figure 18.2 show a commercially available pulsed UV-light unit, which generates $1.27 \text{ J/cm}^2/\text{pulse}$ of radiant energy at 1.8 cm below the quartz window. The system produces three pulses with $360\text{-}\mu\text{s}$ duration per second. The UV-light strobe was 5.8 cm above the quartz window of the chamber. The distance of the sample to be treated from the quartz window can be adjusted by using variable tray levels.

Pulsed UV light may have some shocking effect on the cell wall of bacteria in addition to the effect of high-intensity pulses. Takeshita et al. (2003) investigated the mechanisms of damage of yeast cells induced by pulsed light and continuous UV light. The authors reported that the DNA damage induced by continuous UV light is slightly higher than that of pulsed light. However, protein elution due to pulsed UV light was higher than that resulting from continuous UV light. But more research has to be done before arriving at a conclusion. Localized heating of bacteria is induced by pulsed UV light as the heating and cooling rate of bacteria and the surrounding matrix

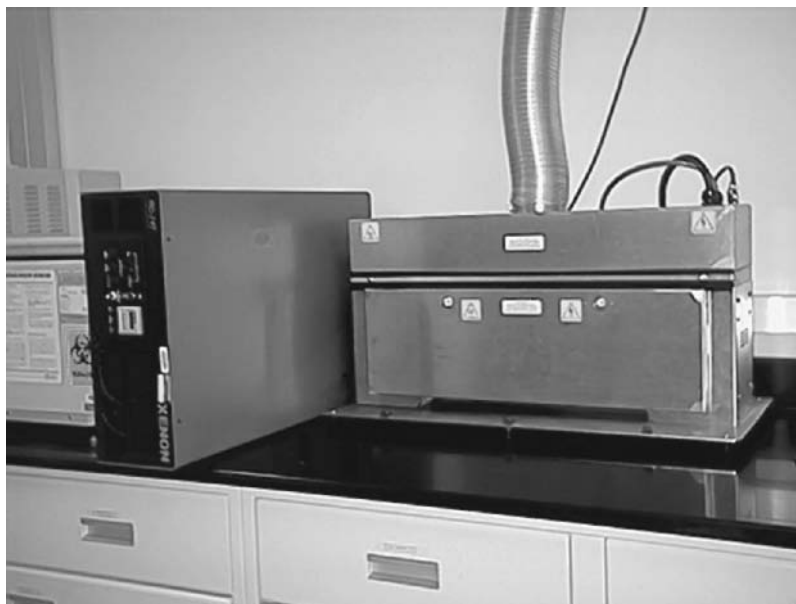


Figure 18.2. SteriPulse®-XL 3000 pulsed UV-light sterilization system (Xenon Corporation®, Wilmington, MA). (For color details, please see color plate section.)

are different (Fine and Gervais, 2004).). Wekhof (2000) suggested that the inactivation mechanism of pulsed UV light includes both the germicidal action of UV-C light and the rupture of microorganism due to thermal stress caused by UV components. Pulsed UV light is gaining attention in recent years because it can provide sufficient antimicrobial inactivation and commercial sterilization with no toxic by-products (FDA, 2000). It can be effectively used to inactivate pathogens on the surface of food or packaging materials.

5. UV-Light Penetration and Absorption

UV light has a limitation for penetration capacity. UV light can penetrate up to several millimeters in solid food material depending upon the optical properties of the food materials. However, it can easily penetrate through water since it is transparent to the wavelengths produced by UV light. However, foods such as sugar solutions have a limited penetration. Oppenlander (2003) suggested some of the possible

arrangements of the UV-light lamp in a photoreactor, which can be utilized to enhance the effectiveness of absorption by target material. For example, a falling film photoreactor design might be essential for effective penetration in milk. Other designs which reduce the thickness of food material to be treated would be crucial for the commercial success of this technology for use in opaque liquid foods. Addition of some absorption enhancing agents such as edible colorants will also enhance the penetration (Palmieri et al., 1999).

The absorption coefficient of liquid food increases as the color or turbidity of liquid increases. The penetration capacity of UV light reduces as the absorption coefficient increases (Guerrero-Beltran and Barbosa-Canovas, 2004). Shama (1999) reported the coefficient of absorption for various liquid foods including water, syrup, wine, beer, and milk. The coefficient of absorption increases as the suspended solid content increases. For example, white wine and milk had a coefficient of absorption of 10 and 300 cm^{-1} , respectively. As expected, the dark colored food materials absorb more and hence result in larger coefficient

of absorption. For instance, the coefficients of absorption of clear and dark syrup were 2–5 and 20–50 cm^{-1} , respectively (Shama, 1999). Therefore, for UV-light treatment of foods with higher coefficient of absorption, optimization of the system which will enhance the penetration depth will be beneficial. Furthermore, treatment of these food products in the form of a thin film may result in better penetration and thus increase the efficiency of microorganism inactivation.

6. Applications of by Pulsed UV Light

McDonald et al. (2000) compared the effectiveness of a continuous UV-light source and a pulsed UV-light source for the decontamination of surfaces and reported an almost identical level of inactivation of *B. subtilis* with $4 \times 10^{-3} \text{ J/cm}^2$ of pulsed UV-light source and $8 \times 10^{-3} \text{ J/cm}^2$ continuous UV-light source.

The effect of pulsed UV-light emission with low or high UV content on inactivation of *L. monocytogenes*, *E. coli*, *S. enteritidis*, *P. aeruginosa*, *B. cereus*, and *S. aureus* were investigated by Rowan et al. (1999). Bacterial cultures were seeded separately on the surface of Tryptone soya-yeast extract agar and treated with a low UV content pulsed UV-light source (Heraeus Noblelight XAP series: type NL4006) or high UV content pulsed UV-light source (Heraeus Noblelight XFP series: type NL4320). The high UV content light source emitted higher energy especially at 200–450 nm. Reductions of 2 and 6 $\log_{10}$ were obtained with 200 light pulses (approximate pulse duration = 100 ns) for low and high UV content, respectively. Sonenshein (2003) investigated the effect of high-intensity UV light on inactivation of *B. subtilis* spores. *B. subtilis* spores were diluted to give concentrations of 1×10^9 (Sample A), 1×10^8 (Sample B), or 1×10^7 (Sample C) using sterile deionized water. A 50- μL sample of each concentration were placed at three different positions (Position 1, on the lamp axis and at the midpoint of the lamp; Position 2, 1 cm above the lamp axis and at the midpoint of the lamp; and Position 3, 1 cm above the lamp axis and 172 mm to the right of the midpoint of the lamp) and treated with pulsed UV light. Three pulses

(1 second) of UV -light resulted in more than 6.5 $\log_{10}$ CFU/mL (Sample B) and 5.5 $\log_{10}$ CFU/mL (Sample C) reductions when the samples were placed at the lamp axis and at the midpoint of the lamp.

In order to show effectiveness of pulsed UV-light, Krishnamurthy et al. (2004a) treated *S. aureus* as suspended cells in phosphate buffer and agar seeded cells. A 12-, 24-, or 48-mL of *S. aureus* suspension in phosphate buffer was treated under pulsed UV-light for 1–30 seconds. As expected, the $\log_{10}$ reduction increased, treatment time increased. After a 5-second treatment time, the $\log_{10}$ reduction obtained was about 7.50 $\log_{10}$ CFU/mL. The effect of the sample depth was determined to be significant. There was no growth after 1-second treatment of 12 mL sample. However, after 1-second treatment, only 4.6 $\log_{10}$ reduction and 1.5 $\log_{10}$ reduction were obtained in case of 24- and 48-mL samples, respectively. The temperature of the sample increased, as the treatment time increased. Also, there was no significant temperature increase during the first 5-second treatment. Though, the pulsed UV-light treatment is supposed to be non-thermal for inactivation, there was a significant increase in the temperature for longer treatment times due to the energy absorbed. During a 20-second treatment, the temperature increase was about 20°C for a 12-mL phosphate buffer sample. For agar seeded cells, *S. aureus* in peptone water was surface plated on Baird-Parker agar plates and the plates are treated under pulsed UV light for 1, 2, 3, 4, 5, 10, 15, or 30 seconds. Again, a 5-second treatment inactivated all *S. aureus* cells. The temperature of the agar increased during long treatment times. However, there was no significant increase in the temperature during the first 5 seconds in which complete inactivation occurred. Therefore, the inactivation was mainly due to the pulsed UV light, not due to synergistic effect of temperature increase. The pulsed UV light was very effective in inactivating *S. aureus* in colorless solutions and solid surfaces.

Krishnamurthy et al. (2004b) also studied inactivation of *S. aureus* in milk as a model to represent an opaque food. Milk contaminated with *S. aureus* was treated with pulsed UV light based on the experimental design suggested by the surface response method. The milk samples with various depths were

treated with pulsed UV light for varying times at various distances. In general, the $\log_{10}$ reduction during pulsed UV-light treatment increased with time and decreased with the distance from UV-light source and the depth of sample. A 12, 30, or 48-mL of cell suspension in milk was treated under pulsed UV light for 30, 105, or 180 seconds. The reduction obtained varied from 0.16 to 8.55 $\log_{10}$ CFU/mL demonstrating the ability of pulsed UV light to inactivate *S. aureus*. Complete inactivation was obtained at (i) 8-cm sample distance from quartz window, 30-mL sample volume, and 180-second time combination and (ii) 10.5-cm sample distance from quartz window, 12-mL sample volume, and 180-second treatment time combination. Due to the turbidity of the milk sample, the penetration of UV light is not effective as in the case of phosphate buffer. The reason for less penetration or less absorption of UV energy is the reflection of energy and/or absorption of energy by other milk components.

The efficacy of pulsed UV light for continuous-flow milk treatment for the inactivation of *S. aureus*, a pathogenic microorganism frequently associated with milk, was investigated by Krishnamurthy et al. (2005). The effect of sample distance from the quartz window, number of passes, and flow rate were investigated. A response surface method was used for design and analysis of the experiments. Milk was treated at 5-, 8-, or 11-cm distance from UV-light strobe at 20, 30, or 40 mL/minute flow rate and treated up to three times by recirculation of milk to find the effect of number of passes on inactivation efficiency. $\log_{10}$ reductions varied from 0.55 to 7.26 $\log_{10}$ CFU/mL. Complete inactivation was obtained in two cases and growth was not observed mostly following the enrichment protocol; (i) 8-cm sample distance from quartz window, 30-mL sample volume, and 180-second treatment time combination and (ii) 10.5-cm sample distance from quartz window, 12-mL sample volume, and 180-second treatment time combination.

Another interesting application of pulsed UV was on honey in which honey inoculated with of *Clostridium sporogenes* spores were treated with pulsed UV-light treatment (Hillegas and Demirci, 2003). The number of pulses, the distance between honey and

lamp, and the depth of honey were investigated. In general, increasing the number of pulses (which also means increasing the treatment time) increased the percent reduction. The results showed an increase from 0% reduction at 15 and 135 pulses (5 and 45 seconds) to 89.4% reduction at 540 pulses (3 minutes). The percent reduction increased as the depth of honey was decreased. For instance, reductions of 0.0 and 39.5% were obtained for 8 and 2-mm -deep honey, respectively, for a 45-second treatment at 20 cm below the quartz window. When the depth of honey was changed from 8 to 2 mm, an increase in reduction becomes evident. The trend that emerges from decreasing the shelf height, or decreasing the distance between the honey and the UV light, can be seen by examining the percent reduction for different shelf heights at the same number of pulses and the same depth of honey. In conclusion, raising the shelf (or decreasing the distance between the honey surface and the UV lamp) demonstrated an increase in spore reduction. Increasing the number of pulses (or treatment time) also appeared to be effective at inactivating the spores. When the depth of honey was decreased from 8 to 2 mm at the 20-cm shelf height, the spore kill increased for the same number of pulses. Even though varying these three parameters enhanced the percent inactivation of *C. sporogenes*, it failed to inactivate the spores completely. It appears that the UV light has a limited penetration in the honey. The heat generated within the pulsed UV light does not appear to have a synergistic effect on the inactivation of *C. sporogenes* in honey. The heat generated within the pulsed UV light does not appear to have a synergistic effect on the inactivation of *C. sporogenes* in honey, because the spores survived.

Pulsed UV-light system was used to inactivate fungal spores of *Aspergillus niger* in corn meal (Jun et al., 2003). Response surface methodology was utilized as the experimental design. Three parameters for the process were evaluated; processing time (20–100 seconds), voltage input (2,000–3,800 V), and distance from UV lamp (3–13 cm). The voltage range of 2,000–3,800 V yielded energy output range of 1.8–5.7 J/cm² per pulse at 1.8 cm below the lamp surface. The optimal values of the three parameters based on response surface model were a treatment

time of 50 seconds, a sample distance of 8 cm from the UV lamp, and an input voltage of 3,800 V to yield a $3.12 \log_{10}$ reduction of fungal spores.

E. coli O157:H7 inoculated alfalfa seeds were treated with pulsed UV light (Sharma and Demirci, 2003). To determine the extent of inactivation of *E. coli* O157:H7 on alfalfa seeds, 1, 3, 5, and 10 g of inoculated seeds with corresponding layer thicknesses of 1.02, 1.92, 3.61, and 6.25 mm were subjected to pulsed UV light for 5, 10, 30, 45, 60, 75, and 90 seconds at 8-cm distance from the UV lamp. Complete elimination of the pathogen was achieved at the longest treatment interval with reductions in population higher than $4 \log_{10}$ CFU/g. As the thickness of the seed layer increased, the population reduction decreased significantly for most time intervals. In order to study the effect of distance from the UV lamp, seeds with 6.25 mm layer thickness were treated at distances of 3, 5, 8, and 13 cm from the UV lamp. The reduction in population of *E. coli* O157:H7 for varying distances ranged from 0.07 to $4.89 \log_{10}$ CFU/g, with reductions being significantly higher at shorter distances and longer treatment times. Overall, the germination of seeds treated at various thicknesses was not significantly reduced except for 60- and 75-second treatment of 1.92 mm layer and 90-second treatment of 3.61 mm layer thickness. Seeds treated at different distances from the UV lamp had germination percentage over 81% up to 60-second treatment relative to a germination rate of 86% for untreated seeds. However, germination reduced to about 75% for 75- and 90-second treatments. Hence, pulsed UV-light treatment resulted in complete elimination of *E. coli* O157:H7 without significantly reducing alfalfa seed viability.

Ozer and Demirci (2005) investigated the efficacy of pulsed UV light to inactivate of *E. coli* O157:H7 and *L. monocytogenes* Scott A on salmon fillets by evaluating the effects of treatment times and distance from the UV strobe. Skin or muscle side inoculated salmon fillet (8 cm $\times$ 1.5 cm) in a Petri dish was placed on shelf at three different distances from the quartz window; 3, 5, and 8 cm. At each distance, the pulsed UV-light treatment was performed for 15, 30, 45, and 60 seconds. For *E. coli* O157:H7, maximum $\log_{10}$ reduction was $1.09 \log_{10}$ CFU/g on mus-

cle side at 8 cm for 60-second treatment, whereas $0.86 \log_{10}$ CFU/g reduction on skin at 5 cm for 30-second treatment. For *L. monocytogenes* Scott A, maximum reduction was $1.02 \log_{10}$ CFU/g at 8 cm for 60-second treatment on skin side, whereas $0.74 \log_{10}$ CFU/g reduction on muscle at 8 cm for 60-second treatment. The fillet's surface temperature increased up to 100°C within 60-second treatment time. Therefore, some fish samples were overheated after 30 and 45 seconds at 3- and 5-cm distances from quartz window, respectively, which resulted in visual color and quality changes. Overall, this study demonstrated that about 1 log reduction ($\sim 90\%$) of *E. coli* O157:H7 or *L. monocytogenes* could be achieved within 60 seconds at 8-cm distance. The study indicated the potential of pulsed UV-light technology for decontamination of muscle foods.

Woodling and Moraru (2005) recently studied the influence of surface topography on the effectiveness of pulsed UV-light treatment for the inactivation of *Listeria innocua* on stainless steel surfaces. Pulsed UV-light treatment resulted in a high level of sublethal injury, which holds promise for its use in a hurdle approach, wherein another type of treatment (i.e., sanitization of food contact surfaces) could be applied to the sublethally injured cells immediately after the pulsed UV-light treatment. The follow-up treatment could impede cell recovery and thus lead to a much higher level of reduction than would result from the individual application of each microcidal technique. Overall, the fact that a significant level of microbial reduction was achieved after a very short treatment time in their study indicates much promise for the use of pulsed UV-light as a quick and relatively inexpensive method of reducing the microbial load on a range of different surfaces in food processing environments.

Efficacy of pulsed UV light for inactivation of *B. subtilis* spores in water was investigated using a flow-through pulsed UV-light chamber (Demirci and Krishnamurthy, 2005). The authors reported that complete inactivation of *B. subtilis* spores obtained up to 14 L/minute flow rates. There was no growth observed when treated samples were incubated both under light or no-light conditions, indicating that the spores were beyond repair by both dark repair and

photorepair mechanisms. The corresponding inactivation obtained was $5.5 \log_{10}$ CFU/mL. The authors also noted that after pulsed UV-light treatment, absorption at 254 nm reduced significantly suggesting reduction in the turbidity.

7. Effect of UV Light on Food Components and Quality

In the presence of air, depolymerization of starch occurs under UV light (Tomasik, 2004). Presence of sensibilizers (metal oxides, particularly ZnO) enhances this process. Usually, photooxidation followed by photodecomposition occurs. UV-light exposure initiates free radical oxidation and catalyzes other stages of the process. UV light forms lipid radicals, superoxide radicals (SOR), and H_2O_2 (Kolakowska, 2003). SOR can further induce carbohydrate cross-linking, protein cross-linking, protein fragmentation, peroxidation of unsaturated fatty acid, and loss of membrane fluidity function. UV radiation may also denature proteins, enzymes, and amino acids (especially amino acids with aromatic compounds) in milk, leading to textural changes. Water also absorbs UV photons and produces OH- and H+ radicals, which in turn aids changes in other food components. Therefore, UV-light treatment not only changes the chemistry of food components, but also may lead to product quality deterioration when it is applied at high doses. However, most of the changes in the components are detrimental to microbial growth. Therefore, proper optimization of the disinfection process is necessary in order to maintain the quality of food products while ensuring its safety. Normally, microbial inactivation can be achieved within seconds to minutes depending upon the opacity of the food products and microorganism type. Therefore, food quality may not be affected negatively when the process is done at optimized conditions.

Light-induced flavor is caused due to activation of riboflavin, which is responsible for the conversion of methionine from methanol which leads to a burnt-protein-like, burnt-feather-like, or medicinal-like flavor. Peroxides produced during UV-light exposure may also attack fat-soluble vitamins and colored

compounds and may lead to change in nutritional quality and/or discoloration. Furthermore, long treatments with UV light may increase the temperature of food product which will lead to temperature related quality changes such as cooked flavor, change in color due to nonenzymatic browning. UV light can also degrade vitamins by photodegradation. Especially vitamins A, C, and B₂ are affected by UV light.

Prolonged treatment with UV light can result in discoloration of food materials. Cuvelier and Berset (2005) reported that paprika gels started to fade upon UV-light exposure after 3 hours. Fat soluble vitamins and colored compounds can be affected by the peroxides produced during extended UV-light treatment. Undesirable changes in food may occur when food is treated with UV light for extended period of time. Foods may need to be treated for a less time to achieve the desired decontamination level, and hence there will not be any adverse change in food quality, which can be possible with pulsed UV light.

The in-package UV-light treatment of white bread slices with PureBright® system resulted in bread slices with fresh appearance for more than 2 weeks; however, the control slices dried out and got moldy (Rice, 1994). The author also suggested that the quality of tomatoes treated with pulsed light was acceptable up to 30 days of storage at refrigerated temperature.

The efficacy of pulsed UV light for decontamination of minimally processed vegetables was investigated by Gomez-Lopez et al. (2005). They conducted sensory evaluation with a semi-trained panel of four to six people. The panelists evaluated the quality of minimally processed white cabbage and iceberg lettuce. Off-odors were present for the pulsed light treated white cabbage just above the acceptable limit. This limited the shelf life of white cabbage to a maximum of 7 days. The panelists described the off-odor as “plastic,” which was distinctive immediately after the pulsed light treatment, but the off-odor faded away after couple of hours in the storage. Therefore, the off-odor can be assumed to disappear before consumption by the consumers. It is interesting to note that pulsed UV light treated iceberg lettuce received better scores than the control samples for off-odor,

taste, and leaf-edge browning. This clearly indicates pulsed UV-light treatment helps in preserving the lettuce quality.

In general, UV-light treatment of food may not cause any adverse effect if applied in moderate amounts, which is required for microbial inactivation as strongly suggested by previous studies. However, modification and optimization of the UV-light treatment might be necessary for successful implementation of the process in some foods.

8. Pathogen Inactivation Modeling for Pulsed UV Light

UV dose-response curves for dispersed or free-floating microorganisms can be represented by first-order kinetics (Severin et al., 1984; EPA, 2003; Liu, 2005). Generally, an UV-light inactivation curve is sigmoidal (CFSAN-FDA, 2000) with a shoulder and tail. Shoulder effect is due to delayed response of a microorganism to UV light because of injury (CFSAN-FDA, 2000; EPA, 2003). Photoreactivation, dark repair, and resistance of bacteria are factors influencing shoulder effect. Tailing effect occurs because of the shielding of external particles, clumping of bacteria, and resistant microorganisms. Pulsed UV light has very high instantaneous energy as compared to continuous UV light. Even a second treatment results in significant inactivation of pathogenic microorganism. Several researchers suggested that inactivation of pathogens occur within seconds because of increased energy availability. Therefore, pulsed UV-light inactivation curves may not have a shoulder because of high instantaneous energy available for microbial inactivation. Pulsed UV light may also not have tail effect for inactivation of pathogens in a clear solution. However, while treating a complex food matrix with particles, one may need to take into account these effects. Inactivation models developed for UV light can be utilized for pulsed UV-light modeling since significant portion of the energy delivered in a pulsed UV-light system is in the UV range (typically, more than 50%).

The first-order inactivation equation (Severin et al., 1984) can be represented as:

$$N = N_0 \exp^{-(k \times I \times t)} \quad (18.3)$$

where N_0 and N are concentrations ($\log_{10}$ CFU/mL) of viable microorganisms before and after UV-light treatments, respectively, for a treatment time of t seconds. k is the first-order inactivation coefficient (cm^2/J) and I is the intensity of UV-light energy applied (J/cm^2).

When the microorganisms exhibit a shoulder effect, the above equation can be modified as:

$$N = N_0(1 - (1 - \exp^{(-kD)})^d) \quad (18.4)$$

where d is the intercept of the exponential phase of the dose-response curve with the y-axis (EPA, 2003). Similarly, Equation (18.2) can be modified to take into account the tailing effect as follows:

$$N = N_0 e^{(-kD)} + N_p e^{(-k_p D)} \quad (18.5)$$

where N_0 is the concentration of dispersed microorganisms present, N_p is the concentration of particles containing microorganisms, and k_p is the inactivation constant for microorganisms associated with particles (EPA, 2003).

Further investigation on modeling has to be done as pulsed UV light also comprised visible and infrared heating. Therefore, inclusion of the effect of both visible and infrared regions would result in better prediction.

9. Economics of Pulsed UV Light

Compared to other available disinfection technologies, the cost of pulsed UV-light disinfection systems is competitive or some times cheaper. Dunn et al. (1997) estimated that the treatment cost at $4 \text{ J}/\text{cm}^2$ with PureBright® pulsed UV-light treatment system is $0.01\text{¢}/\text{m}^2$ of treated area. The cost includes conservative estimate of electricity, maintenance, and equipment amortization. Lander (1996) also estimated $0.01\text{¢}/\text{m}^2$ as the cost of treatment with PureBright® system, where the estimated cost includes the electricity, maintenance, and investment in a hooded high-intensity lamp and power unit.

The processing cost for 4 log reduction of *E. coli* in primary waste water by UV light, electron beam, and gamma irradiation were, 0.4, 1.25, and $25\text{¢}/\text{m}^3$, respectively (Taghipour, 2004). This clearly indicates

that the UV-light treatment is cost-effective for inactivation of pathogenic microorganisms.

10. Conclusions and Future Trends

As studies indicated, pulsed UV light has potential to be utilized for food processing. Pulsed UV light can provide a cost-effective alternative for inactivation of pathogens. Pulsed UV light can effectively inactivate both vegetative cells and spores. In several studies, the quality changes during pulsed UV-light treatment were negligible or comparable to conventional treatments. However, quality changes are dependent upon the type of food product being treated with pulsed UV light.

As a novel technology, pulsed UV light is still in development. Therefore, several improvements can be made. By monitoring the actual energy absorbed by the food sample at different depth levels can result in better model development and process validation. During prolonged pulsed UV-light treatment, temperature of the food sample increases. The effects of thermal inactivation should be monitored and recognized. For certain products, temperature buildup can be detrimental to food quality. Hence, filtering out the wavelengths causing temperature increases could help preserve the quality of the food. As a broad-band spectrum light, pulsed UV light also includes the wavelength range from 330 to 480 nm which is responsible for photoreactivation, a mechanism to repair the DNA damage caused by UV light. It will be crucial to filter out this wavelength region in order to ensure the complete inactivation of pathogenic microorganisms. For continuous treatment of liquid food by pulsed UV light, a mechanism has to be developed to treat a food material as a thin film for reducing treatment time and enhancing quality. For opaque food materials, treatment with some photocatalyzer may enhance effectiveness of pulsed UV-light treatment. Provision of a heat sink such as cold air can be beneficial to preserve the quality of the food material by avoiding quality changes caused by temperature buildup. For liquid food treatment, annular pulsed UV-light system with lamp at the center with reflective inner surfaces will ensure maximum absorption of the energy.

In regard to pulsed light technology, National Advisory Committee on Microbiological Criteria for Foods (NACMF) recently published some of the future research needs for pulsed light (NACMF, 2006) as: (i) data on pulsed light effectiveness for specific commodities, (ii) comparison of resistance of specific pathogens, including bacteria, viruses, and parasites exposed to pulsed light, (iii) identification of critical process factors and their effect on microbial activation, (iv) optimization critical process factors and development of protocols to monitor critical factors, (v) suitability of the technology for solid foods and nonclear liquids, (vi) differences between pulsed light technology and UV (2,554 nm) light treatment, especially with respect to mechanism of inactivation.

In conclusion, extensive studies on pathogen inactivation have to be continued in order to identify the most resistant pathogen. An easier and faster way to determine the adequacy of the pulsed UV-light treatments by measuring absorbed UV-light energy has to be developed. Successful design of the pulsed UV-light lamp and the treatment chamber may result in better microbial inactivation.

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Chapter 19

Ultraviolet-C Light Processing of Liquid Food Products

J.A. Guerrero-Beltrán and G.V. Barbosa-Cánovas

1. Introduction

Emerging new technologies in food processing are being used more and more to treat foods and have the capacity to deliver the safe and high-quality products that consumers demand today. Ultraviolet (UV) light processing is one novel technology that has been used to process foods. In this technology, UV-C (“C” denotes UV type) light (wavelength 254 nm) is being used specifically as a disinfection method to inhibit or inactivate food-borne microorganisms in liquid food products. Generated by UV mercury lamps, UV-C light can have a germicidal effect on many types of microorganisms (bacteria, viruses, protozoa, molds, and yeasts) and enzymes. However, the effect of UV-C on microorganisms in liquids may depend on variables such as density of liquid, types of microorganisms, liquid type, UV-C absorptivity of liquid, and solids (suspended or soluble) in the liquid. On the other hand, enzyme inactivation may depend on the amount and types of enzymes in the liquid food and the UV-C dose.

A monochromatic UV light (wavelength 254 nm) can be obtained (Anonymous, 2007a) using two low-pressure mercury (LPM) germicidal lamps (Figure 19.1). Subsequently, the UV light region in the electromagnetic spectrum of the wavelength can be used as a radiation source to inactivate microorganisms in foods (liquids or solids). In general, wavelengths ranging from 100 to 400 nm can be used for UV light

processing (Figure 19.2) (Bintsis et al., 2000; Sastry et al., 2000 Anonymous, 2007b). Four types of UV light exist: UV-A, UV-B, UV-C, and UV-V; they are differentiated by wavelength range as well as other characteristics, and each acts in a different manner (Table 19.1).

Wavelengths between 220 and 300 nm (UV-C) have a germicidal effect and can effectively destroy microorganisms in many foods (Morgan, 1989; Sizer and Balasubramaniam, 1999; Bintsis et al., 2000). In this range, the maximum effect is best achieved between 250 and 270 nm. However, the effect decreases as wavelength increases (Bachmann, 1975). Therefore, a wavelength of 254 nm (generated by UV LPM lamps) can be used to disinfect many types of materials (Bintsis et al., 2000), including liquids and a variety of solid foods.

2. Effect of UV Light on Microbial Inactivation and DNA

The reduction of microorganisms with UV-C light can be achieved in one of the two ways: application at low intensity with long treatment times or application at high intensity with short treatment times (Bachmann, 1975; Morgan, 1989). Due to the many different types of microorganisms, species, and strains that can infect food, in this case liquid foods, the dosage required for sterilization or disinfection will depend on the type of food and initial load of microorganisms. For example, large microorganisms such as fungi and yeasts are more resistant to UV-C light; therefore, it is important to take into account the proper UV-C light dosage needed for disinfection

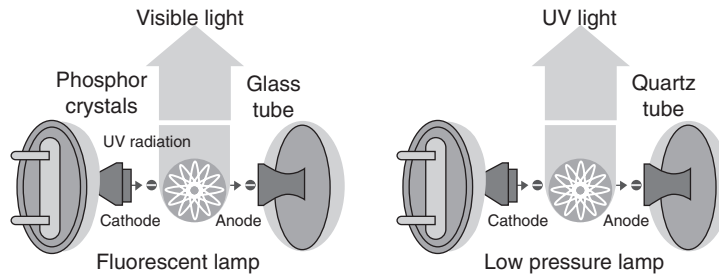


Figure 19.1. UV germicidal lamps. (Adapted from Anonymous, 2007a.)

(Bachmann, 1975) or sterilization. Bacteria suspended in water are also more resistant to being targeted with UV-C light than bacteria floating in the air (Bintsis et al., 2000), which is due to the penetration effect of UV-C light through different types of media with varied composition.

During UV treatment of food, UV-C light is absorbed by DNA in the cells, which in turn depletes the growth of cells and results in cell death (Liltved and Landfald, 2000). Microorganisms exposed to UV-C light are also affected at the DNA level (deoxyribonucleic acid). In this case, the compromised reproduction system of microorganisms leads to their death (Gardner and Shama, 2000); the DNA

bonds affected by the UV-C light split, which is due to the physical shifting of electrons that occurs (Anonymous, 2007a) with exposure. Additionally, UV-C radiation (i.e., UV-C light) can create a cross-linking between thymine and cytosine (pyrimidin bases) in the same DNA strand (Figure 19.3). For this reason, the main effect of UV-C radiation is in the reproduction system of microorganisms (Wright et al., 2000). Consequently, DNA transcription and replication are barren at this level. Cell functions are thus compromised and eventually cells may die (Snowball and Hornsey, 1988; Sastry et al., 2000). However, caution should be taken to avoid reactivation of UV-C compromised cells.

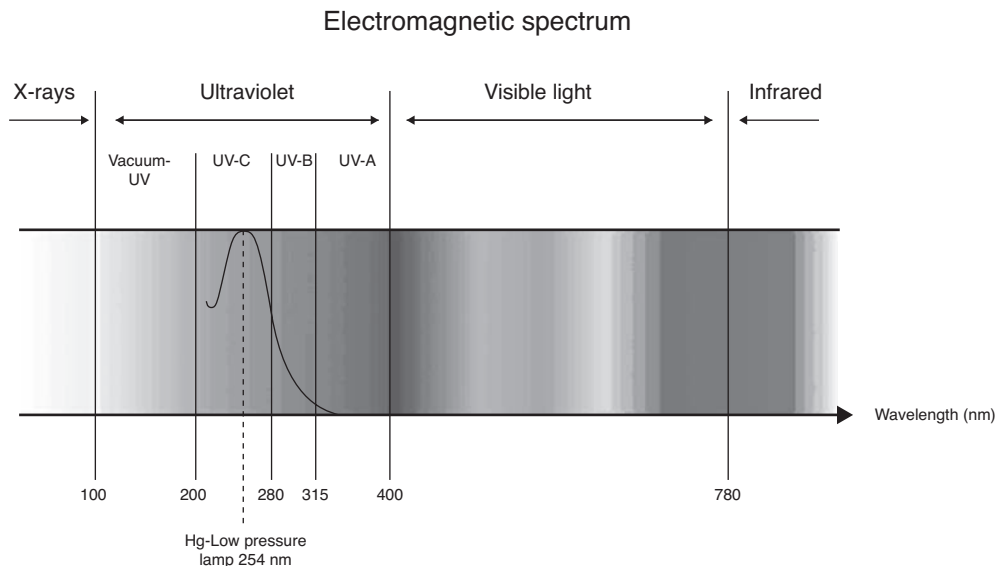
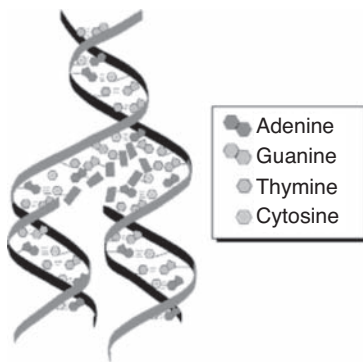


Figure 19.2. Electromagnetic spectrum. (Adapted from Anonymous, 2007b.) (For color details, please see color plate section.)

Table 19.1. Types of UV light available and wavelength ranges

Type	Wavelength	Range	Characteristics
UV-A	Long	320–400 nm	Causes skin changes (tanning)
UV-B	Medium	280–320 nm	Causes skin burning (cancer)
UV-C	Short	200–280 nm	Has germicidal properties (microorganisms)
UV-V		100–200 nm	Vacuum UV range

Photoreactivation of UV-C irradiated microorganisms in processed foods may be used to repair damaged DNA. The injured DNA in cells is repaired when exposed to UV light in the range 330–480 nm (UV-A). The reparation effect has been correlated with the exposure time to light intensity (Liltved and Landfald, 2000) since low intensity for short duration, for instance, does not injure the cells enough to inactivate their reproduction system. It has been observed that the reparation effect is due to protein factors, such as DNA repair genes (Yajima et al., 1995), where the split nucleic acid is repaired because of the activation of the photolyase, which monomerises the dimmer species obtained during UV-C radiation (Stevens et al., 1998). However, the reparation of DNA in UV-C treated cells can be neglected, depleted, or diminished if microbial cells are maintained in a dark environment where visible

**Figure 19.3.** UV-light effect at DNA level. (Adapted from Anonymous, 2007a.) (For color details, please see color plate section.)

light cannot penetrate (Stevens et al., 1998). On the other hand, UV-C processed foods must be stored in dark environments at low temperature to ensure conditions are similar to that of pasteurized products.

3. UV Light Penetration into Liquid Food Products

UV light processing is easy to use, and when used as a sterilization or disinfection method does not yield chemical residues. Besides being a process that is inexpensive, nonthermal, simple to use, and efficient in comparison to other sterilization processes (Bachmann, 1975), UV-C light does not generate radioactive products or gamma radiation (ionizing radiation) during processing. Nevertheless, UV-C light is effective only on food surfaces or in clear liquids. Thus, application is limited in use to sterilization of surfaces, due to its low penetration power (Morgan, 1989). Further, UV-C light is not effective on any type of material (including foods) stored in dark environments, or in the pores and orifices of the material (Bachmann, 1975). For that reason, every material, liquid or solid has to be tested based on its composition to determine the dosage needed. The color and nonsoluble solids content of the food system is important as well, since UV-C light transmission through the material improves if it is transparent or colorless. Therefore, the UV-C light source should be placed as close to the target as possible (Anonymous, 2002). This will yield better results in the disinfection or sterilization of the food system. However, UV-C light can be used to treat colored or turbid food liquids if adequate equipment is built. In this case, a very narrow film of liquid should be used, or else flowing of the liquid in a turbulent regime to ensure that all of the liquid is targeted equally by the UV-C light.

4. UV-C Light Uses

4.1. Liquid Food

The application of UV-C technology in processing of liquid food products is especially important, as shown in a study by Barbosa-Cánovas et al. (1998);

however, the UV light dosage is of paramount importance in delivering microbiologically safe liquid food products with good sensory characteristics. Research of UV-C light treated liquid food materials has shown that UV light penetrates into juices at an average of 1 mm and absorbs only 90% of the light (Sizer and Balasubramaniam, 1999). Wright et al. (2000) used an array of ten chambers to treat apple cider inoculated with five strains of *E. coli* O157:H7; they used selected flow rates and doses. However, reduction was only 3.8 log cycles (CFU/mL), which is not enough to achieve 5 log reductions as recommended by the FDA (1997). Guerrero-Beltrán and Barbosa-Cánovas (2006) treated mango nectar inoculated with *S. cerevisiae* at selected flow rates and doses; the maximum log reduction obtained was 2.94 (CFU/mL) after 30 minutes of UV-C light treatment at 451 mL/minute. Farid et al. (2001) treated orange juice flowing down on a small wall structure to form a thin film; they found that the shelf life of treated juice doubled without changes in color and flavor. Guerrero-Beltrán et al. (2006) treated grape, grapefruit, and cranberry juices inoculated with *S. cerevisiae* at selected flow rates and doses. They pointed out that the maximum log reduction of yeasts was 0.51, 2.42, and 2.39 (CFU/mL) for grape, grapefruit, and cranberry juices, respectively, after 30 minutes of UV-C light treatment at 1.02 L/minute. It can be seen from this information that the composition and color of the fruit juice is critical in the germicidal effect of UV-C light. On the other hand, as already stated by Hoyer (1998) and Sastry et al. (2000), to avoid photoreactivation of irradiated cells, the treated liquid food should be refrigerated and stored in dark packages or environments.

4.2. Water

UV-C light is commonly used to disinfect water (Bintsis et al., 2000). UV-C light technology has been used successfully for many years to obtain potable water that is free of microorganisms (Bachmann, 1975; Wright et al., 2000). Its application to water disinfection includes water for drinking, water for cooling towers in the chemical industry, water for production of pharmaceuticals, water for clean-

ing of microchips, and use in other industries where chemical-free water is used (Cruver, 1984).

Using UV-C light for water disinfection is quite advantageous as it does not change the color, odor, flavor, or pH of water (Anonymous, 2002). For instance, in the brewery industry, UV-C light treated water is required to ensure a final product without altered taste (Morgan, 1989). Further, UV-C light could potentially treat water waste and the water used to clean tubing systems in the food industry, instead of using chlorinated water (Liltved and Landfald, 2000), which can leave residuals that confer flavor to foods processed in such systems.

4.3. Surfaces

UV-C light has been used as a disinfection method for treatment of many types of materials in addition to liquids. For instance, the pharmaceutical, electronic, and aquaculture industries use UV-C light as a disinfection medium to deliver nonpolluted media (Anonymous, 2002). It has also been used to sterilize surfaces of nonbiological materials, such as packaging materials (Shama, 1999), and materials not resistant to heat (Bachmann, 1975), such as plastic pouches, bottles, and caps (Bintsis et al., 2000).

4.4. Air

UV-C light has been used to clean air in buildings, hospitals (Morgan, 1989; Shama, 1999), and egg-hatching cabinets (Bailey et al., 1996) as a disinfection method. In doing that, no other toxic chemicals, such as chlorine or other cleanings, need to be spread to disinfect these delicate environments in which person or animals used to stay for long periods.

5. UV Light Equipment

A number of UV-C disinfection units have been designed, at laboratory level, to treat liquid foods with nonpathogenic microbial loads. For optimal disinfection, the UV-C unit should deliver microbiologically safe liquid foods with good sensory characteristics; UV-C light should also reach all parts of the treated liquid food to ensure it has been treated

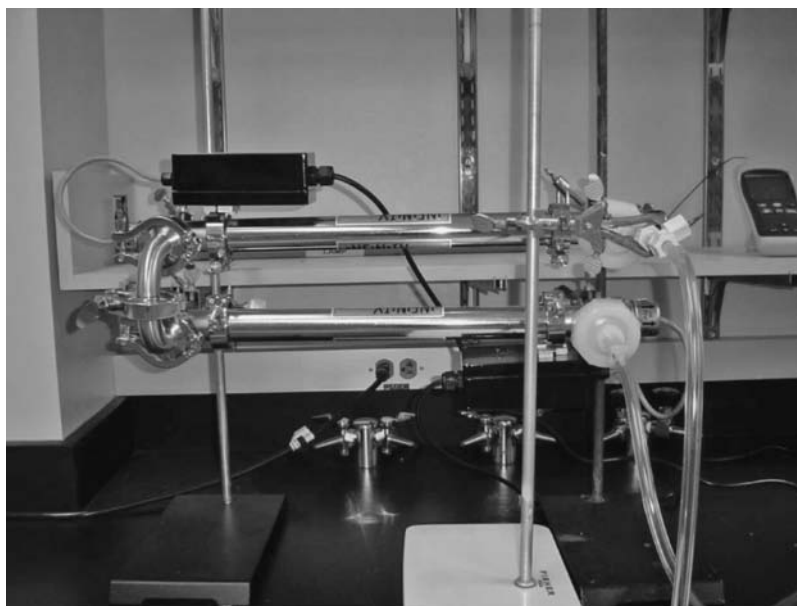


Figure 19.4. Two UV-C assembled systems by Guerrero-Beltrán, J.A. and Barbosa-Cánovas, G.V. (Washington State University). (For color details, please see color plate section.)

thoroughly. The easiest way to obtain a UV-C unit for liquid food treatment is to build one with simple materials; the unit can be constructed using a concentric tubing system made of sanitary food plastic or stainless steel tubing (the tubing holds the UV-C lamp), pumps, and refrigeration system (Guerrero-Beltrán and Barbosa-Cánovas, 2004). In this array, the liquid passes through the annular part of the tubing and is recirculated as many times as needed to obtain a germicidal effect, delivering a safe UV-C treated liquid food product. Other types of UV-C units are reported elsewhere. Examples include the UV-C arrangements reported by Shama (1992) for disinfection of drinking water. Also, Shama (1992) and Shama et al. (1996) developed a thin film UV-C disinfection system containing a nozzle to deliver the liquid, which then forms a film in a bell shape that spreads downward from the upper part. One UV-C lamp is erected in the center of the system and five more lamps surround the liquid film flowing downward to be UV-C treated. Another approach has been reported in which a tube in a coil arrangement is surrounded by UV-C mercury lamps, to deliver the light and treat the liquid flowing through the tube

(Anonymous, 1999). Thus, it can be seen that any UV-C unit is intended to treat liquid foods with a nonpathogenic microbial load.

Another type of system, such as the dual system depicted in Figure 19.4, also can be assembled to treat liquid foods. A compartment or an UV-C lamp holder made of quartz exists inside each of two single UV-C systems with one system attached to another; alternatively, a bank or a complex UV-C array can be built to treat liquid foods, avoiding recirculation. Thus, using any of the above methods, a UV-C treated liquid food product can be obtained and packaged in a sterile package. Nevertheless, it is mandated by the FDA that UV-C treated food be packaged in a dark environment and stored in a low temperature system to avoid reactivation of partially injured nonpathogenic microorganisms, since the UV-C process is considered to be a method of pasteurization not sterilization. Since UV-C treatment can increase the temperature of the liquid during circulation, the liquid food should be fed into the system at low temperature at the beginning of the UV-C unit operation, and lowered when the liquid passes from one system to the next.

6. UV Light Variables in Processing Liquid Foods

The effect of UV-C radiation on microorganisms depends on a number of variables:

- *Flow behavior and flow rate:* Since UV-C light does not penetrate deeply into liquids, unless the liquid is transparent and colorless, a turbulent flow behavior needs to be created; alternately, a very narrow film of liquid inside the treatment system can be generated (Anonymous, 1999; Shama, 1999). Flow rate on the other hand is important, as low flow rates are recommended to ensure an adequate UV-C germicidal effect (Liltved and Landfald, 2000).
- *Type of liquid and UV-C absorptivity:* The penetration of UV-C light depends on type and absorptivity of the liquid, which in turn depends on color, transparency, and type and amount of soluble and/or suspended solids (Shama, 1999; Bintsis et al., 2000). Therefore, in UV-C treatment of transparent and clear liquids less dosage could be required to obtain microbiologically safe liquid food products. On the contrary, thick liquids or liquids containing suspended solids may need more doses for adequate UV-C treatment (Guerrero-Beltrán et al., 2006). The germicidal effect is commonly reduced in the case of unclear liquids. Salts of calcium, magnesium, iron, and manganese are a disadvantage in the process since they can block the absorptivity of a liquid that has been UV-C treated (Snowball and Hornsey, 1988).
- *Density and type of microorganism:* Besides color and UV-C light blocking materials in liquids, the type of microorganism targeted is important during UV-C light processing. Even though large microorganisms, such as yeasts and molds, are more likely to be reached by UV light, they are less sensitive to radiation due to their size and shape. Thus, more doses are required to inactivate large microorganisms and to deliver a stable and microbiologically safe liquid product.
- *Geometric configuration:* The geometric configuration of the UV-C unit is also important. The unit needs to be designed to generate either of two pos-

sible situations as required: (1) forming of a very thin film and/or (2) creating of a turbulent flow to ensure that all the liquid is treated uniformly, and as a result, all microorganisms are reached by the germicidal light.

- *UV-C mercury lamps:* The intensity of UV-C light may decrease during UV light processing. Therefore, the intensity of the light source should be monitored frequently to ensure that the desired dose has been applied during processing to obtain a germicidal effect. Therefore, the length of time using the lamp should be recorded during operation. Measurement of the lamp's intensity should be done frequently with a dosimeter to ensure that the dosage required is delivered correctly by the lamp to the product.

7. Dosage Measurement

The ultraviolet light intensity emitted by mercury lamps may change during operation. UV-C lamps are useful for an average of 10,000 hours. This limited lifespan is the main reason why the UV-C lamp's intensity needs to be measured constantly, and this will ensure that the lamp delivers the correct dose during processing. For this purpose, UV sensors (called radiometers) and chemical actinometers can be used to address this problem. Radiometers can be either thermal or photonic and measure UV irradiance. On the other hand, actinometers can measure concentrations of products with well-characterized energies delivered from photochemical reactions. The concentration of those products is related to the UV light absorbed by the treated product (Shama, 1999). Also of paramount importance is the standardization of UV sensors to obtain repeatable measurements, as standardization is commonly obtained by actinometry (Sastry et al., 2000).

8. Modeling

8.1. UV-C Light Absorption in Liquids

Ultraviolet light is absorbed in liquids in different ways. The transmission of the intensity of a monochromatic UV-C light will depend on type of medium and content of suspended or soluble solid

Table 19.2. Coefficient of absorption of UV-C (254 nm) in liquid foods

Liquid Food	αd (cm ⁻¹)
Distilled water	0.007–0.01
Drinking water	0.02–0.1
Clear syrup	2–5
White wine	10
Red wine	30
Beer	10–20
Dark syrup	20–50
Milk	300

Adapted from Shama, 1999.

in the liquid. The Lambert–Beer law describes the attenuation or transmission of light through a liquid medium:

$$I = I_0 e^{-\alpha d} \quad (19.1)$$

where I is the attenuated intensity (W/m²), I_0 is the incident intensity (W/m²), d is the depth reached by the light (cm), and α is the absorption coefficient (cm⁻¹) of the liquid (Shama, 1999). Table 19.2 depicts the absorption coefficients for selected liquid foods (Shama, 1999). The absorption coefficient increases as solid content and turbidity increases.

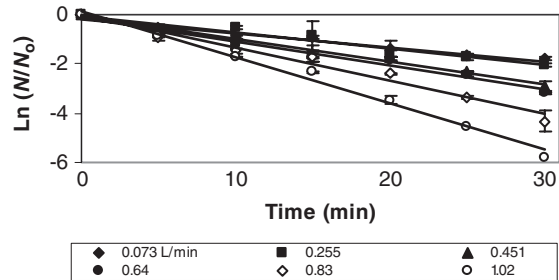
8.2. Dosage

The dosage used to inactivate microorganisms or enzymes depends on the UV-C exposure time and intensity. The UV-C dose is given as:

$$D = I_{254} \cdot t \quad (19.2)$$

where I_{254} is the UV intensity flux or irradiance, or dosage rate (W/m²), D is the dose or radiant exposure (J/m²), and t is the exposure time (seconds) (Chang et al., 1985; Morgan, 1989; Stevens et al., 1999; Bintsis et al., 2000). On the other hand, when a liquid is flowing inside UV-C tubing, the exposure time is defined as (Guerrero-Beltrán and Barbosa-Cánovas, 2004):

$$t = \frac{\text{Volume of chamber}}{\text{Flow rate}} \quad (19.3)$$

**Figure 19.5.** First-order kinetic representation for *Saccharomyces cerevisiae* inactivation in UV light treated cranberry juice. (Adapted from Guerrero-Beltrán et al., 2006.)

8.3. First Order Kinetics Modeling

The microbial and enzymic reduction in liquid food products can be modeled using a first-order kinetics model. Several authors have used the first-order kinetics model to plot the survival of microorganisms (or remaining amount of enzymes) as a function of doses:

$$\ln\left(\frac{N}{N_0}\right) = -kIt = -kD \quad (19.4)$$

where N is the survival microbial load (CFU/mL) or remaining enzyme activity units (EAU) after some exposure time, N_0 is the initial microbial load (CFU/mL) or initial enzyme activity units, and k is the inactivation constant rate (m²/J). The dose is also referred to as “fluence” (F) (Shama, 1999). Figure 19.5 depicts a first-order kinetics representation for *Saccharomyces cerevisiae* inactivation in UV light treated cranberry juice.

8.4. Decimal Reduction Dose

The decimal reduction rate is the dosage (or time) needed to inactivate 90% of microorganisms or enzyme activity at a given flow rate. This is expressed as follows:

$$D_{UV} = -\frac{2.303}{k} \quad (19.5)$$

where D_{UV} is the decimal reduction rate (J/m² or minute). Table 19.3 presents D_{UV} values for

Table 19.3. D_{UV} values for UV microbial and enzyme inactivation in fruit products

Flow Rate (L/minute)	D_{UV} (minute)				
	<i>S. cerevisiae</i> ^a	<i>L. innocua</i> ^a	<i>E. coli</i> ^a	<i>S. cerevisiae</i> ^b	PPO ^c
0.073	40.4	16.6	16.8	26.0	199.4
0.255	34.0	12.5	9.8	12.9	180.6
0.451	25.4	9.2	6.3	11.8	156.7

^aApple juice (Guerrero-Beltrán and Barbosa-Cánovas, 2005).^bMango nectar (Guerrero-Beltrán and Barbosa-Cánovas, 2006).^cPolyphenoloxidase (PPO) in mango nectar (Guerrero-Beltrán and Barbosa-Cánovas, 2006).

inactivation of selected microorganisms and polyphenoloxidase in fruit products.

8.5. Other Models

The first-order kinetics model is described by a simple straight line; however, some survival microbial curves may present a sigmoid shape or a curve with shoulders or a plateau region. These curves have to be modeled using more complicated mathematical models. The initial plateau segment indicates that the microbial load has been injured; the second segment indicates that the injured microorganisms have increased; and the last segment, or tailing region, represents the remaining survival microorganisms due to suspended or soluble solids impeding UV light passing through the material (Hoyer, 1998; Sastry et al., 2000). Examples of such are the Peleg model (Peleg, 1995) (Equation (19.6)), Weibull model (Gacula and Singh, 1984) (Equation (19.7)), Logistic model (Gutfreud, 1998) (Equation (19.8)), and Gompertz model (Gutfreud, 1998) (Equation (19.9)), among other more complex models. However, at present, the first-order kinetics modeling is the most common way to describe microbial or enzymic inactivation versus exposure time.

$$\log_{10} \left(\frac{N}{N_o} \right) = \frac{-t}{k_1 + k_2 t} \quad (19.6)$$

where k_1 and k_2 are constants. If $k_2 > 0$ the curve has an upward concavity, but if $k_2 < 0$ there is a

downward concavity, and a linear relationship when $k_2 = 0$.

$$\log_{10} \left(\frac{N}{N_o} \right) = -bt^n \quad (19.7)$$

where b and n are constants. If $n < 1$ the curve has an upward concavity, but if $n > 1$ there is a downward concavity, and a linear relationship when $n = 1$.

$$\log_{10} \left(\frac{N}{N_o} \right) = -\log \{1 + \exp(kt)\} \quad (19.8)$$

where k is a temperature-dependent coefficient.

$$\left(\frac{N}{N_o} \right) = -\exp(a + kt) \quad (19.9)$$

where k and a are constants.

9. Concluding Remarks

UV-C light processing is a novel technology that can be used to deliver microbiologically safe liquid food products with good sensory characteristics. However, much more research needs to be done regarding the type of liquid processed, the inactivation of microorganisms and/or enzymes, UV-C dosage, flow rates, and aromas, including flavor characteristics. Also, UV-C processed liquid foods such as liquid dairy products, vegetable juices, and fruit juices (including ciders), among other liquid foods, should be tested to assess the nutritional characteristics of the final product. Further research should include the use of low temperature for cooling liquid foods before entering through the UV-C processing equipment. UV-C unit arrays also have not yet been designed to process liquid foods at a low price compared to products obtained using conventional methods, such as thermal treatment or pasteurization. Finally, specially designed UV-C systems are required to ensure that the gap through which the liquid passes is as narrow as possible, permitting all of the liquid to be processed.

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Chapter 20

Nonthermal Plasma as a Novel Food Processing Technology[§]

Brendan A. Niemira and Alexander Gutsol

1. Introduction

With the increasing complexity of food products offered in the modern marketplace, and increasingly demanding standards for food safety and quality, the development and/or adaptation of effective technologies for nonthermal food processing are a priority. The industry standard of microbial inactivation for successful interventions, applied singly or in combinations, is a 5 log₁₀ (or 99.999%) reduction in microbial load. The specific requirements for microbial inactivation and cleanliness vary from commodity to commodity (Anonymous, 2003). In recent years, nonthermal plasma (NTP) has been increasingly investigated as a tool to sanitize delicate, heat-sensitive surfaces such as electronics, medical devices, and the soft tissues of mouth and other human tissues (Garate et al., 1998; Moisan et al., 2002; Fridman et al., 2005; Laroussi and Lu, 2005). These efforts have led to a number of different technologies to produce NTP, each with different advantages. The rapid advance of this field has prompted the interest of the food processing industry. Adaptation of mature technologies

from one application to another inevitably requires a reorientation of the technology, the difficulty and complexity of which is determined by how dissimilar the new application is from the original. It must be noted that even within the context of the biomedical and dental applications for which NTP was originally developed, the technology has only begun to mature into commercial-scale applications (Fridman et al., 2006); NTP food processing, although a subject of increasing scrutiny, is still experimental. The application of NTP to foods holds promise as a nonthermal sterilization treatment, but research data to address fundamental questions is still largely lacking. The bulk of published studies on how NTP inactivates microorganisms are related to nonfood applications (Laroussi et al., 2003); these data are instructive, but interpretation within the context of potential applications to food requires a measure of extrapolation. Where studies with foods have been conducted, NTP has been shown to be an effective antimicrobial process, with minimal impact on food sensory qualities under optimized treatment (Montenegro et al., 2002; Deng et al., 2005; Niemira et al., 2005a, 2005b).

This chapter will present an introduction to NTP as it pertains to food processing applications. This will include a brief primer on NTP terminology, a summary of the mechanisms by which NTP is created, and a description of how it interacts with treated substrates. Several of the most mature NTP technologies will be described and their potential advantages and disadvantages for food processing will be discussed. The data on antimicrobial efficacy of NTP will be reviewed along with the available data on

[§]Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the US Department of Agriculture.

reduction of microorganisms on NTP-treated foods. Finally, a brief economic analysis of NTP processing will be presented, along with key areas for future research.

2. Physical and Chemical Properties of Plasma

2.1. *What is a Plasma?*

As materials acquire energy, they change state, from solid (lowest energy) to liquid and then to gas. At extremely high energies, matter undergoes a further transformation into a plasma, the “fourth state” of matter. In the broadest sense, a plasma is an ionized gas consisting of neutral molecules, electrons, positive and negative ions, with the concentrations of each balanced such that the plasma is electrically neutral overall. It is necessary to apply significant energy to create plasma from normal gases or liquids. This energy can be imparted as heat, electricity, radiation, fast adiabatic compression (as in shock tubes or sonoluminescence), laser light, or by other means. This energy is retained by the plasma for some defined period of time, and the amount of energy a given volume of plasma contains is related to its chemical composition, density, and temperature. It should be noted that, from a technical standpoint, the different components of the plasma (electrons, ions, and neutral molecules) can each have different temperatures, that is different energy levels. Charged particles within the plasma allow it to conduct electricity and make it possible to manipulate the plasma using electrical and magnetic fields (Fridman et al., 2005; Lieberman and Lichtenberg, 2005).

In extremely hot plasmas resulting from thermonuclear reactions, temperatures are so high (millions of Kelvins) that all atoms are completely ionized (so-called fully or completely ionized plasmas). Incompletely ionized plasmas are usually divided into two classes: thermal (“equilibrium”) and nonthermal (“nonequilibrium”) plasmas. In thermal plasmas (e.g., welding arcs, lightning), the temperature of all components is sufficiently high (about 1 eV $\approx$ 11,600 K) that all of the particles are in thermo-

dynamic equilibrium, and energy is not transferred among the particles. In contrast, in NTP (e.g., neon signs, plasma televisions), the average energy (temperature) of the electrons is much higher than that of heavy particles (molecules and ions) that make up the rest of the plasma. This means that the electrons can transfer some of their energy to the heavy particles with every collision, resulting in excitation of the heavy particle. These excited particles are very reactive, and emit light in the visible and/or UV range. The spectrum and the intensity of light emission depend on plasma energy and chemical composition.

From a practical standpoint, thermal plasma would be detrimental to the quality of food products after an extremely short exposure. Until recently, NTPs were realized at low pressures and very small scales (Lieberman and Lichtenberg, 2005). In order for a plasma to be of use in the context of food processing, it must not impart damaging levels of heat to the food being treated (nonthermal), and it must operate at or near one atmosphere. A number of different technologies have been developed to generate these NTPs, each with its own degree of applicability or adaptability to food processing. These technologies will be discussed in more detail later in the chapter, within the context of antimicrobial efficacy and physical effects on treated substrates.

2.2. *Terminology*

The nomenclature for NTPs found in the scientific literature is varied. In some cases, the plasma is referred to by the specific technology used to generate it (“gliding arc,” “plasma needle,” “plasma jet,” “resistive barrier discharge,” etc.), while other names are more generally descriptive, based on the characteristics of the plasma generated (“one atmosphere uniform glow discharge plasma,” “atmospheric plasma,” “ambient pressure nonthermal discharges,” “nonequilibrium atmospheric pressure plasmas,” etc.). The two features which distinguish NTP from other mature, industrially applied plasma technologies, is that they are (1) nonthermal and (2) operate near atmospheric pressure. In these two salient characteristics lies the potential of these

technologies for application to the specific needs of the food processing industry.

The term “cold plasma” has been recently used as a convenient descriptor (Moisan et al., 2001; Wang et al., 2003; Laroussi and Lu, 2005; Niemira et al., 2005a, 2005b) to distinguish these technologies from other plasmas (operating at hundreds or thousands of degrees above ambient). In practical use, however, within the context of food processing the term “cold” can engender misleading images of refrigeration requirements as a part of the plasma treatment. In order to foster clarity and consistency when discussing plasma technologies suitable for application to foods, the general term “nonthermal plasma” (NTP) will be used throughout this chapter to distinguish from extremely hot, thermal plasmas, and from low-pressure plasmas that are mostly used in micro-electronic industry and in light-emitting devices. It should be noted that, although NTPs operate at atmospheric pressure, the feed gas composition can be air, a pure gas such as He, Ar, N₂, etc., or a defined gas mix, depending on the application.

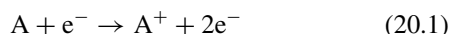
3. Plasma Physics—A Beginner’s Guide

In order to establish the applicability of NTP to foods and food processing, it is first necessary to briefly summarize the mechanisms by which plasmas are created and sustained, and how they interact with foods and food-borne microorganisms. Although a full description of the physics which govern plasmas, and the mathematics used to describe them, is beyond the scope of this chapter, these topics have been reviewed and discussed by Raizer (1991), Polak and Lebedev (1999), Fridman and Kennedy (2004) and Lieberman and Lichtenberg (2005).

There are two processes that occur during the creation of plasmas which give them their unique properties: (1) ionization and (2) recombination. In stationary plasmas, these processes are balanced. The first process dominates during plasma development (e.g., electrical breakdown of gas molecules). The second process dominates during plasma decay, when the ionization energy of plasma converts to heat, chemical energy, light, etc.

3.1. Ionization

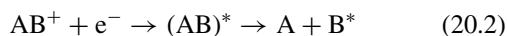
Ionization involves the separation of at least one electron from an atom or molecule, a very energy-intensive process. In NTPs, the major ionization mechanism is electron impact, when a high-energy electron, driven by the electric field of the NTP device, “knocks out” a valence electron from the molecule:



This process can happen only if the kinetic energy of the impacting electron is higher than the ionization potential. There are other ionization processes: (1) stepwise ionization by electron impact, when a high-energy electron “knocks out” an electron from electronically excited atoms or molecules; (2) ionization by collision of two heavy particles; and (3) photoionization, when a neutral atom absorbs a very energetic photon. These processes are discussed in detail by Fridman and Kennedy (2004).

3.2. Recombination

When electrons and positive ion come together, the resulting neutral molecule contains all of the ionization energy that was originally present. Unless the molecule simply ionizes again, this energy must be released in another process. One of the possible mechanisms is dissociative recombination:



During this process, an electronically excited intermediate molecule is briefly formed (AB)*, which rapidly reacts to form the neutral molecule A and the excited molecule B*. In this case, the net effect is to fracture the original molecule AB into smaller reactive chemical species. This has led to the application of NTPs in pollution mitigation (Sharma et al., 2000). As large organic molecules are exposed to the NTP, either as a gas flowing through the NTP emitter, or in a solution which is surface-treated with NTP, the target molecules are cracked by the reactive NTP.

Another common mechanism is radiative recombination:



This process is the opposite of photoionization. The energy imparted to the ion A^+ from the combination with the electron results in the formation of the unstable intermediate A^* . To release this energy, the intermediate releases a photon, which, depending on the energy levels involved, may be in the visible or UV range.

In addition to these two major processes (ionization and electron–ion recombination), there are other processes that are especially important in plasmas of different molecular gases and gas mixtures: electron attachment and detachment processes that result in the formation and destruction of negative ions, respectively; ion–ion recombination processes that result in mutual neutralization of positive and negative ions; electron, vibrational, and rotational excitation of molecules and atoms; different chemical reactions; surface recombination of charged particles and chemical radicals on the boundary of plasma. As with ionization, these processes are discussed in greater detail elsewhere (Fridman and Kennedy, 2004).

4. NTP Sterilization: Overview of Mechanisms and Technologies

A growing body of data is available on the antimicrobial efficacy of NTP, as generated and employed by the various technologies. Depending on the field of inquiry and the intent of the study, the subject of NTP treatment may be inert surfaces, pathogenic and spoilage microorganisms, or human tissues. These data are instructive in considering NTP treatment of foods, although not universally applicable. For each application, the general mechanisms of sanitization are defined by the physical and chemical nature of plasmas, rather than by the nature of the substrate.

4.1. Mechanisms of Microbial Inactivation

NTP inactivates microbes by three primary mechanisms (Moisan et al., 2002): (1) chemical interaction

of cell membranes with radicals (O , OH , etc.), excited or reactive molecules (O_2^* , O_3 , NO , etc.) or charged particles (electrons, and atomic or molecular ions); (2) erosion of cell membranes and cellular constituents by UV radiation; and (3) destruction of DNA by UV. The precise role and relative efficiency of each of these mechanisms is the subject of ongoing research, but it is generally accepted that a plasma which results in a combination of multiple mechanisms will have the greatest sanitizing efficacy (Moisan et al., 2002; Laroussi et al., 2003). UV radiation can be reabsorbed by the ambient gas surrounding the plasma; this very energetic UV that can propagate only through very low pressure gas (“vacuum UV”) was believed to be of more consequence than the less energetic UV that can propagate relatively far from atmospheric-pressure plasmas. Vacuum UV and active oxygen species were shown to have a synergistic effect for polymer etching (Ponomarev et al., 1989). Later, it was shown that the joint action of radicals and UV photons also facilitates bacterial inactivation (McDonald et al., 2000). The antimicrobial properties of UV radiation are well known (Laroussi et al., 2003); however, an understanding of the contribution that UV radiation could make to the overall antimicrobial efficacy of NTP in a food processing system awaits further investigation.

4.2. NTP Technologies

From the standpoint of the mechanisms involved in NTP interaction with the treated surfaces, NTP sterilization systems can be divided into three groups. NTP systems of the first (Table 20.1) group, remote treatment, generate plasma which is then moved by the flow of the feed gas onto the surface to be treated. One of the advantages of this type of system is that the surface to be treated can be physically separated from the electrodes used to generate the plasma, which simplifies the design and operation of the device. However, the chemical species with the highest chemical activity are also those that have the shortest living time, which means that this flow of “decaying” plasma (sometimes called an “after-glow”) exposes the surface predominantly to the flux

Table 20.1. Summary of factors relating to three general classes of NTP technologies

	NTP Technology Class		
	I. Remote Treatment	II. Direct Treatment	III. Electrode Contact
Nature of NTP applied	Decaying plasma (afterglow)—longer lived chemical species	Active plasma—short and long-lived species	Active plasma—all chemical species, including shortest lived and ion bombardment
NTP density and energy	Moderate density—target remote from electrodes. However, a larger volume of NTP can be generated using multiple electrodes	Higher density—target in the direct path of a flow of active NTP	Highest density—target within NTP generation field
Spacing of target from NTP-generating electrode	Approx. 5–20 cm; arcing (filamentous discharge) unlikely to contact target at any power setting	Approx. 1–5 cm; arcing can occur at higher power settings, can contact target	Approx. ≤ 1 cm; arcing can occur between electrodes and target at higher power settings
Electrical conduction through target	No	Not under normal operation, but possible during arcing	Yes, if target is used as an electrode OR if target between mounted electrodes is electrically conductive
Suitability for irregular surfaces	High—remote nature of NTP generation means maximum flexibility of application of NTP afterglow stream	Moderately high—NTP is conveyed to target in a directional manner, requiring either rotation of target or multiple NTP emitters	Moderately low—close spacing is required to maintain NTP uniformity. However, electrodes can be shaped to fit a defined, consistent surface.
Examples of technologies	Remote exposure reactor, plasma pencil	Gliding arc; plasma needle; microwave-induced plasma tube	Parallel plate reactor; needle-plate reactor; resistive barrier discharge; dielectric barrier discharge
References	Gadri et al., 2000 Laroussi and Lu, 2005 Montie et al., 2000	Lee et al., 2005 Niemira et al., 2005a, 2005b Sladek and Stoeffels, 2005 Stoffels et al., 2002	Deng et al., 2005 Kelly-Wintenberg et al., 1999 Laroussi et al., 2003 Montenegro et al., 2002 Niemira et al., 2005a, 2005b

of the lower activity, long-living chemical species (Gadri et al., 2000). The lower concentration of ions that exist in afterglow are known to generate active chemical species and UV photons during recombination, but their concentration is much lower than in “active” plasma (plasma supported by electric field) (Fridman and Kennedy, 2004).

NTP systems of the second group, direct treatment, are characterized by different types of discharges which periodically supply “active” plasma directly to the treated surface. These systems provide higher concentrations of active agents in pulsed mode, (short pulses, delivered hundreds or thousands

of times per second), theoretically resulting in higher antimicrobial efficacy. The level of UV radiation which reaches the treated surface is higher for these technologies than for those of the first group, as there is essentially no intervening normal atmosphere between the plasma and the surface. One of the technical challenges with “active” plasma systems is that food materials with a high moisture content can be made to conduct electricity at sufficiently high voltages. Extended exposures of sensitive food surfaces can result in electrical conduction through that surface, with the potential for damage, should the electrical conduction become concentrated in a single

point. These NTP systems are therefore somewhat more challenging to build and operate than those of the first group.

The third group of NTP systems places the surface to be sterilized in very close contact with one of the electrodes, and may act as an electrode itself. In these systems, microorganisms are exposed to the broadest combination of active antimicrobial agents, at the highest possible intensity. Even more than with NTP technologies of the second group, the positioning of the food surface to be treated must be carefully adjusted to avoid point discharges, which can cause damaging levels of heat to be concentrated at a single localized area. Although apparently the most promising of the types of NTP from an antimicrobial efficacy standpoint, these systems are among the most technically challenging. From a technical standpoint, it should be noted that an NTP system which uses the target *as one of the electrodes* should be distinguished from a system which has the target *between two mounted electrodes*. Both systems expose the target material to the most intense possible plasma density (free electrons, radicals, ions, and UV radiation), but the former also passes current through the target material, while the latter may not. Thus, from a strict technical standpoint, the latter system is more properly placed within the second group of NTP technologies. From a practical standpoint, however, in describing the systems later in this chapter, these will be considered as functionally equivalent. Systems which treat material placed between two electrodes are necessarily physically constrained by the spacing between them. This spacing is determined by the specific material chosen for the electrodes, the atmosphere, and the desired characteristics of the plasma (Gadri et al., 2000). Material to be sanitized must fit between the electrodes for the system to be functional, which limits the types of material this system can treat (Deng et al., 2005).

Each of these three general approaches (treatment with afterglow, treatment with active plasma, and treatment by electrode contact) have been evaluated within the contexts of more conventional applications of NTP, such as biomedical and dental applications (Chirokov et al., 2005; Laroussi and Lu, 2005; Fridman et al., 2006), to varying degrees of efficacy

and applicability. Ongoing research is establishing the criteria by which the general approach and the specific NTP technologies will be evaluated for suitability to any given food processing application.

5. Antimicrobial Efficacy

The ability of NTP to sanitize surfaces is related to a wide range of factors, the most significant of which are the power level used to generate the NTP, the volume and speed of the NTP that is applied, the position of the treated surface within the NTP field, the atmosphere used as a feed gas, and the nature of the NTP technology. In discussing the literature which describes the efficacy of the treatments, consideration of data from nonfood applications must necessarily be done with due care (Figure 20.1).

5.1. Remote Treatment ("afterglow") Technologies

One atmosphere uniform glow discharge plasma (OAUGDP) has been tested in a variety of configurations and applications (Montie et al., 2000). One version of this NTP technology (the Remote Exposure Reactor) uses a set of electrodes arranged in three or more plates to generate a relatively high volume of NTP, which is moved by the flow of the feed gas to the target material, some 20 cm downstream (Figure 20.1a). The power density in this system is approximately 0.44 W/cm² of plasma panel surface. *Escherichia coli* and *Staphylococcus aureus* were inoculated ($\sim 10^8$) on polypropylene and exposed to NTP in this device, using air as the feed gas. A treatment of 10 seconds reduced *E. coli* by approximately 4 log₁₀ CFU, but longer treatment up to 25 seconds did not cause further reductions. For *S. aureus*, the 10-second treatment caused a reduction of approximately 2 log₁₀ CFU, with a total reduction of approximately 3 log₁₀ CFU for the longest treatment time (25 seconds) (Figure 20.2).

This NTP system has the capability of recirculating the feed gas, which has been suggested to enhance the efficacy of the process overall (Gadri et al., 2000). When the downstream flow of gas was recirculated into the feed-gas intake, the plateau effect seen for

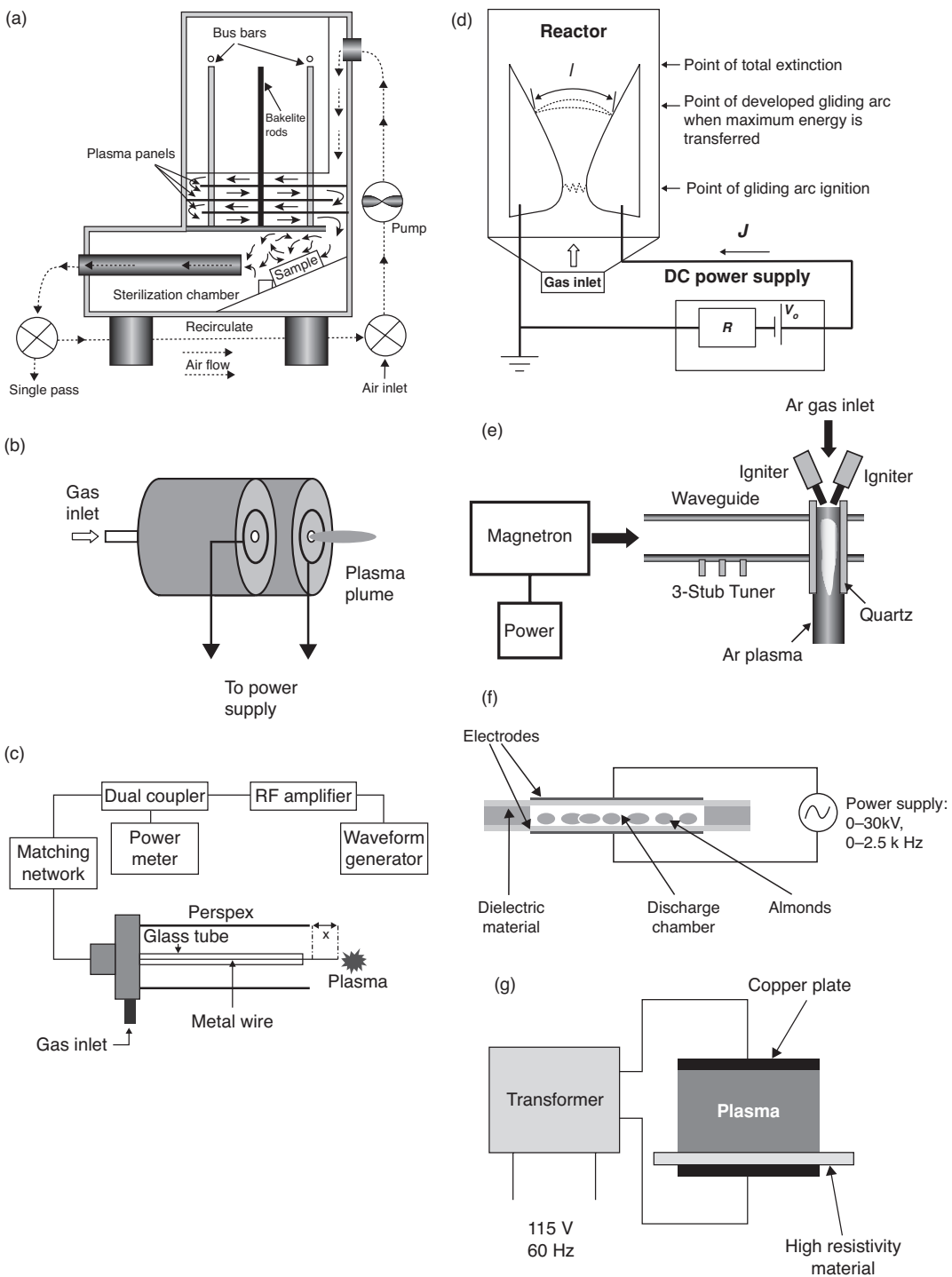


Figure 20.1. Illustrative diagrams of various NTP technologies. (a) remote exposure reactor (Gadri et al., 2000); (b) plasma pencil (Laroussi and Lu, 2005); (c) plasma needle (Sladek and Stoeffels, 2005); (d) gliding arc (Niemira et al., 2005a); (e) microwave plasma tube (Lee et al., 2005); (f) dielectric barrier discharge (Deng et al., 2005); (g) resistive barrier discharge (Laroussi et al., 2003). Figures are not to scale with respect to each other. (All images are used with the permission of the original publishers, who retain the respective copyrights thereto.)

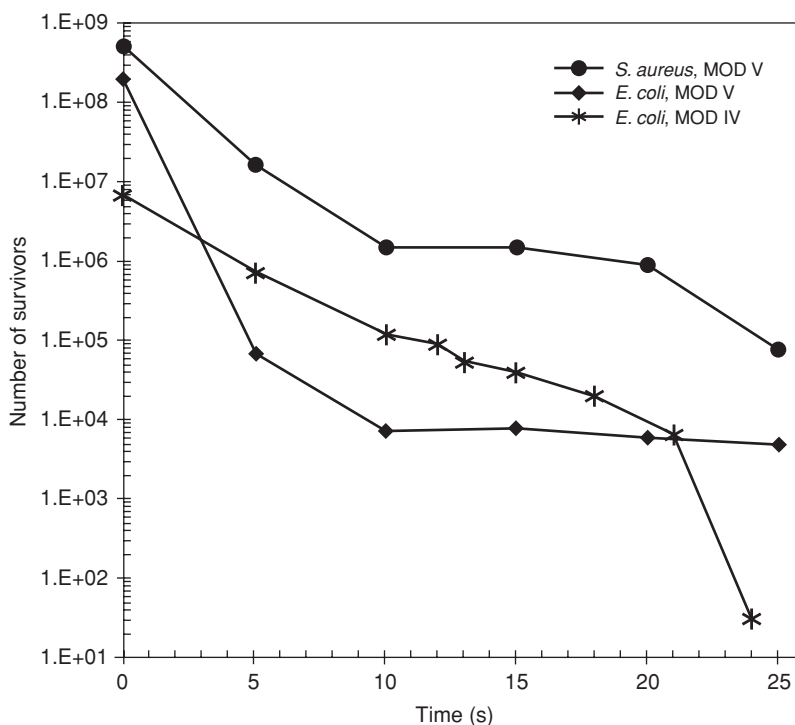


Figure 20.2. Survival of *E. coli* and *S. aureus* following treatment with the remote exposure reactor (Mod V) and the parallel plate reactor (Mod IV). (Figure © 2000 IEEE, reproduced from Montie et al. (2000), with permission.)

E. coli and *S. aureus* was reduced. The number of survivors for both bacteria was reduced as treatment time was extended. Reductions at the longest treatment (25 seconds) were approximately $5 \log_{10}$ CFU for *E. coli*, and approximately $4 \log_{10}$ CFU for *S. aureus* (Gadri et al., 2000).

The plasma plume (Laroussi and Lu, 2005) is based on thin, annular copper electrodes mounted on perforated glass disks 0.5–1 cm apart. The electrode supports are inside a nonconductive tube (called a “plasma pencil”) suitable for handheld use (Figure 20.1b). This system uses submicrosecond electrical pulses (6 kV, 3 kHz). The feed gas (He, approximately 4 L/minute) pushes the NTP away from the electrodes, creating a plume approximately 1 mm in diameter and 5 cm long. Because of the small size of the NTP emitter apparatus, the projection of the plume to a convenient working distance and its ability to operate for extended periods without heating

either the apparatus or the resultant plasma plume, the authors conclude that it has potential for dental and/or medical applications. Treatment times with this device are described as approximately 1 minute. Given the relatively narrow NTP produced with this device, application of this particular NTP technology to the needs of food processing would require multiple “plasma pencils,” emitting a series of NTP plumes.

5.2. Direct Treatment (“active plasma”) Technologies

The plasma needle (Stoffels et al., 2002) generates a 2–3 mm NTP at the tip of a fine, sharpened steel wire, which is coaxial within a grounded metal cylinder (1 cm diam.) (Figure 20.1c). The system is RF-driven (0.2–0.5 kV, 13.56 MHz) with a total power consumption of approximately 20 mW–3 W, depending

on the treatment parameters. Increased power levels caused an increase in the resultant NTP temperature. The feed gas is primarily He (approximately 0.3 L/minute); trials with gas mixtures of He with Ar, N₂, H₂, or air also tended to cause the temperature of the resultant NTP to increase. At optimized power levels, the resultant NTP is at room temperature and may be applied to sensitive tissues (e.g., dental structures, human skin) without discomfort. Treatment of *E. coli* cultures (Sladek and Stoeffels, 2005) using 180 mW at a distance of 1 mm showed a reduction from control of approximately 4.6 log₁₀ CFU after 10 seconds, and 5.1 log₁₀ CFU after 60 seconds. Treatments longer than 60 seconds did not result in additional inactivation at this power level and spacing. By increasing the power to 320 mW, the 60 seconds treatment (1 mm spacing) was slightly more effective (5.3 log₁₀ CFU reduction). As the space between the NTP emitter and the treated culture was increased, antimicrobial efficacy was reduced, until at 10-mm spacing, no reductions were observed at any power level tested. The authors observed that at higher power levels (>200 mW), the temperature of the plasma rises above 47°C.

Lee et al. (2005) treated cultures of *E. coli* (ATCC 8739) and methicillin-resistant *S. aureus* with NTP induced in Ar by a microwave system (1 kW, 2.45 GHz) (Figure 20.1e). Samples on glass slides were placed directly under the NTP outflow (Ar at 100 L/minute). The intensity of UV (254 nm) generated in this system ranged from 65 to 94 W/cm², measured at the point of treatment. The authors report sterilization of the samples (> 7 log₁₀ reduction) with a treatment as brief as 1 second. SEM images of treated bacteria show extensive cellular damage after 1 second, with complete disruption after 5 seconds of treatment (Figure 20.3).

A gliding arc generates NTP by dispersing electrical discharge plasmas to reduce their temperature to nonthermal levels (Figure 20.1d). A gliding arc NTP approximately 12 cm by 15 cm by 1 cm was applied to golden delicious apples inoculated with *Listeria innocua* (Niemira et al., 2005a). The 10 kV system operated at power levels from 50 to 450 mA, using air as the feed gas, at approximately 300 L/minute. The treatment was applied for various times up to

4 minutes. Treatments of 2 minutes at 115 mA and 4 minutes at 150 mA yielded reductions of 0.39 and 1.10 log₁₀ CFU/mL, respectively, with no damage to the apple initially, or during storage at 8°C for up to 35 days. These power levels caused no rise in temperature at the apple surface. In trials of higher power levels, application of 260 mA for 2 minutes resulted in a 1.58 log₁₀ CFU/mL reduction. Increasing power resulted in an increased propensity for filamentous discharge (Figure 20.4).

These concentrated electrical arcs within the plasma field caused heating and pinpoint damage to the apple surface. A 30-second treatment at 190 or 260 mA or 450 mA raised the apple surface temperature by a comparable amount (13°C or 11°C, respectively), while treatment with 450 mA raised the temperature by 30°C. The temperature increase was due to the filamentous arc discharges. Nonconducting spacers which raised the apples above the NTP apparatus by 1.5 cm served to partially, although not completely, mitigate this issue. When the lower power settings were retested with this arrangement, with the apple farther from the densest part of the NTP, the efficacy of the 4 minutes at a 150-mA treatment was reduced to a 0.55 log₁₀ reduction. Additional trials were performed at a range of power levels to explore the phenomenon of arcing, and to establish treatment parameters which either promoted or subdued this undesirable phenomenon. The authors concluded that the shape of the gliding arc NTP emitter could be further optimized to provide sanitizing efficacy of apples while avoiding a collapse of the NTP into filamentous discharges.

Using the same gliding arc NTP apparatus described above, Niemira et al. (2005b) treated cantaloupe and eggs inoculated with *E. coli* 25922. On cantaloupe, 260-mA gliding arc reduced the population by 1.0 or 1.3 log₁₀ CFU after treatment of 1 or 3 minutes, respectively. These treatments increased the temperature at the area of treatment by 17°C. No initial damage to the surface or internal tissue was evident at this treatment level, or after storage at 8°C for 6 days. The design of the apparatus placed the cantaloupe nearer to the top of the gliding arc electrodes, resulting in a noticeable level of filamentous discharge, even at low power levels (115 mA).

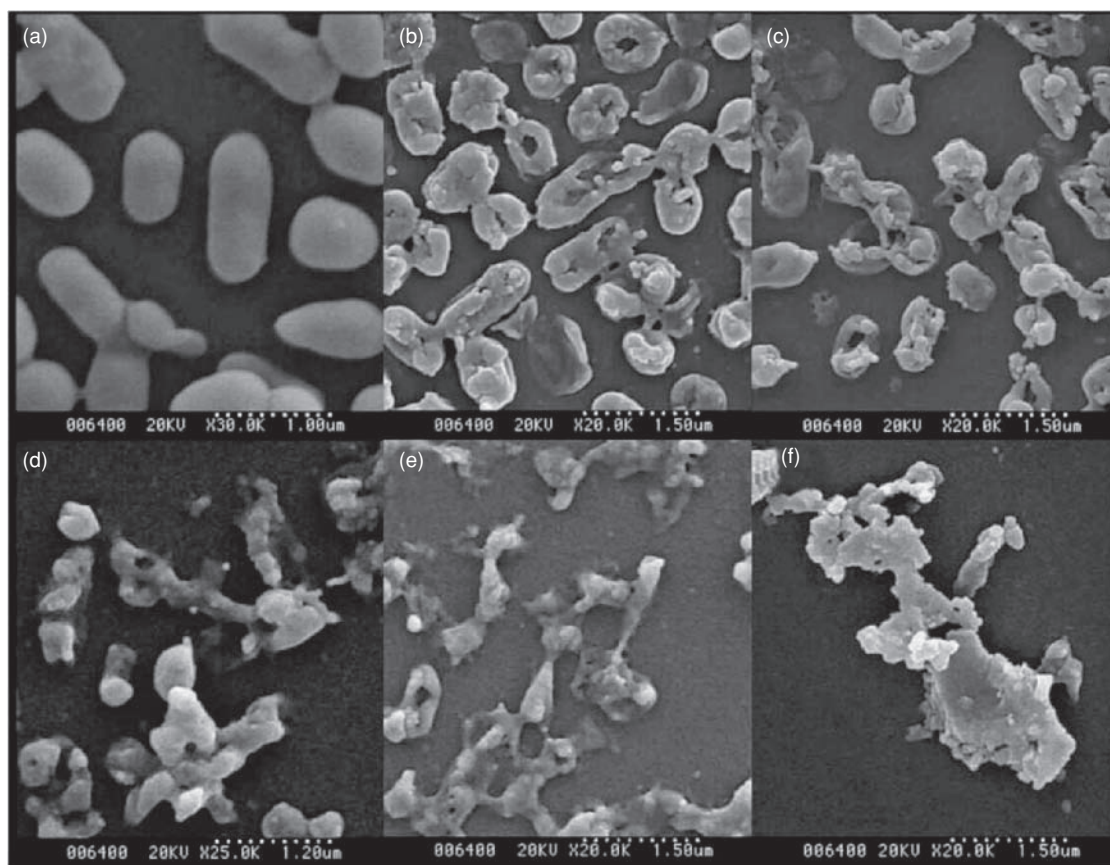


Figure 20.3. SEM photomicrographs of *E. coli*: untreated control (a) spores exposed to the microwave-induced argon NTP at atmospheric pressure for 1 (b), 2 (c), 3 (d), 4 (e) and 5 (f) seconds. (Image reproduced from Lee et al. (2005), with permission.)

However, the thicker skin and larger mass of the melon relative to apples made the impact of this inhomogeneous NTP field negligible.

On eggs, a gliding arc NTP treatment of 260 mA reduced the population by 0.4 or 1.0 $\log_{10}$ CFU after 1 or 2 minutes, respectively (Niemira et al., 2005b). The longer treatments increased the temperature at the area of treatment by 12°C. No damage to the egg surface or internal egg albumen was evident at this treatment level. The eggs, being smaller than the apples tested, were farther from the gliding arc electrodes during NTP treatment, and filamentous discharge was not observed until the power was set to the highest level (450 mA). These results

(Niemira et al., 2005a, 2005b) highlight the necessity for optimization, not only of the operational parameters of treatments, but of the physical design of the NTP apparatus with respect to the commodity being treated.

5.3. Electrode Contact Treatments

Using a modified version of the OAUGDP known as the Parallel Plate Reactor, a variety of microorganisms were treated between the electrodes (18 cm $\times$ 15 cm, variable gap spacing) of a RF-driven NTP (5 kV, 7 kHz) using air as the feed gas (Kelly-Wintenberg et al., 1999). Microorganisms were held

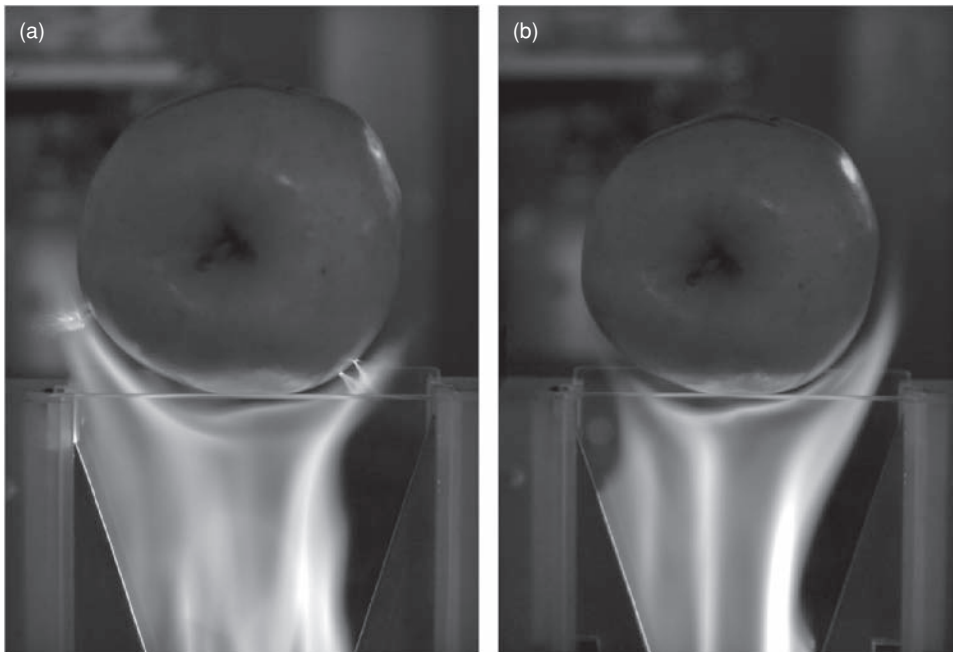


Figure 20.4. Gliding arc NTP treatment of Golden Delicious apples. NTP produced using air as feed gas (300 L/minute), operating at 10 kV. Treatments pictured are of power levels 450 mA (a) and 190 mA (b). (Image courtesy B.A. Niemira, US Department of Agriculture.) (For color details, please see color plate section.)

on several different substrates, and treated for various times to determine D values (Table 20.2).

NTP was able to sterilize the inoculated surfaces for all microorganisms and materials tested, with treatment times ranging from 30 seconds to 15 minutes. The researchers reported that the kill curves for the microorganisms tested were biphasic, with a distinct shoulder at the shorter treatment times. NTP was very effective against wet mounted bacteria on polypropylene, but required longer exposures to achieve the same kill levels when the inoculum was mounted on glass and allowed to dry. Bacteria embedded within agar plugs required still longer treatments for the same level of kill. Bacterial spores, yeasts, and viruses required longer treatment times than the vegetative bacterial cells.

Laroussi et al. (2003) summarized a series of NTP studies using a resistive barrier discharge to treat *E. coli* and *Bacillus subtilis* (Figure 20.1g). This system operates with an atmosphere of He at approxi-

mately 3 kV, with total power of 50–300 W, and a gap spacing of up to 5 cm, depending on the treatment parameters. This system was found to be effective in inactivating bacteria, with greater than 4 log₁₀ CFU reduction of vegetative cells of *B. subtilis* following a 10-minute treatment, with reduced efficacy in treating endospores. Electron microscopy of NTP-treated *E. coli* cells shows extensive damage to the cell membranes, a damage which was not apparent with NTP-treated *B. subtilis*. The authors concluded that while NTP-initiated cell lysis by membrane disruption is a major factor in the antimicrobial efficacy of NTP, nondestructive alterations in membrane permeability may be an equally important factor (Laroussi et al., 2003). From the standpoint of food processing, this finding raises the intriguing possibility of combining NTP treatment with a subsequent chemical-based antimicrobial intervention, introduced as a post-NTP application or as part of the product formulation.

Table 20.2. Response of microorganisms to exposure to one atmosphere uniform glow discharge plasma (OAugDP) in the parallel plate reactor (PPR) configuration

Microorganisms (concentration)	Surface ^a	Exposure Time	Log ₁₀ Reduction	<i>D</i> Values ^b
Bacteria				
<i>Escherichia coli</i> (7×10^6)	Polypropylene	30 seconds	$\geq 10^5$	<i>D</i> ₁ = 6 seconds <i>D</i> ₂ = 2 seconds
<i>E. coli</i> (1×10^6)	Glass	70 seconds	$\geq 10^5$	<i>D</i> ₁ = 33 seconds <i>D</i> ₂ = 10 seconds
<i>E. coli</i> (8×10^7)	Agar	5 minutes	$\geq 10^6$	<i>D</i> ₁ = 70 seconds <i>D</i> ₂ = 17 seconds
<i>Staphylococcus aureus</i> (1×10^7)	Polypropylene	60 seconds	$\geq 10^6$	<i>D</i> ₁ = 7 seconds <i>D</i> ₂ = 2 seconds
<i>S. aureus</i> (1×10^6)	Filter paper	30 seconds	$\geq 10^5$	ND ^c
<i>Pseudomonas aeruginosa</i> (2×10^7)	Polypropylene	30 seconds	$\geq 10^6$	ND ^c
Endospores				
<i>Bacillus pumillus</i> spores (1×10^6)	Paper strips	3 minutes	$\geq 10^5$	<i>D</i> ₁ = 1.8 minutes <i>D</i> ₂ = 12 seconds
<i>B. niger</i> spores (1×10^6)	Paper strips	5.5 minutes	$\geq 10^5$	<i>D</i> ₁ = 5.5 minutes <i>D</i> ₂ = 12 seconds
Yeast				
<i>Saccharomyces cerevisiae</i> (1.5×10^6)	Glass slides	5 minutes	$\geq 10^5$	<i>D</i> ₁ = 3 minutes <i>D</i> ₂ = 30 seconds
<i>Candida albicans</i> (1.5×10^6)	Glass slides	3.5 minutes	$\geq 10^5$	<i>D</i> ₁ = 2.1 minutes <i>D</i> ₂ = 30 seconds
Viruses				
Bacteriophage Phi X 174 (2.5×10^7)	Glass slides	15 minutes	$\geq 10^6$	<i>D</i> ₁ = 6.8 minutes <i>D</i> ₂ = 1.2 minutes

Reprinted from Kelly-Wintenberg et al., 1999. Used with permission.

^aPolypropylene was used to simulate microorganisms deposited on porous surfaces and glass slides were representative of nonporous surfaces.

^b*D* value is described as the time necessary to reduce the population of cells 1 log₁₀ or 90%. These values were determined from plots of the number of survivors versus time. Kill curves were biphasic in nature; *D* values are presented for the first (*D*₁) and subsequent (*D*₂) portions of the curves.

^cNot determined.

Montenegro et al. (2002) used electrodes in a needle-plate arrangement to generate an NTP with a conic distribution within a treatment chamber. Electrode spacing was 3 mm, and sample volume was 0.8 mL. The system was powered with a 150 kV DC, coupled to a high-speed switching system. Apple juice was inoculated with approximately 10^7 CFU/mL *E. coli* O157:H7 and was treated in contact with the electrodes under varying conditions of pulse number (100–4,000), pulse frequency (0–10,000

Hz), pulse width (100 ns–2 seconds), and peak voltage applied (0–15 kV). Using 9 kV, 100 Hz, the authors were able to obtain increasing log₁₀ reductions with increasing pulse numbers, up to sterility (>7 log₁₀) with 4,000 pulses. At this level, the most aggressive treatment, the temperature of the juice increased by only 2.9°C, affirming the nonthermal nature of the treatment. This treatment combination required less than 40 seconds to implement. Interestingly, when the pulse number was held at 4,000

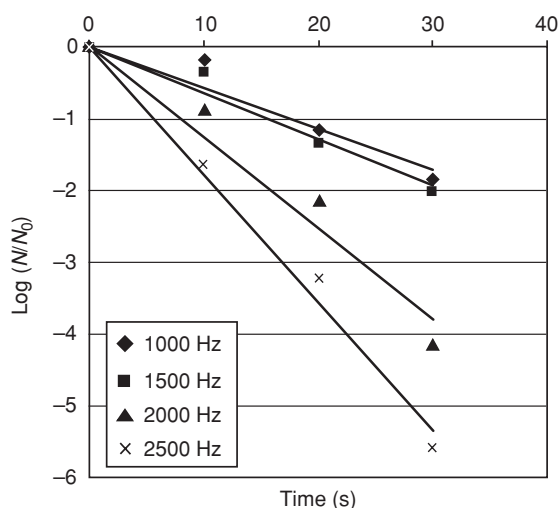


Figure 20.5. Survival of *E. coli* on almonds following treatment with dielectric barrier discharge NTP. Voltage fixed at 25 kV, frequency range of 1.0–2.5 kHz. (Image reproduced from Deng et al. (2005), with permission.)

(9 kV), and the pulse frequency was increased, the efficacy of the process decreased, with approximately 3 log₁₀ reductions at 200 Hz and only 1 log₁₀ reduction in the 400–1,000 Hz range. This response of *E. coli* O157:H7 in apple juice to the shortened pulse interval has yet to be explained.

Deng et al. (2005) treated almonds inoculated with *E. coli* 12,955 by placing them between the electrodes of a dielectric barrier discharge (DBD) apparatus, 20–30 kV, 1–2.5 kHz (Figure 20.1f). Studies were conducted in air. Reductions of approximately 4 log₁₀ CFU/g were obtained for treatments of 30 seconds at 25 kV, 2 kHz. Increasing the frequency to 2.5 kHz increased the reduction to approximately 5 log₁₀ CFU/g for the same 30 seconds of treatment (Figure 20.5).

The effect on the quality of the treated almonds was negligible. The authors concluded that NTP treatment for the sanitization of almonds using the DBD apparatus was a feasible approach.

Niemira et al. (2005b) treated Golden Delicious apples which were spot inoculated with *L. innocua*. Direct NTP discharge from a DBD apparatus (20 kV at 12 kHz) was applied to the food surfaces across

a separation distance of 2–3 mm. Activated electrode area was approximately 2 cm², and the power density was approximately 1 W/cm², applied for intervals up to 60 seconds. The study was conducted in air. Treatment for 60 seconds was the most effective as an antimicrobial treatment, resulting in a 2.0 log₁₀ reduction ($P < 0.05$). This treatment increased the temperature at the area of treatment by 9°C. The irregular apple surface resulted in filamentous discharges from the smooth DBD electrode. In treatments of 15 seconds and 30 seconds, these did not cause discoloration, but treatment for 60 seconds resulted in browning. During subsequent storage at 8°C for 10 days, the apples treated with 15 seconds or 30 seconds remained unblemished.

Cantaloupe melon were inoculated with *E. coli* ATCC 25922 and treated using the same direct DBD system as above (Niemira et al., 2005b). As with apple, filamentous discharges were evident. A treatment of 2 minutes reduced the population by 1.0 log₁₀ CFU and raised the temperature at the area of treatment by 6°C without causing noticeable damage to the melon surface. A 4-minute treatment increased the temperature by 26°C at the plasma contact point, but the relatively thick melon skin prevented appreciable sensory damage. As with apples, the interface of the flat DBD emitter with the irregular melon surface led to microdischarges within the plasma field. However, sensory damage to the melon surface was more limited than with apple.

Eggs were also inoculated with *E. coli* ATCC 25922 and treated with direct DBD as part of that study (Niemira et al., 2005b). A treatment of 15 seconds significantly ($P < 0.05$) reduced the population by 1.0 log₁₀ CFU. The longest treatment, 60 seconds, reduced the population by >2.0 log₁₀ CFU (below the detectable level). The 60-second treatment increased the temperature at the area of treatment by 6°C. During the course of this study, trials conducted with eggs positioned on a grounded plate resulted in charge conduction across the egg surface and subsequent point discharges to ground during treatment. These point discharges caused small pinhole burns at the base of the egg, resulting from the concentration

of electrical current and heat. In subsequent trials, the apparatus was modified such that eggs were positioned with a conductive diffuser mat interposed between the egg and the grounded plate, which eliminated the point discharges. Subsequent investigation of the inside of the shell following this modification showed no appreciable change in egg albumen.

5.4. General Considerations Regarding Feed Gas Composition

In testing an air-based DBD system, Deng et al. (2005) repeated the most effective treatments under conditions of defined feed gases, instead of air as in the initial trials. The researchers saw no effect of substituting N_2 , but processing under CO_2 or Ar reduced the efficacy of the process, yielding only a $\sim 2 \log_{10}$ reduction following a 30-second treatment. It has been suggested that an oxygen-free feed gas for the NTP would be desirable in some applications, as it precludes the possibility of ozone formation (Laroussi and Lu, 2005). Lassen et al. (2005) treated *Bacillus stearothermophilus* spores with NTPs derived from O_2 , Ar/ H_2 (50/50, 15/85, 25/75, and 9/95%), O_2/H_2 (50/50 and 95/5%) and O_2/CF_4 (88/12%) in an RF-plasma system, operating at 100 or 400 W (13.56 MHz). The most effective feed gas for spore inactivation was Ar/ H_2 , 15/85%, although O_2/CF_4 was seen to be more reactive with the inert substrate. Stoffels et al. (2002) showed that, where power levels were held constant, the choice of feed gas composition influenced the temperature of the resultant plasma. These results suggest that the best choice of feed gas/processing atmosphere is application-dependent, and must be optimized for individual commodities (Deng et al., 2005; Laroussi and Lu, 2005; Lassen et al., 2005; Stoffels et al., 2002).

One possibility which is currently being explored in NTP research is the possibility of introducing relatively high molecular weight compounds, such as volatile oils, to enhance antimicrobial efficacy of NTP (Babko-Malyi et al., 2002). In treatments of *E. coli* and *S. aureus*, Gaunt and Hughes (2004)

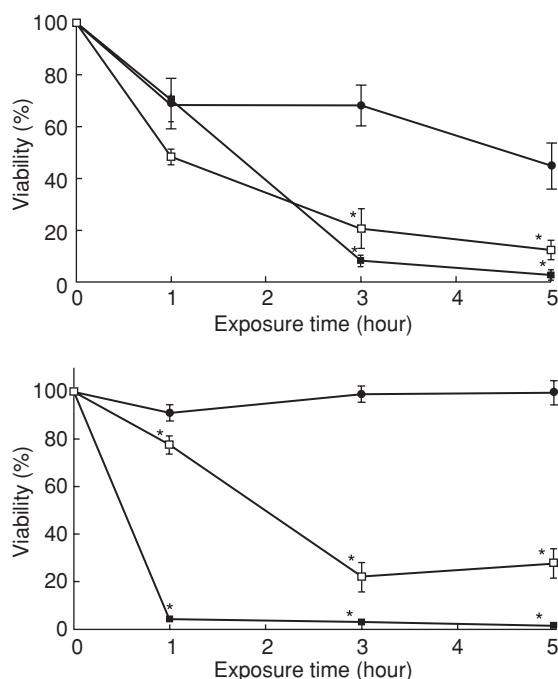


Figure 20.6. Survival of *E. coli* (top) and *S. aureus* (bottom) following exposure to volatilized essential oils. Plasma discharge treatments are nonamended control (●), β -pinene amended (■), or orange oil amended (□). Significant difference from control indicated by single asterisk (*). Error bars = SE, $n = 6$. (Image reproduced from Gaunt et al. (2005), with permission.)

showed that the antimicrobial efficacy of an NTP discharge was enhanced by the introduction of ethyl alcohol or cinnamon oil. In contrast, volatilized tea tree oil reduced the antimicrobial efficacy of the NTP. In a later study using a thermal plasma (Figure 20.6) discharge, Gaunt et al. (2005) treated *E. coli* and *S. aureus* with volatilized β -pinene and orange oil. Both volatilized compounds were shown to be effective in reducing the viability of the bacteria following extended exposure, 1 hour in the case of *S. aureus*, 3 hours in the case of *E. coli*. Ionized products, rather than electrically neutral products, were shown to be the most effective component of the plasma discharge. These suggest that the introduction of antimicrobial chemical agents into the

NTP feed gas could be an important area of future research.

6. Plasma Treatment of Food Contact Surfaces: Materials Processing

Conventional plasmas have been used to improve the characteristics of a variety of materials, through vapor deposition of specific compounds, ablation, etching, and other processes. For the food industry, plasma treatment of food contact surfaces and packaging materials using advanced plasma systems may provide benefits for safety, quality, and profitability in food processing and at point-of-sale food distribution. It should be noted that, although researchers in this field of materials processing refer to “cold plasma,” this process of plasma treatment can be conducted at 150°C (Wang et al., 2003), well beyond the range of “nonthermal” as it is defined in this chapter. However, a brief overview of this field is warranted; additional information may be obtained via the following references, and the literature cited therein.

Treatment of stainless steel coupons with di(ethylene glycol) vinyl ether in an RF-driven plasma system resulted in the deposition of a poly(ethylene glycol)-like layer (Wang et al., 2003). This protective layer reduced the ability of *Listeria monocytogenes* to adhere and form biofilms. Related work by Dong et al. (2005) describes the antifouling properties of the poly(ethylene glycol)-like coating on plasma-treated stainless steel specifically within the context of food contact surfaces. These researchers observe that the plasma treatment system is suitable for application to polymers, rubber, ceramics, and other materials as well as steel. Jiang et al. (2004) used RF-driven plasma to deposit silver nanoparticles on silicone rubber, which were then inoculated with *L. monocytogenes*. After 12 hours, no viable cells were recoverable from the plasma-treated material, a reduction of 4.5 log₁₀ CFU relative to the untreated control. Plasma modification of food packaging polymers results in improved performance, durability and antimicrobial properties (Ozdemir et al., 1999).

7. Economic Analysis

Given the very early stage of development of NTP, estimates of the cost of implementation are necessarily based on preliminary data. The significant differences in technology and intended application between NTP and conventional thermal plasmas mean that one cannot meaningfully relate their expected capital costs and operational expenses. Deng et al. (2005) refers to the low equipment and operational cost of NTP processing as advantages of this technology, but does not provide an economic analysis with this assessment. Lee et al. (2005) described a microwave-induced NTP system as “low-cost and reliable,” but does not elaborate on this description. Adler et al. (1998) determined that low-temperature plasma sterilization was more cost-effective than chemical sterilization of delicate medical devices. Pilot- or commercial scale NTP food processing systems will necessarily present a higher level of capital cost than the laboratory bench-scale devices that have been developed to date. How capital costs will scale for application to food processing will be influenced by the specific NTP technology used, and the intended application of the NTP, although details remain unclear.

Operational costs specific to NTP equipment will be based on power consumption of the NTP emitters and associated equipment (power and control electronics, monitoring, cooling, etc.), the nature and flow rate of the gas used, wear and tear on electrodes, and other factors. Systems which use air as the feed gas will be less expensive than those which use a pure gas (Ar, He, etc.), or a defined gas mixture. Bench-scale systems have widely varying power consumptions, depending on the scale of the system and the NTP technology used. For air-based systems, a minimum voltage of approximately 3 kV is required to achieve ionization. Reported values for experimental systems range from ~15 W for a plasma plume approximately 0.1 cm in diameter by 5 cm long (Laroussi and Lu, 2005), ~500 W for a glow discharge reactor with a pair of 18 cm × 15 cm electrodes (270 cm², operating at “less than a few watts/cm²”) (Montie et al., 2000) to ~1 kW for a

gliding arc system producing a plasma discharge approximately 12 cm × 15 cm by 1 cm thick (Niemira et al., 2005a). Depending on the intended application, the potential exists for multiple plasma emitters, perhaps using several different NTP technologies in combination, to be deployed as part of a given food processing system, with consequently increased gas flow, power consumption, etc. Other ancillary costs such as installation, training, maintenance, etc., will be related to the novelty and complexity of the equipment, costs which typically decline as technology matures.

8. Key Areas for Future Research

The technology to produce NTP is developing rapidly, driven by the potential benefits of NTP in a variety of applications. From the standpoint of the food processing industry, a number of important questions must be addressed with NTP research to identify the most appropriate applications for this technology. Some of these key areas for future research are summarized below:

- Influence of complex surfaces, and the efficacy against protected or subsurface contaminants (e.g., chicken skin, apple stem and/or scar area, seed coats)
- NTP processing in high-moisture environments
- Applicability of “chemical-free” NTP to high-value commodities (e.g., organic foods)
- Volatile antimicrobial compounds within NTP feed gas
- Control of feed gas composition to control UV production, potential for utilization for antimicrobial efficacy, and sensory impact of NTP processing on foods
- Efficacy against biofilm-associated microorganisms
- Sensory impact of NTP on meats, seafood, or produce
- Potential for application to beverages or liquids
- Applicability of newer NTP technologies (e.g., microwave-driven, GHz-rate RF, magnetically guided gliding arc)
- Regulatory methods—approval of medical devices for food, confirmation of toxicological safety of NTP-treated foods, etc.
- Economics—scale-up, capital costs, commercial-scale throughput, etc.

9. Conclusions

NTPs, and the wide range of devices used to create them, represent a diverse, innovative and flexible group of rapidly evolving tools for sanitizing surfaces. This promising technology will continue to be investigated for potential use in food processing, to address important questions regarding antimicrobial efficacy, food sensory impact, and the technical issues of integration of NTP into food processing systems.

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Section III

Other Nonthermal Processes

Chapter 21

Basics of Ozone Sanitization and Food Applications

Ahmed E. Yousef, Mustafa Vurma, and Luis A. Rodriguez-Romo

1. Introduction

Interest in ozone use in food production and processing has been increasing steadily. This trend is driven by industry's need for potent antimicrobial agents and the news about recent successful implementations of this sanitizer. Ozone has been effectively used in the production of bottled drinking water. Additionally, many water treatment plants currently use ozone as a better alternative to chlorine. Processors of fresh-cut produce who are considering ozone use in their facility are encouraged by the positive experience of few small companies that have already integrated ozone into their production lines. Costs of implementing ozone in food processing are not excessively prohibitive and removal of excess sanitizer does not represent a disposal hurdle.

In spite of these successes, many food processors are carefully analyzing the economic benefits and risks associated with ozone implementation. Compared to other sanitizers, the gas has limited solubility in water and, thus, aqueous applications require efficient gas injection systems and closed treatment vessels. Careful monitoring of ozone dissolution and residues in processing water and the potential for off-gassing may add technical complexities to processing lines. Corrosiveness of ozone makes it difficult to use with old pieces of equipment (e.g., pumps) in an ozone-upgraded facility.

Interestingly, some of the ozone properties that are considered undesirable in a given application make

the sanitizer suitable for other applications. The limited solubility of ozone in water can be advantageous, and the gaseous state of the sanitizer may have beneficial implementations in food processing. Additionally, some of the drawbacks (e.g., corrosivity) correlate with the potency of ozone as an effective sanitizer. This chapter covers the science and technology of ozone sanitization. The authors emphasize the antimicrobial properties of ozone and potential uses to control spoilage and pathogenic microorganisms in food. Advantages and limitations of ozone use in food processing are also addressed.

2. Ozone Chemistry and Physics

Ozone is a triatomic molecule (O_3) and a very reactive form of oxygen. It is commonly produced in nature by interactions of molecular oxygen (O_2) with chemicals, electric discharges during lightning, or short ultraviolet (UV) radiation from the sun (Figure 21.1). These interactions cause rearrangements of atomic oxygen and the formation of the triatomic molecule of ozone. The gas has a characteristic pungent odor that is readily detectable by the human nose at concentrations as low as 0.02 mg/L. Gaseous ozone is colorless at low concentrations and has a bluish color at high concentrations (Rice et al., 1981).

In the stratosphere, small amounts of ozone (0.05 mg/L) are formed at 15–50 km of altitude by photochemical reactions involving the action of solar UV radiation (<240 nm) on molecular oxygen. The troposphere, at approximately 15 km of altitude, contains about 10% of atmospheric ozone. Longer

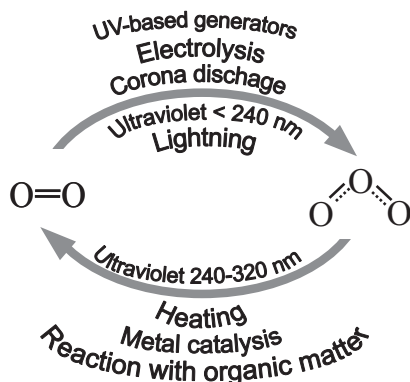


Figure 21.1. Formation and decomposition of ozone in nature or through industrial processes (O_2 , oxygen; O_3 , ozone; inner reactions occur in nature; outer reactions are industrially generated).

UV radiation (240–320 nm) decomposes ozone to oxygen (Figure 21.1). Although very low ozone concentrations are naturally present on the earth surface, higher ozone levels can be detected in urban areas as a result of reactions of atmospheric oxygen with pollutants (i.e., carbon monoxide, hydrocarbons, nitrogen oxides) released by combustion engines and industrial activity (Horváth et al., 1985; Kim et al., 1999; Kim et al., 2003).

Ozone has a molecular mass of 48 and consists of three oxygen atoms arranged in an obtuse angle (116.8°) with a molecular bond length ($1.278 \text{ \AA}$) slightly larger than that of a double oxygen bond ($1.207 \text{ \AA}$) (Mahapatra et al., 2005). Ozone gas can absorb infrared, visible, and UV radiation with maximum and minimum absorption in the UV range at approximately 260 nm and 200 nm, respectively (Horváth et al., 1985). Ozone can be frozen with boiling liquid hydrogen (-252.8°C at atmospheric pressure), forming blue to violet crystals with a melting point of -192.5°C under atmospheric pressure (101.3 kPa). Liquid ozone becomes a gas at -111.9°C (its boiling point) under the conditions just described. The density of gaseous ozone (2.14 g/L) is higher than that of air (1.28 g/L) at 0°C and 1 atmosphere (Horváth et al., 1985; Kim et al., 2003).

Ozone is the strongest oxidant currently available for food applications. It has an oxidation potential of

Table 21.1. Selected physical properties of ozone (Horváth et al., 1985; Wojtowicz, 2005)

Property	Value
Melting point	$-192.5 \pm 0.4^\circ\text{C}$
Boiling point	$-111.9 \pm 0.3^\circ\text{C}$
Heat of formation	142.7 kJ/mol
Critical temperature	-12.1°C
Critical pressure	5532.3 kPa
Density (gaseous phase) ^a	2.14 g/L

^aDetermined at 0°C and 1 atmosphere (101.3 kPa).

2.07 V, which is higher than that of hypochlorous acid or chlorine (Rice and Browning, 1980; Kim et al., 2003). A list of selected physical properties of ozone is shown in Table 21.1.

2.1. Ozone Solubility

Since many of ozone applications in the food industry involve aqueous ozone, solubility of the gas in water will be addressed in the section. Gaseous ozone is more soluble in water than oxygen and nitrogen, but it is less soluble than chlorine and carbon dioxide. Dissolution of ozone occurs according to Henry's law, which states that at a given temperature, the solubility of a gas is directly proportional to the pressure it exerts above the liquid (Horváth et al., 1985). Water temperature is probably the most important parameter affecting ozone solubility, and the dissolution of the gas increases at lower temperatures. The solubility of ozone in water can vary at atmospheric pressure from 1,130 mg/L at 0°C to 307 mg/L at 60°C (Figure 21.2) (Horváth et al., 1985; Bablon et al., 1991). For practicality reasons, solubility of ozone in water can be expressed as solubility ratio (S_r) using the formula:

$$S_r = \frac{\frac{\text{mg } O_3 \text{ in water phase}}{\text{water volume (L)}}}{\frac{\text{mg } O_3 \text{ in gaseous phase}}{\text{gaseous phase volume (L)}}}$$

A negative logarithmic relationship occurs between S_r and water temperature in the range of 0.5 – 43°C (Bablon et al., 1991).

The design of ozone–water contactors could considerably affect the solubilization rate of the gas. For

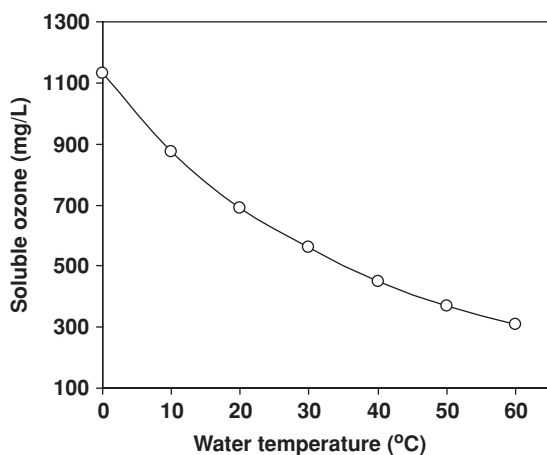


Figure 21.2. Solubility of gaseous ozone in water at atmospheric pressure (101.3 kPa) as a function of temperature. (Data from Horváth et al., 1985.)

example, high ozone dissolution can be achieved by injecting the gas into water and generating small bubbles (e.g., 1–3 mm diameter), in combination with appropriate gas mixing or turbulence (Katzenelson et al., 1974; Schultz and Bellamy, 2000). On the other hand, organic matter, metals, and other impurities in water can consume ozone or catalyze its decomposition and, therefore, decrease the apparent gas solubilization rate (Khadre et al., 2001). High pH enhances molecular ozone decomposition into hydroxyl radical and also interferes with the ozone solubility rate (Alder and Hill, 1950; Kim et al., 2003).

Solubility of ozone gas in water for food processing can be improved by (i) decreasing water temperature, (ii) increasing water purity, (iii) increasing ozone concentration delivered, (iv) increasing the pressure of the gas above the water, (v) decreasing the size of gas bubbles in the injection systems, (vi) improving gas mixing and distribution, and (vii) extending ozone residence time (Horváth et al., 1985; Khadre et al., 2001).

2.2. Ozone Stability

Ozone is more stable in the gaseous than in the aqueous phase (Stumm, 1958). It has been calculated that the theoretical half-life of ozone gas (1.5% wt/wt O_3 in O_2) at 25, 100, and 250°C is 19.3 years, 5.2 hours,

and 0.1 seconds, respectively. Pure mixtures of ozone in oxygen are stable at ambient temperatures in the absence of catalysts and light. However, ozone produced at industrial scale is less stable due to the presence of impurities. It is considered that the half-life of ozone gas at atmospheric pressure, dry conditions, and room temperature is approximately 12 hours, and its decomposition to oxygen depends on a series of factors that include temperature, light, organic matter, reactivity with surfaces (e.g., metals), concentration, and pressure (Rice and Browning, 1980; Bablon et al., 1991; Koike et al., 1998; Weavers and Wickramanayake, 2001). The relative stability of ozone gas makes it possible to generate it at one location of a facility and to pipe it some distances, without substantial loss (Rice and Browning, 1980). Explosion of ozone in the gas phase by shock wave may occur at $\geq 15\%$ wt/wt O_3 in O_2 at 25°C, and detonation may be enhanced by electrical sparks, heat, or intense light flash. However, in practice, the use of ozone gas at such concentrations is not common in food processing, and explosions are extremely rare (Guzel-Seydim et al., 2004b).

In the aqueous phase, the half-life of ozone could vary from seconds to hours depending on water quality and temperature (Weavers and Wickramanayake, 2001; Kim et al., 2003). For example, it is generally considered that the half-life of ozone in distilled water at 20°C is 20–30 minutes (Rice and Browning, 1980; Khadre et al., 2001). Kim (1998) reported that the half-life of ozone in deionized and tap waters at 25°C was 12 and 6 minutes, respectively.

The stability of aqueous ozone is influenced by the presence of ozone-demand material in water, as well as ozone concentration, temperature, pH, incidence of UV radiation, mechanical stirring, and the presence of metal ions and radical scavengers (Horváth et al., 1985; Weavers and Wickramanayake, 2001; Kim et al., 2003). The pH has a considerable effect on the stability of aqueous ozone. Kim (1998) indicated that the stability of ozone in solution decreased as the pH increased with highest stability at pH 5.0 and undetectable ozone levels at pH 9.0. In food processing, aqueous ozone stability is greatly influenced by the water source, and this factor must be considered while using ozone in processing plants

where water contains readily oxidizable organic and inorganic materials.

Ozone in aqueous phase continuously decomposes to oxygen in a stepwise mode with the generation of free radical species, which include hydroperoxyl ($\text{HO}_2^\cdot$), hydroxyl ($\cdot\text{OH}$), and superoxide anion ($\cdot\text{O}_2^-$) radicals (Hoigné and Bader, 1975; Grimes et al., 1983; Bablon et al., 1991). For example, the decomposition of 1 mole of aqueous ozone results in the generation of approximately 0.5 mole of $\cdot\text{OH}$ (Jans and Hoigné, 1998). The generated free radicals have a strong oxidizing power, a half-life of microseconds, and are responsible for the high reactivity of ozone (Kim et al., 2003). A schematic representation of the sequence of ozone decomposition reactions to oxygen and free radicals is shown in Figure 21.3. The relative instability of ozone, in gaseous and aqueous phases, prevents its storage for subsequent use. Therefore, ozone must be generated on-site and at the concentration required for particular food processing applications.

2.3. Ozone Reactivity

In the aqueous phase, molecular ozone acts as a dipole with electrophilic and nucleophilic properties, and reacts with organic and inorganic compounds (Khadre et al., 2001). As outlined in Figure

21.3, decomposition of ozone is a chain of reactions that includes initiation, promotion, and inhibition, where various compounds are involved (Staehelin and Hoigné, 1985; Jans and Hoigné, 1998). Free radicals such as superoxide anions ($\cdot\text{O}_2^-$) are formed during the initiation step with initiator compounds such as hydroxyl (OH^-) and hydroperoxide ions (HO_2^-), cations (e.g., Fe^{+2}), and organic substances (e.g., glyoxylic acid, formic acid). The UV radiation at 254.7 nm and hydrogen peroxide (H_2O_2) can also initiate the free radical formation process.

During the promotion step, superoxide and hydroperoxide radicals are regenerated from hydroxyl radicals (Figure 21.3). Organic and inorganic compounds such as formic acids, aryl groups, glyoxylic acid, primary alcohols, humic acids, and inorganic phosphate species are common promoters. The reaction rate of superoxide anion radical and ozone is very high; therefore, the radical can also act as a promoter.

The inhibition step involves the consumption of hydroxyl radicals without regeneration of the superoxide anion (Figure 21.3). Bicarbonate and carbonate ions, alkyl groups, tertiary alcohols, and humic compounds are common inhibitors (Staehelin and Hoigné, 1985). Chain ozone decomposition reactions are also blocked by antioxidants found in food by scavenging the free radicals during decomposition (Kim et al., 2003).

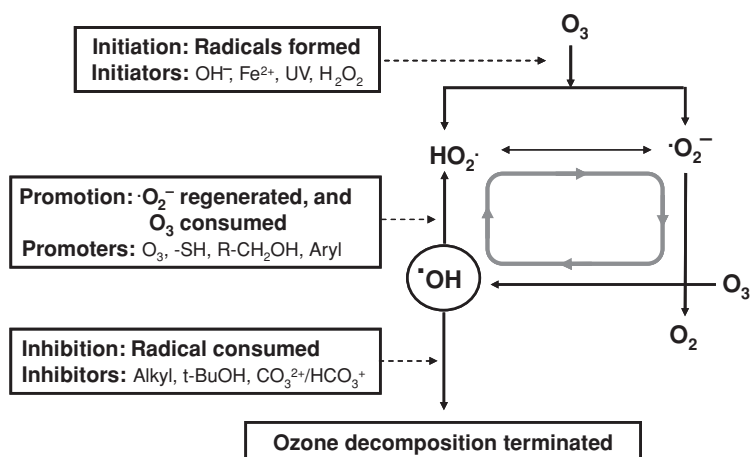


Figure 21.3. Ozone decomposition and free radical formation reactions (adapted from Khadre et al., 2001) (O_2 , oxygen; O_3 , ozone; $\cdot\text{OH}$, hydroxyl radical; $\cdot\text{O}_2^-$, superoxide anion radical; $\text{HO}_2^\cdot$, hydroperoxide radical).

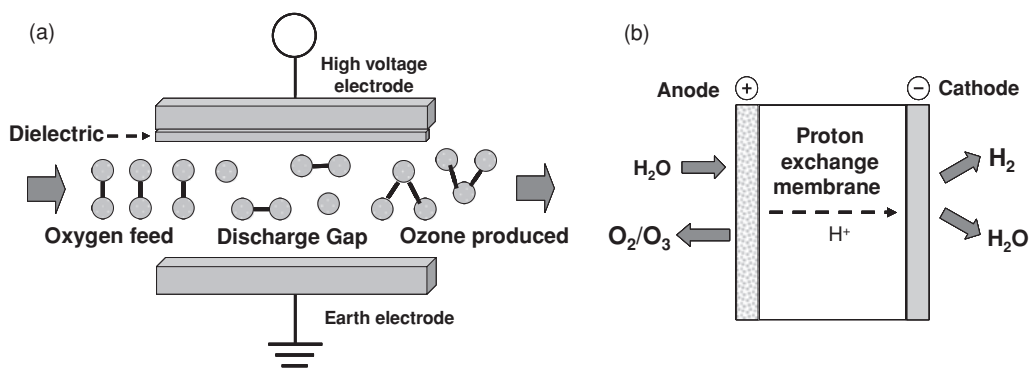


Figure 21.4. Ozone generation by corona discharge method (adapted from Rice et al., 1981) and electrolysis of water (Lynntech, 1998) (a, corona discharge; b, electrolysis).

3. Ozone Production

Ozone is usually generated for industrial applications at the point of use and in closed systems by electric discharge methods. Ozone can be produced by various other methods including chemical, photochemical, thermal, chemonuclear, electrolytic, and electrochemical procedures (Horváth et al., 1985). A low concentration of ozone (0.03 ppm) was produced by reaction of oxygen with 185-nm wavelength radiation in high-transmission UV lamps (Ewel, 1946). UV-based ozone generators are commercially available and these may be suitable for applications that require small quantities of ozone.

Electric discharge systems (corona discharge) are widely used for the industrial production of large amounts of ozone (Horváth et al., 1985). Production of ozone by corona discharge involves applying a high voltage between two electrodes separated by a dielectric material in the presence of oxygen or dry air (Figure 21.4a). High-voltage alternating current excites oxygen electrons and induces splitting of oxygen molecules into atoms, which combine with other oxygen molecules to produce ozone. The concentration of ozone produced in this system depends on the voltage, current, frequency, dielectric material, discharge gap, absolute pressure within the discharge gap, and the nature of the gas passing through the electrodes (Horváth et al., 1985). Efficiency of ozone production increases at low temperatures, and varies

from 1–3 to 2–6% by weight when air and oxygen are used as feed gases, respectively (Weavers and Wickramanayake, 2001). Corona discharge ozone generators that can produce up to 16% (wt/wt) ozone in oxygen gas mixture are currently available (<http://www.degremont-technologies.com/>).

Andrews and Murphy (2002) indicated that ozone could be generated efficiently by an electrochemical procedure. Electrochemical ozone generation involves electrolysis of water into hydrogen and oxygen atoms (Figure 21.4b). Hydrogen gas is vented from the gas–water mixture, and oxygen atoms are combined to produce ozone in an oxygen mix. Electrochemical generation is self-pressurized (≤ 20 psig), and ozone attains a concentration of 12–14% (w/w) in oxygen (Lynntech, 1998).

4. Ozone Measurement

4.1. Aqueous Phase

There are approximately 20 analytical procedures proposed to measure aqueous ozone using chemical, physical, and physicochemical methods (Horváth et al., 1985; Weavers and Wickramanayake, 2001). Reactivity, instability, volatility, and the effects of interfering substances with ozone should be considered when choosing a method to measure its concentration. Chemical methods are based on the quantification of products resulting from the reaction

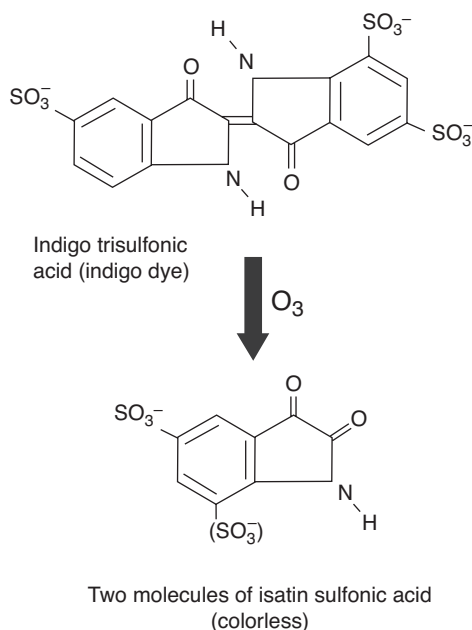


Figure 21.5. Reaction between ozone and indigo dye. (Modified from Bader and Hoigné, 1981.)

of ozone with an appropriate reagent. Oxidation of iodine solution by aqueous ozone has been used to analytically determine ozone concentration (Shechter, 1973). However, iodometric methods have variations in sensitivity and accuracy because they do not measure ozone alone, but total oxidants in solution (Alder and Hill, 1950). Bader and Hoigné (1981) developed the indigo method, a procedure based on the reaction of ozone with sulfonated indigo dye. In this method, ozone reacts with the carbon-carbon double bond of the dye, resulting in its decolorization (Figure 21.5). The change in color is determined spectrophotometrically (at 600 nm), and the measured absorbance is used to calculate ozone concentration with a minimum detection limit of 0.005 $\mu\text{g/mL}$. The indigo method permits less interference, due to other oxidant compounds, than iodometric procedures, and it is recommended as a standard to measure residual ozone (Gordon et al., 1988; APHA, 1995). Concentration of ozone in pure aqueous solutions may also be measured by a spectrophotometric method at wave length of 258 nm.

4.2. Gaseous Phase

Several methods have been proposed to measure gaseous ozone using iodometry, UV absorption, and chemiluminescence procedures (Weavers and Wickramanayake, 2001). However, only the UV spectrophotometric method was recommended to measure ozone in gas phase accurately (Gordon et al., 1988). Instruments that measure ozone concentration on the basis of UV absorption are widely used in various applications. In addition, instruments to measure ozone based on calorimetry and amperometric methods are commercially available (Khadre et al., 2001).

5. Antimicrobial Properties of Ozone

Ozone has strong antimicrobial activity against bacteria, fungi, protozoa, and spores from bacteria and fungi when these microorganisms are present in low ozone demand environments (Khadre et al., 2001). Ozone is also effective against most viruses tested. The mechanisms involved in microbial inactivation by ozone are attributed to its oxidation reactions with cellular components of microorganisms. Oxidation activity of ozone is either directly associated with its molecular form or its decomposition by-products, also known as reactive oxygen species, such as hydroxyl ($\cdot\text{OH}$), superoxide anions ($\cdot\text{O}_2^-$), and hydroperoxyl (HO_2^-) radicals (Kanofsky and Sima, 1991; Hunt and Marinas, 1997). Complex oxidation reactions occur against unsaturated lipids in the microbial cell envelope, intracellular enzymes, and genetic material (Khadre et al., 2001; Kim et al., 2003). According to Giese and Christenser (1954), the primary target of ozone is the microbial cell surface. Reactions between ozone and double bonds of unsaturated lipids in the cell envelope lead to leakage of cellular constituents and microbial lysis (Scott and Leshner, 1963). Komanapalli and Lau (1996) examined the effect of gaseous ozone at 600 mg/L on *Escherichia coli* K-12 for up to 30 minutes. The authors found that viability of *E. coli* K-12 decreased, membrane permeability was compromised, and intracellular proteins were degraded progressively when the ozonation time increased. Cellular damage of

ozone-treated microorganisms was also observed by electron microscopic analysis (Dave, 1999; Khadre and Yousef, 2001b). It was apparent that ozone caused damage to the membranes of gram-negative bacteria which resulted in loss of its cellular components. On the other hand, ozone caused less visible damage to the cell wall of gram-positives and intercellular damage was the main reason for ozone-mediated inactivation of these cells (Kim, 1998).

In addition to the damage to microbial cell envelope, ozone inactivates certain enzymes by oxidizing its sulfhydryl groups (Barron, 1954). According to Dillon et al. (1992), ozone may induce mutagenic effects on *Salmonella* Typhimurium, leading to cell injury or inactivation. Young and Setlow (2004) studied mechanisms of *Bacillus subtilis* spore inactivation and resistance to aqueous ozone. The authors found that DNA damage was not the reason for ozone inactivation of these sporeforming microorganisms. It has been suggested that ozone-mediated inactivation was due to the damage of *B. subtilis* spore inner membrane. Resistance to ozone was attributed to the spore coat.

5.1. Inactivation of Bacteria

Susceptibility of bacteria to ozone varies among genera and species (Table 21.2). In general, bacterial spores show greater resistance to ozone treatments than vegetative cells. Broadwater et al. (1973) studied the ozone inactivation of vegetative and spore forms of *Bacillus cereus*. The authors reported that 0.12 mg/L ozone decreased the population of *B. cereus* vegetative cells by 2 log units whereas 2.29 mg/L were needed to achieve the same inactivation level for the spores of this bacterium. In another study, Khadre and Yousef (2001b) tested the resistance of spores from eight *Bacillus* spp. to aqueous ozone and the authors reported that *B. stearothermophilus* was the most resistant whereas *B. cereus* was the most sensitive. It has been suggested that *B. stearothermophilus* spores may be used as an indicator for sanitization efficacy of ozone on various food-contact surfaces.

Contradictory results have been reported on the differences in resistance of gram-positive versus

gram-negative bacteria to ozone treatments. Kim and Yousef (2000) studied the inactivation kinetics of ozone treated gram-positive and gram-negative bacteria in a batch-type reaction system. According to these authors, ozone was very effective in inactivating *E. coli* O157:H7, *Pseudomonas fluorescens*, *Leuconostoc mesenteroides*, and *Listeria monocytogens* and among these microorganisms *E. coli* O157:H7 was the most resistant while *L. monocytogens* was the most sensitive. In another study, gram-negative bacteria were more sensitive to aqueous ozone than were the gram-positives (Restaino et al., 1995). Similarly, Sobsey (1989) reported that gram-positives were more resistant to ozone treatments than were the gram-negatives.

Environment where bacteria are present is also very important for the inactivation efficacy of ozone treatment. When *Salmonella* Enteritidis was treated with 1.5 ppm ozone in distilled water, the population of the pathogen decreased 6 log units (Kim et al., 1999). Only 1 log reduction in population of *Salmonella* Enteritidis was achieved when the pathogen was inoculated on poultry skin and treated with gaseous ozone at 8% (wt/wt in air) for 15 seconds (Ramirez et al., 1994).

Inactivation of *Shigella sonnei* by ozone was assessed when this pathogen was inoculated in water and shredded lettuce (Selma et al., 2007). Treatment of *S. sonnei* with 1.6 and 2.2 ppm aqueous ozone for 1 minute decreased the population of the pathogen by 3.7 and 5.6 log units, respectively. Inactivation of this pathogen on shredded lettuce was only 1.8 log units after treatment with 5 ppm of ozonated water for 5 minutes. Inactivation of *Yersinia enterocolitica* in water or on potato surface with ozone was evaluated (Selma et al., 2006). Aqueous ozone treatments at 1.4 and 1.9 ppm for 1 minute decreased *Y. enterocolitica* count in water (10^8 CFU/mL, initially) by 4.6 and 6.2 log units, respectively. When this pathogen was inoculated on potato surface ($\sim 2 \times 10^5$ CFU/g), the ozonated water treatment at 5 ppm for 1 minute decreased the population by 1.6 log only.

The sporicidal effect of ozone against *Bacillus* and *Clostridium* was enhanced when the spores were treated in an acidic medium (Foegeding, 1985). The

Table 21.2. Inactivation of bacteria by ozone

Bacteria	Inactivation (log ₁₀ CFU units)	Treatment Conditions			References
		Time (minutes)	Concentration (mg/L)	Medium/Food	
<i>Escherichia coli</i>	4.0	1.67	0.23–0.26	Water	Farooq and Akhlaque (1983)
<i>E. coli</i> O157:H7	~3.7	3	21–25	On apple surface	Achen and Yousef (2001)
	~0.6	3	21–25	In stem/calyx	
<i>Listeria monocytogenes</i>	0.7 to ~7.0	0.5	0.2 to 1.8	Water (pH at 5.9)	Kim and Yousef (2000)
<i>Shigella sonnei</i>	5.6	1	2.2	In water	Selma et al. (2007)
<i>S. sonnei</i>	1.8	5	5	In shredded lettuce	
<i>Yersinia enterocolitica</i>	4.6	1	1.4	Water	Selma et al. (2006)
	6.2	1	1.9	Water	
	1.6	1	5	Potato surface	Ramirez et al. (1994)
<i>Salmonella enteritidis</i>	1.0	0.25	8% (wt/wt)	Broiler carcass	
<i>Salmonella enteritidis</i>	0.6 to ~4.0	0.5	0.5 to 6.5	Water	Dave (1999)
<i>Salmonella typhimurium</i>	4.3	1.67	0.23–0.26	Water	Farooq and Akhlaque (1983)
<i>Bacillus cereus</i>	>2.0	5	0.12	Water	Broadwater et al. (1973)
<i>B. cereus</i> (spores)	>2.0	5	2.29	Water	
<i>B. cereus</i>	6.1	1	11	Spore suspension	Khadre and Yousef (2001b)
<i>B. stearothermophilus</i>	1.3	1	11	Aqueous ozone mix	
<i>Legionella pneumophila</i>	>4.5	20	0.32	Water	Edelstein et al. (1982)
Fecal streptococci	>2.0	19	2.2	Raw waste water	Joret et al. (1982)

degree of inactivation is also affected by the physiological status of treated bacterium. Cells in their exponential phase were more sensitive to ozone than cells in their stationary phase (da Silva et al., 1998; Kim et al., 2003).

5.2. Inactivation of Fungi

Ozone in aqueous and gaseous states is a potent antifungal agent and its fungicidal action varies among species (Table 21.3). Beuchat et al. (1999) investigated the susceptibility of conidia of aflatoxigenic aspergilli to ozone. *Aspergillus flavus* and *Aspergillus parasiticus* conidia were treated with 1.74 ppm ozone in phosphate buffer at pH 5.5 and 7.0. The authors reported that the *D*-values were 1.7

and 1.5 minutes for *A. flavus* and 2.1 and 1.7 minutes for *A. parasiticus* at pH 5.5 and 7.0, respectively.

The antimycotic effect of aqueous ozone against *Candida parapsilosis* was studied by Farooq and Akhlaque (1983). Treatment of *C. parapsilosis* with ozonated water at 0.23 to 0.26 mg/L for 1.67 minutes decreased the population of this microorganism by 2 log units. Kawamura et al. (1986) reported that the counts of *Candida tropicalis* decreased by 2 log units when the yeast cells were treated with aqueous ozone at 0.02 mg/L for 20 seconds or at 1 mg/L for 5 seconds.

In another study, ozonated water containing ~0.19 mg/L ozone instantaneously decreased *Cis-tus albanicus* and *Zygosaccharomyces bailii* populations by 4.5 log units, but the same concentration

Table 21.3. Inactivation of fungi by ozone

Fungi	Inactivation (log ₁₀ CFU units)	Treatment Conditions			References
		Time (minutes)	Concentration (mg/L)	Medium/Food	
<i>Aspergillus flavus</i> (conidia)	1.0	1.72	1.74	Buffer (pH 7.0)	Beuchat et al. (1999)
	1.0	1.54	1.74	Buffer (pH 5.5)	
<i>A. parasiticus</i> (conidia)	1.0	2.08	1.74	Buffer (pH 7.0)	
	1.0	1.71	1.74	Buffer (pH 5.5)	
<i>A. niger</i> (spores)	<1.0	5.0	0.188	Water	Restaino et al. (1995)
<i>Candida</i> <i>parapsilosis</i>	2.7	1.67	0.23–0.26	Water	Farooq and Akhlaque (1983)
<i>C. tropicalis</i>	2.0	0.30–0.08	0.02–1.0	Water	Kawamura et al. (1986)
<i>C. albicans</i>	>4.5	Immediate	0.188	Water	Restaino et al. (1995)
<i>Zygosaccharomyces</i> <i>bailii</i>	>4.5	Immediate	0.188	Water	Restaino et al. (1995)

decreased *Aspergillus niger* spores by <1 log after 5-minute treatment (Restaino et al., 1995). The fungicidal action of aqueous ozone, at 0.3–0.5 mg/L, was evaluated by Naitoh and Shiga (1982). According to these authors, the threshold values of fungicidal ozone activity against spores of *Aspergillus*, *Penicillium*, and *C. paraceticus* were 90–180, 45–60, and 5–10 minutes, respectively. Additionally, the antimicrobial efficacy of ozone increased against all the tested microorganisms with decreasing pH and temperature of treatment water.

Susceptibility of six different yeasts to gaseous ozone at different temperature and humidity conditions was investigated by Naitoh (1993). Cells of *Hansenula anomala*, *Saccharomyces rosei*, *Pichia farinose*, *C. parapsilosis*, *Kluyveromyces marxianus*, and *Debaryomyces hansenii* var. *hansenii* were treated with gaseous ozone (~5 ppm) for 1–5 hours at 30–60°C and 25–90% relative humidity. Populations of *C. parapsilosis* and *K. marxianus* decreased >1 log with ozone treatment for 5 hours at low temperature; however, this treatment did not affect the microbial counts of other tested yeasts. The author reported that the fungicidal effect of ozone was enhanced with increasing treatment temperature, humidity, and time. Mycelial growth of *Botrytis cinerea* was slower when this plant pathogen was inoculated on potato dextrose agar and stored at 2°C in

ozone-enriched (1.5 µg/L) environment (Nadas et al., 2003).

5.3. Inactivation of Protozoa

Protozoan parasites such as *Giardia*, *Cryptosporidium*, and *Cyclospora* have been implicated in a number of waterborne disease outbreaks worldwide (Clark et al., 2002; Erickson and Ortega, 2006). According to Clark et al. (2002), ozone is a more effective chemical disinfectant than chlorine or chlorine dioxide against protozoan parasites in water systems. Selected studies describing inactivation of protozoa by ozone are summarized in Table 21.4. Widmer et al. (2002) investigated the effect of ozone on *Giardia lamblia* cysts in gerbils using an infectivity assay and by scanning electron microscopy, immunoblotting, and flow cytometry techniques. Cysts were treated with ozone at 1.5 mg/L for 0, 30, 60, and 120 seconds. The authors reported that ozone exposure for 60 seconds or longer effectively inactivated cysts of *G. lamblia* and the treatments caused extensive protein degradation and profound structural modifications to the cyst wall.

Differences in resistance of cysts and oocysts of protozoan parasites to ozone treatments have been reported (Erickson and Ortega, 2006). According to Wickramanayake et al. (1984), cysts of *Naegleria*

Table 21.4. Inactivation of protozoa by ozone

Protozoa	Inactivation (log ₁₀ CFU units)	Treatment Conditions			References
		Time (minutes)	Concentration (mg/L)	Medium/Food	
<i>Giardia lamblia</i>	2.0	1.1	0.7	Water	Wickramanayake et al. (1984)
<i>G. lamblia</i>	>3.0	1.0	1.5	Water	Widmer et al. (2002)
<i>G. lamblia</i>	>3.0	2.0	1.7	Buffer (pH 6.85)	Finch et al. (1993)
	>4.0	5.0	1.9	Buffer (pH 6.85)	
<i>G. muris</i>	2.0	2.8	0.5	Water	Wickramanayake et al. (1984)
<i>G. muris</i>	~4.0	5.0	0.6	Buffer (pH 6.70)	Finch et al. (1993)
<i>Cryptosporidium parvum</i>	>1.0	5.0	1.0	Water	Korich et al. (1990)
<i>Naegleria gruberi</i>	2.0	2.1	2.0	Water	Wickramanayake et al. (1984)

gruberi were more resistant to ozone than were *Giardia muris* cysts. The author reported that ozone treatment at 0.2 mg/L for 7.5 minutes inactivated 2 log units whereas only 1.05 minutes was sufficient to achieve similar inactivation for the cysts of *G. muris*. The population of *Cryptosporidium parvum* oocysts decreased >1 log when the parasite was exposed to 1 mg/L ozone for 5 minutes (Korich et al., 1990). The researchers also reported that *C. parvum* oocysts were 30 times more resistant to ozone than were *Giardia* cysts when these parasites were treated under the same conditions.

5.4. Inactivation of Viruses

Ozone is an effective virucide. This is evident from results of selected studies on inactivation of viruses including bacteriophages and human/animal viruses as summarized in Table 21.5. Ozone at low concentration levels and short contact times is generally sufficient for inactivation of viruses when present in low ozone demand media. When the ozone demand of the medium is high (e.g., in wastewater), long contact time and high ozone concentration are required to inactivate viruses (Kim et al., 1999).

Variation in viruses susceptibility to ozone has been reported (Khadre et al., 2001). It appears that

bacteriophages such as f2, MS2 are the most susceptible viruses to ozone. Resistance of viruses to ozone was greater for hepatitis A than for poliovirus (Herbold et al., 1989). Susceptibility of human rotavirus to ozone was tested (Khadre and Yousef, 2002). A rotavirus suspension at high titer ($\sim 10^{11}$ TCID₅₀/mL) was treated with ozone at 5.2–25 mg/L for 1 minute. These treatments decreased the infectivity of these microorganisms by 2–8 log units TCID₅₀/mL. Poliovirus type 1 (Mahoney) was treated with 0.25 mg/L of ozone for 5 minutes and this exposure yielded 2-log inactivation (Harakeh and Butler, 1985). Farooq and Akhlaque (1983) reported 2.5-log reduction of poliovirus type 1 (Mahoney) when the virus suspension was ozonated at 0.23–0.26 mg/L for 1.67 minutes.

6. Ozone and Food Applications

6.1. Food Properties and Ozone Applicability

Reactivity, solubility, and disinfection efficacy of ozone are affected by many factors such as temperature, pH, humidity, and presence of ozone-demanding materials in the treated medium. In addition, microbicidal effect of ozone is highly dependent on its accessibility to target microorganisms without

Table 21.5. Inactivation of viruses by ozone

Viruses	Inactivation (log ₁₀ CFU units)	Treatment Conditions			References
		Time (minutes)	Concentration (mg/L)	Medium/Food	
Bacteriophage f2	2.0 to 7.0	0.08	0.09 to 0.8	Water	Kim et al. (1980)
Bacteriophage MS2	2.96	1.0	0.6	Phosphate buffer	Finch and Fairbairn (1991)
Bacteriophage MS2	>3.0	0.17	0.37		
Norwalk	>3.0	0.17	0.37		
Poliovirus type 1	>3.0	0.17	0.37	Water	Shin and Sobsey (2003)
Poliovirus type 1	2.5 to 3.0	1.67	0.23 to 0.26	Water	Farooq and Akhlaque (1983)
Poliovirus type 3	1.63	1.0	0.6	Phosphate buffer	Finch and Fairbairn (1991)
	~1.0	0.80	0.10		
Hepatitis A	2.7	0.02	0.25		
	>2.8%	-	0.38	Phosphate buffer	Herbold et al. (1989)
Hepatitis A	3.9	0.08	0.3 to 0.4	Phosphate buffer	Hall and Sobsey (1993)
Rotavirus human	3.0	6.0	0.1 to 0.3	Phosphate buffer	Vaughn et al. (1987)
Rotavirus SA 11 simian	3.0	6.0–8.0	0.1 to 0.25		
Rotavirus Wa human ATCC	Up to 1.0	1.0	2.1 to 4.2	Water	Khadre et al. (2001)
Rotavirus Wa human Wooster	Up to 5.0	1.0	1.9 to 15.9		
Coxsackie virus A9	>1.7	0.16	0.035	Water	Boyce et al. (1981)
Coxsackie virus B5	2.0	2.0	0.32	Sludge effluent	Harakeh and Butler (1985)
	4.0	2.5	0.40		

interacting with the food components (Figure 21.6). Microorganisms are generally not found in readily accessible location in food as they are in pure water. Microorganisms that are strongly attached, internalized, or organized as a biofilm on food surfaces or those embedded in the food matrix are not readily inactivated by ozone treatments. Achen and Yousef (2001) reported that washing with aqueous ozone was more effective in inactivating microorganisms on the surface of apples than in decontaminating the calyx and stem areas. The authors also pointed out that the ozone efficacy was reduced when *E. coli* O157:H7 was allowed to attach to the apple surface.

Readily available high-ozone-demand compounds may compete with microorganisms for ozone. High-fat-containing foods such as meat require higher ozone concentration than low-fat

foods such as fruits and vegetables (Kim et al., 2003). The ability of ozone to reduce microbial load in the presence of whipping cream, locust bean gum, soluble starch, and sodium caseinate was investigated (Guzel-Seydim et al., 2004a). The authors pointed out that the food components had influenced the bactericidal action of ozone against the treated microorganisms. Compared to control buffer, starch did not provide any protective effect against microbial inactivation of ozone while moderate or strong protective effects were observed in the presence of other tested components. Similarly, Restaino et al. (1995) reported that ozone inactivation of microorganisms was not affected by the presence of soluble starch in the treatment medium while addition of bovine serum albumin (BSA) reduced the microbial inactivation. Achen (2000) demonstrated

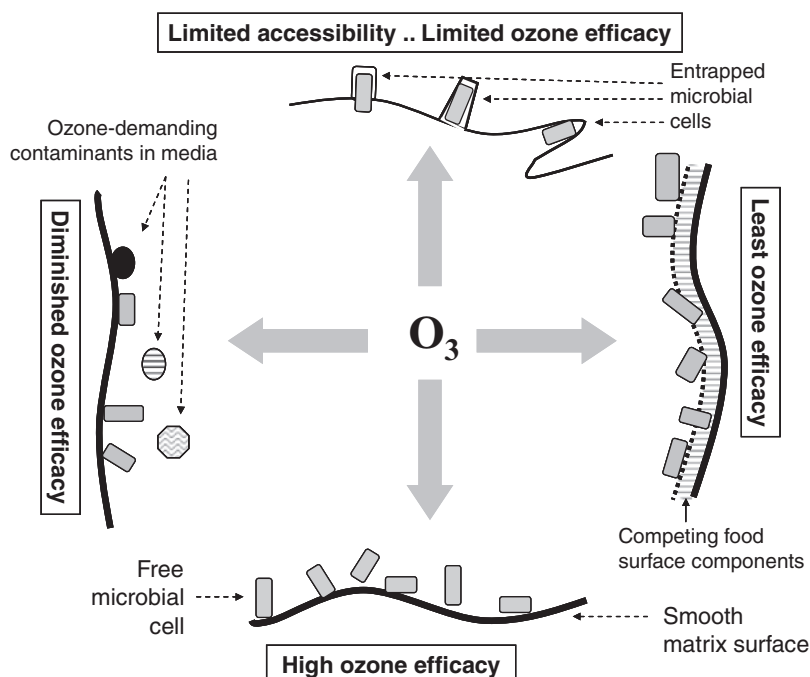


Figure 21.6. Schematic representation of accessibility of ozone to target microorganisms as related to the efficacy of the sanitizer.

that the inactivation of bacteria with ozone was dependent on the concentration of BSA added to the medium.

6.2. Ozone as an Alternative Sanitizer in Food Processing

Ozone was introduced as a disinfectant in the treatment of drinking water for the first time in 1893 at Oudshoorn, Netherlands (Rice et al., 1981). Subsequently, ozone was used for water disinfection in many European countries (Bryant et al., 1992). Ozone may be used in the gaseous or aqueous state in food processing. In general, gaseous ozone is applied for storage applications whereas the aqueous form is used for surface decontamination of foods, equipment, or packaging materials.

6.3. Ozone Treatment System

Ozone can be applied in gaseous or aqueous states for food applications. Sanitization of fresh or fresh-

cut vegetables is an example of processes that can make use of both forms of ozone. Figure 21.7 shows conceptual aqueous and gaseous ozone systems that may be applicable to fresh produce treatments. Essential components for an ozone treatment system for food applications include:

- A gas feed system
- An ozone generator with electrical power supply
- An ozone contactor for aqueous ozone applications, or a treatment vessel for gaseous ozone treatments
- Ozone measurement devices
- An ozone off-gas destruct system

High-purity oxygen or dry air can be used to generate ozone. Commonly, corona discharge generators are used to produce ozone for food applications, and these generators require a high-voltage electric supply units. Gaseous ozone should be dissolved into water for aqueous ozone treatments of foods. Transfer efficiency of ozone from gaseous state to liquid

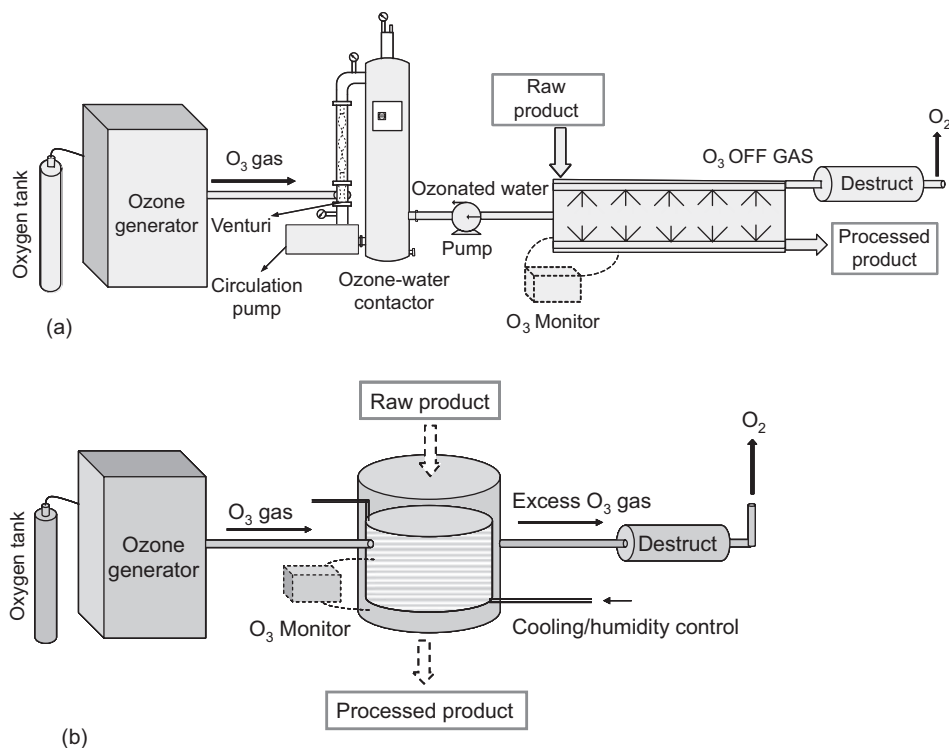


Figure 21.7. Conceptual (a) aqueous and (b) gaseous ozone treatment systems that may be relevant to food applications.

form is an important factor that could affect the process feasibility. There are several ozone dissolution methods for increasing transfer efficiency of this gas into water; these include conventional fine bubble diffusers, turbine mixers, injectors, packed columns, spray chambers, porous plate diffuser contactors, and submerged static radial turbine contactors (Bellamy et al., 1991). Ozone and the matrix (e.g., food) to be treated are brought together in a treatment vessel. This vessel should be leak proof and equipped with monitoring devices and an excess gas destruction unit. The treatment vessel should be designed to permit efficient contact between ozone and the matrix. Automatic control units can be used in conjunction with process flow meters and monitors to maintain the target ozone concentration in the process and control ozone generation. There are thermal and catalytic destruction units commercially available to convert excess ozone to oxygen prior to its release in the at-

mosphere. Ozone detectors should be employed in the working environment to routinely monitor the concentration of this gas for employees' safety. Concentration of ozone and time of exposure are critical parameters that determine ozone efficacy during the treatment. For water applications, efficacy of the treatment is commonly expressed as ozone concentration (mg/L) and contact time (minutes) that are sufficient to inactivate a given microbial population (e.g., 2 log decrease). The product of multiplication of these two parameters is termed *Ct* value.

Interaction of ozone with processing equipment and packaging material should be considered for efficacy of the treatment as well as the corrosion stability of the materials used. Ozone's corrosive effect is most pronounced at high concentrations commonly found inside the ozone generator or in the ozone-to-water contacting system. Materials most frequently used in the food processing industry are

resistant to ozone at moderate concentrations. The materials with high resistance to corrosion by ozone include austenitic (300 series) stainless steel, glass, PTFE (Teflon), hypalon, and concrete. In addition, plastics commonly used in food industry, such as polyvinylchloride (PVC) and polyethylene (PE), are generally resistant to low ozone concentrations. The use of copper alloys and natural rubber should be avoided because they are prone to oxidation and rapid disintegration, respectively. When designing and manufacturing a treatment system, all equipment materials, including the seals, gaskets, and lubricants that come into contact with this sanitizer should be selected from materials of high ozone resistance (Kim et al., 2003).

7. Selected Food Applications

Potential applications of ozone as an antimicrobial agent in food industry have been extensively studied (Kim et al., 1999; Khadre et al., 2001; Kim et al., 2003). Ozone has been tested on food products such as meat, poultry, fish, fruits and vegetables, and cheese. Other ozone applications tested include the decontamination of food packaging materials, food contact surfaces, and removal of residual pesticides on fruits. Ozone has the advantage of decomposing spontaneously to a nontoxic product, that is, O_2 .

7.1. Fruits and Vegetables

Fresh fruits and vegetables are susceptible to contamination with pathogenic and spoilage microorganisms, beginning with the preharvesting stage throughout postprocessing. Food-borne disease outbreaks linked to minimally processed fruits and vegetables have increased during the past few decades (Sivapalasingam et al., 2004). Microbial contamination of fresh fruits and vegetables not only poses significant risks to public health but also affects the industry financially by decreasing product shelf life.

Ozone has been explored for treating agricultural commodities because it provides more disinfecting power than other sanitizers (e.g., chlorine) and removes a myriad of contaminants including microorganisms resistant to chlorine treatment

(Graham, 1997). Sanitation of fresh produce is one of the most promising applications of ozone. The food industry is strongly interested in using this sanitizer to enhance the shelf life and safety of these perishable products and in exploring new applications of the sanitizer.

Efficacy of ozone against natural microflora on lettuce was tested (Kim et al., 1999). Mesophilic and psychrotrophic natural contaminants of shredded lettuce were inactivated by 1.4 and 1.8 log units, respectively, when aqueous ozone was applied at 1.3 mM for 3 minutes. When the ozonation time was increased to 5 minutes, the counts of these microorganisms decreased by 3.9 and 4.6 log units, respectively. The authors suggested that bubbling gaseous ozone into wash water was necessary to increase the efficacy of ozone against microorganisms on lettuce. In a more recent study, Beltran et al. (2005) concluded that sanitization of fresh-cut lettuce using ozonated water was a good alternative to chlorine treatment. Washing shredded lettuce with ozonated water and storing the treated produce under modified atmosphere packaging reduced its microbial populations and extended its shelf life.

Ozone delivery method affects ozone efficacy against microorganisms on fruit surfaces. Achen and Yousef (2001) investigated the inactivation of *E. coli* O157:H7 on apple surfaces by bubbling ozone during washing or by dipping in preozonated water. Counts of *E. coli* O157:H7 decreased by 3.7 and 2.6 log units when the apples were ozone-washed by bubbling and dipping, respectively. When the pathogen was inoculated in the stem-calyx region of apples, <1 log inactivation was achieved by the ozone treatments with both delivery methods. The authors suggested that using a surfactant, such as tetrasodium pyrophosphate, to rinse the apples prior to ozone treatment might have enhanced fruit decontamination.

Gaseous ozone has been studied for inactivation of microorganisms on various fresh fruits and vegetables during storage. Storage of blackberries under 0.1–0.3 ppm gaseous ozone suppressed the fungal growth for 12 days without causing any damage to the tested fruit (Barth et al., 1995). Storage of onions, potatoes, and sugar beets under ozone-enriched atmosphere at 3 mg/L with 6–14°C temperature and

93–97% humidity reduced the spoilage and microbial population of treated products without affecting their chemical composition or sensory quality (Baranovskaya et al., 1979).

Ozone treatments reduced the fungal decay and extended shelf life of table grapes (Sarig et al., 1996). When the grapes were treated with ozone before or after inoculation with *Rhizopus stolonifer*, significant decrease in decay was achieved. It appeared that the ozone treatment also induced plant resistance against fungal decay. Depending on treatment conditions, ozone may also cause physiological injury and damage to treated fruits and vegetables (Horváth et al., 1985).

7.2. Raw Poultry and Meats

Presence of pathogenic microorganisms such as *Campylobacter* spp., *L. monocytogenes*, *Salmonella* spp., and *E. coli* O157:H7 in raw poultry and meat constitutes significant public health hazards. Pathogenic and spoilage microorganism may contaminate poultry and meat products during slaughtering, handling, processing, and distribution (Zhao et al., 2001). Chemical sanitizers such as chlorine have been tested to decontaminate carcasses and cut meat but the efficacy of these sanitizers was questionable. Therefore, ozone has been tested as an alternative sanitizer in these raw products.

Antimicrobial effects have been demonstrated when ozone was used to treat meat surface (Greer and Jones, 1989; Mitsuda et al., 1990), poultry carcasses (Jindal et al., 1995), poultry-processing chiller water (Diaz et al., 2001), and hatchery equipment (Whistler and Sheldon, 1989). However, variable results were reported when ozone was used to decontaminate beef and beef brisket fat (Kim et al., 2003). Using ozone at low concentration may not be suitable for decontamination of high-ozone-demand foods such as meat while ozone at high concentrations may change the chemical compositions and sensory qualities of these types of products (Khadre et al., 2001).

7.3. Shell Eggs

Contamination of raw shell eggs with *Salmonella* Enteritidis is a widespread public health problem.

Several chemical and physical decontamination methods have been developed to inactivate this pathogen in shell eggs but the microbicidal effectiveness of these treatments was variable (Rodriguez-Romo and Yousef, 2005). Additionally, some of these treatments have deleterious effects on egg quality. Yousef and Rodriguez-Romo (2004) developed a method for inactivation of *Salmonella* Enteritidis in shell eggs using combinations of mild heat and gaseous ozone. These treatments synergistically inactivated this pathogen in shell eggs by more than 6.3 log units without affecting the quality of egg contents.

Gaseous ozone treatment of hatching quail eggs at 10 ppm for 6 hours resulted in more than 3 log inactivation of *Salmonella* (Ito et al., 1999). Koidis et al. (2000) investigated aqueous ozone against *Salmonella* Enteritidis on shell eggs using two concentrations (1.4 and 3.0 ppm) and two temperatures (4 and 22°C). Ozone treatments at 22°C with 1.4 ppm inactivated this pathogen by 1 log in 90 seconds, whereas treatments at 4°C using 3.0 ppm resulted in 2 log reduction in the same time period.

7.4. Fish and Seafood

Haraguchi et al. (1969) investigated the bactericidal effects of gaseous ozone on the microbiological qualities of fresh jack mackerel (*Trachurus trachurus*) and shimaaji (*Caranx mertensi*). Viable bacterial counts decreased by 2–3 log units when the skin of gutted fish was treated with 0.6 ppm ozone in 3% NaCl solution for 30–60 minutes. Ozone treatments extended the shelf life of the fish by 20–60%. Shelf life of ozone-washed (1 mg/L for 90 minutes) shucked vacuum-packed mussels was extended by 3 days when compared to control counterparts (Manousaridis et al., 2005). Aqueous ozone treatment (4.5 ppm for 10 seconds) significantly reduced natural microbiota of both live catfish entering the plant and finished catfish fillets (Sopher et al., 2007).

7.5. Dry Food and Food Ingredients

Efficacy of gaseous ozone against microbial contaminants on dry food and food ingredients depends on

surface properties of the treated products, ozone concentration, humidity in the environment, temperature, and water activity of the product (Kim et al., 2003). Ozone more effectively detoxified aflatoxins in pistachio kernels than in ground pistachios (Akbas and Ozdemir 2006). Similarly, higher ozone concentration and longer treatment time were needed for cereal flour and ground pepper to achieve microbicidal effect comparable to that for the whole cereal and pepper (Naitoh et al., 1989; Zagon et al., 1992).

Antimicrobial effect of gaseous ozone against *Bacillus* spp. and *Micrococcus* spp. on cereal grains, peas, beans, and spices was tested (Naitoh et al., 1988). The authors reported that ozone inactivated these microorganisms by up to 3 log units and that efficacy of the treatment depended on ozone concentration, treatment temperature, and environment relative humidity. Gaseous ozone was found to be an effective antifungal fumigant to preserve stored wheat (Wu et al., 2006). Fungal spores associated with wheat were inactivated by 96.9% within a 5-minute ozone treatment (0.33 mg O₃/g wheat × minute). The authors also pointed out that the fungicidal effect of ozone was improved by increasing both water activity and temperature of the wheat.

7.6. Packaging Material and Food Contact Surfaces

A small number of microbial contaminants are usually present on food packaging materials. Several treatments are currently utilized to achieve sterility of packaging materials including H₂O₂ alone or in combination with other sanitizers, heat, and UV radiation (Stefanovic and Dickerson, 1986; Yokoyama, 1990; Gardner and Shama, 1998). Sterilization of packaging materials by H₂O₂ is a common practice, yet the process has several disadvantages (Yokoyama, 1990). Unacceptable amount of this sanitizer may remain after the treatment, and this residue may interact with some compounds in the package material (Castle et al., 1995). Alternative sterilization methods have been sought by the food and packaging industries. Ozone in gaseous and aqueous states has been explored for surface disinfection of packages and equipment (Pascual et al., 2007).

Ozone was tested on surfaces of multilaminated aseptic food packaging and stainless steel, against natural bacterial contaminants, bacterial biofilms of *P. fluorescens*, and dried films of *B. subtilis* spores (Khadre and Yousef, 2001a). Sterility was achieved when uninoculated multilaminated packaging was treated with 5.9 µg/mL aqueous ozone for 1 minute. Population of *P. fluorescens* on multilaminated packaging material was inactivated by up to 8 log units per 12.5 cm² surface when the inoculated materials were repeatedly treated with 3.6 µg/mL ozone. Counts of *B. subtilis* spores (10⁸/6.3 cm²) decreased below detection level by washing with 13 µg/mL ozone for the multilaminated packaging material and 8 µg/mL for the stainless steel. The authors found that ozone inactivated *P. fluorescens* biofilms more effectively on stainless steel than on the multilaminated packaging material.

Ozone has been effectively applied to disinfect oak barrels used for aging wine in Australia. A spoilage yeast (*Brettanomyces* spp.) causes off-taste and other defects in wines and ozone has been proven to control this fungus effectively (Day, 2004).

7.7. Pesticides on Agricultural Commodities

Residues of pesticides such as herbicides, insecticides, and fungicides are widely spread in produce and other agricultural commodities. Persistence of these residues and the associated potential adverse health effects raise concerns among consumers and health authorities. Oxidizing agents such as chlorine, chlorine dioxide, peracetic acid, and ozone have been successfully tested to remove several pesticides from apples (Ong et al., 1996; Hwang et al., 2002). The degradation of four pesticides, diazinon, parathion, methyl-parathion, and cypermethrin, by aqueous ozone in water and on the surface of Chinese cabbage (*Brassica rapa*) was investigated (Wu et al., 2007). Ozone treatment at 1.4 mg/L for 30 minutes was effective in oxidizing 60–99% of the 4 tested pesticides. Ozonated water at 1.4–2.0 mg/L was the most effective in removing up to 60% cypermethrin from the tested vegetable. The authors pointed out that the efficacy of residual pesticides removal by ozone was

influenced by its concentration, treatment temperature, chemical properties of pesticides, and properties of the fresh produce. Ozone alone or in combination with H_2O_2 , UV, or H_2O_2 and UV has been found effective in detoxifying and degrading pesticides in water and wastewater (Ikehata and El-Din, 2005).

8. Combination Treatments

Combination of ozone with other technologies has been extensively investigated in order to enhance the treatment's microbicidal efficacy. Ozone-based advanced oxidation processes is a developing technology that utilizes powerful oxidizing intermediates such as hydroxyl radicals, resulting in disinfection efficacy greater than that of ozone alone (Kim et al., 2003). Combination of ozone with H_2O_2 , UV, and electron beam facilitates the hydroxyl radicals generation (Sommer et al., 2004). Selected examples of combination treatments are addressed in this section.

8.1. Ozone and Hydrogen Peroxide

Relatively low amounts of aqueous H_2O_2 are effective in initiating ozone decomposition and consequently generating hydroxyl radicals (Khadre et al., 2001). Water quality, temperature, pH, and the ratio of H_2O_2 to ozone are also important factors to be considered so as to reach optimum oxidative performance (Kim et al., 2003). Antimicrobial effectiveness of ozone and H_2O_2 combination was evaluated against viruses, *E. coli*, and spores of *B. subtilis* in water (Sommer et al., 2004). Applying initial dosages of 2.5 mg/L ozone and 1.5 mg/L H_2O_2 inactivated 6 log units of 3 tested viruses and *E. coli* after the 4-minute treatment. However, only 0.4 log inactivation of *B. subtilis* spore was achieved with the same treatment. Increasing the contact time to 10 minutes enhanced the sporicidal activity of the combination treatments, causing 1.5 log decrease in spore viability.

8.2. Ozone and Chlorine

Chlorine is found as hypochlorous acid or hypochlorite in water under neutral pH. Ozone oxidizes

hypochlorites to form the weaker oxidizers, chlorates, and chlorides (Bablon et al., 1991). Therefore, possible interactions between chlorine and ozone should be taken into consideration when these sanitizers are applied simultaneously or sequentially. Synergistic action of ozone and chlorine, in sequential applications, against various microorganisms has been reported (Rennecker et al., 2000; Li et al., 2001; Cho et al., 2003). Li et al. (2004) treated *G. lamblia* cysts with a combination of ozone and free chlorine and investigated the morphological changes of the parasite using transmission electron microscopy (TEM). The TEM results revealed that pretreatment of the cyst wall with the first sanitizer improved the penetrability of the second sanitizer through the cytoplasm of the trophozoite, demonstrating the synergy of the combination treatment.

8.3. Ozone and Other Gases

Sequential or simultaneous application of ozone with other gases such as carbon dioxide, argon, and chlorine dioxide may potentially increase the microbicidal efficacy of the treatment. Vurma (2009) tested the feasibility of reducing natural microbial flora and extending shelf life of strawberries using combinations of ozone and carbon dioxide. The researchers found that the combination of these two gasses was more effective in decreasing the natural microbial counts and preserving the quality of the treated strawberries than was either ozone or carbon dioxide treatment alone. Mitsuda et al. (1990) reported the synergistic effect of ozone and carbon dioxide on microbial inactivation in foods. The synergy was believed to be due to the quenching action of carbon dioxide on the chain decomposition reaction of ozone; this increased the stability and bactericidal effectiveness of the ozone in the treatment environment.

8.4. Ozone and Heat

Although rapid decomposition of ozone occurs with elevated temperature, sequential application of ozone and heat could be beneficial (Kim et al., 2003). Perry et al. (2008) applied mild heat and gaseous ozone in sequence to inactivate *Salmonella*

Enteritidis inside shell eggs using a prototype 300-L vessel. Shell eggs, internally contaminated with *Salmonella* Enteritidis ($\sim 10^5$ CFU/g egg), were heated in water at 57°C for 21 minutes, transferred to the processing vessel, placed under vacuum (67.5 kPa), and subsequently treated with ozone gas (~ 140 g O₃/m³ and 184–198 kPa) for 40 minutes. Combined treatment of contaminated eggs significantly decreased internal *Salmonella* population by 4.2 log units.

Preozonation of *B. subtilis* spores at sublethal concentration (6 ppm for 1 minute) greatly sensitized these spores to subsequent heat treatments (Kim et al., 2003). The *D*-values observed during heat treatment of spores were 294, 74.6, and 27 minutes at 85, 90, and 95°C, respectively. When the spores were pretreated with ozone, the *D*-values decreased to 26.3, 9.3, and 4.0 minutes for the corresponding heat treatments. Similarly, Novak and Yuan (2004) reported that ozone pretreatment decreased the resistance of both vegetative and spore forms of *Clostridium perfringens* on beef surfaces to mild heat treatment.

8.5. Ozone and UV Radiation

Naitoh (1992) investigated the synergistic antimicrobial action of gaseous ozone and UV against the spores of four *Bacillus* and six *Clostridium* strains. Sporicidal activity was improved when the concentration of ozone or time of exposure to UV increased. Spores for these microorganisms were more sensitive to both ozone and UV treatments when the relative humidity was high. Applying ozone and UV combination reduced the treatment time needed for spore inactivation. Similarly, ozone and UV combination enhanced the inactivation of *Bacillus* spores in water circulation system in the presence of organic compounds such as tryptophan and ribose (Urakami et al., 1997).

Magbanua et al. (2006) studied the inactivation kinetics of *E. coli* in ozone demand-free media using ozone and UV separately or simultaneously. Combination treatments produced synergy in microbial inactivation; generation of highly reactive hydroxyl radicals via ozone photolysis was believed

to contribute to these results. Using UV irradiation followed by gaseous ozone treatment on externally contaminated shell eggs was synergistic against *Salmonella* Enteritidis (Rodriguez-Romo and Yousef, 2005).

8.6. Ozone and Pulsed Electric Field (PEF)

Combining selected nonthermal technologies in a sequential or simultaneous mode may have synergistic lethality against microorganisms of concern. Potential synergy between PEF and ozone, when applied in sequence, against *E. coli* 0157:H7, *L. monocytogenes*, and *Lactobacillus leichmannii* was investigated (Unal et al., 2001). The authors reported a synergistic antimicrobial action against the tested microorganisms when the cells were pretreated with ozone, followed by PEF treatment. Oshima et al. (1997) investigated the simultaneous treatment of ozone and PEF against *E. coli* and data from their study showed an additive inactivation effect.

9. Limitations, Safety Considerations, and Regulatory Status

Ozone is a powerful and effective sanitizer when applied against microorganisms in low ozone-demand media, such as, relatively pure water or buffer systems (Kim et al., 2003). However, the oxidizing power of this sanitizer may limit its use in certain food applications. Ozone may cause sensory defects such as discoloration and undesirable odors on some treated products. In addition, food's nutritional components such as vitamins, amino acids, enzymes, essential fatty acids may be altered as a result of oxidation by ozone (Kim et al., 2003). Ozone may also cause physiological tissue damage to treated fruits and vegetables. Adverse effects of ozone depend on the food composition, applied ozone dose, and treatment conditions (Kim et al., 1999). Ozone may lead to the formation of some undesired disinfection by-products (DPB) such as bromate when the treated water contains bromide (von Gunten, 2003). Thus, bromide quantity in treatment water should be monitored to prevent the generation of DBP in ozone-washed products.

Ozone could be an extremely hazardous gas and its toxicity depends on the concentration and exposure time. The characteristic odor of ozone is detectable at concentrations as low as 0.02 ppm, and higher concentrations of this sanitizer exert acute symptoms in humans. Severe irritation to the upper and lower respiratory tracts is caused by ozone exposure. Symptoms resulting from exposure to high concentrations of ozone include headaches, dryness of the throat, and pain in the chest. Higher level of exposures may be fatal for humans (Kim et al., 2003).

In the United States, current permissible ozone exposure level time-weighted average (PEL-TWA) in the workplace environment is 0.1 ppm (0.2 mg/m³), as recommended by the National Institute for Occupational Safety and Hazards (NIOSH) and adopted by the US Occupational Safety and Health Administration (OSHA). According to OSHA, short-term exposure limit (STEL) is 0.3 ppm (0.6 mg/m³) for an exposure of less than 15 minutes and four times per day. The immediately dangerous to life or health (IDLH) concentration of ozone in air is 5 ppm (NIOSH, 2005).

Use of ozone in food application involves generation of the gas on site and maintaining the off-gas in a closed system until it is destructed. Ozone has relatively short half-life and it decomposes to harmless oxygen. When ozone is generated and used in food applications, precautions and personal safety always must be taken in consideration. Excess ozone should be degassed and separated from the water stream or the treatment vessel and converted to oxygen prior to its release to the atmosphere. Ambient ozone level should be monitored in the working environment and destruction systems, and respirators are needed for the safety of workers in food processing facilities.

The use of ozone in gaseous state was approved by the US Food and Drug Administration (FDA) in 1975 for meat aging coolers. The application of ozone for disinfection of bottled water was approved by FDA in 1982 (FDA, 1982). Recently, FDA approved the use of ozone in its gaseous and aqueous phase as an antimicrobial agent in food (Code of Federal Regulations, 2001). Furthermore, there is no labeling requirement for ozone-treated products.

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Chapter 22

Case Studies of Ozone in Agri-Food Applications

Rip G. Rice, Dee M. Graham, and Charles D. Sopher

1. Introduction

Since ozone was approved by the US Food and Drug Administration in June 2001 (US FDA, 2001) as an antimicrobial agent for direct contact with foods of all types, many ozone systems have been installed at commercial food processing and agriculture facilities. Agri-Food applications for ozone are based primarily on its ability to control microorganism growth. Since microorganisms can and do grow anywhere along the food chain, from growing crops to processed, packaged, and stored products, ozone has the potential for multitudinous applications in the Agri-Food industry. In this chapter, some recent case studies of commercial applications of ozone are discussed.

Prior to FDA approval of ozone (2001), very few case studies were available. Although many studies focusing on the use of ozone in food applications have been published, not many studies are complete enough to be classified as true case studies.

What constitutes a true case study? Such studies must be conducted under controlled conditions and must be conducted in sufficient detail for review by experts in the fields involved.

In 2003, the International Ozone Association's Pan American Group (IOA/PAG) formed an Agri-Food Task Force to focus on the efforts of many IOA members in this area of evolving uses for ozone. One of the Task Force's efforts is the creation of user

success reports (USRs). These are brief documents, essentially case studies, which explain why ozone was selected to perform some of the food growing, processing/preservation tasks, and cover a discussion of its performance and cost and other benefits that have been derived from the use of ozone. These USRs are posted on the IOA web site (<http://www.io3a.org>) and some of them are discussed in this chapter.

1.1. Position of the IOA Regarding Ozone for Contacting Foods

Upon formation of the IOA/PAG Agri-Food Task Force, the IOA issued a statement indicating its support for the work of this Task Force, under the following conditions:

1. IOA supports the applications of ozone in the Agri-Food industries as long as such applications are made under conditions that are protective of workers involved and of the safety and improvement of product or process qualities to which ozone is applied.
2. IOA will *not* support the indiscriminate application of ozone in any manner that endangers the health of workers or products/processes.
3. IOA will provide space on its web site(s) and will sanction publication in IOA documents to present information on known procedures of applying ozone to agri-food products (or air) under conditions stated in items (1) and (2) above.

Having these as its guiding principles, the IOA/PAG Agri-Food Task Force began its work and has

developed several case studies, now termed as USRs; these are described below.

2. Agriculture and Food Storage Case Studies

2.1. Bulk Storage and Curing of Harvested Onions

Refer to IOA (2006a) for more details.

2.1.1. The Problem Onions, classically, have been bagged and left in the field to cure. This method is labor-intensive, and recent approaches have been aiming at storing bagged onions in covered storage bins. Bulk storage prior to bagging could further decrease labor costs. However, bulk storage increases problems caused by storage diseases. If storage of onions in an ozone-containing atmosphere can mitigate the effects of storage diseases, considerable savings can accrue to the onion farmer.

In 2003, at a West Coast (USA) onion-growing facility, 240,000 bags of onion crops were stored in a single shed. Of these, 158,500 bags came from a single field. An estimated 30% of the 158,500 bags were contaminated with decay and a mold-caused disease of onions termed “neck rot” that could not be detected on the sorting table. On the basis of past experiences, only 20–30% of these contaminated bags would be expected to result in marketable onions, at best, but the entire stored volume of onions might have been lost had nothing been done to change the expected outcome.

2.1.2. The Ozone Solution Onions are mechanically harvested, and brought to storage in bulk trucks with belt unloading. The onions are unloaded onto a machine where dirt, debris, and damaged or spoiled onions are removed. The onions, then, are conveyed into the storage building on conveyor belts and piled into storage. One of the conveyors (the O3Zone Tunnel—see www.O3Co.com) is provided with a cover to contain the ozone in that conveyor and allow an uneven flow of onions to pass through. The ozone is contained within the O3Zone Tunnel so that workers are not exposed to ozone. Ozone concentra-

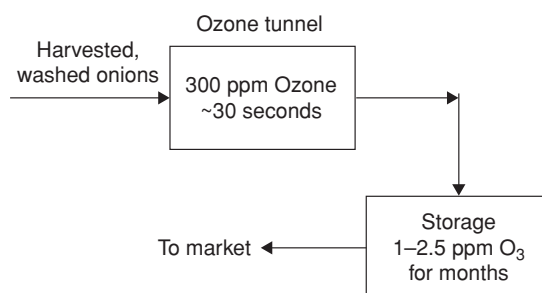


Figure 22.1. Schematic diagram of gas phase ozone treatment and storage process.

tions in the O3Zone conveyor Tunnel are maintained above 300 ppm (see Figure 22.1).

Once the onions are placed into storage, ozone then is applied through the ventilation system to maintain a low concentration of ozone (about 1–2.5 ppm) surrounding the onions throughout the storage period. The premise is to reduce, significantly, the pathogen population on the onions going into storage (by exposure to approximately 300 ppm of ozone for 15–30 seconds) and then keep the pathogens under control during storage with ozone at about 1–2.5 ppm. The storage ozone concentration is kept low so that no damage occurs to the onions, yet is high enough to keep the pathogenic microorganisms under control.

Temperature within the storage area is maintained within 0.5–1°F of the set point. Each storage room has a large air plenum the entire length. Air is delivered from the plenum under the onions by cross tubes or ducts in the floor that have openings to allow the air to move up through the pile. About 2 cfm of air per hundred pounds of onions is provided. The onions are stored in bulk from 10 to 20 ft deep or in bins stacked 20 ft high. In either case, ventilation air is provided to control the temperature and gas buildup.

2.1.3. The Cost Savings Had the contents of the storage shed not been treated with ozone and had been lost to mold growth during storage, the loss in marketable onions would have amounted to a value of about \$750,000. Had only the lot from the single field been lost to rot, the market value would

have been about \$300,000. Without ozone treatment, only some 30% of the 158,000 bags (47,550 bags) of onions were expected to be marketable. Instead, ozone treatment resulted in an additional 55,500 bags of onions being marketable at an additional income of \$166,500.

The capital cost of the Ozone Tunnel was \$116,000, including ozone generation and control equipment. Operating costs: 1 lb of ozone requires 10–15 kWh to produce. The ozone generating unit produces approximately 3 lbs of ozone per day. At $\$0.10/\text{kWh} \times 15 \text{ kWh} \times 24 \text{ hours} \times 3 \text{ lbs/day} = \$108/\text{day} = \text{approximately } \$20,000 \text{ over 6 months}$. The income realized from the additional 55,000 bags of onions, saved by ozone storage, was \$166,500, meaning that this extra income from this one crop more than paid for the ozonation equipment, which is being used on subsequent crops of stored onions.

2.2. Storage of Potatoes—Walker Farms, Menan, Idaho, 2002 Crop

Refer to IOA (2006b) for more details.

2.2.1. The Problem Walker Farms grows over 6,000 acres of potatoes each year. In 2002, Walker Farms produced 160 acres, about 80,000 cwt (Hundred Weight), of potatoes with over 5% of these potatoes infected with *Phytophthora erythroseptica*, or “Pink Rot.” Upon placing an initial 8,000 cwt of potatoes into the cellar, Walker Farms determined that the entire 80,000 cwt would not store. Due to the significant amount of Pink Rot, Walker Farms felt the risk of losing the entire field was too high; therefore, the decision was made not to harvest the infected potatoes and to move on to the next field.

2.2.2. The Ozone Solution Upon completing the harvest of this 2002 crop, Walker Farms returned to the infected field and harvested the remaining potatoes that were filled with Pink Rot. They placed these potatoes in a separate cellar and ran the potatoes through an O3Zone Tunnel (see above—“onion storage”) exposing the potatoes to approximately

300 ppm of gaseous ozone. During storage for several months, the potatoes were exposed constantly to approximately 1–2.5 ppm of ozone, similar to the storage of onions (see Figure 22.1). No ozone was applied to the original 8,000 cwt of potatoes.

The potatoes were harvested in the last week of September, 2002. By mid-November, the first potatoes, not put through the O3Zone Tunnel, were rotted to the point that they had to be abandoned, as expected. These potatoes were hauled away for disposal.

The remaining potatoes that were put through the Tunnel and stored with ozone did not show signs of Pink Rot spreading among the potatoes. The original Pink Rot on 5% of the stored potatoes was still present; however, none of the other potatoes had become infected. In April and May, 2003, these potatoes were sold in the normal marketing order. Ozone had extended the storage life of these potatoes and created a financial gain for Walker Farms.

2.2.3. Cost Savings There was 72,000 cwt of potatoes that were sold for \$4.00/cwt (\$288,000). Had they been abandoned, the cost to haul and dispose of them would have been about \$0.25/cwt (\$18,000). The ozone treatment, therefore, was worth \$306,000 (\$288,000 + \$18,000).

Capital cost for the O3Co ozonation equipment and O3Zone Tunnel, including control equipment, was \$116,000. This means that the ozone equipment and Tunnel was paid for in about 4 months.

Operating costs: 1 lb of ozone requires 10–15 kWh to produce. The O3Co ozone generating unit produces approximately 3 lbs of ozone per day. At $\$0.10/\text{kWh} \times 15 \text{ kWh} \times 24 \text{ hours} \times 3 \text{ lbs/day} = \$108/\text{day} = \text{approximately } \$27,000 \text{ over 8 months}$.

2.2.4. Reduction in Spoilage Without ozone treatment, this crop was not deemed worth storing, due to Pink Rot infection.

2.2.5. Maintenance of Product Quality Quality of the potatoes not infected by Pink Rot initially was maintained over the 8 months of ozone storage.

2.3. Tomatoes Grown Hydroponically in Ozone-Containing Water

Refer to Knueve (2002) for more details.

2.3.1. The Problem At a Florida farm at which tomatoes are grown hydroponically (in water in the absence of soil), groundwater is supplied from a 100 ft well at 2 gal/minute (gpm) continuous flow (8 gpm peak flow) without treatment. Blossom end rot (a well-documented disease afflicting tomatoes) caused rejection of about 40% of the tomato crop.

When faced with situations such as these, growers normally turn to their fertilizer suppliers, who also market pesticides and other chemicals used in hydroponic facilities. The fertilizer supplier had exhausted all alternatives directed at resolving the 40% crop rejection issue.

At the same time, the tomato grower was faced with deteriorating ground water quality and decided to install a state-of-the-art ozone water treatment system. The groundwater contains 60 ppm hydrogen sulfide (H_2S), has a pH of 7.8 and oxidation-reduction potential (ORP) readings of -177 mV (a negative ORP reading indicates the absence of dissolved oxygen).

2.3.2. Ozone Solutions After installation of an ozonation system to cope with the H_2S , the groundwater quality improved as follows:

- H_2S levels were reduced from 60 mg/L to 0.
- pH was lowered from 7.8 to 7.04 (by reducing the organic load and producing sulfuric acid, H_2SO_4).
- ORP readings increased from -177 mV to $+225$ mV (due to addition of dissolved oxygen during ozonation).

Thus, the ozone treatment system had done its intended job of improving the groundwater quality. But then, a remarkable improvement was found in the reduction of blossom end rot and other crop production parameters as follows:

- Crop rejection rates were reduced from 40 to less than 3%.
- Time to first harvest was reduced by 28 days.

- Total yield of tomatoes increased by more than 300% in a single season.
- Fertilizer use was reduced by $\sim 25\%$ (possibly by being better absorbed at the lower pH).

These benefits from the installation of ozone resulted in a payback time for the ozonation system of less than 6 months.

3. Food Processing Plant Case Studies

3.1. Garlic Processing Plant—Spray Bar Rinse System

Refer to IOA (2006d) for more details.

3.1.1. The Problem A US garlic processor serves large companies as a food ingredient supplier and also serves prisons, schools, restaurant chains, etc. The plant products are whole peeled garlic, private-label garlic purées, and jalapeño pepper products. In the past, the firm sprayed garlic cloves with solutions of sodium hypochlorite by means of a spray bar. Before installation of the ozone system, sodium hypochlorite solution was employed to maintain cleanliness of the spray bar rinse system. For whole peeled garlic, the objectives of the hypochlorite solution were to reduce aerobic plate counts (APCs) from about 100,000 (measurable counts per mL) to below 10,000, and to reduce levels of lactic acid (spoilage) bacteria and mold. Spray solutions contained 100–125 ppm of sodium hypochlorite.

Numerous maintenance problems were encountered with the use of hypochlorite, including pitting of the stainless steel rollers, and the high levels of total dissolved solids plugged the spray bar holes, corroding the feed pump and plumbing. Finally, sodium hypochlorite was leaving a residual in the wastewater pond, which is located directly over a source water aquifer. Granular activated carbon (GAC) scrubbing of the air was required, to remove and destroy odors from 100 to 125 ppm sodium hypochlorite solutions. Also, sodium hypochlorite reacts with organics, causing a strong ammonia odor, and has the potential for imparting a hypochlorite (or reaction product) residual on the plant products.

3.1.2. The Ozone Solution Replacement of the hypochlorite system by an ozone/water system eliminated the maintenance costs of hypochlorite, the pitting and corrosion of equipment, the need for GAC scrubbing of odors, and lowered the processing costs significantly.

A ClearWater Tech (HDO3-II skid-mounted) ozone delivery system was installed to replace the hypochlorite approach on the garlic spray bar rinse system. The skid-mounted, preplumbed, and prewired ozonation system includes the following components:

- A 20 g/hour, variable output corona discharge ozone generator
- A 15 SCFH oxygen concentrator capable of 90%+ purity and -100°F dew point
- A 1 hp, stainless steel booster pump
- A Kynar[®] injector
 - Back-flow prevention (J-break)
 - Stainless steel contact vessel
 - Contact vessel vent valve (stainless steel)
 - Fully integrated dissolved ozone monitor
 - Full instrumentation
- Electricity use is 1 kW/hour

The plant water supply comes from a deep well on the property, and is fed directly to the HDO3 system @ 20 psi. The HDO3 unit adds 1.3 ppm dissolved ozone to water, which is fed directly to the spray bar at 13 gpm @ 20 psi. Spray heads were increased in size to accommodate the lower psi of the ozone system.

A 4–20 mA signal from the integrated dissolved ozone monitor controls output from the ozone generator. The system also includes a 10 gpm wash-down wand and an ambient ozone monitor ensures the safety of workers (the monitor is designed to trigger an alarm and shut off power to the ozone generator if the ambient ozone level ever were to rise above regulated standards).

3.1.3. Results Obtained with Ozone

3.1.3.1. Attainment of Microbial Goals The ozone system produced an overall 20–30% reduction in APCs (checked twice a month). This percentage re-

duction is not a full log. Either hypochlorite or ozone applied individually reduced the plate count levels from about 100,000 CFU/mL to 5,000 CFU/mL. The plant's requirement is plate counts below 10,000 CFU/mL.

3.1.3.2. Equipment and Maintenance Effects Maintenance costs were reduced and pitting of the stainless steel rollers was eliminated. Spray bar plugging (formerly caused by the high total dissolved solids (TDS) of sodium hypochlorite) and corrosion was eliminated. This saved \$500–600/month in materials and maintenance costs. The air scrubbing system has been shut down completely, saving approximately \$150/month in operating and maintenance costs.

Annual maintenance costs for the ozonation system are estimated at about \$450, less than the maintenance costs for 1 month with the former hypochlorite system.

3.1.3.3. Wastewater Effects Wastewater from the spray bar system now contains less than 1 ppm of sodium hypochlorite (too low to leave a residual in the wastewater pond).

3.1.3.4. Improved Product Quality Because of the replacement by ozone, there is no potential for hypochlorite-derived chemical residuals on the plant garlic products. Neither are ozone-derived chemical residuals known to contaminate the plant products.

3.1.3.5. Process Reliability The company reports more consistent sanitation results with the reduced system maintenance requirements.

3.1.3.6. Product Marketing Since the installation of ozone treatment, it is now easier to certify the plant product as “organic” under the USDA/NOP final rule, subsection 205.605. Ozone is an allowed ingredient used in or on an organic product (garlic coming to the plant from the field).

3.1.3.7. Cost Benefits Before installing the ozonation system, chemical costs for sodium hypochlorite totaled \$3,000 annually. Maintenance costs

on the hypochlorite system were \$500–600 per month (average \$550/month), and the air scrubbing system cost \$150/month to operate. These costs (\$700/month) = \$8,400 annually (plus \$3,000 for the NaOCl = \$11,400) were eliminated when the ozonation system was installed.

The ozonation system cost \$16,500, plus \$2,500 for installation (total = \$19,000). First year savings to the plant totaled \$11,400. On this basis, the return on investment is estimated to be about 17 months.

3.1.4. Employee Health and Safety Issues Food safety is an important issue and ozone helps in this regard, both in employee safety (less chemical handling since sodium hypochlorite is no longer in use) and increased food quality (reduced spoilage).

By its nature, garlic processing requires a good deal of ventilation to protect the plant workers. Garlic odors react rapidly with ozone in the ambient air. Since garlic odor levels in the plant are quite high, any stray ozone is quickly quenched. Additionally, the wall-mounted ambient ozone monitor checks to ensure that ambient ozone levels, if any, are below OSHA requirements.

3.1.5. Additional Comments The plant management is so pleased with the replacement of hypochlorite with ozone, that they plan to use the ozone system for equipment wash down and hard surface cleaning (a 10 gpm spray wand is in place). Also, ozone is being considered for spray bar rinsing in the jalapeño processing line and in garlic purée processing.

3.2. Treatment of Food Processing Plant Chiller Water for Reuse

At this midwestern US food processing plant, bean dip, salsa, and other related products are cooked at 180°F, then packaged in 5–8 lb lots in polyethylene bags, which then are sealed and chilled in a 5,000 gallon chilling water loop and storage tank at 35–40°F for approximately 2 hours until the product has reached 60–65°F. Refer to IOA (2006e) for more details.

3.2.1. The Problem Microbial contamination (from slight spillage on the bags during filling and/or occasional bag breakage) caused excessive drain and refill of the chiller water, requiring the chiller system to be drained and refilled anywhere from 1 day to 1 week. An ozone system was installed to treat the chiller water in an attempt to reduce the frequency of draining and refilling the chiller water loop. The goal is to keep water in chill water loop safe from microbial contamination for a longer period of time, thus saving on water consumption, labor, and reducing process downtime.

3.2.2. The Ozone Solution This application uses the HDO3–1 ozonation system supplied by Clearwater Tech LLC (San Luis Obispo, CA). An independent side stream loop was created so as not to interfere with the chiller and its hydraulics. Dissolved ozone (1.15 ppm) in solution is delivered to the 5,000-gallon tank. System features include stainless steel construction, integrated dissolved ozone monitor, adjustable ozone output, and air-cooled, stainless steel ozone reaction chambers. The unit is skid-mounted and comes preplumbed and prewired. A process schematic diagram is shown in Figure 22.2.

The chiller water prior to installation of the ozone system could only be kept for reuse for periods from 1 day to 1 week, depending on organic loading, primarily spillage on the outside of bags during filling, before microbial contamination caused the need to drain the 5,000 gallon tank. Annual process water consumption prior to ozone was an estimated 1,123,000 gallons. After ozone system installation, the chiller water has been kept for reuse up to 6 months in some of the company's plants, reducing the annual process water consumption to 72,000 gallons and related refrigeration cost savings due to conservation of chilled water.

3.2.3. Cost Benefits The company has installed the system in four plants total, which has resulted in a savings of 1,051,000 gallons of water annually at the four plants overall. This water savings converts to cost savings of \$1,600 (total of the four plants).

As a result of the extended use time of the process water, the four plants have been able to reduce

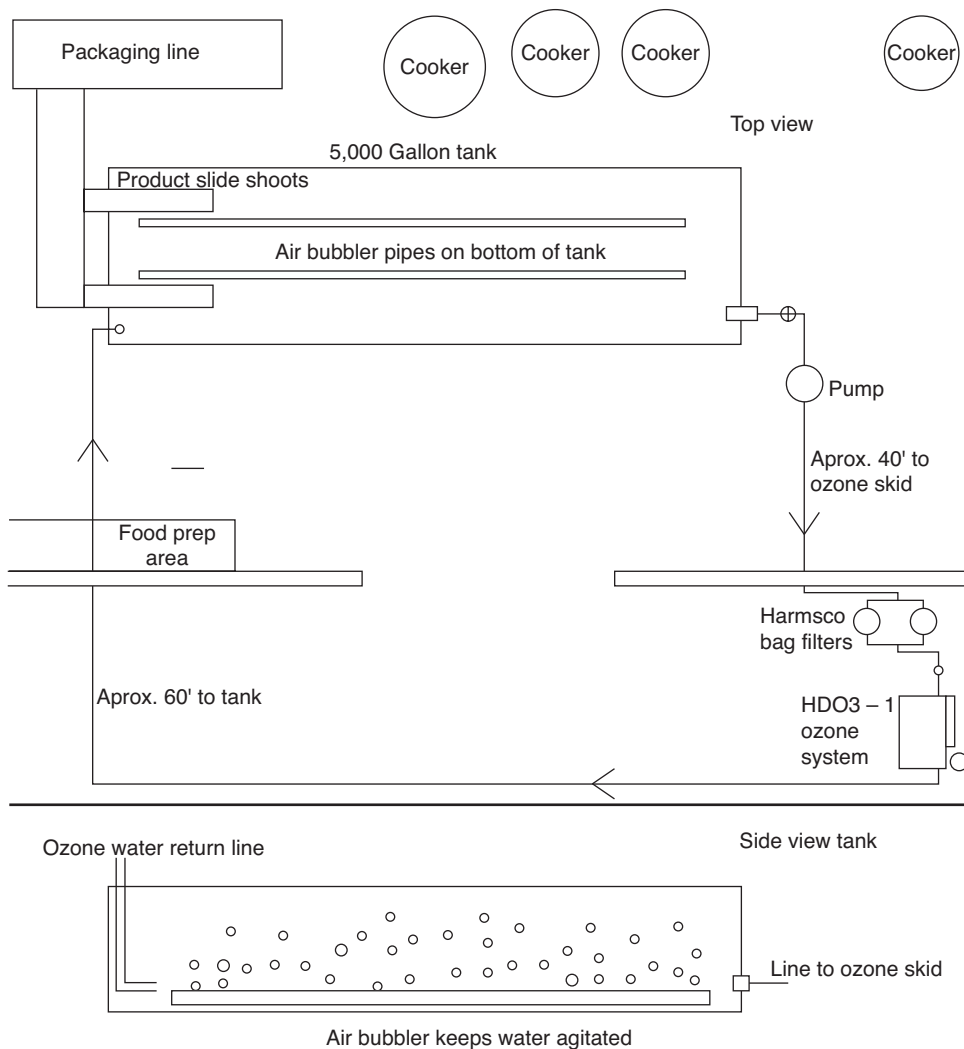


Figure 22.2. Process schematic at Midwestern food processing plant. (Courtesy of ClearWater Tech, LLC, San Luis Obispo, CA.)

maintenance costs associated with the cleaning and maintenance of the chiller water tanks. These maintenance cost savings are estimated at \$9,000 annually (total of the four plants).

Total annual savings in water and maintenance at the four plants equals \$10,600. Costs for the ozonation equipment and their installation in the four plants totaled \$70,000 (\$25,000 for plant 1 and \$45,000 for

plants 2, 3, and 4). This results in a return on investment of 6.61 years.

However the local city water department offers grant funds for companies that are able to implement systems to reduce water usage. The city water department has monitored water savings as a result of ozone system implementation, and has awarded the company an \$11,000 grant to offset the cost of ozonation

system installation. The \$11,000 grant, thus, reduces the R-O-I from 6.61 to 5.57 years.

3.2.4. Employee Health and Safety Issues The only concern was that the chilling water tank was directly in the middle of the plant area containing the food cookers. Employees work in a circle around the tank, so off-gassing of ozone from the open top tank was a concern. The skid system delivers the ozone completely dissolved in the chilled water. Because of this, and the cold water temperature (35–40°F), and the fact that employees are about 10–20 ft from the tank, no ozone can be measured in the air around the employee stations. Additionally, the plant air contains odors of cut onions and garlic, which quickly react with any transient ambient ozone.

3.3. *Strawberries and Frozen Strawberry Topping*

This Fresno, CA, strawberry processor installed ozone in June, 1998. Ozone-containing water is sprayed on fresh strawberries as a sanitizing agent to reduce the overall microbial load before freezing of the berries and also to prepare frozen strawberry toppings. Typical data are presented in Tables 22.1 and 22.2. Washing raw strawberries with water containing an average of 2.7 ppm of ozone reduces levels of *E. coli* and Coliform organisms, standard plate counts, and yeast and mold counts. For example, standard plate count (SPC) organism levels are reduced on average from 17,767 in raw strawberries to 987. Likewise, yeast/mold counts are reduced from an average of 56,500 to 1,304. Refer to Lyons-Magnus (1999) for more details.

3.4. *Ozone for Treatment and Storage of Grain*

Grain is produced in open fields and, as such, arrives at a processing mill carrying foreign vegetative material in addition to plant leaves, dust, and stalks. It also may be contaminated with bird and rodent droppings. The milling process separates the grain from the other material and grinds it into flour. Chlorinated

water normally is used to control bacteria and mold during this process.

Milling begins with the grain being mechanically separated from stalks and foreign matter. The next step is to increase the moisture content of the grain. This is necessary because dry wheat is too hard to grind into flour. Consequently, water is added to increase the grain's moisture content from below 12 to approximately 15% (this latter number varies within the industry). Moisturizing is accomplished via automated temper systems, which are large augers that mix and move the grain while a carefully regulated stream of water is added. The plant where this case study was conducted (Harvest States Amber Milling, Huron, OH) has three augured temper systems.

Chlorine is added to the water to control bacteria and mold which typically are present in the grain. At the Harvest States plant, chlorinated water (450 ppm) is applied at a flow rate of 4 gallons per minute (GPM) in each temper system or a total of 12 GPM for the 3 units together. The temper systems convey the moistened grain into large concrete tempering tanks or bins, which are approximately 45 ft high and 8 ft in diameter. The grain flows continuously, and average residence time in the bins is approximately 18–24 hours, which allows the added water to be absorbed.

Next, the grain is conveyed through tubes to the wheat rolling mill where the grain is abraded to loosen the bran, which then is separated by sifters and air purifiers. The grinding continues until the grain reaches the proper level of fineness, at which point it is packaged or shipped in bulk. Refer to IOA (2006f) for more details.

3.4.1. Problems with the Current Process

3.4.1.1. Contamination of Grain Prior to and during Processing Wheat and other grains collect dust, insects, and other foreign matter, such as soil and feces, in the fields where they are grown. When the wheat is harvested, inevitably some foreign matter accompanies the grain and plant material. The grain is exposed to additional contamination from bacteria and foreign material through transportation vehicles, storage containers, fertilizers, rain, and

Table 22.1. Raw strawberries and frozen strawberry topping micro data. Week of July 6–11, 1999 (Lyons-Magnus, 1999, cited in US Food & Drug Administration, 2001)

	Time	Raw			Finished			Tsunami	Ozone
		<i>Escherichia. coli</i> - <i>coli</i>	SPC	Yeast/-Mold	<i>E. coli</i> / <i>coli</i>	SPC	Yeast/Mold		
Date	Specs	Neg/<10	<50,000	<2,000	Neg/<3	<10,000	<2,000	ppm	ppm
6 July	6:45 AM	Neg/TNTC ^a	40,000	TNTC	Neg/<10	600	1,100	4	2.56
	9:15 AM	Neg/25,000	60,000	TNTC	Neg/<10	10	<10	5	2.56
	1:15 PM	Neg/8,000	6,000	1800	Neg/<10	30	20	6	2.62
	5:30 PM	Neg/2,500	8,000	900	Neg/<10	100	20	8	2.59
7 July	1:05 PM	Neg/5,000	800	TNTC	Neg/<3	3	<3	8	2.77
	2:35 PM	Neg/40	1,200	24000	Neg/<3	12	<3	8	2.77
	5:22 PM	Neg/4,000	10,000	35000	Neg/<3	6	<3	5	2.93
	7:20 PM	Neg/20	30,000	TNTC	Neg/<10	800	1,900	6	2.98
8 July	2:30 AM	Neg/<10	600	71,000	Neg/<10	650	1,600	6	2.98
	6:50 AM	Neg/<10	5,000	TNTC	Neg/<10	2,000	1,400	6	2.79
	9:20 AM	Neg/<10	1,200	20,000	Neg/<10	<10	<10	7	2.82
	2:50 PM	Neg/<10	6,000	40,000	Neg/<10	<10	<10	7	2.72
	5:40 PM	Neg/400	TNTC	TNTC	Neg/<10	180	500	5	2.70
9 July	1:20 AM	Neg/300	4,000	12,000	Neg/<10	900	2,500	5	2.88
	5:15 PM	Neg/150	TNTC	TNTC	Neg/<10	800	700	5	2.51
	7:30 PM	Neg/TNTC	20,000	TNTC	Neg/<10	580	1,100	5	2.55
	9:35 PM	Neg/TNTC	18,000	TNTC	Neg/<10	900	800	5	2.51
	11:00 PM	Neg/1,500	600	90,000	Neg/<10	1,900	1,700	5	2.76
10 July	1:15 AM	Neg/TNTC	TNTC	TNTC	Neg/<10	2,200	1,500	5	2.76
	2:20 AM	Neg/<10	80,000	TNTC	Neg/<10	1,480	2,000	5	2.81
	7:00 AM	Neg/TNTC	TNTC	TNTC	Neg/<10	1,000	800	5	2.78
	8:30 AM	Neg/TNTC	TNTC	TNTC	Neg/<10	750	600	6	2.78
	2:30 PM	Neg/TNTC	TNTC	TNTC	Neg/<10	200	150	7	2.78
	7:40 PM	Neg/TNTC	TNTC	TNTC	Neg/<10	350	1,020	5	3.09
11 July	1:15 PM	Neg/<10	TNTC	TNTC	Neg/<10	100	1,700	6	3.01
	7:40 PM	Neg/<10	TNTC	TNTC	Neg/<10	300	900	5	2.68
	10:05 PM	Neg/<10	TNTC	TNTC	Neg/<10	200	500	4	2.36
	Average		17,141	32,744		642	1,074	6	2.74
	Std. Dev.		23,063	30,496		654	678	1	0.17
	Maximum		TNTC	TNTC		2,200	2,500	8	3.09
	Minimum		600	900		3	20	4	2.36

^aTNTC: Too Numerous To Count.

moisture in the air. The result is a potentially high level of bacteria and mold on the preprocessed grain surfaces. Further, exacerbating the problem is the necessary addition of water to the grain to increase its moisture content, creating warm, moist tempering conditions before milling. This moist grain supports growth of mold and bacteria. It is to combat these inherent problems of contamination that grain processors treat the grain with chlorinated water during

the tempering process when water is added to the grain to increase its moisture content.

3.4.1.2. Effectiveness of Chlorine Treatment Although problems of mold and bacteria in grain traditionally are addressed by the use of chlorinated water, this technique frequently is inadequate for removing these contaminants. As a result, tempering bins, holding containers, and processing lines

Table 22.2. Raw strawberries and frozen strawberry topping micro data. Week of July 13–18, 1999 (Lyons-Magnus, 1999, cited in U.S. Food & Drug Administration, 2001)

	Time	Raw			Finished			Tsunami	Ozone
		<i>Escherichia. coli/-coli</i>	SPC	Yeast/-Mold	<i>E. coli/-coli</i>	SPC	Yeast/Mold		
Date	Specs	Neg/<10	<50,000	<2,000	Neg/<3	<10,000	<2,000	ppm	ppm
13-July	7:00 AM	Neg/<10	4,000	TNTC ^a	Neg/<10	1,000	800	21	2.52
	9:30 AM	Neg/3000	45,000	TNTC	Neg/<10	1,300	900	21	2.52
	5:15 PM	Neg/TNTC	TNTC	TNTC	Neg/<10	2,000	1,100	21	2.34
	7:30 PM	Neg/200	600	12,000	Neg/<10	1,400	1,300	21	2.6.2
	9:50 PM	Neg/15000	50,000	160,000	Neg/<10	330	1,700	21	2.47
	11:15 PM	Neg/<10	800	TNTC	Neg/<10	300	1,200	21	3.04
14 July	2:00 AM	Neg/200	TNTC	TNTC	Neg/<10	60	700	21	2.88
	2:35 PM	Neg/12000	TNTC	TNTC	Neg/<10	800	1,900	12	2.36
	5:30 PM	Neg/120	1,400	9,000	Neg/<10	1,500	950	4	2.36
	7:45 PM	Neg/2100	TNTC	TNTC	Neg/<10	600	750	4	2.30
	9:35 PM	Neg/1300	TNTC	TNTC	Neg/<10	300	1,400	3	2.38
15 July	1:20 PM	Neg/7000	TNTC	TNTC	Neg/<10	60	600	9	2.86
	2:20 PM	Neg/900	80,000	16,000	Neg/<10	210	500	9	2.86
	3:20 PM	Neg/700	TNTC	TNTC	Neg/<10	4,100	2,000	8	2.86
	5:35 PM	Neg/800	TNTC	TNTC	Neg/<10	140	1,600	3	3.15
	7:45 PM	Neg/1200	TNTC	TNTC	Neg/<10	520	1,400	3	2.43
	9:45 PM	Neg/60	TNTC	TNTC	Neg/<10	600	1,600	5	2.71
16 July	6:45 AM	Neg/1200	TNTC	TNTC	Neg/<10	200	900	18	2.75
	9:45 AM	Neg/400	11,000	TNTC	Neg/<10	150	1,800	14	2.75
	3:05 PM	Neg/80	16,000	TNTC	Neg/<10	50	400	3	2.36
	5:45 PM	Neg/1800	7,600	TNTC	Neg/<10	1,000	1,700	4	2.54
	7:25 PM	Neg/300	2,700	90,000	Neg/<10	500	2,000	5	2.43
	9:25 PM	Neg/200	14,000	70,000	Neg/<10	900	2,100	5	2.71
17 July	6:50 AM	Neg/TNTC	TNTC	TNTC	Neg/3,000	1,200	1,800	5	2.96
	4:00 PM	Neg/TNTC	TNTC	TNTC	Neg/900	2,000	1,300	4	2.85
	5:05 PM	Neg/TNTC	TNTC	TNTC	Neg/500	1,800	1,100	4	2.71
	7:15 PM	Neg/TNTC	TNTC	TNTC	Neg/400	1,500	2,500	4	2.73
	9:25 PM	Neg/10	30,000	80,000	Neg/3,500	5,400	2,700	4	3.14
	11:10 PM	Neg/TNTC	TNTC	TNTC	Neg/1,200	700	1,200	5	2.78
18 July	6:55 AM	Neg/200	400	TNTC	Neg/<10	300	450	11	2.79
	8:25 AM	Neg/12,000	TNTC	TNTC	Neg/200	700	400	11	2.79
	11:25 AM	Neg/200	3,000	TNTC	Neg/<10	900	700	12	2.79
	3:20 PM	Neg/TNTC	TNTC	TNTC	Neg/<10	700	1,400	4	2.80
	5:05 PM	Neg/60	TNTC	15,000	Neg/<10	350	1,500	4	2.91
	Average		17767	56,500		978	1,304	9.5	2.7
	Standard Deviation		23,538	53,676		1,118	596	6.9	0.2
	Maximum		TNTC	TNTC		1,000	1,500	21.0	2.9
	Minimum		4000	15,000		350	800	4.0	2.5

^aTNTC: Too Numerous To Count.

become contaminated and have to be cleaned. This requires complete shutdown of the plant and results in production and financial losses.

3.4.1.3. Problems with Chlorine Chlorine is a reactive chemical and causes corrosion in metal parts in the mixing and grain transfer equipment. In addition, it is a hazardous chemical to store and handle. Furthermore, chlorine chemically reacts with some materials and organics, and the resulting chlorinated compounds sometimes remain in the final product as contaminants. Some of these compounds, trihalomethanes (THMs) and halogenated acetic acids (HAAs) have suspected carcinogenic properties.

3.4.1.4. Returned Shipments In addition, elevated bacterial and mold counts sometimes cause final products to fail to meet microbial standards of grain purchasers. Returned shipments can cost up to \$5,000 per rail car plus reprocessing costs.

3.4.2. Case Study Project The case study was conducted at the Harvest States Amber Milling plant in Huron, OH, USA, under sponsorship of the Electric Power Research Institute (EPRI) and was designed to test the effectiveness of ozone (generated by corona discharge and by UV radiation at 185 nm and applied simultaneously with UV radiation (at 254 nm) as an antimicrobial agent for flour milling. Ozone was used in three ways in the project:

1. In steeping water applied to wheat during the flour milling process (CD-generated ozone)
2. In gaseous phase in conjunction with UV light and hydroxyl radicals during tempering
3. In gaseous phase as a facility-cleaning agent for further bacteria and mold control.

The project study was conducted in 2000 with process design, equipment specification, and installation performed by RGF Industries (West Palm Beach, FL, USA). Testing of the ozonation process began in May, 2000.

3.4.2.1. Details of Ozone System and Application The treatment system used in the study consisted of ozone (generated by corona discharge, absent

UV radiation) treatment supplemented by “photo-ionization” (a term coined by RGF Industries), delivering both UV light and ozone to create hydroxyl radicals for the control of microorganisms, mold, and yeast on the wheat grain and the processing equipment. The system has three major components:

1. A skid-mounted corona-discharge ozonation system with a dry air feed to dissolve ozone in the water used to temper the grain.

This system is placed in an explosion-proof room adjacent to the existing water tank. It is sized to provide sufficient ozone for 12–15 to 4 GPM for each of the three temper systems, even though it was used to supply only one tempering auger during this study. The major components of the system include a corona discharge ozone generator with a pressure swing adsorption air drying system, Venturi mixer, chlorine destruction filter, ozone destruction for ozone in contactor off-gas, recirculating stainless steel pump/motor assembly, and pressurized contact vessel. An integrated dual metering system is used to measure and control the levels of dissolved ozone in the water and to measure and control the levels of ozone present in the ambient air.

2. A stainless steel photo-ionization chamber mounted over the grain-mixing auger to supply UV light and UV-generated ozone.

The purpose of this combined ozone/UV system (as stated by RGF Industries) is to create hydroxyl radicals to attack the surface of the grain. The ionization hood consists of a series of ozone-emitting UV bulbs covered for protection in an FDA-approved plasticized shroud targeted on titanium dioxide (TiO_2). The hoods are hinged-mounted to the top of the existing temper augers and employ a safety system to automatically shut down the UV lamps if the hood is opened by plant personnel.

3. An ozone generator to supply gaseous ozone in elevated concentrations to the grain storage tempering bin and the roll bin to suppress bacteria and mold growth.

A dried-air-fed corona discharge system generates the ozone gas and delivers it to the application

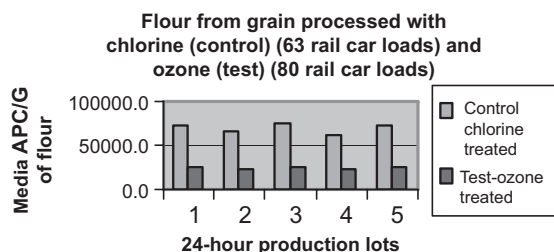


Figure 22.3. Comparison of grain treated with chlorinated water versus ozone/UV. (Courtesy of Int'l. Ozone Assoc., 2006f.)

points via Teflon tubing. It, too, is accompanied by a dual metering system to monitor ozone levels in the bin as well as in ambient air surrounding the bins.

3.4.2.2. Evaluation and Data Gathering To determine the effectiveness of ozone treatment, more than 1,000 samples of flour ready for shipment were evaluated. These samples were tested for total plate count bacteria. To establish a base of comparison, 365 of the samples were taken from flour processed using traditional chlorinated water disinfection. The remaining 650 samples were taken from flour processed using ozone disinfection. Figure 22.3 summarizes the many data points obtained during this study.

Because the Harvest States plant does not perform mold counts, visual inspections were used to determine the effectiveness of ozone for mold control in comparison to chlorinated water. In addition to microbial analysis and mold estimation, the study included an evaluation of plant operation using ozone-containing water versus chlorinated water.

3.4.3. Case Study Results

3.4.3.1. Antimicrobial Action Data gathered during the project indicate a potential 75–80% reduction in total plate count bacteria in comparison to conventional treatment with chlorinated water. The APC of one group of flour samples from grain treated with chlorinated water averaged 181,675 CFU/g. In comparison, the average APC for flour from ozone-treated grain was 42,627 CFU/g, a reduction of 77%.

Later data on 195 samples of flour processed during late July and August had 75 lots (38%) with APC of less than 10,000 CFU per gram.

Although the project did not include mold counts to quantify the effectiveness of ozone over chlorine in mold abatement, visual inspection of equipment and lines by plant staff indicated a similar reduction of mold growth in the equipment.

3.4.3.2. Cost Savings/Return on Investment According to RGF Industries, the company supplying ozonation and UV equipment for the project, there is a cost savings of about \$40,000 per year by using ozonation and UV in place of chlorination. (Electrical costs to operate the ozone system are \$0.18 per hour or approximately \$1,600 per year.) This rate of savings would pay out the capital investment in the ozonation and UV equipment in 30 months. This analysis does not include potential additional savings from some reduction in maintenance and plant downtime previously required to remove mold from lines and equipment, plus costs for reprocessing grain failing to meet microbial standards when train car loads are returned.

3.4.4. Employee Health and Safety Issues The hoods are hinge-mounted to the top of the existing temper augers and employ a safety system to automatically shut down the UV lamps if the hood is opened by plant personnel, thus avoiding inadvertent exposure of plant personnel to ultraviolet radiation.

The levels of ozone in the work place at the points of application are monitored by ambient ozone monitors designed to effect complete system shutdown if excess levels of ozone are sensed.

3.4.5. Equipment Compatibility The ozone system is located on the top floor of the flour mill near the existing chlorine tank—a floor away from the milling operation to avoid any potential from dust explosions. The ozone gas and water plumbing go to the floor below where the grain processing and storage equipment are located. The ozonation system is completely independent of the plant chlorine system so that the plant still has access to this method of sanitation.

3.4.6. Commercialization Harvest States has installed the ozone/UV grain milling system on full-scale in its plant.

3.5. PlumRose Meat Products

Refer to EPRI (1999) for more details.

3.5.1. The Problem PlumRose USA, Inc., a processor and packager of ham, turkey, chicken, and deli meats, wanted to ensure the highest possible level of sanitation and protection from potential pathogens and spoilage microbes while minimizing the need to store and handle hazardous chemical cleaners and sanitizers at its processing plant in Booneville, Mississippi. Like all companies using chlorine-based cleaners and sanitizers, this PlumRose facility faced increasingly stringent regulations and expense in meeting ever stricter environmental standards. Effluent burden, while not a problem in 1999, could become a problem in the future.

3.5.2. The Ozone Solutions In October, 1999, this plant replaced its chlorinated detergent cleaners and chlorine-based sanitizers with an ozone-containing water system. To prepare for the proposed system changes, extensive microbiological data, collected routinely in daily quality testing, were compiled to establish a baseline for evaluating the new system's performance.

The state-of-the-art ozonation system installed by PlumRose provides ozone-containing water on demand for several sanitizing operations within the plant. The system includes a corona discharge ozone generator using oxygen generated on-site by PSA (pressure swing adsorption) as the feed gas, an ozone injector, ozone contact tank, distribution pipes and pumps, fully automated controls, air quality sensors, and several proprietary application systems designed especially for the PlumRose operation. The ozone generator is centrally located and isolated from normal work areas. Ozone-containing water is delivered in closed piping under low pressure to appropriate points of use within the plant. Including supporting gear and distribution pumps, the total power require-

ment for the ozonation system is 50 amps from a 240-volt, Three-phase system.

3.5.3. Results The first use for the new ozone system was for cleaning and sanitizing stainless steel racks used for smoking hams. The soiled racks travel through a three-part sanitizing machine. The first step is an alkali cleaner. Originally, this was followed by a first rinse and final rinse with chlorinated water. Now, water containing 1 mg/L (ppm) of ozone is used for both rinses.

As a result of the successful rack cleaning operation, additional uses for ozone-containing water were implemented throughout the plant, including sanitizing plastic storage tubs, and stainless steel walk-in coolers. As an added benefit, cooler sanitation now includes cold (ozone-containing) water. This reduces heating costs, load on the HVAC units, and condensation.

3.5.3.1. Fewer Chemical Cleaners Prior to installation of the ozonation system, a large inventory of 30% hypochlorite solution was kept in locked storage. Leaking or damaged drums was an ever-present danger. During the day, periodic rinses containing 100 ppm of chlorine were used on equipment surfaces. Now, water containing 1 ppm of ozone is used for cleaning equipment in various parts of the plant.

3.5.3.2. Recycled Water Because of the design of the new system, water from the final rinse in the rack washer is recovered, reozonated, and used for the first rinse. Recycling reduces water usage, effluent burden, and treatment and disposal costs.

3.5.3.3. Microbiological Safety Table 22.3 presents data showing that equal or better sanitation levels are obtained before and after the processing change was made.

3.5.3.4. Costs and Cost Savings The complete ozonation system, including data logger and fully automated controls, was built and installed for \$73,800. Extensive microbiological testing by PlumRose Quality Laboratories cost \$20,000. At the time

Table 22.3. Comparison of chlorine versus ozone sanitation at PlumRose (EPRI, 1999) using contact plates

Racks Sampling	Number of Checks	% with Colonies	% with No Colonies
4 th Qtr 1998	216	15	93
1 st Qtr 1999	188	31	84
2 nd Qtr 1999	196	14	93
3 rd Qtr 1999	208	13	94
Ozone System Installed October 6, 1999			
Week 1	20	1	95
Week 2	50	3	94
Week 3	50	2	96

the EPRI document was written (late 1999), the ozonation system was reducing the \$9,000 per quarter that PlumRose formerly spent on hypochlorite. Other cost savings had not yet been calculated (cooler sanitation water, effluent burden + treatment and disposal savings).

3.6. Ready-to-Eat Meats

This Eastern US meat processing plant wished to lower the levels of microorganisms on processing equipment and plant surfaces, and on the surfaces of processed food products (fresh and smoked pork sausage, bacon, skinless and natural-casing wieners, lunch meat, and hams). The old process used an organic acid for sanitation, which is costly. Ozone was tested for efficacy and for cost savings, with great success, and has been installed in the full-scale treatment plant.

Ozone-containing water is used as an equipment sanitizer of hard surfaces for preoperational sanitation, in-process sanitation, in-process antimicrobial product wash (casing soak, product spray), and for post-lethality treatment. All products are showered directly with ozone-containing water before packaging.

Ozone can be used in conjunction with a residual chemical sanitizer as necessary. The overall application in this case is clean, ozone rinse, quaternary ammonium sanitizer @ 400 ppm.

Figure 22.4 is a schematic diagram of the ozone system installed at this plant. Refer to Hamil (2005) for more details.

3.6.1. Cost Benefits This plant implemented the centralized ozone system for hard surface and direct product sanitation at a total cost of approximately \$30,000. The customer is realizing \$124,000 annual savings through the elimination of organic acid use.

3.6.2. Product Quality Improvements Results from preoperation ATP Tests (2003 vs. 2004) show ozone to be effective in providing 79.96% reduction in ATP counts over 2003 and 58.24% reduction in the number of swab failures over 2003 (see Figure 22.5).

3.6.3. Additional Benefits The new aqueous ozone system has allowed the plant to replace potable water sprays on belts, reduce/prevent bacteria build-up on production belts, and prevent product surface contamination.

The ozone/water system can be easily adapted for use on cutting equipment, belts, etc. The use of ozone does not affect organoleptic quality of RTE meat products.

3.7. Poultry Drinking Water Ozonation

Refer to Sopher et al. (2004) for more details.

3.7.1. The Problems As with any livestock industry, chickens require a clean and uncontaminated source of drinking water to make maximum daily gains and maintain flock health. Water sources high in minerals such as calcium and magnesium can cause problems simply through calcification of equipment and emitters. Plugged emitters reduce water intake and feed conversion as well as being a nuisance to keep clean and in working condition. High calcium and magnesium in the evaporative cooling systems reduce the usable life of the systems and increase maintenance costs. High microbe levels lead to diseases and mortality in the most severe cases and poor flock performance at lower levels of contamination.

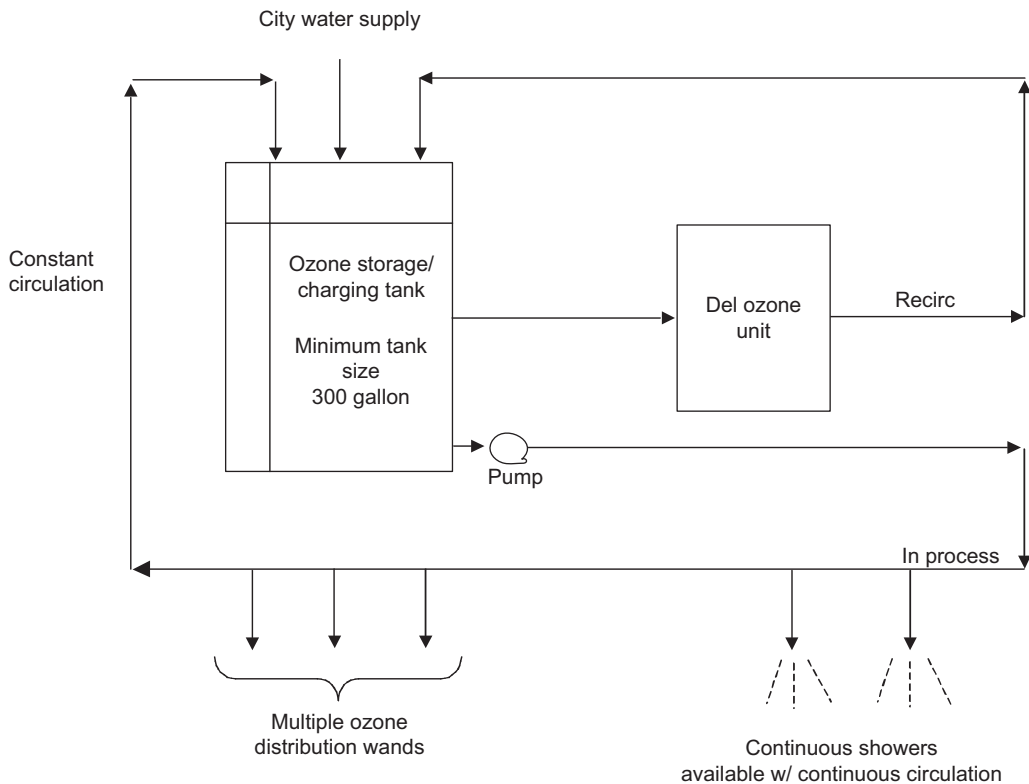


Figure 22.4. Schematic diagram of ozonation system installed at Eastern US meat processing plant. (Del Ozone, San Luis Obispo, CA.)

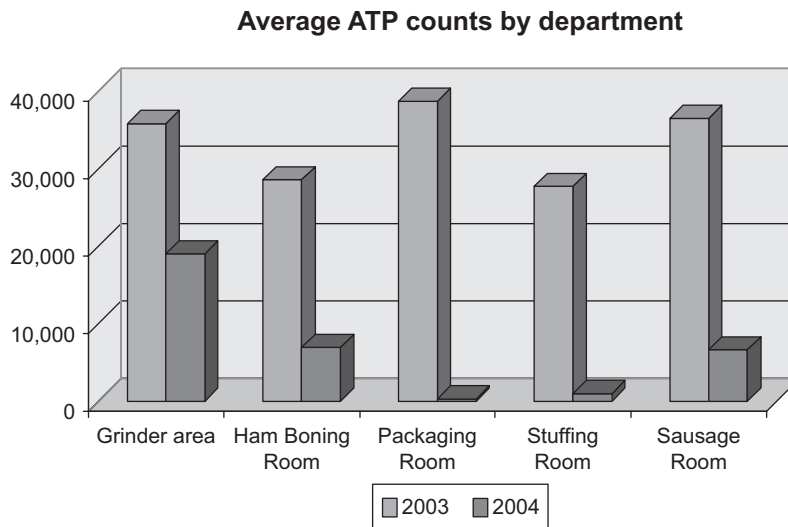


Figure 22.5. Average ATP counts at Eastern US meat processor before (2003) and after (2004) installation of ozone systems (Hamil, 2005).

The removal of microbes through chlorination of the drinking water is not always a satisfactory solution as most types of poultry do not react well to high chlorine levels. The chlorine in the digestive tract of poultry is thought to reduce levels of beneficial microorganisms in the gizzard, and, thus, reduce feed conversion and possibly immunity to diseases.

In the Southeastern part of the United States, iron, sulfur, and manganese can reduce water quality to the level that the chickens limit water intake; in turn, feed conversion and weight gains are reduced. In high enough concentrations, manganese can cause mortality rates to increase and profits to decrease.

3.7.2. The Ozone Solutions To overcome the problems of poor quality water, some producers are installing water purification systems based on ozone and filtration that provide poultry water at the level of national drinking water standards. Ozone has the capability of purifying the water by removing microbes and pesticides and neutralizing the harmful effects of iron and manganese through precipitation. Sulfur (as sulfide ion) can be oxidized to harmless sulfites and sulfates. Some sulfate salts (i.e., calcium) are insoluble and precipitate. Once the iron, manganese, and some sulfate compounds have precipitated, they can be removed by filtration. Because ozone has a very short life, it does not leave residuals in the water; thus, treated water can be recontaminated with microbes.

3.7.3. Case Study A study utilizing ozone and filtration (sponsored by TVA and EPRI's Global Energy Partners) was conducted at the Steve Cumberland Farm, Neshoba County, Mississippi. Mr. Cumberland and his wife operate six broiler houses. Most broilers are produced in flocks of 25,000 birds in a single broiler house.

The situation at start-up consisted of a 365 ft deep well outfitted with a 2-hp pump capable of providing 30 GPM. The iron content of the water was high and the water smelled of H_2S . Sulfide odors were particularly noticeable when water was sprayed at 60 pounds of pressure.

A 32 g per hour ozone generator was installed along with three filtration tanks. The filtration tanks

are timed to flush at night and expel any precipitated iron, manganese, and sulfate compounds. Prior to installation of the ozonation equipment, the well water was sampled by the TVA Central Laboratory Services and analyzed for inorganic contaminants. These water analyses were used as guides in developing specifications for the needed ozonation and filtration equipment. Water samples also were collected from the municipal water source at the farm. Treated water samples were collected once the ozonation and filtration equipment were installed.

3.7.4. Cost Factors Involved with Poultry Drinking Water Broiler houses in the Southern United States usually are constructed at a cost of approximately \$6.50/ft². The density of birds placed in a poultry house varies depending on the final market weight desired. As a rule of thumb, each bird usually occupies 0.75 ft² of house space. This translates into an initial capital cost of \$5 per bird or \$125,000 per poultry house. As the industry moves to heavier chickens at processing, the space required per chicken will increase to 0.80–0.85 ft² of space per chicken. This increase in space requirement per chicken will be reflected in the amount of fixed costs needed to produce a flock. These costs can be calculated when the needed broiler size of bird is determined.

Most producers calculate that they need 1 gallon of water per bird per day for a flock that is nearing maturity during the summer months. Using this rule of thumb, water systems are sized to meet this need. A producer with six producing houses will need a water supply capable of supplying 150,000 gallons of water per day for full production. Most integrators will require a dependable water supply that provides at least 15 GPM per house. Utilizing these requirements, the needed water for a 6-house production unit would be a minimum of 130,000 gallons of water per day. Regardless of whether one uses the grower's rule-of-thumb of 1 gallon per day per bird, or the integrator's requirement of 15 GPM per house, the needed water per day for 6 houses is 130,000–150,000 gallons per day. To achieve this water capacity a farmer must have a minimum sustained well pumping capacity of 90 GPM. One well

with this capacity is risky because power outages and breakdowns could limit water supplies to the flock or cause disasters whereby entire flocks are lost. For this reason most farmers either have standby wells and generators or have access to a municipal water source for emergencies.

3.7.5. Cost Savings Realized Variable costs for water decreased by nearly \$20,000 for the six bird houses after ozone filtration was installed. Fixed costs for well drilling and maintenance were not given by Sopher et al. (2004), but were stated to be “quite low compared to the cost of municipal water.”

3.7.6. Other Benefits Potential problems of chlorine in municipal water supplies were avoided. Emitter fouling due to precipitants decreased.

Based on this study, Mr. Cumberland indicated that he would drill an additional well or wells and utilize ozonation to treat the water for the poultry houses.

3.8. Fresh-Cut Lettuce, Salads, and Vegetables (Strickland et al., 2010)

The Strickland Produce Company, Nashville, TN, installed in early 1999 an ozonation system to explore recycling salad mix wash waters (EPRI, 2002). Since 1981, Strickland Produce has packed fresh cut vegetables and lettuce in bags for the ready-to-eat (RTE) market. Most of the produce, including lettuce and cabbage, is trucked from California, sorted, cut, washed, packed in plastic bags, and distributed under refrigeration. Flume water, which is used for washing, and transporting the produce through the plant, is recycled in a closed loop. In follow-up of numerous plant tests, laboratory studies were conducted at the University of Tennessee (Garcia, 2001; Garcia et al., 2003), to confirm the process conditions, conduct storage studies, and sensory panels on finished product. Strickland installed the recommended process for a full-scale, commercial fresh-cut salad plant in 2001 (Garcia et al., 2003). Refer to Strickland et al. (2010) for more details.

3.8.1. The Problem In the past, Strickland used only chlorine for sanitizing the flume water. Chlorine was added to an 8,000-gallon water tank at 100 ppm level that fed the flume. At this level, worker discomfort was experienced and headaches were common. Since the chlorine provided antimicrobial activity but did not clean the water, the flume water quickly became discolored, laden with organic residues and needed to be replaced as often as every 2–3 hours. Water costs and wastewater disposal costs are expensive and often can mean that the difference between profit and loss in the highly competitive produce business. Also, produce that was treated immediately before the water was replaced ran the risk of contamination from the higher levels of organic compounds in the flume water.

Because of the water and wastewater costs combined with the risk of increased contamination, Strickland Produce was searching for a technology that could provide the antimicrobial protection of chlorine and at the same time would clean the flume water as it recirculated.

3.8.2. State-of-the-Art Technology Installed

With support from Tennessee Valley Authority (TVA), Nashville Electric Service, and EPRI, Strickland Produce installed a state-of-the-art water cleaning and ozonation system (an ozone generator capable of producing 3.6 lbs/day of ozone at 6% concentration (in oxygen)). To reduce the suspended solids load, the system has a self-cleaning 50- μ m Ronningen-Petter DCF “wedge-wire” filter installed in the 200-GPM flume in front of the water chiller.

After the chiller, a side stream of 50 GPM is diverted from the flume and ozonated (6 mg/L dosage) at 50 psig pressure in an Osmonics-designed ozonation system. Side stream ozonation is a technique that has been developed and used for many years in European drinking water treatment plants. All of the ozone required (6 mg/L) for the total volume of water to be ozonated is added to 25% of the water flow. The ozonated side stream then is returned to the flume and mixed with the remainder of the moving flume water. This provides a total ozone dosage for all of the water being treated of 1.5 mg/L. A primary advantage of side-stream ozone injection is that a

much smaller contactor and contact chamber is required to treat 25% of the total water than to ozonate the total water volume.

The ozone gas is injected into the side stream with a Mazzei injector followed by a pressurized contactor tank. Before returning the ozonated side stream water back to the flume stream, entrapped gases are removed by a GDT Corporation centrifugal separator with catalytic destruction of any excess ozone. Thus, ozone is not allowed to escape into the plant environment (a stringent EPA and OSHA requirement).

This is a second major advantage of side-stream ozonation—removal of ozone off-gases is accomplished with much smaller sized equipment on the side stream volume (25%) than on the total flume volume.

Once excess ozone has been removed, the cleaned flume water continues through the plant, washing cut vegetables. Near the end of the ozone wash cycle, chlorine is added for residual sanitation and antimicrobial protection while the product is being packaged and stored. The amount of chlorine added since installation of ozone is proprietary, but is estimated to be less than 33% of what was required prior to ozonation being installed.

At the end of the flume, the washed products are screened and excess water is removed through centrifugation. Because a small amount of water is lost in the process, necessary fresh water is added to bring the system to capacity and the flume water is recirculated to wash additional vegetables.

The resulting product is both appealing and has several side attributes that make it very innovative and in keeping with the desires of modern consumers. The product is RTE and fits into the needs of a “society on the go.”

3.8.3. Benefits from Ozone Treatment

3.8.3.1. Improved Product Quality A 30-member panel at the Department of Food Science and Technology at the University of Tennessee (UT) in Knoxville analyzed refrigerated samples taken from the Strickland Produce operation. The results of the

sensory analysis showed the superior quality and longer shelf life of the ozone-treated samples, even after 25 days of storage. The reduced use of chlorine in combination with an effective ozone treatment improved the taste and appearance of the fresh-cut produce considerably compared to the use of chlorine or ozone alone (see Figure 22.6).

3.8.3.2. Water Savings Turbidity analyses of the flume water showed that the ozone-treated water stayed clearer over an extended operating time, thus allowing a full day of operation without the need for water replacement. Water savings in the order of 60% have been achieved. Savings of this magnitude are very important in both water usage and sewage treatment costs.

3.8.3.3. Improved Shelf Life and Better Assurance of Food Safety Microbiological analyses performed by UT scientists confirmed the plate count level reduction for aerobic bacteria, which explains the longer shelf life of ozone-treated produce. This shelf-life extension is quite significant, reducing the amounts of discarded products due to spoilage, increasing shipping distances, and increasing storage life times.

3.8.3.4. Additional Observed Benefits

- Improved cleanliness of the recirculated water in the flume system (no brown off-color, better sanitation, and reduced bacterial count in the water).
- Flume water replacement is required only once a day instead of every 2–3 hours, reducing water usage and reducing the volume of plant effluent.
- Reduced usage of chlorine, thus better tasting produce, and better appearance.
- Lower bacterial count on the produce, thus longer shelf life and better assurance of food safety.

3.8.3.5. Cost to the Producer The total cost of the system was approximately \$200,000.

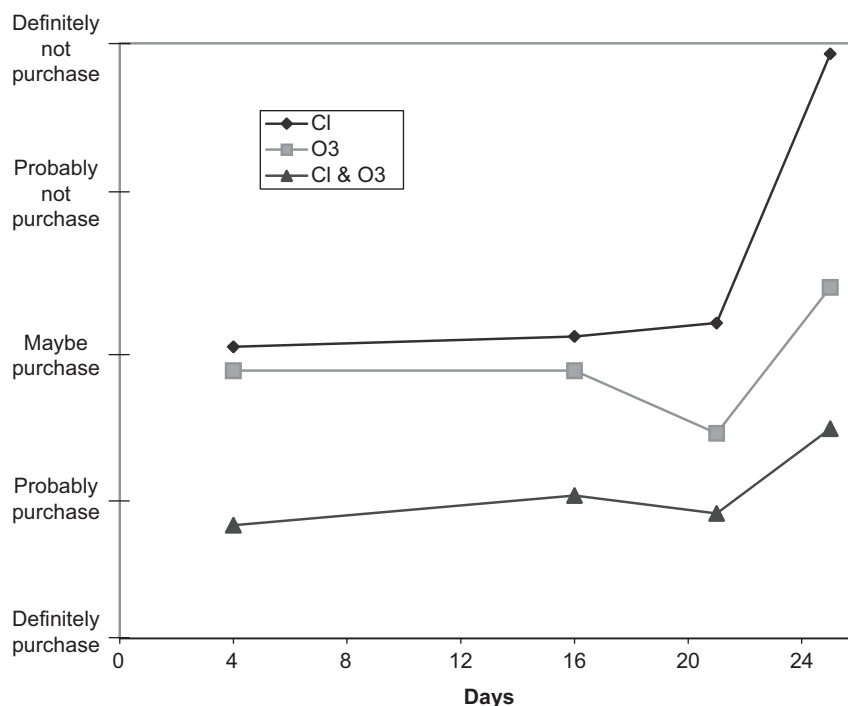


Figure 22.6. Panelists' willingness to purchase commercial salad samples sanitized with chlorine (Cl), ozone (O₃), or a combination of both and based on appearance of the salads (5, definitely not purchase; 4, probably not purchase; 3, maybe purchase; 2, probably purchase; 1, definitely purchase) (Garcia et al., 2003, cited in Strickland et al., 2010).

3.9. Other Fresh-Cut Vegetable Ozone Installations

This type of ozone/water wash water/recycling system is in use in many fresh-cut vegetable plants throughout the United States. Caldwell & Son, Inc., a large Los Angeles producer of fresh produce, installed a wall mounted ozonation system costing \$25,000 in 2003 for washing fresh-cut vegetables (Thomas, 2005).

The ozone system replaced chlorine washing, not because of major problems with chlorine washing, but because the plant management wanted to provide an additional product safety step. The dissolved ozone concentration in the wash water is maintained at about 1.3 mg/L, and this level can be adjusted quickly at the ozone generators. City water pressure is at 46 psi, but the ozonation and water wash dis-

tribution system needs a minimum of 70 psi to keep ozone gas in solution. To achieve this, a high-pressure pump has been incorporated into the ozonation system (Thomas, 2005).

3.10. Utilization of Ozone in Catfish Processing

Catfish processors are concerned about raw product prices (live catfish), labor, and processing costs such as packaging materials, cold storage, and waste disposal costs. A major concern with processors is food product safety and quality. Food poisoning outbreaks caused by organisms such as *E. coli* and/or *Salmonella* can require production stoppages and recalls and can even force an operation into bankruptcy. To ensure that food poisoning outbreaks do not happen, catfish processors are continually looking for

new technologies that will safely remove or reduce microbial levels and provide a safe product.

The utilization of chlorine products in processing plants is standard practice, with levels up to over 100 ppm found in vegetable processing plants. Chlorine at these levels will provide antimicrobial protection. It also will cause a strong chlorine odor in the plant, employee discomfort (eyes and nose burning), and can cause off-flavors in meat and fish products. These factors coupled with the recent finds of the harmful effects of THMs and haloacetic acids, has made high levels of chlorine unpopular in processing plants. In vegetable plants, it is often used with ozone treatments to provide residual antimicrobial effects while ozone destroys the microorganisms.

Although there is great interest in the use of ozone in catfish processing, there are numerous logistics problems. Equipment manufacturers are cautiously developing packaged systems to introduce ozone into catfish processing plants. This “one size fits all” or claiming that a few standardized ozone systems will work in all types of plants has produced a mixture of results ranging from excellent to terrible. In visiting several catfish processing plants utilizing ozone, it has been observed that installations include improperly sized systems, unmaintained systems, improperly used systems, and unsafe systems.

To demonstrate the use of ozone in catfish processing systems, a consortium of TVA (Tennessee Valley Authority) Global Energy Partners, LLC, C&S AgriSystems, Inc., and ClearWater Tech LLC conducted an onsite demonstration of the use of ozone at the Superior Fish Products processing plant in Mississippi. Processes studied for this report were as follows:

- The application of ozone on live fish as they entered the stun chamber
- Utilizing an ozonated wash on the fillet machines
- Washing finished fillets in ozone-containing water
- Cleaning equipment with ozone-containing water
- Demonstrating the effects of ozone to reduce odors in the offal room.

The goals of this demonstration were to:

- measure the effects of ozone as an oxidizing agent to reduce the microbial load brought into the plant by live catfish
- determine if an ozone-containing water wash would reduce the microbial load on fillets coming from the fillet machine to the chiller
- determine if a significant reduction in microbial load could be obtained by washing finished fillets in ozone-containing water
- determine if ozone washing of equipment that had been cleaned previously utilizing Best Management Practices could further reduce microbial load
- qualitatively evaluate the effect of gaseous ozone on air quality in the offal room.

Refer to Sopher et al. (2007) for more details.

3.10.1. Plant Procedures Live fish are transported to the plant and held in oxygenated tanks until being released into a pit and conveyed into the plant. After the holding tank is released into the pit, the live fish are conveyed into the plant where they are stunned electrically. After the fish are stunned, they are conveyed to the plant fillet lines. From the fillet machine, the whole fillets are finished by hand and conveyed into the chiller. As the fillets pass through the chiller, they are dumped onto a finishing line as the chiller rotates. From the chiller, the fillets are separated from the other portions of the fish and placed into tote boxes. The fillets are fed onto a slotted belt and moved to the grader.

All nonedible portions of the fish, including the heads, bones, and skins, are conveyed to the offal room for accumulation in trailers. These trailers are delivered to processors of animal feed and other products. Although this room is temperature controlled, it can become highly odorous in the summer months. Many call it the *awful* room.

3.10.2. Materials and Methods

3.10.2.1. Laboratory Analyses A local contract microbiological laboratory (Standard Laboratory, Inc., Starkville, MS) was engaged to perform microbial analyses, and assisted with the

development of sampling procedures. Surfaces were sampled by utilizing swabs placed in 25 mL of buffered water (Butterfield buffering agent— K_3PO_4 — $MgSO_4$ —*Standard Methods*) after swabbing the surface. The dilutions used were 1–25 and 1–250 mL. Counts were reported per mL for swab samples.

The procedure for fish fillets was a rinse. A 100-g sample was taken and 100 mL of buffered water was added for the rinse. The dilutions were 1–1,000 mL. The results were reported per gram of fish.

Fillets from both the fillet machine and the final product after the chiller were sampled by bagging whole fillets. All samples were iced immediately after sampling and delivered to the laboratory the next day.

Micro analyses were performed according to *Standard Methods*, 14th Edition.

3.10.2.2. Live Fish Sampling Live fish from the conveyor going to the plant were selected randomly and swabbed on both sides for microbial samples. A manifold, then, was placed over the conveyor. The manifold was PVC pipe drilled with 1/8th inch holes on 2-inch centers to allow a gentle but fairly large amount of ozone-containing water to pass over the fish. Pressure on the manifold was 22–25 psi. Ozone-containing water was passed over the fish for 10 seconds and 5 fish selected at random were swabbed for microbe samples.

3.10.2.3. Ozonation at the Fillet Machine Although fillet machines are operated with running water directed around the fillets, filleting is still a portion of the operation that can contain high amounts of fecal matter from ruptured intestines and is an area that contributes a high microbial load to the total microbial load of any plant.

Fillets from the fillet line were selected randomly and bagged for microbial analyses. Ozone-containing water then was directed onto the fish moving through the line and random samples were taken for microbial comparison with the untreated samples.

3.10.2.4. Ozonation of the Final Fillets from the Chiller Applying ozone-containing water to fillets from the chiller presented a problem because the fillets from the chiller are dumped onto the final grading belt in small piles of about 3–5 lbs. These piles do not allow for the fillets to come into contact with the ozone-containing water. To overcome this problem, a tote box was cleaned and rinsed with ozone-containing water containing 4.8 mg/L of ozone, and the fillets were rinsed under a stream of ozone-containing water. All fillets were given a 10-second wash in ozone-containing water at 22–25 psi. An ozone level of 4.5 ppm was used.

3.10.2.5. Washing of Equipment after Daily Cleaning The plant management requested that ozone be tested as a final rinse for equipment cleaned after each daily production cycle. Three areas of production were chosen for the test: cutting boards, final sorting conveyor, and fillet sorter for the final product. Each area was swabbed after cleaning and then rinsed 10 seconds with ozone-containing water (5.0 ppm of ozone) and swab samples again were taken. The cleanliness of this area is a company concern.

The cleaning operation utilizes a soap that contains chlorine and also uses a final “sanitizing” rinse that contains chlorine. Residual chlorine is present in the plant and on the equipment after cleaning. For this reason, unpredictable results were expected.

All the cleaned surfaces were washed using a 5/8-inch hose that was operated at 20–25 psi with ozone-containing water (5.0 ppm of ozone). Swab samples were taken before and after the ozone rinse. The cutting board surfaces were tested and the underside of the chiller conveyor was swabbed in five of the grooves for each sample. This conveyor was visibly quite clean on the top surface, but residues of food grade lubricant were found on the underside. The sorting conveyor was swabbed under the flipper mechanism that removes the fish from the belt. For each treatment on each piece of equipment, five samples were taken.

All aqueous ozonation treatments were made utilizing an HDO3-III ozonation system from Clearwater Tech, LLC (San Luis Obispo, CA). An oxygen concentrator in conjunction with the system was utilized as an oxygen source. Untreated well water was utilized as the water source.

3.10.2.6. Ozonation of the Offal Room Air Treatment of this room was a simple demonstration of how ozone can destroy odors. The oxygen concentrator utilized for producing ozone for use in the aqueous ozone tests was switched to an air dryer and gaseous ozone was piped to the ceiling of the offal room utilizing Tygon tubing. The generator was operated for two 15-minute periods. The ozone level in the room was checked using a hand held aeroQUAL Ozone Meter, series 300. Levels were watched very carefully to ensure that 0.045 ppm of ozone in the air was not exceeded.

3.10.3. Data Obtained, Statistical Analyses, and Interpretations For all laboratory analyses, five samples were taken on both untreated and treated (ozonated) samples. Each experiment was analyzed utilizing a two-factor analysis of variance (ANOVA) without replication. An *F*-test was utilized to determine statistical significance. Each test is presented and interpretations provided:

3.10.3.1. Live Catfish—Unwashed versus Washed with 4.5 ppm Ozone-Containing Water in Feed Conveyor

Total plate counts (CFU) from swabbed fish

Unwashed	Washed
75,000	2,100
41,000	1,800
20,000	650
35,000	300
15,000	700

Groups	Count	Sum	Average	Variance
Unwashed	5	186,000	37,200	559,200,000
Washed	5	5,550	1,110	623,000

ANOVA

Source	SS	df	MS	F	P-value
B/T gp	3.26 e ⁺⁷	1	3.26 e ⁺⁷	11.6	0.009213
W/n gp	2.24 e ⁺⁷	8	0.028 e ⁺⁶		
Total	5.50 e ⁺⁷	9			

Washing the catfish in the conveyor before the fish enter the stun chamber significantly reduced the microbial load going to the plant. For the plant to utilize this technique, it is suggested that the present wash lines over the conveyor be charged with ozone-containing water at 5.0 ppm ozone. To ensure that off-gassing does not cause worker safety problems with high levels of ozone in the air from the water, ozone monitors should be installed over the conveyor and in locations occupied by plant workers. Because the conveyor is subject to stopping, ozonation and water flow should be stopped when the conveyor stops. Since the study herein performed only one test at one ozone concentration, further testing is required to develop the exact ozone level needed and the wash time necessary to ensure a reduced microbial load. This testing can be completed whenever an ozone generator is installed in the plant.

After observing the process used to stun the fish, it was concluded that a wash after the stun box would be more effective than before the stun equipment. This wash would be effective in removing fecal materials that are released when the fish are stunned; thus microbial levels in the plant should be reduced.

3.10.3.2. Fillets from the Fillet Machine—Unwashed versus Rinsed with 4.5 ppm Ozone-Containing Water

Total plate counts from fillets

Unwashed	Washed
6,000	3,600
8,500	3,200
5,700	7,000
3,200	2,800
8,500	2,000

Groups	Count	Sum	Average	Variance
Unwashed	5	31,900	6,380	4,927,000
Washed	5	18,600	3,720	3,712,000

ANOVA

Source	SS	df	MS	F	P-value
B/T gp	1.77 e ⁺⁵	1	17.7 e ⁺⁵	4.1	0.077618
W/n gp	34.6 e ⁺⁵	8	4.32 e ⁺⁴		
Total	52.2 e ⁺⁵	9			

Although the ozone wash reduced the plate counts at the fillet machine, the reduction was not significant at the 5% level of probability.

Care should be taken to avoid worker exposure to ozone off-gassed from the washing treatment at the fillet machine. If ozone-containing water is used at this point, an air sensor that is capable of reducing the water flow or automatically shuts off the ozone generator needs to be installed to ensure worker safety.

3.10.3.3. Fish Fillets from Chiller—Unwashed versus Washed 10 Seconds in 4.5 ppm Ozone-Containing Water

Total plate counts

Unwashed	Washed
37,000	86,00
55,000	13,000
37,000	10,000
43,000	10,000
72,000	16,000

Groups	Count	Sum	Average	Variance
Unwashed	5	244,000	48,800	222,200,000
Washed	5	57,600	11,520	8,852,000

ANOVA

Source	SS	df	MS	F	P-value
B/T gp	3.47 e ⁺⁷	1	3.47 e ⁺⁷	30.1	0.0006
W/n gp	9.24 e ⁺⁶	8	1.16 e ⁺⁶		
Total	4.40 e ⁺⁷	9			

The ozonation of the catfish fillets at this point in the process provided highly significant reductions in microbial loads. The average plate counts/mL dropped from 48,800 to 11,520. The standard deviations for the total plate counts within the groups were reduced from 14,906 to 2,975 with the ozone-containing water treatments. Installing ozonation at this point in the process line will take planning and innovation. A water bath conveyor system is suggested as it can be covered and to prevent off-gassing to the work area if high levels of ozone are utilized.

Because the action of ozone in the water bath will be very rapid, the bath can be fairly short in length but should provide excellent microbial control. In developing the system, the catfish processing plant also could consider perforated belts and sprays from both sides. This technique can lead to off-gassing and worker safety problems, and may require a hood to remove off-gasses.

Washing the cutting boards with ozone-containing water actually increased the microbial load on the boards. The cutting boards are washed each day with foaming soap and a sanitizer. Both the soap and the sanitizer contain chlorine. Washing the boards with ozone-containing water after using the soap and sanitizer removes the residual chlorine and provides a medium for microbe growth. The present sanitation techniques for the daily cleaning of the cutting boards should be continued. If ozone-containing water is made available in the

plant, beneficial effects of ozone probably could be measured if equipment rinses are utilized during break and lunch periods. Ozonation during these times works well as it does not leave a residue and processing can restart as needed.

3.10.4. Cleaning of the Offal Room Air quality modification with ozone is an emerging technology that has tremendous promise. With this promise will come an equipment that can perform the following functions:

- Oxidize air pollutants
- Ensure worker safety with ozone levels of less than 0.05 ppm ozone in the workplace

The test conducted in the offal room did reduce odors. While the test was being conducted, an aero-QUAL unit was utilized to monitor ozone levels. The ozone in the offal room reached 0.045 ppm within 15 minutes and would have climbed much higher if the ozone generator had not been stopped. The test was successful since it showed that ozone released into the room will quickly reduce the level of fish odors.

If plant management wishes to ozonate the room, any of the three approaches below can be taken:

1. UV lights can be installed to provide ozone at low levels and provide continuous air cleaning.
2. Small ozone generator(s) can be mounted in the ceiling and utilized to reduce the odor levels.
3. If ozone is utilized in the plant, it may be possible to take a small side stream to treat the air in the offal room.

Warning—regardless of the method adopted, the following must be included:

- Monitors must be in place to turn off the ozone generator(s) if and when the ozone concentration in ambient air rises above 0.045 ppm when personnel are present.
- If higher levels are allowed, personnel must not be present and ozone levels must have dissipated before the room is opened for reoccupancy.

- If the room is allowed to have high levels of ozone, particular attention should be paid to oxidizing rubber items (tires) and air handling equipment.

These warnings are provided because many individuals utilize ozone under the principle that if a little ozone does a little good—a lot must do a lot of good. In ozone technology, the *proper amount* works very well. Lesser and higher amounts should be avoided.

3.10.5. Conclusions and Recommendations

3.10.5.1. General Conclusions Based on the tests conducted at this catfish processing plant, the following was concluded:

- Ozone-containing water rinses and washes can provide statistically significant reductions in microbe levels in catfish processing plants.
- Gaseous ozone can be employed to reduce odors and create a more pleasant workplace environment.
- Ozone-containing water rinses utilized in conjunction with sanitizers and soaps containing chlorine can actually remove the chlorine and reduce the residual effect of soaps and sanitizers.

3.10.5.2. Future Recommendations and Considerations In developing future growth plans and processes, the following may be considered:

- Ozonation of fish being hauled to the plant.
- Ozone-water washes of the fish trucks transporting fish to the plant.
- Utilizing a daily clean-up for 4–6 hours each day and run two 8-hour shifts with a pressure washing followed by an ozone wash between shifts. (This is an excellent method of spreading depreciation).
- Utilize ozone-water for drain cleaning and waste conveyor cleaning.

3.10.6. Response of Plant Management to this Case Study Based on the results of this detailed case study and the recommendations made therein, the catfish processor was made aware of the many benefits of ozone in the process. The demonstration

was conducted just prior to Hurricane Katrina striking the area, and the project is on hold. It will be considered and installation of ozone will take place in the future.

3.11. Storage of Apples in Air Containing Ozone

In 2003, a large Washington State apple grower installed ozone equipment in several storage rooms to determine the impact of ozone treatment of air in controlled atmosphere apple storage rooms, and to compare these results with results of storage in controlled atmosphere rooms not supplied with ozone. Sufficient tests were conducted to provide statistical confidence. Apples had been in storage approximately 4 months, and paired rooms were compared. Samples of refrigerated packed apples and the ambient packing room air during operation also were examined (Graham, 2005).

3.11.1. Procedure Air samples were drawn through the porthole of sealed controlled atmosphere storage rooms, from the air space of refrigerated packed apple storage rooms, and from three areas in the packing room during normal operation.

1. Air samples were collected with a SAS Super 100 volumetric air sampler programmed to collect 100 L of room air in 60 seconds from the storerooms and 50 L of air in the packing area.
2. Air samples were collected on 90 mm PDA agar plates for yeast and mold counts, drawing six replicate samples from each of ten storerooms plus three locations in the ambient temperature packing area.
3. Air samples were collected through a porthole in the door, using precautions to avoid air from outside the storeroom. Inoculated Petri plates were covered and inserted into sterile sleeves for transport to a laboratory in Walnut Creek, CA, for 5-day incubation and counting. Representative plates also were photographed.
4. Plates were identified with the storage room number, and three locations in the packing area.
5. The SAS collection head was swabbed with alcohol between rooms but not between replicate plates. Any cracked plastic plates were discarded before sampling.
6. This sampling protocol yields a total of 66 inoculated Petri dishes.
7. Temperature in the sampling area ranged from 31 to 40°F. Ambient temperature ranged from 35 to 60°F during the sampling period.

3.11.2. Results and Comments The data were compiled on an Excel Spread sheet and analyzed for *sum*, *average*, and *standard deviation* (Table 22.4). The counts in the packaging room were excessive, exceeding 187,000 cfu/ft³ at all three packing room locations (Table 22.5). All mold and yeast counts in the apple storerooms were very low, less than 3,000 cfu/ft³, with minimal differences between paired rooms without and with ozone. Representative samples of mature Petri plates were photographed to illustrate results graphically.

The low (3,000 cfu/ft³) mold and yeast counts in the controlled atmosphere rooms are so low that addition of ozone to the controlled atmosphere rooms is not likely to yield much benefit.

In current practice, the apples in storage have some residue of as many as three different chemical treatments as well as the gas mixtures maintained in controlled atmosphere storage. Ozone may be a good candidate for replacing one or more of these aggressive chemical treatments. This approach could be explored on small lots of apples in tightly controlled test rooms.

Ozone could be effective and is a more environmentally friendly agent than the currently used antifungal drench used on all truckloads of apples in bins.

The greatest benefit from ozone is likely to be found in cleanup of the heavily burdened flume water at the bin dump stations and the packing area air.

4. Concluding Remarks

The case studies discussed in this chapter are but a few of the many examples of successful applications of ozone in the Agri-Food industry known to date.

Table 22.4. Mold and yeast counts (cfu/100 L air sample) in paired apple storage rooms (Graham, 2005)

Room Number, Apple Variety, Ozone?	Mold, CFU	Yeast, CFU	Room Number, Apple Variety, Ozone?	Mold, CFU	Yeast, CFU
57, Fuji, yes	1	0	69, Fuji, no	5	0
	4	0		1	0
	11	0		3	0
	5	0		2	0
	1	0		5	0
	6	0			
<i>Sum</i>	28	0	<i>Sum</i>	16	0
<i>Average</i>	4.7	0	<i>Average</i>	3.2	0
<i>Standard deviation</i>	3.7	0	<i>Standard deviation</i>	1.8	0
25, boxed fruit, yes	6	2	48, boxed fruit, no	1	0
	2	0		2	0
	7	1		2	1
	5	0		1	1
	1	0		6	0
	5	0		4	0
<i>Sum</i>	26	3	<i>Sum</i>	16	2
<i>Average</i>	4.3	4.3	<i>Average</i>	2.7	2.7
<i>Standard deviation</i>	2.34	2.3	<i>Standard deviation</i>	2.0	2.0
38, Golden, yes	1	0	39, Golden, no	1	0
	2	0		0	11
	1	1		0	1
	4	0		0	1
	0	0		2	0
	0	0		0	2
<i>Sum</i>	8	1	<i>Sum</i>	3	15
<i>Average</i>	1.3	1.3	<i>Average</i>	0.5	0.5
<i>Standard deviation</i>	31.5	1.5	<i>Standard deviation</i>	0.8	0.8
40, Gala, no	0	9	41, Gala, yes	3	7
	1	0		3	0
	0	0		17	0
	1	1		1	2
	2	17		3	0
	1	6		6	1
<i>Sum</i>	5	33	<i>Sum</i>	33	10
<i>Average</i>	0.8	5.5	<i>Average</i>	5.5	1.7
<i>Standard deviation</i>	0.8	6.7	<i>Standard deviation</i>	5.9	2.7
55, Fuji & Rome, no	6	0	58, Fuji & Rome, yes	1	0
	4	0		0	0
	2	0		0	0
	6	0		1	0
	2	0		1	0
	11	1		1	0
<i>Sum</i>	31	1	<i>Sum</i>	4	0
<i>Average</i>	5.2	0.2	<i>Average</i>	0.7	0
<i>Standard deviation</i>	3.4	0.4	<i>Standard deviation</i>	0.5	0

Table 22.5. Mold and yeast counts in ambient temperature apple packing area during normal operations (Graham, 2005). Duplicate 50-L air samples from each area

Area Number	Description	Mold, CFU	Yeast, CFU
1	Air above bin dump	>487	5
2	Air above brush dryer	>487	5
		>487	3
		>487	6
3	Air above packing station	>487	12
		>487	10
		>487	10

Many firms using ozone today are hesitant to divulge any information about their commercially successful applications of ozone for fear of losing their competitive position. Other firms simply have no incentive to tell the world how they are using ozone, preferring to spend their time producing and marketing higher quality food products.

Some food applications of ozone are decades old, such as the cold storage of eggs, mold control in European breweries and wine cellars, and depuration of shellfish in Mediterranean countries.

In Japan, since about the mid 1980s, multiple applications for ozone in the food industry have been developed, from preservation and storage of fresh-caught fish at sea, to treatment of raw agricultural products entering food processing plants, to packaging of processed foods (Naito and Takahara, 2006). The Japanese even install ozone-generating UV bulbs in ceilings of food processing plants. These are turned on only at night (in the absence of plant workers) to cope with *Listeria* in the plant. Ozone is generated in reasonable quantities by UV radiation at 185 nm, and being heavier than air, falls slowly to ground level where it can find its way into cracks and crevices of walls, floors, and food processing equipment and do its antimicrobial work. The UV lamps are turned off automatically (by means of a timer) about an hour before the morning shift enters, so that any remaining ozone will dissipate.

As interesting as these long-time applications for ozone in various segments of the Agri-Food industry

are, there are no published case studies to provide specific details of use for those interested in taking advantage of many of the benefits of ozone.

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Chapter 23

Ozone Pathway to Commercialization

James T.C. Yuan

1. Introduction

The challenge of commercializing ozone is the design of a complete system that can be integrated into the existing operation environment. In many people's mind, ozone processing is all about choosing a good and efficient ozone generator but unfortunately, that perception only counts for a small portion of a complete ozone processing system. From an ozone generator to an ozone processing system is like an engine to a car—it is crucially important, but it is not the only part that makes car move from point A to point B. A successful ozone system would deliver a powerful sanitation/disinfection function with cost-effective and environment-friendly features toward food safety initiatives. Few good concise overviews of ozone which were published by Khadre and others (2001), Kim and others (1999), and other chapters of this section already well covered ozone treatment basics and ozone applications in case studies. This chapter mainly illustrates the key factors that industry would consider for the commercialization of ozone technology.

2. A System Concept

As mentioned in the Introduction, the first point to keep in mind when considering implementation of ozone technology is the “system” concept, and not just few components fitting together. The other im-

portant points are (1) every commodity and finished product is different, and (2) every processing facility is different. Therefore, there is no cookie cutter solution for this technology, and we have to look into each unique situation and come up with a solution. With those said, however, basic components mentioned below lay a good foundation of consideration for the commercialization.

3. Power Supply

Ozone requires energy to split the oxygen and form an ozone molecule, and electricity is usually the source of energy. We often need to make sure we have enough electrical power allocated to the ozone system, which means high-voltage and/or high-frequency power supply. We also need to be aware that about 70% operating cost of ozone is from energy consumption, and we have to build this cost factor into our final planning.

4. Feed Gas

Ozone is an active (or unstable) form of oxygen, so the source of oxygen is important. The source of oxygen (feed gas) could come from either air or pure oxygen, and could be generated on-site or from a cylinder, as long as it meets two basic requirements:

1. Dry
2. Oil and particle free (at -60°C dew point)

Furthermore, an air/oxygen filtration system is necessary to remove particulate matter and moisture from the air or oxygen stream entering the generator.

Any contaminants may affect the electrical discharge. When using a corona discharge (CD) type of ozone generator, dry air or oxygen is required for the feed gas, since moisture in the feed gas will cause nitric acid to form inside the generator and will decrease the efficiency of ozone production or even lead to generator failure. Moisture can be removed by molecular sieves, activated alumina, silica gel, or a combination of refrigeration and desiccation. A simpler solution for food processors is to use the air or oxygen supply from an industrial gas company.

5. Ozone Generator

The popular types of ozone generators are built on ultraviolet (UV) or CD, and some important factors to be considered for choosing a good ozone generator are as follows:

- Oxygen fed
- Safety interlocks
- Automatic control
- Sturdy construction with good compatible materials

The UV ozone generator produces ozone from the irradiation of air with UV light at wavelengths below 200 nm, specifically 185 nm. Wavelengths above 250 nm (254 nm optimum) destroy ozone rather than produce it. High-energy UV light splits oxygen molecules (O_2), freeing unstable oxygen radicals (O) ready to react with other oxygen molecules (O_2) and creating triatomic oxygen or ozone (O_3). Low airflow rates are necessary to ensure greater UV absorption by the volume of air, leading to a higher efficiency of conversion of oxygen to ozone. The maximum ozone concentration that can be produced using UV is less than 0.1% by weight. The more efficient method used for ozone production is CD. This type of ozone generator, as exemplified in Figure 23.1, requires a high-voltage electrical discharge (5,000 volts minimum to 14,000 volts maximum). The electrical discharge or corona causes O_2 in the air stream to split and collide with other oxygen molecules, forming O_3 in much the same way as in the UV generation. The CD ozone generator normally contains from 2 to 4% (by weight) ozone



Figure 23.1. A good ozone generator is an essential element to the integrated system. (For color details, please see color plate section.)

using dry air, and up to 14% (by weight) ozone using high-purity oxygen as the feed gas. Oxygen-fed systems increase generator efficiency by making more O_2 molecules available and reducing maintenance.

6. Ozone Contact/Transfer

This is an essential part that determines the efficiency of an ozone system, but yet the area has often been overlooked. Factors affecting mass transfer include the following:

- Gas phase concentration
- Gas to liquid ratio (bubble surface area)
- Pressure within the contacting system
- Water temperature
- Mixing (gas/liquid interface)
- Contact time
- Instantaneous O_3 demand

Gaseous ozone is a more effective and reactive sanitizer in humid environments. If the application

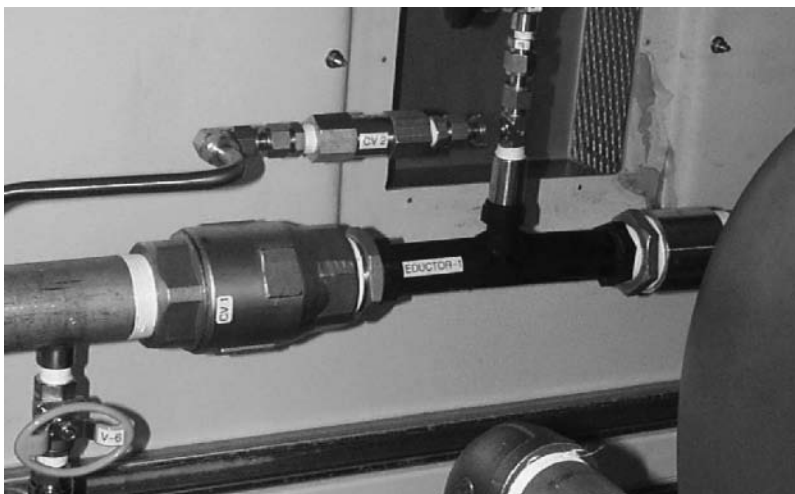


Figure 23.2. To achieve a good mass transfer of ozone gas into the aqueous system, Venturi injector is one of solutions. (For color details, please see color plate section.)

of ozone includes use in water sanitation, there is a need for optimizing gaseous ozone solubility in the aqueous phase. To this end, several variables become significant including water temperature, pressure, filtration, and pH. Generally speaking, the colder the temperature, the higher the pressure, the less the ozone demand (through filtration), and the lower the pH would increase the mass transfer or solubility of ozone. In addition, the concentration of ozone in the water will be influenced by the concentration of ozone in the carrier gas and the mode of ozone injection into the water. One of the most effective means of introducing ozone into a water stream involves the use of a Venturi injector (Figure 23.2). The water stream is used to produce a vacuum. Ozone is drawn into the Venturi by the vacuum and mixed with water. The small bubbles (and the large contacting surface area) of ozone-containing gas produced enable enhanced ozone solubility in water.

7. Ozone Safety: Off-gas Removal/Destruction

When designing an ozone-integrated system, it is important that all plumbing, tubing, and hardware coming into contact with ozone be carefully selected

(see Table 23.1, ozone compatibility table). Ozone is capable of oxidizing most metals (e.g., iron, copper) leading to corrosion. Rubber and plastics, when oxidized, will harden and crack. All piping, gaskets, and sealing compounds must be resistant to this strong oxidant, and careful planning must be executed to ensure longevity of the sanitizing system. The permissible exposure level of ozone to which workers may be exposed is 0.1 ppm averaged over a 40-hour workweek. The short-term exposure limit is 0.3 ppm over 15 minutes, allowed twice per 8-hour workday. The concentration of 5 ppm ozone in air is generally accepted as immediately dangerous to life or health. In order to reduce worker risks, the ozone integrated application system needs to be well contained. The ozone generator and associated equipment should be housed in a specified area with appropriate ventilation including a minimum of six air changes per hour as measured six inches from floor level. The exhaust must be ventilated away from building openings and should not exceed the air supply. It is essential to strategically use ambient ozone monitors and equipment to detect ozone levels in the room and application areas, as well as for internal system checks. It is also necessary to have an effective ozone destruction system incorporated to remove or destroy reactor

Table 23.1. Material compatibility with ozone

Materials	Gaseous Ozone (Dry)	Gaseous Ozone (Humid)	Aqueous Ozone
<i>Metals</i>			
Copper	*	*	*
Brass	*	*	*
316L Stainless steel	****	****	****
<i>Plastics</i>			
ECTFE (Halar [®])	***	***	***
PTFE (Teflon [®])	****	****	****
PVC (rigid)	***	***	***
PVC (flexible)	**	**	***
PVDF (Kynar [®])	***	***	****
<i>Elastomers</i>			
FPM (Viton [®])	***	***	***

Sources: 8th International Symposium on Corrosion in the Pulp and Paper Industry. Stockholm, Sweden, May 16–19, 1995. Pulp and Paper Industry Corrosion Problems, Volume 8.

off-gas before venting to the atmosphere. Off-gas may be removed under pressure by use of a degassing separator. Installation of a fan downstream can be used to draw the off-gas through the system. A water trap could be used to collect condensate or liquid escaping from the contact or degas tank. The ozone in the off-gas could be destroyed by 5-second exposure to 220°C, irradiation with UV light at 254 nm, or passage through a bed of dry manganese dioxide catalyst material.

8. Other Considerations

Putting together all of the above components may not be enough for all applications. There are other items that are also vital to the system. For example, a proper water filtration system is almost as important as the ozone generator for water reclamation or recycling applications. Because ozone consumption is correlated with high concentrations of suspended matter, the majority of organic constituents (ozone demand) must be filtered out in order to prevent ozone consumption prior to contact with the microorganisms. Filtration of aqueous systems to reduce organic loads will increase the efficiency of the ozone system in its intended use. Another key factor is reliable dissolved ozone monitoring to ensure reproducibility and regulation of dissolved ozone once the optimal ozone concentration or dose has been established for a specific

application. The regulation of ozone concentrations is accomplished through efficiently designed monitoring and application systems. Additional components should include an adequate power supply to feed electricity to the system as well as emergency shut-off switches external to the system and adequate lighting.

9. Secret to a Successful Commercialization

Ultimately, every integrated ozone system will need a careful thought and expert engineering and design to accommodate the specific application with optimum efficiency. Main reason that I keep mentioning of customizing an ozone system to a specific

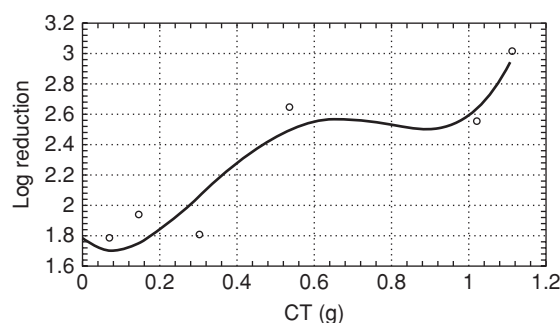


Figure 23.3. Ozone kinetics: the kinetics of ozone biocidal efficacy.

Table 23.2. Example of CT (ozone concentration × ozone contact time) and impact on microbial reduction.

	E. coli		Salmonella	
	2 ppm	5 ppm	2 ppm	5 ppm
Original	6.65	6.69	6.46	6.44
After 30-second wash	4.22	3.98	4.71	4.28

application is the well-kept secret: each and every application is unique in its processing nature and it requires special attention in building an optimum system. For example, Figure 23.3 clearly demonstrates that because microbial reduction kinetic of ozone is biphasic, even with increased CT (product of ozone concentration and ozone contact time), the microbial reduction is not necessary in the linear relationship. More data confirms the ozone biocidal kinetics that

increased CT not necessary represents more of microbial kill (Table 23.2). So, we have to be careful on where and what do we plan to do with ozone in our facility. Otherwise, we may end up with either not putting enough CT to kill unwanted microorganisms or use too much CT and waste our resource (also translate to the bottom line). Be cautious on your product, process, and production line; do understand how ozone can be applied to your need, conduct your investigation (or together with a reputable vendor), and set up your own ozone system and CT, then the success will be on your side.

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Chapter 24

Effects of Dense Phase CO₂ on Quality Attributes of Beverages

Sibel Damar and Murat O. Balaban

1. Introduction

Dense phase CO₂ (DPCD) technology is a cold pasteurization method that uses antimicrobial effects of CO₂ under high pressure. DPCD has been shown to inactivate pathogens, certain spoilage organisms, and enzymes affecting the quality of food products (Kamihira and others, 1987; Balaban and others, 1991a; Dillow and others, 1999; Shimoda and others, 2001; Sims and Estigarribia, 2002; Kincal and others, 2005). It is applied to liquid foods under temperatures lower than 60°C, mostly below 35°C and using pressures below 50 MPa. A limited number of studies on the quality effects of DPCD show that this method can effectively inactivate pathogens and certain enzymes while retaining fresh-like physical, sensory, and nutritional quality of food products (Arreola and others 1991; Kincal 2000; Ho 2003; Folkes, 2004; Damar and Balaban, 2005; Kincal and others, 2005; Del Pozo Insfran et al., 2007).

DPCD technology has been investigated over the past 50 years. Fraser (1951) was first to show the inactivation of bacterial cells under high pressure. Although the exact mechanism of microbial inactivation by DPCD is still not clear, studies show that there are several possible means of inactivation. CO₂ can lower the pH when dissolved in the aqueous part of a food by forming carbonic acid,

and can also lower the internal pH of microbial cells by permeating through the cell membrane. Once the internal pH is reduced, cells are inactivated by inhibition of metabolic enzymes (Daniels and others, 1985; Ballestra and others, 1996). Some researchers suggested that molecular CO₂ can inhibit certain bacterial enzymes by forming a bicarbonate complex (Weder, 1990; Weder and others, 1992), and others suggested that calcium- or magnesium-binding proteins could be precipitated by the carbonate ion, resulting in lethal change (Lin and others, 1993). There are studies that show the physical rupture of microbial cells by DPCD (Isenschmid and others, 1995; Ishikawa and others, 1995; Dillow and others, 1999; Hong and Pyun, 1999; Spilimbergo and others, 2003). This was explained by the rapid expansion of CO₂ during pressure release to cause disruption of the cells. Another suggested mechanism of microbial inactivation is the modification of cell membrane and extraction of intracellular components due to the enhanced solvent properties of CO₂ under high pressure (Kamihira and others, 1987; Isenschmid and others, 1995; Hong and Pyun, 1999, 2001).

Although which of the mechanisms on microbial inactivation by DPCD is the most important is unknown, researchers agree in the governing role of CO₂. Generally, any effect that increases CO₂ solubility in a treatment medium and, therefore, penetration of CO₂ into the cells enhances microbial inactivation by DPCD. The type of treatment system, treatment conditions (pressure, temperature, treatment time, CO₂ level), treatment medium

(water activity, presence of dissolved materials, pH) and type of microorganisms are the factors affecting the microbial inactivation by DPCD. Depending on these factors, microbial reduction achieved in vegetative cells ranged from 2 to 12 logs (Damar and Balaban, 2006).

Studies on enzyme inactivation indicate good potential for the use of DPCD, especially in fruit and vegetable processing where certain enzymes cause quality deterioration if not inactivated. These enzymes can be inactivated at various levels by DPCD, depending on the type and source of the enzyme, and DPCD treatment conditions. Balaban and others (1991a, 1991b) showed that DPCD can inactivate certain enzymes at temperatures where thermal inactivation is not effective. Pectinesterase (PE), polyphenol oxidase (PPO), lipoxygenase (LOX), and peroxidase (POD) are among the enzymes that were treated with DPCD and shown to be inactivated at certain levels at temperatures below 55°C (Balaban and others, 1991a; Chen and others, 1992, 1993; Tedjo and others, 2000; Park and others, 2002).

In the last two decades, the number of research and patents on DPCD increased and commercial-

ization efforts intensified and various types of batch, semicontinuous, and continuous systems were developed. Today, there are two commercial systems available, a continuous-flow system developed by Praxair Co., and a continuous membrane contact system offered by Air Liquide Co. The food products that were treated with DPCD include orange juice (OJ), apple juice, grape juice, coconut water, watermelon juice, mandarin juice, carrot juice, juice blends, beer, kava kava, milk, and sugar cane juice.

This chapter reviews the DPCD treatment systems and the effects of DPCD on the food quality.

2. DPCD Treatment Systems

Several batch, semicontinuous, and continuous systems have been used in DPCD applications. Early studies used batch systems where CO₂ and treatment medium are stationary in a container during DPCD treatment. The system includes a CO₂ gas cylinder, treatment chamber, pressure regulator, water heater, and CO₂ release valve (Figure 24.1). The sample is first placed into the chamber and heated to the desired temperature, and then CO₂ is introduced to the

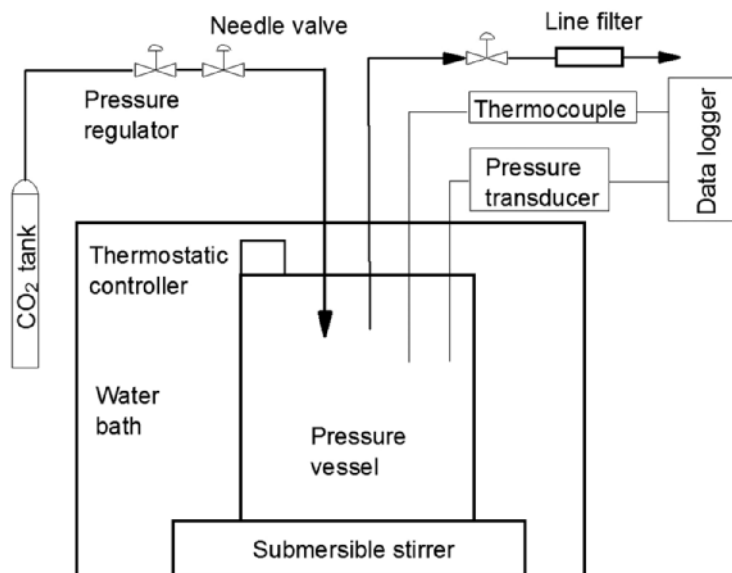


Figure 24.1. A typical batch DPCD treatment system (Hong and Pyun, 1999).

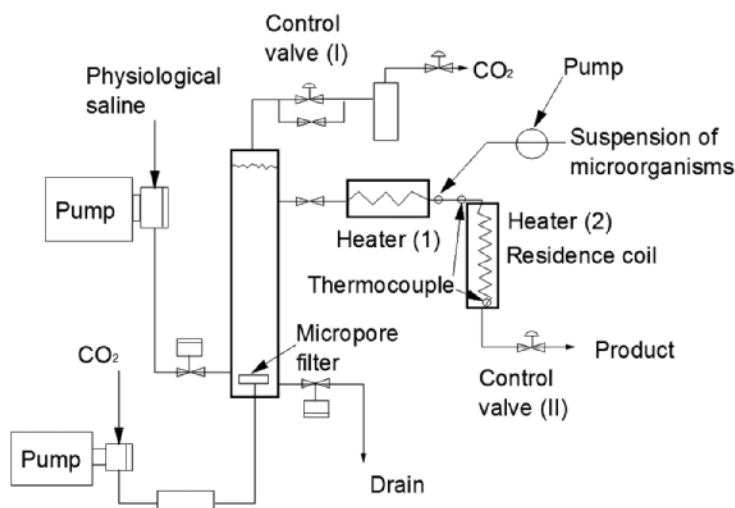


Figure 24.2. A continuous microbubble DPCD system (Shimoda and others, 2001).

chamber until the desired pressure is achieved. Treatment time in these systems can take hours to achieve the saturation of treatment medium with CO₂. Some systems use an agitator to decrease saturation time.

A semicontinuous microbubble CO₂ system was developed by Ishikawa and others in 1995, where a cylindrical filter was used to microbubble CO₂ entering the pressure vessel. The use of the filter accelerated the saturation of treatment medium and provided three times more inactivation of enzymes than without using it. In 2001, Shimoda and others developed a continuous microbubble system (Figure 24.2). In this system, liquid CO₂ and treatment medium were pumped continuously through a cylindrical vessel. Liquid CO₂ was first changed to gas and entered the vessel through a stainless steel mesh filter and moved upward through the treatment medium and saturated it.

A continuous membrane contactor system was developed by Sims (2001), where a large CO₂-liquid contact area was provided using hollow membrane modules. In the membrane contactor, CO₂ diffused into the liquid to reach saturation levels instantaneously (Figure 24.3). CO₂ was recycled back and reused in this system. This system has been shown to be effective in inactivation of several microorgan-

isms in short period of times (Sims and Estigarribia, 2002).

Praxair Co. developed a continuous flow DPCD system in 1999 (Figure 24.4). Liquid CO₂ and the food product are pumped through the system and mixed before passing through the high pressure pump, where the pressure is brought to the desired levels. Temperature is controlled and residence time is set by flow rate adjustment through the holding coils. A back pressure valve releases CO₂ pressure at

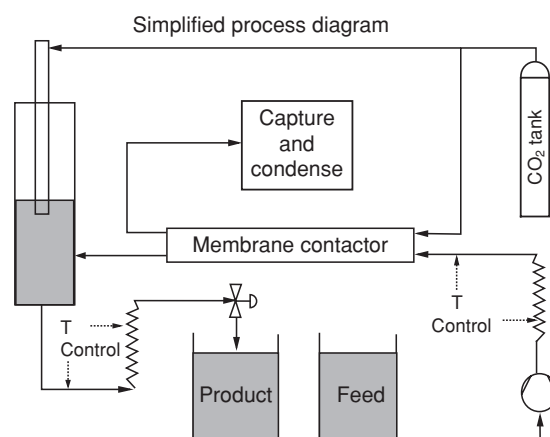


Figure 24.3. A continuous CO₂ membrane contactor system (Novak, 2005).

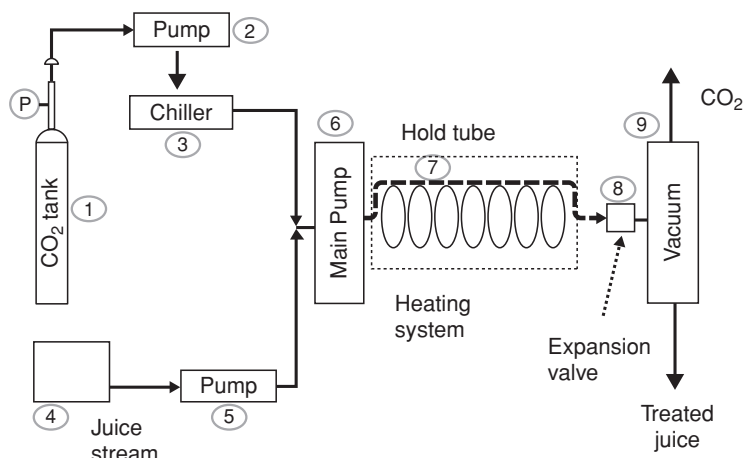


Figure 24.4. A continuous-flow DPCD system.

the end of the process. This system has been shown to be effective in killing pathogens and spoilage bacteria in short treatment times (Folkes, 2004; Damar and Balaban, 2005; Kincal and others, 2005; Lecky, 2005).

3. Orange Juice Quality after DPCD Treatment

Traditionally, OJ is thermally processed. The primary objective of this is to inactivate the enzyme PE that causes loss of cloud. Typically, pasteurization is done at 90°C for 10 seconds. However, this causes undesirable flavor and aroma changes.

Arreola and others (1991) measured quality attributes (pH, Brix, cloud stability, total acidity, color, ascorbic acid (AA) content, and sensory attributes) of OJ treated with supercritical-CO₂ (SC-CO₂) under 7–34 MPa, 35–60°C and 15–180 minutes in a batch system. OJ was placed into a vessel, flushed, and pressurized with CO₂ and brought to the desired pressure and temperature. Samples were withdrawn from the chamber periodically without affecting the pressure. The results showed that there was no significant difference in pH or Brix of the original juice and SC-CO₂ treated juice ($p < 0.01$). This showed that although pH of the juice was lowered during SC-CO₂ treatment, as soon as pressure was released, CO₂ left the system and pH returned to the original value.

AA retention was significantly higher in SC-CO₂-treated samples compared to temperature controls, samples held at the same experimental temperature as DPCD treated samples for the same amount of time ($p < 0.01$). Results showed that AA retention was about 71–95% in DPCD samples while it was only retained by 62–83% in temperature control samples (Figure 24.5). Higher AA retention in DPCD samples was explained by the higher stability of AA under low pH provided during DPCD and also under the O₂-excluded environment. AA oxidizes easily when oxygen is present.

Cloud was enhanced in DPCD samples from 1.27 to 4.1 times regardless of treatment temperature (Figure 24.6), and was stabilized even in the presence of residual PE. This showed that PE inactivation is not the only reason for cloud enhancement and stability by DPCD. Shearing and homogenization through the expansion valve might have caused size reduction in suspended particles and higher solubility of these particulates in the juice. Cloud stability of DPCD samples (29 MPa, 50°C, 4 hours) was retained after 66 days of refrigerated storage, whereas temperature control (50°C, 4 hours) and room temperature control (25°C, 4 hours) lost the cloud completely (Figure 24.7).

Instrumentally measured color scores showed higher brightness of DPCD-treated OJ compared to fresh untreated OJ. Sensory evaluations of untreated,

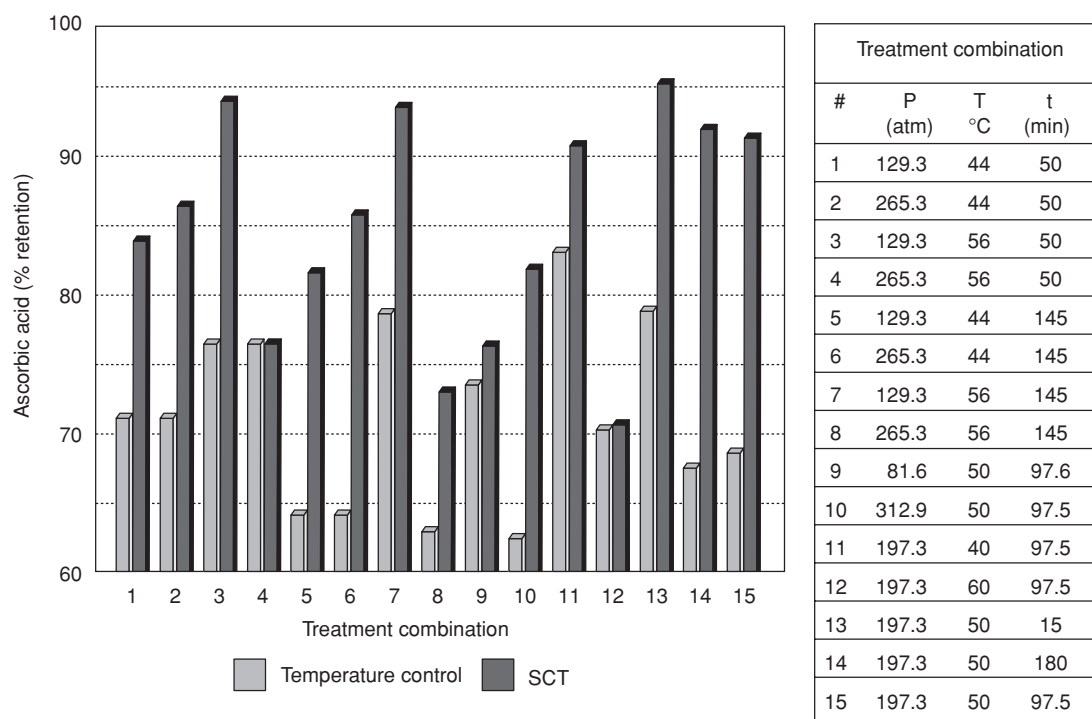
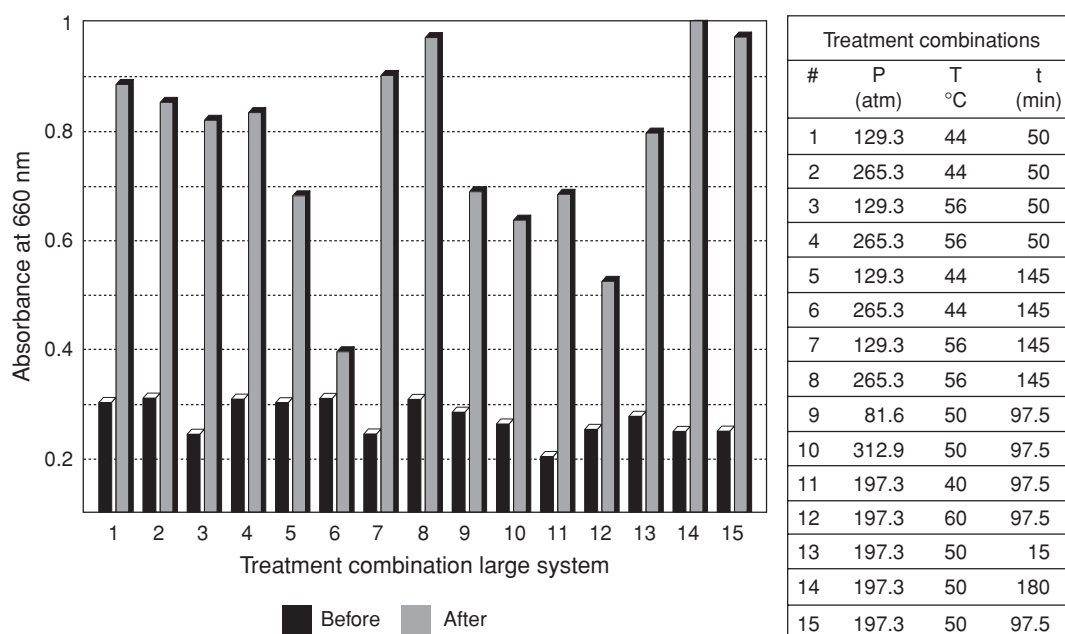


Figure 24.5. Ascorbic acid retention (%) of DPCD-treated and temperature controls of single strength orange juice.



Cloud is expressed as absorbance at 660 nm

Figure 24.6. Cloud of single strength orange juice before and after DPCD treatment.

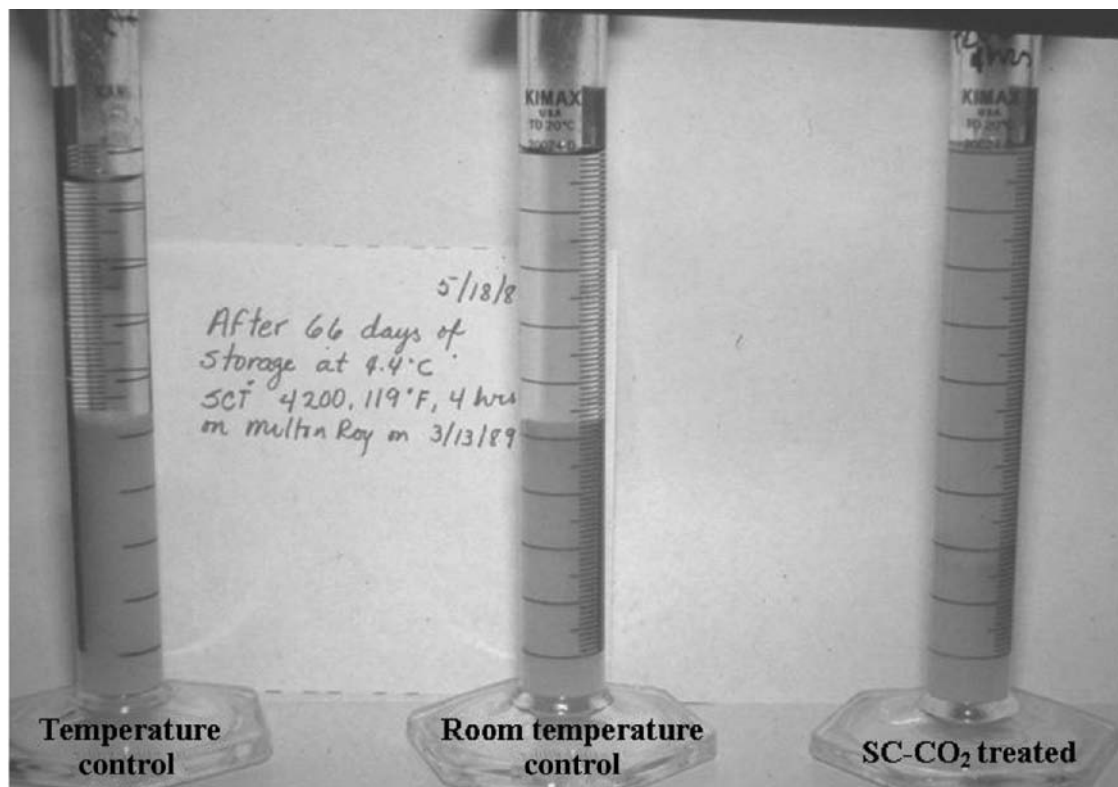


Figure 24.7. Cloud stability of temperature control, room temperature control, and DPCD treated orange juice samples after 66 days of refrigerated storage at 4.4°C. (For color details, please see color plate section.)

DPCD and commercially pasteurized (thermal) samples by 30 untrained panelists showed that overall acceptability, flavor, and aroma of fresh and DPCD-treated OJ were not significantly different (Figure 24.8). Commercial samples had significantly less acceptability, flavor, and aroma. The color, cloud, and appearance of DPCD-treated OJ were liked more than fresh or commercial OJ.

The sensory, nutritional, and physical quality enhancement in OJ by DPCD was also shown by Kincal (2000) in a continuous flow system. Treatment conditions were pressures of 38, 72, and 107 MPa, CO₂/juice ratios of 0.40–1.18, and residence time of 10 minutes. Cloud of OJ was enhanced from 446 to 846% after DPCD treatments compared to controls. No significant changes were observed in pH and °Brix. %TA of DPCD treated juice was sig-

nificantly higher than control juice ($p < 0.01$). Small but significant increases in L^* and a^* values were obtained after DPCD. No significant differences were observed for sensory attributes between fresh OJ and DPCD treated OJ after 2 weeks of refrigerated storage at 1.7°C.

4. Grape Juice Quality after DP-CO₂ Treatment

Grape juice is usually preserved by thermal pasteurization and addition of chemical preservatives in grape juice products and wine making. However, these methods may have adverse effects on the nutritional and sensory quality of grape juice. There are many studies showing that DPCD can effectively inactivate yeasts that would cause spoilage of the

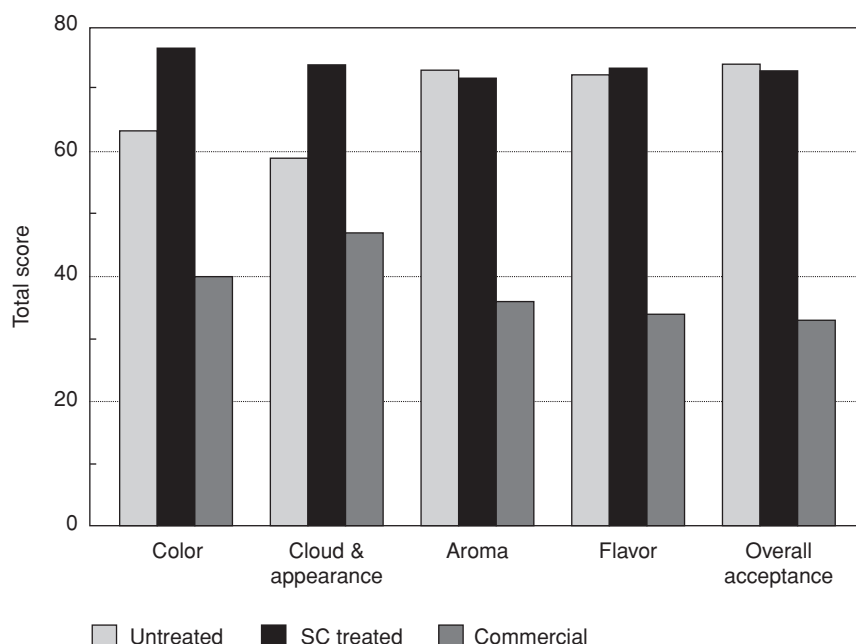


Figure 24.8. Sensory evaluation scores of control (untreated), DPCD-treated (Supercritical (SC)-treated) and commercial orange juice samples.

juices, and formation of off-flavors during fermentation of the wines (Gunes and others, 2005; Del Pozo Insfran et al., 2007). Del Pozo Insfran et al. (2007) pasteurized muscadine grape juice with DPCD (34.5 MPa, 8 or 16% CO₂) and heat treatments (75°C, 15 seconds), and measured the amount of phytochemicals, antioxidant capacity, and sensory attributes in comparison to unprocessed and heat-pasteurized grape juice. He showed that thermal pasteurization decreased anthocyanins by 16%, soluble phenolics by 26%, and antioxidant capacity by 10%, whereas DPCD-treated grape juice retained fresh-like levels of these (Table 24.1). He also showed that DPCD-treated grape juice retained higher amounts of total anthocyanins, antioxidant capacity (Figure 24.9), and total soluble phenolics (Figure 24.10) than thermally pasteurized juice at the end of 10 weeks refrigerated storage at 4°C. DPCD-treated grape juice, however, showed increase in the microbial counts starting from 5 weeks storage whereas there was no increase in microbial counts of heat-pasteurized sample.

Sensory evaluations of DPCD and heat treated, and unprocessed control grape juices were done by 60 untrained panelists. A continuous line scale from 0 = no difference to 10 = extremely different was used to rate the difference of each sample from the reference (fresh-control) for the color, flavor, and

Table 24.1. The effect of heat (75°C, 15 seconds) or DPCD (DP-1: 34.5 MPa, 8% CO₂; DP-2: 34.5 MPa, 16% CO₂) pasteurization on the total anthocyanin, soluble phenolic, and antioxidant content of unprocessed muscadine grape juice

Treatment	Total Anthocyanins ^a (mg/L)	Soluble Phenolics ^a (mg/L)	Antioxidant Capacity ^a (μM TE/mL)
Unprocessed	1,105 ^a	2,211 ^a	22.1 ^a
DP-1	1,077 ^a	2,213 ^a	20.7 ^a
DP-2	1,102 ^a	2,157 ^b	21.7 ^a
HTST	866 ^b	1,859 ^c	18.2 ^b

^aValues with similar letters within columns are not significantly different (LSD test, $p > 0.05$).

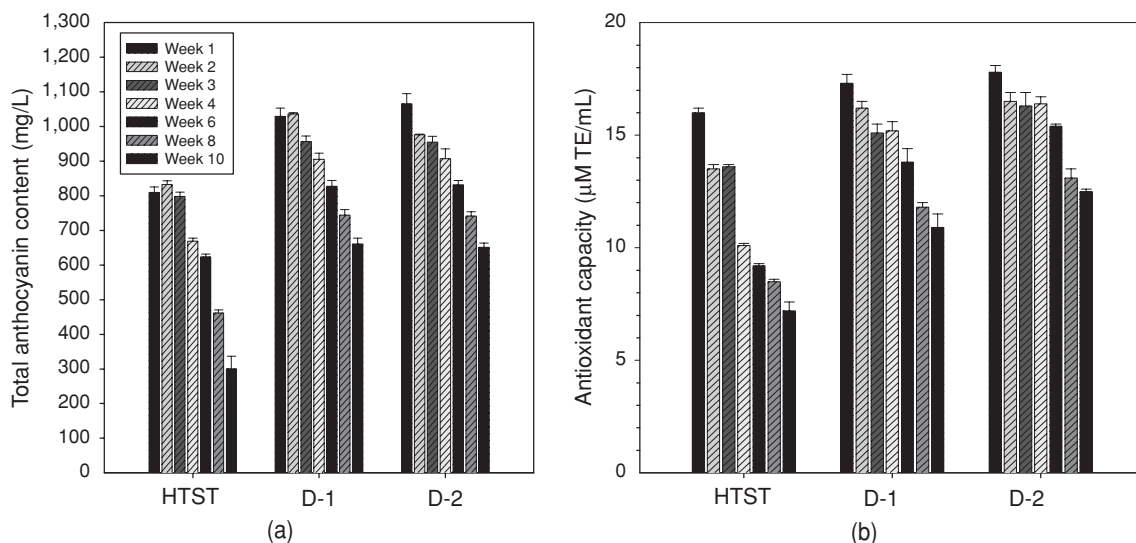


Figure 24.9. Total anthocyanin content (mg/L) and antioxidant capacity ($\mu\text{M TE/mL}$) of heat treated (High temperature short time (HTST):75°C, 15 seconds) and DPCD treated (D-1:34.5 MPa, 8% CO_2 , D-2:34.5 MPa, 16% CO_2) muscadine grape juice during 10 weeks of refrigerated storage (4°C).

aroma. Overall likeability of each sample was rated in a nine-point hedonic scale. Results showed that panelists liked DPCD-treated samples significantly more than heat-treated samples, and with no significant difference between the DPCD and fresh sample (Table 24.2). Flavor and aroma of DPCD-

treated grape juice was not significantly different than fresh juice, whereas heat-pasteurized samples were significantly different than fresh grape juice. Color of the fresh, heat-treated and DPCD-treated grape juice were not significantly different from each other. Therefore, DPCD treatment provided

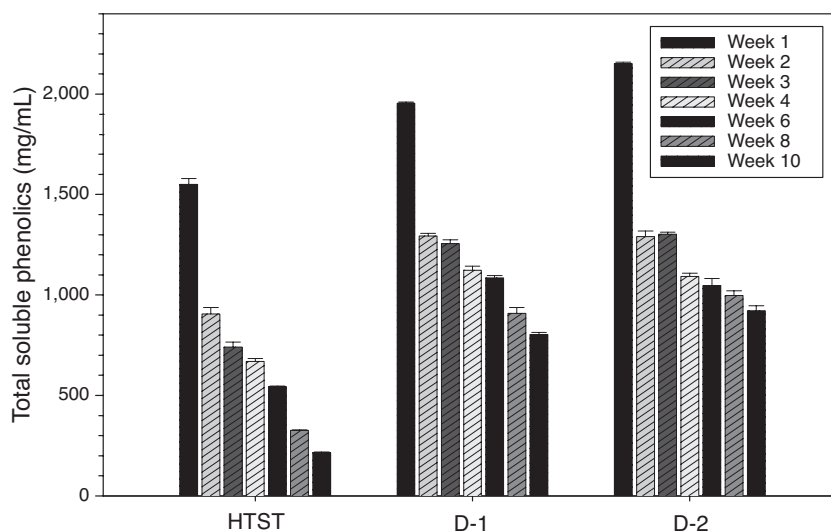


Figure 24.10. Total soluble phenolics (mg/mL) content of heat treated (HTST:75°C, 15 seconds) and DPCD-treated (D-1:34.5 MPa, 8% CO_2 , D-2:34.5 MPa, 16% CO_2) muscadine grape juice during 10 weeks of refrigerated storage (4°C).

Table 24.2. Differences in sensory attributes between fresh (hidden reference), heat-treated (HTST: 75°C, 15 seconds), and DPCD-treated (DP-1: 34.5 MPa, 8% CO₂, DP-2: 34.5 MPa, 16% CO₂) muscadine grape juice

	Difference in Color ^a	Difference in Aroma ^a	Difference in Flavor ^a	Overall Likeability ^a
Hidden reference	1.48 ^a	2.38 ^a	2.46 ^a	6.23 ^a
DP-1	2.00 ^a	2.74 ^a	4.32 ^a	5.98 ^a
DP-2	1.67 ^a	2.20 ^a	3.92 ^a	5.95 ^a
HTST	1.78 ^a	5.11 ^b	8.47 ^b	4.02 ^b

^aValues with similar letters within columns are not significantly different (LSD test, $p > 0.05$).

fresh-like quality in the first 5 weeks of refrigerated storage and offered advantages over thermal pasteurization.

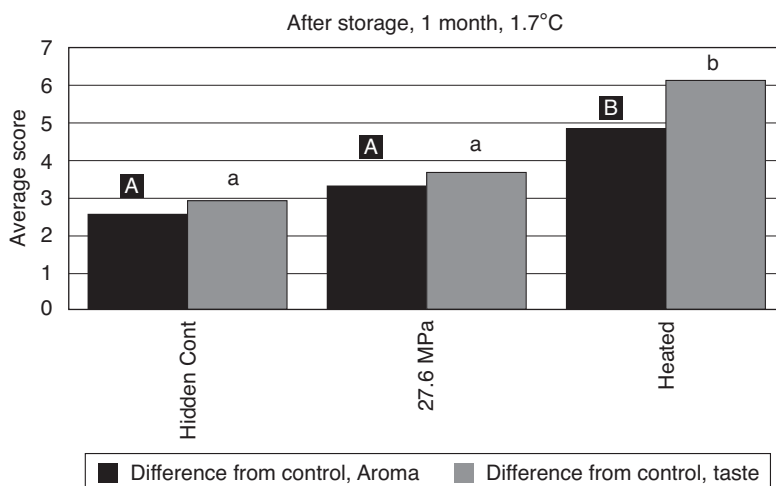
5. Quality of Beer after DPCD Treatment

Beer is a delicate and heat labile beverage. Off flavors are easily formed during heat pasteurization. Since freshness is top priority to brewers, the use of a nonthermal method in order to inhibit the growth of spoilage microorganisms is of great importance for quality. Sterile filtration is a nonthermal method that removes the spoilage bacteria and yeast without altering the color and flavor. However, this method requires extensive monitoring and is labor-intensive. Folkes (2004) pasteurized beer with a continuous DPCD system (27.6 MPa, 21°C, 5% CO₂, 5 minutes)

and evaluated the physical and sensory quality attributes such as foaming and foam stability, haze formation, aroma, and flavor. Sensory tests showed that aroma and taste of DPCD pasteurized beer were not significantly different from fresh beer after 1 month of storage (1.7°C) whereas those of heat pasteurized beer (74°C, 30 seconds) were significantly different ($p > 0.01$) (Figure 24.11). Beer haze was significantly reduced by DPCD. Foam capacity and stability was significantly less after DPCD treatment, but not at levels detrimental to the quality.

6. Sensory Evaluation and Flavor Profile of Coconut Water after DPCD Treatment

Our work at the University of Florida investigated consumer likeability and flavor profile of a

**Figure 24.11.** Taste and aroma difference from control scores of untreated (hidden control), DPCD treated (27.6 MPa) and heat-treated (heated) beer after 1 month of storage at 1.7°C.

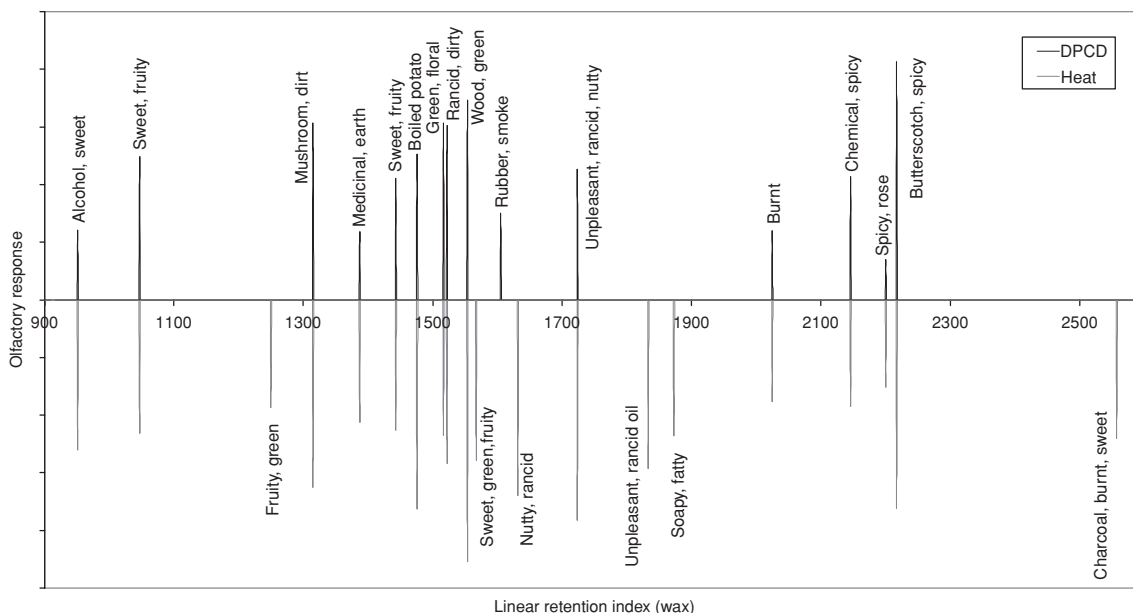


Figure 24.12. Comparison of aromagrams of DPCD (25°C, 34.5 MPa, 13% CO₂, 6 minutes) and heat (74°C, 15 seconds) processed coconut water beverages obtained from GC-olfactory port responses (2 weeks storage at 4°C).

DPCD-processed (34.5 MPa, 25°C, 13% CO₂, 6 minutes) coconut water beverage in comparison to that of fresh-untreated, and heat-processed (74°C, 15 seconds) samples. Sensory panels that were conducted throughout 9 weeks of refrigerated storage (4°C) showed that DPCD-treated and fresh coconut water beverages were liked similarly whereas heat-treated coconut water beverage was liked significantly less ($p > 0.05$). Aroma difference from control (untreated-fresh) scores of heat and DPCD-treated samples were not significantly different, but taste difference from control scores were significantly different ($p > 0.05$). Heat-treated samples were rated with significantly higher off-flavor scores than fresh or DPCD-treated samples ($p > 0.05$). The gas chromatography-olfactory analysis of flavor compounds in DPCD and heat-processed coconut water beverages were conducted, and an aromagram was developed by taking average area of the olfactory responses with the aroma descriptors given by the sniffers. This aromagram showed that there were differences in the aroma profiles of DPCD and heat-treated samples (Figure 24.12). Heat-treated samples had more aroma com-

pounds that were described as green, fruity, nutty, rancid, unpleasant, fatty, and burnt aromas. These aromas could be possibly developed by decomposition of aroma compounds due to heating.

7. Future Outlook

To date, there is no commercial food product processed by DPCD. There are two different commercial DPCD systems available; a continuous flow system developed by Praxair Co. (Chicago, IL) and a continuous membrane contactor system developed by Air Liquide Co. (Countryside, IL). The success of this technology on the market depends on the justification of the added process cost by the quality improvement and shelf-life extension, exploring niche areas where DPCD has advantages over other competing nonthermal technologies, and resolution of regulatory issues.

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Chapter 25

Chlorine Dioxide (Gas)

Lindsey A. Keskinen and Bassam A. Annous

Chlorine dioxide (ClO_2) is an oxidizing agent commonly used in water treatment to control odor and as a sanitizer for treatment of drinking water, by the pharmaceutical industry for the sterilization of equipment and surfaces, and is also used for the bleaching of flour and paper (Table 25.1; Encyclopedia of Chemistry, 2007). The predominant industrial use of ClO_2 is as a bleaching agent for wood pulp, applied by paper manufacturers; its bleaching action is, at least partially, due to its ability to inhibit the activity of polyphenol oxidase (Fu et al., 2007).

ClO_2 exists as a dissolved gas in water and it does not react with water molecules. As a potent oxidizing agent, ClO_2 will participate in oxidation reactions, but not in chlorination reactions with organic compounds present in water (Fukayama et al., 1986). This is in contrast to chlorine (Cl_2), which participates in chlorination reactions leading to the formation of potentially carcinogenic and teratogenic trihalomethanes and haloacetic acids (Stevens, 1982). Unlike ozone, another powerful oxidant and disinfectant, ClO_2 does not participate in reactions resulting in the formation of bromates, which are also considered carcinogenic (Encyclopedia of Chemistry, 2007). Relative to other oxidizing agents, ClO_2 is a strong oxidant and also a strong disinfectant (Tables 25.2 and 25.3; Rice and Gomez-Taylor, 1986).

ClO_2 will not remain in water over long periods of time and has an estimated half-life of 25 min-

utes as a dissolved gas in aqueous solution (EPA, 2006). In water, between pH 4–10, the dissolved gas exists as a free radical (EPA, 2006). At pH > 10, aqueous ClO_2 will dissociate to chlorate and chlorite ions (EPA, 2006). ClO_2 has a reddish-brown color in the liquid phase and a yellowish-green to orange color in the gaseous phase (EPA, 2006). Due to its color, chlorine dioxide gas (CDG) can be measured with an ultraviolet-vis spectrophotometer (Czarneski and Lorcheim, 2005). Dissolved ClO_2 in water can be measured by a variety of colorimetric methods (e.g., indirect amaranth dye method (ATOFINA), lissamine green B for ClO_2), amperometric titration, potassium iodide titration, or ion chromatography (Gordon, personal communication).

The US Occupational Safety and Health Administration set a time-weighted average of 0.1 parts per million (ppm; 0.3 mg/m^3) exposure limit (based on an 8 hours/day, 40 hours workweek) for CDG in the workplace, with a short-term exposure limit of 0.3 ppm within 15 minutes (OSHA, 2007). ClO_2 has a Cl_2 -like odor which is detectable by humans at a threshold of 0.1 ppm. Exposure to levels of CDG higher than a time-weighted average of 0.003 ppm for 8 hours may result in respiratory irritation (EPA, 2006). Ingested aqueous ClO_2 is rapidly absorbed in the gastrointestinal tract and is slowly cleared from the blood, and is then primarily excreted as chloride (EPA, 2000). The US Environmental Protection Agency (EPA) has set a limit for the residual concentration of ClO_2 to 3 ppm for postharvest application to fruits and vegetables which will not be sold as raw agricultural commodities (e.g., subsequently blanched, cooked, or canned) (EPA, 2006).

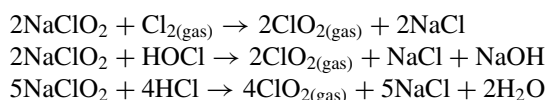
Nonthermal Processing Technologies for Food, Edited by
Zhang, Barbosa-Cánovas, Balasubramaniam, Dunne, Farkas, and Yuan.
This chapter remains with the U.S. Government.

Table 25.1. Chemical and physical properties of chlorine dioxide

CAS Registry Number	10049-04-4
Molecular formula	ClO ₂
Molecular weight	67.46
Melting point	−59°C
Boiling point	11°C
Water solubility	3.0 g/L at 25°C and 34 mmHg
Specific gravity	1.642 g/mL at 0°C (liquid); 3.09 g/L (gas)

(Encyclopedia of Chemistry, 2007).

CDG is considered highly unstable and is explosive at concentrations exceeding 10% in air (EPA, 1999). As a result, ClO₂ is not allowed to be shipped in the United States, and must be generated onsite (EPA, 1999). Three different ClO₂ generation reactions are predominant in commercially marketed ClO₂ generation systems (EPA, 1999):



One method of generation involves the mixing of two dry solid chemicals in gas permeable sachets,

Table 25.2. Relative oxidation power of various oxidizing agents (Rice and Gomez-Taylor, 1986)^a

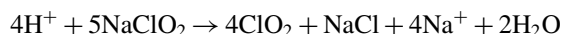
Species	Oxidation Potential (V) ^b at 25°C	Relative Oxidation Power ^c
Hydroxyl free radical	2.80	2.05
Ozone	2.07	1.52
Hydrogen peroxide	1.77	1.30
Permanganate ion	1.49	1.10
Hypochlorous acid	1.49	1.10
Chlorine	1.36	1.00
Hypobromous acid	1.33	0.98
Chlorine dioxide	1.275	0.94
Monochloramine	1.16	0.85
Hypoiodous acid	0.99	0.73

^aReproduced with permission from “*Environmental Health Perspectives*.”

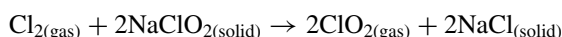
^bRelative to the hydrogen electrode.

^cBased on chlorine as reference (= 1.00).

allowing the CDG to pass through the sachet and into the surrounding air or water (ICA TriNova, LLC, Forest Park, GA). The combination of the two dry solids produces ClO₂ via the following reaction (ICA TriNova, LLC):



Another method is a gas:solid method which is commercially available (CDG Technology Inc., Bethlehem, PA). It generates ClO₂ through the following reaction between Cl₂ gas and thermally stable solid sodium chlorite, resulting in the formation of CDG:



1. Calculating Concentration of CDG

Once ClO₂ is generated, the concentration of the gas must be calculated in order to determine whether it falls within the guidelines specified by various regulatory agencies for use—these regulations are typically listed in ppm. In the case of aqueous ClO₂, this calculation is quite simple—1 mg/L of ClO₂ is equal to 1 ppm. However, the calculation for converting the concentration of CDG from mg/L to ppm is more complicated. In general, 1 mg/L of CDG is equal to 365.7 ppm, at standard temperature and pressure (21.1°C or 70°F and 101.325 Pa). This is calculated by using a factor calculated from the Ideal Gas Law (V/n):

$PV = nRT$, rewritten as:

$$V/n = RT/P$$

where P, the absolute pressure in Pascal (101.325 Pa is standard atmospheric pressure); V, volume (in L or m³) of the container containing n moles of the gas; n, the amount of the substance (in moles); R, the Ideal Gas Constant, 8.3145 (m³ × Pa)/(mol × K); and T, temperature of the gas in degrees Kelvin (to convert from Celsius to Kelvin, add 273.15 to the temperature in Celsius)

Once V/n is calculated, the following equation may be used to convert mg/L ClO₂ to ppm:

$$\begin{aligned} &[(X \div 1,000 \text{ mg/g}) \div M] \times (V/n) \times 1,000,000 \\ &= \text{ppm ClO}_2 \end{aligned}$$

Table 25.3. Comparison of relative effectiveness of oxidants/disinfectants (Rice and Gomez-Taylor, 1986)^a

Oxidant	Disinfecting Efficiency	Oxidizing Efficiency	Halogenation Capability
Chlorine	High	High	Low
ClO ₂ (pure)	High	High	Low
ClO ₂ (made from NaClO ₂ + excess chlorine)	High	High	Variable, depending on amount of free chlorine
ClNH ₂	Low	Low	Low
Ozone	High	High	Zero, except when bromide is present
KMnO ₄	Low	High	Zero
H ₂ O ₂	Low	Moderate	Zero
Bromine	High	Low	High
Iodine	High	Very low	Low

^aReproduced with permission from “*Environmental Health Perspectives*.”

where:

X, ClO₂ in mg/L; M, molecular weight of ClO₂ in g/mol; V/n, volume taken up by 1 g of gas in L/mol, calculated based on temperature of room from Ideal Gas Law (24.5 L/mol at standard temperature and pressure)

As an example, to convert 1 mg/L ClO₂ at 21.1°C to ppm:

$$[(1 \text{ mg/L ClO}_2 \div 1,000 \text{ mg/g}) \div 67 \text{ g/mol} \times (24.5)] \times 1,000,000 = 365.7 \text{ ppm ClO}_2$$

2. ClO₂ as a Surface Sanitizer

Aqueous ClO₂ was registered as a surface disinfectant and sanitizer by the EPA under the Federal Insecticide, Fungicide, and Rodenticide Act in 1967 for use in livestock barns, bottling plants, food processing plants, and other manufacturing and storage facilities (EPA, 2007). In 1988, the EPA registered CDG as a sterilant for use in manufacturing, laboratory equipment, environmental surfaces, tools, and clean rooms (EPA, 2007). Since then, CDG has been increasingly used by manufacturers and the pharmaceutical and medical device industry as a sterilant for clean rooms and to apply to pharmaceutical equipment in isolator decontamination (Czarneski and Lorcheim, 2005).

One high-profile use of CDG occurred after the *Bacillus anthracis* (anthrax) mailings in the United States in 2001, when the EPA granted a crisis exemption allowing government agencies to use aqueous and gaseous ClO₂ to decontaminate affected buildings to mitigate any public health threat (EPA, 2007). The EPA granted an initial exemption which allowed the use of aqueous ClO₂ to decontaminate the Hart Senate Office Building in Washington, DC. Although the application of aqueous ClO₂ was effective on hard surfaces at a concentration of 500 mg/L after 30 minutes exposure time, it was ineffective on porous surfaces, such as carpeting and fabrics (EPA, 2007). This finding prompted the EPA to amend the initial crisis exemption to limit the use of aqueous ClO₂ to hard surfaces only. EPA approved the use of CDG in building decontamination at a final concentration–time of 9,000 ppm–hours at 70°F and $\geq 65\%$ relative humidity. This concentration–time combination could be achieved in various combinations of time and CDG concentrations, such as 750 ppm exposure for 12 hours or 3,000 ppm for 3 hours (EPA, 2007).

Following the anthrax attacks of 2001, further research was conducted on the efficacy of various means of environmental decontamination of bioterror agents, or their research surrogates (e.g., *Bacillus atrophaeus* used as a nonpathogenic research surrogate for *B. anthracis*) from rooms. In one such study,

CDG and a decontamination foam (in which the active ingredient was hydrogen peroxide) were tested for their effectiveness in inactivating *B. atrophaeus* on wood laminate, painted metal, and vinyl tile (Buttner et al., 2004). The foam treatment was the most effective on these hard surfaces, with no culturable *B. atrophaeus* spores found in any of the 27 samples collected. The CDG treatment (1,400 ppm for 4 hours in a room held at 24°C and 84% relative humidity) resulted in 3 samples (11%) testing positive for culturable spore (Buttner et al., 2004). However, the foam treatment resulted in damage to the vinyl tile and the wood laminate, whereas the only damage attributed to treatment with CDG was the corrosion of an aluminum laboratory cart over the course of several treatments (Buttner et al., 2004). CDG treatment (10 mg/L for 30 minutes) of tanks used for aseptic juice storage achieved complete inactivation of the spoilage microorganisms *Lactobacillus buchneri*, *Leuconostoc mesenteroides*, *Eurotium* spp., *Penicillium* spp., *Candida* spp., and *Saccharomyces cerevisiae* (Han et al., 1999).

Foschino et al. (1998) compared the efficacy of aqueous ClO_2 in inactivating *Escherichia coli* cells in aqueous suspension or adhering to AISI 304 stainless steel and polyvinyl chloride (PVC). They reported that aqueous ClO_2 was capable of reducing the viable cells in water by 5 log CFU (colony forming unit)/mL within 30 seconds of exposure to 1.4 mg/L. However, 5 log CFU/mL reductions in *E. coli* cells attached to stainless steel was possible following exposure to 7 mg/L aqueous ClO_2 for 6 minutes or 14 mg/L for 4 minutes (Foschino et al., 1998). They also reported that aqueous ClO_2 treatment (14 mg/L for 8 minutes) failed in achieving a 5 log reduction in *E. coli* cells attached to PVC. Scanning electron microscopy images showed that the porous surface of the PVC had provided harborage sites that protected the attached *E. coli* cells from the aqueous ClO_2 sanitizer (Foschino et al., 1998).

3. ClO_2 Use in the Produce Industry

ClO_2 has been studied by a number of researchers for its usefulness as a treatment to minimize microbial contamination of raw and minimally processed pro-

duce. Aqueous ClO_2 has been tested as a potential produce wash, while CDG has been investigated as a potential intervention to be applied during storage and shipment of produce.

Rodgers et al. (2004) reported that only ozonated water (3 ppm) was as effective a wash step as aqueous ClO_2 (5 ppm) at achieving greater than 5 log reduction in populations of *Listeria monocytogenes* and *E. coli* O157:H7 on apples, lettuce, and cantaloupe. Populations of *L. monocytogenes* and *E. coli* O157:H7 did not rebound after 9 days of storage; however, yeasts and molds rebounded to their initial levels over the same storage period (Rodgers et al., 2004). Lee et al. (2004b) reported that a 120 ppm for 1 minute or a 40 ppm for 4 minutes aqueous ClO_2 wash was required to achieve at least a 4.8 log reduction in *Alicyclobacillus acidoterrestris* spores on apples within a 1-minute treatment time. Aqueous ClO_2 treatment (15 ppm for 10 minutes to 2 hours) of artificially inoculated blueberries yielded a 3–4 log reduction in populations of *L. monocytogenes*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Staphylococcus aureus*, and *Yersinia enterocolitica* (Wu and Kim, 2007). However, only a 2.8 log reduction in the populations of naturally occurring yeasts and molds present on the blueberries was achieved following two-hour treatment (Wu and Kim, 2007).

Aqueous ClO_2 (25 mg/L) treatment for 5 minutes of alfalfa seeds inoculated with *E. coli* O157:H7 resulted in <1 log CFU/gm in the pathogen populations, and an increase in treatment time or ClO_2 concentration (50 mg/L) did not yield significantly higher log reductions (Singh et al., 2003). Furthermore, subsequent sprouting of the treated alfalfa seeds supported the growth of *E. coli* O157:H7 population to 8 log CFU/g, resulting in ultimate contamination of the product which was not significantly different from the control seeds which were simply rinsed in sterile deionized water (Singh et al., 2003). Similarly, aqueous ClO_2 was not an effective intervention for suppressing the growth of *Erwinia carotovora*, *Fusarium* spp., or *Helminthosporium solani*, common causes of decay of stored potatoes and lesions on potatoes, even though in laboratory trials on agar aqueous ClO_2 was able to decrease the numbers of viable spores (Olsen et al., 2003).

Commercial hydrocooling water containing ClO_2 (1.3 ppm) which was used in processing pickled cucumbers was effective in controlling the population of aerobic bacteria and Enterobacteriaceae in the water (Reina et al., 1995). However, the tested concentrations of aqueous ClO_2 did not result in a significant reduction in the microbial load of the rinsed cucumbers (Reina et al., 1995). Higher concentrations of aqueous ClO_2 (2.8 and 5.1 ppm) were deemed infeasible for use in that particular application because of the resultant “excessive ClO_2 -like odor” (Reina et al., 1995). ClO_2 has also been used as a means of controlling the bacterial load in poultry chiller water and humidification water in the poultry houses at levels of no more than 3 ppm for water which will be in direct contact with poultry carcasses (EPA, 2006).

The use of gaseous ClO_2 to inactivate human pathogen on strawberries, blueberries, and raspberries has yielded promising results. Exposure of strawberries inoculated with *E. coli* O157:H7, *Salmonella enterica*, or *L. monocytogenes* to 5 mg/L of CDG for 10 minutes resulted in excess of 4 log reductions per strawberry for all of the bacterial pathogens examined (Mahmoud et al., 2007). In addition to this, the shelf life of the strawberries was lengthened to 16 days, as compared to an 8-day shelf life for the untreated strawberries, with no effect on the color of the strawberries (Mahmoud et al., 2007). Similar log reductions in *S. enterica* population on strawberries exposed to 8.0 mg/L CDG for 120 minutes (Sy et al., 2005). The same study also examined the treatment of blueberries and raspberries inoculated with *S. enterica*, resulting in reductions of 2.4–3.7 log CFU/g on blueberries and 1.5 log CFU/g on raspberries (Sy et al., 2005). Reductions of yeast and mold populations ranged from 2.5 log CFU/g on blueberries to 4.2 log CFU/g on strawberries, when treated with 8.0 mg/L of ClO_2 for 120 minutes (Sy et al., 2005). Strawberries and blueberries treated with 4.1 mg/L ClO_2 for 30 minutes had over 2 log CFU/g less *S. enterica* than untreated berries, and were not significantly different in sensory attributes to untreated berries (Sy et al., 2005).

CDG treatment to inactivate *S. enterica* population on raspberries was less effective than similar treatments of blueberries and strawberries. This was

attributed to several factors, including a lower relative humidity achieved during treatment influenced by the higher respiration rate of the raspberries compared to blueberries and strawberries, and the nature of the surface of raspberries, which have less accessible areas in between the drupelets of the berry which provide potential harborage sites to protect microbial contaminants from treatment (Sy et al., 2005). Unlike the findings in the study by Mahmoud et al. (2007), Sy et al. (2005) did not observe significant gains in shelf life of any of the treated berries.

Studies of CDG treatment of apples have shown that ClO_2 is capable of killing significant numbers of bacteria on apple skin without negative effects on product quality (Du et al., 2003; Sapers et al., 2003). In a study comparing effectiveness of glacial acetic acid vapors, hydrogen peroxide vapors, ClO_2 vapors, and CDG at inactivating generic *E. coli* on apples, ClO_2 gas achieved the highest log reduction of 4.5 log CFU/g, and had the least detrimental effect on the sensory attributes of Golden Delicious apples (Sapers et al., 2003). In a study examining the effect of the site of apple decontamination, it was found that a treatment of 7.2 mg/L for 30 minutes was capable of lowering the population of *E. coli* O157:H7 on apples by 6.5 log CFU on the apple calyx, and by 4.1 log CFU on the apple stem cavity (Du et al., 2003). However, exposure to 7.2 mg/L for 10 minutes was adequate to achieve at least a 5 log CFU reduction of *E. coli* O157:H7 on apple skin, and complete inactivation of the inoculated bacteria could be achieved on apple skin after 20 minutes (Du et al., 2003). Complete inactivation was also achieved with exposure to 12 mg/L for 10 minutes or 3.3 mg/L CDG for 20–30 minutes (Du et al., 2003).

CDG has also been studied as a potential treatment for vegetables. When lettuce inoculated with *E. coli* O157:H7, *S. typhimurium* and *L. monocytogenes* was exposed to CDG (0.435 mg/L, 22°C, 3 hours), log reductions in excess of 5 log CFU/g were observed for all pathogens without deterioration of the visual quality of the lettuce leaves (Lee et al., 2004a). However, treatment of carrots and potatoes has not yielded such promising results. In a study to determine whether treatment of grated carrots with CDG would result in an extension of product shelf life,

a gain in shelf life of 1 day was observed (Gomez-Lopez et al., 2007). However, the highest concentration of CDG achieved was 1.33 mg/L, and this concentration had fallen to 0 mg/L within 6 minutes of treatment; so, more research is required to determine an optimal concentration and exposure time to further extend the shelf life of carrots (Gomez-Lopez et al., 2007).

To summarize, CDG is a promising sanitizing treatment for a variety of food products; however the treatment has to be optimized for each individual product. Gaseous ClO_2 treatment is more effective than the aqueous treatment in inactivating human pathogens and spoilage microorganisms attached to inaccessible sites on the produce surfaces (Annous et al., 2010). Aqueous ClO_2 is useful in certain applications as well, particularly as a means of maintaining a low bacterial load in wash water in various food processing applications. While ClO_2 is a powerful oxidizer, it does not participate in the formation of potential carcinogens (trihalomethanes, bromates, etc.) upon reaction with organic compounds.

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Chapter 26

Electrolyzed Oxidizing Water

Ali Demirci and Katherine L. Bialka

1. Introduction

Traditionally, chemicals have been used as antimicrobials, such as oxidizing and reducing agents, salts, and organic acids in order to improve food quality and microbial safety, because heat may not be a good choice for certain food products, especially for minimally processed foods such as fruits, vegetables, raw meat, etc. It was demanded that these chemicals be effective against a broad spectrum of microorganisms, nontoxic, and nonirritating. There has been an increase in demand for novel technologies that can be applied to minimally processed foods, which will maintain their freshness and wholesomeness while ensuring microbial safety. While achieving this, the sanitizer should be user and environmentally friendly, readily available, and inexpensive in order to be competitive in the marketplace. In recent years, we have seen applications to utilize emerging technologies such as ozone, supercritical CO₂, and chlorine dioxide. Another technology, named electrolyzed oxidizing (EO) water, has emerged, which is produced by the electrodialysis of a dilute sodium chloride solution in an electrodialysis chamber. It offers not only an effective solution for the inactivation of microorganisms, but it is also user and environmentally friendly; it needs only electricity and table salt to produce it onsite, which eliminates transportation and storage of hazardous cleaning and sanitiz-

ing solutions. In this chapter, EO water's production, properties, and some applications will be reviewed.

2. Electrodialysis and EO Water Production

EO water is produced using a process called electrodialysis, which is a membrane separation process driven by an electrical current. More specifically, electrodialysis involves the dissociation of an ionic solution using a DC current to attract the negatively charged ions to the anode and positively charged ions to the cathode (American Water Works Association, 1995)

In the production of EO water, a relatively weak sodium chloride solution (0.1%) is passed through an electrodialysis chamber where the anode and cathode are separated by a membrane (Figure 26.1). When a current is passed through this chamber, the solution is allowed to dissociate into the two streams: acidic and alkali EO water. The acidic portion of EO water comprise hydrochlorous acid and is produced at the anode. Negatively charged ions of the salt and water, Cl⁻ and OH⁻, respectively, are attracted to the anode where they bind with dissociated water molecules (H⁺ and O₂) to form HCl, HOCl, Cl₂, OCl⁻, and O₂ (Hsu, 2005).

The alkaline solution is produced at the cathode, where water dissociates into hydroxyl ions and hydrogen gas, and then binds with sodium to form sodium hydroxide. Various companies manufacture EO water generators. Figure 26.2 shows a typical EO water generator, their production capacity changes depending on the manufacturer and model.

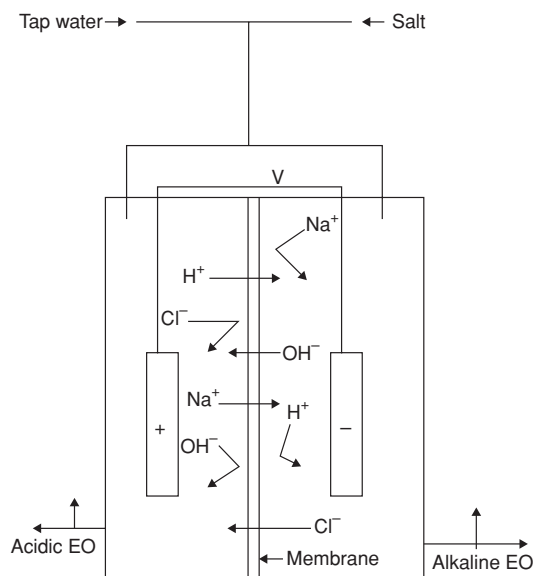


Figure 26.1. Schematic of EO water generator (Kim et al., 2001).

3. EO Water Properties

EO water is composed of two solutions with very different uses, properties, and modes of action. Alkaline EO water has $\text{pH} > 11$ and a strong reducing capacity, which makes it an ideal solution for clean-

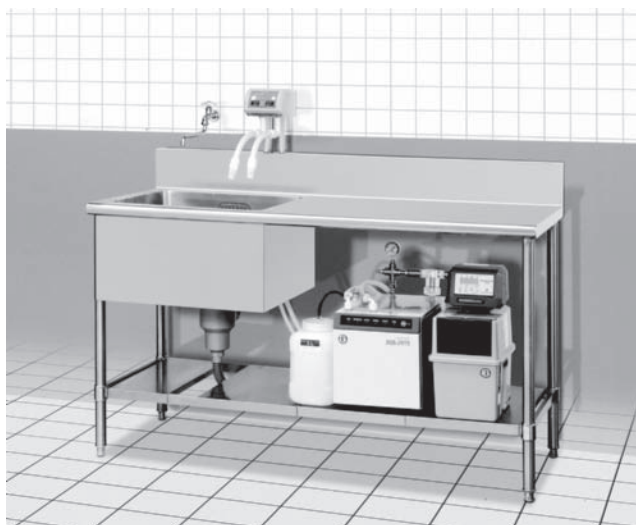
ing (Hsu, 2005; Walker et al., 2005a). On the other hand, acidic EO water is an ideal biocide with a high oxidation–reduction potential, low pH ($\sim \text{pH } 3$), and moderate levels of free chlorine (Kim et al., 2000a; Park et al., 2002a).

3.1. Alkaline EO Water

The alkaline portion of EO water is generated at the cathode and mostly consists of sodium hydroxide ions, giving it a basic pH of 11.5 and an oxidation–reduction potential (ORP) of -795 mV . Because of the presence of sodium hydroxide and a negative ORP, it can be used as detergent replacement. However, not much research has been dedicated to the efficacy of alkaline EO water as either a detergent or a sanitizer.

3.2. Acidic EO Water

Acidic EO water had a pH of 2.5–3.5, an ORP of 1,000–1,200 mV, and 30–90 ppm free chlorine depending on the amperage used for its production. The higher the amperage, the lower the pH and higher ORP and free-chlorine concentration obtained. For example, 10 Amp amperage yields acidic EO



(a)



(b)

Figure 26.2. EO water generators (Hoshizaki Electric Co. Ltd, Japan). (For color details, please see color plate section.)

water with a pH of 2.6, ORP of 1,150 mV, and about 90 ppm free chlorine.

3.3. Inactivation Mode of Acidic EO Water

Acidic EO water works similarly to chlorine, which includes the inhibition of glucose oxidation (mainly aldolase enzyme), disruption of protein synthesis, reactions with nucleic acids, unbalanced metabolism after destruction of key enzymes, induction of DNA lesions, and inhibition of oxygen uptake and oxidative phosphorylation (Marriott, 1999). The efficacy of acidic EO water has been linked to a combination of its high ORP, low pH, and chlorine content (Len et al., 2000; Park et al., 2002a). It has also been reported that acidic EO water may contain reactive oxygen species like O_3 , O , and OH , which are known as strong oxidants and therefore good antimicrobials (Stan and Deaschel, 2003). ORP refers to the ability of a solution to oxidize or reduce and has, by some, been used as a measure of disinfection capability. Microorganisms require specific ranges of ORP in order to survive, usually between +200 mV and +800 mV, which clearly falls outside of the ranges of ORP produced by EO water (Jay, 2000). Kim et al. (2000a) reported that the ORP of a treatment solution is the primary factor that affects microbial inactivation and can be a much better indicator of a sanitizer's efficacy than free-chlorine concentration. However, Len et al. (2000) found a high correlation ($r = 0.95$) between hypochlorous acid (HOCl) content of EO water and its antimicrobial activity. They suggest that HOCl is the primary bactericidal agent due to its neutral charge, which would allow it to better penetrate the cell wall of a microorganism.

Other researchers have attributed its disinfection properties to a combination of the properties possessed by EO water. The bactericidal properties of acidic EO water were explained by Venkitanarayanan et al. (1999a) as a combination of high ORP, free chlorine, and low pH. They hypothesized that the low pH of EO water sensitizes the outer membrane of the microorganism, allowing for the more efficient transfer of hypochlorous acid into the cell. Kim et al. (2000b) found that the interaction of residual

chlorine, ORP, and pH was necessary to achieve inactivation of food-borne pathogens.

3.4. Membrane Free Electrolysis—Neutralized EO Water

Membrane-free electrolysis uses the same basic electrolytic principles, but the anode and cathode products are combined inside the cell to produce a more neutral 8.5 pH solution. Electrolytic cells lacking a membrane are both easier to maintain, since membrane fouling issues are eliminated, and are more readily scalable. This allows the technology to be used in a wider array of applications where neutralized EO water needed. The combined solution has similar biocidal properties as compared to the acidic EO water, with good cleaning and degreasing properties. Combined flow solutions are also believed to be less corrosive and more stable than acidic EO water. Commercial systems are currently available for production of the combined flow hypochlorite product for clean-in-place applications (Figure 26.3).

3.5. Generation Conditions and Effect of Storage

The effect of generation conditions on the properties of EO water has been extensively studied by (Hsu, 2003, 2005; Hsu et al., 2004). Factors such as flow rate, temperature, dilution, and salt concentration have been of great interest. The effects of these factors were studied on the ORP, total residual chlorine, dissolved oxygen, and electrical conductivity of acidic EO water. It was observed that when the salt concentration was increased, so did the conductivity of the solution as well as the concentration of chlorine, with approximately 46% of the chlorine ion remaining in the EO water after electrolysis. It was also noted that as the flow rate was increased, the ORP and chlorine concentration were decreased. There was no effect on the pH of EO water or the dissolved oxygen content when these factors were manipulated.

The effects of storage on EO water properties have also been of great interest. Len et al. (2002) studied the effects of storage on pH and chlorine in acidic

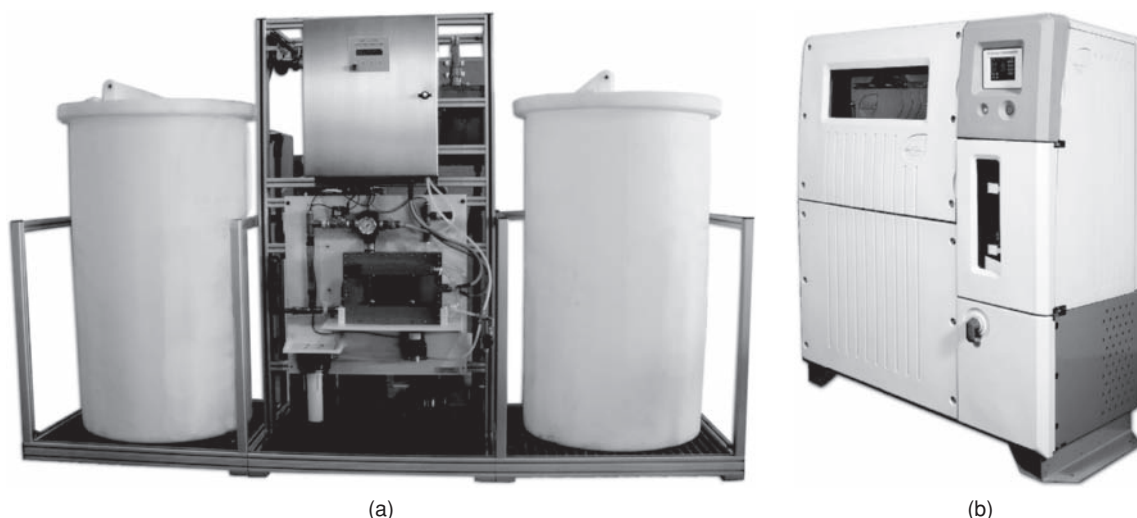


Figure 26.3. Neutralized EO water generator (MIOX Corporation, Albuquerque, NM). (For color details, please see color plate section.)

EO water. They found that chlorine loss followed first-rate kinetics and decreased approximately five-fold when the solution was agitated for 30 hours, and when not agitated, all chlorine was lost due to evaporation after 100 hours of open storage. Degradation of chlorine was not so drastic when EO water was stored in a closed vessel. It took approximately 1,400 hours for the chlorine concentration to decrease by 60%. Hsu and Kao (2004) also looked at the effects of storage of EO water properties. After 12 days of semi-open storage, they found that the pH remained stable but the ORP dropped from 1,150 to 1,124 mV and that the chlorine concentration decreased by 81%. They noted that the length of storage time is not as important as whether EO water has been exposed to the atmosphere.

4. Inactivation of Suspended Cells by EO Water and the Effect of Temperature

The efficacy of EO water has been documented for the purpose of inactivating microorganisms. Both bacteria and fungi are effectively inactivated primarily by acidic EO water. Inactivation of microorganisms using acidic EO water is very dependent

on free-chlorine concentration, ORP, temperature, and pH.

Venkitanarayanan et al. (1999a) evaluated the effects of acidic EO water at four different temperatures: 4, 23, 35, and 45°C on suspensions of *Escherichia coli* O157:H7, *Salmonella enteritidis*, and *Listeria monocytogenes*. At a temperature of 4°C, complete inactivation of *E. coli* O157:H7 was observed after 10 minutes at a pH of 2.4, an ORP of 1,150 mV, and a free-chlorine concentration of 86 ppm. At a pH of 2.5 and free-chlorine level of 83 ppm, complete inactivation was achieved after 10 minutes at 4°C. For *L. monocytogenes*, complete inactivation at 4°C was achieved after 10 minutes at a lower level of free chlorine. Only 43 ppm of free chlorine was needed for complete inactivation at a pH of 2.6 and an ORP of 1,160 mV. When the temperature was increased to 23°C, there was no observable difference in the amount of time needed for complete inactivation of the microorganism. However, when the temperature was increased to either 35°C or 45°C, there was a noticeable difference in the time required for complete inactivation. At 35°C and a free-chlorine concentration of 84 ppm, only 2 minutes of exposure was needed to completely inactivate *E. coli* O157:H7. Four minutes, at a pH of 2.4

and free-chlorine concentration of 79 ppm, was required to completely inactivate *S. enteritidis*. And 2 minutes was required to inactivate *L. monocytogenes* at a pH of 2.5 and a free-chlorine concentration of 73 ppm. At 45°C, a 1-minute exposure to acidic EO water at a pH of 2.4 and a free-chlorine concentration of 85 ppm was required to inactivate *E. coli* O157:H7. A 3-minute exposure to EO water at a pH of 2.4 and a free-chlorine concentration of 79 ppm was required for complete inactivation of *S. enteritidis* as well as *L. monocytogenes* but at a free-chlorine concentration of 73 ppm. Stan and Deaschel (2003) found a 6.6 log₁₀ CFU/mL reduction of *Salmonella enterica* in an aqueous system. *Campylobacter jejuni* was successfully inactivated in solution after 10 seconds of exposure to acidic EO water (Park et al., 2002b).

Park et al. (2004) evaluated the effects of acidic EO water in terms of both residual chlorine concentration and pH on the inactivation of *E. coli* O157:H7 and *L. monocytogenes*. Residual chlorine concentrations between 0.1 and 5.0 ppm and pHs of 3.0, 5.0, and 7.0 were assessed. They found that the level of residual chlorine of EO water be maintained at 1.0 ppm or higher for complete inactivation of these microorganisms. The residual chlorine was the more important characteristic at pHs of 3.0 and 5.0. Only at a pH of 7.0 did the residual chlorine level need to be increased to 2.0 ppm in order for complete inactivation to be achieved.

The individual properties of EO water have also been evaluated with respect to their impact on microorganisms. Kim et al. (2000a) assessed ORP, pH, and residual chlorine levels on *E. coli* O157:H7, *L. monocytogenes*, and *B. cereus*. In order to reduce the ORP readings of acidic EO water, they added iron and found that very little inactivation of the microorganisms was possible. The same results were observed when the chlorine of acidic EO water was reduced by the addition of neutralizing buffer. They found that the interaction of residual chlorine, ORP, and pH was necessary to achieve inactivation of food-borne pathogens. They also found that spore-forming bacteria were much more resistant to treatment with EO water than non-spore forming bacteria. Complete inactivation of *E. coli* O157:H7 and *L. monocytogenes* was achieved after 10 seconds of exposure,

while *B. cereus* spores were never completely inactivated. After 120 seconds of treatment with 56 ppm residual chlorine EO water, a reduction of 3.52 log₁₀ CFU/mL of spores was achieved.

Fabrizio and Cutter (2003) found that when compared to other solutions, acidic EO water was much more effective at inactivating the food pathogens *L. monocytogenes* and *S. typhimurium*. They compared acidic EO water, alkaline EO water, acidic EO water that had been aged for 1 day, and chlorinated water (20 ppm). They found a reduction of 5.07 log₁₀ CFU/mL of *S. typhimurium* and a reduction of 6.76 log₁₀ CFU/mL of *L. monocytogenes*. This was significantly higher than the reductions achieved using chlorinated water, which produced reductions of 0.03 and 0.17 log₁₀ CFU/mL of *S. typhimurium* and *L. monocytogenes*, respectively, after 15 minutes of treatment. They also found that alkaline EO water was not effective at reducing either microorganism as well as that aged acidic EO water did not lose any biocidal activity.

The effectiveness of EO water has also been studied for fungi. Okull and LaBorde (2004) looked at the effect of acidic EO water on *Penicillium expansum* in suspension. They found a 4.85 log₁₀ CFU/mL reduction of *P. expansum* spores after 30 seconds of exposure to acidic EO water compared to a reduction of 3.59 and 3.88 log₁₀ CFU/mL of spores using 100 and 200 ppm chlorine solutions, respectively. Buck et al. (2002) evaluated the effectiveness of acidic EO water on a wide variety of fungal species. All tested species of fungus either failed to germinate or had reduced levels of germination. They found that thin-walled species, like *Botrytis* and *Monilinia*, were killed after 30 seconds of exposure and that thicker-walled species, like *Curvularia*, required 2 minutes to kill. They also found that the addition of surfactants eliminated the germicidal activity of EO water.

5. Applications of EO Water

5.1. Treatment of Surfaces

There has been great interest in using EO water as a sanitizer for food and nonfood contact surfaces for a

variety of reasons. Ayebah and Hung (2005) attribute this to its “high antimicrobial activity, low cost, and ease of production and use.” Kim et al. (2001) investigated EO water for the inactivation of *L. monocytogenes* biofilms on stainless steel surfaces. They found out that the adherent cell population on the stainless steel coupons was reduced by about 9 log cycles after 300 seconds of EO water treatment. Park et al. (2002a) evaluated the effectiveness of inactivating *Staphylococcus aureus* and *Enterobacter aerogenes* on a variety of surfaces. They examined glass, stainless steel, glazed ceramic tiles, unglazed ceramic tiles, and vitreous china. They found reductions greater than 2 log₁₀ CFU/cm² of *E. aerogenes* on all surfaces and no surviving bacteria remained in the wash solutions after 5 minutes of treatment with acidic EO water. Reductions of *S. aureus* were slightly lower, averaging about 1.73 log₁₀ CFU/cm² on all surfaces, and again there were no surviving bacteria in the wash water after 5 minutes. However, when the materials were treated with agitated acidic EO water for 5 minutes at 50 rpm, there were no remaining bacteria on any of the surfaces for both *E. aerogenes* and *S. aureus*. The increased efficacy of EO water with agitation was attributed to the increased removal of the cells from the surface and subsequent death in solution, increased penetration, aided by agitation, of the EO water, and more efficient interaction of chlorine and cells due to agitation.

Liu et al. (2006) investigated acidic EO water's effectiveness against *L. monocytogenes* on seafood processing equipment surfaces. Stainless steel, ceramic tile, and floor tile were contaminated with crabmeat residue and inoculated. Log₁₀ reductions of 2.33, 2.33, and 1.52 CFU/chip were achieved on stainless steel, ceramic tile, and floor tile, respectively. They found that the presence of organic material (crabmeat) greatly decreased the efficacy of acidic EO water.

Walker et al. (2005a) applied both alkaline and acidic EO water to materials typically used in milking systems such as stainless steel sanitary pipe, PVC hose, rubber, and polysulfone plastic for treatment times between 5 and 20 minutes for both alkaline and acidic water at temperatures between 25 and 60°C. They found that both the alkaline EO water treatment

and the acidic EO water treatment were significant in the inactivation of microorganisms on each of the materials they used. Later on, they tested EO water technology on a pilot-scale pipeline milking system by soiling the system with raw milk inoculated with common microorganisms and then washing it with alkaline EO water followed by acidic EO water (Walker et al., 2005b). After cleaning, the system was evaluated by ATP bioluminescence and microbiological analysis for cleanness and sanitation check, respectively. A 10-minute wash with 60°C alkaline EO water followed by a 10-minute wash with 60°C acid EO water successfully removed all detectable bacteria and ATP from the non-porous milk contact surfaces. Shorter treatment times (5 and 7.5 minutes) with EO water were also evaluated, along with a control treatment using conventional dairy cleaning chemicals. There were no significant differences between the 10-minute and 7.5-minute EO water treatments and the conventional treatment.

E. coli O157:H7 and *L. monocytogenes* were successfully inactivated on kitchen cutting boards with acidic EO water (Venkitanarayanan et al., 1999b). They were able to completely inactivate both *E. coli* O157:H7 and *L. monocytogenes* at all treatment times and temperatures. Temperatures ranged from 23 to 55°C and times from 5 to 20 minutes.

One possible downside of sanitizing processing materials, such as stainless steel, with acidic EO water is that it has the possibility to be corrosive, like most chlorine-based sanitizers. Ayebah and Hung (2005) investigated EO water's corrosiveness on carbon steel, copper, aluminum, PVC, and stainless steel. They found that EO water did not have any undesirable effects on stainless steel, but did cause pitting corrosion on carbon steel, copper, and aluminum. When EO water was neutralized, acidic water increased to a higher pH, it did not have a significantly different effect than deionized water.

The use of neutralized EO water has also been evaluated for its efficacy at inactivating food-borne microorganisms on surfaces. Deza et al. (2005) evaluated it for the purpose of inactivating *E. coli*, *L. monocytogenes*, *Pseudomonas aeruginosa*, and *S. aureus* on stainless steel and glass. They found log₁₀ reductions of more than 7 CFU/mL of all

microorganisms tested with neutralized EO water, which proved to be just as effective as treating these surfaces with sodium chlorite. However, neutralized EO water, as well as acidic EO water, has the advantage of being safe to handle and being produced on site.

5.2. Treatment of Produce

Much research has been concentrated on the use of EO water to wash fresh produce. Alfalfa seeds and sprouts have received a lot of attention due to their notoriety in *E. coli* O157:H7 outbreaks and difficulty in decontamination (NACMCF, 1999). Kim et al. (2003) evaluated acidic EO water and two other sources of chlorine, chlorine water, and calcium chlorite, on their ability to disinfect alfalfa seeds and sprouts inoculated with *Salmonella*. After 10 minutes of treatment with each solution, a reduction of 1.09 log₁₀ CFU/g was achieved using EO water, which was not significantly different than the reductions with the two chlorine treatments. Stan and Deaschel (2003) also evaluated the effect of acidic EO water on *Salmonella enterica*. They found reductions of 2.04 and 1.96 log₁₀ CFU/g for seeds treated for 15 and 60 minutes, respectively. It was also noted that no *Salmonella* could be recovered in the wash solution after treatment. Sharma and Demirci (2003) evaluated the use of acidic EO water to inactivate *E. coli* O157:H7 on alfalfa seeds and sprouts in conjunction with mechanical agitation. They achieved a maximum reduction of 96.9% on alfalfa seeds after treatment for 64 minutes and 99.8% on alfalfa sprouts after 64 minutes using a solution with 64.5 ppm free chlorine.

Bari et al. (2003a) evaluated the effect of EO water in conjunction with dry heat and sonication on *E. coli* O157:H7 on a variety of seeds: alfalfa, radish, and mung bean. Acidic EO water was compared to distilled water, a chlorine solution, and a sanitizer called califresh-S. For all treatments, acidic EO water resulted in the highest log₁₀ reductions. When combined with dry heat and a solution temperature of 50°C, a reduction of 3.42 CFU/g was reached, when this was combined with sonication the reduction of *E. coli* O157:H7 was increased to 4.56 CFU/g on

mung bean seeds. Reductions of *E. coli* O157:H7 on radish seeds were less than those on mung bean seeds. A maximum reduction of 1.94 log₁₀ CFU/g was achieved when the seeds were treated with dry heat, sonication, and hot EO water. The results for alfalfa seeds are comparable to those for mung bean seeds, with a maximum reduction of 4.29 log₁₀ CFU/g, resulting from a treatment that consisted of dry heat, sonication, and hot EO water.

Koseki et al. (2004a) assessed the efficacy of acidic EO water and alkaline EO water, followed by acidic EO water for the purpose of decontaminating cucumbers and strawberries. When washing with acidic EO water alone for 10 minutes, reductions of 1.5 log₁₀ CFU/g, 1.8 log₁₀ CFU/g, and 2.1 log₁₀ CFU/g were obtained for aerobic mesophilic bacteria, coliform bacteria, and fungi, respectively, on cucumbers. When acidic EO water was combined with alkaline EO water each at 5-minute treatment, the reduction in aerobic mesophilic bacteria was significantly increased to 2 log₁₀ CFU/g; however, the reductions in coliform bacteria and fungi were not significantly increased. On strawberries, after 10 minutes of treatment with acidic EO water, reductions of 1.6, 2.4, and 1.7 log₁₀ CFU/g were obtained for aerobic mesophilic bacteria, coliform bacteria, and fungi, respectively. When combined with alkaline EO water, reductions were not significantly increased. Aerobic mesophilic bacteria were reduced by 1.1 log₁₀ CFU/g and fungi by 0.8 log₁₀ CFU/g; there was no change in coliform bacteria reduction, since both treatments resulted in below detectable levels.

The efficacy of EO water has also been extensively studied for its application on lettuce. Koseki et al. (2004b) washed lettuce with both alkaline and acidic EO water, which resulted in a log₁₀ reduction of 2 CFU/g of aerobic bacteria. Koseki et al. (2004b) also used acidic EO water as ice to inactivate *L. monocytogenes* and *E. coli* O157:H7 on ice. Acidic EO water was frozen and allowed to emit chlorine gas at concentrations of 30, 70, 150, and 240 ppm. A maximum reduction of 2.0 log₁₀ CFU/g of *E. coli* O157:H7 was achieved at a chlorine concentration of 150 ppm without any adverse quality affects. *L. monocytogenes* was reduced by 1.5 log₁₀ CFU/g at 240 ppm of chlorine. Park et al. (2001)

also evaluated the effects of acidic EO water on lettuce. When treated for 3 minutes, reductions of 2.41 and 2.65 log₁₀ CFU/leaf were achieved for *E. coli* O157:H7 and *L. monocytogenes*, respectively.

Tomatoes washed with acidic EO water showed significantly greater reductions in pathogenic microorganisms than 200 ppm chlorine water. Bari et al. (2003b) found reductions of 7.85 log₁₀ CFU/tomato of *E. coli* O157:H7, 7.46 log₁₀ CFU/tomato of *Salmonella*, and 7.54 log₁₀ CFU/g of *L. monocytogenes*. Reductions obtained using chlorine water only resulted in a reduction of approximately 4.7 log₁₀ CFU/tomato. Furthermore, no adverse quality effects were observable on tomatoes treated with acidic EO water. Deza et al. (2003) used neutral EO water to decontaminate tomatoes inoculated with *E. coli* O157:H7, *S. enteritidis*, and *L. monocytogenes*. After treatment for 60 seconds, reductions of 4.92, 4.30, and 4.74 log₁₀ CFU/cm² of *E. coli* O157:H7, *S. enteritidis*, and *L. monocytogenes* were observed on the surfaces.

Acidic EO water was also used to extend the shelf life of pears inoculated with *Botryosphaeria berengeriana* (Al-Haq et al., 2002). They found that after immersion in EO water and storage, disease incidence and severity were significantly decreased. Okull and LaBorde (2004) demonstrated substantial reduction for cross-contamination of spores of *P. expansum* via wounded apples from decayed fruit or by direct addition of spores to a simulated dump tank.

5.3. Treatment of Animal Products

The surfaces of chicken eggs have been successfully treated with EO water. Russell (2003) inactivated *L. monocytogenes*, *S. aureus*, and *E. coli* using EO water applied with an electrostatic spraying system. Park et al. (2005) evaluated alkaline and acidic EO water on eggs inoculated with *S. enteritidis* and *L. monocytogenes*. Treatment with alkaline EO water resulted in reductions of 0.94 and 3.13 log₁₀ CFU/egg for *L. monocytogenes* and *S. enteritidis*, respectively, after 5 minutes. Washing with acidic EO water resulted in reductions of 4 and 3.48 log₁₀ CFU/egg of *L. monocytogenes* and *S. enteritidis*, respectively, af-

ter 5 minutes. When alkaline and acidic EO water were combined, reductions of 4.39 and 3.66 log₁₀ CFU/egg of *L. monocytogenes* and *S. enteritidis*, respectively, were achieved after treatment for 2 minutes total. Bialka et al. (2004) evaluated the effects of alkaline and acidic EO water on inactivating *S. enteritidis* and *E. coli* K12, respectively, in both an in-vitro and pilot-scale setting. Reductions of >2.15 and 2.31 log₁₀ CFU/g of *S. enteritidis* and *E. coli* K12 were achieved in an in-vitro system with 3 minutes of alkaline followed by 3 minutes of acidic EO water washing. In a pilot scale washing scenario, a log₁₀ reduction of >2.95 CFU/g of *E. coli* K12 was achieved after washing with alkaline EO water for 3 minutes and acidic EO water for 1 minute 23 seconds. They also found that there was no additional effect on the quality of the egg caused by EO water compared to conventional treatment.

The effect of acidic EO water was evaluated for the purpose of inactivating pathogenic microorganisms during the washing of poultry. Park et al. (2002b) found that after 10 minutes at 23°C, a reduction of 1.82 log₁₀ CFU/g for *Campylobacter jejuni* was possible, and after 30 minutes, a reduction of 2.01 log₁₀ CFU/g on chicken was possible when washed with acidic EO water. They also found that there were no detectable bacteria in the wash water after treatment. Fabrizio et al. (2002) compared EO water with various antimicrobial interventions, including ozonated water, acetic acid, and trisodium phosphate to reduce *S. typhimurium* on poultry. They found that acidic EO water reduced *S. typhimurium* approximately 0.86 log₁₀, whereas trisodium phosphate and acetic acid reduced *S. typhimurium* 1.41 log₁₀ immediately after the treatment. However, after 7 days of storage at refrigerated temperature, *S. typhimurium* was detectable only after selective enrichment for acidic EO water as well as trisodium phosphate and acetic acid, which clearly demonstrated that EO water has a great potential for poultry especially following extended refrigerated storage, because trisodium phosphate and acetic acid are usually costly and may adversely affect the environment.

Ozer and Demirci (2006) evaluated acidic EO water to inactivate *E. coli* O157:H7 and *L. monocytogenes* Scott A on salmon fillets. A maximum

reduction of 1.07 log₁₀ CFU/g *E. coli* O157:H7 was achieved at a treatment temperature and time of 35°C and 64 minutes and a maximum reduction of 1.12 log₁₀ CFU/g *L. monocytogenes* Scott A.

Pathogens on fresh pork have also been reduced using acidic EO water. Fabrizio and Cutter (2004) found reductions of 0.41, 0.98, and 1.06 log₁₀ CFU/cm² of total viable counts, *E. coli*, and total coliforms after 7 days of storage following treatment. Reductions of 1.36, 1.39, and 0.65 log₁₀ CFU/cm² of *S. typhimurium*, *L. monocytogenes*, and *E. coli* resulted after 7 days of storage following treatment. The hides of cattle have also been decontaminated using EO water. Bosilevac et al. (2005) treated hides with both alkaline and acidic EO water, with reductions of 1.0 and 0.9 log₁₀ CFU/cm² of aerobic plate count and *Enterobacteriaceae*, respectively, as well as prevalence of *E. coli* O157:H7 on hides was reduced from 82 to 35% following EO water treatment. Alkaline and acidic EO water were also used to improve the quality and decontaminate frozen shrimp. Loi-Braden et al. (2005) found that when shrimp were treated with alkaline EO water for 5 minutes and acidic EO water for 2 minutes and stored for 119 days, reductions of 1.65 and 1.36 log₁₀ CFU/g of *E. coli* O157:H7 and *Salmonella*, respectively, were obtained. It was also noted that there were no discernable quality changes on shrimp treated with EO water.

6. Conclusions and Future Trends

EO water shows great potential to be used as a cleaning and sanitizing agent for the food industry. The two solutions that comprise EO water offer very different properties and benefits. Acidic EO water works well as a sanitizing agent due to its low pH, high ORP, and concentration of chlorine. Alkaline EO water with its high pH and low ORP makes for an attractive cleaning agent. EO water has been successfully shown to inactivate a variety of pathogenic and spoilage microorganisms in solution and has been effectively used to decontaminate both food-contact surfaces as well as a variety of foods. Its ease of production and handling, as well as its proven biocidal properties, make it an especially attractive product

and a suitable alternative to hazardous sanitizers and cleaning compounds.

However, a greater understanding of EO water is still needed. The roles and interaction of ORP, pH, and free chlorine are not completely understood, and the exact biocidal component of EO water is not completely understood and deserves more investigation. The role of the reactive oxygen species present in acidic EO water needs to be investigated. The effects and value of alkaline EO water have been overlooked by many researchers for both contact surface and food decontamination and should be further investigated. The long-term effect of EO water on processing equipment needs to be investigated. Finally, the environmental effect of used EO water needs to be documented. With more research and understanding, EO water will find its place as an alternative tool in agricultural, food, and even pharmaceutical industries.

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Section IV

Combination Processes

Chapter 27

Novel Technologies in Combined Processes

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1. Introduction

Food preservation techniques are continuously developing to adapt to consumer's changing requirements. Present life habits and an increased demand for healthier foods are perhaps the most important reasons for promoting this development, but there are some other driving forces in the permanent evolution of food preservation methods.

During the 1970s, the involvement of certain psychrotrophic microorganisms in food poisoning outbreaks was clearly established, as well as their facility to grow in refrigerated foods, even faster than spoilage microflora. These microorganisms were denoted as emerging pathogens and included species such as *Listeria monocytogenes*, *Yersinia enterocolitica*, and *Aeromonas hydrophila*, amongst others. These facts lead to the conclusion that it was convenient to pasteurize some fresh products before their refrigerated storage. Heat treatments would not be an appropriate choice in these products since the degree of modification of the nutritional and sensory properties would make them unacceptable as such fresh-like foods.

The economic development after the 1970s crisis was the origin of a different profile of consumer of high purchasing power, and furthermore, better informed about health, nutrition, and, generally speaking, food. This kind of consumer demanded fresh-like, nutritive, and safer products.

On the other hand, during the 1980s and the 1990s, there was a trend to gradually reduce the amount and type of chemical preservatives in foods, and available food preservation technologies could not compensate for the total absence of preservatives. Finally, market globalization has increased the volume of interchange of fresh foods, fact that represents a potential danger of dissemination of diverse diseases transmitted by foods.

To respond to the needs of food industry, Food Technology is exploring new approaches. On one hand, an effort is being made to improve current thermal treatment, trying to reduce heating lag phases and trying to improve the homogeneity and intensity of treatments, even in solid foods. On the other hand, there is a continuous search for new methods for microbial and enzymatic inactivation, which guarantee stability and safety and at the same time cause lesser side effects on food characteristics. A last approach is the design of new combined processes of lower intensity but equivalent or even higher degree of stability and safety in foods.

Amongst the nonthermal technologies developed in the last decades, ionizing radiation (IR), high hydrostatic pressures (HHP), pulsed electric field (PEF), and ultrasound (US) are perhaps the most promising ones. Each technology has its own advantages and drawbacks that will determine, according to them and also to the characteristics of the products, such as composition or main agents of deterioration, the particular applications in food processing and preservation.

Food is spoiled by the action of diverse agents of physical-chemical or biological nature. Among the

biological agents, enzymes are of particular interest in foods of plant origin, whereas microorganisms are of interest almost in every kind of food due to their diversity and adaptation ability. Bacteria are perhaps the most important group of microorganisms, since they are frequently involved not only in food spoilage but also in food poisoning. Bacteria of interest in food preservation may be classified into two large groups: those cells with active metabolism, also denoted in general terms as vegetative cells, and bacterial spores, which are mainly characterized by their lack of metabolic activity and their elevated resistance to almost every agent of physical or chemical nature. From a practical point of view, current thermal treatments are classified into pasteurization or sterilization treatments according to their potential to destroy vegetative cells or spores and enzymes, respectively, to a given level. These two types of thermal treatments render a product that needs refrigeration or by contrast is stable at room temperature, respectively.

Nonthermal methods for food preservation investigated so far are normally efficient to destroy vegetative cells, and therefore suitable for pasteurization-like processes, but unfortunately their inactivating effect on enzymes is scarce, fact that limits their future applications. In addition, spore inactivation by these technologies, if possible, generally requires very intense treatments that either provoke unacceptable changes in food properties or that are not presently achievable by available industrial equipments.

In order to minimize the limitations of the nonthermal technologies mentioned above, nowadays there is a growing interest in the development of combined processes that include these technologies. This chapter will review current knowledge in this field.

2. Combined Processes

A brief study about the effects of the various preservation methods shows that the relative resistance of each spoilage agent may change depending on the technology studied. For instance, if we compare the relative resistance to heat and to IR of two serovars

of *Salmonella*, *senftenberg* 775 W, and *typhimurium*, we observe that the first one is much more resistant to heat than the second one, whereas exactly the opposite happens with IR (Álvarez et al., 2006). Thus, the conclusion is obvious: a process can be designed by applying both technologies at low intensity without affecting food quality. A mild heat treatment would inactivate the heat sensitive serovar *S. typhimurium*, and low dose IRs would be able to inactivate the thermoresistant but radiosensitive *S. senftenberg*. This process would show, at least, an additive effect, since the total efficacy would be equal to the addition of the lethal effects of each treatment applied. This is the basis of the so-called combined processes or hurdle technology.

On the other hand, most of the inactivation technologies produce structural or physiological changes on microbial cells, independently on whether they are finally inactivated or not. These changes may sensitize them to a simultaneous or subsequent treatment with other technology. For instance, it has been demonstrated (García et al., 2004) that a PEF treatment sensitizes *Escherichia coli* cell toward acidity. Hence, the application of a PEF treatment followed by the exposition of cells to acidic medium results in a synergistic effect; in other words, the total lethal effect achieved is greater than the mere addition of the individual lethal effects of each one of the hurdles, PEF and acidity. Synergistic effects are often related to homeostasis maintenance, damage, and cellular recovery phenomena. Microbial growth and survival requires specific physico-chemical cytoplasmatic parameters, such as a given pH and osmotic pressure, which are highly dependent on the environment. In order to avoid undesirable changes in cytoplasmic conditions when bacteria are exposed to adverse environmental conditions, they trigger specific adaptive responses, in the so-called homeostatic adaptation, to resume growth and assure survival. When the intensity of the treatment goes beyond a certain limit, the ability of microorganisms to counteract cellular changes is exceeded. Therefore, growth is inhibited and inactivation may occur. It has been proved (Montville and Matthews, 2001) that cells became easily inactivated if more homeostatic mechanisms are required for survival, and also if the production of

energy or synthesis of proteins is compromised; this will explain some of the synergistic effects observed with different combinations. For instance, microbial survival in low water activity media is reduced if the pH is also decreased simultaneously, or when a thermal treatment is applied over acidified foods.

The first studies of the inactivation kinetics of microorganisms exposed to a preservation technology corresponded to heat. Heat inactivation can be described by first-order kinetic, fact which seemed to indicate that inactivation was due to the modification of only one key target, which was expected to be the DNA molecule. However, later it has been proved that before irreversible denaturation of DNA, heat induces a large amount of structural alterations within the cells, also known as thermal damages. When the treatment finishes, usually uninjured, injured, and death cells are found amongst the treated microbial population. Injured cells, which may correspond to more than 90% of the survivors (García et al., 2005a), may either repair heat damage or not, depending on the environmental conditions. Therefore, a combined process will show a synergistic effect when one of the hurdles prevents the repair of the damage produced by another, as it usually happens when protein or energy synthesis is compromised within the cell.

Sometimes, an antagonistic effect between hurdles has been observed. For instance, it is well known that the low water activity of a particular food slows down microbial growth, but it also results in an increased resistance to almost any physical inactivation method, such as heat treatments. Antagonistic effects may be related to physical phenomena, as in the previous example, but also with the development of resistance toward one or various stresses, *via* adaptation responses. Applying a hurdle at a low intensity can cause cellular damage, which is desirable, but may also trigger the expression of some genes or the activation of alternative metabolic pathways that can provide a better microbial survival. If these adaptive responses are unspecific, the proteins, enzymes, etc., synthesized can also improve the survival of microorganisms toward the next hurdle applied. For instance, an acid shock has been proved to protect cells against a subsequent acid, oxidative, or heat treatment (Farber and Pagotto, 1992; Lou and Yousef,

1997). If the occurrence of this kind of adaptive responses is foreseen, a design of hurdles applied simultaneously is expected to offer a better result than sequentially.

Until recent times, most of the combined processes designed had an empirical basis, but the remarkable advances in the knowledge of the mechanisms of inactivation, damage and recovery, and stress adaptation offer the possibility of designing new combined processes with a solid scientific basis. This review will emphasize on the mechanisms of inactivation, damage and adaptation of the various emerging preservation technologies, and their consequences for the development of adequately designed combined processes. We will also discuss the combinations already studied, and we will try to explain the results obtained according to the current scientific knowledge.

3. Combined Processes Based on New Technologies

New preservation technologies have the general objective of being less deleterious to the sensorial and nutritional characteristics of foods than traditional processing. They also have some limitations, especially related to the lack of sufficient lethal effect on bacterial spores. That is the reason why their application filed will probably be broaden if they are used as part of combined process. Each of the emerging technologies presents different modes of action on microorganisms. Therefore, the appropriate combinations might be different for each one.

3.1. Ultrasound

US is defined as sonic waves with frequencies above 16–18 kHz, which are over the threshold for human hearing. US with frequencies between 20 and 40 kHz are defined as high-energy or power US, whereas those whose frequency ranges between 40 kHz and 1 MHz are known as low-power (high-frequency) US. Finally, those ranging from 0.5 to 20 MHz are classified as diagnostic US (Mason and Paniwnyk, 1996).

Ultrasonic waves are defined by its frequency (number of oscillations per second), their amplitude (maximum elongation value), and their wavelength (distance between two consecutive points vibrating in the same phase). The energy associated to a wave is proportional to the amplitude of the wave to square, and the amplitude is inversely proportional to the distance to the source of emission to square. The intensity is defined as the energy transmitted by the wave per second and surface unit, and the pressure amplitude corresponds to the difference between the pressure originated by the wave and the resting pressure (Suslick, 1990).

When US propagates through a liquid media, it creates alternating compression and expansion cycles. When the negative pressure in the liquid, created by the expansion cycle, is low enough to overcome intermolecular forces and reduce the absolute pressure below vapour pressure within the liquid, small bubbles are formed. During subsequent expansion/compression cycles, gas bubbles expand and contract. This phenomenon is known as cavitation.

Depending on the intensity, ultrasonic waves can produce two different types of cavitation: “stable cavitation” and “transient cavitation.” Stable cavitation, originated by high-frequency-low-power US, produces small bubbles, whose size oscillates regularly during thousands of cycles. It also causes microstreaming phenomena in the liquid media. By contrast, in transient cavitation originated by high power US, the bubbles—that must have a minimum size known as resonance size—absorb the energy more efficiently, and their volume gradually increase in consecutive expansion cycles (McClements, 1995). The resonance size depends on the frequency, and at 20 kHz is of approximately 170 μm (Suslick, 1990). At a certain point, the bubble reaches a critical size where it cannot absorb any more extra energy from the acoustic wave. At this point, the bubble becomes unstable, its content suddenly condenses, and as a result, a relative vacuum is generated, provoking its implosion (Neppiras, 1980; Berliner, 1984).

As a consequence of the implosion, molecules violently hit each other, and as a result, shock waves originate, creating spots of very high temperature

and pressure. During implosion, temperature and pressure increases in these spots at very fast rates (10⁹°C/s, Neppiras, 1980) attaining in 1 microsecond peaks of up to 5,000°C and 100 MPa (Suslick, 1988). These extreme conditions usually induce water sonolysis, resulting in the appearance of highly reactive radicals.

3.1.1. Biological Effects of US Lethal effects of US on microorganisms are known since 1929 (Harvey and Loomis). Since then, notable advances have been made, and some general statements regarding the relative resistance of various microorganisms can be listed. In this sense, it has been reported that the size and the shape of the cells are important factors determining their resistance to US. It is well known that cells are more sensitive to US the bigger their size (Kinsloe et al., 1954; Ahmed and Russell, 1975) and that coccoid cells are usually more resistant than rod-shaped bacteria (Jacobs and Thornley, 1954; Alliger, 1975). The physiological state of the culture also plays a role, and cells in exponential growth phase are more sensitive than those in the stationary phase (Kinsloe et al., 1954). Gram-positive bacteria are more resistant than Gram-negative bacteria. Moulds present an intermediate resistance (Earnshaw et al., 1995; López-Malo et al., 2005), and bacterial spores are almost not affected by sonication treatments (Sanz et al., 1985).

Lethality of US depends on the amount of energy transferred into the medium (Raso et al., 1999), which mainly depends on the amplitude of the ultrasonic waves, on its frequency, and on the physicochemical characteristics of the medium. It has been reported that the lethality of ultrasonic treatments increases exponentially with the amplitude (Raso et al., 1998b; Raso et al., 1998c; Pagán et al., 1999a; Mañas et al., 2000).

Although previous existing data seemed to indicate that microstreaming generated during stable cavitation is able to inactivate some big sized and red blood cells (Hughes and Nyborg, 1962; Williams et al., 1970), its lethal effect is very low and most authors agree in attributing the lethality of US to transient cavitation (Kinsloe et al., 1954; Davies, 1959; Raso et al., 1998b; Condón et al., 2005). As

described above, the implosion of bubbles results in a sudden increase of temperature in particular spots or regions of the medium, but according to published data, these “hot spots” are not responsible for the bactericidal effect of US. It has been demonstrated that heat induces cellular damages, which are not present in US-treated cells. Moreover, Raso et al. (1998b) developed an equation that allows the prediction of the lethal effect of a combined treatment of heat and US, considering the lethal action of each agent on bacterial cells as independent from the other. This equation has proven to accurately predict the inactivation rate of various microorganisms (Pagán, 1997; Mañas, 1999; Pagán et al., 1999a,b,c; Álvarez et al., 2000). These facts indicate that the mechanisms of inactivation of heat and US are intrinsically different.

The high temperatures and pressures produced in the implosion spots of the bubbles can generate the dissociation of water molecules into OH^* radicals and H atoms (Suslick, 1990) that might be responsible for the inactivation of bacterial cells by oxidative damage. However, it has been demonstrated that microbial cells subjected to oxidant chemicals suffer cellular damages before they became inactivated (Virto et al., 2005), contrary to what it happens with US, where no cellular damages are detected. Moreover, the addition of a free radical scavenger, for example cysteamine, does not change the microbial resistance towards US (Allison et al., 1996; Pagán, 1997; Raso et al., 1998b). These results suggest that microbial inactivation by US is not due to reactive species generated from water sonolysis.

Shock waves produced during transient cavitation might also break the cellular envelopes provoking bacterial inactivation. In fact, according to the opinion of most authors (Davies, 1959; Pagán, 1997; Raso, 1995; Valero et al., 2007), this is the ultimate mechanism responsible for the microbial inactivation by US. Raso (1995) demonstrated by phase contrast microscopy that after a heat treatment, which inactivated 99% of a *Yersinia enterocolitica* population, almost all the cells retained their integrity, whereas after being inactivated by US, cells were completely disintegrated. Guerrero et al. (2001) also observed by microscopy the breakage of yeast cells after an US treatment. In addition, it has been proven that there

are not damaged cells, detected by selective plating media techniques, among the populations subjected to an US treatment (Pagán et al., 1999a). These results confirmed that US inactivate cells through envelope breakdown in an “all or nothing” type mode of action.

The main problem for industrial application of this technology lies in its low lethality. Data already published (Sherba et al., 1991; Allison et al., 1996; Raso et al., 1998b; Pagán et al., 1999a; Pagán et al., 1999b) show decimal reduction time values that vary between 1 and more than 10 minutes for vegetative bacteria; thus, to reach the “performance criterion” nowadays established would require, in most of the cases, very long treatments. This is the reason why many attempts to develop combined processes that increase the lethality of US have been made.

3.1.2. Combined Processes with US The combined processes proposed to date to increase the efficacy of US have either a physical or a biological basis, depending on whether they aim to increase the intensity of the cavitation phenomena or to sensitize bacterial cell to US. So far, combinations tested include pressure, temperature, and antimicrobial substances.

3.1.2.1. Combining US with Pressure The combinations of US with pressure can be classified in those that use moderate pressures, up to 100 kPa, and those that use HHP, up to 1,000 MPa. The first combination was named manosonication (MS) by our research group (Raso et al., 1998b, 1998c), and it is based on the increase of intensity of the cavitation phenomena resulting from sonication in pressurized media. As previously pointed out, the cavitation intensity depends, besides the sonic wave properties, on the physico-chemical characteristics of the treatment media. An increase of pressure results in a higher US intensity threshold necessary to induce cavitation, but once this threshold is exceeded, the energy released as a consequence of the bubble collapse is higher (Alliger, 1975; Raso, 1995). On the other hand, the amount of energy transferred to the medium by US due to the increase in pressure is

not dependent on the amplitude of ultrasonic waves (Raso et al., 1999).

From published data (Raso et al., 1998b; Pagán et al., 1999a; Mañas et al., 2000), it can be deduced that there is an exponential relationship between pressure and D_{MS} , up to a given value, where further increases in the external pressures do not increase the lethality of the combined treatment in the same magnitude. That value is close to 300 kPa for vegetative cells and to 500 kPa for bacterial spores (Condón et al., 2005). In some cases, an increase in pressure in the range above that critical value could even reduce the lethality of US.

As described above, ultrasonic waves propagate within the media, generating alternative cycles of compression and expansion. If, during the latter ones, the ultrasonic intensity is high enough, intramolecular forces are overcome and bubbles are formed, or, more commonly, if there is a bubble already in the liquid medium, its size increases. As it can be expected, the increase of hydrostatic pressure makes the formation and growth of bubbles more difficult. If ultrasonic intensity is high enough, the bubble grows by means of a higher energetic consumption but releasing more energy when it implodes and, thus, increasing its lethal effect. If pressure is too high, bubble growth is completely inhibited and transient cavitation ceases, therefore diminishing the lethality of the treatment.

Pressurization of media presents another additional advantage of physical nature. The temperature increase causes an increase of the vapour pressure of the medium, which should facilitate cavitation, but it should also reduce the intensity of implosion, as the vapour within the bubbles acts as a cushion (Alliger, 1975). Obviously, when boiling temperatures are reached, cavitation ceases, but application of hydrostatic pressure makes possible the cavitation phenomenon in media at temperatures over 100°C: This phenomenon also makes possible the design of combined processes involving heat and US in order to inactivate bacterial spores (sterilization processes).

The combination of HHP and US has only been explored by Lee et al. (2003). These authors subjected *L. monocytogenes* and *E. coli* cells to an ultrasonic treatment and after that the suspensions were

exposed to HHP. This combination did not present any synergistic effect, neither in *L. monocytogenes*, nor in *E. coli*. These results can be easily explained, because given the fact that US inactivation is an “all or nothing” phenomenon that mainly affects the cellular envelopes, it seems reasonable that the ultrasonic treatment does not sensitize cells to HHP. On the contrary, it can be foreseen that the application of the two agents in the inverse order, HHP followed by US, could render a synergistic effect, because HHP, as we will review later in this chapter, may induce structural changes in the membrane, which could modify cell resistance to US. However, this combination would be less interesting for the food industry, since it suppresses one of the main advantages of high pressure, the possibility of treating packaged foods, as the authors point out.

It has to be noted that the combination of US and HHP as conceived by Lee et al. (2003) may present synergistic effects if applied to inactivate bacterial spores, because it has been proven that US causes damage to their cellular envelopes before inactivation takes place (Raso, 1995).

3.1.2.2. Combining US with Heat As previously described, an increase in the treatment medium temperature results in a decrease in its viscosity and vapour pressure, leading to lower cavitation intensity. Thus, an antagonistic effect would be expected from the combination of temperature and US, as it has been reported for US applied at temperatures close to that of boiling water (García et al., 1989). However, at mild temperatures (40–70°C), most authors have verified that the lethal efficacy of the combination (thermoultrasonication) increases with increasing temperatures (Ordóñez et al., 1984; Earnshaw et al., 1995; Hurst et al., 1995). The synergistic effect observed has been attributed to the fluidization of the cytoplasmic membrane, resulting in a lower resilience of the cellular envelopes.

Thermoultrasonication effect on bacterial spores is limited. This is not surprising, since the cellular envelopes of the spores are much more resistant to mechanical stress than those of vegetative cells, and on the other hand, temperatures needed for sporeformers

thermal inactivation are close to water boiling point, conditions where cavitation ceases.

In 1992, Sala et al. suggested that the application of mild pressures would prevent medium boiling above 100°C and would widen the range of temperatures where cavitation occurs. Moreover, external pressure increase would result in higher energy consumption and, therefore, in an increase in the amount of energy transferred to the medium during cavitation (Berliner, 1984). The combination of US, temperature, and pressure was named “Manothermosonication (MTS),” whereas the application of US under pressure at room temperatures was denoted “Manosonication (MS).”

At temperatures below 40–50°C approximately, decimal reduction times corresponding to MS/MTS treatments become independent of the processing temperature. At temperatures above this threshold, decimal reduction time values increase exponentially with treatment temperature. Figure 27.1 shows the effect of temperature on $D_{MS/MTS}$ values of *Salmonella senftenberg*. This inactivation kinetic is accurately described by an equation developed by Raso et al. (1998b), where an independent lethal action of heat and US is assumed. In other words, regarding vegetative cells inactivation, this combined process shows an additive effect. There are, however, some exceptions reported. MTS shows a synergistic effect when it is applied to very thermoresistant cells such as

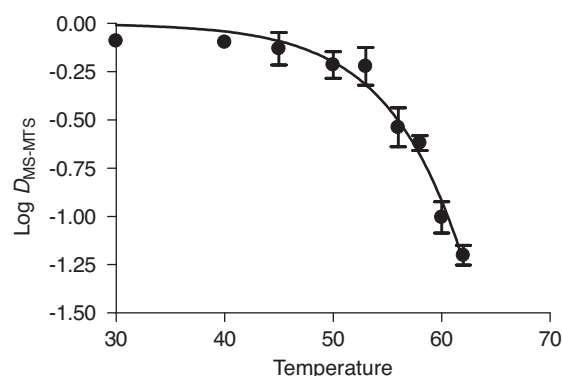


Figure 27.1. Effect of temperature on the inactivation of *Salmonella typhimurium* by ultrasound under pressure (117 μ m, 200 kPa, pH 7.0).

streptococci (Pagán et al., 1999c), cells subjected to a previous heat shock (Pagán et al., 1999b), and cells treated in low water activity media (Álvarez 2000). Condón et al. (2005) suggested that this synergistic effect could be due to the fluidization of the membrane, resulting in a lower mechanical resistance toward ultrasonic shock waves, as previously suggested for thermoultrasonication. We are trying to verify both hypotheses at the moment. MTS also presents a synergistic effect in the inactivation of bacterial spores (Raso, 1995; Raso et al., 1998c), and this has been attributed to an alteration in spore envelopes, which may cause the rehydration of the protoplast and loss of thermal resistance. The application of this combined process would be very efficient energetically, because almost all the energy produced during cavitation is transformed into heat.

3.1.2.3. Combining US with Antimicrobial Agents

The number of publications available about this topic is very limited and sometimes the results reported are contradictory, which might be attributed to the different modes of action of the antimicrobial agents involved. Ahmed and Russell (1975) demonstrated that the efficacy of some chemical disinfectants is higher under an ultrasonic field. Ultrasonic waves disperse cell aggregates, and therefore the cell surface exposed to the disinfectant increases, and so does the efficacy of the combined treatment. This application might be very useful for the sanitization of surfaces in contact with food and pasteurization of water. Recently, Virto (2005) has demonstrated that washing vegetables with sodium hypochlorite solutions under an ultrasonic field increases the microbial lethal efficacy of the process by a factor of 100- to 1,000-fold. This increased efficacy could be partly attributed to the mechanism described by Ahmed and Russell and partly to the removal of air bubbles from the plant structure, which may probably impede the contact between disinfectant and microbes.

The application of US and chemical preservatives, like sorbates and benzoates, also seems to increase the lethality of the process (Arce-García et al., 2002). However, as previously mentioned, cavitation causes cellular inactivation by an “all or nothing”

phenomenon. For this reason, the total expected lethal effect would be of small magnitude unless the chemical preservative lowers the mechanical resistance of the cellular envelopes. Bacterial spores represent an exception to this behaviour, because they suffer some structural damage before becoming inactivated. Raso et al. (1998c) demonstrated that spore suspensions subjected to a MS treatment are sensitized toward lysozyme. This possibility might allow designing sterilization processes, although further research is required in this aspect.

3.2. Ionizing Radiation

IR as a food preservation method was proposed more than a century ago when in 1896, Minsch suggested this technology to inactivate the microorganism responsible for food spoilage (Molins, 2001). Since then, IR has been proven as an effective means to inactivate pathogenic and spoilage bacteria, parasites, and to a lesser extent, viruses. Although many investigations have been carried out on this technology, especially in the 1950s and 1960s, nowadays some important aspects of microbial inactivation through IR still remain unclear. This is an important aspect to solve in order to facilitate the industrial implementation of IR and also for the design of combined processes based on this technology. An especially important question is the type of radiation applied. For instance, the level of inactivation attained is known to be dependent on the applied dose, which is measured with different dosimeters, but it remains unknown if the microbial behaviour is the same for the different types of radiation (γ and β) available for the food industry. If it were not the same, direct comparisons between data obtained by different researchers and data obtained under different experimental conditions could not be made.

3.2.1. Biological Effects of IR We should keep in mind that the total effect of IR on biological systems is the result of the addition of its direct and indirect effects. The direct effect is referred as the chemical changes produced on the molecules after the absorption of radiating energy; in other words,

it is the consequence of ionization—displacement of electrons from their habitual orbitals resulting in the appearance of ions, free radicals, and excitable particles. This energy absorption is, theoretically, greater the higher the number of electrons of the atoms of a molecule is, thus, the greater its molecular weight is. Since the nucleic acids are the more complex molecules at the cellular level, the possibility of genetic material suffering the direct action of IR is high. That is the reason why DNA is one of the key targets of this direct action of IR. For instance, it has been estimated that a dose of 0.1 kGy dose would damage 0.0005% of the amino acids, 0.14% of the enzymes, and 2.8% of the DNA of a particular cell (Pollard, 1966). As a consequence, it seems logical that the irradiation lethal dose decreases with the increase in complexity of the DNA of an organism.

On the other hand, the indirect action of IR is attributed to the interaction between free radicals formed from the direct action of IR on various cellular and medium components, especially on water. The indirect mechanism of action is, according to most authors (Urbain, 1978; Thomas, 1988; Moseley, 1989; Loaharanu, 1995; Rahman, 1997; Calderón García, 2000), mainly responsible for the effects of IR. This indirect effect is greater the higher the water content of the treatment medium. Furthermore, the molecular oxygen dissolved in water can react with hydrogen radicals or with free electrons, resulting in the formation of hydroperoxide radicals and superoxide anions. Among the many radicals formed, the most reactive by far is the hydroxyl radical (OH^*) (Moseley, 1989).

Whatever the mechanism involved, damage to the double DNA helix interferes with the expression of a number of genes and biosynthesis of enzymes, thus affecting cellular division, both in prokaryotic and eukaryotic cells. For this reason, IR is useful not only for microorganism control, but also to inhibit various physiological processes, such as ripening and germination, in foods of plant origin. The survival or death of an irradiated cell will depend on the intensity of the damage induced and on its ability to repair the damaged DNA, this later aspect being very variable amongst the different organisms (Moseley, 1989).

IR can cause the breakage on only one or on both DNA strands. The occurrence of double strands is believed unlikely to occur because it requires a very specific orientation of the DNA molecule with respect to the irradiation source. It can be also produced by breakages located in very close points of both strands, another fact that is not very frequent (Molins, 2001). It is generally acknowledged that approximately 20% of the free radicals affect the sugars of the DNA, whereas the remaining 80% affect the bases (Moseley, 1989). The occurrence of double strands represents an irreversible damage usually irreparable for the cell. On the contrary, the more common damages, those that affect sugars or bases in only one strand, may be repaired in most cases, proven the recovery conditions are appropriate. An average of 2.1 breakages per strand of DNA are estimated to occur as the result of exposure to a radiation dose of 10 kGy in the presence of oxygen, and the damages produced by a radiation of 120 kGy are considered as nonreparable.

Repair of bacterial DNA is carried out through two different mechanisms (Neidhart et al., 1990). The first one is a quick response mechanism that takes place within 1 or 2 minutes at 20–37°C or alternatively in 10 minutes at 0°C. This mechanism is independent of the recovery medium and requires two enzymes: firstly, the DNA polymerase-I deletes and replaces the damaged bases, and thereafter the enzyme polynucleotide-ligase joins the ends of the broken DNA strand. The second mechanism is slower; it takes between 40 and 60 minutes at 37°C, is highly dependent on the recovery media and involves a recombination process, which uses the parental DNA strand as template.

It is important to emphasize that, as a consequence of the repair mechanisms taking place after IR, mutations in bacterial cells may occur, particularly when only one strand has been affected (Muller, 1928). Fortunately, so far the induction of virulence has not been reported. On the contrary, it has been observed that some pathogenic species may reduce or even lose some virulence markers as a consequence of the exposure to IR (Sommers et al., 2002).

Besides the effects on the DNA, IR can also affect other components and cellular structures, such as

enzymes, various cytoplasmic components, and the envelopes, whose permeability may be modified with the corresponding consequences on cellular viability. In this sense, Yatvin et al. (1987) demonstrated that radicals formed from water radiolysis can react with polyunsaturated fatty acids of cellular membranes, affecting enzyme and protein membrane-linked activity. This may cause loss of intracellular components and even cause the breakage of the membrane and subsequent death within a given specific treatment. This means that IR may injure the cells at different levels, fact that plays a very significant role on microbial survival even if no irreversible damages have occurred (Molins, 2001).

Microbial radio-resistance widely varies, although as it has been described above, resistance toward irradiation decreases as the genetic complexity of the microorganism increases. Thus, viruses, due to their lower DNA content, their smaller size, and lower water content are the most resistant, followed by bacteria, moulds, and yeast (Gerwen et al., 1999). Within bacteria, vegetative cells are approximately between 10 and 50 times more sensitive than spores, possibly because their higher water content (a 70% in vegetative bacteria as compared to less than 10% in spores). Amongst vegetative cells, Gram positives are more resistant than Gram negatives (Farkas, 1998). This is a very general classification, and many exceptions to these general observations have been reported. It is well known that the case of *Deinococcus radiodurans* shows a resistance even greater than bacterial spores, probably due to its complex DNA reparation mechanism. DNA repair enzymes from this microorganism are very efficient and quick, and in addition, each cell contains between 4 and 10 copies of its own chromosome, which assures the integrity of at least one of them after the IR treatment (Narumi, 2003). Fortunately, this particular species has no significance either in public health or in food spoilage.

Sensitivity of microorganism to IR also depends on the environmental conditions, such as the nature of the treatment medium, the temperature of the product, the composition of the atmosphere surrounding the food, or the presence of chemical preservatives (Patterson and Loaharanu, 2000).

3.2.2. Combined Processes with IR From a practical point of view, the existence of sublethal damage as a consequence of IR treatments is of great interest for Food Technology, because it provides the possibility of developing combined processes. These processes would be, logically, of lower intensity and would allow attaining a given level of microbial inactivation with the minimum impact on the organoleptic and nutritional properties of foods. Among the combinations already studied, irradiation at high and low temperatures, combinations with modified atmospheres, and the irradiation in the presence of chemical preservatives are perhaps the most promising ones.

3.2.2.1. Combining IR with Temperature As it happens with dehydrated products, the low water availability of frozen foods reduces the indirect effects of IR. That is the reason why microbial resistance to IR is higher in frozen products. For example, it has been observed that the D value of *Campylobacter jejuni* in minced meat is 0.32 kGy at -30°C , 0.16 kGy at $0-5^{\circ}\text{C}$, and 0.17 kGy at 30°C (Lambert et al., 1992). These results also demonstrate that it is the physical state of water molecules rather than the temperature itself, the factor that determines microbial sensitivity to IR. It can be summarized that freezing plus irradiation is a combination with antagonistic effect, and therefore, apparently, not particularly interesting from a practical point of view. However, in some cases, this might be the most appropriate choice for the industry, also because the deleterious effects of irradiation on the sensory and nutritional characteristics of food are of a much lower magnitude. For instance, the maximum irradiation dose above which modifications of the sensorial characteristics (off-odours) of liquid and frozen eggs are detected are 2 and 4 kGy, respectively. These doses allow the inactivation of 5 and 8 logarithmic cycles of *Salmonella* spp. populations, respectively (Álvarez et al., 2006). In other words, the application of an IR treatment to frozen egg allows obtaining a product with appropriate sensorial characteristics and a higher safety margin with respect to *Salmonella*.

Between freezing and room temperature, approximately, no influence of the treatment temperature

has been described (Lambert et al., 1992). However, at temperatures above a certain threshold, the lethality of the process is enhanced. The combination of irradiation and heat has been studied by applying both agents simultaneously and successively. Kim and Thayer (1996) observed that an application of a thermal treatment and an irradiation successively resulted in an additive effect. This probably means that the inactivation mechanisms of heat and irradiation are different and that injuries produced by heat do not sensitize microorganisms to irradiation. For instance, this would indicate that thermal treatments would not affect the enzymes in charge of DNA repair, which play an essential role in irradiation survival. This result is somehow surprising since, as it is generally well known that heat-damaged cells are more sensitive to oxidative stresses (Martin et al., 1976; Brewer et al., 1977; Hurst, 1984; Stringer et al., 2000).

The simultaneous application of heat and irradiation is known as thermoradiation and has been studied by different researchers (Pallas and Hamdy, 1976; Schaffner et al., 1989; Ama et al., 1994). Normally, a synergistic effect is described for this combination. Pallas and Hamdy (1976) observed that D values of *Staphylococcus aureus* decreased from 0.098 to 0.053 kGy when temperature was raised from 35 to 45°C . Since a previous heat treatment does not sensitize microorganism toward irradiation, it can be deduced that in thermoradiation, the IR is the agent that damages cells and enhances the effect of heat. The synergic effect is also observed after the combination of a previous irradiation and a subsequent thermal treatment (Shamsuzzaman et al., 1990; Welt et al., 2001), fact that reinforces the hypothesis mentioned above. Figure 27.2 illustrates the effect of this combination for *Salmonella senftenberg*. One can expect that damages to DNA or to cellular envelopes induced by irradiation sensitize microorganisms to heat, although this aspect has not been yet clarified. This sensitizing effect has also been demonstrated with bacterial spores (Shamsuzzaman et al., 1990), which opens the possibility to use this process to sterilize foods.

Although the lethality of IR is greater the higher the temperature, thermoradiation presents some

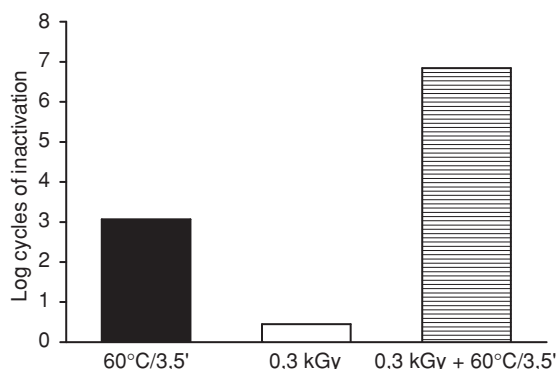


Figure 27.2. Log₁₀ cycles of inactivation of *Salmonella senftenberg* after a heat treatment (60°C/3.5 min), an irradiation treatment (0.3 kGy) and the combination of both.

limitations. In some cases, the increase in temperature may promote some of the deleterious effects on food properties. For instance, IR increases the rate of lipid oxidation faster at higher temperatures (Monk et al., 1994). For this reason, before the industrial implementation of this technology, it will be necessary to better study the effects of irradiation on microorganisms and on sensory and nutritional properties of foods in order to attain the maximum degree of inactivation while minimizing the impact of the treatment on food properties. In other words, it still needs a better optimization of the treatment conditions, aspect not sufficiently studied so far.

3.2.2.2. Combining IR with Modified Atmospheres

The presence of oxygen generally increases microbial sensitivity toward IR, up to threefold, approximately. The oxygen molecule has an uncoupled pair of electrons and, therefore, can be source of free radicals. It has also been demonstrated that oxygen enhances the appearance of peroxide radicals, which also impair repair of cellular injuries (Moseley, 1989).

The presence of oxygen during irradiation may produce undesirable changes in sensorial quality of foods, such as lipid oxidation. To minimize these adverse effects, the application of irradiation treat-

ments under vacuum or modified atmosphere conditions has been suggested, conditions that reduce the incidence of lipid oxidations but hardly change microbial resistance. For example, D values reported for *Escherichia coli* treated in chicken meat were 0.35, 0.29, 0.27, and 0.23 kGy under atmosphere composed of air, 100% CO₂, vacuum, and nitrogen, respectively (Patterson, 1988). Nowadays, the combined treatment consisting of a low dose (less than 3 kGy) irradiation treatment followed by a storage under modified atmospheres or vacuum conditions is one of the more suitable preservation strategies using this technology (Raso and Barbosa-Cánovas, 2003). In fact, it has been reported that irradiation (1.75 kGy) of pork chops packaged in a modified atmosphere containing 75% of N₂ and 25% of CO₂ extends the shelf-life at 4°C from 8 to 12 days (Grant and Patterson, 1991).

3.2.2.3. Combining IR with Antimicrobial Agents

The presence of different chemical preservatives like sodium benzoate, sodium sorbate, sodium chloride, nisin, EDTA, etc., seems to increase the lethality of IR (Monk et al., 1994). Álvarez et al. (2007) observed that the addition of 1 g/L of sorbic acid to liquid whole egg reduced the D values of *Salmonella enteritidis* from 0.5 to 0.31 kGy. The increased lethality of irradiation when chemical preservatives are added is reasonable, if we take into account that, apart from the effects on ADN, the irradiation treatment also provokes damages in other cellular structures, including the membrane. This synergic effect might be the result of a double mechanism: on one hand, the presence of free radicals might impair the repair of cellular injuries; and on the other hand, the damage produced on the envelopes might alter their permeability, facilitating the entrance into the cytoplasm of hydrophobic compounds with antimicrobial activity (Helander et al., 1997). Combinations of chemical preservatives plus thermoradiation or irradiation under modified atmospheres will probably present synergistic effects since heat also modifies cellular envelopes' permeability, although these combinations have not yet been investigated.

3.3. Pulsed Electric Fields

PEF treatments consists of the application of high-intensity electric field (10–50 kV), short-duration (1–100 microseconds) pulses to a food placed between two electrodes.

High-intensity electric fields induce the formation of pores in cellular membranes in a phenomenon known as electroporation. Reversible electroporation is a very useful tool in biotechnology and molecular genetics because, due to the modification of cellular permeability, it allows introducing different molecules like proteins and DNA inside the cell or promotes the cellular fusion (electrofusion). By contrast, irreversible electroporation leads to cell inactivation. Food preservation by PEFs is based on this later phenomenon.

3.3.1. Biological Effects of PEF Permeabilization of cellular envelopes as a result of PEFs application is a well-known fact. However, the intrinsic mechanism leading to the formation of pores is not completely understood yet. Among the proposed theories, Zimmermann's is the most generally accepted (Zimmermann et al., 1974). According to this theory, the cellular membrane would behave like a capacitor, composed of a material with a low dielectric constant as compared to that of both the intra- and the extracellular media where they are suspended. Free charges tend to accumulate in the inner and outer surface of the membrane, generating a transmembrane potential of about 10 mV. When an external electric field is applied, as in PEF treatment, a higher amount of free charges of opposite charge accumulate at both membrane surfaces, resulting in compression of the membrane. When the external electric field exceeds a critical value or threshold (approximately 1 v), the membrane is unable to withstand the compression forces and pores are formed. The size and amount of pores depend on the electric field strength and the duration of the treatment. Also, depending on the treatment intensity, pores can be either reversible, that is, they reseal after the treatment, or irreversible.

There are, however, other theories that try to explain the occurrence of pores in membranes under an electric field. Tsong (1991) attributes the perme-

abilization to the effect of the electric field on both the protein and the lipid fraction. According to this author, the transmembrane potential generated by an external electric field induces the aperture of the protein channels. Inside these channels, a Joule heating or other electric modifications may occur and proteins might be irreversibly denatured, resulting in the formation of pores. The external electric field also induces the reorientation of membrane lipids, resulting in the formation of hydrophilic pores. Furthermore, the electric current passing through the already existing pores of the lipid fraction may also result in a change of membrane fluidity as a consequence of localized Joule heating. This increased membrane permeability could induce an increase in cell size and its breakage as a result of the entry of water and low molecular weight solutes, as well as the leakage of cytoplasmic contents such as ATP or nucleic acids. It has also been observed that these treatments change some of the membrane functions. This is the case of its ability to maintain the intracytoplasmic pH and saline homeostasis.

Electronic microscopic observations of different microbial species exposed to PEF have shown the presence of broken cells and pores and other alterations in the membrane (Harrison et al., 1997). Nevertheless, the number of cells with membrane alterations is usually lower than the number of inactivated cells, what suggest the existence of other inactivation mechanisms (Barsotti and Cheftel, 1999).

The occurrence of sublethal damage in PEF-treated cells has been a matter of controversy for years. Although the presence of transient pores suggest the possible existence of sublethal damage, data published in the late 1990s suggested the contrary (Simpson et al., 1999; Dutreux et al., 2000a,b; Unal et al., 2001; Ulmer et al., 2002; Wuytack et al., 2003; Aronsson et al., 2004) and only occasionally was reported the occurrence of injured cells, in an small percentage of the microbial population (Damar et al., 2002). The absence of sublethal injury strongly suggested that microbial inactivation by PEF was an "all or nothing" phenomenon, and so was generally assumed. Later on, investigations carried out in our laboratory have made important contributions in this matter. Nowadays, we know that, under specific

treatment conditions, more than the 99.9% of the surviving population may be sublethally injured (García et al., 2003; García et al., 2005a). We also know that the occurrence of sublethal injury depends on the treatment media pH and on the bacterial species. For instance, Gram-positive bacteria present a higher proportion of cells damaged when the PEF treatment is carried out at pH 7.0, whereas for Gram-negative bacteria, a higher proportion of damaged cells are detected after treatments at pH 4.0 (García et al., 2005a). Moreover, it has also been reported that cells repair the damages inflicted by PEF by a mechanism that requires energy and lipid synthesis, but not protein, peptidoglycan or RNA *de novo* synthesis.

The numerous factors affecting microbial inactivation by PEF, together with the diversity of equipments used in different laboratories, make difficult the comparison of results obtained by the different authors. Already published data suggest that neither the medium conductivity nor the pulse width influences PEF lethality on microorganisms. Frequency is another nonessential parameter; proven lethal temperatures are not reached (Hülshager et al., 1981; Jeantet et al., 1999; Raso et al., 2000). Efficacy of the treatment strongly depends on the electric field strength and on the treatment time. Although an exponential relationship between the lethality attained and the energy applied (Heinz and Knorr, 2000) has also been reported, the studies carried out to date (Schoenbach et al., 1997) show that, for the same energetic consumption, treatments applied at higher voltages are more effective.

PEF treatments are able to inactivate vegetative bacteria, moulds, and yeast. Among them, yeast are the most sensitive, and Gram-positive bacteria are generally more resistant than Gram-negatives, at pH 7 (Sale and Hamilton, 1967; Hülshager et al., 1983; Zhang et al., 1994; Qui et al., 1998; Vega-Mercado et al., 1996; Wouters and Smelt, 1997; Qin et al., 1998). Most of the studies show that bacterial spores are, under usual treatment conditions, resistant to PEF, even after germination (Pagán et al., 1998).

Cellular size determines microbial inactivation by PEF since a smaller cellular size implies that a lower transmembrane potential is induced by a given electric field strength, and therefore, smaller cells are

more resistant to the treatment (Zimmermann et al., 1974; Hülshager et al., 1983). Various studies have also demonstrated that cells in exponential growth phase are more sensitive to PEF than those in stationary phase (Jacob et al., 1981; Hülshager et al., 1983; Pothakamury et al., 1996; Barbosa-Cánovas et al., 1998; Wouters et al., 1999; Álvarez et al., 2000).

The effect of the treatment medium pH is controversial (Sale and Hamilton, 1967; Hülshager et al., 1981; Zhang et al., 1994; Vega-Mercado et al., 1996; Wouters et al., 1999; Álvarez et al., 2000) and will be analyzed later in this chapter with more detail. Regarding the influence of water activity, Álvarez et al. (2000) observed that a 22 kV/cm treatment decreased 4 log cycles of the population of *S. senftenberg* in pH 7.0 McIlvaine buffer, whereas when water activity of this media was lowered until a value of 0.93 (53% of sucrose added), less than 1 cycle was inactivated. Finally, also the chemical composition of foods, apart from its physical characteristics, influences microbial resistance to PEF (Zhang et al., 1994; Grahl and Märkl, 1996; Keith et al., 1998).

3.3.2. Combined Processes with PEF The low efficacy of PEF on bacterial spore inactivation, together with the decrease in the inactivation rate as the treatment time increases—PEF inactivation does not follow an exponential kinetic of inactivation, and PEF inactivation graphs show a concave upwards shape—and the existence of sublethal damage, makes this technology particularly suitable for combined processes. Combinations with PEF already tested include thermal treatments at moderated temperatures, acidification, and the addition of natural antimicrobials. Although data available about its combination with HHP and MS are scarce, these combinations will be mentioned here too.

3.3.2.1. Combining PEF with Heat PEFs are able to inactivate microorganisms at nonlethal temperatures. However, it has been reported that the lethality of PEF increases with increasing treatment temperatures, above a temperature threshold. This effect has been demonstrated both at lethal and nonlethal temperatures.

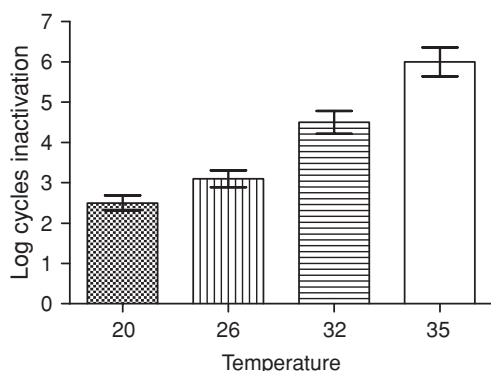


Figure 27.3. Log₁₀ cycles of inactivation of *Listeria monocytogenes* after a PEF treatment at 36 kV/cm of 12 microseconds ($E = 26$ kJ/Kg) at different temperatures.

From a practical point of view, the increase in food temperature as a result of PEF processing might result favourable, proven that nondeleterious temperatures for food properties maintenance are reached. This heating would allow reducing the processing time or alternatively increasing the inactivation degree attained. Moreover, heat is generated as a result of the Joule effect, due to the electric current passage. Thus, the design of an anisothermic combined heating, within the appropriate temperature range, would be energetically more efficient.

Figure 27.3 demonstrates the effect of temperature on *L. monocytogenes* inactivation by PEF at a given electric field strength, and Figure 27.4 demonstrates the effect of temperature at two different elec-

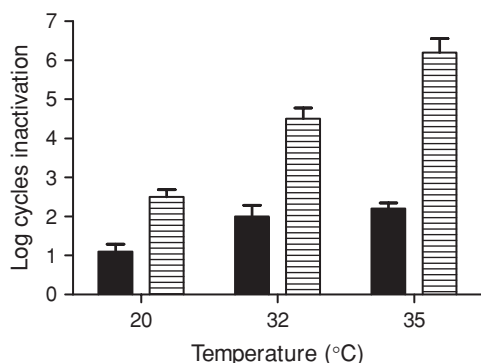


Figure 27.4. Influence of temperature on *Listeria monocytogenes* inactivation by PEF at 28 kV/cm (black bars) and 36 kV/cm (striped bars).

tric fields strengths. From these figures, it can be deduced that there is a remarkable sensitizing effect to PEF caused by the increase in temperature and that this sensitizing effect is of a greater magnitude at higher electric fields strengths.

Evrendilek and Zhang (2003) observed that a previous heating did not sensitize *E. coli* O157:H7 toward a subsequent PEF treatment. This result is not surprising, and, in fact, this combination could even present an antagonistic effect. It is well known that low intensity heat treatments induce the synthesis of heat shock proteins, which protect cells toward other technological treatments. Little is known about the effect of heat shock on PEF resistance, but García (personal communication) has demonstrated that the exposure of *E. coli* to a previous heat shock does not protect cells against a PEF treatment. The application of the treatments in the opposite order did not either result in a higher lethality (Floury et al., 2006). These results suggest that the inactivation mechanism of heat and PEF is different and largely independent and that the synergic effect of its simultaneous application may lie the membrane fluidity changes induced by heat, resulting in a membrane sensitized toward PEF, as it has been previously suggested (Jayaram et al., 1992).

3.3.2.2. Combining PEF with Acidification As previously quoted, early data about pH effect on PEF resistance were contradictory. While some authors found that PEF resistance decreased with decreasing pH, others found that it increased, and even others reported that it had no influence (Álvarez et al., 2000; Aronsson and Rönnér, 2001; Wouters et al., 2001a,b). Recently published works demonstrate that pH effect on PEF resistance in a particular species depends on the type of microorganism and, more specifically, on the structure of their envelopes. García et al. (2004) have reported that a PEF treatment that attained 1 log cycle inactivation of a *L. monocytogenes* population at pH 7.0 inactivated 4 log cycles at pH 4.0. By contrast, a treatment able to inactivate 4.5 log cycles of an *E. coli* O157:H7 population at pH 7.0 only inactivates 1 log cycle of the cells in a pH 4.0 medium (Figure 27.5). This different behaviour can be, according to available data, considered as characteristic

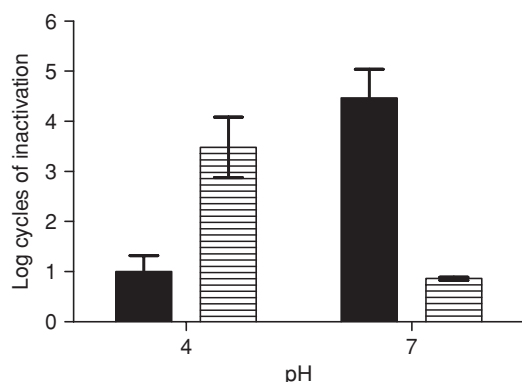


Figure 27.5. Log₁₀ cycles of inactivation of *Escherichia coli* O157:H7 (black bars) and *Listeria monocytogenes* (striped bars) after a PEF treatment at 25 kV/cm for 300 exponential decay pulses in citrate-phosphate buffer of pH 7.0 and 4.0.

for Gram-positive and Gram-negative bacteria, respectively. In addition, the same researchers have found that both groups present sublethal damage at the pHs where they show a higher PEF resistance and that the ability to repair this damage is the reason for its increased PEF resistance.

Various practical conclusions can be drawn according to these investigations. If a combined process is going to be based on the combination of acidification and PEF, it must be taken into account that the limiting microorganism will probably change: if at neutral pH, usually a Gram-positive would be the target microorganism; in an acid/acidified food, it will probably be a Gram-negative. If we aim to design a more complex combined process, this different behaviour will have even more importance, since in acidified foods the addition of lysozyme or nisin will have little utility, because these compounds are more effective against Gram-positive bacteria.

Another interesting aspect is the finding that microorganisms suffer damage in those conditions in which they show their higher PEF resistance, opening the possibility for designing combined processes with synergistic effects. In this sense, García et al. (2004) have shown the increased sensitivity toward acid pH of PEF-damaged cells. In other words, the lethal effect attained by the PEF treatment increases during the subsequent storage in an acidified

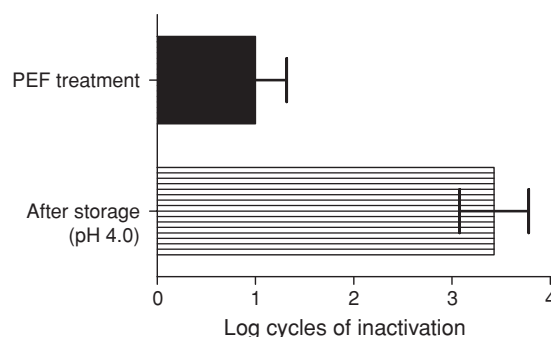


Figure 27.6. Log₁₀ cycles of inactivation of *Escherichia coli* O157:H7 after a PEF treatment at 25 kV/cm for 300 exponential decay pulses with (black bars) and without (striped bars) a subsequent holding for 2 hours in citrate-phosphate buffer at pH 4.0.

medium. Figure 27.6 illustrates this effect. These results suggest that a process consisting of the application of a PEF treatment to food with a neutral pH and its subsequent acidification previous to packaging will probably have a synergistic effect. In addition, the results show that the lethality of a PEF treatment applied on acidic conditions should not be quantified immediately after its application but after storage of a few days, because cells will progressively die as a consequence of cellular damage. In order to be able to predict the effect of this combination, we can also recover the cells in a media with the maximum non-inhibitory concentration of NaCl, which, as it is well known, will prevent the repair of damaged cells. In any case, it has to be taken into account that the lethality of a PEF treatment applied to an acidified product will be 10- to 100-fold more effective than the apparent lethality observed immediately after the treatment.

3.3.2.3. Combining PEF with Antimicrobial Agents
Combinations of PEF and antimicrobials have been proven as effective to obtain an important degree of inactivation of pathogen and spoilage microorganisms. The addition of nisin reduces PEF resistance both in Gram-positive and gram-negative species (Kalchayanand et al., 1994; Calderón-Miranda et al., 1999a, 1999b, 1999c; Dutreux et al., 2000b; Terebiznik et al., 2000; Pol

et al., 2001a, 2001b), probably because electroporation of external envelopes caused by PEF allows the inclusion of the antimicrobial agent in the cytoplasmic membrane, which is its preferential target. The addition of combinations of nisin, lysozyme, and carvacrol also increases, even more, the lethality of PEF.

The lethality of PEF treatments also increases in the presence of benzoic and sorbic acid at pH 3.4 but not at pH 6.4 (Liu et al., 1997). These results are quite surprising. It is generally accepted that these acids can only enter the cell at low pHs, when they are undissociated. However, it was accepted that electroporation produced by PEF allowed them to enter even in its dissociated form. In such case, a synergistic effect even at neutral pHs should be expected. Data from Liu et al. (1997) indicate that either the dissociated fraction cannot pass through the pores or the anion does not have any specific lethal effect.

Apart from the combined processed already mentioned, there are a few more combinations sporadically reported in literature. Perhaps, the combination of PEF and HHP deserve special attention and will be explained in more detail later in the chapter.

3.4. High Hydrostatic Pressure

Treatment of food by HHP is carried out in stainless steel cylinders filled with water. Food is packaged inside flexible films, and it is subjected to pressures between 100 and 1,000 MPa for a given time, generally of various minutes. One of the most important advantages of this method is that the duration of the treatment is independent of the amount of product to be processed and also that once the desired pressure is reached, no additional extra energy is needed to maintain treatment conditions. Its main disadvantage is that the treatment process is discontinuous and that the amount of product that can be processed is limited by the treatment chamber size.

HHP allows extending food shelf life due to its inactivation effect on microorganisms and enzymes, but it also provokes certain changes on food constituents, especially on its texture characteristics. For this reason, HHP may be applied in Food Technology

mainly with two different applications: food preservation/pasteurization and food processing.

3.4.1. Biological Effects of HHP The mechanisms behind vegetative cell inactivation by HHP are not fully known; however, in recent years, there have been important advances in this field of research. Mackey and Mañas (2008) have thoroughly described current knowledge to date and have proposed an inactivation mechanism for *Escherichia coli* as a model for Gram-negative cells.

It is generally accepted that HHP inactivation is multitarget in nature. HHP affects cells envelopes, causes cytoplasmic protein aggregation, DNA clumping, enzyme inactivation, and ribosome conformational changes, among other modifications. Despite some authors having found relationships between cells inactivation and protein denaturation (Sonoike et al., 1992) or have reported changes in nucleoid conformation (Mañas and Mackey, 2004), according to Mañas and Mackey (2004), neither DNA nor protein condensation would be lethal changes if the membrane functionality and integrity is properly maintained upon decompression, proven the recovery conditions are adequate.

It has been demonstrated that in *E. coli*, a linear relationship can be found between the ribosome-associated enthalpy and loss of viability (Niven et al., 1999). However, as discussed by the authors, the loss of integrity of ribosomes cannot be solely attributed to the direct effect of high pressure. In such case, during the first moments of the HHP treatment, it would be expected certain ribosome degradation previous to the onset of loss of cell viability, given the fact that each bacterial cell contains a number of ribosomes higher than that needed for survival. Niven et al. (1999) found a relationship between *E. coli* inactivation and loss of ribosome conformation; however, this later was partially recovered when treated cells were incubated in a magnesium enriched medium after decompression. According to these authors, the loss of ribosome conformation could be partially due to the loss of magnesium across the membrane during the HHP treatment. In fact, Perrier-Cornet et al. (1999) observed a direct relationship between loss of viability of a yeast population and loss of

cytoplasmic contents, and some other authors have reported the occurrence of loss of various intracellular solutes and even macromolecules (Mañas and Mackey, 2004). In summary, although ribosome destabilization could be definite, even leading to cell death by HHP, it seems to be a secondary cellular event caused by the previous alteration of envelope permeability that takes place during pressurization.

Early investigations on the mode of action of HHP on microbes already suggested the cytoplasmic membrane as the most important key target (Hoover et al., 1989; Cheftel, 1995; Smelt, 1998; Patterson, 1999; Mañas and Pagán, 2005). High pressure promotes lipid phase transition from liquid crystalline to gel phase (McDonald, 1993), and therefore its lipid composition and physical state determines cellular HHP resistance (Casadei et al., 2002). Various researchers have shown that HHP treatments render cells that take up vital dyes such as propidium iodide (Benito et al., 1999; Pagán and Mackey, 2000; Mañas and Mackey, 2004), and some other alterations, including formation of vesicles and invaginations, loss of intracellular components, and loss of osmotic responsiveness (Mañas and Mackey, 2004). However, attempts to correlate loss of viability and loss of membrane functions and/or integrity have not been completely successful. This might be partly explained by the well-established fact that vegetative cells are able to reseal some of the pores induced by pressure in their envelopes, even once they have already been inactivated (Pagán and Mackey, 2000; Mañas and Mackey, 2004). A direct relationship has been reported between the cytoplasmic membrane permeabilization, measured by propidium iodide uptake after decompression, and loss of viability in exponential-phase *E. coli* cells, but not in stationary phase cells (Mañas and Mackey, 2004; Mañas and Pagán, 2005). These results suggest that other mechanisms are also involved in stationary phase cells inactivation by HHP. Another important remark is that the outer membrane of Gram-negative cells is also permeabilized by HHP treatments (Hauben et al., 1996; Gänzle and Vogel, 2001).

It has been demonstrated that HHP cause damages in various cellular structures, fact which determines

the existence of a great proportion of sublethally injured cells after a given treatment (Patterson et al., 1995; Kalchayanand et al., 1998; Alpas et al., 2000). Sublethal injuries will be repaired only if the recovery conditions are appropriate (Mackey, 2000). For instance, condensation of the nucleoid and massive protein aggregation has been reported to occur in pressurized *E. coli* cells (Mañas and Mackey, 2004), although both alterations were reversed in a short time in the majority of the population. As described above, the conformation of the ribosomes is also recovered in cells incubated in a medium with magnesium ions added (Niven et al., 1999).

Also, envelopes' selective permeability may be regained after decompression, in a different mode depending on the type of membrane. The outer membrane barrier function is automatically and almost immediately regained upon decompression, whereas the cytoplasmic membrane repair is slower and more demanding and requires some biosynthetic activities, including RNA and protein synthesis (Hauben et al., 1996; Chilton et al., 2001).

Finally, it has to be noted that recent investigations in the field of the mode of action of HHP have added interesting data about the involvement of oxidative bursts in vegetative cells (Aertsen et al., 2005), fact that supports the view of HHP inactivation as a multitarget and complex process.

There is a minimum pressure for onset of inactivation of vegetative cells of approximately 100–150 MPa. Above this threshold pressure, the lethal effect depends on the pressure applied and time of pressurization. Increases in pressure above that threshold value produce an increase in the lethality of the treatment. With regards to time response, in this case, kinetics of inactivation is normally not exponential (Smelt, 1998).

Microbial resistance to HHP varies widely. Gram-positive microorganisms are generally more barotolerant than Gram-negatives, and yeast shows an intermediate resistance (Shigehisa et al., 1991; Hoover et al., 1989; Smelt, 1998). Available results seem to indicate that pressure and heat resistance are not related, that is, the most heat-resistant cells such as *S. senftenberg* are not particularly barotolerant (Metrick et al., 1989).

Information available about the influence of the treatment medium composition and characteristics on pressure resistance is sometimes contradictory. Some researchers have observed that neither the pH of the medium (Raso and Barbosa-Cánovas, 2003) nor the type of acid used (Ogawa et al., 1990) influence the lethality of HHP, whereas others have reported the opposite conclusions (Pandya et al., 1995). Lowering the water activity of the medium seems to decrease the lethality of the HHP treatments, protecting microorganisms (Knorr, 1993). The composition of foods has some influence; for instance, it has been reported that *L. monocytogenes* is more resistant to HHP in milk (Styles et al., 1991), and *S. senftenberg* in chicken meat (Metrick et al., 1989) than in buffers. However, *Vibrio parahaemolyticus* was more sensitive in oysters than in buffer (Styles et al., 1991). To date, the component/s responsible for these effects are not known, although some authors have suggested that calcium and other divalent cations could exert a protective effect on microorganisms (Hauben et al., 1996).

Bacterial spores are highly resistant to inactivation by HHP; however, it is well known that a prolonged exposure to pressure induces spore germination and therefore a notable loss of barotolerance (Gould and Sale, 1970; Gould, 1973; Heinz and Knorr, 2001). Unfortunately, it has been demonstrated that in many spore populations, a fraction of spores remain ungerminated after pressurization (Sale et al., 1970).

Also, enzymes show a high tolerance to HHP. Despite some exceptions, data in literature indicate that enzymatic inactivation by HHP at room temperatures cannot be attained with pressures within the range achievable in industrial equipments. For instance, only 50–60% of the pectinmethyl esterase is inactivated after a HHP treatment of 600 MPa for 20 minutes at room temperature, and residual activity of peroxidase after the same treatment is even higher (Mertens and Knorr, 1992).

3.4.2. Combinations with HHP The high barotolerance of bacterial spores and the scarce efficacy of HHP on enzymes constitute an important limitation for the industrial application of this technology

for the preservation of many foods. Nevertheless, it is finding applications for particular cases, where the decrease of the rate of inactivation with time represents a problem or a limitation. For these reasons, and given the ability of HHP treatment to provoke cellular injuries at different levels, the development of combined processes including HHP seems very appropriate.

This technology has been deeply studied in the last years, including several combinations with other emerging technologies (IR and PEF) and with traditional preservation technologies (acid pH, water activity decrease, antimicrobial substances, modified atmospheres, low temperatures). Results obtained are diverse.

3.4.2.1. Combination of HHP with Low pH Available data about the effect of HHP treatments on acid foods are limited and no practical conclusions can be drawn. Also, it has to be considered that since the mechanisms of inactivation of spores and vegetative cells by HHP are different, the effects of the various combinations could also differ. It is remarkable that even within the same microbial groups diverse results have been reported.

It has been demonstrated that HHP inactivate membrane bound ATP-ase in vegetative cells, which plays an essential role in intracytoplasmic pH regulation of vegetative cells. This fact would explain the lowered barotolerance of vegetative cells in acid media (Wouters et al., 1998). According to this theory, several researchers have reported that the inactivation of various vegetative species by HHP increases with decreasing pH (Mozhaev et al., 1994; Mackey et al., 1995; Stewart et al., 1997; Linton et al., 1999; Alpas et al., 2000; Koseki and Yamamoto, 2006). Other authors have reported no effect of the pH (Patterson et al., 1995; Barbosa-Cánovas et al., 1998). These apparent contradictions cannot be attributed to methodological reasons. Maggi et al. (1993) reported that some enterobacteria strains were more barotolerant at acid pHs, whereas others were more tolerant at neutral pH. Alpas et al. (2000) studied the HHP resistance of four Gram-positive and four Gram-negative microorganisms in media of different pH. They concluded that Gram-negative cells

were less resistant to HHP in acid media than Gram-positive cells and that within each bacterial group, the most barotolerant strains were also more resistant to acid conditions. In the same investigation, it was shown that both bacterial groups were sublethally injured at every pH. Perhaps some of the contradictory results exposed above could be explained by the sublethal injury phenomena described here, similarly to the combination of PEF and acidification. In fact, various other researchers have reported the occurrence of sublethal injury in a high percentage of the survival population to HHP treatment, and that injured cells become particularly sensitized against acidic conditions during storage (Pagán et al., 2001). Figure 27.7 shows the survival curves at pH

7.0 (a) and 4.0 (b) of *Staphylococcus aureus* to HHP (450 MPa), recovered in a nonselective media (TSA) and a selective one (TSA + NaCl). In other words, from a practical point of view, the number of surviving cells decreases along the storage period, and therefore the total lethality of the combined process is greater than initially expected. As in the case of PEF plus acidity, these results also indicate the convenience of evaluating the efficacy of the process after a storage period after the HHP treatment.

The mode of action of the combination of HHP plus acidification on spores could be explained as suggested by Gould and Jones (1989) for HHP and IR. Acid media could provoke a loss of divalent cations from the cortex, and alternatively its protonization. In both cases, cortex would suffer a profound alteration, with the subsequent protoplast rehydration and loss of barotolerance. In this case, most authors describe that the combination is effectively more efficient at more acidic pHs (Timson and Short, 1965; Roberts and Hoover, 1996; Stewart et al., 2000), and only occasionally it has been reported the opposite effect (Fornari et al., 1995; Raso et al., 1998a). Ross et al. (2003) remarked that during pressurization, carboxylic acids show enhanced dissociation and become less effective because undissociated acid forms are believed to be the effective antimicrobial entities. This theory would also explain the occasional loss of efficacy of the combined process in vegetative cells. Another interesting aspect not considered, as far as we know, is the influence of the sporulation temperature on the efficacy of the combined process. Sporulation temperature strongly determines heat resistance of *Bacillus* in media of different pHs, and therefore it seems probable that it could also influence spore resistance to the combined process HHP/acidification.

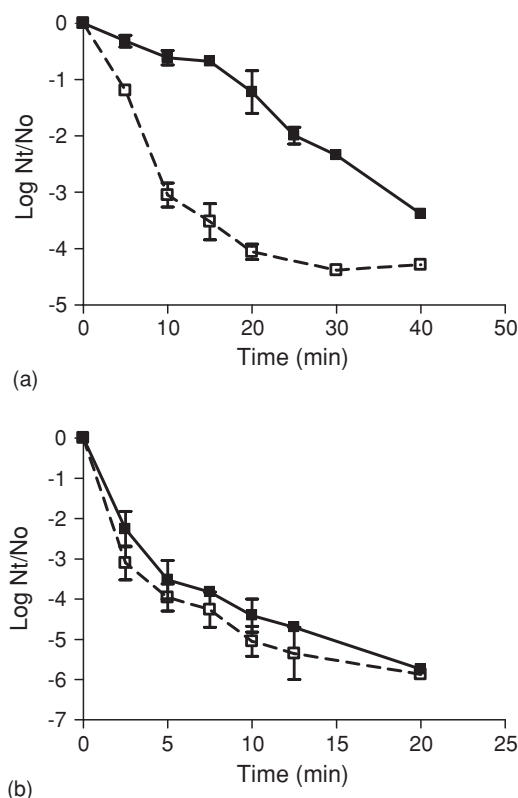


Figure 27.7. Survival curves of *Staphylococcus aureus* 4220 to HHP (450 MPa) at pH 7.0 (a) and 4.0 (b) recovered in TSA (continuous line) and TSA added with 11% w/v of sodium chloride (discontinuous line).

3.4.2.2. Combining HHP with Heat The lethality of HHP also changes with treatment temperature. The higher bacterial resistance has been reported to be at temperatures around 30°C, and treatment temperatures either below or above this maximum lead to a faster inactivation (Mackey and Mañas, 2008). For bacterial spores, the higher lethality of HHP at elevated treatment temperatures has been attributed

to the germination and subsequent loss of heat resistance (Raso and Barbosa-Cánovas, 2003). A summary of the combinations of HHP and temperatures needed to inactivate 5 log cycles of various spore-forming bacteria can be found in the review by Raso and Barbosa-Cánovas (2003). For vegetative cells, the different barotolerance observed at different temperatures has been related to changes in the cytoplasmic membrane. At low temperatures, the higher sensitivity to HHP has been attributed to a decrease in membrane fluidity, which would be difficult to reseal after upon decompression and at temperatures higher than 30–35°C to phase transition of membrane lipids (Mackey and Mañas, 2008). It is also probable that cellular injury and recovery phenomena might also be involved in these cases, since both agents, heat and high pressure, provoke oxidative damage in cells (Aertsen et al., 2005). This aspect has not been sufficiently investigated.

Despite the undeniable interest of the combination of HHP and heat, and from a practical point of view, its application has some technical difficulties. On the other hand, the application of the two technologies in a successive mode could not result as advantageous, since it has been clearly established that both of them induce adaptive phenomena through the synthesis of cross-protective proteins (Wemekamp-Kamphuis et al., 2002; Ananta and Knorr, 2004).

3.4.2.3. Combining HHP with Antimicrobial Agents

Combinations of HHP and natural antimicrobials have been extensively studied and revised in detail by Ross et al. (2003) and Raso and Barbosa-Cánovas (2003). Most authors have found that the addition of nisin, lysozyme, pediocin, lactoperoxidase system, etc., to the pressurizing medium increases the lethality of the treatment (Kalchayanand et al., 1994; Hauben et al., 1996; Roberts and Hoover, 1996; Kalchayanand et al., 1998; Garcia-Graells et al., 1999; Morgan et al., 2000; Masschalck et al., 2001a, 2001b; Ray, 2001). The mode of action of the combination may be different for each antimicrobial. In Gram-negative cells, where the cytoplasmic membrane is protected from the external medium through the external membrane, it has been demonstrated that the permeabilization of this later during pressur-

ization facilitates the entrance of the antimicrobials to their target structure. Therefore, these antimicrobial substances exert the action on the membrane or cell wall, for nisin and lysozyme, respectively, which otherwise would be unaffected. Moreover, it has also been demonstrated that the immediate re-sealing of pores formed in the outer membrane upon decompression prevents the antimicrobial action of lysozyme (Hauben et al., 1996; Masschalck et al., 2001a,b). For Gram-positive microorganisms, the increase in efficacy of these combinations may be related to a more efficient entrance of the antimicrobial substances to the target structures or alternatively to a less efficient damage repair mechanisms in the presence of such antimicrobials in the recovery medium after treatment. In any case, and from a practical approach, the maintenance of HHP-treated cells under unfavourable recovery conditions, such as in the presence of acids or high salt concentrations, amongst others, allows obtaining a higher lethal effect (Pagán and Mackey, 2000; Pagán et al., 2001).

The combination of HHP and nisin also attains a higher lethal effect on bacterial spores (López-Pedemonte et al., 2003). This effect can be attributed to the permeabilization of the proteic external layers of spores, which would facilitate the contact between nisin and the cytoplasmic membrane (Raso and Barbosa-Cánovas, 2003).

3.4.2.4. Combining HHP with IR

The application of HHP and IR either simultaneously or successively has been studied. In this case, the aim was to design sterilization processes by the combination of these two technologies, which have little lethal effect on spores if applied independently. Both modes of application attain some degree of spore inactivation, including species of the genus *Bacillus* and *Clostridium*, but the total lethal effect is not intense enough to implement it as a method for industrial sterilization. Gould and Jones (1989) suggested that HHP induced spore germination, with the consequent loss of irradiation resistance, and that IR produced alteration in the spore cortex, rehydration of the protoplast, and loss of resistance.

In HHP-induced spore germination, it would be expected that the rehydration of the protoplast would

increase the indirect action of the IR treatment, but even with a rehydrated protoplast, the resistance of germinated spores remains higher than that of vegetative cells. In fact, a germinated spore is still very different from a vegetative cell, especially with regards to the DNA, which is highly stabilized in spores by several low molecular weight proteins, and with regards to its peptidoglycan layer, which is much thicker than that of vegetative cells. After germination, spores regain the vegetative cell physiology, but they require a relatively long period, fact that probably explains the results described above.

3.4.2.5. Combining HHP with PEF Another combination that has been explored is HHP plus PEF, also with the objective of inactivating bacterial spores. Pagan et al. reported that a previous HHP treatment induced the germination of up to 5 log cycles of a spore population. This germinated fraction of the spore population is less heat resistant than dormant spores, but on the contrary, it remained resistant to PEF. The authors suggested that the total lethality of the combined process could be increased by a storage period between HHP and PEF application, in such a way that the cortex rupture and subsequent transformation to vegetative form had time to occur. This combination has also been tested with vegetative cells, but its efficacy was scarce. Shimada and Shimahara (1991) observed a slight increase in HHP sensitivity in *E. coli* cells that had been previously subjected to an electrical current. An antagonistic effect has also been described for this combination (Knorr, 2001). This would indicate that the permeabilization of the membrane by both technologies follows different modes of action and that damages inflicted by each technology do not sensitize cells against the other. It is also possible that the application of sublethal HHP treatments would increase the minimum transmembrane potential for electroporation, and this aspect requires further attention.

4. Concluding Remarks

Recent attempts to find alternative technologies for current food preservation methods have been only relatively successful, mainly because their lethality

on microorganisms is limited by the deleterious effects on food components (irradiation, US) or because the rate of inactivation decreases with treatment time (PEF, HHP). In addition, some of them are particularly inefficient against bacterial spores and/or enzymes. For all these reasons, there is a growing interest in designing combined processes that increase the efficacy and field of application of these new technologies.

Many of the combinations already tested were initially aimed to find effective combinations for direct industrial application. For this reason, these early empirical results were sometimes contradictory. Nowadays, the objective is to design adequate combinations according to the mode of action of the different agents. However there are still some aspects about the mechanisms of resistance, inactivation, damage, and recovery that remain obscure, the advances in these fields are being remarkable. Therefore, it can be foreseen that in next years, new combined processes with a strong physiological basis will be designed, to obtain safer and fresher foods.

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Chapter 28

Nonthermal Processes as Hurdles with Selected Examples

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1. Introduction

Consumers increasingly demand food products that, besides retaining their natural characteristics, that is, flavor, color, and texture, further contain fewer additives than the traditional products available in the market. In response, one of the most recent developments in the food industry has been the implementation and design of nonthermal processing technologies, to obtain safe foods with a limited impact on nutritional and sensory quality (Table 28.1).

Nowadays, intense research on multiple nonthermal methods of food preservation is being conducted to evaluate their potential as alternative or complementary processes to those traditionally used by the food industry. Nonthermal processes allow treatment of foods at temperatures below those habitually used in thermal processes, thus preventing the undesirable effects of heat treatments on food quality. Nevertheless, besides preserving the quality of food, new nonthermal technologies need to achieve an equivalent or preferably enhanced safety level, as compared with the replaced techniques. Overall, most nonthermal preservation techniques are highly effective in inactivating vegetative forms of bacteria, yeasts, and molds (Cheftel, 1995; Wouters and Smelt, 1997; Smelt, 1998). However, some problems still remain in achieving inactivation of both bacterial spores

and enzymes, which strongly conditions their use for preservation of certain food products. To extend the use of nonthermal techniques in food processing, intelligent combinations of these technologies, with either traditional treatments or other emerging technologies, are currently under consideration (Raso and Barbosa-Cánovas, 2003). This approach, known as “hurdle technology,” has already been successfully applied using traditional techniques of food preservation (Leistner and Gorris, 1995).

2. Combinations of Nonthermal Processes and Heat

Nonthermal technologies aim to destroy microorganisms, thus ensuring produce safety and stability, while avoiding dramatic losses of sensory and nutritional properties. To achieve this goal, food is held at temperatures below those normally used in thermal processing (Barbosa-Cánovas et al., 1998). However, temperature during nonthermal treatments is an important factor that influences microbial destruction. A higher microbial inactivation rate is usually observed when nonthermal treatments are applied above room temperature. The combination of mild heat treatments with nonthermal processing techniques is of great practical interest because equivalent microbial inactivation levels can be achieved at lower treatment intensities and/or for shorter periods without substantial changes in the fresh-like properties of food (Barbosa-Cánovas et al., 2005).

Table 28.1. Nonthermal processing technologies (National Advisory Committee on Microbiological Criteria for Foods 2006)

Technology	Intensities of Pasteurization	Applications with Success	Limited Use or Efficacy
HHP	Hydrostatic compression 100–1,000 MPa	Ready-to-eat meats, seafood, marinated raw meats, fruit, and vegetable products	Spores
Irradiation	In United States, upper treatment dose is limited to 10 kGy, with exception of spice treatment	Fresh or processed fruits and vegetables, poultry, cereals and derived products, seafood, spices, germinated sprouts, ready-to-eat meals	Negative organoleptic changes in some dairy products. Damage or softening induced in some vegetable products at excessive doses
PEF	High intensity (typically, 20 to 80 kV/cm) electrical pulses of short duration (μ s). Destruction of microbial cells is result of the electroporation of cell membranes	Fruit juices and beverages, fluid dairy products, sauces	Further research required to understand inactivation mechanisms causing lethal or sublethal injury
Ultrasound	Power intensities 1–1,000 W/cm ²	Microbial inactivation in liquid foods, applications in food quality control, hydrogenation of oils, modification of crystallization, aging of alcohol, coalescence of particles, texture modification of meat products	Potential of ultrasound as a sole treatment to pasteurize foods is minimal; more research is needed to elucidate mechanistic insight of changes induced by ultrasounds

HHP: high hydrostatic pressure; PEF: pulsed electric fields.

2.1. High Hydrostatic Pressure (HHP) with Heat

An important difference is found between the vegetative and spore forms of bacteria with regard to the pressure sensitivity of microorganisms. Bacterial spores are more baroresistant than vegetative bacteria (Cheftel, 1992) and may survive pressures above 1,200 MPa (Sale et al., 1970). However, combination of HPP with mild heat treatments can be an effective method of spore inactivation (Mallidis and Drizou, 1991; Seyderhelm and Knorr, 1992; Roberts and Hoover, 1996). Pressure above 600 MPa combined with mild or moderate heat is required to inactivate bacterial spores (Hayakawa et al., 1994; Mills et al., 1998).

There is such a strong synergism between pressure and heat that even the spores of some species can be inactivated at pressures as low as 100 or 200 MPa if the pressure is raised at the same time the heat is applied. The combination of moderate

heat and long-time pressurization to inactivate bacterial spores in buffer solution and model liquid food systems has been studied by some researchers (Furukawa and Hayakawa, 2000, 2001; Furukawa et al., 2001). Raso et al. (1998a) investigated the inactivation of *Zygosaccharomyces bailii* ascospores suspended in apple, orange, pineapple, cranberry, and grape juices and observed that the population of ascospores decreased between 0.5 and 1 log cycles after 5 minutes of treatment at 300 MPa. Moerman et al. (2001) observed the reduction in total plate count of sporulated microorganisms like *Bacillus subtilis* and *Stearothermophilus* in meat batters subjected to combined temperature-high-pressure treatments. Gao et al. (2006) studied the inactivation of *Bacillus subtilis* spores and reported that the optimum process parameters for a 6 log cycle reduction were 87°C, 576.0 MPa, and 13 minutes.

As outlined above, temperature is another important factor affecting the inactivation level of

Table 28.2. Selected examples of inactivation of vegetative bacterial cells by HHP in combination with heat

Microorganism	Food	Comments	Reference(s)
<i>E. coli</i> O157:H7 (NCTC12079)	Milk	400 MPa/50°C/15 min treatment for 5 log ₁₀ reduction	Patterson et al., 1995; Patterson and Kilpatrick, 1998
<i>E. coli</i> O157:H7	Orange juice	6 log reduction at 550 MPa/30°C/5 min	Linton et al., 1999
<i>S. aureus</i> , <i>Bacillus spp.</i> , <i>L. monocytogenes</i> , <i>E. coli</i> O157:H7, <i>Salmonella</i> <i>enteritidis</i> , <i>S. typhimurium</i>	Juices and organic acid liquids	8 log reduction at 345 MPa/50°C/5 min except for <i>S. aureus</i> . Most vegetative cells are sensitive to 700 MPa.	Alpas et al., 2000
<i>Enterobacteriaceae</i> and <i>Listeria</i>	Sausages	500 MPa/80°C/5 min	Yuste et al., 2000
<i>L. monocytogenes</i>	Milk	Growth at 43°C increases resistance to HHP	Bull et al., 2005

vegetative bacteria. In fact, it is well documented that elevated temperatures (30–50°C) promote pressure inactivation of microorganisms (Patterson and Kilpatrick, 1998), but the effect of low temperatures (<20°C) on inactivation is less clear. In ewe's milk, for example, *P. fluorescens*, *L. helveticus*, and *L. innocua* showed higher resistance at 25°C than 4°C, while the opposite behavior was reported for *E. coli* and *S. aureus* (Trujillo et al., 2002). Moreover, in ewe's milk, *E. coli* appeared to be most pressure resistant at 10°C, whereas barosensitivity of *P. fluorescens* was optimal at 25°C (Gervilla et al., 1997). Inactivation of vegetative bacteria cells with combination of HHP and heat has been investigated in different media. Some selected examples are described in Table 28.2.

On the other hand, inactivation of vegetative forms of yeast and molds with combination of HHP and heat has been barely investigated, probably because the microorganisms involved are very sensitive to mild HHP treatments at room temperature (Ogawa et al., 1990; Pandya et al., 1995; Palou et al., 1997; Parish, 1998; Raso et al., 1998b). However, inactivation of mold ascospores requires combining HHP and moderate temperatures (60 to 70°C) (Butz et al., 1995).

The effects of HHP treatments on enzymes may be related to reversible or irreversible changes in protein structure (Cheftel, 1992). However, loss of catalytic activity can differ depending on the type of enzyme, nature of substrates, temperature, and

length of processing (Cheftel, 1992; Kunugi, 1992; Cano et al., 1997).

Some key enzymes in fruit and vegetable processing include polyphenoloxidase, lipoxigenase (LOX), pectinmethylesterase, and peroxidase (POD). High pressure inactivation of these enzymes has been extensively studied in the last few years (Ogawa et al., 1990; Asaka and Hayashi, 1991; Anese et al., 1995; Knorr, 1995; Quaglia et al., 1996; Indrawati et al., 2000, 2001; Soysal et al., 2004; Akyol et al., 2006). LOX inactivation in green beans due to high-pressure treatment was studied in the pressure–temperature ranges of 0.1–650 MPa and 10–70°C by Indrawati et al. (2000). At ambient pressure, whole green beans LOX was less thermostable than the enzyme in the green bean juice at temperatures below 68°C, whereas the stability hierarchy was reversed at temperatures above 68°C. The pressure needed to obtain the same rate of LOX inactivation at a given temperature was lower in whole green beans than in juice. Soysal et al. (2004) investigated the combined effects of pressure and temperature on the POD activity of a carrot extract in the pressure range 0.1–600 MPa and temperature 25–45°C. At lower pressures (<396 MPa), carrot POD stability increased in comparison to nonpressurized samples. Treatments at 600 MPa and 25°C for 15 minutes attained a POD inactivation of 55.3%, whereas 91.2% inactivation was obtained at 600 MPa and 45°C.

Quality retention and preservation of physicochemical properties can be achieved with high

pressure treatments applied at either room temperature or moderately elevated temperatures (Knorr, 1993). High-pressure processing at moderate temperature is currently being used commercially for the pasteurization of products such as fruit juices, guacamole, jams, oysters, and ham (Nguyen et al., 2003). Studies have shown that the vitamin content, color, stability, pectic substances, and antioxidants of fruit and vegetable products can be maintained by applying high-pressure treatments at moderate temperature conditions for short treatment times (5–10 minutes) (Quaglia et al., 1996; Kim et al., 2001; Nienaber and Shellhammer, 2001; Islam et al., 2003).

2.2. Irradiation with Heat

The possibility of using heat in combination with irradiation was first suggested in the 1950s, when synergistic effects of the combined application of both technologies were observed in a variety of biological systems, including bacteria (Grant and Patterson, 1995). More recently, it has been suggested that irradiation in combination with heat could be used to control spoilage and to increase the microbiological safety of certain foods (Farkas, 1990). Although the inactivation effect of heat treatments followed by irradiation is additive (Kim and Thayer, 1996), a synergistic effect has been observed when the heat treatment is applied after irradiation or when both treatments are applied simultaneously (i.e., thermoradiation) (Raso and Barbosa-Cánovas, 2003). Several studies have demonstrated that irradiation has a significant effect on the destruction of vegetative bacteria (Licciardello, 1964; Okazawa and Matsuyama, 1978; Szczawinska, 1981; Thayer et al., 1991) and bacterial spores (Licciardello and Nickerson, 1962; Gombas and Gomez, 1978; Minnaar et al., 1995). For example, no *C. sporogenes* spores survived the heat, irradiation, and heat-irradiation combined treatments of mushrooms in brine (Minnaar et al., 1995).

A synergistic effect on the inactivation of vegetative bacteria and bacterial spores can be achieved with thermoradiation (Schaffner et al., 1989; Thayer et al., 1991; Ama et al., 1994; Grant and Patterson, 1995). In particular, Thayer et al. (1991) investi-

gated the effects of heat and ionizing radiation on *Salmonella typhimurium* in mechanically deboned chicken meat and reported that irradiation treatments at doses 0.9 kGy caused heat-sensitization in *S. typhimurium*. The effect of heating after exposure to ionizing radiation (60, 65, or 70°C/0.8 kGy) on the destruction of *L. monocytogenes* and *S. typhimurium* in artificially inoculated minced cook-chill roast beef and gravy was investigated by Grant and Patterson (1995). They found evidence for radiation-induced heat-sensitization in *L. monocytogenes*.

Combining heat and irradiation at less severe conditions may allow production of high-quality, shelf-stable food products (Farkas, 1990), for instance, mushrooms in brine (Minnaar et al., 1995), cooked chicken breast meat (Graham et al., 1998), or bitter melon (Khattak et al., 2005).

2.3. Pulsed Electric Fields (PEF) with Heat

Synergistic interactions between PEF and thermal treatments have been reported in both lethal and non-lethal processing temperature ranges (Wouters et al., 1999; Heinz et al., 2003; Hermawan et al., 2004; Bazhal et al., 2006; Amiali et al., 2007). Bazhal et al. (2006) achieved an extra 2 log reduction of *E. coli* O157:H7 in PEF-treated liquid whole egg compared with the thermally treated product; treatment was 9–15 kV cm⁻¹ combined with mild temperature of 60°C. PEF treatments performed at 65°C were more effective in inactivating *E. coli* in apple juice than the same treatments at 35°C. Furthermore, the energy consumption used to achieve the same level of microbial inactivation could be reduced in the range of 100 kJ/kg or more to less than 40 kJ/kg (Heinz et al. 2003). Hermawan et al. (2004) reported a maximum of 4.3 log reductions in *S. enteritidis* counts after subjecting inoculated, prewarmed (55°C) liquid whole egg to a 25 kV cm⁻¹ treatment at 200 Hz with 2.12 μs pulses totaling 250 μs.

Inactivation of enzymes with PEF treatment also may ensure the stability of the final product (Yang et al., 2004). A dose of 100 U nisin/mL added to orange juice in conjunction with 20 pulses of applied field at 80 kV/cm, at 44°C, and pH of 3.5, resulted in a 92% reduction of pectinmethylesterase activity

(Hodgins et al., 2002). Yeom et al. (2002) observed a 90% pectinmethylesterase inactivation in orange juice with PEF treatment, at 25 kV/cm and water bath temperature of 50°C.

Pulsed electric field pasteurization does not significantly impair the major attributes of the final product. Hodgins et al. (2002) combined temperature, acidity, and PEF to maximize microbial inactivation in orange juice without affecting its quality. The effect of combining PEF with the addition of nisin, lysozyme, or a combination of both, on the quality of orange juice was also investigated. Optimal conditions, applying 20 pulses of an electric field of 80 kV/cm at pH 3.5 and 44°C, with 100 U nisin/mL added, resulted in over a 6-log cycle reduction in the microbial population, whereas 97.5% of vitamin C content was preserved.

2.4. Ultrasound with Heat

The lethal effect of ultrasound was increased substantially after applying this technology in combination with thermal treatment (Burgos et al., 1972; García et al., 1989), but the inactivation of bacterial spores remained low. It was demonstrated that microbial inactivation with ultrasound increases when treatment is applied under pressure (Manosonication, MS) (Raso et al., 1998c). Thus, the application of MS treatment simultaneously with heat treatment (Manothermosonication, MTS) led to higher microbial inactivation.

Some researchers have investigated the inactivation effect of MS and MTS (Ciccolini et al., 1997; Raso et al., 1998c, 1998d; Pagán et al., 1999; Valero et al., 2007). Ciccolini et al. (1997) studied the combined effect of low-frequency ultrasounds (20 kHz) with temperature on the survival of a strain of *Saccharomyces cerevisiae* suspended in water. Their results showed that ultrasonic waves do not destroy yeast cells, but instead inflict damage, thus increasing their sensitivity to heat. The lethal effect of ultrasonic waves (UW) on *Yersinia enterocolitica* at different static pressures (MS) and combined heat-UW under pressure treatments (MTS) was investigated by Raso et al. (1998c). They found that static pressure is a very efficient means of increasing the lethality of

UW (MS). This increase becomes greater when the amplitude of UW is higher. Between 50 and 58°C, the lethality of heat can be increased by combining heat treatments with UW under pressure (MS). The lethality of this treatment (MTS) is equivalent to the additive lethal effect of heat and UW. Pagán et al. (1999) found that the inactivation of *L. monocytogenes* by high-power ultrasonic waves (20 kHz, 117 mm) at ambient temperature and pressure was low ($D = 4.3$ minutes). The inactivation rate obtained by MS was not influenced by treatment temperature up to 50°C. However, at higher temperatures, the lethality of this combined process (MTS) increased considerably. The influence of ultrasonication and conventional heating under different processing conditions on the inactivation and potential subsequent growth of microorganisms in orange juice was investigated by Valero et al. (2007). Although a limited level of microbial inactivation (≤ 1.08 log CFU mL⁻¹) was attained by selected batch ultrasonic treatment (500 kHz/240 W for 15 minutes), microbial growth was observed in the substrate after 14 days of storage at both refrigeration (5°C) and mild abusive (12°C) temperatures.

MS and MTS allow the possibility of inactivating enzymes without detrimental effects on the quality attributes (López et al., 1994; López et al., 1998; De Gennaro et al., 1999; Kuldiloke, 2002; Cruz et al., 2006; Valero et al., 2007). Kuldiloke (2002) investigated MTS treatment on fresh lemon juice and strawberry juice, showing the great potential of this new technology in maintaining juice properties, such as cloud stability, color, pH, and conductivity. However, in terms of nutritional value, ascorbic acid undergoes degradation during MTS treatment as well as through storage. Valero et al. (2007) did not find ultrasound-related detrimental effects on the quality attributes of juice (i.e., limonin content, brown pigments, and color). The resistance of tomato pectic enzymes to MTS was likewise studied by López et al. (1998). Pectinmethylesterase (PMF) and polygalacturonases (PG I and PG II) were inactivated much more efficiently with MTS treatment than by simple heating. In MTS inactivation of these enzymes, the effect of heat and ultrasonic waves was synergistic. The D values (time required for original enzyme activity

to decrease by 90%) for PME heat inactivation at 62.5°C were reduced 52.9-fold by MTS, and for PG I at 86°C and PG II at 52.5°C reduction was 85.8- and 26.3-fold, respectively.

3. Combinations of Nonthermal Processes and Acidification

3.1. HHP with Low pH

Low pH enhances the inactivation of vegetative bacteria under high-pressure treatment. According to Mozhaev et al. (1994), sorbic and benzoic acids enhance the effect of high-pressure on microorganisms, enabling lower pressures and shorter treatment times to achieve inactivation. Balasubramaniam et al. (2001) achieved an extra 2.5-log reduction for *B. subtilis* spores at 827 MPa and 75°C, when pH of treatment buffer was reduced from 7.0 to 3.0. Roberts and Hoover (1996) also achieved greater inactivation of *Bacillus coagulans* spores at just 400 MPa, when pH was lowered from neutral to 4.0. Other authors, however, have reported that altering the pH has little effect on HHP inactivation of yeasts and bacterial spores (Patterson et al., 1995; Raso et al., 1998d; Marcos et al., 2005; Moerman, 2005). Raso et al. (1998d) reported that optimum pH for germination and inactivation of *B. cereus* depended on sporulation temperature. At 250 mPa, the extent of germination increased with higher pH. At 690 mPa, the pH barely affected germination of *B. cereus* sporulated at 20°C (3 log cycles), but inactivation increased as pH of the medium was lowered. After treatment, the germination of *B. cereus* sporulated at 30 and 37°C (6–7 log cycles) around neutral pH. Higher inactivation was obtained at pH 6. Moerman (2005) studied the microbial spoilage of pork Marengo (a low acidic, partially prepared stew of pork pieces, carrots, and peas), evaluated after a high-pressure treatment of 400 MPa for 30 minutes at 20 and 50°C. Several *Clostridium* spp. and *Bacillus* spp. survived the treatment; the Gram-positive cocci *Enterococcus faecalis* and *Staphylococcus aureus* were more pressure resistant than *Saccharomyces cerevisiae*, and the Gram-negative bacteria *Pseudomonas fluorescens* and *Escherichia coli*. Marcos et al. (2005) manufactured low-acid

fermented sausages (fuet and chorizo) to evaluate the combined effect of high-pressure processing (HPP) and ripening on food-borne pathogens. Raw sausages inoculated with a 3-strain cocktail of *Salmonella enterica* and a 3-strain cocktail of *L. monocytogenes* were pressurized at 300 MPa for 10 minutes at 17°C. Application of HPP as an additional hurdle during the ripening process produced a greater decrease in *Salmonella* population, showing lower counts (3 MPN/g) in ripened sausages. By contrast, values of *L. monocytogenes* counts in non-treated (NT), sausages were lower than in pressurized sausages due to the delay of pH drop caused by HHP inactivation of endogenous lactic acid bacteria.

3.2. Irradiation with Low pH

The lethal effect of ionizing radiation is not greatly enhanced by mild acidification. Namely, destruction of bacteria and, consequently, the shelf life of irradiated chicken and ground beef were not significantly improved by low concentrations of infused or added acetic acid (Hausam et al., 2002; Ouattara et al., 2002). In another study, the differences in pH of five commercial orange juice formulations (from pH 3.87 to 4.13) had no influence on the radiation dose required for 90% inactivation of a *Salmonella* Enteritidis strain isolated from a citrus juice associated with an outbreak of salmonellosis (Niemira, 2001). Farkas and Andrassy (1993) investigated irradiation combined with pH reduction (with different acidulants) for their effects on the shelf life of a chilled and temperature-abused beef/pork product. Enterobacteriaceae were either destroyed or injured by irradiation, in which case, the injured cells were unable to grow at acidic pH, even at abuse temperatures. Radiation-resistant lactic acid bacteria subsequently became the dominant microflora, suggesting that the selection of lactic acid bacteria in irradiated foods might serve as an intrinsic factor for control of the growth of certain pathogens (Farkas and Andrassy, 1993).

3.3. PEF with Low pH

PEF technology is gaining commercial application most rapidly with juices and other fruit-derived

products (Qiu et al., 1998; Jia et al., 1999; Leistner and Gould, 2002), as the hurdle of low pH exists naturally in raw materials. Nevertheless, much more research is needed before this technology can be adapted for production of shelf-stable low-acid foods (Yousef, 2001).

Some researchers have studied the combined effect of PEF with low pH in different media. For example, Vega-Mercado et al. (1996) found that up to 2.2 log reductions in plate counts of *E. coli* in SMUF solution can be achieved when both pH and electric field are modified, from 6.8 to 5.7 and 20 to 55 kV/cm, respectively. The PEF inactivation of *Escherichia coli* O157:H7 in a 10% glycerol solution was enhanced synergistically by lowering pH from 6.4 to 3.4 using benzoic or sorbic acids (Liu et al., 1997). Similarly, adjusting the pH of skim milk and liquid eggs with organic acids has been shown to provide additive and synergistic inactivation of bacteria when combined with PEF treatment (Góngora-Nieto et al., 1999; Fernández-Molina et al., 2001a, 2001b). In contrast, PEF plus acidification with hydrochloric acid resulted in no extra inactivation of microflora in raw milk, compared with PEF alone (Smith et al., 2002). Evrendilek and Zhang (2001) found that exposing *E. coli* O157:H7 to pH 3.6 before PEF treatment resulted in lower inactivation than exposure to pH 5.2 or 7.0. It was concluded that adaptation of *E. coli* to the acid stress resulted in greater survivability during PEF treatment. Aronsson et al. (2004) studied the growth of *Escherichia coli* and observed that the level of inactivation increased with higher electrical field strength and decreased pH values.

3.4. Ultrasound with Low pH

Pagán et al. (1999) found that the effect of ultrasonic treatment (20 kHz, 117 μ m) on *Listeria monocytogenes* under sublethal pressure (200 kPa) was not greatly influenced by a pH reduction of 7.0–4.0. The acidic conditions had a much greater effect on the organism's resistance to heat than its sensitivity to ultrasonication. Similar findings were reported much earlier by Kinsloe et al. (1954), who exposed bacterial and yeast cells to a sonic field in saline suspensions of varying pH. Lowering the pH from

neutrality to 4.0 did not alter the death rates of *Pseudomonas aeruginosa* or *Saccharomyces cerevisiae* under sonication. For *E. coli*, *Serratia marcescens* and *Micrococcus varians*, higher death rates were observed when a higher treatment temperature (45°C) was combined with both sonication and lowered pH (Kinsloe et al., 1954).

4. Combinations of Nonthermal Processes and Antimicrobial Agents

The addition of naturally occurring antimicrobials has proven to be an effective hurdle when combined with nonthermal processing techniques. Nisin is currently the only bacteriocin approved for use in food by the World Health Organization, although bacteriocins, such as those produced by lactic acid bacteria, are legally considered natural if used in concentrations equal to or below those found in foods naturally fermented with bacteriocin-producing starter cultures (Pol et al., 2000; Cleveland et al., 2001).

Two extensively studied natural antimicrobial compounds with potential to control food-borne microorganisms are nisin and lysozyme, but they are normally only effective against Gram-positive organisms; however, lysozyme, nisin, and other bacteriocins have been shown to act on several species of Gram-negative bacteria, because the barrier properties of their outer membrane are firstly disrupted (Ross et al., 2003).

4.1. HHP with Antimicrobials

Combining antimicrobials with HHP can enhance the effectiveness of pressurization with added advantages in product quality and safety (Raso and Barbosa-Cánovas, 2003).

Diverse antimicrobials have significantly enhanced the destruction of both Gram-positive and Gram-negative bacteria in different media under HHP. For instance, HHP inactivation of *Escherichia coli* and *Listeria innocua* inoculated in liquid whole egg was significantly improved with nisin addition at concentrations of 1.25 and 5 mg/L. A reduction of almost 5 log units in *E. coli* counts and more than 6 log units for *L. innocua* were obtained after a

treatment at 450 MPa with addition of 5 mg/L of nisin (Ponce et al., 1998). Morgan et al. (2000) observed a synergistic killing effect of HHP in the presence of bacteriocin lactacin 3147. HHP treatment at 250 MPa caused a 2.2 log reduction of *Staphylococcus aureus* in reconstituted skim milk, while 10,000 AU/mL lactacin 3147 alone caused a 1 log cycle reduction. Both treatments combined, however, resulted in destruction of more than 6 log cycles of the pathogen. Lactacin 3147 exerts a bactericidal effect by disturbing the selective permeability of the membrane of sensitive cells, and it was found that as pressure is increased, the effect of lactacin 3147 becomes more pronounced. Inactivation of the spores of *C. gloeosporioides* in saline solution using high hydrostatic pressures in combination with citral and lemongrass oils was studied by Palhano et al. (2004). *C. gloeosporioides* spores were efficiently inhibited after a pressure treatment of 350 MPa for 30 minutes. When *C. gloeosporioides* spores were treated with 0.75 mg mL⁻¹ citral or lemongrass oil, the pressure needed to achieve the same spore inhibition was 150 MPa. This work suggests the use of high hydrostatic pressure and plant essential oils as an alternative control for fruit diseases.

Timing of antimicrobial addition is important for additive or synergistic effects to occur with HHP. For example, Hauben et al. (1996) found that *E. coli* was not sensitive to nisin or lysozyme after HHP treatment, despite a significant degree of sublethal injury. Addition of lysozyme and nisin during pressurization treatment, however, increased lethality in an additive manner. Masschalck et al. (2001) observed the same phenomenon when using nisin on one Gram-positive and seven Gram-negative bacterial strains exposed to HHP. Fenice et al. (1999) treated sporangiospores with high hydrostatic pressure and/or fungal chitinase in order to study the inhibition of germination and growth of the food spoiling mold *Mucor plumbeus*. Total fungal inhibition was obtained either at 4.0 kbar or with 10 U/mL of chitinase from *Penicillium janthinellum*. A pretreatment with 1 U/mL of the same chitinase reduced the pressure needed to obtain a complete spore inhibition of 3 kbar.

The method of application of HHP treatment can also influence the bactericidal efficiency of added an-

timicrobial compounds. For example, García-Graells et al. (1999) found that pulsed HHP application (i.e., 10-min HHP treatments interrupted by brief decompressions) significantly increased the sensitivity of four *E. coli* strains to both nisin and lysozyme in milk, as compared with exposure to the antimicrobials during a single, continuous HHP application.

4.2. HHP with Carbon Dioxide

Many studies investigated the effects of using carbon dioxide (CO₂) under pressure (dense phase CO₂, DPCD) on pathogenic and spoilage organisms, vegetative cells and spores, yeasts and molds, enzymes and their activities, and food quality attributes. DPCD is a cold pasteurization method that affects microorganisms and enzymes through the molecular effects of CO₂ under pressures below 50 MPa, without exposing foods to the adverse effects of heat and thus retaining their fresh-like physical, nutritional, and sensory qualities. Tables 28.3 through 28.5 summarize the studies carried out in the last few years on inactivation of various microorganisms, spores, and enzymes by DPCD (Damar and Balaban, 2006).

DPCD has been applied mostly to liquid foods, particularly fruit juices (Damar and Balaban, 2006). There are a limited number of published studies discussing its effects on the quality of foods. One of the first food applications of DPCD is treatment of whole fruits such as strawberry, honeydew melon, and cucumber to inhibit mold growth. However, DPCD may cause severe tissue damage in some fruits even at low pressures (Haas et al., 1989).

Studies with orange juice (OJ) show that DPCD treatment can improve some physical and nutritional quality attributes (Arreola et al., 1991; Kincal, 2000; Ho, 2003). For example, Kincal (2000) obtained up to 846% cloud increase in OJ treated by DPCD (38 MPa, room T, 10 minutes). There were no significant changes in pH and Brix of treated samples. A small but insignificant increase in *L** and *a** values occurred. Sensory evaluations of DPCD-treated and untreated OJ were not significantly different. Ho (2003) used a continuous flow system and reported that there were no significant differences between

Table 28.3. Studies on inactivation of various microorganisms by high pressure dense phase carbon dioxide

Medium	Microorganism	Pressure (MPa)	Time	Temperature (°C)	System	Log Reduction	Reference
TSB w/polymers	<i>Bacillus cereus</i>	20.5	4 h	60	Batch	8 (C)	Dillow et al., 1999
	<i>Listeria innocua</i>	20.5	0.6 h	34		9 (C)	
	<i>S. aureus</i>	20.5	4 h	40		9 (C)	
	<i>Salmonella salford</i>	20.5	4 h	40		9 (C)	
	<i>Pseudomonas aeruginosa</i>	20.5	4 h	40		8 (C)	
	<i>E. coli</i>	20.5	0.5 h	34		8 (C)	
	<i>Proteus vulgaris</i>	20.5	0.6 h	34		8 (C)	
	<i>Legionella dunnifi</i>	20.5	1.5 h	40		4 (C)	
	<i>Lactobacillus plantarum</i>	13.8	30 min	30		>6 (C)	
Growth medium	<i>L. monocytogenes</i>	6	75 min	35	Batch	6.98 (C)	Erkmen, 2000a
PS with broth	<i>Enterococcus faecalis</i>	6.05	18 min	35	Batch	8 (C)	Erkmen, 2000b
Fruit juice-milk			3–6 h	45		5 (C)	
PS	<i>Brobothirix thermosphacta</i>	6.05	100 min	35	Batch	5 (C)	Erkmen, 2000c
Skinned meat			150 min				
MRS broth	<i>L. plantarum</i>	7	100 min	30	Batch	>8	Hong and Pyun, 1999
PS	<i>Salmonella thyphimurium</i>	6	15 min	35	Batch	7 (C)	Erkmen and Karaman, 2001
PS with broth	<i>S. thyphimurium</i>	6	140 min	25	Batch	7 (C)	Erkmen, 2001
Whole milk	<i>E. coli</i>	10	6 h	30		6.42 (C)	
Skim milk	<i>E. coli</i>	10	6 h	30		7.24 (C)	
PS	<i>Bacillus subtilis</i>	7.4	2.5 min	38	Semi-continuous	7 (C)	Spilimbergo et al., 2002
Sterile water	<i>Pseudomonas aeruginosa</i>	7.4	2.5 min	38	Continuous membrane	8.7	Sims and Estigarribia, 2002
	<i>E. coli</i>	7.5	5.2 min	24			
Orange juice	<i>L. monocytogenes</i>	38	10 min	25	Batch	6	Park et al., 2002
Orange juice	<i>E. coli</i> O157:H7	107	10 min	25		5	
Apple juice	<i>E. coli</i> O157:H7	20.6	12 min	25		5.7	
Carrot juice	Aerobic plate count	4.9	10 min	5		4	
Watermelon juice	Aerobic plate count	34.4	5 min	40	Continuous flow	6.5	Lecky, 2005
Mandarin juice	Aerobic plate count	41.1	9 min	35	Continuous flow	3.47	Yagiz et al., 2005
Coconut water	Aerobic plate count	34.5	6 min	25	Continuous flow	>5	Damar and Balaban, 2005
Orange juice	<i>E. coli</i> O157:H7	107	10 min	25	Continuous flow	5	Kincal et al., 2005
	<i>S. typhimurium</i>	21				6	
	<i>L. monocytogenes</i>	38				6	

TSB, tryptic soy broth; PS, physiological saline; MRS, De Man Rogosa Sharpe; C, complete inactivation.

Table 28.4. Summary of studies on spore inactivation by dense phase carbon dioxide

Solution	Microorganism	Pressure (MPa)	Time	Temperature (°C)	System	Log Reduction	Reference
Sterile distilled water	<i>Bacillus megaterium</i>	5.8	30 h	60	Batch	7	Enomoto et al., 1997
Physiological saline	<i>Bacillus polymyxa</i>	30	60 min	45	Micro-bubble	6	Ishikawa et al., 1997
	<i>Bacillus cereus</i>			50			
	<i>B. subtilis</i>			55			
Sterile ringer solution	<i>B. subtilis</i>	5	1 h	80	Batch	3.5	Ballestra and Cuq, 1998
	<i>Byssoschlamys fulve</i> ascospores						
Sterile water	<i>Bacillus stearothermophilus</i>	20	2 h	35	Batch	0	
Orange juice	<i>Saccharomyces cerevisiae</i> ascospores	15	<10 min	45	Continuous membrane filter	>6	Sims and Estigarribia, 2002
	<i>Alicyclobacillus acidoterrestris</i> spores	7.5					
	<i>Geobacillus stearothermophilus</i>	30	2 h	95	Batch	5	Watanabe et al., 2003

physical attributes (pH, Brix, and titratable acidity), nutritional content (vitamin C and folic acid), and aroma profile of untreated and DPCD-treated OJ.

Yagiz et al. (2005) treated mandarin juice with DPCD (13.8 to 41.4 MPa, 25°C to 45°C, 7 to 9 minutes). DPCD enhanced cloud stability up to 38.4%, increased lightness and yellowness, and decreased redness of mandarin juice. DPCD-treated samples had higher titratable acidity than the untreated samples, but pH and Brix did not change after treatment.

Folkes (2004) used a continuous DPCD system for pasteurization of beer and compared its physical and sensory attributes with that of fresh and heat-pasteurized beer. Aroma and flavor of DPCD-treated beer was not significantly different from fresh beer after 1 month storage at 1.67°C, but heat-treated beer was significantly different in taste and aroma. DPCD-treated beer had significantly less foam capacity and stability compared to that of heat-pasteurized beer, but not at levels detrimental to product quality.

Table 28.5. Summary of studies on inactivation of enzymes by dense phase carbon dioxide

Enzyme	Source of Enzyme	Pressure (MPa)	Time	Temperature (°C)	System	Loss of Activity	Reference
PE	Orange juice	26.9	145 min	56	Batch	100	Balaban et al., 1991
PPO	Spiny lobster	5.8	1 min	43	Batch	98	Chen et al., 1992
	Brown shrimp		1 min			78	
	Potato		30 min			91	
PPO	Spiny lobster	0.1	30 min	33	Batch	98.5	Chen et al., 1993
LOX	Soybean	10.3	15 min	50	Batch	100	Tedjo et al., 2009
POD	Horseradish	62.1		55		100	
LOX	Soybean	62.1		35		95	
PPO	Carrot juice	4.9	10 min	5	Batch	61	Park et al., 2002
LOX		2.94				>70	

PE, pectinesterase; PPO, polyphenoloxidase; LOX, lipoxygenase; POD, peroxidase.

Damar and Balaban (2005) pasteurized a coconut-water-based beverage with DPCD (34 MPa, 25°C, 13% (w/w) CO₂/juice ratio, 6 minutes) and conducted a shelf-life study after 9 weeks of storage under refrigeration temperature (4°C). Taste panels were conducted to evaluate overall likeability of fresh untreated, DPCD-pasteurized, and heat-pasteurized samples. Overall likeability of DPCD-treated coconut water was not significantly different from that of the untreated, whereas heat-pasteurized samples were rated significantly lower.

The effects of DPCD application on milk have also been investigated (Tomasula et al., 1997; Hoffland et al., 1999; Tisi, 2004). The effects may be desirable or undesirable, depending on the purpose of the process. For example, Tisi (2004) showed that DPCD-treated milk had higher lipolytic activity during storage compared with untreated milk because of the homogenization effect of DPCD on the fat micelles.

4.3. Irradiation with Antimicrobials

The feasibility of using gamma-irradiation and sulphur dioxide to control postharvest diseases and to extend the shelf life of table grapes in cold storage (1–2°C) was studied by Al-Bachir (1998) using Baladi and Helwani Syrian cultivars. He concluded that gamma-irradiation in combination with sulphur dioxide was the best method of preserving the two varieties of table grapes. Ouattara et al. (2001) evaluated the combined effect of low-dose gamma irradiation and antimicrobial coating on the shelf life of precooked shrimp (*Penaeus* spp). Antimicrobial coatings were obtained by incorporating various concentrations of thyme oil and *trans*-cinnamaldehyde in coating formulations prepared from soy or whey protein isolates. Coated shrimps were stored at 4 ± 1°C under aerobic conditions and were periodically evaluated for aerobic microorganisms and *Pseudomonas putida*. Results showed that gamma irradiation and coating treatments had a synergistic effect in reducing total aerobic and *P. putida* populations, leading to at least 12 days of extended shelf life. Giroux et al. (2001) studied the effect of ascorbic acid concentrations (0.03 to 0.5%) and irradiation

doses (0.5 to 4 kGy) on microbial growth of beef patties during storage at 4 ± 1°C. Ascorbic acid was also compared to citric acid at a similar pH value to differentiate the effects of ascorbic acid from those of pH reduction. Results showed a significant reduction of aerobic bacteria and total coliforms, and a significant interaction between ascorbic acid and irradiation dose was observed. Kuo and Chen (2004) evaluated the effect of gamma-irradiation at 0, 3, or 5 kGy on Chinese sausages with added sodium lactate. The authors found that irradiation at 5 kGy alone or in combination with 2% sodium lactate as well as irradiation at 3 kGy could completely inhibit lactic acid bacterial growth. Fan et al. (2005) investigated the effects of calcium ascorbate (CaA) and ionizing radiation on the quality of Gala apple slices under modified atmosphere packaging, and found that the combination of CaA and irradiation enhanced microbial food safety while maintaining the quality of fresh-cut apple slices. Thayer et al. (2006) evaluated the effects of γ -irradiation (0.2 kGy at 20°C) and chlorination (0.5 ppm for 10 minutes), or irradiation followed by chlorination, on *Salmonella*, *Escherichia coli* O157:H7 Ent C9490, and *Listeria monocytogenes* cells suspended on membrane filters. In each case, a synergistic greater inactivation was observed when irradiation was followed by chlorination. Knight et al. (2007) evaluated the microbial safety of frankfurters formulated with 0% or 3% potassium lactate/sodium diacetate solution, inoculated with *Listeria monocytogenes* before and after irradiation treatments (0, 1.8, and 2.6 kGy). The incorporation of lactate/diacetate with or without irradiation had a strong listeristatic effect on aerobically stored frankfurters. Overall, lactate/diacetate retarded the outgrowth of *L. monocytogenes* on frankfurters throughout aerobic storage, and the combination of irradiation and 3% lactate/diacetate reduced and retarded growth of *L. monocytogenes*, especially during the last 2 weeks of vacuum-packaged storage.

4.4. PEF with Antimicrobials

Nisin is the most studied antimicrobial in combination with PEF (Calderón-Miranda et al., 1999a, 1999b; Jagus et al., 1999; Dutreux et al., 2000;

Pol et al., 2000; Sobrino-López and Martín-Belloso, 2006; Gallo et al., 2007). PEF treatment combined with nisin has caused additive inactivation of bacteria in skim milk (Calderón-Miranda et al., 1999a) and liquid whole egg (Calderón-Miranda et al., 1999b). Vegetative cells of *Bacillus cereus* were subjected to low doses of nisin (0.06 mg/mL) and mild pulsed-electric field treatment (16.7 kV/cm, 50 pulses of 2 μ s). The combination of both treatments resulted in a reduction of 1.8 log units more than the effects obtained with the single treatments (Pol et al., 2000).

Death and injury following exposure of *Micrococcus luteus* to nisin and pulsed electric field (PEF) treatments were investigated by Dutreux et al. (2000) in phosphate buffer (pH 6.8, 54.8 ms/cm at 20°C). The application of nisin clearly enhanced the lethal effect of PEF treatment. The bactericidal effect of nisin alone reduced viable counts by 1.4 log units, whereas PEF treatments (50 pulses at 33 kV/cm) resulted in a reduction of 2.4 log units. However, PEF treatments followed by nisin caused a reduction of 5.2 log units, which was similar to the 4.9 log reductions attained when nisin was applied before the PEF treatments. Injury of surviving cells was investigated using media with different concentrations of salt. Sublethally damaged cells of *M. luteus* could not be detected by this means. Sobrino-López and Martín-Belloso (2006) observed that the combined effect of nisin and high-intensity PEF was clearly synergistic in skim milk. However, synergism depended on pH. A maximum microbial inactivation of 6.0 log units was observed at pH 6.8, 20 ppm nisin, and 2,400 μ s of high-intensity PEF treatment, whereas a reduction of over 4.5 log units was achieved when pH, nisin concentration, and high-intensity PEF treatment time were set at 5.0, 150 ppm, and 240 μ s, respectively. Gallo et al. (2007) investigated the combination of nisin and pulsed electric fields (PEF) on *Listeria innocua* in liquid whey protein concentrate. The efficiency of the combined treatment of nisin and PEF was strongly dependent on the sequence of application. The exposure to nisin after PEF application produced an antagonistic effect on *L. innocua* inactivation. In contrast, the addition of nisin prior to PEF treatment exhibited an additive and slightly synergistic effect.

Other researchers (Liang et al., 2002; Smith et al., 2002; Wu et al., 2005) have also observed synergistic inactivation of Gram-positive and Gram-negative bacteria when PEF treatments are combined with nisin and lysozyme addition. The inactivation of naturally occurring microorganisms in raw skim milk by PEF treatments, alone or combined with nisin and lysozyme, added both singly and together, was investigated by Smith et al. (2002). A highly synergistic 7.0 log microbial reduction was achieved in raw skim milk by combining PEF treatment (80 kV/cm, 50 pulses) with mild heat (52°C) and by adding nisin (38 IU/mL) and lysozyme (1,638 IU/mL). On the other hand, in red grape juice containing nisin and lysozyme (1:3 lyso:chrisin; 0.4 g/100 ml), the application of 20 pulses of 65 kV/cm (peak-to-peak) at 50°C resulted in a reduction of more than 5 log cycles. The same treatment applied to white grape juice produced a decrease in counts of 4.4 log cycles, whereas a 6.2 log reduction was achieved in red grape juice at 51°C when 20 pulses of 80 kV/cm were applied (Wu et al., 2005).

4.5. Ultrasound with Antimicrobials

Ahmed and Russell (1975) found that a treatment combining ultrasonication and hydrogen peroxide was several times more lethal to *Bacillus* and *Clostridium* spores than with each treatment separately. It is believed that ultrasonic waves improve the lethality of hydrogen peroxide by increasing permeability of cells; this increases the rate of reaction between hydrogen peroxide and cell components and disperses cell aggregates, which increases the surface contact area. Arce-García et al. (2002) reduced the intensity and duration of ultrasound treatments required to inhibit *Zygosaccharomyces rouxii* by 67 and 33%, respectively, by incorporating potassium sorbate, sodium benzoate, or eugenol into the recovery media. The authors suggested that the different action targets of ultrasonication, mild heating (45°C), and antimicrobial hurdles were responsible for the observed inhibition. Guerrero et al. (2005) investigated the resistance of the yeast *Saccharomyces cerevisiae* to ultrasounds (600 W, 20 kHz, 95.2 lm wave amplitude) at 45°C in Sabouraud broth

(pH 5.6) containing 1,000 ppm low weight chitosan. Incubation of the inoculated systems for 30 and 90 minutes prior to ultrasonication was also evaluated. It was found that incubating the yeast with chitosan prior to ultrasonication increased inactivation before and after the ultrasonic treatment. After 30 minutes of exposure to chitosan, a reduction of approximately 1 log cycle in yeast populations was obtained, but followed by 30 minutes of ultrasonic treatment, resulted into a reduction of more than 3 log cycles. Ninety minutes of exposure to chitosan, however, resulted in minimal changes in yeast reduction.

López-Malo et al. (2005) evaluated the combined effect of simultaneous applications of heat treatments and low-frequency ultrasounds (20 kHz) at different amplitudes on *Aspergillus flavus* and *Penicillium digitatum* spores viability suspended in laboratory broth formulated at different a_w (0.99 or 0.95) and pH (5.5 or 3.0), with or without vanillin or potassium sorbate. In general, D values were lower for thermosonication treatments than for thermal treatments. When potassium sorbate or vanillin was added and thermosonication treatment was applied at increased amplitude, lower D values were obtained.

5. Other Combinations

5.1. HHP with Modified Atmosphere Packaging (MAP)

The shelf life of fresh-like refrigerated products can be extended by combining HHP and MAP packaging. However, specific studies should be conducted to define the best combinations for any given food (Raso and Barbosa-Cánovas, 2003). Juliano et al. (2006) evaluated different methods of improving the texture profile and water retention of scrambled egg patties after high-pressure high-temperature (HPHT) treatment. The effects of water addition (0 to 15%), 3 levels of vacuum packaging, 4 preheating rates, and 2 HPHT conditions (700 MPa/105°C/5 min; 700 MPa/121°C/3 minutes) on texture and water retention of egg patties were also compared for selected formulations. The authors observed that use of low vacuum packaging for less porous patties, modified with xanthan gum, maintained original texture values

after treatment at 700 MPa/105°C/5 minutes. Testing the mechanisms of texture and water retention improvement in HPHT treated patties could contribute to meeting quality requirements not fulfilled by conventional thermal processing in the development of shelf-stable scrambled egg products.

5.2. Irradiation with Low Temperature and MAP

Packaging is a critical factor affecting the quality of irradiated foods, which can vary depending on packaging and storage conditions. For instance, an appropriate combination of aerobic- and vacuum-packaging conditions can be effective in minimizing deterioration in irradiated foods during storage (Licciardello et al., 1984; Przybylski et al., 1989; Lambert et al., 1992; Hagenmaier and Baker, 1997; Ahn et al., 2003; Nam and Ahn, 2003; Ahn et al., 2005). Fresh iced catfish fillets, processed with low dose irradiation (50–100 krad) in combination with modified atmosphere packaging, were sampled every 10 days during a 30-day storage period and analyzed for microbial load, color, and 2-thiobarbituric acid values (TBA) as indices of change in quality (Przybylski et al., 1989). No difference was observed between microbial counts of packages flushed with elevated carbon dioxide atmospheres (80:20 CO₂/air, 100% CO₂) and controls flushed with 100% air. Therefore, irradiation treatments, with or without elevated carbon dioxide-modified atmospheric packaging, significantly reduced the bacterial load and extended shelf-life from 5–7 to 20–30 days.

The effects of irradiation dose (0, 0.5, and 1.0 kGy), headspace oxygen (0, 10, and 20% O₂, balance nitrogen), and storage temperature (5, 15, and 25°C) on the physical, chemical, and sensory changes in fresh pork were studied by Lambert et al. (1992) using factorial design experiments. Irradiation in the absence of oxygen extended the sensorial shelf life of pork to 9–26 days at 5°C and <2 to 2 days at 25°C. Oxygen in the package headspace combined with irradiation adversely affected the physical, chemical, and sensory characteristics of the end product.

Irradiation of commercially prepared fresh-cut lettuce at a mean dosage of 0.19 kGy resulted in a

product with microbial loads of 290 cfu/g and yeast populations of 60 cfu/g after 8 days storage, compared with 220,000 and 1,400 cfu/g, respectively, for nontreated controls (Hagenmaier and Baker, 1997).

Ahn et al. (2003) evaluated the irradiation and modified atmosphere packaging effects on emulsion-type cooked pork sausage during storage for 4 weeks. CO₂ (100%), N₂ (100%), and 25% CO₂/75% N₂ packaged sausage were irradiated at 0, 5, and 10 kGy, respectively; the residual nitrite, residual ascorbic acid, nitrosomyoglobin (NO-Mb), color values, and their correlation were then observed. The treatments significantly reduced the residual nitrite content and caused partial reduction of NO-Mb during storage. No changes induced by irradiation treatments were observed in ascorbic acid content, whereas a decrease in Hunter color value was reported. CO₂ and CO₂/N₂ packages were more effective in reducing residual nitrite and inhibited the loss of red color compared with N₂ package. Results indicated that the proper combination of irradiation and modified atmosphere packaging could reduce the residual nitrite in sausages with minimal color changes.

Cut Chinese cabbage, processed with air packaging (CO₂ or CO₂/N₂), was irradiated at doses up to 2 kGy, and microbiological and physicochemical qualities were investigated by Ahn et al. (2005) during refrigerated storage for 3 weeks. Irradiation significantly limited microbial growth; additionally, MAP enhanced the reduction of total aerobic and coliform bacteria during storage. Irradiation effectively prevented changes in titratable acidity and pH, while texture did not change significantly. Further, anti-radical and antioxidant activity, as well as phenolic content, were slightly increased by treatments at 0.5 kGy, while at intensities over 1 kGy, the phenolic content was reduced.

5.3. Ultrasound with Low a_w

In general, the presence of solutes in the treatment medium prevents microbial inactivation by different lethal agents (Raso and Barbosa-Cánovas, 2003). For example, adding 57% sucrose to decreased a_w of the treatment medium to 0.94 increased the heat resistance of *L. monocytogenes* at 62°C by 25 times, while

resistance to MS treatment (117 mm, 200 kPa, 40°C) was only doubled (Pagán et al., 1999). Stojanovic and Silva (2007) osmoconcentrated rabbiteye blueberries (*Vaccinium ashei* Reade) in a sucrose solution for 12 hours, and for 3 hours, with and without high frequency ultrasound treatment (CHFUS). The authors found that combining high temperature, high sugar concentration, and oxygen availability had the largest negative influence on color and antioxidant properties (anthocyanins and phenolics) of dehydrated rabbiteye blueberries.

6. Combinations of Nonthermal Processes

Success in combining nonthermal processes depends not only on enhanced microbial inactivation but also on the technical compatibility of selected processes. For example, product characteristics are an important consideration, as PEF and ultrasonication are mainly restricted to liquid products, whereas ultraviolet radiation and pulsed light are limited to applications on food or packaging surfaces (Barbosa-Cánovas et al., 1998; Sizer and Balasubramaniam, 1999; Butz and Tauscher, 2002). The biochemical effects of nonthermal processing on the product should also be considered because molecular and biological modifications caused by some technologies (e.g., HHP) could potentially enhance, neutralize, or reduce the effectiveness of others (Earnshaw et al., 1995).

6.1. HHP with Irradiation

Gould and Jones (1989) reported the effects of simultaneously applying pressure and ionizing radiation to inactivate spores. The two treatments were additive in their sporicidal effect; the intensity of one or both treatments could be lowered while retaining a degree of inactivation. Crawford et al. (1996) found that irradiation sensitized *Clostridium sporogenes* spores to pressure synergistically.

Fully-cooked salmon and catfish fillets were treated by combining ionizing radiation (0, 3, or 6 kGy) and high hydrostatic pressure (HHP) (0, 414, or 690 MPa) at two different temperatures during pressurization (ambient-HHP at approximately

21°C, or heated-HHP at 70°C) (McKenna et al., 2003). The authors found that the tenderness and juiciness scores of salmon and catfish fillets were lower with HHP, heated-HHP, and combination of treatments, whereas irradiation increased tenderness and juiciness scores of salmon and increased flavor intensity of catfish.

6.2. HHP with PEF

Shimada and Shimahara (1991) treated *E. coli* cells with a 50-Hz alternating current separately and before HHP treatment. The survival of *E. coli* was reduced from 50 to 90% with the combination of treatments. Pagán et al. (1998) studied the possibility of germinating *Bacillus* spores using HHP and then inactivating the germinated cells with a PEF treatment. They found that germination of more than 5 log cycles of spores was initiated by pressurization. Although the germinated cells became sensitive to a subsequent heat treatment, they were not as well sensitized to PEF application below 40°C. The combined effects of pulsed electric fields (PEF), high hydrostatic pressure (HHP), and ultrasound on inactivation of *Salmonella enteritidis* in liquid whole egg were investigated by Huang et al. (2006). Treatment combinations only exhibited additive effects. Hence, a combination of high pressure and ultrasound treatments resulted in an optimal 3.2 log microbial reduction, and could be an alternative to severe thermal processing treatments causing protein coagulation when combined with mild pasteurization temperatures.

6.3. HHP with Ultrasound

Raso et al. (1998c) studied the inactivation of *Y. enterocolitica* by combining ultrasonication, pressure, and heat. The lethal effect of ultrasonication (20 kHz, 150 Am) increased with rising pressure until reaching an optimum pressure of 400 kPa, where maximum inactivation occurred. Levels of destruction of *B. subtilis* spores using MTS (20 kHz, 117 Am) followed a similar trend under increasing pressure, with maximum inactivation at a pressure of 500 kPa (Raso et al., 1998d). In both studies, the lethal effect of pres-

surization was significantly more pronounced when the amplitude of ultrasonic waves was increased. Pagán et al. (1999) also found that the ultrasonic (20 kHz, 117 Am) inactivation of *L. monocytogenes* increased dramatically when the pressure was raised from ambient to 200 kPa. Sala et al. (1995) found the lethality of MTS treatments for bacterial cells, spores, and yeasts to be 6–30 times greater than with thermal treatments of equal temperature and concluded that the combined effects of ultrasonication, pressure, and heat were synergistic.

6.4. PEF with Ultrasound

Because of their rigid structures, bacterial spores can survive during food processing and in very harsh environments for a long time. Bacterial spores are highly resistant to pulse electric fields (PEF) treatments. Ultrasonication may be combined with PEF to provide synergistic effect on bacterial spores.

Su et al. (1996) observed that ultrasound enhanced the inactivation of *B. subtilis* spores exposed to PEF treatments. Jin et al. (1998) investigated the effectiveness of PEF and PEF combined with ultrasonication on the inactivation of *Bacillus subtilis* spores. The authors inactivated 4 log cycles of *B. subtilis* spores using sonication at 2,000 Hz combined with PEF at 30–40 kV/cm.

7. Final Remarks

The interest of the food industry in nonthermal food processing technologies and their combination has been fuelled by the consumer's concerns about quality and safety of food products. Although commercialization of these technologies has been slow to date, the above trends, plus improvements in efficiency and reductions in cost, mean that the rate of adoption of nonthermal processes is likely to increase. Hurdles to development of industrial applications include the lack of systematic inactivation of kinetic data, the interpretation of non-linear death kinetics, and the need to establish equivalent control measures for nonthermal treatments in comparison with traditional heat processes. This is especially true for commercial sterilization.

Effective combinations of two or more preservation hurdles may be chosen once the mechanisms of action and cellular targets of each treatment are known. For the intelligent selection of nonthermal processing combinations, therefore, target elements within cells and the effects of treatments on those elements need to be determined. The treatment intensities required for cell inactivation also need quantification and standardization. The number of scientific studies summarized in this review show that combining nonthermal treatments has great potential for improving the safety and quality of foods although many technological and regulatory barriers still need to be overcome before the food supply can receive these benefits.

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Chapter 29

Bacteriocins as Natural Antilisterial Food Preservatives

Li Liu, R. Paul Ross, Colin Hill, and Paul D. Cotter

1. Listeriosis

Listeriosis is a severe food-borne disease caused by the gram-positive pathogenic bacteria *Listeria monocytogenes*, the most dramatic consequences of which are meningitis, encephalitis and, in a relatively high number of cases, death (Cherubin et al., 1991). This acute infection is particularly hazardous to particular high-risk groups such as the elderly, new born, pregnant women, and those whose immunity system is weakened (e.g., those with HIV, leukaemia, etc.) (Schuchat et al., 1991; Low and Donachie, 1997; Rocourt and Bille, 1997). In addition, it has also become evident that *L. monocytogenes* can cause a relatively benign gastroenteritis with diarrhea, headache, and abdominal pain as the major symptoms (Salamina et al., 1996; Frye et al., 2002; Wing and Gregory, 2002).

During the past two decades, there have been many reports of epidemic outbreaks of listeriosis from all over the world (Farber et al., 1991a; Low and Donachie, 1997). A coleslaw-associated outbreak that occurred in 1981 in Halifax, Canada, is generally considered to be the first formally documented example. In that case, 7 adult and 34 perinatal cases of listeriosis, as well as 5 spontaneous abortions and 40 stillbirths, were reported (Schlech et al., 1983). In 1983, 49 cases, 14 of which resulted in deaths, which occurred in Massachusetts, were ascribed to the consumption of a specific brand of pas-

teurized milk that was contaminated with *L. monocytogenes* (Fleming et al., 1985). A number of epidemic outbreaks (California in 1985, Switzerland between 1983 and 1987) have been associated with contaminated soft cheeses (Linnan et al., 1988; Bille, 1989) while non-pasteurized milk, brie-type cheese made from non-pasteurized milk and butter from pasteurized milk were reported to be responsible for outbreaks of listeriosis in Austria (1986), France (1995) and Finland (1998–1999), respectively (Lunden et al., 2004). Since 1998, epidemic outbreaks of the disease have most frequently been associated with processed meats. A multistate outbreak of listeriosis in 1998–1999 in the United States, associated with frankfurters and deli meats, resulted in 101 cases and 21 deaths (CDC, 1999a, 1999b) and in 2000 another multistate outbreak, associated with deli turkey meat, was responsible for 29 cases, 4 deaths, and 3 miscarriages or stillbirths (CDC, 2000). Turkey deli meat was also deemed responsible for 46 cases, 7 deaths, and 3 stillbirths or miscarriages in the northeastern United States in 2002 (CDC, 2002). The most recent outbreaks of listeriosis occurred in the Czech Republic in 2006. Seventy-five cases, including 12 deaths, 2 miscarriages, and 14 cases of neonatal disease, were reported, and geographically, almost half of the country was represented (Vit et al., 2007).

Generally, the frequency with which the disease occurs is between 0.2 and 0.8 cases per 100,000 annually (Gellin et al., 1991; Kela and Holmström, 2001; Lukinmaa et al., 2003). While, in relative terms, this incidence is not high, the disease and the pathogen responsible have been the focus of much research due to the fact that the associated mortality rate is

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extremely high, that is, typically 20–30% and, in some instances, up to 50% (Gellin and Broome, 1989; Schuchat et al., 1991; Low and Donachie, 1997; Rocourt and Bille, 1997; Mead et al., 1999). These figures contrast dramatically with those associated with other common food-borne pathogens such as *Salmonella enteritidis* (with a mortality rate of 0.38%), *Campylobacter* species (0.02–0.1% mortality), and *Vibrio* species (0.005–0.01% mortality) (Altekruse et al., 1997; Mead et al., 1999). Besides the enormous threat to the well-being of the infected individuals, there can be a tremendous economic burden (Tappero et al., 1995; Goulet et al., 1998). It has been estimated that there are about 2,500 cases of listeriosis in the United States annually and that these result in an associated monetary loss of approximately \$200 million (CDC, 2002).

2. *Listeria monocytogenes*

The genus *Listeria* consists of 6 species: *Listeria monocytogenes*, *Listeria innocua*, *Listeria ivanovii*, *Listeria seeligeri*, *Listeria welshimeri*, and *Listeria grayi* (Rocourt, 1999; Vazquez-Boland et al., 2001). They are small, gram-positive non-sporing rods and facultative anaerobes (Seeliger and Jones, 1986). Other than *L. monocytogenes*, *L. ivanovii* is the only other pathogenic species but significantly causes disease in animals only (Vazquez-Boland et al., 2001).

L. monocytogenes was first described by Murray et al. (1926). This pathogen can grow at a wide range of temperatures (−1.5°C to 45°C; Gray and Killinger, 1966; Junttila et al., 1988; Hudson and Mott, 1994), tolerate a wide range of pHs (4.3 to 9.6; Seeliger and Jones, 1986; Farber et al., 1989), which can be even further widened by induction of tolerance responses (Gahan et al., 1996), grow in environments with a water activity (a_w) of 0.90 (Nolan et al., 1992), tolerate salt concentrations of up to 40% w/v (Liu et al., 2005), and grow in the presence or absence of oxygen (Farber and Peterkin, 1991c). In addition, it is ubiquitous in nature, having been found in silage, sewage, vegetable matter, wild and domestic animals, birds, foods, and even on walls, floors, drains, ceilings, and equipments in food processing environments (Farber and Losos, 1988; McLauchlin

et al., 1990; Greenwood et al., 1991; Wilson, 1995; Fenlon et al., 1996). Strains of *L. monocytogenes* can be subdivided into 13 different serotypes. Although all of these can cause human listeriosis, the majority of human infections are caused by strains of serotype 1/2a, 1/2b, 1/2c or 4b, and 4e (Jay, 1996; Chasseignaux et al., 2002; Thévenot et al., 2005b). *L. monocytogenes* have also been classified into lineages I, II, and III, based on the alleles and ribotype patterns of the virulence genes *hly*, *actA*, and *inlA* (Wiedmann et al., 1997). The relationship between lineages and serotypes of *L. monocytogenes* is as follows: lineage I comprises serotypes 1/2b, 3b, 3c, and 4b; lineage II comprises serotype 1/2a, 1/2c, and 3a; and lineage III comprises serotype 4a, 4c (Nadon et al., 2001) and some unusual serotype 4b isolates (Liu et al., 2006). Of these, the majority of epidemic and spontaneous outbreaks of listeriosis have been caused by lineage I bacteria.

3. Prevalence of *L. monocytogenes* in Foods

As outlined above, *L. monocytogenes* can be found in a variety of environmental sources, including plants, soil, and water (Fenlon et al., 1996). With respect to its contamination of food, the bacteria is sufficiently resilient to tolerate a broad variety of the natural and processing-associated physico-chemical stresses that routinely limit bacterial number and, thus, can persist in sufficient numbers to bring about human infection upon consumption (Rørvik et al., 1995; Unnerstad et al., 1996; Miettinen et al., 1999a). *L. monocytogenes* has been isolated as a contaminant from most of the primary food groups, that is, dairy products, meats, vegetables, fruits, salads, and seafood (Griffiths, 1989; Giovannacci et al., 1999). In the following sections, we will focus on some representative foods from each of these groups.

3.1. Milk

The frequency with which *L. monocytogenes* contaminates raw milk varies from 3 to 6.8% (Gaya et al., 1996; Vitas et al., 2004). In the majority of cases, contamination occurs due to the transfer of

the pathogen from the live cow to its milk, and it should be noted that even healthy cows can act as reservoirs of *L. monocytogenes* (Fenlon et al., 1995). Contamination can also occur through accidental contact with faeces and silages (Husu, 1990a; Fenlon et al., 1995) and insufficient cleaning of the cow or towels between milking (Husu et al., 1990b; Sanaa et al., 1993). As milk is the raw material for many dairy products, the safety of milk is of prime importance. There has been some debate with regard to the ability of pasteurization to remove all *L. monocytogenes* from milk. Although Farber and Losos (1988) found that the pasteurization process could sufficiently eliminate the risk of appreciable contamination, *L. monocytogenes* has been isolated from pasteurized milk in Spain (Fernandez et al., 1986), Turkey (Ahrabi et al., 1998), and the United States (Moura et al., 1993; Frye and Donnelly, 2005). It is unclear whether these incidents are a reflection of a genuine ability to survive pasteurization or if they represent isolated cases where pasteurization has not been carried out correctly.

3.2. Cheeses

Unsurprisingly, cheeses made from raw milk are more frequently contaminated by *L. monocytogenes* (42%) than those from heat-treated milk (2%) (Loncarevic et al., 1995). In addition to contamination of the milk itself, cross-contamination during the manufacturing and postprocessing steps, including packaging, distribution, storage, and preparation by the consumers, can be a major issue (McLauchlin et al., 1990; Schaffner et al., 2003; Vitas et al., 2004). Although various studies have estimated that between 1 and 15% of cheeses are contaminated by this organism (Pini and Gilbert, 1988; Greenwood et al., 1991; Loncarevic et al., 1995; de Silva et al., 1998; Rudolf and Scherer, 2001), these values can vary dramatically depending on the type of cheese in question (e.g., in one case, 41% of a homemade Brazilian soft white cheese was found to be contaminated; de Silva et al., 1998) and the location of its manufacture, that is, Italy 16–17.4%, Germany 9.2%, Austria 10%, France 3.3–14%, Cyprus 10%, and the

United Kingdom 4% (Pini and Gilbert, 1988; Rudolf and Scherer, 2001).

3.3. Meat and Poultry

Meat and poultry have been shown to be high-risk foods with respect to *L. monocytogenes* contamination (Skovgaard and Morgen, 1988; Norrung et al., 1999; Levine et al., 2001; Vitas et al., 2004). Contamination of meat is often linked to the fact that the intestinal tract of animals and poultry can harbour *L. monocytogenes*, a problem that is amplified when animals or poultry are slaughtered in insufficiently hygienic conditions (Autio et al., 2000; Peccio et al., 2003; Salvat and Fravallo, 2004). Genotyping and other such studies have also revealed that cross-contamination can occur during processing or postprocessing (Gombas et al., 2003; Peccio et al., 2003; Salvat and Fravallo, 2004; Van Coillie et al., 2004; Thévenot et al., 2005b; Lin et al., 2006). The extent to which contamination occurs varies dramatically. In one study, 28–40% of raw meats, including pork, beef, and poultry, were found to contain *L. monocytogenes* (Ryu et al., 1992; MacGowan et al., 1994; Chasseignaux et al., 2001; Vitas et al., 2004; Thévenot et al., 2005b). However, in a number of other studies, both lower (5–34%; Rørvik and Yndestad, 1991a; de Simón et al., 1992; Inoue et al., 2000) and higher contamination rates (60–71.6%; Pini and Gilbert, 1988; Van Coillie et al., 2004; Thévenot et al., 2005b) have been reported. The highly variable rate of contamination is unsurprising as the values undoubtedly reflect differences with respect to the source of the samples and the levels of hygiene pre- and postslaughtering (Nesbakken et al., 1996). There would, however, seem to be a particularly high association between *L. monocytogenes* with poultry (Wilson, 1995; Inoue et al., 2000; Rørvik et al., 2003) and contamination rates of up to 84% in raw chicken have been reported (Lawrence and Gilmour, 1994; Franco et al., 1995; Uyttendale et al., 1999).

Because the risk associated with the presence of *L. monocytogenes* in raw meat is negated if the food is cooked, the presence of the pathogen in ready-to-eat (RTE) meat products is regarded as a

much more serious issue. This fact is reflected in the number of epidemic outbreaks of listeriosis associated with processed meat products. Significantly, between 1993 and 1996, *L. monocytogenes* was isolated from 16% of tongue, 9% of “pâté,” 11.7% of “rilletes,” 6.02% of salami, and 13.1% of hams in France (DGCCRF, 1996). While the corresponding figures from other countries are variable, and generally lower, there is still great cause for concern, that is, during the 1990s, *L. monocytogenes* was found in 0.31% of jerky, 0.10% of cooked poultry, 0.07–0.2% cooked sausage, 0.22% of cooked beef, and 3.25% dry fermented sausages (Levine et al., 2001) in the United States, in Canada, 3–5% of processed meat products, including fermented sausage, processed turkey breast, beef, and chicken wieners contain the pathogen (Bohaychuk et al., 2006), while 8.1% of precut RTE meat in a Hellenic market have been found to be *L. monocytogenes* positive (Angelidis and Koutsoumanis, 2006).

3.4. Sea Foods

L. monocytogenes has been isolated from a wide range of seafood including crustaceans, molluscan shellfish, salmon, fish, cooked crab meat, shrimp, cold-smoked mussels, and other finfish (Hudson et al., 1992; Ben Embarek, 1994; Simon et al., 1996; Jinneman et al., 1999). Thus, it is not surprising that a number of outbreaks of listeriosis have been linked to the consumption of seafood (Ericsson et al., 1997; Brett et al., 1998; Farber, 2000a, 2000b). As with other foods, the prevalence of *L. monocytogenes* in raw fresh fish varies greatly from one study to another but, in general, is within a range from 0 to 30% (Ben Embarek et al., 1997; Jinneman et al., 1999; Hoffman et al., 2003; Markkula et al., 2005). The following data reflects this trend in that *L. monocytogenes* was recovered from 11% of raw shrimps, 1.5% of retailed fresh-water fish, and 13% of fish products, RTE shrimps, crab, and smoked salmon sampled at the wholesale level (Rocourt and Bille, 1997). With respect to raw salmon, the incidence rate in Europe and America ranges from 7.4 to 29.5% (Fonnesbech Vogel et al., 2001; Norton et al., 2001; Hoffman et al., 2003) and the rate of occurrence in fresh and

thawed rainbow trout are 14.6 and 8.8%, respectively (Miettinen and Wirtanen, 2005). Outbreaks generally result as a consequence of consumption of the food in its raw form, insufficient processing procedures, or contamination at the associated facilities (Rørvik et al., 1997; Autio et al., 1999; Johansoon et al., 1999; Norton et al., 2001). Although the smoking of seafood is frequently employed to improve its safety, it has been established that *L. monocytogenes* is frequently present in cold-smoked salmon. This problem has been stressed by many researchers (Farber 1991b; Rørvik et al., 1991b; Rørvik et al., 1995; Loncarevic et al., 1996; Jorgensen and Huss, 1998; Johansoon et al., 1999; Inoue et al., 2000; Dauphin et al., 2001; Dominguez et al., 2001; Gombas et al., 2003; Vitas et al., 2004). However, considering the high frequency with which *Listeria* is found in smoked fish, the number of cases of listeriosis due to smoked fish have been relatively few (Dillon and Patel, 1993; Dillon et al., 1994; Fuchs and Nicolaides, 1994).

3.5. Other Foods

Cabbage contaminated with *L. monocytogenes* was the source of the first documented outbreak of listeriosis (Schlech et al., 1983). Unsurprisingly, the microorganism's ability to grow in raw cabbage and cabbage juice was subsequently confirmed (Beuchat et al., 1986), and it has been established that the pathogen was present on 3% of cabbages tested in the United States in 2002 (Prazak et al., 2002). Similarly, lettuce, broccoli, asparagus, carrot, cucumber, and cauliflower have been found to harbour *L. monocytogenes* (Sizmur and Walker, 1988; Steinbruegge et al., 1988; Berrang et al., 1989; Beuchat and Brackett, 1990; Dhokane et al., 2006) and although tomatoes and tomato products were found not to be good substrates for the growth of *L. monocytogenes*, the microorganism can remain viable on raw whole and chopped tomatoes and in commercial tomato juice and sauce for longer periods than their normal expected shelf life (Beuchat and Brackett, 1991). Again, the prevalence of *L. monycytogenes* contamination can vary with geography and the specific food involved, for example, 7.8% of vegetables in Spain

have been reported to be contaminated (de Simón et al., 1992), while a study carried out with pre-cut fruit, sprouted seeds, and unpasteurized fruit and vegetable juices in the United Kingdom revealed a 0.1% prevalence rate (Little and Mitchell, 2004). *L. monocytogenes* has also been reported to exist in products containing peanut or chocolate including peanut-based beverage, chocolate milk, chocolate-peanut spread, and peanut butter (Kenney and Beuchat, 2004). In a recent survey in United Kingdom, it has been revealed that butter can harbour *L. monocytogenes* although it is usually regarded as low-risk food (Lewis et al., 2006), and soy milk and yoghurt have also been reported to be good substrates for the growth and survival of *L. monocytogenes* at 8°C (Tipparaju et al., 2004). Finally, *L. monocytogenes* can be viable in both internal tissue and on the surface after extended storage in a refrigerator (Kim et al., 2005a) and has been detected in sauerkraut pickle with added salt after 35 days fermentation and in Kimchi at 10°C (Inatsu et al., 2004; Niksic et al., 2005).

4. Legislation and Management Systems Directed Toward the Control of *L. monocytogenes* in Foods

As stated above, the frequency with which *L. monocytogenes* has been isolated from foods and the associated health risks are of major concern, and as a consequence, criteria or legislation has been established in many countries to minimize the potential risk to the public. The EU position concerning the concentration of *L. monocytogenes* in RTE foods (European Commission, 2002) states that “a level of <100cfu/g *L. monocytogenes* at the point of consumption poses a low risk” and similarly in Canada a level of 100 cfu/g of *L. monocytogenes* in food is permitted (Robin et al., 2006). The most recent regulations from the US Department of Agriculture with respect to *L. monocytogenes* in RTE meat products state that “establishments that produce RTE meat and poultry products that are exposed to the environment after lethality treatments and that support the growth of *L. monocytogenes* will be required to have, in their hazard analysis and critical control point (HACCP)

plans, or in their sanitation standard operating procedures or other prerequisite programs, controls that prevent product adulteration by *L. monocytogenes*. The establishments must share with FSIS data and information relevant to their controls for *L. monocytogenes*” (FSIS, 2003). In addition to the introduction of cut-off points with respect to the presence of the pathogen, there has also been a concerted effort directed toward the development of effective control and prevention strategies to minimize the likelihood that the pathogen will be present in a food at all. On the basis of FAO/WHO risk assessment principles, general international guidelines for controlling *L. monocytogenes* in foods have been generated (CCFH, 2003). In addition, the Australian Dairy Authorities Standards committee has drafted the *Listeria* Manual for use in the Australian dairy industry (ADASC, 2000). In order to control *L. monocytogenes* in final food products, certain internationally recognized measures including Good Hygiene Practices (GHP), Good Manufacturing Practices (GMP), food safety/hygiene training, and the implementation of HACCP systems are routinely employed by food manufacturers (Mengoni and Apraiz, 2003; Stecchini and Del Torre, 2005). The implementation of HACCP and other such systems by food business and the provision of food safety training for all food employees is a legal requirement in many countries and areas (European Commission, 2002; FAO/WHO, 2002; CCFH, 2004d; European Union, 2004; Food Safety Authority of Ireland, 2004). These measures are designed to control *L. monocytogenes* throughout the entire processing procedure by, for instance, controlling environmental temperatures and maintaining high hygiene levels in workers, sanitizing equipment, and the processing line by cleaning with the suitable detergent and separating raw products (Gibson et al., 1999; Chasseignaux et al., 2001; Marsden et al., 2001; Sagoo et al., 2003; Thévenot et al., 2005b, 2006).

5. Techniques (Nonbacteriocin) Used to Control *L. monocytogenes* in Foods

The nonbacteriocin-associated techniques utilized to control *L. monocytogenes* in foods are those

described elsewhere in this book and thus are merely summarized here.

5.1. Physical Techniques

The physical techniques used to control *L. monocytogenes* in foods can be classified into two groups: thermal treatment and nonthermal treatment. The nonthermal treatments include irradiation, high-hydrostatic pressure (HHP), packaging, electrolyzed oxidizing water, ultrasound, and ultraviolet light.

5.1.1. Irradiation Irradiation as described here encompasses gamma rays from radionuclides, high energy electrons, and x-rays. The effectiveness of irradiation in controlling *L. monocytogenes* in foods, and in frequently contaminated foods such as RTE meats, vegetables, and seafood, in particular, has been the subject of a number of reviews (Farkas and Andrassy, 1993; Clardy et al., 2002) and recent studies (Bari et al., 2005; Sommers and Boyd, 2005; Dhokane et al., 2006; Mintier and Foley, 2006; Robertson et al., 2006). Although irradiation has been officially approved for the purpose of the removal of microbes from food (FDA, 1997; FSIS, 1999), concerns regarding its safety, efficiency, environmental impact, and suitability have been expressed (Farkas and Andrassy, 1993). Irradiation can also have an adverse effect on the odour of many foods, because of the formation of obnoxious volatiles during peroxidation and breakdown of some chemical constituents of foods (Ananthakumar et al., 2006; Rababah et al., 2006), and irradiation can cause discoloration and bring about lipid oxidation. The latter side effect can adversely impact on other properties, and in some situations may be hazardous to the health of consumers (Gray et al., 1996).

5.1.2. High Pressure (HP) HP is a nonthermal technique that controls and eliminates *L. monocytogenes* in foods by adversely affecting microbial membranes, enzymes, and other structures and molecules, while minimally impacting on the product itself (Mackey et al., 1994; Carminati et al., 2004; Fonberg-Broczek et al., 2005). HP processing can impact on the foods to some extent and both

beneficial (pressure tenderization and gelation) and detrimental impacts (discoloration and lipid oxidation) can result. HP treatment of foods has been the focus of an excellent recent review (Patterson, 2005).

5.1.3. Packaging Of the packaging methods employed to control *L. monocytogenes* in food, that is, vacuum packaging, modified atmosphere packaging (MAP), and active/intelligent packaging, MAP has been the focus of most research. The antilisterial impact of MAP is due to growth suppression as a consequence of the use of various combinations of oxygen, carbon oxide, and nitrogen. In response to the consumer-driven requirement for natural preservation, this system has been widely implemented to protect meat, vegetables, fruits, seafood, and other foods (Kader et al., 1989; Stammen et al., 1990; Tewari et al., 1999) and its effect on *L. monocytogenes* in meat and other foods has been reviewed (Defernando et al., 1995; Phillips, 1996). Briefly, MAP-associated advantages include respiration reduction, increased shelf life, and the replacement/reduction in levels of other preservatives. However, disadvantages include the temperature-dependent nature of the process, the added costs involved, the requirement for special equipment, and, in some cases, a deleterious effect on food quality, that is, certain components can cause discoloration of some foods and sometimes even stimulate the growth of specific pathogens (Lee et al., 1996; Cutter, 2002).

5.1.4. Electrolyzed Oxidizing Water Electrolyzed oxidizing (EO) water is generated by the electrolysis of a low level NaCl (0.05–0.25 M) solution using separated anode and cathode electrodes in an electrolysis chamber (Deza et al., 2003). As a consequence of this treatment, the water is imparted with strong bactericidal and virucidal properties. Two types of electrolyzed oxidizing water can be used, i.e., acidic electrolyzed water (AEW) and neutral electrolyzed water (NEW). Although this technique is still in the early stages of development, it has been shown to effectively reduce the level of *L. monocytogenes* on the surfaces of tomatoes (Bari et al., 2003; Deza et al., 2003),

eggs (Russell, 2003; Park et al., 2005), and lettuce (Koseki et al., 2004).

5.1.5. Ultrasound and Ultraviolet Light The application of these two methods to control *L. monocytogenes* in foods has not been extensively reported. Nonetheless, it has been established that ultrasound, when used in combination with other preservation techniques, can be lethal to *L. monocytogenes* (Pagan et al., 1999a, 1999b; Zenker et al., 2003; D'Amico et al., 2006). It is important to note that the decontamination with ultrasound is limited to nonviscous liquid products as it is impeded by viscous liquids and solids. With respect to ultraviolet (UV) light, there have been a number of reports describing its use to kill *L. monocytogenes* (Stermer et al., 1987; Yousef and Marth, 1988; MacGregor et al., 1998; Rowan et al., 1999; Matak et al., 2005; De Reu et al., 2006; Ingham et al., 2006). However, UV-light can only be used to kill the bacterium on food surfaces as its penetration into foods is weak. Unfortunately, in a study of vegetables, UV-light did not extend the shelf life of products but caused deleterious effects

on sensory properties (Gómez-López et al., 2005a, 2005b).

5.2. Chemical Techniques

Chemicals are frequently used to control *L. monocytogenes* in foods. A wide range of chemicals have been assessed, singly or in combination, to determine their effectiveness in controlling *L. monocytogenes* in foods. The chemicals involved can be categorized as acid, other chemical, plant extract, smoke, and synthesized peptide (Table 29.1).

In addition to the individual treatments described above, the use of a combination of two or more of them in combination can be particularly fruitful (Earnshaw et al., 1995; Gould, 1996, 2000).

6. The Use of Bacteriocins to Control *L. monocytogenes* in Foods

Although physical and chemical treatments can efficiently inhibit the food pathogen *L. monocytogenes*, each treatment has associated limitations and many can have certain adverse effects on the target foods.

Table 29.1. The chemicals utilized for the control of *L. monocytogenes* in foods

Type	Source	Preservative	Adverse Effect
Acid	Weak organic acid and ester	Propionate, sorbate, benzoate, benzoate esters, monolaurin	Unwanted residuals; Increased resistance of <i>L. monocytogenes</i> to hurdles; Effects on food sensory
	Organic acid acidulant	Lactic, citric, malic, acetic, diacetate, tartaric, erythobate, methyl paraben	
	Inorganic acid	Sulphite, nitrite, hydrochloric, phosphoric, Ethanol, hydrogen peroxide, salt, chlorine dioxide, monoglycerides, EDTA, SDA	
Other chemical			
Plant extracts	Hop, pimento leaf, green tea, horseradish, rosemary, clove, oregano, laural, lavender, fennel	Eugenol, cloves, cinnamaldehyde, thymol, limonene, furanocoumarins, carvacol, epicatechin gallate, epigallocatechin gallate, epicatechin, and caffeine	Higher cost; Unclear adverse effects
Smoke		Isoeugenol, phenols	Hazard residuals; Organoleptic changing
Synthesized peptide		Peptide-containing leucine and lysine	

With the increasing demand from consumers for more natural foods, bacteriocins produced by food-grade bacteria, which are active against *Listeria*, have attracted great interest. An extensive array of bacteriocin-based studies have been carried out to determine if they could ultimately act as a substitute for traditional physical and chemical treatments with respect to the control of *L. monocytogenes* in foods. Because the lactic acid bacteria (LAB) are commonly found in foods and enjoy GRAS (generally regarded as safe) status, the bacteriocins that they produce are those that are most frequently assessed with respect to food applications. In fact, because bacteriocin production is such a common trait among LAB, it is certain that humans have unknowingly benefited from the antibacterial activities of bacteriocins since the first fermented foods/drinks were made.

Bacteriocins are ribosomally synthesized peptides with narrow- or broad-spectrum antibacterial activity produced by a wide range of bacteria, which are themselves immune to the activity of the bacteriocin that they produce. A vast amount of research has been carried out to enhance our understanding of bacteriocins, and this data has been frequently reviewed (Abee et al., 1995; Daw and Falkner, 1996; Moll et al., 1999; McAuliffe et al., 1999; Hechard and Sahl, 2002; Cotter et al., 2005).

On the basis of their structure and action mode, bacteriocins produced by LABORATORY have been divided into two main classes in a recent review (Cotter et al., 2005). Class I bacteriocins (or lantibiotics) are characterized by the presence of posttranslationally modified (β -methyl) lanthionine residues. The antimicrobial activity of these small heat-stable antimicrobials is realized through the dissipation of membrane potential and the efflux of metabolites from the target cell, caused by the formation of pores and/or the inhibition of enzymatic reactions (for review, see Chatterjee et al., 2005). Class II bacteriocins are also small heat-stable peptides that, in general, cause leakage of components from the target cell as a result of membrane permeabilization but differ from Class I peptides in being unmodified (Nes and Holo, 2000). The Class II bacteriocins have been divided into four subclasses: IIa (pediocin-like bacteriocins), IIb (two-peptide bacteriocins), IIc (cyclic

bacteriocins), and IId (nonpediocin single linear peptides), with Class IIc being further divided into c(i) and c(ii). Of the Class II subclasses, Class IIa peptides are, in general, regarded as having the most potent antilisterial activity. It should be noted that a new classification scheme for enterocins has been recently proposed (Franz et al., 2007).

6.1. Class I Bacteriocins

6.1.1. Single Peptide Lantibiotics

6.1.1.1. Nisin Nisin, first discovered in the 1920s and produced by *Lactococcus lactis* (Rogers, 1928), is the only bacteriocin currently approved for use as a biopreservative in foods (Delves-Broughton, 1990). Nisin is a broad range bacteriocin but, as a consequence of being easily broken down by enzymes in the gastrointestinal tract, does not impact on the natural gut biota. It was first used as preservative in cheese products in the United Kingdom and since then numerous other applications in foods have been identified. It can be introduced into foods in a number of different ways and, like other preservatives, has been used in conjunction with other antimicrobial technologies.

Nisaplin is a commercial nisin concentrate. Its ability to inhibit *L. monocytogenes* was first assessed in a trial involving meat cubes when 2.6 and 2.45 log unit reductions in pathogen numbers were apparent at room temperature and 5°C, respectively (Chung et al., 1989). Nisaplin also efficiently inhibits the growth of *L. monocytogenes* in ricotta-type cheeses (Davies et al., 1997). A favourable impact of nisin on the control of *L. monocytogenes* was also reported in pasteurized liquid egg products (Knight et al., 1999).

Nisin can also be introduced into fermented food through the use of nisin-producing strains during the fermentative process. In a study of a Camembert cheese fermented with a nisin-producing starter culture, the numbers of *L. monocytogenes* artificially added into cheese was reduced by 2.4 log units (Maisnier-Patin et al., 1992). In another study, antilisterial activity was apparent when a nisin-producing transconjugant (i.e., a strain that became a nisin-producer as a consequence of the natural acquisition

of the nisin genes) was used as a starter culture for cheddar cheese making (Zottola et al., 1994).

In addition to its own strong antilisterial activity, the impact of nisin can be augmented through synergism with other treatments such as lactoperoxidase (Rodriguez et al., 1997; Zapico et al., 1998; Bous-souel et al., 2000), sodium lactate (Nykanen et al., 2000), tert-butylhydroquinone in sausage (Chung et al., 2005), and thymol (Ettayebik et al., 2000). *L. monocytogenes* in vacuum-packaged fresh beef can be significantly inhibited when nisin is combined with EDTA (Zhang and Mustapha, 1999; Ari-vaptipun et al., 2000), while the antilisterial effectiveness of Nisaplin is enhanced greatly by the presence of acetic acid, lactic acid, or potassium benzoate in bologna, ham slices, and commercial frankfurters (Geornaras et al., 2005, 2006). It is important to note that the synergistic activity of nisin and lactates is greatly dependent on the presence of metal cations (McEntire et al., 2003). In addition to these chemical preservatives, it has also been reported that the use of protective cultures such as *Enterococcus faecium* BFE 900–6a and *Lactobacillus sakei* Lb 706–1a successfully enhanced the ability of nisin to suppress the proliferation of *L. monocytogenes* in tofu (Schillinger et al., 2001). Synergistic effects were also apparent when nisin was used in raw and cooked pork in combination with MAP (Fang and Lin, 1994a, 1994b) and when it was combined with radio frequency treatment to synergistically control *L. monocytogenes* in caviar (Al-Holy et al., 2004).

As an alternative to the direct incorporation of nisin into food products, nisin has also been impregnated into edible packaging (e.g., corn zein or soy-based films; Hoffman et al., 2001; Dawson et al., 2002; Lungu and Johnson, 2005) or released into foods through packaging (Cooksey, 2005). Although packaging coated with a cellulosic solution containing nisin was successful in terms of controlling *L. monocytogenes* on the surface of hot dogs (Franklin et al., 2004), it was less successful when incorporated into cellulose casings for frankfurters that were subsequently vacuum-sealed and stored at 4°C (Luchansky and Call, 2004). When incorporated into a composite HPMC (hydroxypropylmethylcellulose) film containing stearic acid and other hydrophobic

compounds, the antilisterial activity of film was variable, most likely as a consequence of electrostatic interactions between the cationic nisin and these other compounds (Coma et al., 2001).

In conclusion, studies assessing the effectiveness of nisin in the control of *L. monocytogenes* in foods have demonstrated the merits of combining nisin with other antibacterial hurdles.

6.1.1.2. Lacticin 481 Lacticin 481 is a lantibiotic produced by *Lactococcus lactis*, which was first purified and characterized in the early 1990s (Piard et al., 1990, 1992). It exhibits a medium target range, inhibiting other LABORATORY and *Clostridium tyrobutyricum* via a bacteriolytic mechanism of action (O'Sullivan et al., 2002). Investigation of the potential use of lacticin 481 in food applications has focused primarily on its ability to enhance flavour (Mills et al., 2002; O'Sullivan et al., 2002, 2003; Avila et al., 2005, 2006; Garde et al., 2006) rather the inhibition of spoilage/pathogenic bacteria. Nonetheless, lacticin 481 exhibits antilisterial activity (Akcelik et al., 2006), and food grade lactococcal starters producing both lacticin 481 and the two-peptide lantibiotic lacticin 3147 strongly inhibited *Listeria* (O'Sullivan et al., 2003). The related lacticin 481-like peptide, variacin, also inhibits *L. monocytogenes* (Pridmore et al., 1996) but its use as a food preservative has not been investigated.

6.1.2. Two-Peptide Lantibiotics

6.1.2.1. Lacticin 3147 Lacticin 3147 was firstly isolated from Irish kefir grain (Ryan et al., 1996) and is a two-component broad spectrum lantibiotic that is active against *Listeria*. As a consequence of its heat stability and activity over a wide pH, a variety of studies have been carried out to reveal its potential for use as a food preservative (for reviews, see Ross et al., 1999; Cotter et al., 2005; Guinane et al., 2005). Because of an off-odor associated with the original natural lacticin 3147 producer, DPC3147, the plasmid responsible for the production of lacticin 3147 has been naturally conjugated to commercial starter strains or adjuncts for food applications. The incorporation of lacticin 3147 in this way can bring about

a 99% reduction of *L. monocytogenes* in cottage cheese within 5 days at 4°C (McAuliffe et al., 1999) and also accelerates cheese ripening while allowing proper acidification of the curd (Martinez-Cuesta et al., 2001). The use of a lacticin 3147-producing starter culture can also significantly reduce *Listeria* levels during beaker sausage making, a phenomenon that is further enhanced in the presence of added manganese and magnesium (Scannell et al., 2001). Lacticin 3147-producing live cultures can also be sprayed onto the surface of smear-ripened cheese to efficiently inhibit *L. monocytogenes* (O'Sullivan et al., 2006).

As an alternative to its introduction through production by a bacterial strain introduced into/onto food, lacticin 3147 can also be added in the form of a whey or milk powder generated following fermentation with a lacticin 3147-producing strain (Morgan et al., 1999), or in the form of a purified, concentrated powder. The addition of lacticin 3147-containing whey powder (10%) to natural yogurt and cottage cheese resulted in a 99% and 85% reduction, respectively, of *L. monocytogenes* levels within 2 hours (Morgan et al., 2001). The success of lacticin 3147 in inhibiting *Listeria* can be further enhanced through its combined use with other treatments. When combined with acid, the antilisterial activity of lacticin 3147 is greatly enhanced in fresh pork sausage (Scannell et al., 2000a) and a greater than 6 log kill of *L. innocua* in milk and whey results when lacticin 3147 is combined with a 250 MPa high-pressure treatment (Morgan et al., 2000).

6.2. Class II Bacteriocins

While it is apparent from the above that a number of Class I bacteriocins could be utilized as antilisterial compounds, there has been an even greater focus on the use of Class II bacteriocins for this purpose.

6.2.1. Class IIa Bacteriocins The Class IIa bacteriocins are the largest and most extensively studied subclass of bacteriocins. They are produced by various LAB, including *Lactobacillus*, *Enterococcus*, *Pediococcus*, *Leuconostoc*, and *Carnobacterium* sp. and are frequently strong inhibitors of *L. monocy-*

togenes. Indeed, they are also frequently named as the “anti-*Listeria*” bacteriocins. While most of these strains produce a single bacteriocin, some can produce multiple bacteriocins. Here again, we will focus on the use/potential use of Class IIa bacteriocins to control *L. Monocytogenes*, but for an excellent review of the structure, function, mode of action, and biosynthesis of Class IIa bacteriocins in general, see Drider et al. (2006).

6.2.1.1. Pediocins There are a number of pediocins (i.e., bacteriocins produced by pediococci) within subclass IIa, and these have been the focus of much research, especially in terms of the inhibition of *L. monocytogenes* in foods. Pediocin PA-1, associated initially with *Pediococcus acidilactici* PAC-1.0, was the first such bacteriocin to be identified and continues to be the most extensively studied pediocin (Gonzalez and Kunka, 1987). It was established that PA-1 is identical to another pediocin known as pediocin AcH. The effectiveness of pediocin PA-1/AcH in inhibiting the growth of *L. monocytogenes* was first reported in beef wiener exudates (Yousef et al., 1991). Similar trends were also apparent in other foods including cottage cheese, half-and-half cream, and cheese sauce when pediocin PA-1 was mixed into prepared food samples in the form of a dried powder preparation. An obvious decline in pathogen numbers was apparent in all trials over the pH range 5.5–7.0 at both 4 and 32°C (Pucci et al., 1988). Alternatively, pediocin PA-1 can be introduced into a food by a live bacteriocin-producing culture. This approach was employed in a cheddar cheese trial whereby *L. monocytogenes* was present at a level of 10³ cfu/mL. A significant antilisterial effect (2 log₁₀ cfu/g decrease) was apparent (Buyong et al., 1998). The pediocin AcH-producing strain *P. acidilactici* H JBL1095 has also been used as a starter culture for the production of turkey summer sausage spiked with 10⁵ cfu/g *L. monocytogenes*. It was apparent that the production of pediocin PA-1 did not impact on acid production, that is, the fermentation process, and the counts of *L. monocytogenes* were reduced to a greater extent (3.4 log₁₀ unit decrease) in the bacteriocin-containing sausage than in its pediocin negative counterpart (0.9 log₁₀ unit decrease).

(Luchansky et al., 1992). This trend was also repeated in the case of dry fermented sausage inoculated with 10^5 cfu *L. monocytogenes*/g (Foegeding et al., 1992) and in chicken sausage containing 10^7 cfu *L. monocytogenes*/g (Baccus-Taylor et al., 1993). Pediocin AcH producers have also been added as adjuncts, that is, when a pediocin AcH-producing strain was applied as a co-culture with a nonbacteriocin-producing strain on red smear cheese, the *L. monocytogenes* ($2 \log_{10}$ cfu/g) present was almost completely eliminated (Loessner et al., 2003). Finally, when a preparation of pediocin AcH together with its producer bacteria was dropwise applied onto cooked sausage slices containing $2.7 \log_{10}$ cfu/g *L. monocytogenes*, pathogen counts decreased by over 0.7 $\log_{10}$ cfu/g within 6 days without adversely impacting on either pH or flavour (Mattila et al., 2003).

Unsurprisingly, pediocin PA-1 has also frequently been used in combination with other antilisterial treatments. Synergistic effects were apparent when it was combined with sodium diacetate and sodium lactate in beef frankfurters (Uhart et al., 2004) or with nisin, or a variety of chemical acids, for the treatment of cabbage, broccoli, and mung bean sprouts (Bari et al., 2005). It should be noted that the development of resistance to pediocin AcH was also detected in this study, thus highlighting the need for care with regard to the over-use of this bacteriocin.

Other, less well-known pediocins have been the subject of some research. Pediocin PO2 exhibits a very strong antilisterial activity, inhibiting *L. monocytogenes* in heat-treated whole milk and pasteurized egg when added as a whey permeate (Liao et al., 1993), and in salami (Foegeding et al., 1992) and frankfurters (Berry et al., 1991; Yousef et al., 1991) when introduced via bacteriocin-producing cultures. When combined with postpackaging thermal and irradiation treatment, pediocin ALTA2341 effectively controlled *L. monocytogenes* in frankfurters without negatively impacting on food quality (Chen et al., 2004a, 2004b).

6.2.1.2. Enterocins The enterocins (bacteriocins produced by enterococci) are another large group of bacteriocins, and there are a number of examples of Class IIa enterocins that exhibit strong antilisterial

activity. Enterocin A is one of the most commonly produced enterocins and, although it has a narrow antimicrobial spectrum, it has a high specific activity against *L. monocytogenes* (Aymerich et al., 1996). Although some strains (e.g., *E. faecium* CCM4231) produce enterocin A alone (Foulquie Moreno et al., 2003), enterocin B is very frequently co-produced with enterocin A and together they act synergistically against the pathogen (Casaus et al., 1997). As a consequence of being co-produced, and synergistically active, enterocin A and B are usually used in combination. However, it should be pointed out that enterocin B is not an example of a Class IIa bacteriocin but belongs to Class IIc. In a study of the antilisterial activity of enterocin A and B in dry fermented sausage, it was established that production of enterocin A and B was optimal between 25 and 35°C and when the initial pH was between 6.0 and 7.5. However, it was also apparent that certain ingredients, such as sodium chloride and pepper, greatly inhibited the production of enterocin A and B (Aymerich et al., 2000a). Because of problems with respect to the use of enterococci in food, the introduction of enterocin A and B as additives has been investigated as an alternative. Such an approach brought about a 1.13 $\log$ reduction in *L. monocytogenes* counts in dry fermented sausage (Aymerich et al., 2000a) and following this success has been similarly applied to the control of *L. monocytogenes* in other meat products including ham, minced pork meat, deboned chicken breasts, pate, and fermented sausage. Remarkable levels of pathogen inhibition were apparent in all of these cases (Aymerich et al., 2000b). In addition, enterocin A alone (alternatively named as enterocin CCM4231; Foulquie Moreno et al., 2003) has also been widely applied in foods to control *L. monocytogenes*. In soy milk, a 4.96–5.15 $\log_{10}$ unit reduction of pathogen numbers was obtained when purified enterocin A was added at a level of 3,200 AU/ml (Laukova and Czikova, 1999a). When the same level of enterocin A was used in Saint-Paulin cheese, maximal inhibition of the pathogen was reached after week 1 and a 4.8 $\log_{10}$ unit difference existed between the experimental and control cheeses (Laukova et al., 2001). When used in dry fermented Hornad salami

at a level of 12,800AU/g, a 3 log₁₀ unit difference between the experimental and control treatments was apparent after one week (Laukova et al., 1999b).

For the other remaining enterocins, relatively fewer food-related studies have been carried out. Enterocin CRL35 (enterocin35) was firstly isolated from *E. faecium* CRL35 and the possibility of using it as bio-preservative in frozen seafood to control artificially contaminated *L. monocytogenes* was confirmed (Concha et al., 1999). It has also been tested, in combination with purified lactocin 705 and/or nisin, in meat products. Although the initial inhibition of *L. monocytogenes* was followed by regrowth of the pathogen when this bacteriocin was used on its own, a more significant reduction in viable counts of *L. monocytogenes* was observed when treatments employing lactococcin 705 or nisin were utilized and, finally, *L. monocytogenes* was completely eliminated when the three bacteriocins were in combination (Vignolo et al., 2000). Disappointingly, when the *Enterococcus mundtii* strain that produces the closely related enterocin mundticin AT06 (Bennik et al., 1998) was added to fresh mungbean sprouts under 1.5%O₂/20%CO₂/78.5%N₂ at 8°C, no inhibition of *L. monocytogenes* was evident. Nevertheless, the potential benefits of mundticin AT06 as a biopreservative were revealed when it was incorporated into a washing step for dipping vegetables prior to their exposure to *L. monocytogenes* (Bennik et al., 1999) and when used in a gelatin coating for the vegetable thereafter (Bennik et al., 1999).

6.2.1.3. Carnocins (i.e., Bacteriocins Produced by Carnobacteria) Carnocins have also been the subject of a large number of investigations. It was noted that *Carnobacterium piscicola* CP5, isolated from a French soft cheese, and partially purified preparations of the antimicrobial it produces, designated carnocin CP5, inhibit the growth of *L. monocytogenes* in skimmed milk. This impact was enhanced by increasing the incubation temperature from 4°C and at 7°C a protective effect was apparent for up to 7 days (Mathieu et al., 1994). It was ultimately established that carnocin CP5 in fact comprised two bacteriocins: carnocin CP51, which shares similarity with another carnocin, that is, carnobacteriocin

BM1 (B1), and carnocin CP52, similar to carnobacteriocin B2 (Herbin et al., 1997). All four carnocins are examples of Class IIa bacteriocins. It should be noted that when *L. monocytogenes* was exposed to semi-purified carnobacteriocin B2, regrowth of the pathogen eventually became evident, again, indicating the need for care with respect to the overuse of such peptides (Nilsson et al., 2004).

6.2.1.4. Carnobacterium piscicola A9b is another example of a bacteriocin producer. When a mixture of bacteriocin positive and negative cultures was used to protect cold-smoked salmon, it was established that although the salmon juice displayed antilisterial activity in both cases, the level of activity was almost twice as great (6 log unit reduction of pathogen) when the bacteriocin producer was employed (Nilsson et al., 2004).

The antilisterial activity of divercin V41 isolated from *C. divergens* V41 (Metivier et al., 1998), in a food system was firstly examined by adding the bacteriocin, either through in situ production or as a crude bacteriocin preparation, to vacuum-packed, refrigerated, cold-smoked salmon. The presence of the bacteriocin resulted in the delayed growth of the pathogen at 8°C and its inhibition at 4°C. Importantly, no adverse effects were detected (Duffes et al., 1999a). Further studies were carried out to specifically assess the contribution of divercin V41. The benefits of its presence were apparent from studies involving a smoked salmon model medium, where in situ production brought about the inhibition of *L. monocytogenes* (Richard et al., 2003), and from studies with French cold-smoked salmon (Brillet et al., 2004).

Piscicolin 126 was first obtained from *C. piscicola* JG126, which was isolated from spoiled ham (Jack et al., 1996). As with so many other Class IIa bacteriocins, piscicolin 126 was found to very strongly inhibit *L. monocytogenes*. In milk challenged with *L. monocytogenes*, the addition of piscicolin 126 immediately reduced the viable counts of the pathogen. As usual, this effectiveness depended on the level of bacteriocin and pathogen present. Although this bacteriocin was equally successful when applied to Camembert cheese, some

degree of *L. monocytogenes* recovery was apparent in both cases, presumably caused by the emergence of piscicolin 126-resistant isolates (Wan et al., 1997).

Finally, piscicocin CS526, produced by *C. piscicola* CS526, is an efficient inhibitor of *L. monocytogenes* in cold-smoked salmon at different temperatures (Yamazaki et al., 2003). When whey powder fermented with the piscicocin CS526 producer was added to ground meat (10% w/v), levels of *L. monocytogenes* rapidly decreased from 10^5 cfu/g to below detectable levels within 5 days. This bacteriostatic effect was also found to be enhanced by lower temperature (Azuma et al., 2007). *C. piscicola* V1, the producer of piscicocin V1a and piscicocin V1b, was shown to be bactericidal against *L. monocytogenes* in vacuum-packed cold-smoked salmon. Both bacteriocin-producing strains and associated crude bacteriocins can effectively prevent the growth of *L. monocytogenes* in fish system and did not pose any adverse effect on products in relation to their sensory properties (Duffes et al., 1999b).

6.2.1.5. Leucocins Leucocin A and C are produced by *Leuconostoc carnosum* 4010. This producer strain was used in vacuum-packed meat sausage and its presence resulted in the reduction of *L. monocytogenes* below detectable levels. The absence of the pathogen continued to be evident after 21 days at 5°C, thus indicating that this strain, and the bacteriocins it produces, merited further investigation (Budde et al., 2003). As a result, the ability of the strain to control *L. monocytogenes* in a sliced cooked meat product was tested. While the presence of the bacteriocin producer had a beneficial impact regardless of the way in which it was introduced, it was apparent that its introduction onto the surface of the product through sprinkling after heat-treatment was more effective than treatments involving partially purified bacteriocins or the use of a live culture prior to the heating step (Jacobsen et al., 2003).

6.2.1.6. Class IIa Bacteriocins Produced by Lactobacilli There are a variety of lactobacilli that produce Class IIa bacteriocins. Studies have been carried out to reveal the effect of environmental factors, including temperature, pH, salt concentration,

spices, and nutrients, on the production and activity of curvacin A, a Class IIa bacteriocin produced by *Lactobacillus curvatus* LTH1174, in vitro using media modified to mimic real food environments. The bacteriocin-producing strain was shown to be sensitive to sodium nitrite but this effect was masked when the culture was grown anaerobically. Interestingly, paprika increased the maximum growth rate of the strain and garlic stimulated the production of the bacteriocin. Temperature and pH ranges, 20–27°C and pH 6.0–7.0, respectively, were found to be optimal for growth and bacteriocin production. It should be noted that the production of curvacin A by this strain can be adversely affected by a number of different environmental factors (Verluyten et al., 2003, 2004a, 2004b).

Sakacin A and its producer, *Lb. sakei* Lb 706, have been found to inhibit *L. monocytogenes* in pasteurized minced meat. This killing effect was even more pronounced in laboratory broth, suggesting that there is room for further optimization of bacteriocin production in food. In comminuted cured pork stuffed into casings, the growth of *L. monocytogenes* was prevented by the addition of the sakacin A producer at pH 6.3, and its numbers were reduced by 1 log at pH 5.7 (Schillinger et al., 1991).

The purified form of another sakacin, sakacin P (produced by many *Lactobacillus sakei* strains), exhibited an antilisterial activity in chicken (Katla et al., 2002). It was found that when another sakacin P positive strain, *Lb. sakei* I151, was used in a sausage fermentation study, it colonized the ecosystem rapidly. Sakacin P production was detectable throughout the whole procedure and efficient inhibition of *L. monocytogenes* was observed. Moreover, the addition of the sakacin P producer strain did not pose any adverse effects on product quality; in fact, sakacin P-containing sausage received a higher grading from taste panellists than its nonbacteriocin-containing counterpart (Urso et al., 2006). Curiously, at least one sakacin P producer, *L. sakei* 5, was shown to also produce two other bacteriocins, sakacin T and X (Vaughan et al., 2003).

Bavaricin A was first isolated from strain *Lb. bavaricus* MI401. Its ability to control *L. monocytogenes* in brined shrimp, together with nisin Z

and carnocin UI49, has been tested. Although not as successful as nisin, bavaricin A was found to extend the shelf life of the product by 6 days (Einarsson and Lauzon, 1995). Bavaricin MN is produced by *Lb. bavaricus* MN. Following initial successes in a model meat gravy system (Winkowski and Montvilie, 1992), this producer was inoculated into beef gravy cubes with a view to control the *L. monocytogenes*. The pathogen was inhibited or killed in all cases, with efficacy being dependent on the inoculum level of the bacteriocin producer. A combination of a high level of *Lb. bavaricus* MN inoculum and low refrigeration temperatures were found to be most effective (Winkowski et al., 1993).

6.2.1.7. Other Class IIa Bacteriocins *Lactococcus lactis* CCMM/IAV/BK2 was isolated from jben cheese and shown to produce a bacteriocin that has been preliminarily categorized as a Class IIa bacteriocin. It was used as a starter culture for jben cheese making, resulting in an evident reduction of *L. monocytogenes* (Benderroum et al., 2000). Though not established definitively, it is claimed that *Lc. lactis* subsp. *lactis* LMG21206 and *Lb. curvatus* LBPE also produce Class IIa bacteriocins. When these bacteria were used as adjunct cultures to control *L. monocytogenes* in dry-fermented sausages, *L. monocytogenes* levels dropped below detectable limits after 4 hours of fermentation and 15 days of drying in the presence of *Lb. curvatus* LBPE and *Lc. lactis* LMG21206, respectively. No regrowth of *L. monocytogenes* was found in either case (Benkerroum et al., 2005).

Other Class IIa bacteriocins that exhibit antilisterial activity but that have not been studied from a food application perspective are listed in Table 29.2.

6.2.2. Class IIb Bacteriocins Many Class IIb bacteriocins have been reported to possess antilisterial activity. However, with the exception of lactocin 705, plantaricin UG1, and brochocin B, the application of these bacteriocins against the pathogen in foods has not been extensively studied.

6.2.2.1. Lactocin 705 This bacteriocin is the product of strain *Lb. curvatus* CRL705. It has been employed to control *L. monocytogenes* levels in

ground beef (Vignolo et al., 1996) with killing of the pathogen being most apparent when the associated meat slurry was heated (Vignolo et al., 1996). More recently, the spraying of this bacteriocin, and its producer, onto the surface of a meat slice that was then vacuum-packed was found to be effective (Castellano and Vignolo, 2006). Plantaricin UG1, the second example of Class IIb bacteriocin, has been successfully employed in beef (Enan et al., 2002), and finally, the presence of the brochocin B producer *Brochothrix campestris* ATCC 43754 resulted in a 2–3 log reduction in *L. monocytogenes* in prok adipose tissue discs (Greer and Dilts, 2006).

Other Class IIb bacteriocins/bacteriocin-producers with antilisterial activity, but which have not been used in foods, are listed in Table 29.3.

6.2.3. Class IIc Enterocin AS-48 is a Class IIc enterocin with strong activity against *Listeria*. There are numerous examples of the successful application of enterocin AS-48 and the corresponding producing strains to inhibit the growth of *L. monocytogenes* in foods. A few examples are described here. The introduction of a semi-purified preparation of the bacteriocin and two different live, producing cultures has been shown to bring about a significant reduction in *L. monocytogenes* in sausage. A concentration of 450AU/g was regarded as being a significant figure with regard to preventing regrowth of the pathogen (Ananou et al., 2005). The impact of enterocin AS-48 on the survival/proliferation of *L. monocytogenes* in vegetable foods has also been studied. For these tests, the vegetables, which included alfalfa sprouts, soybean sprouts, and green asparagus, were immersed in solutions containing prepared enterocin AS-48 for 5 minutes at room temperature, with immersion in distilled water being used as a control. The presence of AS-48 had a positive impact on the alfalfa and soybean sprouts (2.0–2.4 log unit of reduction) but did not dramatically impact on the pathogen when present on asparagus. The efficacy of this treatment in general was also found to be temperature-dependent, with 15°C being optimal. The benefits of treatment with enterocin AS-48 in this way can also be further enhanced through its use in combination with other chemical preservatives (Molinos et al., 2005). As

Table 29.2. Other class IIa bacteriocins with activity against *Listeria* without documented applications

Bacteriocin	Producer	Reference
Divergicin M35	<i>C. divergicin</i> M35	Tahiri et al., 2004
Sakacin M	<i>Lb. sake</i> 148	Sobrinho et al., 1992
Sakacin G	<i>Lb. sake</i> 2512	Simon et al., 2002
Sakacin K	<i>Lb. sake</i> CTC 494	Leroy and de Vuyst, 1999
Mesentericin Y105	<i>Leuconostoc mesenteroides</i>	Hechard et al., 1992
BifidocinB	<i>Bif. bifidum</i> NCFB1454	Yildirim and Johnson, 1998a
Coagulin	<i>B. coagulans</i> I4	Hyronimus et al., 1998
Curvacin FS47	<i>Lb. curvatus</i> FS47	Garver and Muriana, 1994
Mundticin KS	<i>E. mundtii</i> NFRI 7393	Kawamoto et al., 2002
Leucocin AUL187	<i>Leuconostoc gelidum</i>	Hastings et al., 1991
Piscicolin 61	<i>C. piscicola</i> LV 61	Schillinger et al., 1993
Ent. mundtii QV2	<i>E. mundtii</i> QV2	Zendo et al., 2005
Piscicolin 126	<i>C. piscicola</i>	Jack et al., 1996
Lactococcin MMFII	<i>L. lactis</i> MMFII	Ferchichi et al., 2001
Piscicocins V1a/V1b	<i>C. piscicosa</i> V1	Cintas et al., 1998
Carnocin CP5	<i>C. piscicola</i> CP5	Mathieu et al., 1994
Bavaricin MN	<i>Lb. sake</i> strain	Kaiser and Montville, 1996
Auriocin A70	<i>Staph. aureus</i>	Netz et al., 2001
Acidocin A	<i>Lb. acidophilus</i> TK9201	Kanatani et al., 1995
Acidocin B	<i>Lb. acidophilus</i> M46	ten Brink et al., 1994
Plantaricin C19	<i>Lb. plantarum</i> C19	Atrih et al., 2001
Penocin A	<i>P. pentosaceus</i> ATCC 25745	Diep et al., 2006
Plantaricin 423	<i>Lb. plantarum</i> 423	Van Reenen et al., 1998
Pediocin 5	<i>P. acidilactici</i> UL5	Huang et al., 1996
Pediocin A	<i>P. Pentosaceus</i> FBB61	Daeschel and Klaenhammer, 1985
Mesenterocin 52A/B	<i>Leuconostoc mesenteroides</i> FR52	Revol-Junelles et al., 1996
Bacterocin T8	<i>E. faecium</i> T8	De Kwaadsteniet et al., 2006.
Pediocin JD	<i>P. acidilactici</i> JD1-23	Christensen and Hutkins, 1992
Pediocin L50	<i>P. acidilactici</i>	Cintas et al., 1995
Pediocin AcM	<i>P. acidilactici</i> M	Elegado et al., 1997
Pediocin N5p	<i>P. pentosaceus</i>	Strasser de Saad, 1993
Pediocin P	<i>P. pentosaceus</i> Pep1	Osmanagaoglu et al., 2000
Pediocin ACCEL	<i>P. pentosaceus</i> ACCEL	Wu et al., 2004
Pediocin PD-1	<i>P. damnosus</i> NCFB1832	Bauer et al., 2005
Pediocin SJ-1	<i>P. acidilactici</i> SJ-1	Schved et al., 1993
Bacteriocin 31	<i>E. faecalis</i> Y1717	Tomita et al., 1996
Bacteriocin RC714	<i>E. faecium</i> RC714	del Campo et al., 2001
Enterocin SE-K4	<i>E. faecalis</i> K-4	Eguchi et al., 2001
Durancin L28-1	<i>E. durans</i> L28-1	Yanagida et al., 2005

bacteriocins can suffer from either reduced activity or decay in certain food environments, most notably in certain fluid or semi-fluid foods due to an interaction with food components, the stability of enterocin AS-48 in different fruit and vegetable juices was assessed. Using this approach, it was established that this bacteriocin was remarkably stable. In vegetable juices, including cabbage, cauliflower, lettuce, green

beans, celery, and avocado juice, it was stable for 24–48 hours under refrigeration and in freshly made fruit juices, including orange, apple, grapefruit, pear, pineapple, and kiwi, it remained stable for 15 days at 4°C, a figure which extended to 120 days under 4°C for commercial fruit juices. The enhanced stability in commercial juices may be attributable to the fact that commercial juices have been physically or

Table 29.3. Class IIb, class IIc, and class IId bacteriocins with antilisterial activity, which to date have not been tested in foods

Bacteriocin	Producer	Reference
Class IIb		
Lactococcin G/M	<i>L. lactis</i> LMG 2081	Nissen-Meyer et al., 1992
Lactococcin Q	<i>L. lactis</i> QU4	Zendo et al., 2006
Gassericin T	<i>Lb. gasseri</i> SBT 2055	Kawai et al., 2000
Lactacin F	<i>Lb. acidophilus</i> 11088	Muriana and Klaenhammer, 1991
Lactobin A	<i>Lb. amylovorus</i> LMGP-13139	Contreras et al., 1997
Gassericin X	<i>Lb. gasseri</i> LF221	Majhenic et al., 2004
Thermophilin 13	<i>Strep. thermophilus</i>	Marciset et al., 1997
Unnamed	<i>Lb. acidophilus</i> YIT 0154	Yamato et al., 2003
Cerein 7	<i>B. cereus</i>	Oscariz et al., 1999
Plantaricin EF/JK	<i>Lb. plantarum</i> C11	Anderssen et al., 1998
Plantaricin NC8	<i>Lb. plantarum</i> NC8	Maldonado et al., 2003
Plantaricin S	<i>Lb. plantarum</i> LPCO10	Jimenez-Diaz et al., 1995
ABP-118	<i>Lb. salivarius</i> UCC118	Flynn et al., 2002
Class IIc		
Durancin L28-1A (entB)	<i>E. durans</i> L28-1	Yanagida et al., 2005
Lactococcin 972	<i>L. lactis</i> IPLA 972	Martinez et al., 1999
Enterocin RJ-11	<i>E. faecalis</i> Rj-11	Yamamoto et al., 2003
Enterocin 1071A/B	<i>E. faecalis</i> BEF1071	Balla et al., 2000
Class IId		
Lactococcin A	<i>L. lactis cremoris</i>	Holo et al., 1991
Divergicin A	<i>C. divergens</i> LV13	Worobo et al., 1995
Millericin B	<i>Strep. milleri</i> NMSCC 061	Beukes et al., 2000
Class III		
Helveticin J	<i>Lb. helveticus</i> 481	Joerger and Klaenhammer, 1990
Enterocin MMT05	<i>E. faecalis</i> MMT05	Ghraiiri et al., 2004
Unnamed	<i>Lb. delbrueckii</i> 1043	Miteva et al., 1998
Enterolysin A	<i>E. faecalis</i> LMG2333	Nilsen et al., 2003

chemically treated and as a consequence some component with which the bacteriocin would otherwise interact has been removed. This was further supported by the fact that the activity of enterocin AS-48 in sterile distilled water was retained for 120 days from 4 to 28°C, although activity loss was enhanced as storage temperatures were increased further (Grande et al., 2005).

Enterocin EJ97, isolated from *E. faecalis* EJ97, is another Class IIc enterocin that has demonstrated a concentration- and temperature-dependent activity against *Listeria* in broth. When the enterocin EJ97-producing strain was inoculated into half-skimmed milk, its capacity to control *L. monocytogenes* was still detectable but lowered (Garcia et al., 2004). Similarly *E. faecium* F58, a producer of enterocin

L50 (identical to enterocin I), has also been successfully used in reduced fat and whole goat's milk and goat's jben cheese for the control of *L. monocytogenes* (Achemchem et al., 2006).

Reuterin is produced by a number of strains of *Lactobacillus reuteri*. Lyophilized reuterin produced by strain 12002 was found to inhibit *L. monocytogenes* in cottage cheese, an impact which was further enhanced by the use of UHT skim milk or the addition of 3% salt (el-Ziney and Debevere, 1998). Lyophilized reuterin has also been employed to protect meat and meat products. In cooked pork, the bactericidal effect of reuterin on *L. monocytogenes* was observed immediately upon the addition of reuterin and a 0.63 log unit reduction in pathogen numbers resulted. In raw ground pork, reuterin at a

concentration of 250 AU/g resulted in a 3 log unit reduction in viable *L. monocytogenes* (El-Ziney et al., 1999). Reuterin has also been successfully employed when added to the surface of beef sausage (Kuleasan and Cakmakci, 2002).

6.3. Bacteriolysins (Class III Bacteriocins)

This class of antimicrobials have recently been reclassified as bacteriolysins (Cotter et al., 2005). However, as a consequence of their historical classification as bacteriocins, they are discussed here. Though not established definitively, it has been proposed that linocin M18, produced by *Brevibacterium linens* M18, is a Class III bacteriocin (Klaenhammer, 1993). If linocin M18 is indeed a bacteriolysin, it is the only bacteriolysin to date that has been shown to inhibit *Listeria* in food. The linocin M18-producing strain was used as starter culture in a red smear cheese to evaluate its ability to inhibit *L. monocytogenes* in situ. While a 1–2 log unit reduction in the growth of *L. monocytogenes* was achieved, it is unclear to what extent this impact is attributable to bacteriocin production (Eppert et al., 1997).

6.4. Unclassified Bacteriocins

There exist a number of bacteriocins that have been successfully employed to control *L. monocytogenes* in food but have not themselves been characterized sufficiently to determine the class of bacteriocins to which they belong or indeed whether they are genuinely “novel.” A selection of these is listed here.

6.4.1. Bacteriocins Produced by *Lactobacilli* *Lb. sake* 2a, producing a bacteriocin-like substance, has been found to inhibit the growth of *L. monocytogenes* on Brazilian sausage (De Martinis and Franco, 1998). A bacteriocin produced by *Lactobacillus curvatus* CWBI-B28 has been used in a number of different ways to control *L. monocytogenes* in cold-smoked salmon (Ghaffi et al., 2006). When used as a starter culture, the bacteriocin-producing strain *Lb. plantarum* MCS decreased *L. monocytogenes* numbers in salami to below detectable level by the end of the maturation period (Campanini et al., 1993). Finally, a

bacteriocin produced by *Lb. curvatus* 32Y was introduced into polythene through soaking, spraying, and coating and then placed onto pork steak and ground beef contaminated by *L. monocytogenes*. Growth of the pathogen was inhibited and antimicrobial activity was highest after 24 hours at 4°C (Mauriello et al., 2004).

6.4.2. Enterocins The producer of enterocin 226NWC was first isolated from natural whey cultures used as mozzarella cheese starters and was found to efficiently inhibit the growth of *L. monocytogenes* in reconstituted skim milk (Villani et al., 1993). The producer of enterocin 416K1 was isolated from Italian sausages and, when utilized in a purified form in the manufacture of Italian sausage (cacciatore), brings about a significant inhibition of *L. monocytogenes*. When combined with the natural producer, this effect was augmented, resulting in the elimination of the pathogen (Sabia et al., 2003).

6.5. Other Bacteriocins

C. piscicola C2 and two other *C. piscicola* strains have been reported to prevent the growth of *L. monocytogenes* in cold-smoked fish juices (surubim and salmon) (Alves et al., 2005), while the bacteriocin-producer *Streptococcus salivarius* subsp. *thermophilus* B was utilized, in a co-culture based approach, to reduce the levels of *L. monocytogenes* in yogurt to below detectable levels within the first 24 hours of processing (Benkerroum et al., 2002).

For a more extensive list of the bacteriocins that fall into this category, see Table 29.4.

7. Conclusion

Although great efforts have been made to minimize the contamination of foods and food processing facilities with *L. monocytogenes*, the pathogen is still frequently detected in foods and outbreaks of listeriosis continue to occur (Schlech III, 2000; CDC, 2005). The great ability of the pathogen to adapt to a myriad of harsh environmental conditions, included those associated with processed food, continues

Table 29.4. Unclassified and partially classified bacteriocins with antilisterial activity

Bacteriocin	Producer	Reference
Plantaricin D	<i>Lb. plantarum</i> BFE 905	Franz et al., 1998
Plantaricin LP84	<i>Lb. plantarum</i> NCIM 2084	Suma et al., 1998
Plantaricin NA	<i>Lb. plantarum</i>	Olasupo 1998
Unnamed	<i>Lb. plantarum</i> N014	Rattanachaikunsopon and Phumkhachorn, 2006
Plantaricin 35d	<i>Lb. plantarum</i> 35d	Messi et al., 2001
Mesentericin ST99	<i>Leuconostoc mesenteroides dextranicum</i> ST99	Todorov and Dicks, 2004
Acidocin A (Class II)	<i>Lb. acidophilus</i> TK9201	Kanatani et al., 1995
Lactococcin R	<i>L. lactis cremoris</i> R	Yildirim and Johnson, 1998b
Garviecin L 1-5	<i>L. garvieae</i>	Villani et al., 2001
Unnamed	<i>E. avium</i>	Audisio et al., 2005
Unnamed	<i>B. lentus</i> NG121	Sharma et al., 2006
Unnamed	<i>B. amyloliquefaciens</i>	Lisboa et al., 2006
Unnamed	<i>B. subtilis</i>	Zheng and Slavik, 1999
Unnamed	<i>B. cereus</i> 8A	Bizani and Brandelli, 2002
Unnamed	<i>B. licheniformis</i> P40	Cladera-Olivera et al., 2004
Thuricin 7	<i>B. thuringiensis</i> BMG1.7	Cherif et al., 2001
Entomocin 9	<i>B. thuringiensis entomocidus</i> HD9	Cherif et al., 2001
Unnamed	<i>L. lactis</i> H-559	Lee et al., 1999
Unnamed	<i>Lb. acidophilus</i> 30SC	Oh et al., 2000
Enterocin I	<i>E. faecium</i> 6T1a	Floriano et al., 1998
Unnamed	<i>Brevi. linens</i> ATCC9175	Motta and Brandelli, 2002
Diacetin B	<i>L. lactis</i> UL 720	Ali et al., 1995
BLIS213	<i>C. piscicola</i> 213	Khouiti and Simon, 1997
Gassericin KT7	<i>Lb. gasseri</i> KT7	Zhu et al., 2000
Micrococcin G05	<i>Micrococcus</i> sp. G05	Kim et al., 2005b
Enterocin MR99	<i>E. faecalis</i> MR99	Sparo et al., 2006
GM005 (Class I)	<i>L. ap.</i> GM005	Onda et al., 2003
Thermophilin 347	<i>Strep. thermophilus</i>	Villani et al., 1995
Thermophilin A	<i>Strep. thermophilus</i> ST134	Ward and Somkuti, 1995
Thermophilin T	<i>Strep. thermophilus</i> ACA-DC 0040	Aktypis et al., 1998
Bovicin HC5 (Class I)	<i>Strep. bovis</i> HC5	Mantovani et al., 2002
Aureocin A53 (Class II)	<i>Staph. Aureus</i>	Giambiagi-Marval et al., 1990
Unnamed	<i>Strep. CNCM</i> I-841	Gomez et al., 1997
Brevicin 286	<i>Lb. brevis</i> VB286	Coventry et al., 1996
Carnosin 44A (Class II)	<i>Leuconostoc carnosum</i> LA44A	van Laack et al., 1992
Leucocin F10	<i>Leutocostoc carnosum</i>	Parente et al., 1996
Carnosin H (Class II)	<i>C. 377</i>	Blom et al., 2001
Enterocin ON-157	<i>E. faecium</i> NIAI 157	Ohmomo et al., 2000
curvaticin L442	<i>Lb. curvatus</i> L442	Xiraphi et al., 2006

to facilitate its survival in many cases. Although many approaches have been taken to eliminate this pathogen, many of the treatments that most successfully eliminate/inhibit growth of the pathogen are relatively harsh and thus can have an adverse impact on the associated food. Thus, it is not surprising that the utilization of bacteriocins as food biopreservatives has attracted ever-greater attention. The fact

that these antimicrobials are food-associated, natural, and highly active has contributed to this interest. As discussed above, a large number of bacteriocins control *L. monocytogenes* levels in food effectively. This efficacy can be even further enhanced through the coordinated use a bacteriocin (or bacteriocins) with other physical or chemical hurdles. Another approach is to bring about heterologous production

of a bacteriocin with antilisterial activity (Makrides, 1996). On occasion, the heterologous production of a bacteriocin can yield higher levels of production than associated with the native host. It also provides an attractive means by which the factors limiting the application of a particular bacteriocin, for example, adverse traits attributable to the native host can be avoided through expression in a commercially useful strain (Rodríguez et al., 2003). A number of successful examples exist, including heterologous expression of acidocin A/B, carnobacteriocin B2, nisin, colicin A, divergicin A, helveticin J, lactacin F, lactacin 3147, lactocin S, lactococcin A, leucocin A, mesentericin Y105, pediocin PA-1, brochocin C, and enterocin A/B in LABORATORY (Rodríguez et al., 2003). More recently, high-level production of enterocin P (Gutiérrez et al., 2005) and plantaricin 423 (Van Reenen et al., 2003) has also resulted from heterologous expression.

As well as selecting the most appropriate bacteriocin and producer, one of the key decisions to make with respect to the use of bacteriocins in food is to decide on the most appropriate way in which they can be incorporated. As described above, the options include its production by starter or adjunct nonstarter strains or as raw, semi-, or fully purified additives. In the former cases, the bacteriocin producing strain is regarded as one of the components of the food, and therefore no legal issue needs to be addressed. However, in the latter case, extracted bacteriocin is regarded as a chemical additive and its industrial application in food has to be officially approved. The "natural" property of bacteriocins produced by genetically modified producers, for example, heterologous production of a bacteriocin by nonnatural means, is also an issue. So far no bacteriocin, other than nisin, has been approved for use as a food additive. However should *L. monocytogenes* continue to be the cause of such great health and economic costs, it may be only a matter of time before bacteriocins are similarly employed.

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Chapter 30

Antimicrobial Packaging

Dong Sun Lee and Jung H. Han

1. Basic Principles

The main functions of food packaging are protection and preservation of packaged foods. Packaging attempts to isolate foods from the surrounding environment during distribution and storage until consumers open the package. Food packaging systems attempt to maintain the initial quality of foods and reduce the deterioration of the quality due to oxidation or microbial growth.

Microbial growth and food spoilage can occur on the surface of packaged foods since food surfaces are more likely to be contaminated during processing and package filling. Aerobic microorganisms can grow on the food surface if a supply of oxygen is present in the headspace gas. In the case of liquid foods, mixing and diffusion of headspace oxygen may accelerate microbial growth. Packaging can be designed to protect foods from environmental gases, moisture loss or gain, and light. Additionally, packages protect foods from physical quality changes, such as textural and structural loss. Oxygen-scavenging chemicals can be incorporated into packaging systems to maximize the protective barrier functions of the packaging. Active oxygen removal from the package headspace, by an oxygen scavenger, helps to preserve the food by preventing possible oxidative reactions after packaging and can inhibit the growth of molds.

Antimicrobial packaging can inhibit or delay microbial growth on food surfaces and, in some applica-

tions, in foods (Han, 2000). Even though antimicrobial substances incorporated in the packaging materials can diffuse into liquids or semisolid foods, their main target for microbial inhibition is food-package surfaces and/or food-headspace interfaces.

Maintaining a high enough concentration of an antimicrobial agent in the packaging materials can be a limitation of antimicrobial substances incorporated in the packaging structure. The initial concentration of the antimicrobials to be incorporated would need to be very high. This is not desirable and, often, not feasible. Thus, antimicrobial packaging systems are best used where they can control surface proliferation of microbes (Han, 2003, 2005). Antimicrobial packaging works best for foods with a low microbial contamination when packaged. Antimicrobial packaging cannot improve the storage stability of foods which have not been processed under sanitary conditions and are highly contaminated.

1.1. Direct Contact and Transfer by Migration Systems

Antimicrobial packaging can help preserve foods by direct contact of the packaging material with the food surface, or by volatile or gaseous antimicrobial agents diffusing from the packaging material to the food surface. Direct contact packaging materials may contain antimicrobial agents, or their precursors, which can be chemically modified by contact with food. Migration is the main mechanism controlling the effectiveness of the antimicrobial packaging system. Table 30.1 summarizes types of antimicrobial food-packaging systems.

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Table 30.1. Migration/contact matrix for mechanistic principle for antimicrobial packaging

	Migration	Nonmigration
Direct contact	Diffusion and dissolution	Natural antimicrobial material Antimicrobial-immobilized polymers
Noncontact	Transfer of volatile active component through headspace	Not applicable

Three major transfer factors control the migration rate, and hence the concentration of active substances transferred to a food surface. These are (1) the diffusion rate of the compound or compounds in the packaging material to the package surface; (2) the dissolution rate of the compound or compounds at the interface between the packaging material and food; and (3) the diffusion rate in the food. Han (2000) analyzed the diffusion and dissolution process and measured active component concentrations at interface and in the food. The objective was to see if necessary levels of antimicrobial compounds could be maintained on the basis of the diffusivity and interfacial partition coefficient of the antimicrobial agent in the antimicrobial packaging system. Han (2005) investigated the possible length of shelf-life extension by the use of antimicrobial packaging. The main problem appeared to be the control of the rate of release of the active compound so as to maintain an active concentration at surface of the food as long as possible.

Volatile compounds such as ethanol, chlorine dioxide, and allyl isothiocyanate (AITC), entrapped in packaging layers or sachets, can diffuse to the food surfaces. These compounds vaporize and, through gaseous diffusion, build a concentration on food surfaces in the package. The rate of volatile release of the active substances can be controlled by the design of the encapsulation system. The equilibration concentration in the package headspace may be maintained with high-barrier packaging materials. High-barrier materials block the permeation loss of the preservation compound through the packaging film.

Some packaging materials can be modified physically or chemically to yield antimicrobial effectiveness (Steven and Hotchkiss, 2003). Active compounds such as bioactive peptides and enzymes can be covalently linked to the polymer surface or immobilized into a polymer matrix. However covalent bonding can prevent migration into the contacting food. To be effective, these polymers must have exposed, antimicrobial, functional groups on their surface and the food must contact the active surface.

As an example, antimicrobial activity can be created on a nylon surface by exposure to ultraviolet irradiation. Radiation produces amides (Steven and Hotchkiss, 2003). Because the antimicrobial activity is on the surface and does not migrate, its application requires intimate contact with the food surface.

1.2. Tailoring Packaging Materials to Specific Food Properties

Antimicrobial packaging must be tailored to the type and preservation requirements of the packaged food. Solid foods require close contact between the food surface and the packaging material for both migratory and nonmigratory antimicrobial compounds. For example, any exposed surface should be covered by a contacting antimicrobial wrapping film.

With migrating antimicrobial systems, the diffusion and partition coefficients of the active agent in the package material determine the surface concentration. Han (2000) analyzed the effect of these variables (i.e., diffusivity in packaging material, diffusivity in food, and partition coefficient at the interface) on the time-variable distribution of the migrating antimicrobial concentration.

Natural convection will allow homogenous distribution of an antimicrobial compound diffusing from the package wall surrounding liquid foods. However, an extremely high initial load of the active agent may be required in the package wall to provide an effective concentration in the contents. Nonmigratory antimicrobial packaging materials may inhibit microbes which contact the surface through convective mixing. Aerobic microbial growth can be inhibited by inert gas flushing, vacuum packaging, and minimizing the package headspace.

Piece-form solid foods and porous foods require gaseous antimicrobial agents to be released inside the package because food surfaces may not contact the package wall. Gas-phase antimicrobial agents can diffuse into the food. An effective concentration level in the package can be achieved by a controlled release system that is sensitive to the concentration in the void spaces.

The effectiveness of antimicrobial packaging can be improved by oxygen scavenging or by the use of modified atmosphere packaging. Removal of oxygen and flushing with carbon dioxide, nitrogen, or other inert gases can increase the effectiveness of an antimicrobial packaging system. Antimicrobial packaging is a preservation technology which may be more effective in combination with other hurdles.

2. Antimicrobial Compounds and Methods of Incorporation in Packaging Materials

Suitable packaging materials and methods for incorporating antimicrobial compounds in these materials inhibitory are reviewed by Appendini and Hotchkiss, 2002; Han, 2000, 2003, 2005; and Quintavalla and Vicini (2002). Compounds are usually added at the 0.1–5.0% level. Cha and Chinnan (2004) and Han (2005) reviewed various antimicrobial agents that are used for packaging applications.

Procedures for adding antimicrobial compounds to packaging materials must protect the activity of the compounds. In a similar manner, the compounds should not unduly influence the functionality and protective properties of the packaging material. Table 30.2 lists suitable fabrication methods for antimicrobial packaging systems for various antimicrobial agents and for various packaging materials. Fabrication processes using high temperature, such as extrusion and injection molding, can be applied to heat-stable compounds, such as zeolite, zirconium, and some organic acids. Extrusion has been used to incorporate antimicrobial compounds into polyethylene (PE). Low-melting point PE has also been used for incorporating antimicrobial compounds (Weng and Hotchkiss, 1993; Han and Floros, 1997; An et al.,

1998; Lee et al., 1998; Cha et al., 2003). Synthetic preservatives have been incorporated in polyethylene terephthalate (PET) and polystyrene (PS) (Vartiainen et al., 2003). Organic acid derivatives, ceramic powder, nisin, and plant extracts have also been incorporated by extrusion and molding.

Antimicrobial agents can be attached to the surface of packaging materials using treatments to increase the bonding affinity of the compounds to the surface. The antimicrobial molecules may remain on the surface or may be detached on contact with foods. The following antimicrobial agents have been attached to packaging surfaces: bacteriocins, peptides, enzymes, polyamines, and organic acids (Appendini and Hotchkiss, 2002). Benzoic and sorbic acids have been bonded to polymer surfaces after pretreatment with HCl or NaOH solutions (Weng et al., 1997; Weng et al., 1999). Bacteriocins, peptides, or enzymes can be adsorbed or bound onto the surfaces of synthetic or natural polymers. They can be released to the contacting liquid or wet food to retard microbial spoilage (Daeschel et al., 1992; Appendini and Hotchkiss, 1997; Appendini and Hotchkiss, 2001). Surface adsorption or bonding is usually based on weak chemical bonding or physical attraction. This allows the attached antimicrobial agents to be released in a short time period on contact with food.

Antimicrobial agents, such as peptides and enzymes, can be immobilized on polymer surfaces by covalent bonding. Cross-linkers or ligands are often employed to make a stable linkage between the active antimicrobial compound and the polymer backbone. Polyethylene glycol derivatives are extensively used for retaining the molecular conformation and activity of the antimicrobial compound (Steven and Hotchkiss, 2003). Carbodiimides, glutaraldehyde, and succinimidyl esters are also used for retaining the activity of immobilized antimicrobial groups.

Heat-labile antimicrobial compounds, in aqueous solutions, can be coated on the surface of natural polymers without loss of activity. Edible coatings containing generally recognized as safe (GRAS) or other edible antimicrobial agents can be applied to food surfaces to protect the food from microbial spoilage (Han, 2002; Han and Gennadios, 2005).

Table 30.2. Suitable fabrication methods of antimicrobial packaging systems for various antimicrobial agents and packaging materials

Antimicrobial Substance	Fabrication Methods or Forms				
	Extrusion or Heat Pressing	Surface Immobilization, Adsorption or Chemical Bonding	Solvent Bulk Casting or Coating	Encapsulation or Bulk Physical Adsorption	Plasma or UV Irradiation
Organic acids, their salts or anhydrides	PE, LDPE, PET, PS	Ionomer, PEMA	Chitosan film, EVA, acrylics, PVOH, methyl cellulose, proteins, polysaccharides		
Bacteriocins or peptides	LDPE	PS, silicon, starch	PA layer on LDPE, EVA, acrylics, proteins, polysaccharides, chitosan, PVOH	Paper, PE/PA	
Enzymes		PVOH, nylon	Polysaccharides, PVOH, cellulose acetate		
Nonvolatile plant extracts	LDPE		Polysaccharide, proteins, paper		
Volatiles			Chlorite in anhydride copolymers with olefins	AITC in cyclodextrin, silica gel	
Chitosan				PVOH	
Cationic metal ions	Ag-zeolite in PVC, PP, LLDPE				Ag coating on PE
Other chemical preservatives or functional group			Polysaccharides		Nylon

AITC, allyl isothiocyanate; LDPE, low-density polyethylene, LLDPE, linear low-density polyethylene; PA, polyamide; PE, polyethylene; PEMA, Poly(ethylene-co-methacrylic acid); PET, polyethylene terephthalate; PS, polystyrene; PVC, polyvinyl chloride; PVOH, polyvinyl alcohol; EVA, ethylene vinyl acetate; PA: polyamide.

Heat-labile antimicrobials, suspended in a solution, may be applied as a coating to biological or synthetic polymer packaging surfaces. In this case, the adhesion and stability of the binder coating on the packaging surface can control the release of the antimicrobial agent. Functional coatings have been difficult to develop since adhesion must be balanced with good agent release properties.

A polymer coating designed to release volatile chlorine dioxide from chlorite incorporated in the mixture, can be made from a mixture of a hydrophobic phase (maleic anhydride copolymers with olefins, or grafted maleic anhydride-polypropylene) and a hydrogen bonded phase (a monomeric or poly-

meric amide, or a monomeric or polymeric alcohol). This mixture is applied as a hot melt to the substrate packaging material and solidified by cooling (Wellinghoff, 1994). Chlorine dioxide is released by a reaction of the anhydride or hydrolyzed anhydride with the chlorite anion across the phase boundary. The reaction is activated by moisture adsorbed from the in-package environment.

A volatile compound, such as AITC, can be encapsulated in cyclodextrin. The cyclodextrin can be coated on to the inner walls of the package. AITC is volatilized into the headspace of the package in response to the moisture of the in-package environment. Biological antimicrobials labile to heat, such

as bacteriocins and chitosan, can be physically adsorbed onto bulk polymers or plastic resins. These materials can be fabricated into sheets or granules. The treated sheets or granules allow a controlled rate of antimicrobial release when they contact food.

UV irradiation or plasma treatment of polymer surfaces may be used to form domains with antimicrobial activity. Experimental trials have been made with a PE film coated with antimicrobial, nanosize, silver clusters, using a plasma deposition process. Coating takes place in a diethyleneglycol dimethylether vapor in argon gas (Cannarsi et al., 2003).

While many antimicrobial compounds have been tested in the food-packaging applications, only a few have successfully survived in the market. Bioactive substances, of natural origin, have been more successful than synthetic preservatives. Some natural antimicrobial agents used in food-packaging systems

include plant extracts such as wasabi extract (AITC activity), grapefruit seed extract, bacteriocins such as nisin, pediocin, lacticin, enzymes (lysozyme), and chitosan (Lee, 2005).

Food packaging with antimicrobial functions can be applied in forms tailored to specific food types and for specific uses. Antimicrobial films are the most common. Sachets can be used to release volatile antimicrobials. Water absorbent pads can release antimicrobial compounds on exposure to a moist environment. Figure 30.1 shows several antimicrobial packaging applications.

Examples of commercially available antimicrobial packaging systems include linear low-density polyethylene (LLDPE) film wrap containing zeolite, PET film with wasabi-extract, silica oxide sachet with encapsulated ethanol, polyolefin film evolving ClO_2 , and cellulosic films containing plant extracts.

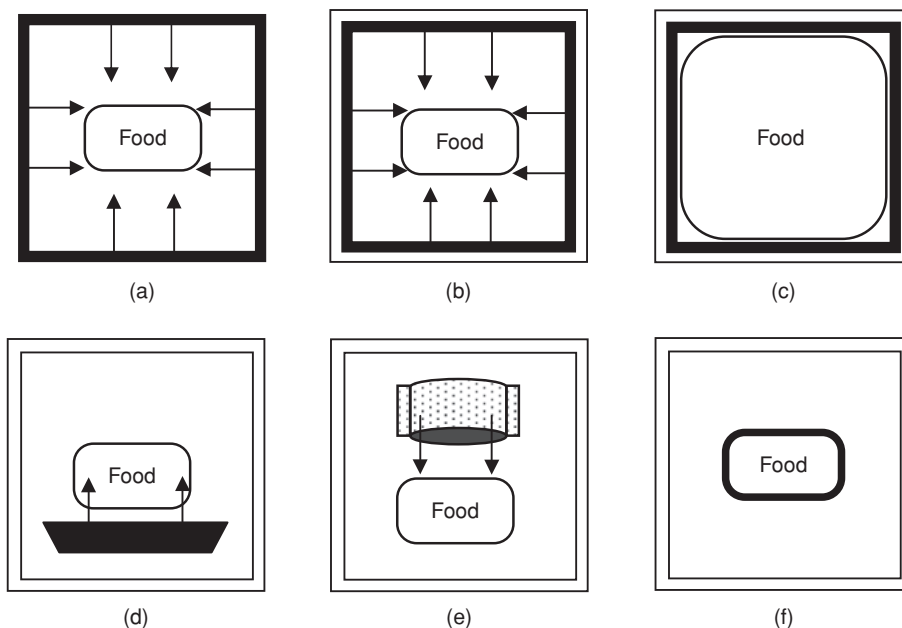


Figure 30.1. Several possible types of antimicrobial packaging: (a) antimicrobial films or bags of whole structure; (b) antimicrobial coating on conventional packaging materials; (c) immobilized antimicrobial agents in polymeric packaging materials; (d) antimicrobial trays or pads; (e) sachet/insert containing volatile antimicrobial agents; (f) antimicrobial edible coating on food. (From Han 2005.)

3. Microbial Evaluation of the Effectiveness of Antimicrobial Packaging

Controlled release of an antimicrobial compound to the food is one benefit of antimicrobial packaging. However, there are very few studies directed toward optimizing controlled release parameters. Chung et al. (2001) reported that the slow antimicrobial release of propyl paraben from a polymer coating, into a liquid medium, resulted in slow, but consistent, inhibition of a yeast strain. Microbial tolerance of the antimicrobial by the yeast did not develop over time. By contrast, Chi-Zhang et al. (2004) investigated the effect of nisin delivery to a broth containing *Listeria monocytogenes*. Blending with 200 IU/mL of nisin resulted in an almost immediate 4 log reduction of *L. monocytogenes*. This was followed by a subsequent outgrowth of survivors as shown in Figure 30.2. Slow addition of a high concentration of nisin did not dramatically reduce viable cell count, but was able to suppress further microbial outgrowth. Combined delivery, using blending and slow addition, provided the most effective microbial inhibition over time. A slow release of nisin appeared to result in less resistance to nisin by *L. monocytogenes*.

Antimicrobial packaging must maintain a minimum critical concentration of the antimicrobial agent

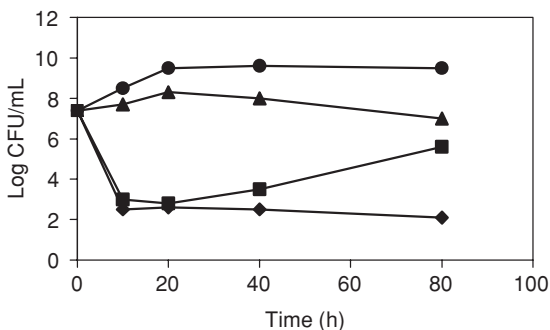


Figure 30.2. Sensitivity of *Listeria monocytogenes* to different modes of nisin delivery. ●, control cultured in the absence of nisin; ■, instant nisin addition at 200 IU/mL; ▲, slow addition of nisin at 1,000 IU/mL; ◆, combination of delivery modes of instant addition at 200 IU/mL and slow delivery of 500 IU/mL. (Reproduced from Chi-Zhang et al. 2004.)

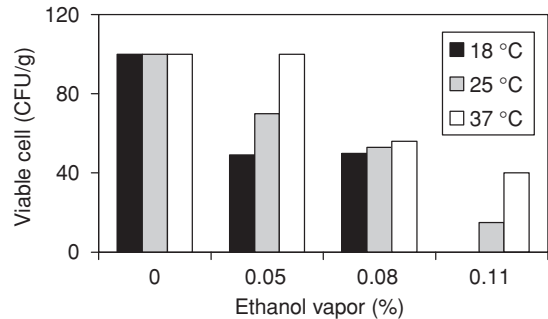


Figure 30.3. Effect of ethanol vapor on the *Escherichia coli* cells inoculated onto Petrifilm® plate and incubated for 48 hours. (Reproduced from Chen et al. 2003.)

in the food to be effective. Proper migration rates and equilibrium concentrations must be achieved in the food. Han (2005) proposed that a permeable membrane system, containing concentrated volatile oil, may be an effective way to deliver a minimum surface concentration.

Gaseous phase antimicrobial delivery systems have attracted interest as a way to deliver critical concentrations of antimicrobial compounds over an extended time period. Ethanol-generating sachets, ClO₂-generating films, and tablets or sheets infused with AITC are typical examples. Ethanol vapor, chlorine dioxide, and AITC are effective in retarding microbial growth on model food surfaces and on packaged food surfaces (Nielsen and Rios, 2000; Chen et al., 2003; Cooksey, 2005; Nadarajah et al., 2005). Figures 30.3 and 30.4 show data on the inhibitory effects of ethanol and AITC on microbial growth, respectively. Maintaining the effective concentration of the active components above the critical inhibitory level, in the headspace, is essential for effective shelf-life extension and prevention of microbial growth.

Currently, the dominant applications of antimicrobial packaging systems are plastic films or cling wrap films containing silver-doped ceramics. These are marketed in Asian countries. However, there are only a few research studies documenting the effectiveness of these types of films for food preservation. Polyvinyl chloride (PVC) cling wrap or PE film, loaded with active ceramic, showed significant bactericidal activity when contacted with pure

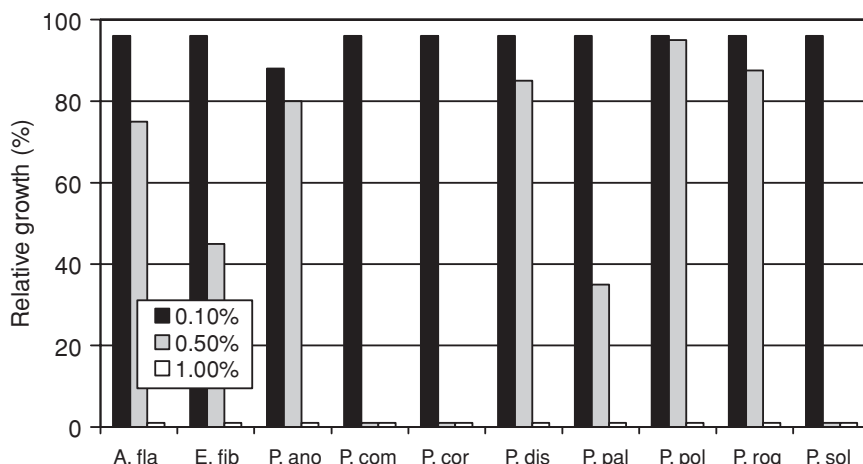


Figure 30.4. Effect of headspace AITC concentration on colony size of fungi (*Aspergillus flavus*, *Endomyces fibuliger*, *Pichia anomala*, *Penicillium commune*, *Penicillium corylophilum*, *Penicillium discolor*, *Penicillium palitans*, *Penicillium polonicum*, *Penicillium roqueforti*, and *Penicillium solitum*) associated to bread relative to an untreated control after 14 days at 25°C. (Reproduced from Nielsen and Rios 2000.)

suspensions of *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella typhimurium*. Their effectiveness was greatly reduced by nutrient-rich foods or by their poor contact with food surfaces (Ishitani, 1995; Sadoru, 1996). When applied as a wrap for cucumbers, the effect on surface microbial growth was marginal as shown in Figure 30.5 (An et al., 1998). It was suggested that microbial growth can be suppressed only by surface contact of the zeolite-

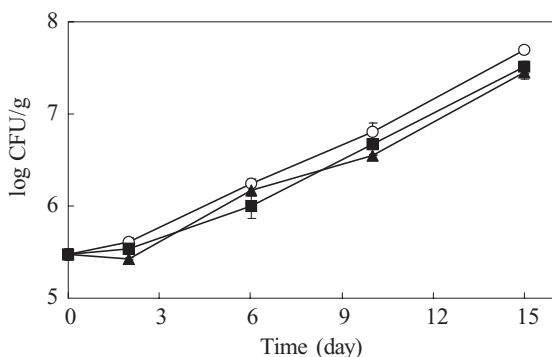


Figure 30.5. Effect of 1% silver zeolite-impregnated LDPE film on total aerobic bacteria count on cucumber individually wrapped and stored at 10°C. Vertical bars indicate standard deviation. ○, control plain film; ▲, film with Ag-zirconium; ■, film with sorbic acid. (Reproduced from An et al. 1998.)

incorporated film. Microbial inhibition can be expected to take place only in nutrient-poor media (Ishitani, 1995). Figure 30.6 also shows the effect of zeolite-impregnated film on microbial growth in Oolong tea, a nutrient-poor drink.

Shelf-life extension attained by antimicrobial packaging addresses only the reduction of quality and safety from microbiological growth. The effect of oxygen concentration, enzyme activity, browning activity, starch retrogradation, light, and storage

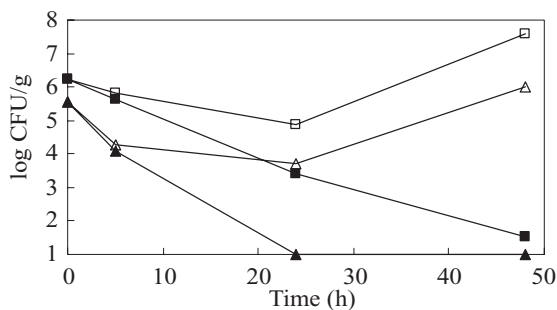


Figure 30.6. Effect of silver-zeolite impregnated PE on total microbial and *E. coli* count on Oolong tea stored at 25°C. □, total count of control; ■, total count for silver-zeolite impregnated PE; △, *E. coli* count of control; ▲, *E. coli* count for silver-zeolite impregnated PE. (Reproduced from An et al. 1998.)

Table 30.3. Extent of shelf-life extension obtained by antimicrobial packaging

Food and Storage Condition	Antimicrobial Packaging System	Shelf-Life Extension Relative to Control	Quality Index or Parameter for Comparison	Reference
Orange juice, 6°C	0.5% hexamethylene-tetramine-added LDPE film	1.4	Yeast count of 10^4 CFU/mL	Devlieghere et al., 2000
Cooked ham, 6°C	0.5% hexamethylene-tetramine-added LDPE film	1.7	Aerobic bacterial count 10^6 CFU/g	Devlieghere et al., 2000
Chicken breast in N ₂ package, 3°C	Slow release ClO ₂ sachet	1.7	Aerobic bacterial count 10^6 CFU/breast	Cooksey, 2005
Chicken breast in 75% N ₂ /25% CO ₂ package, 3°C	Slow release ClO ₂ sachet	1.2	Aerobic bacterial count 10^6 CFU/breast	Cooksey, 2005
Milk, 3°C	3% nisin/3% chitosan incorporated polymer coating	1.9	Specific growth rate of aerobic bacteria	Lee et al., 2004
Milk, 10°C	3% nisin/3% chitosan incorporated polymer coating	1.7	Specific growth rate of aerobic bacteria	Lee et al., 2004
Orange juice, 3°C	3% nisin/3% chitosan incorporated polymer coating	1.1	Specific growth rate of yeasts	Lee et al., 2004
Orange juice, 10°C	3% nisin/3% chitosan incorporated polymer coating	1.5	Specific growth rate of yeasts	Lee et al., 2004

temperature must be considered to obtain a full understanding of quality changes during storage. Table 30.3 summarizes the shelf-life extension attained by the use of selected antimicrobial packaging systems. The quality thresholds used to determine acceptable shelf lives shown in Table 30.3 have been estimated by the researchers cited or by the authors of this chapter. In summary, results show that the shelf lives of perishable foods may be extended by about 1.1–1.9 times by the use of antimicrobial packaging systems when compared to conventional packaging.

4. Future Outlook for the Use of Antimicrobial Packaging for the Shelf-life Extension of Foods

4.1. Tailored Design of Antimicrobial Packaging Systems

The ideal antimicrobial packaging system would be one tailored to protect foods from problematic

spoilage and pathogenic microorganisms normally associated with their production. In that sense, antimicrobial packaging should be designed for individual products to take into account the initial level and type of spoilage and pathogenic microorganisms. Storage conditions, food properties, pH, and water activity, should also be taken into account (Han, 2005). Antimicrobial agents incorporated into the packaging should be selected on the basis of their specificity against target spoilage and pathogenic microorganisms of concern in a particular food. The functional mode and release rate of the antimicrobial agents also should be considered.

4.2. Consumer Studies and Regulations

Wide commercial use of antimicrobial packaging requires that the agents used meet FDA additive regulations and consumers perception of safety. Antimicrobial food packaging has been used to a greater

extent in Korea and Japan than other countries. Regulations and consumers attitudes have allowed the adoption of new food packaging materials and new technologies. As an example, Asian people have a long history of using silver utensils and medicinal plant extracts. Thus, there are fewer objections to the use of silver or plant extracts in packaging materials. In the United States and Europe, food contact substances (food contact materials or FCMs in EU) are carefully controlled. Antimicrobial compounds added to packaging require testing and clearance for approval as new packaging materials. In the United States, antimicrobial packaging compounds that do not migrate into the food, and hence are not considered food additives, can be candidates for approval through the food-contact substance notification process. Compounds that migrate into the food are subject to the food additive petition process (Song and Hepp, 2005). In Europe, all the compounds used in the manufacture of FCMs need to be approved and included in approved lists of FCMs. The FCMs should comply with overall and specific migration limits (de Kruijf and Rijk, 2003). A worldwide effort is needed to harmonize the approval of antimicrobial packaging food-contact substances. Many more science-based research studies are needed to help improve the regulatory process.

In order to achieve the commercialization of antimicrobial food packaging systems described above, more scientific investigations are required. The kinetics of microbial growth inhibition by antimicrobial packaging needs to be understood and verified to be able to predict microbial growth and spoilage under during food distribution. Even though there are still some technical, psychological, and legal barriers inhibiting the wider application of antimicrobial packaging, it is expected that antimicrobial packaging will become widespread.

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Section V

Driving Forces

Chapter 31

Consumer Trends and Perception of Novel Technologies

Christine M. Bruhn

1. Overview

The acceptance of a technology depends on the consumer's perception of benefits and risks. These include the impact of the technology on taste, convenience, nutritional value, the perceived safety of the process or technology, the magnitude of the risk the technology reduces, and the effect of the technology on the environment. Public acceptance is influenced by perceived credibility of data, rigor of regulatory policy, impartial action of regulators, and demonstrated responsibility of industry.

2. Consumer Priorities

Consumers do not ask for technologies, rather they expect the food industry to deliver products with benefits important in their lives. Consumers want great tasting, and convenient and healthy products. Food safety and worker safety are basic expectations. Many passively or actively support food-production methods that are sustainable with minimal environmental impact.

Consumer beliefs, attitudes, and preferences can be assessed through qualitative and quantitative research methods (Salant and Dillman, 1994). Qualitative methods, such as focus groups or individual in-depth interviews, provide insight as to feelings or thoughts on issues (Krueger and Casey, 2000). In this approach, a small number of individuals rep-

resenting the target audience are asked open-ended thought-provoking questions on key issues with the goal of finding a diversity of opinions that can be organized into specific themes. The number of individuals responding to these themes in a population is then assessed through quantitative research. Quantitative methods include telephone, mail, internet, or face-to-face interviews of a large number of consumers (Fink and Kosecoff, 1985; Pope, 1993). Attitudes can be projected nationwide, if the sample is based upon demographic parameters related to the national population. Typically, market research organizations use a sample size of 1,000 persons.

Consumer attitudes do not always predict actual marketplace behavior. Factors other than those addressed in a survey, such as product appearance, price, availability, and quality of substitutes, may alter behavior in either a positive or a negative direction. Further, consumer attitudes are affected by information received. Television, newspapers, magazines, the internet, books, and friends are influential sources of information on food and health (America Dietetic Association, 2000; Cogent, 2006). Information from these sources, in addition to traditional methods of communication like advertising and product labeling, affect purchase decisions. Actual purchased behavior is tracked by supermarkets, manufacturers, and other organizations. Limited data is available publicly, such as that reported by the Food Marketing Institute and the Grocers Association of America, with in-depth analysis offered for purchase by various research organizations.

Good flavor, convenience, and health-enhancing properties are key consumer benefits in today's

marketplace. An examination of the most successful new products in the supermarket indicates that products with a new flavor, unique flavor combination, or new recipe constitute three of the top five supermarket new-product successes (Information Resources Inc., 2004c, 2005a). In a national telephone survey, over 80% of consumers indicate that convenience is an important consideration in purchases (Food Marketing Institute, 2005) and foods with added convenience are among top supermarket sellers (Information Resources Inc., 2004a, 2005b). Dietary fiber, beneficial fatty acids, lycopene, vitamin C, and probiotic cultures are among the top functional foods covered by the media (Center for Media and Public Affairs, 2004). Products with nutritional appeal are also among the most successful new introductions. The growth in the organic market may reflect interest in “natural” products without preservatives or additives or products believed to be produced in a sustainable or environment-friendly manner (Williams and Hammitt, 2001).

New processing technologies can help realize some of the advantages that consumers seek, but the path from introduction to acceptance is not always clear. Some consumers are skeptical of technology and believe a low-technology approach would promote health and environmental sustainability. The introduction of a food processed by a new technology may create concern among these individuals. The public is generally unaware of methods used or safeguards employed in processed food. Any risks associated with a new technology are seen by the public as imposed by the processor and beyond the control of the consumer. In some consumer’s mind, an unfamiliar approach presents unknown risks which could be potentially harmful.

3. Perceived Risks

Several theories have been developed to explain risk perception. Characteristics of risk, such as severity of consequences, involuntary exposure to risk, harm to the environment, exaggerated reports, and adequate regulations, were found to be important for predicting consumer perception of risk (Yeung and Yee, 2005; Yeung and Morris, 2006). Risks are enhanced

in the public’s mind when imposed by others, when not accompanied by clear benefits, or when viewed as unfair (Slovic, 1987).

Consumer research can identify the questions consumers have about a new technology. People want to know what risk may be reduced by a new technology and what risk is imposed by the technology. People are primarily interested in knowing how the new process or technology affects them. For example, more people are interested in knowing how eating irradiated food affects human health than in knowing how irradiated food tastes (Food Marketing Institute, 1998; Information Resources Inc., 2005c). Taste can be determined by personal experience, but the long-term effect on health requires additional input beyond the individual’s capabilities. Similarly, more people are interested in knowing how biotechnology or genetic modification affects food safety rather than in knowing how it affects farming practices or how the technology works (Bruhn and Mason, 2002).

Massachusetts consumers were asked to describe their level of concern on the basis of their hearing the name of specific food technologies. Respondents indicated concern using a Likert-type scale in which 1 indicated no concern and 4, the highest concern. Use of bacteriocins generated the greatest concern, 3.0, followed by irradiation at 2.25, aseptic processing at 2.1, ohmic heating at 2.0, and ultrasound at 1.9 (Cardello, 2003). Although aseptic processing is widely used, the term may be unfamiliar. This suggests that the use of less familiar terms may generate concern. Further, some expressed concern about the common process of heat pasteurization (1.25), indicating that the reference line for minimal concern is not 1.0, but somewhere higher.

Providing information about the technology reduced concern. Focusing consumer response on the method of processing, such as using the term “minimally processed” and “fewer preservatives,” however, generated a negative response from some consumers (Cardello et al., 2007). These studies suggest that communicating about the product and providing information about the process is more likely to address consumer information needs and result in product acceptance compared to simply stating “minimally processed.”

Concern about a technology influences flavor expectations, (Lahteenmaki et al., 2002; Cardello, 2003). Researchers found that flavor ratings were lower when people were told that the product was produced by a new processing method. Flavor ratings increase when people actually see the produce processed by the new technology, when statements about safety are made, and when benefits are described (Schutz et al., 1989; Bruhn, 1995; Frewer et al., 1997; Cardello, 2003). Consistent with these findings, Tuorila and colleagues (Tuorila et al., 1994) found that uncertainty associated with a novel product may introduce a degree of expected disliking, but additional factual information reduces this uncertainty and improves expected liking.

Early users of new technologies have been found to have higher incomes, more prestigious occupations, and more positive self-identities (Rogers and Shoemaker, 1971). Women are generally more likely to express concern about a new process or technology than men (Cardello, 2003; Food Marketing Institute, 2005).

Repeated exposure to neutral or positive information about a technology lowers concern. While examining the effect of an educational intervention on attitudes toward food irradiation, Schutz and colleagues (Schultz, 1994) found that people expressed less concern about irradiation with repeat testing even though they received no educational intervention. Similarly, Cardello (Cardello, 2003) found that postconcern levels for many technologies were reduced by participating in a study in which they sampled products described as processed by a new method.

Generally, negative information is more powerful than positive in influencing public attitudes (McNutt et al., 1986). Market research indicates that positive framing of product attributes, such as meat that is 75% lean rather than 25% fat, results in a more positive evaluation than the reverse negative framing (Donovan and Jalleh, 1999).

4. Product Benefits, a Driving Factor

Research can identify the consumer's view of the most importance product characteristics. Taste is consistently rated as the most important factor that

drives consumption and repeat purchase (Information Resources Inc., 2005c; Cardello et al., 2007). The promise of improved flavor was the driving factor for the introduction of the biotechnology modified Flavr Savr (copyright) tomato. Flavor continues to be viewed positively. About two-thirds of the US consumers surveyed (67%) indicated that they would likely purchase a biotechnologically modified product with improved flavor (Cogent, 2006; International Food Information Council (IFIC), 2008).

Although people cite nutrition and health as important in product selection (America Dietetic Association, 2000), good taste is a more dominant market force. Nutritious products that do not deliver satisfactory flavor do not remain in the market. This is illustrated by the rise and fall in demand for low-carbohydrate foods perceived as helpful in weight management (Information Resources Inc., 2004b, 2004d). Superior flavor is a driving factor for health professionals as well as the general public. Persons with a background in food and nutrition indicated greatest interest in purchasing products processed by a new technology, if the product delivered better flavor (Delgado-Gutierrez and Bruhn, 2008).

The importance of good flavor has increased in recent years. In 1994, a national survey found that 33% of the consumers indicated that they rarely or never gave up good taste for health, while that percentage increased to 43% in 2004. Similarly, in 2004, those who agreed that they always or usually avoided favorite foods to eat healthier fell to 38% compared with 42% in 1994 (Information Resources Inc., 2005c).

Convenience is a driving force in today's market. Convenience, taste, and perceived-health advantages of fresh food compared to frozen or shelf-stable food is increasing the demand for refrigerated meal components (Information Resources Inc., 2005b). Greater convenience is the most important motivation for purchasing minimally processed vegetables (Ragaert et al., 2004).

Demand varies among different demographic groups. The likelihood to buy minimally processed vegetables is higher among better educated consumers and those with young children. In addition to demographic parameters, higher appeal for specific

products can be related to awareness of health benefits. For example, although Belgium consumers rank health and nutritional value relatively low in terms of importance during purchase, consumers with a high awareness of the relationship between food and health attach more importance to these attributes (Ragaert et al., 2004).

Consistent with these trends in the United States, a survey based upon 3,000 personal interviews in the United Kingdom, Germany, and France found that the perception of personal benefits and environmental friendliness were the most important factors affecting likelihood to purchase products processed by high pressure (Butz et al., 2003). The importance of improved flavor appears to differ by culture. About half of the French consumers indicate that they would purchase a product processed by high pressure for better quality, while only 4% of the British consumers indicated that they would purchase for this benefit (Butz et al., 2003). In contrast, almost 40% of the German consumers indicated that they would purchase a product processed by high pressure for better health, while this was a driving factor for only 18% of the French consumers (Butz et al., 2003).

Although consumer research provides valuable insight, an inquiry about a technology's safety may itself create uncertainty about the processing method. When asked about new products, consumers indicate that the potential risk associated with a product is the most important determinant in product use (Cardello et al., 2007). Hicks et al. found that 15% of the volunteers from a national sample admitted that they were fearful of new food-processing technologies (Hicks et al., 2009). Consumers express concern about potentially harmful by-products and unknown health risks. However, when products are in the supermarket, consumers expect them to have been evaluated by regulatory agencies, the supermarket, or others and to have been found acceptable.

5. Communicate with the Public

Continuous consumer communication plays a pivotal role in acceptance. Communication is more than advertising. Effective communication is a two-way

process which involves listening, identifying, and responding to consumer questions.

Information should be presented in a variety of sources, with preferred sources varying by age and gender. Consumers find television, newspaper, magazines, and supermarket brochures convenient. More men and younger consumers prefer web-based sources than women or older persons (Li-Cohen and Bruhn, 2002; Food Marketing Institute, 2008; Hicks et al., 2009).

When deciding about controversial or complex issues, consumers will likely be influenced by opinion leaders, trusted people, or organizations that are knowledgeable about technology (Rogers, 1995). Endorsement by respected experts increases the acceptance of food processed by new methods.

Issues other than those that can be scientifically measured determine if a technology is accepted (Belton, 2001). When message components were segregated, trust in the spokesperson was significantly more important in explaining attitudes than accuracy of information (Bord and O'Conner, 1989). Similarly, Sapp (Sapp et al., 1994), found that word of mouth and trust in government and industry were more important than demographic factors in predicting consumer acceptance of irradiation.

Trust is greatest for groups perceived as knowledgeable, unbiased, and acting with the public's best interest in mind (Frewer et al., 1996). While no one organization is trusted by everyone, patterns of trust have emerged. In the United States, health organizations, such as the American Medical Association and the American Dietetic Association, are viewed as trustworthy by the largest percentage of consumers (American Dietetic Association, 2000; Pew Initiative on Biotechnology, 2004). University scientists and government agencies, such as the United States Food and Drug Administration, are also viewed as trustworthy by a majority of consumers. Industry groups, advocacy groups, and the media are seen as trustworthy by the smallest percentage of consumers. Those who prefer low-technology approaches to food processing appear to have lower levels of trust in government sources. For example, compared with those who select conventional products, those who buy organic foods have a greater feeling of distrust

toward regulatory agencies (Williams and Hammitt, 2001).

To increase trust and the likelihood that communications are understood, educational programs should be built around what the public wants to know, as determined by consumer research. New technology should be described using lay terminology. The new method may be compared to a similar or more familiar technology. Communication should acknowledge that risks can never be completely eliminated. Consumer benefits should be emphasized. Transparency is the operative word, sharing what is known and not known both in regards to risk and benefits. Consumers today are interested in the impact of a technology on worker safety and environmental stewardship, as well as personal welfare.

Developing descriptions of products processed by new technologies should be done systematically. Statements describing new technologies should be evaluated by focus groups before adoption to test message clarity. Messages should anticipate and respond to concerns special interest groups may express. When these approaches are used, public acceptance generally increases (Schutz et al., 1989; Bruhn, 1995; Resurreccion et al., 1995; Bruhn and Mason, 2002; Fox, 2002; Johnson et al., 2004; Zienkewicz and Penner, 2004; Delgado-Gutierrez and Bruhn, 2008).

6. A Case Study: Irradiated Food

Among novel food processing technologies, irradiation has been studied most extensively (Eustice and Bruhn, 2006). Unlike other processing methods, food processed by irradiation must be labeled. The words, “treated by radiation” or “treated with irradiation” must appear on the label at the consumer or food service, or food-processing level. The reason for irradiation, such as to enhance safety or extend freshness, may also be included. Consumer attitude studies indicate that destruction of harmful bacteria was considered a very important benefit by 92% of the consumers while being able to eat ground beef cooked more rare was important to 65% (Food Marketing Institute, 1998).

Consumers want assurance that irradiated food are safe, that irradiation adds value to the product either through enhanced safety, superior quality, or extended freshness, and that nutrition value is not compromised. Consumers have purchased products in the marketplace that meet these criteria.

Marketing irradiated food has been successful, although geographically limited. Mangoes were the first irradiated products marketed in the United States. High-quality fruit from Puerto Rico were sold successfully in Florida in 1986. In March, 1987, irradiated Hawaiian papayas were available for a 1-day trial at two markets in Southern California (Bruhn and Noel, 1987). The more ripe irradiated papayas outsold the conventionally handled fruit by more than ten to one. Carrot Top, a specialty produce market in the Chicago area, introduced a variety of irradiated fruit in 1992 (Marcotte, 1992; Pszczola, 1992). Customers were provided a newsletter as well as point-of-purchase information from sources that endorse and oppose the use of irradiation. Irradiated strawberries were offered for the same price as non-irradiated with a “buy one, get the second free” offer (Pszczola, 1992). While the retailer expected people to select one irradiated and one traditional basket, most consumers selected both baskets of irradiated fruit. Consumers were so pleased with the extended freshness and quality of the irradiated product that it consistently outsold the nonirradiated fruit by more than 20 to 1. The market continued to expand its offering of irradiated fruit. In conjunction with a trial on the effectiveness of quarantine treatment, tropical fruit from Hawaii including papaya, rambutan, and lychee were marketed successfully. Subsequently, an irradiated facility was built on the island of Hawaii to treat fruit before shipment to the mainland. The introduction of irradiated ground beef in Minnesota in 2000 significantly increased public awareness and interest in the technology. Supermarkets in several states offered irradiated ground beef before the closure of the electron beam facility treating the products. Meat and poultry processed by gamma rays continued to be available in supermarkets and through mail order. Market demand is rebuilding with primary use of the technology to enhance safety of meat and poultry, and growing interest in phytosanitary

treatment to disinfest fruits and vegetables. In 2008, irradiation was approved by the US Food and Drug Administration to improve the microbiological safety of spinach and iceberg lettuce (Food and Drug Administration, 2008). At this time, however, the method has not been used, perhaps because irradiation facilities are not located near produce farms and the industry is uncertain as to consumer acceptance.

Attitude studies indicate that most consumers, but not all, will purchase labeled irradiated food when they hear the benefits this technology provides (Bruhn, 1995; Fox et al., 2001; Johnson et al., 2004; Zienkewicz and Penner, 2004; Bhumiratana et al., 2007). Opponents of this process state that foods processed by irradiation contain dangerous products, suffer from significant nutritional loss, and have poor flavor. Fox demonstrated that the effects of inaccurate unfavorable information on food irradiation can be neutralized (Fox, 2002). He advises that educational efforts should address and counter the misperceptions fostered by opponents of new technologies.

7. Summary

Products will have the greatest likeliness of success when developers address consumer needs, respond to consumer concerns, and offer tangible benefits. Researchers have demonstrated that statements about the benefits associated with a particular food or food-processing technique will reduce concerns and increase likelihood of consumption. Factual information from a trusted source, clear statements about safety and benefits, and exposure to a product that delivers quality and convenience will increase the likeliness of consumer acceptance.

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Chapter 32

Consumer and Sensory Issues for Development and Marketing

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Foods processed by nonthermal, novel, or emerging food technologies pose interesting issues for consumer scientists interested in understanding the factors responsible for the choice, purchase, and acceptance of foods. Like most other new products being introduced to the market, optimizing the sensory quality of these foods is critical to their success. However, optimal sensory quality alone will not guarantee this success, because perceptions of food quality do not depend solely on the *intrinsic* sensory characteristics of a product. Rather, they rely heavily on a host of factors that are *extrinsic* to the product. These extrinsic factors include contextual, cognitive, social, ethnic, cultural, and attitudinal variables related to both the product and the consumer of the product. In the case of foods that have been processed by novel or emerging technologies, beliefs about the origins of the food and/or the processing technologies that have been applied to the food may become important considerations for consumers when making choice and purchase decisions. Consumers want to know what these technologies do to the food, what are their potential benefits, and what are their potential risks. As we shall see, consumer conceptions about the perceived risk of the technology can dramatically influence his/her perceptions and expectations of the product, and, ultimately, his/her liking and purchase behavior.

1. Consumer Risk Perception

In order to understand the influence of consumer conceptions and beliefs about novel technologies on the acceptance of foods processed by them, one must first understand consumer perceptions of risk. The study of “risk perception” is a relatively new field. Its roots go back to the early 1970s and the pioneering work of Starr (1969), Fischhoff et al. (1978), and Slovic et al. (1979). The research of these investigators represented a fundamental shift in how scientists viewed and approached the measurement of technological risk. Prior to that time, the risk associated with a new technology was based on expert analyses of risk. These analyses were based on actuarial data on accidents, fatalities, or losses accountable to the technology. However, this *technical/rational* approach to risk was often found lacking when it came to predicting consumer reactions to novel technologies. Frequently, consumers had great concerns about technologies that had relatively low risk from an objective technical/rational standpoint. It was in response to these discrepancies between consumers’ perceived risks and expert analyses of objective risk that researchers adopted a new paradigm by which to understand risk. This new *normative/value* model defined risk as a perceptual construct that can only be evaluated by consumers based on their *subjective* evaluations. In keeping with this focus on *perceived* risk, contemporary studies of technological risk have adopted many of the same psychometric techniques commonly used today to assess consumer perceptions of sensory and image attributes of foods.

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This chapter remains with the U.S. Government.

In a comprehensive study of risk perceptions among consumers (Slovic et al., 1985), a factor analytical approach was used to assess the perceived risk associated with a wide range of everyday hazards, including many “technological” hazards. Figure 32.1 is a representation of the resulting factor structure (Slovic, 1987). The two factors that underlie this space, “dread” risk and “unknown” risk, are defined by a number of characteristics, as shown below the factor space. Several of these characteristics have subsequently been shown to have high predictive validity in determining the perceived risk associated with technological hazards, and it is worthwhile to explore these characteristics more closely in order to better understand their relationship to risk perceptions of foods processed by nonthermal and other emerging technologies.

1.1. Control vs. Lack of Control

In looking at Factor 1, an important characteristic underlying consumer perception of dread risk is the degree to which the risk can be controlled. This is reflected in the fact that on the left side of the factor space are hazards that are primarily under the control of the individual. These potential hazards include the consumption of caffeine or aspirin, the use of electric appliances, the use of trampolines, and the consumption of medicinal drugs. On the right side of the space are hazards that are not under the individual’s control, that is, nuclear weapons, nerve gas accidents, satellite crashes, etc. There is a much greater perception of dread risk if one does not feel “in control” of the hazard, in the same way that people feel more at risk when they are in the passenger seat of a vehicle than when they themselves are driving, even though the actual risks may be the same. This element of “control” is an important factor in the perception of risks associated with foods processed by novel technologies, because the procedures involved in their processing are beyond the control of the consumer. This stands in contrast to what may well be considered more dangerous hazards, for example, bacterial contamination of food that may result from consumers processing foods themselves, for example, cooking, canning, etc. Although, in actuality, the latter may be

a greater risk, it is not perceived as being as risky by the consumer, because the risk is directly under the consumer’s personal control.

1.2. Voluntary vs. Involuntary Risk

Another important factor in dread risk perception is the voluntary versus involuntary nature of the risk. Vaccines provide a good example of this. In Figure 32.1, vaccines fall to the left on Factor 1, indicating that they are perceived as voluntary hazards and of relatively low risk. However, as evidenced by recent reactions of military recruits to the decision by the military to vaccinate troops against possible anthrax exposure, making vaccines involuntary can dramatically change the perceived risk associated with them. In the food arena, high levels of fat or sugar in prepackaged or restaurant food can incite great emotional response and concern among consumers, even though these same consumers may voluntarily add the same or even more fat or sugar to foods they prepare themselves at home.

1.3. Fatal vs. Nonfatal Hazards

As we proceed from left to right on Factor 1, we also see hazards increasing in their likelihood of producing fatal consequences. This characteristic of dread risk perception is highly relevant to novel food processing techniques. For example, while insect infestation of food is seen as an obvious risk to food safety by most consumers, it is generally perceived as a nonfatal risk. However, certain technologies that can be used to prevent insect infestation, for example, pesticides or genetic modification, are associated with potentially fatal consequences in the mind of the consumer.

1.4. Observable vs. Unobservable Risks

If we move to Factor 2, “unknown” risk, we now see a different set of characteristics differentiating the hazards in Figure 32.1. Moving from the bottom to the top of the space, we see a difference in how observable or unobservable are the hazards and/or their consequences. Handguns, automobile and motorcycle accidents, explosions, and aerial construction are

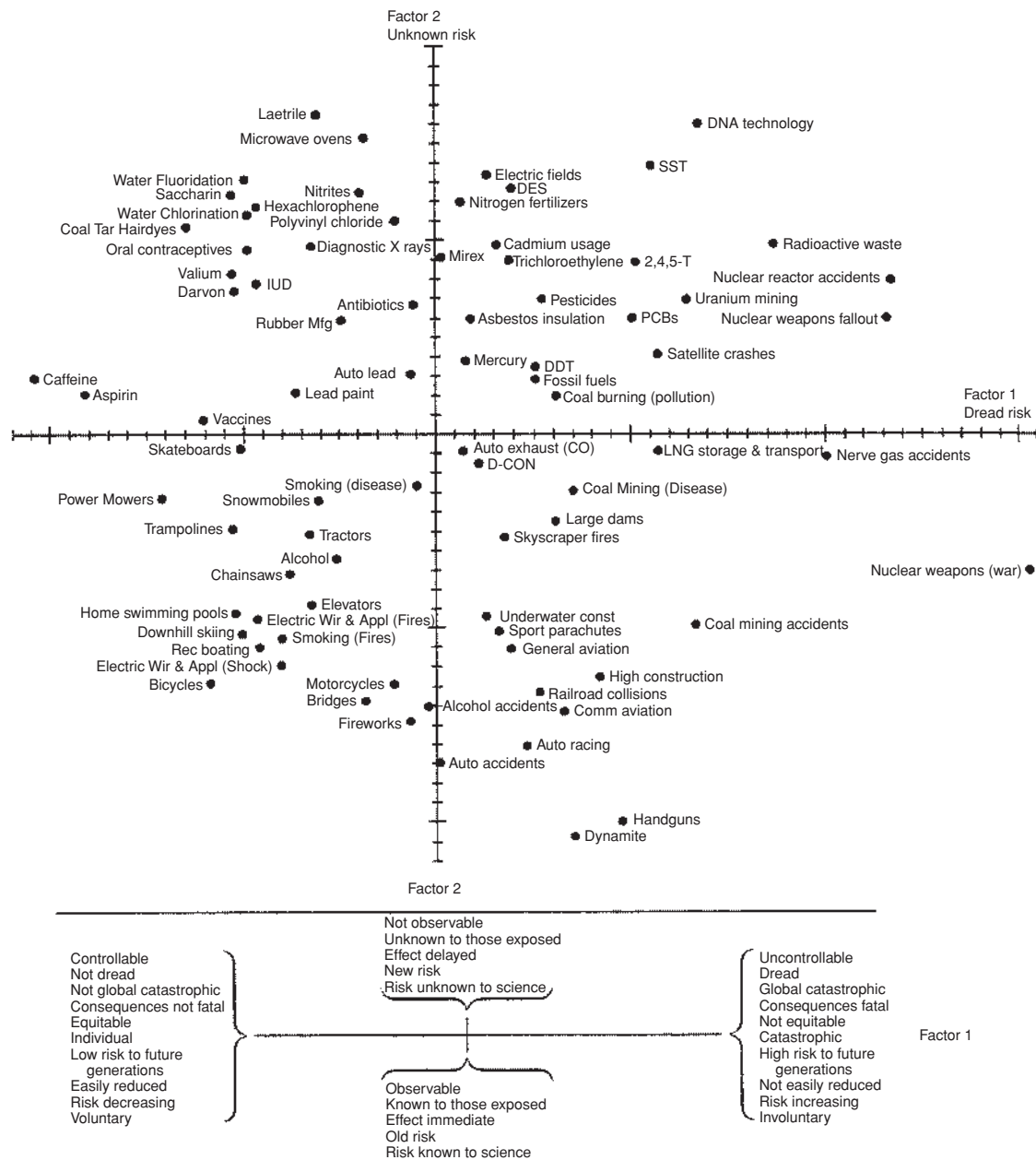


Figure 32.1. Factor analytic space for 81 hazards. Factors 1 and 2 are composites of 18 different risk characteristics. (From Slovic, 1987.)

all highly visible hazards. These stand in contrast to the much less observable hazards of DNA technology, electric fields, water fluoridation, and microwaves. To the consumer, the less observable is the hazard, the greater is its perceived risk. This risk perception factor can also be seen in food safety issues, where mold on food is highly visible, yet it is a low-risk safety issue, whereas preservatives designed to inhibit mold growth are unobservable and, thus, are viewed as more insidious.

1.5. Known vs. Unknown Risks

As you move from the bottom to the top of Factor 2, there is also a divergence in the degree to which the risk is known to science. The hazards of handguns, fireworks, and alcohol consumption are fairly well understood. However, the hazards associated with DNA technologies, electric fields, and antibiotics are far from well understood, even by scientists. In the case of foods, the obvious and known risk of insect infestation can be contrasted with the unknown risks of biotechnology used to engineer pest-resistant plants and produce.

1.6. Immediate vs. Delayed Risks

The last characteristic of perceived risk to consider is how immediate or delayed the effects of the hazard may be. Although the effects of handguns and acci-

dents are immediate, the effects of chemical exposure, radioactive waste, x-rays, and electrical field exposure can be delayed in their appearance for years, decades, or longer. The same contrast between immediate and delayed effects can be seen in the relatively immediate effects of microbial contamination of ingested foods versus the delayed effects of perceived hazards associated with irradiation, PEF, or DNA technologies applied to the same foods for purposes of reducing microbial contamination.

The hazard/risk characteristics reflected in the factor structure of Figure 32.1 exemplify the kinds of attitudinal and belief variables that must be considered when assessing consumer perceptions of risk associated with foods processed by nonthermal and other emerging technologies. Expert testimony concerning “actual,” “objective,” or “known” risks of these technologies may have little bearing on what the consumer perceives as their risk. As depicted in Figure 32.2 from Powell and Leiss (1997), expert opinions of risk can be characterized as being based on scientific knowledge and the study of statistical probabilities of outcomes for population averages. Experts also focus on what is “acceptable” risk, given ever-changing scientific knowledge and comparisons with risks for other outcomes. Lastly, all disasters or fatalities are treated equally by experts. A death is a death. Public perceptions of risk, on the other hand, are based on intuition about the hazards.

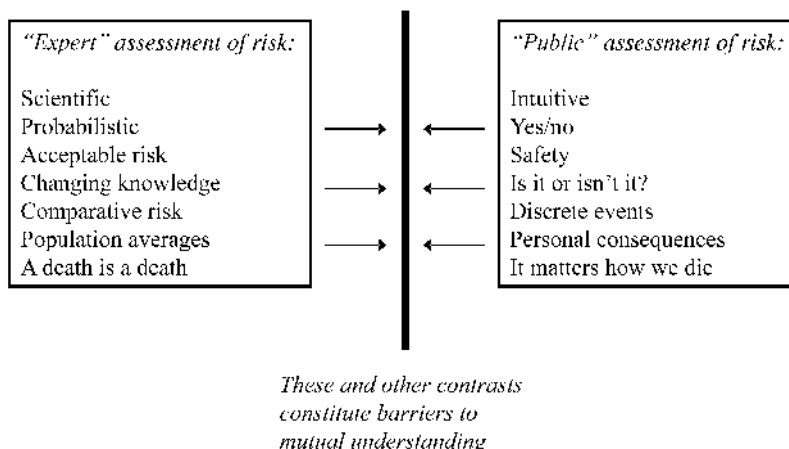


Figure 32.2. Characteristics of expert assessments and public perceptions of risk. (From Powell and Leiss, 1997.)

The perceptions are “all or none,” either the risks are there or they aren’t there, the technology is either safe or it’s not safe, and the level of risk is not as susceptible to changing knowledge. Public perception of risk also tends to focus on specific events and not how these events compare to other outcomes. Lastly, the focus is on the effects as they relate to the individual, and “how” one dies is an important factor in the assessment of risk for any technology.

The difference in risk assessments between scientific experts and the consuming public can be seen as a major barrier to mutual understanding between the two groups. In addition, the degree of trust (or lack thereof) that the public has in scientists and other public spokespersons is a critical element in whether or not consumers attend to the communications about risk and safety that are offered by these sources (Slovic, 1993; Frewer et al., 1996; Eiser et al., 2002). Bridging the gap between the disparate views of experts and consumers regarding risks associated with emerging food technologies is a critical goal of good risk communication practices and a requirement for successful marketing of products produced using nonthermal technologies.

2. Consumer Concerns about Foods Processed by Novel Technologies

Although the mainstream literature on risk perception has identified critical variables important to consumer risk perception for many technological and nontechnological hazards, consumer perceptions of risk for specific food hazards have been left to other investigators. Over the past several years, numerous investigators have utilized the normative/value approach in surveys and studies that have assessed the perceived risks and concerns of consumers toward such food safety issues as irradiated foods, bio-engineered foods, foods containing pesticides, foods processed using laser light sources, and microbially contaminated foods (Bruhn et al., 1987, 1996; Schutz et al., 1989; Bord and Conner, 1990; Moseley, 1990; Sparks and Shepherd, 1991, 1994; Dunlap and Beus, 1992; Wolf, 1992; Frewer et al., 1994, 1997a, 1997b, 1998; Bruhn, 1995; Frewer and Shepherd, 1995; Schutz and Cardello, 1997; Schutz and Wei-

dmann, 1998; Bredahl, 1999; DaCosta et al., 2000; Knox, 2000; Deliza et al., 2003; Zepeda et al., 2003; Grunert, 2005; deJonge et al., 2006; Qin and Brown, 2006; Siegrist, 2006). However, until recently, little comparative data have been collected on consumer concerns for a wide range of novel or emerging food-processing techniques.

As part of a broad research program at U.S. Army Natick RDRE Center to foster the development of a variety of novel, nonthermal processing techniques, data have been collected recently on the concerns of military and civilian consumers toward a wide variety of thermal and nonthermal technologies. In one such study (Cardello, 2007), the consumer research technique of conjoint analysis was used to uncover the factors important to the use of foods processed by various novel and emerging technologies. In this study, three groups of consumers (two civilian and one military sample) completed questionnaires in which they evaluated 49 different food product concepts. Each product concept comprised elements from each of seven different factors. The seven factors were product type, method of food processing, cost, risks, benefits, information type, and the source of product endorsement. Table 32.1 shows the factors and the elements in each factor used in the study.

The food processes were selected to represent a wide range of likely concern levels, ranging from traditional processes of low concern, for example, heat pasteurization, to novel processes that often evoke high concern among consumers, for example, genetic modification and irradiation. Some processing names were selected as alternative labels that could evoke different levels of concern for the same process, for example, ionizing energy versus irradiation, while others were generic process names that could be used to represent a class of processes, for example, cold preservation. Benefits were selected to cover sensory, microbiological, freshness, nutrition, and reduced processing benefits. Endorsing agencies/individuals were selected to represent a range of sources, including various agencies in the US Government, university researchers, international health organizations, consumer groups, and manufacturers/processors. Risks focused on environmental, health, energy, and safety issues, as well as the risk

Table 32.1. Factors and factor levels used in the conjoint study

Processed/Treated By	Product Type	Endorsed By
Irradiation	A meat item	The Food & Drug Administration
Pulsed electric fields	A milk/dairy product	The US Department of Agriculture
High pressure	A bakery item	University researchers
Heat pasteurization	A vegetable	The Surgeon General of the United States
Ionizing energy	A fruit juice	Green Peace
Genetic modification	A sauce/gravy	The World Health Organization
Cold preservation		The manufacturer/processor
<i>Benefits</i>	<i>Cost</i>	<i>Risks</i>
Results in fresher product	Is less expensive	Has an unknown health risk
Is minimally processed	No difference in cost	Is untested
Has lower bacterial risk	Is more expensive	Uses more energy
Is better tasting		Reduces worker safety
Has fewer preservatives	Is more nutritious	May produce harmful by products
<i>Format/Type of Product and Processing Information</i>		
The processing information is printed on the package label		
Product samples are available for tasting in the store		
Processing information is available in pamphlets at the store		
Processing information is available on a website		
Processing information is posted on the store shelf		

of being untested. Product and cost factors were chosen to represent a range in each category.

Consumers rated each product concept for their interest in using it on a 5-point rating scale (1 = not at all interested, 5 = extremely interested).

Figure 32.3 shows an example rating page from the questionnaire.

The data from the study were analyzed by calculating the part-worths or “utility values” for each level of each factor. These utility values indicate the

How interested would you be in using a food product that has the following characteristics?

- A milk/dairy product
- Processed by high pressure
- Reduces bacterial risk
- Is less expensive
- Is untested
- Endorsed by the manufacture/processor
- Processing information available in pamphlets at the store

Using the scale below, indicate how interested you would be in using this product by circling one of the phrases.

Extremely interested
 Very interested
 Moderately interested
 Slightly interested
 Not at all interested

Figure 32.3. Example concept rating page from a conjoint analytic survey on consumer attitudes toward novel food technologies. (From Cardello, 2007)

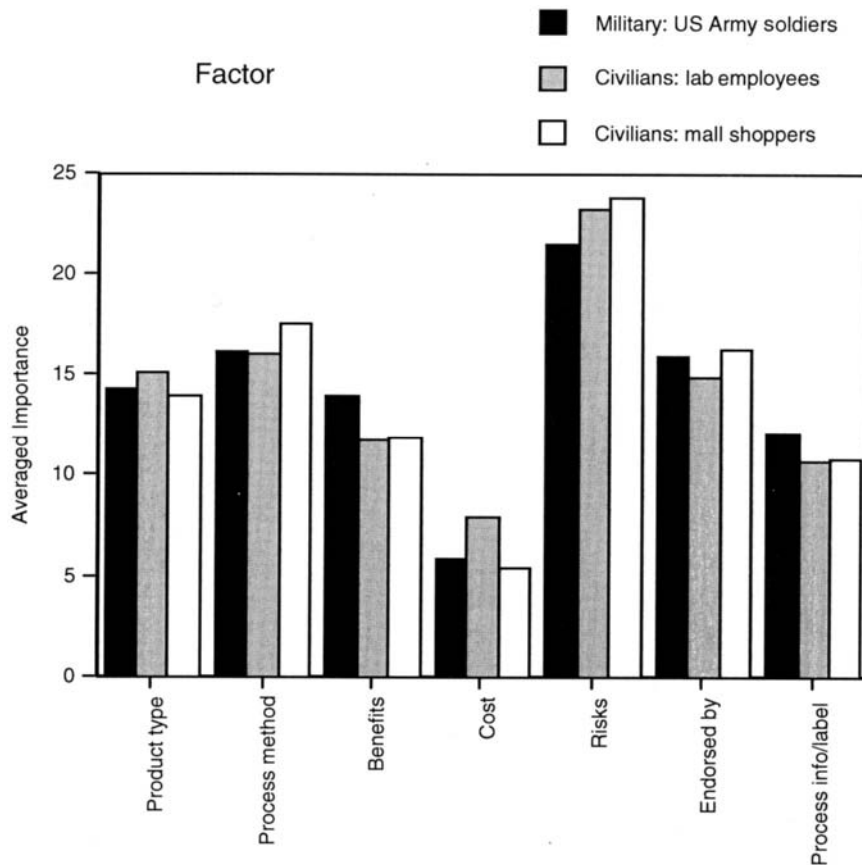


Figure 32.4. Averaged importance values for the seven factors used in the study by Cardello (2007). Data for civilians are in gray (laboratory employees) and white (mall shoppers); data for military are in black.

influence of each factor element on the respondent's ratings. An "importance" value was also calculated for each factor, which reflects the relative range of utility values for the elements within each factor. Figure 32.4 shows the importance values for the seven factors by consumer group.

As can be seen, the potential risk associated with the product had the greatest importance for determining all consumers' interest in use of the products. Post-hoc comparisons showed risk to be significantly more important ($p < .05$) than all other factors in its contribution to interest in using the product. This factor was followed, in approximate order and for all respondent groups, by the processing method, the endorsing agency/individual (not significantly less

important than processing method), and the product type (significantly lower than processing method, but not significantly different than endorsing individual/agency). These were followed by product type and the type/nature of product information, both of which were significantly and progressively lower in importance. Cost was the least important factor and was also significantly less important than all other factors. In comparing differences among the different consumer groups, there were significant differences ($p < .05$) for benefits and cost, but the overall pattern of importances was quite similar.

The above data highlight the importance that risk perceptions can play in the mind of the consumer when considering the purchase, use, or consumption

of foods processed by nonthermal or other novel technologies. Concerns about risk far outweighed considerations related to benefits and even costs.

Although the data in Figure 32.4 only address the *relative* importance of risk perception to other factors in the consumer's mind, other studies have focused directly on the concern that consumers have with specific nonthermal and other food processing technologies. For example, in one survey of military troops (modal age = 20–25 years of age, 98% high school graduates/66% some college), they rated their level of concern about eating foods that had been processed by each of 25 different food-processing techniques. Table 32.2 shows the data from this sur-

Table 32.2. Percent of military respondents ($n = 198$) who indicated that they would be “very concerned” or “extremely concerned” about eating foods that had been processed by each of 25 different food processing techniques (from Cardello, 2000)

Food Processing Method	% Extremely or Very Concerned	% Uncertain
Gene modification	54	17
Irradiation	49	17
Ionizing radiation	48	14
Bacteriocin treatment	44	23
Bioengineering	42	14
Radio frequency sterilization	40	21
Pulsed electric field processing	37	23
Shock wave processing	37	27
Magnetic field processing	33	22
High voltage pulse treatment	30	21
Ohmic heating	30	30
Asceptic processing	29	26
Oxygen scavenger treatment	28	27
Electrical resistance treatment	28	27
Pulsed light processing	28	25
Acidification	27	22
Ultrasound treatment	27	22
Microwave sterilization	25	12
Modified atmosphere processing	24	24
High-pressure treatment	20	18
Microwave processing	18	12
Thermal processing	18	14
Heat pasteurization	13	6
Cold preservation	12	12
Freeze drying	11	7

vey. As can be seen, concern level, as reflected in the percentage of respondents who stated that they were “very” or “extremely” concerned about the use of that technology in the processing of their food, ranged from very low levels (freeze-drying, thermal processing, heat pasteurization, and cold preservation) to very high levels (gene modification, irradiation, and ionizing energy). Certain nonthermal (pulsed electric fields) and thermal (radio-frequency sterilization) techniques also produced relatively high levels of concern, while others (high pressure and ultrasound) produced relatively less concern.

In a more recent study of civilian consumers working in nonfood-related occupations, concern levels for a similar spectrum of food technologies were obtained (Cardello, 2003). Figure 32.5 shows these concern levels and ratings of uncertainty for the 20 food processing technologies that were examined. Again, heat pasteurization, cold preservation, and thermal energy elicited very low levels of concern, whereas genetic engineering, irradiation, the addition of bacteriocins, and pulsed x-rays elicited high levels of concern. Pulsed electric field processing elicited greater concern than hydrostatic pressure and was similar in concern level to ultraviolet light, modified atmospheres, and microwave radiation. In addition, of special concern because of its implications for the purchase of foods processed by nonthermal technologies, there was a large gender difference. Females, the primary food purchasers for families, showed significantly greater concern for all the technologies, than males, a fact that supports previous research on food risk perceptions (Terry and Tabor 1988; Malone, 1990). On the bright side, consumers who were categorized as “technological risk takers” had significantly less concern for all the technologies.

What should be evident from the data in Table 32.2 and Figure 32.5 is that consumer concern levels elicited by different technologies are relatively independent of the technological risk typically associated with these technologies by experts. Rather, consumer perceptions of risk and their concern with these technologies is based on attitudes, beliefs, and stereotypes formed by information (correct or incorrect) gleaned by them from television, print

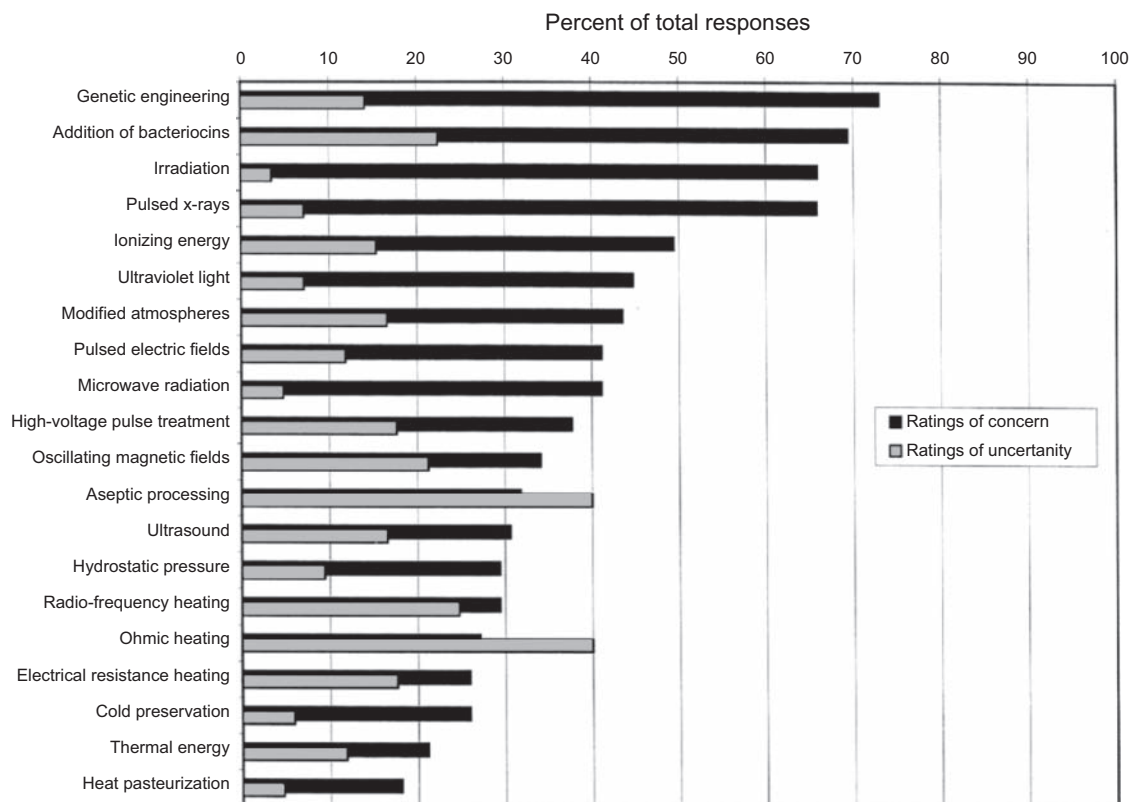


Figure 32.5. Concern levels and ratings of uncertainty for 20 food technologies. (From Cardello, 2003.)

media, personal experience, learning, or hearsay. In many cases, the consumers know relatively little about the technologies. Rather, their concern is associated with their understanding and attitudes toward the actual words used to describe the technology. Thus, in Figure 32.5, the term *irradiation* elicited much greater concern than the term *ionizing energy*, even though both terms refer to the same technology.

Regardless of their origins, consumer concerns about food processing technologies can pose a serious threat to the successful marketing of foods processed by nonthermal and other emerging technologies. Important questions that must be answered are “how do these concern levels influence what the consumer expects of the product?” and “how do these concern-induced expectations influence liking and purchase behavior?”

3. Role of Information and Consumer Expectations on Food Acceptance

As noted previously, information can have a profound effect on consumer liking/disliking of a product, independently of its actual sensory quality. These influences of information are sometimes referred to as “framing” effects, because they are sensitive to the specific context and wording of the information (Kahneman and Tversky, 1974; Tversky and Kahneman, 1984). They are commonly interpreted as operating through cognitive biases that influence the formation of “expectations” about the product (Cardello and Sawyer, 1992; Cardello, 1994, 1995, 2007; Deliza and MacFie, 1996; Schifferstein, 2001). These expectations are either confirmed or disconfirmed by the product, with repercussions for product perceptions, liking, and/or satisfaction.

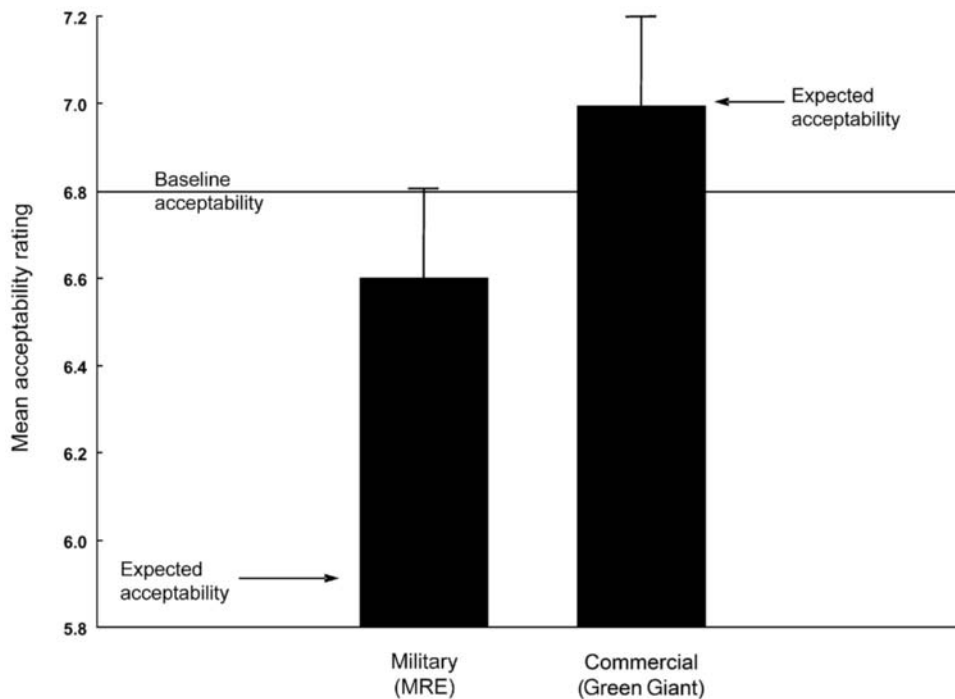


Figure 32.6. Mean baseline acceptability ratings (horizontal line), expected acceptability ratings (arrows), and actual acceptability ratings for identical samples of corn labeled as either a military product or as a commercial brand product. (From Cardello et al., 1996.)

Figure 32.6 is data from a typical study examining the role of information variables and consumer expectations on food product acceptance (Cardello et al., 1996). In this study, the test product was a commercially processed canned corn that was presented under two different informational conditions that were known from a previous study to evoke different quality expectations for the product.

Consumers ($n = 40$) first evaluated the corn in a blind taste test one month prior to the main test. These baseline acceptability ratings are shown by the horizontal line in Figure 32.6. The same consumers returned 6 weeks after the baseline test for two more test sessions, spaced 4 weeks apart. In one session, the consumers were presented with written and visual information, indicating that the canned corn that they were about to taste was a military ration item (MRE). In the other session, they were told that it was a leading commercial

brand of corn (Green Giant brand). After being presented the information, but before tasting the sample, consumers rated how much they expected to like or dislike the corn using a 9-point scale. These mean expected liking/disliking ratings are shown by the arrows in Figure 32.6. The consumers then tasted the corn and rated their liking of it on the same 9-point scale. As can be seen in Figure 32.6, when the consumers were told that the product they were about to taste was a “military” product, they rated its expected acceptability to be significantly lower ($p < 0.01$) than when they thought the product was going to be a commercial brand. More importantly, upon tasting the corn, the consumers rated their actual liking of the corn significantly lower ($p < 0.05$) when they thought it was a military product than when they believed it to be from a commercial source, even though the corn was identical in both conditions.

Of special interest in the data of Figure 32.6 is the fact that the liking ratings of the corn after tasting changed *in the direction of the expected liking*. Although there are a number of different models that predict the effects of disconfirmed expectations on perceived product performance (see Cardello, 1994, 2007, Deliza and MacFie, 1996; Schifferstein, 2001 for reviews), the bulk of empirical research on the role of disconfirmed expectations on product performance has supported the fact that product evaluations change to become more similar to consumers' expectations for the product (Olshavsky and Miller, 1972; Anderson, 1973; Oliver, 1977; Bearden and Teel, 1983). This "assimilation" model was first proposed by Sherif and Hovland (1961), but until the past decade, with rare exceptions (e.g., Olson and Dover, 1976), few studies examined these effects using foods as test products. However, during the past decade, support for an assimilation model of food expectations has been found in a variety of studies (Cardello and Sawyer, 1992; Tuorila et al., 1994; Cardello et al., 1996; Deliza, 1996; Lange et al., 1999, 2000; Schifferstein et al., 1999; Siret and Issanchou, 2000; Caporale and Monteleone, 2001, 2004; Hurling and Shepherd, 2003; DiMonaco et al., 2004; Caporale et al., 2006; Iaccarino et al., 2006; Stefani et al., 2006).

In one early study of this nature on cola beverages (Cardello and Sawyer, 1992), consumers were led to expect that they would receive one of several different cola beverages. For different consumers, the expected cola ranged from their favorite brand to their least favorite brand (as previously determined in blind taste tests). However, in some cases, subjects were given the cola that they expected, while in other cases they were given either a *more* preferred or *less* preferred brand. Pretrial ratings of expected liking and posttrial ratings of actual liking were obtained from all subjects. When the change in product liking from the baseline level was examined as a function of whether the subjects expected a better or worse product than the one they received, there was a strong linear association ($r^2 = 0.66$) in the data, showing that posttest liking moved in the direction of the expectations, as predicted by the assimilation model.

Figure 32.7 shows similar data from a study on yogurts (Schifferstein et al., 1999). Here, consumers were given correct or incorrect information about yogurt using either verbal descriptions or package labels. The information and labels resulted in either positive or negative expectations of the yogurt. The data in Figure 32.7 show the change in the perceived quality and in consumer intentions to purchase the yogurt as a function of the degree to which these expectations were disconfirmed. Expectations that exceeded product performance produced a positive change in quality ratings and in purchase intentions. This assimilation effect occurred regardless of whether the expectations were established through written descriptions or through packaging.

3.1. Implications of the Assimilation Model

The implications of the assimilation model of consumer expectations for foods processed by nonthermal and other emerging technologies are two-fold. First, on the negative side, if consumer expectations of these foods are poor, regardless of the reason, the assimilation model predicts that actual liking of these products will suffer. This effect will be independent of the intrinsic sensory quality of the item. Given the high levels of concern about consuming foods processed by some of these novel technologies (e.g., Table 32.1 and Figure 32.3), it is of some importance to determine how these concerns translate into expected liking or disliking for the product. However, on the positive side, if product expectations can be raised by communications or marketing strategies that overcome negative risk perceptions or other concerns associated with the product, the assimilation model predicts that liking should rebound.

4. Concerns, Expectations, and Liking for Foods Processed by Emerging Technologies

In a study conducted by Cardello (2000), the effect of consumer concerns and expectations on liking of the taste of foods processed by different food-processing techniques was examined. Eighty-eight volunteer consumers were requested to taste and evaluate

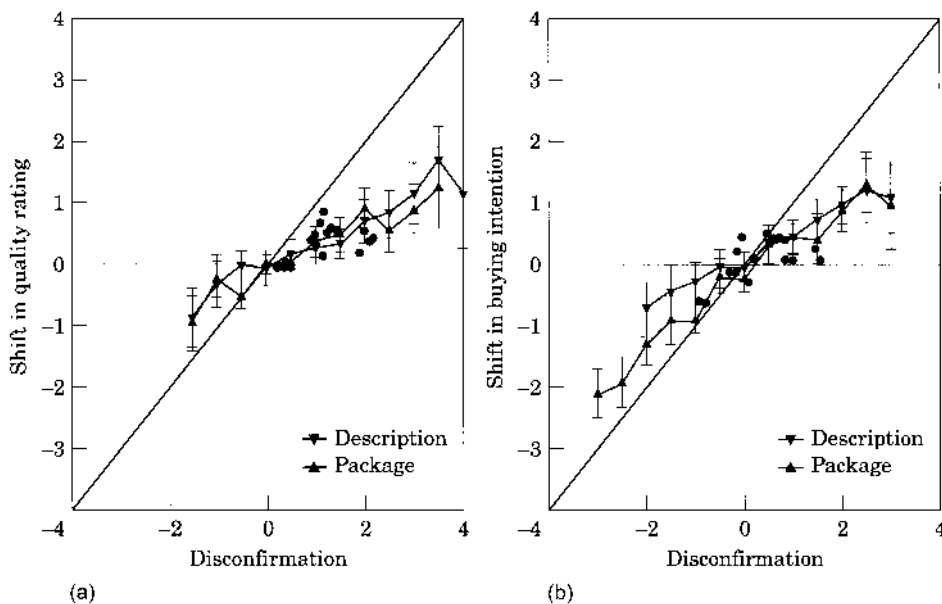


Figure 32.7. Change in (a) quality ratings and (b) buying intention from baseline levels as a function of the degree of disconfirmation between expected and “actual” (baseline) ratings of yogurt following the presentation of product information by either verbal description or package labels. (From Schifferstein et al., 1999.)

various chocolate pudding products, all of which had been thermally processed. The subjects participated in one of three different information conditions. For all three conditions, subjects first participated in a survey session (no samples presented), in which they rated their liking of chocolate pudding and their level of concern for several different food-processing technologies (irradiation, high voltage pulses, hydrostatic pressure, pulsed electric fields, nonthermal preservation, and the addition of bacteriocins). In a second session conducted several weeks later, consumers participated in a taste test in which they were provided information about the chocolate puddings that they were to taste. In one condition, consumers were told that each of the various chocolate puddings that they would taste had been processed by a different food-processing technique. For each of six identical pudding samples, consumers were told that the product they were about to taste had been processed by one of the six different food processing technologies listed above, for example, “this sample was processed by pulsed electric fields.” No other informa-

tion than the name of the technology was provided. In a second condition with different consumers, the information about the name of the technology was accompanied by an objective description of that technology, for example, “in irradiation processing, foods are exposed to a source of ionizing radiation, for example, cobalt 60, for short periods of time.” In a third information condition, consumers were told the name of the technology, given the description of it (as in the second condition), and they were also provided a safety benefit statement, for example, “this process is entirely safe and avoids the thermal damage done to foods by heat pasteurization.” After exposure to the information manipulation, consumers rated their expected liking of the product (prior to receiving the sample). They were then presented the sample, allowed only to look at it, and asked to rate their expected liking again. Lastly, subjects tasted the sample and rated it for overall liking.

Correlations of “concern” ratings for the different food processing technologies with “expectations of liking” for the chocolate pudding that the consumers

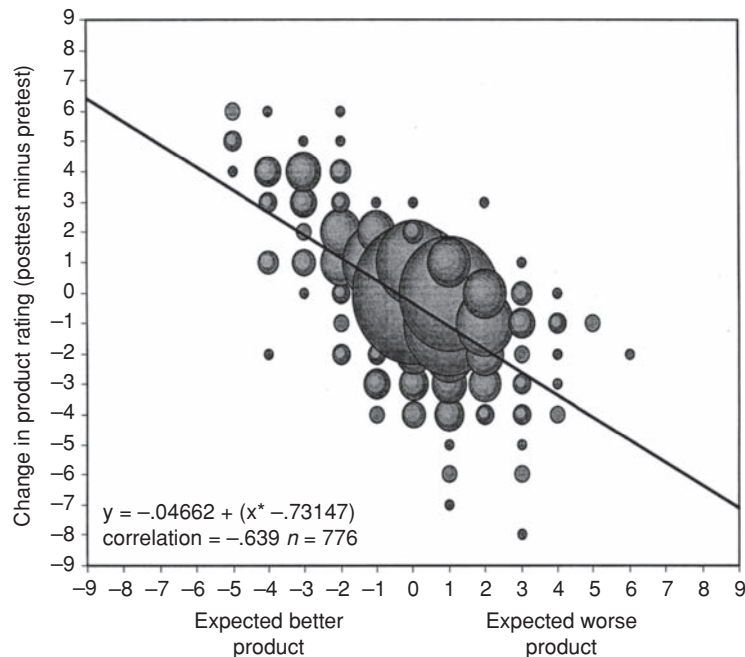


Figure 32.8. Linear regression plot of the change in liking ratings (from baseline) for chocolate pudding as a function of the level and direction of disconfirmation between expected and actual product liking. The sizes of the circles represent the relative number of data points falling at that location. (From Cardello, 2000.)

believed had been processed by that technology were all negative and statistically significant (r 's = $-.45$, $-.31$, and $-.40$) in all three conditions, supporting the hypothesis that as consumers' concerns about the risk of a technology *increase*, expected liking of food products processed by that technology *decrease*. Still more significantly from the perspective of marketing foods processed by these technologies are the data shown in Figure 32.8. Here, the change in product liking from the baseline (blind pretest condition) to the postexpectation trial condition is plotted as a function of whether the consumers expected a better or worse product. As can be seen, the more that consumers expected a better product, the more positive the change in liking, and the more that they expected a worse product, the more negative the change in liking. Overall, the change in liking ratings as a function of expectations was linear with a correlation of $r = -.64$.

On the positive side, the above study also demonstrated that factual product and process informa-

tion, product exposure (merely seeing the product), and a simple safety/benefit statement all had positive effects on expected liking and acceptance of the tasted product. These latter data confirm the fact that good communication practices and effective marketing strategies can be utilized to overcome negative images created by risk perceptions and to significantly improve the probability of market success for foods processed by emerging technologies.

5. Recommendations for Minimizing Consumer Risk Perceptions

There are several recommendations that can be made to product developers and other parties interested in fostering the successful introduction into the marketplace of foods processed by nonthermal and emerging technologies. These include

1. Paying greater attention to the risk perceptions of consumers than the risk assessments of "experts"

when deciding whether a potential market problem exists. As we know from the experience of bringing food irradiation to the marketplace, “risk is in the eye of the beholder.”

2. Being cognizant of different consumer segments in the market and how risk perceptions and product concerns may vary according to market segment (gender, ethnicity, risk-taking, etc.)
3. Testing the potential impact on risk perceptions, product expectations, and liking of different technology names (e.g., irradiation versus ionizing energy), alternative product and process information, and product logos, labels, and brand names.
4. Ensuring that any product that is being planned for market launch has optimal sensory quality (see the section that follows). Nothing will better serve to validate poor expectations about a product than a poor product.
5. Employing formal consumer communication and marketing strategies that take into account recommendations 1–4 above, in order to effectively overcome negative product stereotypes, misconceptions about process risk, and unchallenged beliefs about the product. This is the critical element in closing the gap between *public perceptions* of potential risk and *expert assessments* of those risks.

6. Selection Criteria for Successful Product Introductions

Although consumer risk perceptions and concerns must be assessed early in the development process, so too must early consideration be given to the criteria to be used in selecting a product for market introduction. Having worked on military ration programs for many years, scientists at NSRDEC, including the authors of this chapter, often approach food product development on the basis of meeting military consumer needs. Shelf stability is particularly important. Rations should be as good or better than freshly cooked foods throughout their projected shelf life. With nonthermal and other emerging technologies, NSRDEC researchers have forged military, industrial, and academic partnerships and have continued to develop new research strategies for nonthermal

and emerging technologies to facilitate identification of civilian as well as military markets for the resulting products (Dunne et al., 1997). The operant phrase now is “dual use,” that is every effort is made to avoid developing lines of rations specific only to military operations.

The following guidelines apply to the selection of products for development and potential market introduction of foods processed by nonthermal and emerging processes. First, products should be high preference items in their general food category. Food preference survey data and/or an established consumer market research should support these selections. Selection based on the developer’s specific process capabilities and/or on novelty is risky. One example of such faulty product selection occurred with two pudding-type desserts developed under one of the our PEF contracts. The two products, “chocolate raspberry” and “piña colada” pudding were made from “natural” ingredients and were considered to be of high “technical” quality. Although consumer testing revealed that they were, on average, disliked, this was not due to any aspect of their PEF processing. Rather, the low acceptance was simply due to a low preference for the flavors, independent of how they were processed. This example reinforces a fundamental principle: obtain general consumer acceptability guidance on a product early in the development process as a “reality check” before extensive resources are committed. A product is “only as good as the consumer thinks it is.” A broader principle from the earlier discussion on consumer perception should be remembered: introduction of a product manufactured using novel or nontraditional methods may create positive or negative associations that can significantly influence consumer adoption of that product.

The second criterion for product selection concerns the capabilities and restrictions of the novel process. For example, PEF is capable of processing foods with particle sizes ≤ 3 mm. Although processing cells are presently being designed to handle larger particles, this constraint will continue to apply into the foreseeable future. This means that the process will only be feasible with liquid foods and other foods that are fluid when pumped. HPP, on the other hand,

has different restrictions. Most HPP vessels are quite expensive, thus many are batch systems with relatively small capacities (e.g., 35–215 L). The diameter and depth of the vessel will determine what products fit and the cost of handling, filling, and repeating a process. Furthermore, products containing entrapped air or insufficient moisture may be crushed or may experience an inadequate microbial reduction. The third principle is to choose products for which delivery of fresh-like or cooked-to-order quality has the potential to produce a measurable consumer advantage. Consumers have learned to accept a level of cooked or “heat struck” flavor in such products as orange juice and salsa. The question that needs to be addressed is whether consumers would consider the presence of fresh-like characteristics and/or the absence of this characteristic more acceptable.

7. Sensory Testing of Foods Processed by Nonthermal and Other Emerging Technologies

Any food used in sensory evaluation should be safe for consumption. The testing organization has an obligation to assure that adequate testing, processing, preparation, and handling precedes all consumer testing. In the United States, the USDA and FDA support some limited uses of HPP processes for certain high acid and meat products. However, emerging technologies are just that, “emerging,” thus consideration needs to be given to compliance to current food safety regulations and policies. Some foods are of greater concern than others. For example, the FDA currently treats acid/acidified foods (equilibrium pH or 4.6 or below) more liberally than non-acidified foods. Sensory testing of acid/acidified foods using PEF and HPP processing may have different processing standards than non-acidified food. Additionally, data concerning the effects of these emerging processes on pathogens is constantly changing. It is necessary for those presenting foods for sensory analysis to be proficient in assuring that these foods meet all current regulatory criteria and are safe for consumption. Appropriate microbial safety should be confirmed through testing *prior to* sensory analysis of any products processed by an uncertified process.

Approaches to sensory analysis of products processed by novel technologies are the same as those produced by conventional technologies. Sensory methodology will not be discussed in depth, since details of principles and practices can be found in several textbooks (Gacula, 1997; Lawless and Heymann, 1998; Meilgaard et al., 2006). However, two types of sensory panels are generally appropriate and will be described in greater detail. Sensory panels are valuable tools in guiding product development. Sensory data also serve to corroborate chemical and physical measurements and are invaluable for estimating shelf life of an optimized product.

To begin, “benchmark” products should be used in sensory testing, although new products may not have a suitable “benchmark” counterpart. Benchmarks may be similar products from the consumer market and/or the unprocessed item as prepared prior to the processing treatment. The use of a benchmark or “control” sample enables the developer to determine how close or distant the treated product is to a pre determined or established product, as well as the effect of the process on the product. When premarket consumer research or the existence of an established consumer market suggests that a product has potential for success, development may proceed without a benchmark. When benchmarks are used, the criterion for proceeding with development may be that the experimental product is not statistically different in acceptability and/or the sensory qualities that characterize it. When benchmarks are not used, other measures should be obtained to assure the product is one that the target consumers will purchase. From a consumer viewpoint, the product should be well liked, although no standard minimum degree of liking on a like–dislike scale is appropriate across all foods or commodity classes.

Once a product has been developed that meets or exceeds the benchmark, shelf life trials may be conducted. The US military requires that field rations be shelf stable for at least 3 years at 26.6°C and 6 months at 38°C. This requirement is considerably more stringent than for equivalent products in commercial distribution channels. The microbiological condition of PEF, HHP, or any other unproven process is a major concern. Thus, only limited storage

studies of these products have been conducted at Natick. For example, products previously processed by a proven technology have been further processed with the unproven process to assess the added effect of the new processing. In this way, the effects of processing can be examined without posing undue risks to consumers and sensory panelists. Other studies have utilized (1) microbiological testing of products immediately prior to consumer testing, (2) pasteurized products held in refrigeration for only weeks, or (3) products held for years at both 40°F (control) and 80°F (storage treatment). Again, the caveat for continuing sensory assessments is that the products have remained microbiologically safe up until the time of tasting.

7.1. Physical and Environmental Requirements for Sensory Analysis

The first two factors to consider in developing a sensory testing program are the physical conditions and testing environment of the sensory laboratory. The objective is to create a neutral backdrop that will facilitate panelists' ability to focus their attention on the samples they will be assessing. The sensory laboratory is a deliberate effort to minimize known and unknown environmental variables and their effects. Field tests, that is under the "natural" environmental conditions existing during eating or dining occasions, are *not* the intent of laboratory evaluations, although they are an important class of subsequent tests.

7.1.1. Physical Conditions Any laboratory to be used for sensory testing should be located away from noisy, high traffic areas. It should be centrally located and as close as possible to the source of panelists, who in many organizations are its employees. The sensory panel room area should be odor neutral (free from food and nonfood odors), and it should have the capability to be controlled in terms of air supply, temperature, sound, lighting, and any other factors that may influence panelists' ability to make objective evaluations. Optimal controls should include a slight positive pressure in the panel area to prevent sample preparation odors from entering the panel space, neutral colors on all panel testing area sur-

faces, and wall, floor, and counter surfaces that are functional and easy to clean. Lighting should ideally be adjustable and/or equivalent to the north sky on a uniformly overcast day, since appearance attributes can be an important part of a product assessment.

7.1.2. Environment Effort should be made, both in laboratory layout and on the part of its personnel, to maintain a quiet environment, particularly when panels are in session. For individual assessments, communication among panelists is discouraged by partitioning counters with visual barriers (panels). A sound and sight obscuring wall should separate the laboratory's preparation area from the panel room. Typically a lift door or quarter-round port is used to pass samples from the preparation area to panelists. The sensory testing area should be located so as to separate incoming and waiting panelists from those performing evaluations. When descriptive panels are being used, a room with a "round table" is needed to facilitate group interaction, usually after individual assessment of the samples. A receptionist may be employed to "direct traffic" and provide instructions to panelists. A break area with snacks and beverages for panelist reward may be used, but its location and operation should not interfere with panelists at work.

7.2. Sensory Methods

Although a variety of sensory methodologies might be used in evaluating products processed by emerging technologies, two types of sensory panels are commonly employed: descriptive/analytical and consumer.

7.2.1. Descriptive/Analytical The purpose of this panel type is to develop a group of trained individuals who can objectively guide product development and identify changes that occur in stored or treated products. Important to the effectiveness of this type of panel is that its participants possess the following traits: high interest level, normal sensory acuity, and good verbal ability. A pool of 12–15 highly trained individuals is common, and the panel size on any test can range from 10 to 15 based on availability. Descriptive/analytic panels can be conducted

in “round-table” fashion, without the need for visual partitioning. A panel leader provides additional training in flavor and texture attributes specific to the test product(s) and process(es). This training consists of (1) examining products covering the range of experimental variation expected in the food, including benchmarks; (2) locating and demonstrating ingredients, flavors, or other food materials that exemplify attributes expected to be found in the test samples; and (3) in collaboration with the panel, developing a lexicon or vocabulary of appearance, flavor, and texture terms that may be measured in the product(s).

The panel leader then assigns scales, generally to estimate intensity, to the selected attributes. The scaling method may vary, but often includes verbally anchored category scales, linear graphic rating scales, labeled magnitude scales, or magnitude estimation (see Sidel and Stone, 2004). One or more practice sessions are then conducted with products representing the range of expected variation. Ratings are reported out in the group setting to determine the extent of variability among panelists. Extreme differences among panelists are addressed through additional discussion. Once variability is minimized, subsequent sessions can be conducted without the need for further group interaction. Standard parametric and nonparametric statistics may be used to interpret differences among product treatments or upon completion of a time–temperature storage program.

7.2.2. Consumer panels These panels are distinguished from descriptive/analytical panels as follows: (1) ratings are subjective and panel consistency is not required (there is no right, wrong, or best answer); (2) panelists are untrained but may be experienced in rendering consumer-type judgments—the only requirements to serve on these panels are people’s willingness to participate, that they be target consumers of the product being evaluated, and that they are free of acute or chronic ailments that would affect their sensory abilities; (3) because of the greater expected variability in responses, recommended numbers of panelists are larger, from 36+ and often 100 is a preferred number, although this is difficult to obtain with employee panels; (4) panelists react to stimuli in terms of liking or disliking—it

is not intended that they be able to describe what they are perceiving, although they may be asked to comment on samples to help explain what characteristics of the product produced the ratings obtained; (5) rating scales, such as a 9-point hedonic scale (Peryam and Pilgrim, 1957) or LAM scale (Schutz and Cardello, 2001; Cardello and Schutz, 2004) are used to estimate overall degree of liking or disliking. Sometimes, multiple hedonic scales may be used to estimate acceptability of specific sensory product characteristics, for example, appearance, flavor, and texture. Invariably, when correlational analyses are run, acceptability of flavor is the closest correlate to overall acceptability.

As with descriptive data, collected acceptability ratings are amenable to various parametric and nonparametric statistical treatments (Dijksterhuis, 1997; Lea et al., 1997). In a storage study, testing is typically continued to the next withdrawal if (1) bacterial and yeast/mold counts are in the same range as initially, and (2) changes in sensory descriptive and/or acceptability ratings are not significantly different (decreased) from initial ratings or the preceding withdrawal. Conversely, testing is terminated if (1) microorganism counts increase substantially; (2) clear visual evidence of chemical/physical product deterioration is seen, for example, discoloration; (3) there are either significant decreases in salient product attributes and/or significant increases in uncharacteristic (off) odors and flavors; and (4) if consumer data indicates, on average, significant decreases in overall acceptability or dislike for the product that was initially liked.

A final caveat to this discussion of sensory methods is that, while this section has outlined the environmental requirements and methodologies available to the researcher interested in conducting laboratory sensory assessments of foods processed by nonthermal and emerging technologies, other methods/approaches may be equally valid. Variants of these basic approaches are covered in the textbooks referenced. Furthermore, the results of any studies conducted in a laboratory setting should be considered to be *for guidance only*. No test should be considered definitive until the product is tested with a much larger sample of representative potential users of the product under realistic choice and/or purchase

conditions, because, as noted earlier in this chapter, there are numerous other factors that can alter consumers' perceptions of a product. It is only under realistic use conditions that an assessment can be made of the combined effects of intrinsic (sensory) and extrinsic (contextual and attitudinal) factors on product acceptance and consumer choice/purchase behavior.

8. Conclusions

The development and marketing of foods processed by nonthermal and other emerging technologies require due consideration of consumer beliefs, attitudes, and risk perceptions toward the technologies and processes under consideration and toward the foods processed by them. Currently accepted models of consumer risk perception suggest that a wide variety of factors relevant to nonthermal and emerging technologies can contribute to the perception of risks associated with them. The available data indicate that these concerns are real in the consumer population and vary by food process and by the demographic and psychographic traits of different consumer segments. The data also demonstrate that such concerns translate into lower expectations for foods processed by technologies associated with risk and that these expectations are assimilated into consumers liking or disliking for the products when they are tasted.

Successful development and marketing of foods processed by nonthermal and other emerging technologies will require careful premarket consumer research targeted at understanding consumer beliefs, attitudes, and risk perceptions related to the technology. Such research, combined with a judicious choice of products for market introduction that focus on high preference foods that have undergone successful sensory analysis to ensure optimal sensory quality, will maximize the likelihood of successful market introductions. In addition, good consumer communication practices that effectively utilize labels, advertising, and various forms of media communications to change false impressions, communicate product benefits, and allay concerns will ensure a high likelihood of market success and enable the true benefits of nonthermal and other emerging technologies to be realized by the international consumer market.

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Chapter 33

Effects of High-Pressure Processing and Pulsed Electric Fields on Nutritional Quality and Health-Related Compounds of Fruit and Vegetable Products

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1. Introduction

Consumers are increasingly interested in nutrition and in the consumption of fruits and vegetables due to their healthy characteristics, but not ignoring the safety of the food. Research in human nutrition has shown that a well-balanced diet rich in fruits and vegetables promotes good health and may reduce the risk of certain human diseases (heart disease and cancers) (Liu et al., 2000; Michels et al., 2000; Prior and Cao, 2000; Kris-Etherton et al., 2002; Willcox et al., 2003; Hung et al., 2004).

In general, the composition of fruits and vegetables includes not only nutrients essential to life (carbohydrates, proteins, fats, vitamins, etc.), but also other substances that produce health-beneficial effects and can potentially protect against certain degenerative diseases. These compounds are known as phytochemicals or bioactive compounds (carotenoids, phenolic compounds, vitamins A, C, and E, fiber, glucosinolates, organosulfur compounds, sesquiterpenic lactones, etc.), whose biological activity has been studied by means of assays *in vitro* and *ex vivo* and human intervention studies (Eastwood and Morris, 1992; Giovannucci et al., 1995; Ling and Jones, 1995; Knekt et al., 1997;

Kohlmeier and Su, 1997; Duthie et al., 2000; Knekt et al., 2000; Le Marchand et al., 2000; Piironen et al., 2000; Skibola and Smith, 2000; Simon et al., 2001; Lampe and Peterson, 2002; Sánchez-Moreno et al., 2004b, 2004c, 2004d, 2005b, 2006b; Plaza et al., 2006b).

Moreover, the nutritional quality and the healthy properties of plant products have been also studied by many research groups because of the growing consumer interest in functional foods. Functional foods are foods that in addition to nutrients essential to life contain other substances that help to maintain or improve the general state of health and well-being of consumers. On the basis of this definition, plant foods may be considered functional foods. Moreover, many of the ingredients used in the functional food industry are of plant origin (phytosterols, isoflavones, lycopene, etc.) (Haesman et al., 2004).

Food processing is becoming increasingly sophisticated and complex in response to growing demand for high-quality food.

To meet consumer demand for fresh-like product, food processors employ number of strategies that include modifications to existing food-processing techniques and the adoption of new processing technologies such as nonthermal technologies (Barbosa-Cánovas et al., 2005). Therefore, high hydrostatic pressure or high-pressure processing (HPP) and high-intensity pulsed electric fields (PEF) are being developed as nonthermal emerging technologies for the preservation of foods.

Prior studies have demonstrated that HPP and PEF are efficient to destroy microorganisms and to inactivate food enzymes (total or partially) in plant products, at levels equivalent to those achieved by thermal pasteurization without affecting greatly their nutritional and sensory properties (Tauscher, 1998; Indrawati et al., 2003; Plaza et al., 2003a, 2006a, 2006b; Devlieghere et al., 2004; Barbosa-Cánovas et al., 2005; Cano and De Ancos, 2005; Martín-Belloso and Elez-Martínez, 2005a, 2005b; Torres and Velazquez, 2005; Muñoz et al., 2006; Marsellés-Fontanet and Martín-Belloso, 2007). However, very limited studies investigated the nutritional efficiency of the products treated by various nonthermal technologies.

There are currently two major challenges to food technologists interested in nutrition: (1) knowing the actual contribution of each of the nutrients and bioactive compounds in daily nutrition and its role in health effects and (2) accurately assessing the changes occurring in these compounds as a result of processing and storage technologies. The different stages of vegetable processing (peeling, cutting, washing, sanitation) and the nonthermal treatment parameters (intensity of electric field or pressure, time of treatment, etc.) not only can affect safety and sensory quality but also can cause changes in the nutritional quality and in the healthy properties of processed products.

This chapter summarizes many of the studies conducted on the effects of HPP and PEF in nutritional and bioactive compounds from plant foods.

This chapter also includes results obtained in human intervention studies that have been conducted to investigate the health protective role of fruit and vegetables rich in bioactive compounds that have been processed by nonthermal technologies. These studies also include some results in bioavailability of certain bioactive compounds and the effect on biomarkers of oxidative stress and inflammation. The data summarized in this chapter will be useful for the food processors in identifying the appropriate nonthermal processing parameters for processing microbiologically safe plant foods with high nutritional and functional quality.

2. Fruits and Vegetables Composition: Nutrients and Bioactive Compounds

The nutritional quality of fruit and vegetables are composed by the named macronutrients (also called energy-producing nutrients or energy-yielding nutrients) and micronutrients (which are characterized by their essentiality for human health and the low quantities in which they need to be ingested). Energy-producing nutrients include carbohydrates, fats, and proteins, and micronutrients often refer to vitamins (C, E, B), minerals, fatty acids, and essential amino acids.

From the chemical point of view, fruits and vegetables are food very rich in water, low in proteins (between 1 and 4% in vegetables and lower in fruits, except for soy beans), and lipids (lower content than 0.5–0.6%, except for avocados with 13% lipid content) and with different carbohydrate contents, (fruits, 1–8%, including some fruits with more than 10% of available carbohydrates, while vegetables show values between 1 and 6%). Other group of fruit and vegetable compounds with high nutritional interest is fiber. As dietary fiber is considered, the insoluble fiber (mainly cellulose) and soluble one (pectins) and their ratio (insoluble/soluble) ranges depend on the vegetable species and cultivar. Therefore, fruits and vegetables are very important for health due to the presence of bioactive compounds and micronutrient content (Belitz and Grosch, 1997).

2.1. Micronutrients

Micronutrients, so called because they are needed by the body only in small amounts, play a leading role in the production of enzymes, hormones, and other substances, help to regulate growth, activity, development, and the functioning of the immune and reproductive systems.

2.1.1. Vitamins Vitamins must be supplied in adequate amounts via the diet in order to meet requirements. Scientists are interested in determining the optimal levels of intake for these micronutrients in order to achieve maximum health benefit and the best physical and mental performance.

2.1.1.1. Vitamin C L-ascorbic acid (L-AA, vitamin C, ascorbate) is the most effective and least toxic antioxidant. Numerous epidemiological studies have shown a strong correlation between the healthy effects of consuming fruit and vegetables and the vitamin C content of these (Block et al., 2002). Vitamin C may also contribute to the maintenance of a healthy vasculature and to a reduction in atherogenesis through the regulation of collagen synthesis, prostacyclin production, and nitric oxide (Davey et al., 2000; Sánchez-Moreno et al., 2003a, 2003b). A low intake of vitamin C-rich products results in low levels of vitamin C in the blood (0.3 mg/dL); levels between 0.8 and 1.3 mg/dL, which are considered necessary for good health (Simon et al., 2001), may be attained through a daily intake of 90 mg of vitamin C in adults (Taylor et al., 2000).

Virtually, all of the vitamin C in Western diets is derived from fruits and vegetables. The vitamin C content depends on the species, cultivar, climatic conditions, agricultural practices, ripeness, and postharvest handling. In general, fruits tend to be a good source of this vitamin, for example, blackcurrant (200 mg/100 g fw), strawberry (60 mg/100 g fw), and the citrus fruits (30–50 mg/100 g fw). Also vegetables such as broccoli (90 mg/100 g fw), green pepper (134 mg/100 g fw), or spinach (75 mg/100 g fw) are good source of this vitamin (Lee and Kader, 2000).

2.1.1.2. Vitamin E This vitamin E is the generic term for a family of related compounds, known as tocopherols and tocotrienols. Naturally occurring structures include four tocopherols (α -, β -, γ -, and δ -) and four tocotrienols (α -, β -, γ -, and δ -). α -tocopherol is the predominant tocopherol form found naturally in foods, except in vegetable oils and nuts, which may contain high proportions of γ -tocopherol (Bramley et al., 2000).

The richest sources of vitamin E are vegetable oils and the products made from them, followed by bread, bakery products, and nuts. Vegetables and fruits contain little amount of vitamin E (Bramley et al., 2000).

2.1.1.3. Vitamin B₁, B₂, B₃, B₆, Folate Thiamin (vitamin B₁), riboflavin (vitamin B₂), niacin (vita-

min B₃), and pyridoxine (vitamin B₆) are used as coenzymes in all parts of the body. They participate in the metabolism of fats, carbohydrates, and proteins. They are important for the structure and function of the nervous system (IM, 1998; ASNS, 2004; Lukaski, 2004).

Thiamin serves as a cofactor for several enzymes involved in carbohydrate catabolism. Riboflavin is required for oxidative energy production and is found in a variety of foods. Niacin includes nicotinic acid and nicotinamide. Nicotinamide is a precursor of nicotinamide adenine (NAD) nucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP), in which the nicotinamide moiety acts as electron acceptor or hydrogen donor, respectively, in many biological redox reactions. Vitamin B₆ or pyridoxine hydrochloride and other active forms, pyridoxal, or pyridoxamine, is one of the most versatile enzyme cofactors. Folate is an essential vitamin that is also known as folic acid and folacin. The metabolic role of folate is as an acceptor and donor of one-carbon units in a variety of reactions involved in amino acid and nucleotide metabolism. Excellent food sources of folate from fruits (>55 μ g/day) include citrus fruits and juices (IM, 1998).

2.1.2. Minerals An adequate intake of minerals is essential for a high nutritional quality of the diet and also contributes to the prevention of chronic nutrition-related diseases. Fruits and vegetables are good sources of potassium and also have a very low concentration of sodium. Bananas (450 mg/100 g fw), pomegranate (400 mg/100 g fw), kiwifruit (300 mg/100 g fw), and grapes (200 mg/100 g fw) are fruits that have a high content of potassium. Sodium is present only in trace amounts in plums (Mataix-Verdú et al., 1998).

Potassium is present in vegetables in excess of 100 mg/100 g, as in cauliflower (300 mg/100 g fw) or red beet (500 mg/100 g fw). Sodium is found in concentrations below 30 mg/100 g in vegetables, except spinach with 200 mg/100 g. The iron content in fruits and vegetables is relatively low (1 mg/100 g fw); however, peas, lettuce, fennel, spinach, and Brussels sprouts have more than 2 mg/100 g (Wills et al., 1999). Other minority minerals, such as

copper, zinc, and selenium present in nuts and grains, are included in the group of antioxidant nutrients for its ability to neutralize free radicals.

2.2. Bioactive Compounds

Bioactive compounds or phytochemicals may be defined as chemical substances contained in plant foods in low levels, which endow the food with physiological properties over and above the strictly nutritional function.

Phytochemicals work together with nutrients and dietary fiber to protect against many diseases, including cancer (Hung et al., 2004), heart disease (Djoussé et al., 2004), stroke, high blood pressure (Appel et al., 1997), cataracts (Brown et al., 1999), osteoporosis, and urinary tract infections.

The mechanics of the beneficial effects of consuming fruit and vegetables are not fully understood. Phytochemicals have complementary and overlapping mechanisms of action in the body, including antioxidant effects, modulation of detoxifying enzymes, stimulation of the immune response, modification of inflammatory processes, reduction of platelet aggregation, disruption of cholesterol metabolism, modulation of the concentration of steroid hormones and hormone metabolism, reduction of blood pressure, and antiviral and antibacterial activity (Lampe, 1999). Vitamins (C and E), carotenoids (β -carotene, lutein, lycopene, zeaxanthin, etc.), flavonoids (flavonols, flavones, flavanones, anthocyanidins, isoflavones, flavanols), other phenolic compounds (phenolic acids, stilbenes, lignans), phytosterols, glucosinolates, isothiocyanates, and organosulfur compounds are phytochemical compounds in plant foods with potential health-related properties.

2.2.1. Carotenoids This term summarizes a class of yellow-red pigments found mainly in fruit and vegetables. At present, more than 600 different carotenoids have been identified, although only about two dozen are regularly consumed by humans. Most carotenoids are structurally organized into two substituted or unsubstituted ionone rings, which are separated by four isoprene units containing nine con-

jugated double bonds, such as α - and β -carotene, lutein, zeaxanthin, and α - and β -cryptoxanthin. These carotenoids, along with lycopene, an acyclic biosynthetic precursor of β -carotene, are most commonly consumed and are most prevalent in human plasma (Castenmiller and West, 1998).

On the basis of epidemiological studies, diet rich in fruits and vegetables containing carotenoids, is suggested to protect against degenerative diseases, such as cancer, cardiovascular diseases, and macular degeneration. Much of the evidence has supported the hypothesis that lipid oxidation or oxidative stress is the underlying mechanism in such diseases. To date, carotenoids are known to act as antioxidants in vitro. In addition to quenching of singlet oxygen, carotenoids may react with radical species either by addition reactions or through electron transfer reactions (van Den Berg et al., 2000; Sánchez-Moreno et al., 2003d).

Carotenoid pigments are also of physiological interest in human nutrition, since some of them are vitamin A precursors, especially β -carotene, α -carotene, and α - and β -cryptoxanthin. Carotenoid intake assessment has been shown to be complicated mainly because of inconsistencies in food composition tables and databases. No formal diet recommendation for carotenoids has been established, but some experts suggest intake of 5–6 mg/day. In the case of vitamin A, for adult human males, the RDA is 1,000 μ g retinyl Eq/day and for adult females, 800 μ g retinyl Eq/day (O'Neill et al., 2001; Trumbo et al., 2003).

2.2.2. Flavonoids Flavonoids are the most common and widely distributed group of plant phenolics. Over 5,000 different flavonoids have been described to date and they are classified into at least 10 chemical groups. Among them, flavones, flavonols, flavanols, flavanones, anthocyanins, and isoflavones are particularly common in fruits. Fruits and fruit juices are among the best sources of polyphenols in the human diet due to its high concentration in most plants and also by the relatively large size of the rations that are ingested (100–200 g) (Lotito and Frei, 2006).

Flavonoids are strong antioxidants in vitro, mainly due to their low redox potential and their capacity to donate several electrons or hydrogen atoms. Despite

the strong antioxidant capacity of flavonoids in vitro, their antioxidant efficacy in vivo is limited by several factors. Other biological activities of flavonoids seem to be independent of their antioxidant activity. This is the case of the estrogen-like activity showed by isoflavones.

Factors such as modification on the flavonoid structure or substitution by different sugars or acids could deeply affect the biological activity of flavonoids. In this sense, processing of the fruits and vegetables could produce changes in their structure and therefore influence their beneficial properties for human health.

2.2.3. Phytosterols Plant sterols have been reported to include over 250 different sterols and related compounds. The most common sterols in vegetables and fruits are β -sitosterol and its 22-dehydro analogue stigmasterol, along with campesterol and avenasterol (4-desmethylsterols).

Vegetables and fruits are generally not regarded to be as good sources of sterols as cereals or vegetable oils. The plant sterol content in a food may vary depending on many factors, such as genetic background, growing conditions, tissue maturity, and postharvest changes (Piironen et al., 2000). There are scarce data available on the content of plant sterols in the edible portion of vegetables and fruits (Piironen et al., 2000; Piironen et al., 2003; Jiménez-Escrig et al., 2006).

Since plant sterols competitively inhibit cholesterol intestinal uptake, a major metabolic effect of dietary plant sterols is the inhibition of absorption and subsequent compensatory stimulation of the synthesis of cholesterol. The ultimate effect is the lowering of serum cholesterol owing to the enhanced elimination of cholesterol in stools. Consequently, the higher the dietary intake of plant sterols from the diet, the lower is the cholesterol absorption and the lower is the serum cholesterol level (De Jong et al., 2003; Trautwein et al., 2003).

2.2.4. Organosulfur Compounds Vegetables from the *Liliaceae* (onions and garlic) and *Brassicaceae* (broccoli, cabbage, Brussels sprouts, cauliflower) families are considered to have health-

beneficial properties deriving from the presence of substances whose chemical structure contains sulphur atoms. Allicin (2-propene-1-sulfinothioic acid S-2-propenyl ester $\text{CH}_2=\text{CH}-\text{CH}_2-\text{S}(0)-\text{S}-\text{CH}_2-\text{CH}=\text{CH}_2$) is believed to be the biologically active molecule in garlic when this is macerated or homogenized. Allicin is not present in garlic until its precursor, alliin or (+)-S-allyl-L-cysteine sulfoxide ($\text{CH}_2=\text{CH}-\text{CH}_2-\text{S}(0)\text{CH}_2-\text{CH}(\text{NH}_2)-\text{COOH}$), comes into contact with the enzyme alliinase when the cell membrane is ruptured by chopping or maceration of the garlic. In general, the following beneficial effects have been ascribed to garlic: antioxidant and free radical scavenging (a powerful inhibitor of lipid peroxidation), anti-inflammatory, anticoagulant, fungicide, antiviral (influenza, AIDS), interferon and immune system enhancement, bactericide, reduction of cholesterol levels, anticarcinogenic, and antimutagenic (Lawson, 1998; Seki et al., 2000; Benkeblia, 2004).

Numerous epidemiological studies have shown that a diet rich in vegetables from the *Brassicaceae* family (broccoli, cabbage, Brussels sprouts, and cauliflower) reduces the incidence of some kinds of cancer (Lund, 2003). This anticarcinogenic effect are attributed to a group of organosulfur minority constituents present in them, known as glucosinolates and to the capacity of some of its metabolites, isothiocyanates and indoles, to intervene in biotransformations catalyzed by enzymes associated with the antioxidant systems such as glutathione-S-transferase (Lampe and Peterson, 2002).

3. Effects of HPP on Nutritional and Health-Related Compounds of Fruit and Vegetable Products

Today, there is growing interest in nonthermal processing technologies such as HPP for preserving fruit and vegetable products due to their high content in substances with biological activity beneficial to consumer health. The potential and limitations of HPP have been extensively reviewed, although most of the studies have been focused on microbial and enzyme inactivation (Hayashi, 1990; Hendrickx et al., 1998; Tauscher, 1998; Knorr, 1999; Meyer et al., 2000;

Patterson, 2000; Indrawati et al., 2003; Devlieghere et al., 2004; Barbosa-Cánovas et al., 2005; Cano and De Ancos, 2005; Hogan et al., 2005; Mor-Mur and Yuste, 2005; Torres and Velazquez, 2005).

Technological advances in equipment design and great number of research studies have allowed the development of a very wide range of commercial products mainly based in fruit and vegetables (orange juice, apple juice, avocado paste known as guacamole, fruit jams, onion slices, tomato purée) (Torres and Velazquez, 2005; Rastogi et al., 2007). The main goal to increase the number of commercial HPP plant-derived products is to obtain more scientific evidence about the efficiency of this technology to produce safe fruit and vegetable processed products with all their nutritional compounds, vitamins, and bioactive compounds unchanged or minimally modified. In fact, this technology offers commercial orange juice processed with quality characteristics similar to a freshly squeezed product (Fernández-García et al., 2001a, 2001b; Nienaber and Shellhammer, 2001; Plaza et al., 2003a, 2006a; Sánchez-Moreno et al., 2003a, 2003b, 2005a).

The most recent objectives regarding HPP are to achieve the sterilization of plant products without modifying their sensorial and nutritional quality and their healthy properties. This will allow the preservation of the processed products at room temperature. The application of high pressure using multiple pulses and controlling the temperature above 105°C for a short time have been proposed as an effective combined treatment to achieve sterility with minimal effects on nutrients and bioactive constituents of plant foods (Meyer et al., 2000). Therefore, studies on the effect of HPP on the nutritional quality and stability of bioactive compounds are necessary due to the implementation of combined high pressure/high temperature for food sterilization.

Compared to the research on effect of HPP on the destruction of microorganisms and enzymes, the number of papers published on the effect of HPP on nutritional quality and health-related characteristics of food constituents are relatively few. Several authors have shown that processing different fruit and vegetable products by HPP (orange juice, lemon juice, apple juice, persimmon purée, tomato

purée, strawberry purée, kiwi purée, mixed vegetables soup (“gazpacho”), orange/carrot juice, and apple/broccoli juice) slightly modified their nutritional composition (vitamins, C, A, E, B₁, B₂, and folic acid), bioactive compounds (vitamins A, E and C, carotenoid compounds, and flavonoid), and antioxidant capacity (Donsi et al., 1996; Quaglia et al., 1996; Tauscher, 1998; van Den Broeck et al., 1998; De Ancos et al., 2000, 2002; Kim et al., 2001; Fernández-García et al., 2001a, 2001b; Nienaber and Shellhammer, 2001; Butz et al., 2002, 2003; Indrawati et al., 2003; Plaza et al., 2003a, 2006a; Sánchez-Moreno et al., 2003a, 2003b, 2003c, 2003d, 2003e, 2004a, 2004c, 2004d, 2005a, 2005b, 2006a, 2006b, 2006c; Corrales et al., 2009; Roldán-Martín et al., 2009).

3.1. Effects of High-Pressure Processing on Vitamin C and B Vitamins of Fruit and Vegetable Products

The effect of HPP on the vitamin C content of processed fruit and vegetables is the topic most often studied related to the effects of nonthermal technologies on nutrients and bioactive compounds. Vitamin C (ascorbic acid + dehydroascorbic acid) is one of the most important vitamins in plant foods not only for its nutritional role in human diet but also for its high antioxidant capacity. Many authors have reported that vitamin C in different fruit and vegetable products is not significantly affected by high-pressure treatments (Table 33.1).

3.1.1. Influence of HPP Parameters High-pressure treatments from 400 to 900 MPa and treatment times of 5 and 10 minutes were assayed with green peas as an alternative to blanching previous to freezing process. In this study, increased pressure led to higher retention of ascorbic acid in plant tissue. Maximum retention of approximately 82% of the ascorbic acid content of fresh peas was obtained after applying a combination of 900 MPa/20°C for 5–10 minutes (Quaglia et al., 1996).

Similar results were found in guava purée treated at 400 and 600 MPa for 15 minutes, reaching 100% of the ascorbic acid retention of untreated products (Yen and Lin, 1996).

Table 33.1. Effect of high pressure processing (HPP) on hydrosoluble vitamins' retention of fruit and vegetable products

Product	HP Treatment	Vitamin Retention (%)	Reference
<i>Vitamin C</i>			
Kiwi, strawberry jams	400–600 MPa/10–30 minutes	95–99	Kimura et al. (1994)
Green peas	900 MPa/20°C/5–10 minutes	82	Quaglia et al. (1996)
Guava purée	400 and 600 MPa/15 minutes	100	Yen and Lin (1996)
Strawberry sauce “coulis”	200–600 MPa/30 minutes	89	Sancho et al. (1999)
Orange juice	100/60°C/5 minutes	90	Sánchez-Moreno et al. (2003e)
	400/40°C/1 minute	92–95	Sánchez-Moreno et al. (2003e, 2005a); Plaza et al. (2006a)
	500 MPa/25°C/5 minutes and 800 MPa/25°C/5 minutes	98	Fernández-García et al. (2001a)
Tomato purée	350/30°C/2.5 minutes	100	Sánchez-Moreno et al. (2003e)
	400/40°C/15 minutes	71	Sánchez-Moreno et al. (2006a)
Orange–lemon– carrot juice	500 MPa/25°C/5 minutes and 800 MPa/25°C/5 minutes	96	Fernández-García et al. (2001a)
	500 MPa/5 minutes	97	Houska et al. (2005)
Apple–broccoli juice	500 MPa/20 minutes	72	Houska et al. (2005)
	500 MPa/25°C/1 minute	92	Krebbes et al. (2002)
Green beans	500 MPa/25°C/15 minutes	59	Doblado et al. (2007)
Cowpeas sprout seeds	400 MPa/25°C/1 minute	93	De Ancos et al., 2007
<i>Vitamins B₁, B₆</i>			
Model solution	400–600 MPa/25°C/30 minutes	99–100	Sancho et al. (1999)

Also, the first studies to obtain strawberry and kiwi jams as commercial HPP products (400–600 MPa/10–30 minutes) exhibited a high retention of the ascorbic acid content (between 95 and 99%) of the initial vitamin C concentration in the raw fruit (Kimura et al., 1994). Vitamin C retention in strawberry French sauce “coulis” after high-pressure treatment (200–600 MPa/30 min) has been compared with that induced by classic thermal treatments such as thermal pasteurization (0.1 MPa/72°C). The vitamin C retention achieved after high pressure (88.68%) was similar to that obtained after thermal pasteurization (91.52%); however, sterilization process had a more noticeable effect with only a 67.15% of retention of the initial vitamin C concentration (Sancho et al., 1999).

Orange juice is the principal source of vitamin C daily intake in developed countries. The effects of high-pressure treatments on the ascorbic acid content of orange juice have been studied by several authors (Ogawa et al., 1990; Takahashi et al., 1993;

Donsi et al., 1996; Van Den Broeck et al., 1998; Fernández-García et al., 2001a, 2001b; Nienaber and Shellhammer, 2001; Sánchez-Moreno et al., 2003c, 2003d, 2003e, 2004a, 2005a; Plaza et al., 2006a) (Table 33.1). In general, the conclusion was that ascorbic acid is maintained in the pressure-treated orange juice at similar levels than in a freshly squeezed juice.

Frequently high-pressure treatments were combined with mild temperature. Therefore, combined treatments of high pressure between 100 and 400 MPa and mild temperature (30–60°C) during short treatment (1–5 minutes) produced less than 10% of vitamin C loss. Maximum degradation (up to 10%) was reported when the product was high pressure processed (100–400 MPa) at 60°C. This elevated temperature seems to be the cause of vitamin C degradation (Sánchez-Moreno et al., 2003e, 2005a), because even when extreme pressures were applied (850 MPa), combined with mild temperatures (up to 50°C) during one hour, ascorbic

acid was stable. However, there was significant degradation of ascorbic acid when a combined treatment of 850 MPa and 60–80°C was used in orange juice and tomato juice. The conclusion was that when high-pressure treatments were combined with heat, mild temperatures are necessary to preserve ascorbic acid content in vegetable and fruit products (Van Den Broeck et al., 1998).

Vitamin C retention in orange juice after high-pressure treatment (400 MPa/40°C/1 minute) has been compared with that induced by thermal treatments such as low temperature pasteurization (0.1 MPa/70°C/30 seconds) and traditional pasteurization (0.1 MPa/90°C/1 minute) (Sánchez-Moreno et al., 2005a). High-pressure treatments and thermal treatments such as low pasteurization did not modify vitamin C content of freshly squeezed orange juice; meanwhile, traditional pasteurization produced a slight reduction (7%) of the initial vitamin C content (Sánchez-Moreno et al., 2005a).

Another important issue of the application of HPP is that the retention of vitamin C could depend on food matrix and processing parameters.

In general, the application of HPP (250 MPa/35°C/15 minutes) did not cause a significant change in the ascorbic acid content in fruit and vegetable products, such as carrot juice and tomato juice, as a consequence of the type of fruit juice, ($P > 0.05$) (Dede et al., 2007), but different results were found with other products. Thus, tomato purée suffered a loss of 29% of vitamin C loss after high-pressure treatment (400 MPa/25°C/15 minutes) similar to that observed after thermal pasteurization of tomato purée (70°C/30 seconds and 90°C/1 minute). However, the high-pressure treatment (400 MPa/25°C) applied to tomato purée has produced higher loss of vitamin C (29%) than in orange juice (9%). Thus, although both products (orange juice and tomato purée) were processed with the same pressure (400 MPa), treatment time was different (1 minute for orange juice and 15 minutes for tomato purée); therefore, this treatment parameter influences the retention of ascorbic acid.

Also, the different food matrix could have influence in the rate of vitamin C destruction (9% in orange juice and 29% in tomato purée) (Sánchez-

Moreno et al., 2005a, 2006a). Similar results were found with other products, where parameters of treatment like pressure holding time can produce significant changes in the vitamin C retention. Thus, an apple–broccoli juice pressurized at 350 and 500 MPa during 5 minutes retained the 62 and 97% of vitamin C content of untreated juice, respectively. However, in fresh juices treated at 350 and 500 MPa for 20 minutes about 71–72% of the initial concentration was retained. In this case, the vitamin C content depends on pressure holding time, but independent of the pressure level. This fact suggest that for achieving more retention of vitamin C, it is better to use high pressure and short treatment time than a lower pressure but longer treatment time (Houska et al., 2005).

3.1.2. Influence of Refrigerated Storage

Pressure-treated plant products at or near ambient temperatures must be kept under refrigeration because the pressure treatment is equivalent to a cold pasteurization. Evolution of vitamin C in high pressure-processed plant products during refrigerated storage has also been reported in several publications (Table 33.2).

In general, short storage periods do not affect the vitamin content of pressure-treated plant products. Thus, after 10 days at 4°C, vitamin C content did not show a significant decrease (lower than 9%) in orange juice subjected to various high-pressure treatments (100 MPa/60°C/5 minutes, 350 MPa/30°C/2.5 minutes, 400 MPa/40°C/1 minute) (Sánchez-Moreno et al., 2003e, 2006a). After longer storage period (40 days at 4°C), the pressurized orange juice (400 MPa/40°C/1 minute) suffered a significant decrease up to 14% in the vitamin C content; meanwhile, a decrease up to 18% was achieved in thermal pasteurized orange juice (70°C/30 seconds) when compared with the initial vitamin C concentration in the fresh juice. As a consequence, pressure-treated orange juice maintained better the vitamin C content during long-term storage (40 days at 4°C) than in the thermally pasteurized juice (Sánchez-Moreno et al., 2006a). Similar results were found with tomato juice and carrot juice. The ascorbic acid content of tomato juice and carrot juice treated at

Table 33.2. Vitamin C retention during storage of plant products processed by high pressure (HP)

Product	HP Treatment	Storage Conditions	Vitamin C Retention (%)	Reference
Guava purée	600 MPa/25°C/15 minutes	40 days, 4°C	85	Yen and Lin (1996)
Strawberry nectar	500 MPa/25°C/3 minutes	60 days, 3°C	75	Rovere et al. (1996)
Strawberry <i>coulis</i>	400 MPa/20°C/30 minutes	28 days, 4°C	69	Sancho et al. (1999)
Orange juice	500 MPa/35°C/5 minutes	30 days, 5°C	70	Polydera et al. (2003)
Orange juice cv.Salustiana	500 MPa/20°C/5 minutes	21 days, 4°C	97	Fernández-García et al. (2001a)
Orange juice cv.Salustiana	800 MPa/20°C/5 minutes	21 days, 4°C	94	Fernández-García et al. (2001a)
Orange–carrot–lemon juice	500 MPa/20°C/5 minutes	21 days, 4°C	83	Fernández-García et al. (2001a)
Orange–carrot–lemon juice	800 MPa/20°C/5 minutes	21 days, 4°C	76	Fernández-García et al. (2001a)
Orange juice cv.Valencia Late	100 MPa/60°C/5 minutes	10 days, 4°C	100	Sánchez-Moreno et al. (2003c)
Orange juice cv.Valencia Late	350 MPa/30°C/2.5 minutes	10 days, 4°C	94	Sánchez-Moreno et al. (2003c)
Orange juice cv.Valencia Late	400 MPa/40°C/1 minute	10 days, 4°C	96	Sánchez-Moreno et al. (2003c)
Orange juice cv.Navel	600 MPa/40°C/4 minutes	30 days, 5°C	84	Polydera et al. (2005)
Orange juice Valencia Late	400 MPa/40°C/1 minute	40 days, 4°C	79	Plaza et al. (2006a)
Carrot juice	250 MPa/35°C/15 minutes	30 days, 4°C	70	Dede et al. (2007)
Carrot juice	250 MPa/35°C/15 minutes	30 days, 25°C	45	Dede et al. (2007)
Tomato juice	250 MPa/35°C/15 minutes	30 days, 4°C	70	Dede et al. (2007)
Tomato juice	250 MPa/35°C/15 minutes	30 days, 25°C	70	Dede et al. (2007)

250 MPa/35°C/15 minutes remained over 70% after 30 days of storage at 4°C. When the fruit juices were stored at 25°C, ascorbic acid retention remained over 70% in tomato juice but decreased to 45% in carrot juices. However, tomato juice and carrot juice treated at 80°C for 1 minute displayed a rapid decrease of ascorbic acid when stored at both 4 and 25°C. The carrot juice had no ascorbic acid after 16 and 18 days of storage at 4 and 25°C, respectively, whereas tomato juice maintained approximately the 15% of the initial concentration during the storage at these both temperatures (Dede et al., 2007).

Therefore, it is confirmed that high pressure-treated products have superior quality than thermally processed products during the storage period in terms of ascorbic acid retention, even when extreme high pressure conditions are employed. However, this last statement depends on the food matrix. Thus, orange juice treated at 500 MPa/5 minutes

and 800 MPa/5 minutes and stored for 21 days at 4°C, suffered a slight ascorbic acid decrease up to 2 and 4%, respectively; meanwhile, the nontreated sample has achieved a decrease of about 6%. However, ascorbic acid concentration was lower in a pressurized juice mix of orange/lemon/carrot at the end of storage period, achieving a vitamin C retention up to 83% (500 MPa/5 minutes) and up to 73% (800 MPa/5 minutes) of the initial vitamin C concentration. Again, more evidence have shown that the retention of ascorbic acid concentration in fruit juices after high-pressure treatment and during refrigerated storage depends on the type of food matrix (Fernández-García et al., 2001a).

The ascorbic acid loss was well adjusted to a first-order kinetics during storage of both pressure and thermally pasteurized orange juice products, but ascorbic acid degradation rates were lower for high-pressure treatments (600 MPa/40°C/4 minutes and

500 MPa/35°C/5 minutes) than conventionally pasteurized juice (80°C/60s). Ascorbic acid degradation rate after high-pressure treatments increased when temperature of refrigerated storage increased. This data is in accordance with the statement that high pressure-treated products have superior quality than products thermally processed during the storage period in terms of ascorbic acid retention (Polydera et al., 2003, 2005).

3.1.3. High Pressure Effects on Whole Fruits and Vegetables The influence of high-pressure treatment on vitamin C content of whole fruits and vegetables, legumes, or precut fruit or precut vegetables are scarce in the scientific literature. In green beans, it was observed that vitamin C retention up to 92% after high-pressure treatment (500 MPa/25°C/1 minute) (Krebbes et al., 2002). High pressure is studied as an alternative treatment to improve the safety of germinated seeds and legumes (mung beans, cowpeas, soybeans, and alfalfa) (Muñoz et al., 2006). It is well known that germination increases the vitamin C of seeds and legumes (Plaza et al., 2003b). It seems that high-pressure treatment of seeds before germination significantly reduced the vitamin C content in the germinated products. Thus, it was found that vitamin C content was significantly reduced (between 10 and 28% at 4 days and between 9 and 41% at 6 days) after cowpeas germination, when the seeds were previously treated with high pressure (300–500 MPa/25°C/15 minutes). This vitamin C loss was higher when intensity of pressurization increased (Doblado et al., 2007).

The application of HPP to precut fruit and precut vegetables has been less studied, and the key to the success of this technology is the selection of suitable processing parameters that minimally modified the integrity of the plant cell but preserving the nutrient content and bioactive compounds such as vitamin C. The application of high-pressure treatments (100–400 MPa/25°C/1 minute) did not affect the vitamin C content in precut papaya, and the maximum loss recorded was 7%, considering the initial content of raw fruit (De Ancos et al., 2007).

3.1.4. Influence of HPP on B Vitamins The effect of high pressure on B vitamins (B₁, B₆) has also been studied in model solution, and in this experimental conditions, thiamin and piridoxal were not significantly affected by high-pressure treatments (400–600 MPa/25°C/30 minutes) (Sancho et al., 1999) (Table 33.1).

3.2. Effects of High-Pressure Processing on Carotenoids of Fruit and Vegetable Products

Effects of high-pressure treatments on carotenoid content of fruit and vegetable products were studied in different products (De Ancos et al., 2000, 2002; Fernández-García et al., 2001b; Sánchez-Moreno et al., 2003e, 2004a, 2006a; Plaza, 2004; Plaza et al., 2006b). Orange juice, persimmon fruit, papaya fruit, tomato purée, and tomato-based product (cold soup mix of vegetables called “gazpacho” in Spain) are good dietary sources of carotenoids with provitamin A activity (β -carotene, α -carotene, and β -cryptoxanthin) and radical scavenging capacity (lycopene, β -carotene, α -carotene, β -cryptoxanthin, zeaxanthin, and lutein). In general, carotenoid content of fruit and vegetable products is not destroyed by HPP; moreover, an important increase in carotenoid content extracted has been achieved after the pressurization of some products (Table 33.3).

3.2.1. Influence of HPP Parameters High-pressure treatment at 350 MPa over different treatment times (2.5–15 minutes) produced a significant increase (20–43%) in the amount of extractable carotenoids in orange juice. This trend has been observed also with persimmon fruit products. Also an increase in the concentration of carotenoids with provitamin A activity can be correlated with a significant increase of up to 45% in vitamin A value in persimmon purée treated by high pressure (350 MPa/5 minutes) (De Ancos et al., 2002).

Tomato is the second most consumed in western countries and more than 80% of the lycopene, a potent antioxidant, consumed around the world comes from tomato product. In general, lycopene content in tomato product was unaffected by HPP

Table 33.3. Effect of HPP on carotenoid retention in fruit and vegetable products

Product/Carotene	HPP Treatment	Carotenoid Retention (%)	Reference
<i>Persimmon purée</i>			
Total carotenoid	400 MPa/25°C/15 minutes	116	De Ancos et al. (2000)
Carrot Juice			
α -Carotene	500 MPa/25°C/10 minutes	50.18	Kim et al. (2001)
	500 MPa/60°C/10 minutes	27.60	
β -Carotene	500 MPa/25°C/10 minutes	59.45	
	500 MPa/60°C/10 minutes	40.14	
<i>Tomato purée</i>			
β -Carotene	600 MPa/25°C/60 minutes	100	Butz et al. (2002)
	400 MPa/25°C/15 minutes	136	Sánchez-Moreno et al. (2006a)
Lycopene	600 MPa/25°C/60 minutes	100	Butz et al. (2002)
	500 MPa/25°C/12 minutes	121	Qiu et al. (2006)
	500 MPa/20°C/2 minutes	160	Krebbbers et al. (2003)
	400 MPa/25°C/15 minutes	149	Sánchez-Moreno et al. (2006a)
<i>Orange Juice with pulp</i>			
β -Cryptoxanthin	400 MPa/40°C/1 minute	143.21	Sánchez-Moreno et al. (2005a)
Zeaxanthin	400 MPa/40°C/1 minute	144.52	
Lutein	400 MPa/40°C/1 minute	175.43	
β -Carotene	400 MPa/40°C/1 minute	130.24	
α -Carotene	400 MPa/40°C/1 minute	133.76	
<i>Orange–lemon–carrot juice</i>			
β -Carotene	500 and 800 MPa/25°C/5 minutes	100	Fernández-García et al. (2001a)
α -Carotene	500 and 800 MPa/25°C/5 minutes	100	
<i>“Gazpacho soup”</i>			
Antioxidant carotenoids ^a	400 MPa/60°C/15 minutes	108	Plaza et al. (2006a)
<i>Carrot (whole)</i>			
α -Carotene	600 MPa/25°C/2 minutes	100	McInerney et al. (2007)
β -Carotene	600 MPa/25°C/2 minutes	100	
<i>Green Beans (whole)</i>			
Lutein	600 MPa/25°C/2 minutes	100	McInerney et al. (2007)
<i>Broccoli (whole)</i>			
β -Carotene	600 MPa/25°C/2 minutes	83	McInerney et al. (2007)
Lutein	600 MPa/25°C/2 minutes	90	
<i>Papaya slices(cv Sunrise)</i>			
Antioxidant carotenoids ^b	400 MPa/25°C/1 minute	156	De Ancos et al. (2007)
<i>Papaya slices (cv BH65)</i>			
Antioxidant carotenoids	400 MPa/25°C/1 minute	81	De Ancos et al. (2007)

^aGazpacho antioxidant carotenoids = β -carotene + γ -carotene + lutein + lycopene + lycopene-epoxide.

^bPapaya antioxidant carotenoids = β -cryptoxanthin + β -cryptoxanthin-5,8-epoxide + β -carotene + lutein + zeaxanthin + lycopene + neo lycopene.

combined with ambient or elevated temperature, and in this sense, it is reported that tomato purée treated at 600 MPa/20°C/60 minutes showed no losses in lycopene and β -carotene content (Fernández-García et al., 2001b). Also, in extreme conditions ap-

plied for high pressure sterilization (700 MPa/90°C/30 seconds), high pressure had no effect on the lycopene content (Krebbbers et al., 2003). Several publications showed that high-pressure treatments are able to increase extractable carotenoid amount in

tomato-based products (Krebbbers et al., 2003; Sánchez-Moreno et al., 2004a, 2006a). Moreover, also an important increase in carotenoid content extracted has been achieved after the pressurization of tomato products. Thus, treatment at 500 MPa/20°C/2 minutes increased 58% of the lycopene extracted in tomato purée comparing with untreated product (Krebbbers et al., 2003). The amount of lycopene, β -carotene, and total carotenoid extracted from tomato purée increased up to 14%, 20%, and 10%, respectively, when 400 MPa was applied in comparison to untreated product. Generally, tomato products are formulated with additives such as sodium chloride or citric acid. These additives have a protective effect against high pressure, and a reduction in the amount of carotenoids extraction was observed. The opposite occurred when 400 MPa was applied to tomato purée without additives. An increase in vitamin A value due to the best extraction of carotenoids with provitamin A activity (24% higher than in untreated sample) was reported under such conditions (Sánchez-Moreno et al., 2004a). high-pressure treatment at 400 MPa/25°C/15 minutes significantly increased individual and total carotenoid content extracted from tomato purée compared with that of untreated product. Regarding individual carotenoid content, the increase was 77% in lycopene and 35% in β -carotene. The increase in carotenoid extraction from high pressure-treated orange juice, persimmon purée, or tomato purée could be attributable to various factors such as permeabilization of the plasma membrane cell and denaturation of the carotenoid-binding protein induced by pressure (Sánchez-Moreno et al., 2004a, 2005a).

3.2.2. Influence of Refrigerated Storage Pressurized plant products must be stored under refrigeration because the HP process is equivalent to a cold pasteurization. The behavior of carotenoids during storage of high-pressurized plant products has been less studied than the effect of HPP parameters.

The studies achieved with a tomato-based soup known in Spain as “gazpacho,” showed no significant changes in total carotenoid content after certain high-pressure treatments (150 MPa/60°C/15 minutes

and 350 MPa/60°C/15 minutes), although after long-term storage (40 days at 4°C), significant decrease between 40 and 46% was observed (Plaza et al., 2006b).

high-pressure treatments (350 MPa/30°C/5 minutes) also preserve and increase the carotenoid content extracted and the vitamin A activity during refrigerated storage of orange juice. Thus, after storage for 30 days at 4°C, the orange juices treated at 350 MPa/30°C/5 minutes and 400 MPa/40°C/1 minute had extracted carotenoid concentration levels at 72 and 45%, respectively. These values were higher than in the untreated sample and also higher than in low thermally pasteurized juices (70°C/30 seconds). This total carotenoid concentration remained unchanged until the end of the storage period (40 days at 4°C) (Sánchez-Moreno et al., 2003e; Plaza, 2004). In consequence, vitamin A values showed an increase above 40% of the value of the untreated sample during the refrigerated storage. The inactivation of enzymes that caused losses of carotenoids (peroxidase, lipoxigenase, etc.) during storage and the enhancement in extraction due to high-pressure treatments are some of the reasons attributed by the authors to explain the results (De Ancos et al., 2002).

Other authors showed no significant differences on orange juice carotenoid content after high-pressure treatment (500–800 MPa and 5–15 minutes) and subsequent storage for 21 days at 4°C (Donsi et al., 1996; Fernández-García et al., 2001b).

Similar increase in carotenoid extraction than in orange juice was shown in pressurized persimmon purée (De Ancos et al., 2000). high-pressure treatment (50–400 MPa) on persimmon purée resulted in a slight increase between 9 and 26% of extracted carotenoids when compared with untreated samples. The better extraction of provitamin A carotenoids, β -carotene, and β -cryptoxanthin, was reflected in an 8–16% increase of the vitamin A value in the pressurized persimmon purée (De Ancos et al., 2000).

3.2.3. High Pressure Effects on Whole Fruits and Vegetables The effect of HPP nutritional quality on whole fruits and vegetables are not frequently investigated. The carotenoid content in carrots,

broccoli, and green beans were unaffected by high-pressure treatment, regardless of the pressure level employed (400 MPa/25°C/2 minutes and 600 MPa/25°C/2 minutes) (McInerney et al., 2007). However, other plant products suffered an increase of the extraction of carotenoids after HPP. Therefore, high-pressure treatment at 400 MPa/25°C/1 minute increased carotenoids extraction up to 58% in the precut papaya, although this increase also depended on the fruit variety studied (De Ancos et al., 2007).

In conclusion, nonthermal technologies as HPP could increase the extraction of bioactive compounds of fruit and vegetables and probably improve their bioavailability. From an industrial point of view, HPP can be a reliable alternative to traditional thermal technologies, not only to preserve the nutritional and sensory quality and ensure product microbial safety, but also as a potential tool for obtaining functional foods.

3.3. Effect of High Pressure on Other Health-Related Compounds in Fruit and Vegetable Products

In addition to the well-known vitamin C and carotenoid compounds, fruits and vegetables have other bioactive compounds that significantly contribute to their biological activity in human health such as flavonoids and glucosinolates.

Orange juice is a very rich source of flavanone glycosides (mainly narirutin and hesperidin), which are degraded to aglycones (naringenin and hesperetin) in the human intestinal flora after ingestion, and their beneficial effects to health are mainly consequence of their scavenging free radical capacity (Rice-Evans, 2001). Some studies showed that certain high-pressure treatments, 350 MPa/30°C/2.5 minutes and 400 MPa/40°C/1 minute, increased the hesperetin content up to 34 and 22%, respectively, after the hydrolysis of the extract obtained from the pressurized orange juice. After 10 days at 4°C, total flavanone content extracted from the pressurized orange juice significantly increased between 16 and 19% when compared with the recently treated (Sánchez-Moreno et al., 2003c). Compared to thermal pasteurization (90°C/1 minute) where total fla-

vanone content was unchanged, high-pressure treatment (400 MPa/40°C/1 minute) increased naringenin content by 20% and hesperetin by 40% when compared with untreated orange juice (Sánchez-Moreno et al., 2005a). Flavonol concentration in onions also increased after HPP. Consequently, onion slices treated at 400 MPa combined with 5°C showed an approximately 33% higher extracted quercetin-4'-glucoside content in comparison to the untreated onion (Roldán-Martín et al., 2009). Similar trend was found in the extraction of anthocyanins from grape skin. The total anthocyanin recovery was 58% higher than in the untreated sample when the extractions were assisted by HPP (600 MPa/30 minutes/50% ethanol) (Corrales et al., 2009).

Consumer demand for Brassica vegetables such as broccoli, cabbage, etc., have increased due to their anticarcinogenic characteristics. They contain glucosinolates and their breakdown products, the isothiocyanates, due to the action of the enzyme myrosinase. The main objective of a new technology will be the protection of isothiocyanates, which are the molecules with potential anticancer activity.

One of the first studies with this molecule showed that the combined treatment of 600 MPa with different temperatures (25, 40, 60, and 75°C) increased the rate of degradation of allyl and benzyl isothiocyanates at the same time that the temperature increased, and thus isothiocyanates were degraded four times more than in untreated samples (Grupe et al., 1997).

The effect of high pressure on sulforaphane, one of the most potent isothiocyanate, in water and in minced broccoli was studied. high-pressure treatment at 600 MPa combined with 25°C during 40 minutes has very little effect on sulforaphane content, but if pressure treatment at 600 MPa was combined with temperature (75°C), the concentration of sulforaphane in water strongly decreased. On the other hand, sulforaphane content extracted from minced broccoli increased with increasing pressure and treatment time (150–750 MPa, 20–40 minutes) (Butz et al., 2003). Pressure treatment in combination with high temperature of 60–90°C produced an increasing destruction of sulforaphane at the same time that it was formed after the hydrolysis of the

glucosinolate known as glucoraphanin. Systematic studies combining temperature (60–90°C) and pressure (600–800 MPa) showed first-order kinetics for isothiocyanate degradation. By comparing high pressure with thermal treatment (60–90°C/20 minutes), it was found that isothiocyanates were relatively thermolabile and pressure-stable (Houska et al., 2005; Van Eylen et al., 2007). In conclusion, high pressure combined with elevated temperatures would have limited application to foods with high isothiocyanate content as broccoli.

3.4. Effects of HPP on Antioxidant Activity of Fruit and Vegetable Products

Evaluating the influence of nonthermal processing on the extractability of naturally occurring antioxidants is a key factor in refining the technological conditions necessary to preserve or improve their biological activity and bioavailability. Regarding antioxidant capacity, it must be taken into account the lipophilic (vitamin E, carotenoid compounds) and hydrophilic (vitamin C, phenolic compounds) nature of the major antioxidant compounds and also the ability of the processing technologies to release them from the food matrix. In general, high-pressure treatment increases the antioxidant capacity of fruit and vegetables products, but the results can be dependent on the food matrix, extraction method (solvent), pro-

cessing parameters (pressure, time and temperature), and the method selected to measure the antioxidant capacity (De Ancos et al., 2000; Fernández-García et al., 2000, 2001a, 2001b; Sánchez-Moreno et al., 2003c, 2006a; Plaza et al., 2006b; Corrales et al., 2009; Roldán-Martín et al., 2009). Significant loss of antioxidant capacity in pressure-treated plant products has been observed mainly during longtime refrigerated storage. Table 33.4 summarizes the effect of HPP combined with a refrigerated storage on the antioxidant capacity in different plant products.

Antioxidant capacity (ABTS^{•+} assay) of different juices (orange, apple, peach, and citrus–carrot mix) and tomato purée was not affected after high-pressure treatment combined with low temperature (600 MPa/20°C/60 minutes) (Butz et al., 2003). However, when high-pressure treatment was combined with high temperature and longer treatment time (600 MPa, 60°C, 30 minutes), a decrease in antioxidant capacity (linoleic acid/ β -carotene system) up to 25% when compared with an untreated sample was achieved in a freshly squeezed apple juice; meanwhile, the losses recorded after thermal treatment (60°C/30 minutes) were approximately 10% of the initial value. Therefore, extended pressurization (600 MPa/30 minutes) at 60°C caused higher loss in antioxidant capacity than thermal treatment alone (60°C/30 minutes). However, after a month's storage at 4°C, thermal- and pressure-treated apple

Table 33.4. Antioxidant capacity retention during storage of plant products processed by high pressure (HP)

Product	HP Treatment	Storage Conditions	Antioxidant Capacity Retention (%)	Reference
Orange juice Salustiana	500 MPa/20°C/5 minutes	21 days, 4°C	84	Fernández-García et al. (2001a)
Orange juice Salustiana	800 MPa/20°C/5 minutes	21days, 4°C	84	Fernández-García et al. (2001a)
Orange juice Valencia late	350 MPa/30C/15 minutes	30 days, 4°C	53	De Ancos et al. (2002)
Orange juice Valencia late	400 MPa/40°C/1 minute	10 days, 4°C	95	Sánchez-Moreno et al. (2003c)
Orange juice Valencia late	400 MPa/40°C/1 minute	40 days, 4°C	60	Plaza et al. (2006a)
“Gazpacho soup”	350 MPa/60°C/15 minutes	40 days, 4°C	50	Plaza et al. (2006b)
Tomato juice	250 MPa/35°C/15 minutes	30 days, 4°C	80	Dede et al. (2007)
Tomato juice	250 MPa/35°C/15 minutes	30 days, 25°C	80	Dede et al. (2007)
Carrot juice	250 MPa/35°C/15 minutes	30 days, 4°C	80	Dede et al. (2007)
Carrot juice	250 MPa/35°C/15 minutes	30 days, 25°C	80	Dede et al. (2007)

juices and the untreated sample did not show significant differences in antioxidant capacity (Fernández-García et al., 2000). Contrary to the results with apple juice, a positive synergic effect of high pressure/high temperature has been observed in grape skin extracts. Antioxidant capacity (ABTS^{•+} assay) of the extracts obtained with the combined treatment of 600 MPa and 70°C was significantly higher than that obtained combined with 600 MPa and lower temperature (20°C or 50°C). In this case, the combination of HPP and heat (70°C) improved up to threefold the extraction of anthocyanins, considering the control (Corrales et al., 2009). However, the flavonoid compounds in other products such as onions should not be solely responsible for the antioxidant activity. Accordingly, the combination of 400 MPa and 5°C significantly increased the concentration of quercetin-4'-glucoside but without increasing the initial antioxidant activity of onion (Roldán-Martín et al., 2009).

Orange juice is the plant product widely used in the HPP research studies. Combined treatments of high pressure and moderate temperature (100 MPa/60°C/5 minutes, 350 MPa/30°C/2.5 minutes, 400 MPa/40°C/1 minute) did not cause significant changes on the antioxidant capacity of a freshly squeezed orange juice (DPPH[•] system) (Sánchez-Moreno et al., 2003c; Plaza et al., 2006a). Also, the antioxidant capacity value of high pressure-treated orange juice was not significantly affected after 10 days of storage at 4°C. Only after 40 days at 4°C, a decrease of up to 17% in the antioxidant capacity was observed in pressure-treated orange juice, while the thermally treated (70°C/30 seconds) showed a drop of 23% of the antioxidant capacity. Thus, high-pressure treatment better preserve the antioxidant capacity of orange juice during refrigerated storage than that of low thermal pasteurization (Sánchez-Moreno et al., 2003c; Plaza et al., 2006a). Also, when the antioxidant capacity of orange juice is measured by different method (ABTS^{•+}), similar results was obtained. Thus, orange juice treated at 500 and 800 MPa for 5 minutes and 20°C and stored for 21 days at 4°C also showed a retention of over 84% of the antioxidant capacity (Fernández-García et al., 2001a).

As indicated above, the effect of high pressure on the antioxidant activity depends on the food matrix characteristics (variety, maturity, pH, etc.). Generally, tomato-derived products are formulated with some additive as sodium chloride and citric acid to increase the shelf life of the product. In tomato purée with additives (6 g/L sodium chloride and 20 g/L citric acid), hydrophilic antioxidant capacity (DPPH[•] system) was significantly reduced (up to 29%) after high-pressure treatment (400 MPa/25°C/15 minutes). No significant differences were found between pressurized tomato purée and thermal pasteurized products (70°C/30 seconds and 90°C/1 minute). A good correlation between depletion of vitamin C and decrease of hydrophilic antioxidant capacity was observed in pressure-treated tomato purée. Furthermore, the lipophilic antioxidant activity was not altered with the treatment of high pressure, although there was a significant increase in the concentration of total carotenoids (up to 49%), especially lycopene. Although it was achieved, higher concentration of carotenoids was extracted from tomato purée with no additives (Sánchez-Moreno et al., 2004a; Sánchez-Moreno et al., 2006a). Regarding a tomato product known in Spain as “gazpacho soup,” antioxidant capacity was not significantly affected after high-pressure treatment combined with moderate temperature (150 MPa/60°C/15 minutes and 350 MPa/60°C/15 minutes) (Plaza et al., 2006b).

The same stability in the antioxidant capacity value was observed in the studies with pressure-treated carrot and tomato juices where neither the treatment (250 MPa/35°C/15 minutes) nor the storage period (30 days, 4°C) modified the antioxidant capacity of the untreated product (Dede et al., 2007).

3.4.1. HPP Effects on Whole Fruits and Vegetables HPP is being investigated as an alternative sanitation system to improve the safety of germinated products derived from seeds and legumes (mung bean, cowpea, soybean, and alfalfa) (Muñoz et al., 2006). The increase of antioxidant activity in sprouts seeds could be due to synthesis of vitamin C or polyphenols during germination process. But when seeds were high pressure treated (300 and 400 MPa), the antioxidant capacity of sprouts at days

4 and 6, after the beginning of germination, suffered a significant decrease up to 3–4% and 12–14%, respectively (Doblado et al., 2007).

HPP effects on antioxidant capacity of whole fruit and vegetables are not frequently investigated, and few studies have been reported in the scientific literature. HPP of broccoli (400 MPa/25°C/2 minutes and 600 MPa/25°C/2 minutes) did not affect its antioxidant activity. On the other hand, in pressure (at 400 MPa/25°C/2 minutes)-treated carrots, it was reduced by 21%. Finally, in green beans, hydrophilic antioxidant activity was increased when high-pressure treatments at 400 MPa and 600 MPa were applied during 2 minutes and 25°C. Again, it is shown that the behavior of the antioxidant capacity varied to the type of plant product treated (Doblado et al., 2007). Grape skin samples extracted at 200, 400, and 600 MPa with 50% ethanol at 20°C showed antioxidant capacity value (ABTS^{•+}) three-fold higher than the control samples. The antioxidant capacity value in the extracts obtained at different pressure intensities (200–600 MPa) was not significantly different due to the similar extent of cell membrane disruption caused (Corrales et al., 2009).

In conclusion, HPP are not limited to pasteurization and sterilization, but it can also be used to extract bioactive compounds and increase the antioxidant activity of plant products and as a result of improving its functionality.

4. Effects of PEF on Nutritional Quality and Health-Related Compounds of Fruit and Vegetable Products

PEF processing is a nonthermal processing technology that has been studied in the last few years as an alternative to traditional heat treatments. PEF processing of food is a treatment with very short electric pulses (ms or μ s) at high electric field strengths (kV/cm) and moderate temperatures (Martín-Belloso and Elez-Martínez, 2005a, 2005b).

In comparison to the extensive research devoted to the destruction of microorganisms by PEF and the significant information existing about the effects of PEF on enzymes, there are very limited studies available about the impact of PEF on nutritional qual-

ity and health-related compounds of foods (Martín-Belloso et al., 2005). The investigation of the effect of PEF on bioactive compounds started later than the studies related to microorganisms or enzymes. In the recent years, several studies have been published about the effects of PEF on nutritional and bioactive compounds mainly in foods, especially vegetable juices. These studies investigated PEF effects on vitamins, carotenoids, phenolics, proteins and amino acids, fatty acids, and antioxidant activity.

PEF processing is revealing to be feasible to stabilize fruit juices while maintaining the nutritional and sensorial properties of juices better than heat treatment. Therefore, PEF processing has good prospects for being used in the fruit juice industry (Espach-Barroso et al., 2003).

4.1. Effects of PEF on Vitamin C of Fruit and Vegetable Products

Several investigators studied the effect of PEF on vitamin C content of juices. This is the most studied topic related to PEF and bioactive compounds.

4.1.1. Influence of PEF Processing Parameters

PEF treatments have been reported to cause less change in vitamin content than that in conventional heat treatments. Table 33.5 summarizes the impact of PEF on vitamin C of juices.

Qiu et al. (1998) studied the effects of PEF on vitamin C of orange juice. The study found higher losses of vitamin C in heat-pasteurized orange juice (7–15%) compared with PEF-treated juice (4–5%). PEF-treated orange juice (35 kV/cm, 59 μ s) retained more vitamin C than heat-treated orange juice (94.6°C, 30 seconds) (Yeom et al., 2000). Hodgins et al. (2002) reported that only a 2.5% of the initial vitamin C was lost after treating orange juice with 20 pulses of 80 kV/cm at 44°C. Moreover, Sánchez-Moreno et al. (2005a) studied the impact of PEF (35 kV/cm, 750 μ s) and thermal pasteurization (90°C, 1 minute) on orange juice L-ascorbic acid and total vitamin C content. They observed that both PEF and thermal pasteurization caused a similar decrease in L-ascorbic content ($\sim$ 7.8%) and a total vitamin C decrease of

Table 33.5. Vitamin C retention of juices processed by PEF

Juice	PEF Treatment	Vitamin C Retention (%)	Reference
Orange	$E = 35$, $t = \text{nd}$, $P = \text{nd}$, $f = 800$, $\tau = 1.46$, $T = 30$, b	95–96	Qiu et al. (1998)
	$E = 35$, $t = 59$, $P = \text{nd}$, $f = 600$, $\tau = 1.4$, $T = 60.1$, b	>96	Yeom et al. (2000)
	$E = 80$, $t = 20$ pulses, $P = \text{nd}$, $f = \text{nd}$, $\tau = 2\text{--}3$, $T = 44$, a	>97	Hodgins et al. (2002)
	$E = 40$, $t = 97$, $P = \text{bipolar}$, $f = \text{nd}$, $\tau = 2.6$, $T = 58$, b	100	Min et al. (2003a)
	$E = 35$, $t = 750$, $P = \text{bipolar}$, $f = 800$, $\tau = 4$, $T < 50$, b	91.8	Sánchez-Moreno et al. (2005a)
	$E = 35$, $t = 750$, $P = \text{bipolar}$, $f = 800$, $\tau = 4$, $T < 50$, b	93.2	Plaza et al. (2006a)
	$E = 35$, $t = 1,000$, $P = \text{bipolar}$, $f = 200$, $\tau = 4$, $T < 40$, b	91.2	Elez-Martínez et al. (2006)
	$E = 25$, $t = 400$, $P = \text{bipolar}$, $f = 200$, $\tau = 4$, $T < 35$, b	94.3	Elez-Martínez and Martín-Belloso (2007)
	$E = 35$, $t = 200$, $P = \text{bipolar}$, $f = \text{nd}$, $\tau = 2.5$, $T = \text{nd}$, b	83.1	Torregrosa et al. (2006)
	$E = 35$, $t = 94$, $P = \text{monopolar}$, $f = 952$, $\tau = 1.92$, $T = 26\text{--}27$, b	98	Evrendilek et al. (2000)
Apple	$E = 35$, $t = 94$, $P = \text{monopolar}$, $f = 952$, $\tau = 1.92$, $T = 26\text{--}27$, b	100	Evrendilek et al. (2000)
Apple cider	$E = 35$, $t = 1,500$, $P = \text{bipolar}$, $f = 232$, $\tau = 1$, $T < 40$, b	92	Odriozola-Serrano et al. (2008a)
Strawberry	$E = 40$, $t = 57$, $P = \text{bipolar}$, $f = \text{nd}$, $\tau = 2$, $T = 53$, b	100	Min et al. (2003b)
Tomato	$E = 35$, $t = 1,000$, $P = \text{monopolar}$, $f = 250$, $\tau = 4$, $T < 40$, b	82.6	Odriozola-Serrano et al. (2007)
"Gazpacho"	$E = 35$, $t = 1,000$, $P = \text{bipolar}$, $f = 250$, $\tau = 1$, $T < 40$, b	88	Odriozola-Serrano et al. (2008c)
	$E = 25$, $t = 400$, $P = \text{bipolar}$, $f = 200$, $\tau = 4$, $T < 35$, b	92.3	Elez-Martínez and Martín-Belloso (2007)

E, electric field (kV cm^{-1}); t, treatment time (μs); P, pulse polarity; f, pulse frequency (Hz); τ , pulse width (μs); T, treatment temperature ($^{\circ}\text{C}$); a, Batch mode PEF treatment; b, Continuous PEF treatment.
nd, no data available.

about 8.2%. PEF orange juices showed a significant decrease in L-ascorbic acid content (7.7%) and in total vitamin C content (6.8%) just after treatment when compared with untreated juices. With regard to a minimal heat pasteurization (70°C , 30 seconds), orange juice did not exert any change (Plaza et al., 2006a). The authors reported the loss of vitamin C in orange juice after PEF processing, although these losses were always less than those obtained after a heat treatment. On the other hand, Min et al. (2003a) reported no differences between ascorbic acid

content of fresh orange juice and PEF-treated orange juice when they processed orange juice at 40 kV/cm for $97 \mu\text{s}$, while thermal processing (90°C , 90 seconds) reduced 19% of ascorbic acid. The differences between the results reported in the literature could be mainly due to the differences in PEF operation conditions (electric field strength, treatment time, pulse width, pulse polarity, pulse frequency, temperature, and flow) employed to treat the samples (Table 33.5). Moreover, other factors, such as the different equipment used and the orange variety

and ripeness, could be also responsible for the diversity in vitamin C retention.

In another study about apple-derived products, Evrendilek et al. (2000) studied changes in vitamin C of fresh apple cider treated by PEF (35 kV/cm, 94 μ s) and PEF+heat (35 kV/cm, 94 μ s+60°C, 30 seconds) and of reconstituted apple juice treated by PEF (35 kV/cm, 94 μ s). They concluded that PEF processing was significantly effective in retaining vitamin C content in the juice samples. Vitamin C retention higher than 87% was attained after processing strawberry juice at 35 kV/cm for up to 2,000 μ s (Odriozola-Serrano et al., 2008a).

Effects of PEF processing on the ascorbic acid content of tomato juice were studied and compared with those of thermal processing by Min et al. (2003b). Tomato juice was thermally processed at 92°C for 90 seconds or PEF processed at 40 kV/cm for 57 μ s. Ascorbic acid decreased 10% after thermal processing, but did not decrease significantly after PEF processing. PEF-treated (35 kV/cm for 1,500 μ s) tomato juice exhibited higher vitamin C content just after the treatment than thermally treated

(90°C, 30 seconds and 90°C, 60 seconds) tomato juice (Odriozola-Serrano et al., 2008b).

Though specific conditions that assure microbial inactivation have been investigated in different products, few studies have aimed at evaluating the effect of PEF critical process parameters on vitamins. Elez-Martínez and Martín-Belloso (2007) observed that vitamin C retention depended on process parameters. Bipolar pulses led to higher vitamin C contents. At lower values of the electric field strength, treatment time, pulse frequency, and the pulse width, vitamin C retention increased, in both orange juice and gazpacho (Figure 33.1). Torregrosa et al. (2006) observed a reduction in ascorbic acid content with increased electric field strength and the treatment time. Odriozola-Serrano et al. (2007, 2008c) reported that lower the electric field strength, treatment time, pulse width, or higher the pulse frequency, the greater the vitamin C content of tomato juices processed by PEF.

4.1.2. Influence of Refrigerated Storage Most of the works regarding the retention of vitamin C during storage of PEF-treated products agreed with the fact that juices processed by PEF retained

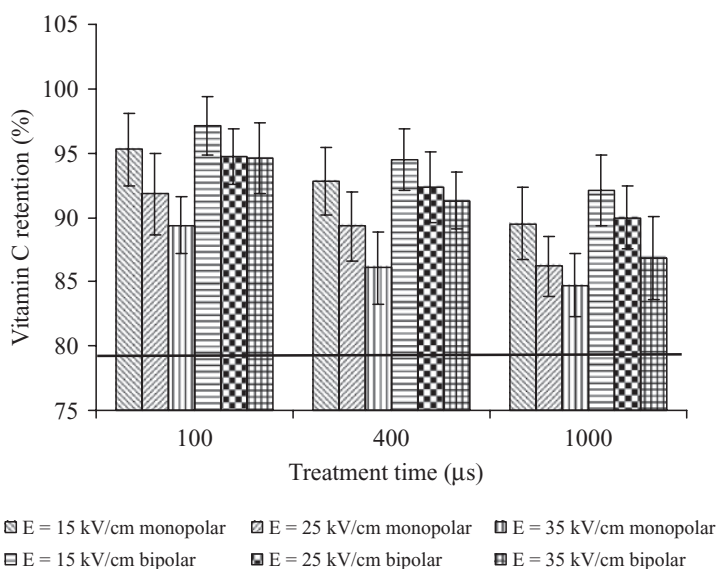


Figure 33.1. Retention of vitamin C in "gazpacho" after exposure to mono- and bipolar PEF at different electric field strengths (E) and treatment times (mean $\pm$ SD). Treatments were performed at 200 Hz and 4- μ s square pulses. Horizontal line corresponds to a thermal treatment (90°C, 1 minute). (From Elez-Martínez and Martín-Belloso, 2007.)

better vitamin C during storage than those processed by traditional thermal treatments. Qiu et al. (1998) observed that the PEF-treated orange juice retained a greater amount of vitamin C (68%) when compared with heat-pasteurized samples (46%) after 90 days at 4°C. Moreover, Yeom et al. (2000) reported that PEF-treated (35 kV/cm, 59 μ s) orange juice retained a higher ascorbic acid content than the heat-pasteurized (94.6°C, 30 seconds) orange juice during 4°C storage. The concentrations of ascorbic acid in thermally processed (90°C, 90 seconds), PEF-processed (40 kV/cm for 97 μ s), and control orange juices decreased with increasing storage time. However, PEF-processed orange juice retained more ascorbic acid than thermally processed orange juice for 84 days at 4°C (Min et al., 2003a). Vitamin C retention was outstandingly higher in orange juice processed by PEF (35 kV/cm for 1,000 μ s; bipolar 4- μ s pulses at 200 Hz) fitting recommended daily intake standards throughout 56-day storage at 4°C, whereas heat-processed (90°C, 1 minute) juice exhibited poor vitamin C retention beyond 14 days of storage (Elez-Martínez et al., 2006). A PEF-processed (35 kV/cm for 750 μ s; bipolar pulses of 4 μ s at 800 Hz) orange juice stored during 40 days at 4°C showed a 32.3% of vitamin C losses at the end of the storage (Plaza et al., 2006a).

PEF processing (35 kV/cm, 94 μ s) increased the retention of vitamin C in apple juice at 4 and 22°C, and this treatment did not change vitamin C retention in apple cider during storage (Evrendilek et al., 2000).

Min et al. (2003b) reported that the concentration of ascorbic acid in tomato juice decreased as the storage time increased regardless of (thermal or PEF) processing methods. However, a higher retention of ascorbic acid in PEF-processed tomato juice than in thermally processed juice was observed during storage until 42 days at 4°C.

4.2. Effects of PEFs on Carotenoids of Fruit and Vegetable Products

4.2.1. Influence of PEF Processing Parameters

A PEF treatment of 35 kV/cm in bipolar mode,

800 Hz, 4 μ s pulse width, and 750 μ s did not modify the profile of individual carotenoids (β -cryptoxanthin, α -cryptoxanthin, zeaxanthin, lutein, β -carotene, α -carotene), nor the total carotenoids content of PEF-processed orange juice (Sánchez-Moreno et al., 2005a). The concentrations of total carotenoids in heat-pasteurized orange juices were lower than in PEF-processed juices (Cortés et al., 2006a, 2006b). Moreover, PEF treatments of increasing duration generally resulted into a significant increase in the concentrations of individual extractable carotenoids (neoxanthin + 9-cis-violaxanthin, antheraxanthin, mutatoxanthin, lutein, zeaxanthin, α -cryptoxanthin, β -cryptoxanthin, cis- β -cryptoxanthin, 9-cis- α -carotene, α -carotene, phytoene + phytofluene, β -carotene, 13-cis- β -carotene, ζ -carotene, 9-cis-b-carotene) in an orange-carrot juice (Torregrosa et al., 2005).

On the other hand, a work conducted by Min et al. (2003b) reflected that the concentration of lycopene in tomato juice did not change significantly after thermal (92°C for 90 seconds) or PEF processing (40 kV/cm for 57 μ s). However, an increase in the lycopene content was observed when tomato juice was PEF-processed for prolonged treatment times (35 kV/cm for 1,500 μ s) (Odriozola-Serrano et al., 2008b). Moreover, higher electric field strength, treatment time, frequency, and pulse width result in a greater lycopene relative content in tomato juices treated by PEF (Odriozola-Serrano et al., 2007, 2008c).

4.2.2. Influence of Refrigerated Storage The carotenoid retention during storage of PEF-treated plant products has been less studied than the effect of PEF processing. Cortés et al. (2006a) reported that PEF treatment (30 kV/cm, 100 μ s) had less effect than conventional thermal treatment (90°C, 20 seconds) on the concentrations of total carotenoids and vitamin A in refrigerated orange juice. With PEF treatment, they observed no significant decrease in the concentration of any carotenoid in comparison to the untreated juice.

Min et al. (2003b) studied the lycopene retention during storage at 4°C of tomato juice processed

by PEF (40 kV/cm, 57 μ s) or by thermal treatment (92°C, 90 seconds). They observed that there was no significant differences in the concentration of lycopene among thermally processed, PEF-processed, and control juices during storage.

4.3. Effects of PEF on Phenolic Compounds of Fruit and Vegetable Products

Little is known about the effect of PEF treatments on the phenolic content of plant-based foods. Apple juice was pasteurized using a PEF treatment (35 kV/cm, bipolar pulses of 4 μ s, 1,200 pulses per second) and compared with a thermal pasteurization (90°C, 30 s). The thermal treatment caused a considerable loss of phenols (32.2%) when compared with the PEF treatment, which only caused a 14.5% reduction (Aguilar-Rosas et al., 2007). Moreover, no significant differences were observed in total phenolic content between untreated and PEF-treated (35kV/cm for 1,500 μ s with 4- μ s bipolar pulses at 100 Hz) tomato juice just after processing (Odriozola-Serrano et al., 2008b).

A continuous PEF system was used to study the impact of PEF on total anthocyanin of cranberry juice. No statistically significant differences were observed in anthocyanin pigment content between PEF-treated samples and controls. However, thermal treatment significantly reduced the anthocyanin pigment content (Jin and Zhang, 1999). On the other hand, the degradation of the anthocyanin cyanidin-3-glucoside isolated from red raspberry and exposed to PEF was increased as the electric field strength and the treatment time increased ($p < 0.05$) (Zhang et al 2007).

In addition, there are almost no reports about the effect of PEF on individual phenolics. A PEF treatment of 35 kV/cm in bipolar mode, 800 Hz pulse frequency, 4- μ s pulse width, and 750 μ s total treatment time (maximum temperature of 50°C) did not modify the naringenin or the hesperetin as well as the total flavanone content of orange juice (Sánchez-Moreno et al., 2005a).

4.4. Effects of PEF on Other Health-Related Compounds in Fruit and Vegetable Products

Little in-depth work has been carried out to date on the effects of PEF on plant food proteins. PEF treatment (35 kV/cm, 1 μ s, 4- μ s bipolar pulses, 1,000 Hz, temperature below 40°C) was evaluated by Garde-Cerdán et al. (2007) to assess their effects on grape juice proteins. The ammonium concentration was not affected by PEF treatments. Treatments applied did not also affect the total concentration of amino acids. In PEF-treated grape juice, the concentrations of histidine, tryptophan, asparagine, phenylalanine, and ornithine were more than that in control samples. Li et al. (2007) studied the effects of PEF treatment (0–40 kV/cm and 0–547 μ s, bipolar pulses of 2 μ s and 500 Hz pulse frequency) on physicochemical properties of soybean protein isolates (SPI). They concluded that PEF treatment caused insignificant change in physicochemical properties and structure of SPI.

PEF treatments have little effect on total fatty acid content of grape juice. Linolenic and oleic acid contents were unaffected by PEF treatment. However, PEF treatment made that the concentration of lauric acid in the grape juice diminished. In a similar way, the processing treatment did not modify the long-chain saturated fatty acids (C14 and C16) (Garde-Cerdán et al., 2007).

4.5. Effects of PEF on Antioxidant Activity of Fruit and Vegetable Products

A PEF processing of 35 kV/cm for 750 μ s with bipolar pulses of 4 μ s width and 800 Hz without exceeding 50°C did not modify the radical scavenging capacity (DPPH• model system) of orange juice. However, a traditional thermal treatment of 90°C for 1 minute led to a significant decrease in the radical scavenging capacity (Sánchez-Moreno et al., 2005a). These authors also found a significant correlation between both L-ascorbic acid and total vitamin C versus antiradical efficiency, whereas no correlation was found between total carotenoids or total flavanones and the antiradical efficiency parameter.

Thus, it seems that the radical scavenging capacity of treated and untreated orange juices towards DPPH[•] is mainly modulated by vitamin C.

Inhibition of DPPH for untreated, traditionally heat-pasteurized (90°C, 1 minute), and PEF treated (35 kV/cm for 1,000 μ s with 4- μ s bipolar pulses at 200 Hz) orange juice was 41.4%, 30.6%, and 35.2%, respectively (Elez-Martínez et al., 2006). Nevertheless, neither PEF processing (35 kV/cm for 1,500 μ s) nor thermal treatments (90°C for 30 seconds or 90°C for 60 seconds) changed the antioxidant activity of fresh tomato juice (Odriozola-Serrano et al., 2008b).

Elez-Martínez and Martín-Belloso (2007) studied the effects of PEF-processing parameters on the antioxidant activity of orange juice and “gazpacho” soup and compared them to those in heat pasteurization (90°C, 1 minute). They found that PEF treatments did not affect the antioxidant activity of

orange juice and “gazpacho,” whereas heat-treated products showed lower values of antioxidant activity (Table 33.6). On the other hand, antioxidant activity of strawberry juice decreased significantly as electric field strength and treatment time increased (Odriozola-Serrano et al., 2008a). Moreover, an increase in pulse width (from 1 to 7 μ s) or pulse frequency (from 50 to 250 Hz) led to a decrease in the antioxidant activity of tomato juice processed by PEF (Odriozola-Serrano et al., 2007).

The antioxidant activity in terms of DPPH radical scavenging capacity of PEF-treated orange juices was studied by Elez-Martínez et al. (2006) and Plaza et al. (2006a) along the storage time. Elez-Martínez et al. (2006) reported that the antioxidant activity of PEF-treated (35 kV/cm, 1,000 μ s, 200 Hz) and untreated orange juice decreased slightly during storage. Moreover, heat treatments (90°C, 1 minute)

Table 33.6. PEF and thermal treatment effects on antioxidant capacity (% inhibition DPPH) of orange juice and “gazpacho”^a (from Elez-Martínez and Martín-Belloso, 2007)

Polarity Mode	Electric Field Strength (kV/cm)	Treatment Time (μ s)	Orange Juice	“Gazpacho”
Monopolar	15	100	41.2 $\pm$ 2.1de	46.6 $\pm$ 2.4bc
		400	37.6 $\pm$ 2.7abcd	44.6 $\pm$ 2.7bc
		1,000	38.1 $\pm$ 2.2abcd	43.1 $\pm$ 2.9abc
	25	100	37.9 $\pm$ 2.6abcd	43.5 $\pm$ 2.8abc
		400	38.4 $\pm$ 1.7abcde	42.6 $\pm$ 3.0abc
		1,000	36.3 $\pm$ 2.1ab	44.3 $\pm$ 2.7bc
	35	100	38.4 $\pm$ 2.2abcde	45.2 $\pm$ 2.6bc
		400	37.4 $\pm$ 2.0abcd	42.1 $\pm$ 2.7ab
		1,000	37.1 $\pm$ 1.9abc	43.5 $\pm$ 3.1abc
Bipolar	15	100	38.5 $\pm$ 2.2abcde	47.1 $\pm$ 2.6c
		400	37.9 $\pm$ 2.4abcd	46.2 $\pm$ 2.9bc
		1,000	37.8 $\pm$ 2.9abcd	43.9 $\pm$ 2.4abc
	25	100	40.2 $\pm$ 2.1cde	46.5 $\pm$ 3.0bc
		400	42.1 $\pm$ 3.5e	43.4 $\pm$ 2.6abc
		1,000	39.6 $\pm$ 2.4bcde	44.3 $\pm$ 3.1bc
	35	100	37.4 $\pm$ 2.6abcd	43.2 $\pm$ 2.9abc
		400	39.8 $\pm$ 2.5bcde	45.9 $\pm$ 2.7bc
		1,000	37.6 $\pm$ 2.2abcd	44.1 $\pm$ 3.1abc
Thermal treatment (90°C/1 minute)			35.4 $\pm$ 2.0 a	39.6 $\pm$ 2.9a
Untreated			39.3 $\pm$ 1.5bcde	45.7 $\pm$ 1.6bc

Different letters in the same column indicate significant differences ($p < 0.05$).

PEF treatments were performed at 200 Hz and pulses of 4 μ s.

^aValues are mean $\pm$ SD from three measurements of triplicate treatments.

resulted in lower free-radical scavenging values but no differences were found between PEF-processed and unprocessed orange juice. Plaza et al. (2006a) observed that, after 40 days of storage at 4°C, although the untreated orange juice showed the highest antiradical efficiency value, there was no differences between PEF-processed (35 kV/cm, 750 μ s, 800 Hz) and low-pasteurized (70°C, 30 seconds) orange juice in terms of antiradical efficiency.

5. Effects of HPP and PEF on Health-Related Properties of Fruit and Vegetable Products

Consumption of fruits and vegetables has been associated with reduced risks of certain diseases (Liu et al., 2000). Current recommendations are that everyone should eat at least five portions of a variety of fruit and vegetables daily to reduce the risk of chronic diseases and possibly delay the onset of age-related problems (Subar et al., 1995; Sorensen et al., 1999; Cullum, 2003). Recent reports suggested that drinking generous amounts of a mixture of various juices improves the blood lipid profile, reduces oxidative stress, and prevents atherogenic modifications of LDL cholesterol and platelet aggregation (Young et al., 1999; Aviram et al., 2000).

5.1. Bioavailability

Bioavailability is defined as the proportion of the nutrients, bioactive compounds, or phytochemicals that is digested, absorbed, and metabolized through normal pathways (Srinivasan, 2001). Consequently, it is not enough to know how much of these compounds are present in the food products, the more important issue is how much of those present are bioavailable. In addition, since the active compound must reach the relevant sites of the body in appropriate concentrations and active form(s), the bioavailability of these compounds is an essential parameter determining their action. Many factors (agronomic conditions, food matrix, processing, storage conditions, etc.) can influence the bioavailability of nutrients, bioactive compounds or phytochemicals. Among them, processing can improve their availability. Porrini et al. (1998) found higher total lycopene plasma concen-

trations after ingestion of tomato purée as opposed to raw tomatoes. Therefore, it is important to evaluate how different processing conditions may affect the bioavailability of nutrients, bioactive compounds, or phytochemicals present in fruit and vegetable products.

5.2. Antioxidants

Antioxidant nutrients have important role in cell function and have been implicated in processes associated with aging, including vascular and inflammatory damage. By acting as antioxidants and at the molecular level, vitamins E and C can participate in preserving cell membrane composition and function, protecting molecules such as cholesterol and DNA from oxidative damage, and consequently improving human health and decrease chronic disease (Van Poppel and van den Berg, 1997; Brigelius-Flohe et al., 2002). Vitamin C may also contribute to the maintenance of a healthy vasculature and to a reduction in atherogenesis through the regulation of collagen synthesis, prostacyclin production, and nitric oxide (Simon, 1992; Ness et al., 1996a, 1996b). The second National Health and Nutrition Examination Survey reported that a low intake of vitamin C is associated with blood concentrations of vitamin C ≤ 0.3 mg/dL (17 μ mol/L) (Simon et al., 2001), whereas blood concentrations in well-nourished persons fluctuate between 0.8 and 1.3 mg/dL (45 and 74 μ mol/L). Fruit and vegetables are the main sources of vitamin C, but 25% of women and $\sim$ 33% men eat < 2.5 servings of fruit and vegetables daily, which provides $\sim$ 80 mg vitamin C/day.

5.3. Health-Related Biomarkers

Because oxidative stress plays an important role in most disease processes and aging, the potential health benefits of fruits and vegetables were largely attributed to their potential antioxidant capacity. However, recent data indicate that the protective effect of fruits and vegetables may extend beyond this antioxidant capacity (Kris-Etherton et al., 2002; Scalbert et al., 2005; Castilla et al., 2006). To investigate the effect of the consumption of orange juice and vegetable soup processed by these novel food

technologies on oxidative stress, authors measured the levels of F_2 -isoprostanes, which provide a meaningful estimation of oxidative stress status in vivo (Pratico, 1999; Block et al., 2002). One of the isoprostanes, 8-epi-prostaglandin $F_{2\alpha}$, was shown to increase with age and chronic disease (Morrow and Roberts, 1997; Roberts and Reckelhoff, 2001), and decrease after the consumption of isoflavones in soy and commercial orange juice and vegetable soup (Wiseman et al., 2000; Sánchez-Moreno et al., 2003b, 2006b).

In addition, hyperuricemia may have a direct injurious effect on the endothelium, altering endothelial cell function and reducing nitric oxide bioavailability, relevant in the development of vascular dysfunction and cardiovascular risk. In fact, elevated serum uric acid concentration appears to be an important risk factor in predicting myocardial infarction (Bickel et al., 2002).

Oxidative stress and inflammation have been implicated in many heart, lung, blood, and sleep disorders, including stroke, atherosclerosis, hypertension, asthma, acute lung injury, heart failure, and sleep apnea (Sánchez-Moreno et al., 2004b; Surh and Packer, 2005). There are several inflammation biomarkers, such as C-reactive protein (CRP), prostaglandin E_2 (PGE_2), adhesion molecules, quimokines, and cytokines.

Plasma CRP levels have recently been identified as an emerging risk factor for ischemic heart disease. Results from cross-sectional and prospective studies have indicated that raised plasma CRP levels are associated with an increased risk for future cardiovascular events among apparently healthy subjects (Ridker et al., 2000).

PGE_2 , produced during inflammation, is a potent inhibitor of T-cell activation and immune response (Harris et al., 2002). Monocyte chemotactic protein-1 (MCP-1) is expressed at high levels in atherosclerotic plaques (Reape and Groot, 1999). Cytokines such as tumor necrosis factor- α (TNF- α), interleukin- 1β (IL- 1β), and interleukin-6 (IL-6) are responsible for the progression of the inflammatory response. Given that systemic low-grade inflammatory activity has a strong prognostic value in cardiovascular disease, some authors consider that

circulating levels of cytokines, such as TNF- α , and IL-6 would be appropriate markers with which to assess the anti-inflammatory effect of antioxidants in vivo (Bruunsgaard et al., 2003). Therefore, associations between these inflammatory and oxidative stress biomarkers in relation to antioxidant concentrations after dietary interventions will provide valuable information regarding the health benefits of diets rich in fruits and vegetables.

The studies by Sánchez-Moreno et al. (2003a, 2003b, 2004c, 2004d, 2005b, 2006b) are the first works assessing the effect of consuming fruit and vegetable products treated by high hydrostatic pressure or pulsed electric field technologies on vitamin C bioavailability and biomarkers of oxidative stress and inflammation in a healthy human population. Specifically, the studies have been carried out with orange juice and a vegetable soup “gazpacho” (Pinilla et al. 2005).

The vitamin C bioavailability study was divided into two components: a dose-response test and a multiple-dose response. For the dose-response evaluation, after a minimum of 12 h of fasting blood was drawn before and every 60 min for 6 h after subjects drank 500 mL of juice or “gazpacho”. For the multiple-dose response, the subjects were instructed to drink the juice or “gazpacho” at home, in two doses, 250 mL in the morning and 250 mL in the afternoon, for 2 consecutive weeks. Blood samples were taken again during the intervention on d 7 and 14 of the study.

The HP (high pressure) treatment for orange juice and “gazpacho” were 400 MPa at 40°C for 1 min and 400 MPa at 40°C for 15 min, respectively. The PEF processing conditions for orange juice and “gazpacho” were 35 kV/cm electrical field applied in a bipolar mode, 800 Hz pulse frequency, 4 μ s pulse width, and 750 μ s total treatment time. Temperature was never more than 50°C. After treatment, HPP-treated and PEF-treated orange juice and “gazpacho” were kept at 4°C until its consumption.

For HP-treated orange juice group, baseline plasma levels of vitamin C were higher ($p = 0.014$) in women than in men (Table 33.7), in agreement with a previous study from our research group (Sánchez-Moreno et al., 2003b).

Table 33.7. Plasma vitamin C, 8-isoprostane (8-*epi*PGF_{2α}), uric acid, C-reactive protein (CRP), and prostaglandin (PG) E₂ concentration at baseline and on days 7 and 14 drinking high-pressurized (HP) orange juice daily for 14 days in men and women^{a,b} (from Sánchez-Moreno et al., 2003a)

	Men			Women		
	Baseline	day 7	day 14	Baseline	day 7	day 14
Vitamin C, ^c μmol/L	42.8 ± 2.1	63.9 ± 2.8	67.7 ± 4.2	55.8 ± 3.8*	79.3 ± 4.7*	73.4 ± 3.4
8- <i>epi</i> PGF _{2α} , ^d ng/L	150.8 ± 12.2	133.3 ± 18.1	111.7 ± 10.0	133.1 ± 4.1	116.5 ± 9.5	112.2 ± 3.8
Uric acid, μmol/L	389.8 ± 32.7	331.0 ± 26.4	346.4 ± 33.3	213.7 ± 11.1*	192.9 ± 18.3*	187.3 ± 17.8*
CRP, mg/L	0.25 ± 0.07	ND ^e	0.15 ± 0.07	0.23 ± 0.08	ND	0.10 ± 0.06
PGE ₂ , ^f ng/L	353.7 ± 18.7	ND	285.9 ± 21.2	314.3 ± 21.4	ND	248.4 ± 12.7

^aValues are means ± SEM, *n* = 6.

^bThere was not a significant sex × time interaction for vitamin C (*p* = 0.671), 8-*epi*PGF_{2α} (*p* = 0.641), uric acid (*p* = 0.746), CRP (*p* = 0.817), and PGE₂ (*p* = 0.959) based on repeated-measures ANOVA.

^cHigher than baseline for men and women combined at both 7 and 14 d (time effect), *p* < 0.05, based on repeated-measures ANOVA (Tukey's est).

^dLower than baseline for men and women at d 14 (time effect), *p* < 0.05, based on repeated-measures ANOVA (Tukey's test).

^eND, not determined.

^fLower than baseline for men and women at d 14 (time effect), *p* < 0.05, based on a Student's *t* test.

*Different from men at that time, *p* < 0.05.

For HP-treated “gazpacho” group, there was no difference (*p* = 0.060) in baseline plasma vitamin C concentrations between men and women (Table 33.8). The plasma vitamin C concentration after drinking the HP vegetable soup increased in both men and women with no differences between days 7 and 14 (Table 33.8). Plasma vitamin C concentrations increased by 26% in men (*p* = 0.01) and by 25% in women (*p* = 0.017) (Table 33.8).

The maximum increase in plasma vitamin C occurred 3–4 hours postdose in both the high pressure- and PEF-treated groups (Figure 33.2).

By comparing with baseline, the vitamin C concentration was significantly higher on days 7 and 14 of the intervention in both men and women (*p* < 0.05) (Figure 33.3).

Plasma 8-*epi*PGF_{2α} concentrations were lower at the end of the study in both groups (for both products). Plasma levels of vitamin C and 8-*epi*PGF_{2α} were inversely correlated also in both groups (for both products) (Figures 33.4–33.6).

For HP-treated orange juice group, plasma CRP concentrations tended to be lower on day 14 than at baseline in men (*p* = 0.317) and women (*p* = 0.235). Plasma PGE₂ was lower at the end of the study in

both men and women (*p* ≤ 0.037) (Sánchez-Moreno et al., 2003a) (Table 33.7).

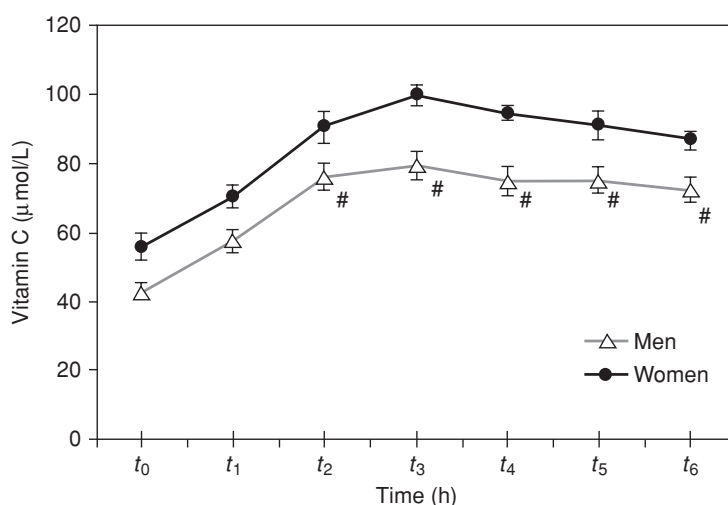
For HP-treated “gazpacho” group, plasma PGE₂ and MCP-1 concentrations decreased in men and women (*p* ≤ 0.05) on day 14, but those of TNF-α, IL-1β, and IL-6 did not change (Sánchez-Moreno et al., 2004d) (Table 33.8).

Consumption of HP-treated orange juice or HP-treated “gazpacho” was associated with an increased plasma concentration of vitamin C and decreased biomarkers of inflammation in both women and men. There was an inverse correlation between concentrations of plasma vitamin C and concentrations of 8-*epi*PGF_{2α}, uric acid, PGE₂, and MCP-1, suggesting that vitamin C may play a critical role in reducing the formation of compounds produced by random oxidation of phospholipids by oxygen radicals involved in the development of oxidative processes and inflammation.

For PEF-treated orange juice (Table 33.9) and “gazpacho” (Table 33.10) groups, vitamin C remained significantly higher on days 7 and 14 in both groups. Plasma 8-*epi*PGF_{2α} concentrations were lower at the end of the study in both groups (Sánchez-Moreno et al., 2004c, 2005b).

Table 33.8. Plasma vitamin C, 8-*epi*PGF_{2α}, uric acid, PGE₂, MCP-1, TNF-α, IL-1β, and IL-6 concentrations in men and women at baseline and on days 7 and 14 of drinking HP “gazpacho” daily for 14d^a (from Sánchez-Moreno et al., 2004d)

	Men			Women		
	Baseline	day 7	day 14	Baseline	day 7	day 14
Vitamin C, ^b μmol/L	44.4 ± 1.1	54.2 ± 1.7	56.1 ± 1.8	51.1 ± 1.3	63.9 ± 2.0	63.7 ± 1.8
8- <i>epi</i> PGF _{2α} , ^c ng/L	195.1 ± 11.6	155.2 ± 16.9	141.5 ± 13.2	137.3 ± 8.3*	114.7 ± 8.6*	98.2 ± 7.9*
uric acid, ^d μmol/L	420.2 ± 21.0	357.0 ± 8.3	362.4 ± 9.2	276.5 ± 11.1*	248.7 ± 13.2*	253.2 ± 10.7*
PGE ₂ , ^e ng/L	321.8 ± 21.2	ND ^f	256.0 ± 18.3	236.4 ± 25.3*	ND	161.5 ± 15.6*
MCP-1, ^g ng/L	618.6 ± 45.5	ND	465.4 ± 43.6	440.0 ± 37.3*	ND	321.5 ± 41.0*
TNF-α, ng/L	5.5 ± 1.7	ND	5.8 ± 1.9	4.1 ± 1.2	ND	3.9 ± 1.1
IL-1β, ng/L	0.9 ± 0.2	ND	1.1 ± 0.2	0.8 ± 0.1	ND	0.6 ± 0.1
IL-6, ng/L	0.37 ± 0.09	ND	0.29 ± 0.08	0.20 ± 0.03	ND	0.12 ± 0.05

^aValues are means ± SEM, *n* = 6.^bHigher than baseline for men and women at days 7 and 14 (time effect), *p* < 0.05, based on repeated-measures ANOVA (Tukey's test).^cLower than baseline for men and women at days 14 (time effect), *p* < 0.05, based on repeated-measures ANOVA (Tukey's test).^dLower than baseline for men at days 7 and 14 (time effect), *p* < 0.05, based on repeated-measures ANOVA (Tukey's test).^eLower than baseline for men and women at days 14 (time effect), *p* < 0.05, based on a Student's *t* test.^fND, not determined.^gLower than baseline for men and women at days 14 (time effect), *p* < 0.05, based on a Student's *t* test.*Different from men at that time, *p* < 0.05.**Figure 33.2.** Plasma vitamin C concentrations in men and women at baseline (*t*₀) and at 1 (*t*₁), 2 (*t*₂), 3 (*t*₃), 4 (*t*₄), 5 (*t*₅), and 6 hours (*t*₆) after drinking 500 mL of orange juice processed under high pressure (HP) containing 250 mg vitamin C. Values are means ± SEM, *n* = 6. There was a significant main effect of sex, but no sex × time interaction (ANOVA). In both men and women, times 0 and 1 differed from all other times, *p* < 0.05. (From Sánchez-Moreno et al., 2003a.)

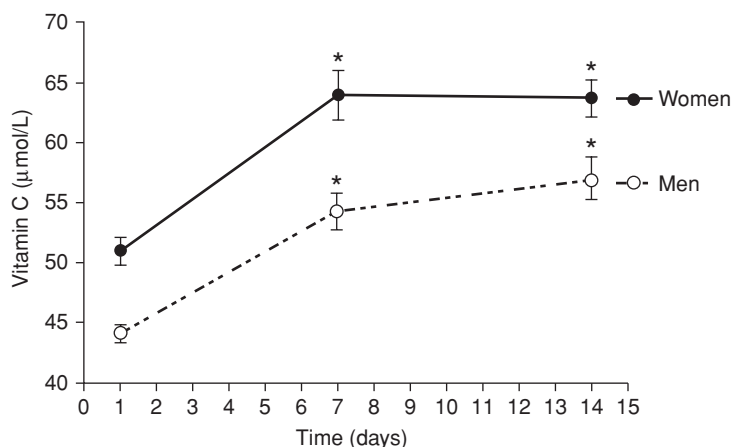


Figure 33.3. Plasma vitamin C concentrations in men and women at baseline and after 7 and 14 days of consuming “gazpacho.” Values are means $\pm$ SEM, $n = 6$. Means for both genders at days 7 and 14 were greater than at day 0, $p < 0.05$. (From Sánchez-Moreno et al., 2004d.)

To summarize the studies carried out by Sánchez-Moreno et al. in this topic, drinking two servings (500 mL) of HP- or PEF-treated orange juice or “gazpacho” daily maintained the vitamin C bioavailability and antioxidant properties of fresh products with a longer shelf life, increasing plasma vitamin C, and decreasing oxidative stress and biomarkers of inflammation in healthy humans.

6. Final Remarks

Nonthermal processing of fruit and vegetable has been revealed as useful tool to extend their shelf life and quality as well as to preserve their nutritional and functional characteristics. In this way, technologies such as HPP and PEF, could increase the extraction of bioactive compounds from fruit and

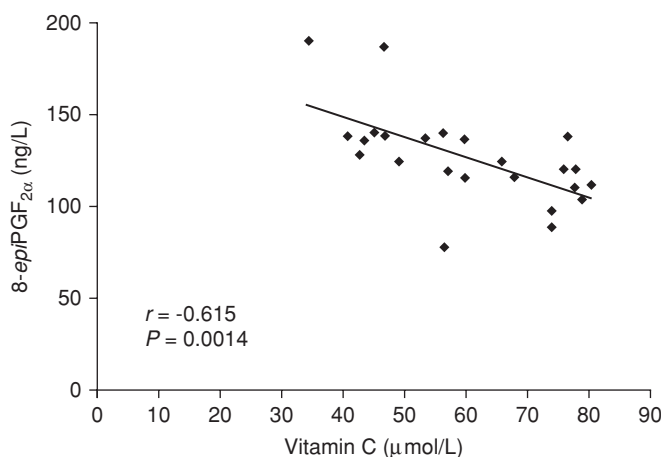


Figure 33.4. Inverse correlation between plasma 8-epiPGF_{2α} and vitamin C concentrations at baseline (t_0) and at day 14 of drinking orange juice processed under high pressure (HP) in 6 men and 6 women ($n = 24$). (From Sánchez-Moreno et al., 2003a.)

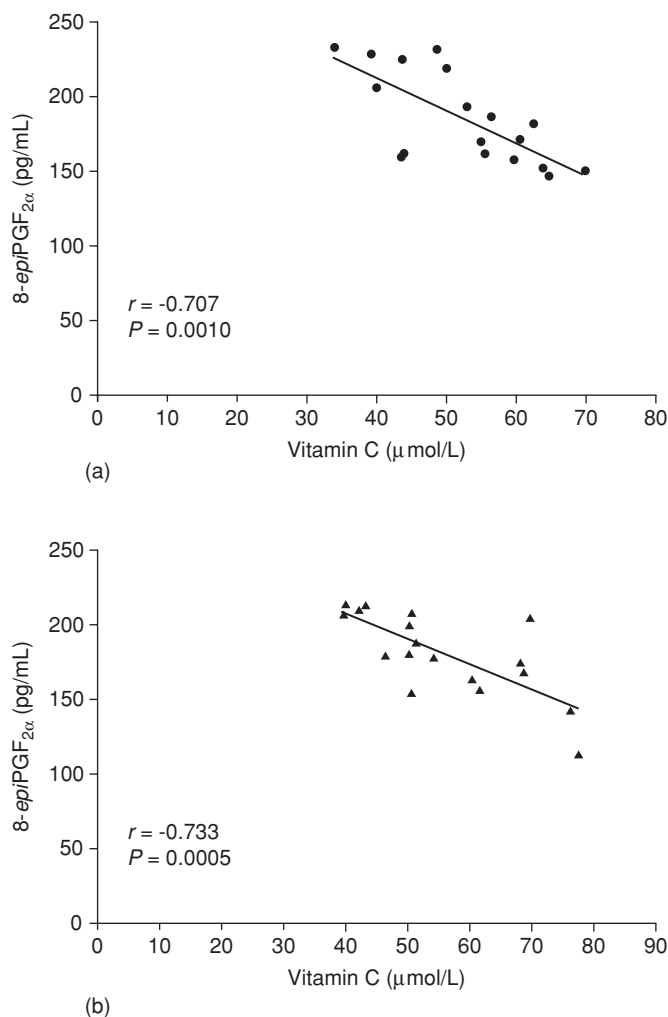


Figure 33.5. Inverse correlation between plasma 8-epiPGF_{2α} and vitamin C concentrations at baseline and at days 7 and 14 of drinking pulsed electric field-treated orange juice ($n = 18$). (a) Study group given pulsed electric fields-treated orange juice. (b) Study group given freshly squeezed orange juice. (From Sánchez-Moreno et al., 2004c.)

vegetables and increase their healthy potential, probably improving their bioavailability. These technologies also enable the food processors to process novel healthy vegetable-derived products by combining these technologies with other food processing treatments or by using them to extract bioactive ingredients from low-cost raw material or vegetable by-products to obtain functionalized products with better sensorial quality and assured safety.

In this regard, the research studies carried out on bioavailability and antioxidant properties of the nonthermal-processed vegetable products, such as orange juice and vegetable soup, indicate a better vitamin C bioavailability and an improved health potential by an increase of plasma vitamin C and a decrease of the oxidative stress and inflammation biomarkers in healthy humans.

Further research will be necessary for exploring new application of nonthermal processing

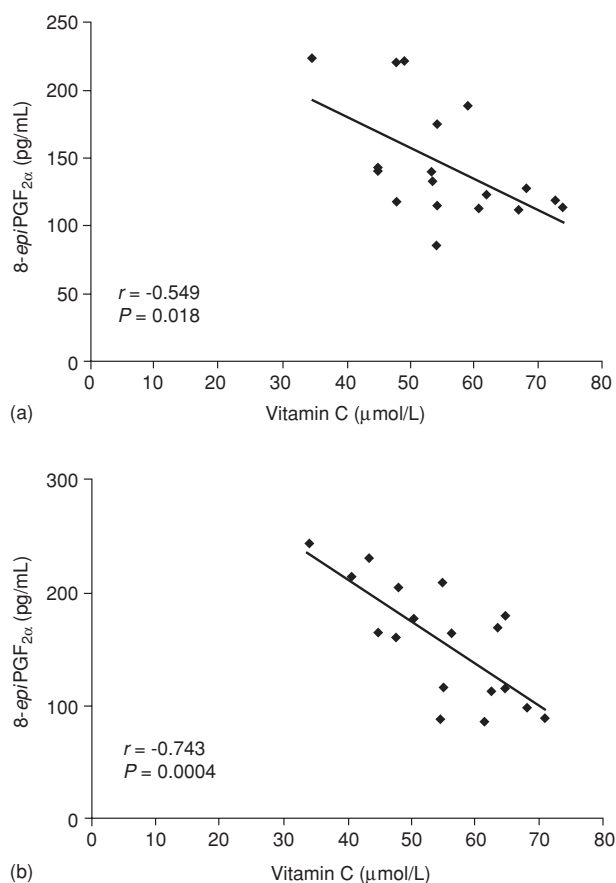


Figure 33.6. Correlation between plasma concentrations of 8-*epi*PGF_{2α} and vitamin C at baseline and at days 7 and 14 of the intervention ($n = 18$). (a) PEF-treated vegetable soup group. (b) Freshly made vegetable soup. (From Sánchez-Moreno et al., 2005b.)

Table 33.9. Plasma vitamin C, 8-*epi*PGF_{2α}, and uric acid concentration at baseline and on days 7 and 14 of drinking pulsed electric fields (PEF)-treated and freshly squeezed (FS) orange juices (from Sánchez-Moreno et al., 2004c)

	PEF Treated			FS		
	Baseline	7 days	14 days	Baseline	7 days	14 days
Vitamin C ($\mu\text{mol/L}$) ^a	43.7 $\pm$ 3.2	57.0 $\pm$ 3.8	56.8 $\pm$ 3.2	45.8 $\pm$ 3.4	61.4 $\pm$ 4.7	60.7 $\pm$ 4.5
8- <i>epi</i> PGF _{2α} (pg/mL) ^b	219.4 $\pm$ 7.6	176.2 $\pm$ 9.7	162.2 $\pm$ 6.7	198.8 $\pm$ 9.5	185.1 $\pm$ 9.7	165.0 $\pm$ 11.7
Uric acid ($\mu\text{mol/L}$)	271.6 $\pm$ 33.7	254.0 $\pm$ 29.1	258.5 $\pm$ 30.5	259.2 $\pm$ 38.8	237.4 $\pm$ 36.5	235.1 $\pm$ 38.8

Values are means $\pm$ SEM (PEF, $n = 6$; FS, $n = 6$). There was no significant treatment $\times$ time interaction for vitamin C ($p = 0.933$), 8-*epi*PGF_{2α} ($p = 0.260$), and uric acid ($p = 0.987$), based on repeated-measures ANOVA.

^aVitamin C significantly higher than baseline for PEF and FS combined at both 7 and 14 days (time effect), $p < 0.05$, based on repeated-measures ANOVA (Tukey test).

^b8-*epi*PGF_{2α} significantly lower than baseline for PEF at both 7 and 14 days and for FS at 14 (time effect), $p < 0.05$, based on repeated-measures ANOVA (Tukey test).

Table 33.10. Plasma vitamin C, 8-*epi*PGF_{2α}, and uric acid concentration, at baseline and on days 7 and 14 of drinking vegetable soup, among PEF-treated and FM vegetable soups^a (from Sánchez-Moreno et al., 2005b)

	PEF-Treated Vegetable Soup (n = 6)			FM Vegetable Soup (n = 6)		
	Baseline	7 days	14 days	Baseline	7 days	14 days
Vitamin C (μmol/L) ^b	49.6 ± 1.0	59.9 ± 2.2	63.5 ± 1.3	49.5 ± 1.5	59.2 ± 1.0	62.6 ± 1.2
8- <i>epi</i> PGF _{2α} (pg/mL) ^c	186.3 ± 18.1	136.5 ± 8.9	112.4 ± 6.4	176.2 ± 22.2	164.4 ± 21.0	128.5 ± 18.5
Uric acid (μmol/L)	329.3 ± 20.8	294.6 ± 22.1	292.2 ± 20.4	315.9 ± 30.2	289.3 ± 31.4	271.4 ± 30.0

^aValues presented as mean ± SEM (PEF, n = 6; FM, n = 6). There was no significant treatment × time interaction for vitamin C ($p = 0.989$), 8-*epi*PGF_{2α} ($p = 0.523$), and uric acid ($p = 0.958$) based on repeated measures ANOVA.

^bVitamin C significantly higher than baseline for PEF-treated and FM vegetable soups at both 7 and 14 days (time effect) ($p < 0.05$) based on repeated-measures ANOVA (Tukey's test).

^c8-*epi*PGF_{2α} significantly lower than baseline for PEF-treated vegetable soup at both 7 and 14 days and for FM vegetable soup at 14 days (time effect) ($p < 0.05$) based on repeated-measures ANOVA (Tukey's test).

technologies not only to improve sensorial quality and stability of foods, but also to obtain healthy minimal processed foods and to design functional foods to get better consumer quality of life.

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Chapter 34

Industrial Evaluation of Nonthermal Technologies

Huub Lelieveld

1. Introduction

Many scientists, technologists, and engineers feel compelled to invent or improve inventions to make life more interesting, easier, or more enjoyable—or they just like to show that things may be done in another way than the traditional one. Having developed something and having seen that it works or may work, or sometimes even that it just might work, they are almost always extremely disappointed by the response of those who they feel should be enthusiastic and ready to provide funds for further research and development. Scientists involved in food science and technology are not different in this respect. Governments, often believing that it is important for the competitiveness of the national industry, may be willing to support such research and development. Then, even after many years of taxpayer funded further work, “the industry” might just be willing to politely listen to your presentation, if you are lucky.

It is very important to realise that companies are commercial enterprises and, therefore, they are interested in you or your projects only if it is clear to them that listening to you may increase the company’s profit, without putting the company—or the person who has to make the decision—at risk, financially or otherwise. In addition, this preferably should be achieved with little or no investments. Although the benefits of implementing new technolo-

gies may be obvious and clear to the food company’s scientists and programme managers—and even to other food safety professionals higher up the corporate decision-making ladder, the benefits often are not as immediately apparent to corporate managers who make the ultimate budget decisions. In smaller companies, making out far more than 90% of the turnover of all food companies in the world, there may not be any food scientist and there may hardly be a hierarchic ladder. There may not be anyone with time to spend on the fancy things that scientists put forward. Nevertheless, however, *if* the owner or director of a small company is convinced that a new technology is of serious interest, the decision process is much faster than in a large company. The larger a company is, the slower is the process; there simply are too many individuals that have the right to influence decisions. They know it and that is one reason why they reorganise so frequently, but it never improves the process. For all of them, there is too much at stake.

Scientists, engineers, and food safety programme managers working in the food industry have the same problem as those working in institutes or universities. They too wonder why it is so hard to get funding for the application of new and better food safety technologies when the benefits are so obvious and clear. Why is there such a vast discrepancy between these two perceptions, and what can the food safety scientist, technician, engineer, or programme manager do to reduce the chasm? By understanding the decision-making process, you may become better prepared to make a plausible business case for the introduction of improved or innovative technologies that support

the mission of the company's food protection programmes and systems.

2. Understanding the Hurdles

There are quite a number of reasons for the differing perceptions between researchers and the responsible management in companies. First, many of us are so enthusiastic about the potential benefits of new food safety technologies that we run the risk of basing our ideas and arguments on incomplete or debatable data. Or, we fail to develop a plausible business case. There also may be hurdles that have nothing to do with the differences in perception, but are of a more practical nature. For example, it may be that a new technology has great potential—but applied somewhere else and/or by others. Perhaps the reality is that the site in which this technology will be used is in an area where expansion of the facility is limited and cannot accommodate new equipment. There may be problems with patents and licensing requirements. And, of course, there may be regulatory issues. No regulator is going to accept a new process if it does not fit into the current regulatory scheme. Below, each of these hurdles is discussed with an eye toward how scientists, engineers, and other food safety programme managers in sanitation, process control, and quality assurance departments can prove the benefits of new technologies to the decision makers.

2.1. *Incomplete or Debatable Data*

If you try to convince a company to invest in a new food safety technology, and you are serious, you must be absolutely certain about your "blanks." Management is going to ask for this information and this can be painful if you aren't prepared with the answers. It is sensible to use only peer-reviewed references pertaining to the technology you are proposing. The peer-review process is a central pillar of research by which independent scientists review findings and identify weaknesses before they are published. Peer-reviewed researches involve rigorous scientific scrutiny of new methods and technologies and, as such, are useful because they provide empirical substantiation of the technology's applica-

tion parameters. It is important to gather enough data to ensure that your proposal is complete. In other words, avoid situations in which you have to explain later, after you have presented the documentation, that perhaps there are some flaws in your story or that you (or worse, a colleague) discovered some unexpected effects that change important findings of your data. Be sure that your case is not based on just one or two lucky shots. Thus, make sure that you present good science.

Never argue that the proposed food preservation technology or process does not alter the product processed. It does, inevitably. It kills the bugs, so why would it leave everything else alone? Heat changes proteins and therefore enzymes, and over time, destroys membranes. Microorganisms do not survive this and neither does the product, which becomes digestible and that is useful. In particular, destruction of the cellular membranes (in the case of vegetable products) is very welcome as it makes nutrients available. Other processes do other things that microorganisms dislike, causing them to die. Whatever the changes are, they should firstly not make the product toxic and secondly, of course, also not distasteful.

An example is the application of pulsed electric field (PEF) technology. Under conditions needed to destroy microorganisms, PEF does not destroy enzymes but does perforate microbial and eukaryotic cell membranes. The cells (hence, the microbes) die and the nutrients become available. Such product changes are shown by Gudmundsson and Hafsteinsson, for salmon cells (Figure 34.1) and by Fincan for onion cells. PEF sends an electric current through the product using electrodes. It is important to realise that some electrochemistry may happen, for example, at the interfaces of the electrodes and the product. Would this change the product and is such a change substantial? How does it affect electrode lifetime (hence, potentially run time as well as maintenance costs)? It may require to rethink materials for the electrodes and process conditions, such as pulse length and frequency. Because of the inertia of the molecules in the product, perhaps more short pulses cause less damage than fewer long pulses. This should be taken into account during the research phase. There must be unambiguous quantitative data

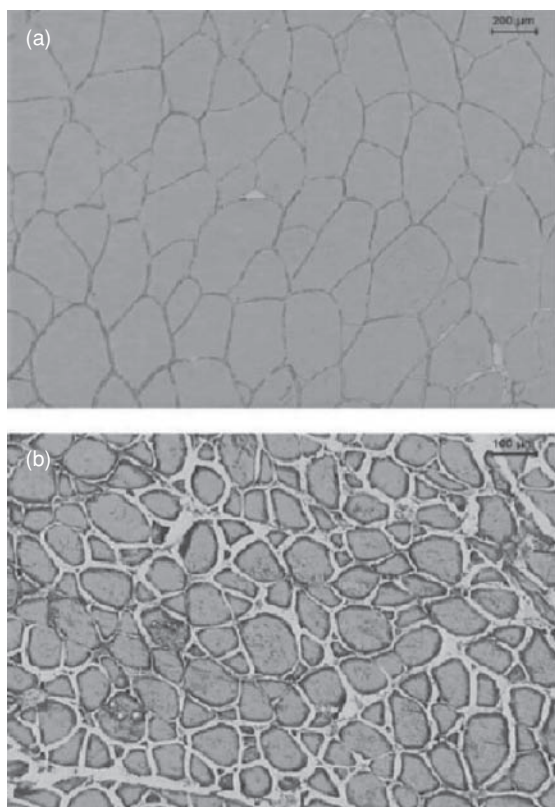


Figure 34.1. These electronmicroscopic photographs show clearly that PEF affects the structure of salmon cells. There is, however, no evidence that it also changes the chemical composition of the fish. (From Gudmundsson and Hafsteinsson, 2001.) (For color details, please see color plate section.).

about any potential changes to the product. With a correct process design, it seems that for many products, there are no significant undesirable effects on the finished product. In 2006, PEF reached industrial application and is being used to make fresh fruit juices microbiologically safe.

It may be necessary to identify research needs pertaining to potential undesired side effects and on eliminating or remedying these in order to supply complete data to decision makers.

2.2. Scaling up

It is not enough that a process works at laboratory scale. It is important to make certain that the technol-

ogy either is readily available (strongly preferred but often not the case) or can be developed at acceptable costs in an acceptable time frame. In other words, it must be demonstrated that the process can be scaled up with convincing arguments. There is no point in proposing a new technology if this is *not* possible. If a company has been convinced that a new technology is worth the risks and investments, then they expect the implementation to happen quickly. The market is here now, and nobody knows about the market 5 years from now. If they learn that it will require much more time for further development, they will be very disappointed and are likely to reverse their decision. If you are working on technologies that you want to be applied in industry, it will be of great help to consider the full-scale application from the start. It may influence the experimental design, think about “scaling down” from production scale in deciding an experimental set-up. What would be needed at full scale? Can I test this at small scale? How does the new process fit in the entire production line? It is of crucial importance to realise what else may have to be changed to apply the new technology. Often, there are consequences for the entire process line. It may have to be drastic, a filling process may have to be placed after the preservation process section, and may have to be aseptic instead of “just” hygienic. Packing material may have to be redesigned and the labelling may have to be changed. Once you have considered this, make certain that you can work with a reliable company that will be able to do the scaling up within a reasonable time. Involve them in your project. If you can’t, try to continue using taxpayers’ money to get that far. Otherwise, your chances are minute. The last resort, of course, is to try it yourself at production scale, become one of the small companies. It is risky, but if you are successful, you get all the attention from the bigger players.

2.3. Justified Investments

It is a tough decision to make a working process line obsolete. This is particularly so, because there is always a possibility that the new process turns out to be less successful than expected and, thus, the old technology would have to be reinstalled. So, leave it

there, just in case? The new technology may become obsolete itself, because of other new technologies seeing the light. Would the investments have been paid back by the time that this may happen? How well are you (or others in your environment) informed about potentially competing novel technologies and how fast may they become established, for example, by the competition? Therefore, is a depreciation time of 7 years realistic, or should it be 5 or 3 years, or even less?

2.4. *Weak Business Case*

A strong and convincing business case is essential and must be made in cooperation with all relevant stakeholders. A weak business case is the one that does not address the concerns of primary stakeholders and relevant decision makers in the company. Their concerns likely focus on costs related to the equipment, implementation, and maintenance of the new technology or system, the capability of the system to increase production efficiencies or to reduce costs in some other way, such as energy or labour savings, and so forth. As a scientist, engineer, or food safety programme manager, your concerns will focus heavily on the effectiveness of the new technology, its capabilities with regard to the reduction of potential food safety hazards, and the ability of personnel to properly operate or troubleshoot the equipment. Both parties will, of course, share the primary concern of producing safe foods. To build a solid business case, firstly, you need to make certain that you know all of the decision makers, in particular those within the company whom you know will be asked to provide input or advice on your proposal, including senior management, regulatory affairs and maintenance, sanitation, or other operational departments on which the new technology will have an impact. So, hopefully you are in good standing with the company, so that you can talk with them before you finalise the proposal. In the next phase, to be organised with your technical contacts, it would be helpful if you could include the marketing and sales department professionals, still during the preparation phase. They will eventually sell the finished product and, hence, you need to be able to articulate how the new technol-

ogy will provide them with, or perhaps limit them from, making certain marketing or labelling claims. After you have identified the potential influencers and decision makers, discuss all relevant information with those in the company who support the idea, paying special attention to any negative aspects and to the limitations of the new food safety technology. The negative effects may prove more important than the positive ones. In these discussions, never try to hide anything and never oversell—it will result in disappointment, which works against the goal. Naysayers, or “antis,” may have more influence than you, so discuss the concerns they raise and listen carefully. You need to have the answers ready—and the answers must be convincing—to effectively address and eliminate those concerns. Examples of typical statements by antis are “Taste is not good, neither are colour and texture,” “The product is not safe and may damage the health of our customers,” “There is no need for the new process, so why accept it?”, “It will only make the product more expensive, because of the huge investments required,” “The new technology puts our personnel at risk,” “The process is probably unreliable,” “The process kills some of the bugs, but chemical changes will spoil the product anyhow.” The critics have the advantage that they need not provide evidence that they are right. You need to provide evidence that they are wrong. Have the convincing answers ready!

A good business case shows the potential of the proposal, complete with all “ifs” and “whens”—and includes pessimistic estimates. If you don’t include the existing or potential downsides of the technology, others will—and may possibly use incorrect information.

2.5. *Other Stakeholders*

In addition to the decision makers in the company, it is important that you identify and consult with other stakeholders—proponents, opponents, neutrals, champions, and colleagues—in regulatory bodies, universities, and industry trade organizations. Carefully listen to their convictions, doubts, and feelings, and then take into account what you learn. If you consider them stakeholders, they probably have

influence in some way that pertains to the adoption of new technology. For example, it is a good idea to consult local or federal regulators to make certain that you know whether there are regulatory issues with the new technology—and whether the company will be able to cope with these issues during implementation. Stakeholders may also include opinion influencers such as consumer organizations, environmentalists, and the mass media or trade press. For example, consumers are strongly influenced by “antis,” as the food industry has witnessed with gamma irradiation and genetically modified food technologies. Although these have much scientific evidence to support their safe use, they have yet to enjoy widespread support of the public due to bad press driven by these groups. Preempt this by working together with consumer organisations at an early stage to promote the use and benefits of the new technology, and make yourself available for interviews and as an editorial resource for the press on the scientific underpinnings and benefits of applying novel or improved treatments.

2.6. Location

A technology may have great potential, but this potential cannot be realized unless it is sited correctly. In other words, the site where you initially believe the technology should be applied to be beneficial to the company may unexpectedly turn out to be somewhere else. The technology may even have to be applied by another company. This will be the case when the product originates from that “somewhere else.” If the raw materials come from the Southern Hemisphere and are sold in the Northern Hemisphere, it may well be that the technology—particularly if it is a preservation technology—has to be applied in the southern location. If so, many difficulties can arise, such as additional regulatory compliance problems because the company must now cope with regulations in two different countries that may be far apart both in terms of geography and policy. To illustrate this, assume that a company, located in a moderate climate area (e.g., Canada or northwestern Europe) produces jams (marmalades) from tropical fruit. The fruit comes from a tropical country, in bulk. To avoid

spoilage, the product is supplied in 10 kg cans, that have been retorted to pasteurise the contents. That thermal process most likely will have done so much damage to the quality of the product that using a novel, less detrimental technology in the receiving location, does not make much sense. In such a case, the novel technology must also be applied in the country of origin.

2.7. Food Processing Patents

Patenting is an expensive process that requires extra research and may require expensive attorneys and lawyers. Despite their clever formulations, most processing patents can be easily circumvented. Worse, patented processes usually take off after the patent has expired. Food preservation by high pressure and PEFs are good examples. Application of HPP technology to preserve food started around 1990 in Japan—almost 100 years after the conception of the idea in 1895. PEF treatment was invented in 1960 (be it for the second time, the idea was conceived 100 years ago), but found its first application in 2006, 46 years later. Meanwhile, many patent applications had been filed and patents expired. PurePulse Technologies in San Diego, CA, did not survive the hurdles. It could be argued that food-processing patents do nothing more than slow down technological progress by diverting resources to lawyers, causing extra experimental work to justify claims, and causing much duplication, or more likely multiplication, of work that could have been avoided if researchers were allowed to cooperate. Patents can become hurdles to the introduction of new technologies—and particularly to making your business case for adoption—in other ways, as well. Examples include wanting patent protection before deciding on an application, or using patents from others that might be infringed. Licensing negotiations also may take quite an effort and a long time. In many cases, the granting of a patent is not really justified. It is virtually impossible for any patent officer to have the expertise to decide whether a technology is new and surprising. It is, therefore, easy to obtain a patent, if you really wish, for whatever reason. A valid reason might be preventing others from patenting the

same, but that may also be achieved by publication in a magazine. Unless patents on your CV is considered of very high value, it is likely that publishing instead of patenting is more valuable. If the invention is really new, keeping it secret for some time, but keeping and filing records with a legal agency, may be more rewarding. That way, patents will not be a hurdle.

2.8. Regulatory and Legislative Disharmony

There are regulatory issues. No regulator is going to accept a new process if it does not fit into the current regulations somewhere and without doubt. If it does not fit, it becomes a significant regulatory hurdle since the development and implementation of new regulations or the modification of existing regulations may take many years, during which you will need to provide evidence on the safety of the process and the product. In many cases, this will cause the proposal to be immediately shelved. Food regulations and legislation exist to protect the consumer, not to deprive the consumer from better food products. However, significant hurdles to the adoption of new or improved technologies are caused by the differences between regulations between countries. These differences often lead to duplication of testing and the arduous task of following different protocols. Animal testing is expensive and adversely affects the reputation of the company. Global harmonisation of regulations and avoiding animal testing are important issues, which need attention and resources—but that is another story, about which you may read online at www.globalharmonization.net.

2.9. The Decision Process

Making decisions means taking risks and (almost) no one knowingly takes unknown risks—and certainly not for proposals that they do not understand in the first place. Of course, you might try to lure people into taking unknown risks unknowingly, but you may be able to do so only once. It is important to realize that there is no need to convince the colleagues who agree with you: They are already your fans. Convinc-

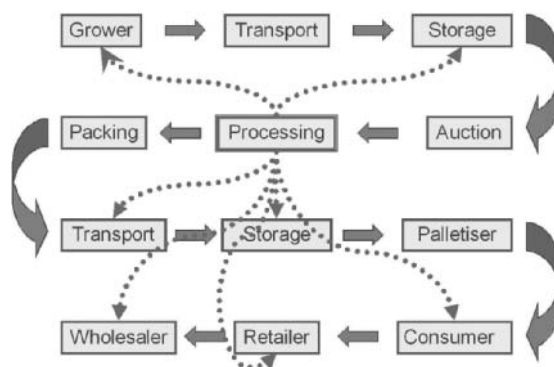


Figure 34.2. The introduction of a new processing technology may have consequences for the entire production chain. (For color details, please see color plate section.)

ing deciders is not the same as teaching motivated students. You need to get the attention of those who decide, even though they may not be interested in technology. They have other interests and based on experience, decision makers often are suspicious because every time someone comes with an “obviously good” proposal for a new process, a whole range of unmentioned consequences pop up. Changes in processing impacts almost every link in the supply chain (Figure 34.2). In addition, there are issues like quality and safety, tracking and tracing, buildings, personnel, energy, and taxes to consider. It all adds to the cost of the proposal, which in turn adds to the unwillingness of the decider. In many cases, the decider does not want to hear about a new process, and certainly not from a scientist. Yes, as a scientist you have an image problem, nicely illustrated by Gary Larson in the *Far Side* cartoon some years ago. The cartoon shows a scientist “melted” (ala Oz’s *Wicked Witch of the West*) on a laboratory bench surrounded by his white-coated colleagues. One of the scientists says, “My God! It is Professor Dickle!... Weinberg, see if you can make out what the devil he was working on, and the rest of you get back to your stations.” In turn, the decider probably presents an image problem to you, as they have other things in mind. They have been educated differently, as editorial cartoonist, Jeff Danziger, understood perfectly when he drew his “*At the Graduate School of Business*” cartoon. The professor at the lecture podium tells the class, “Now, I

know that you all want to make money. . .but today we are going to discuss making. . . things. Actual things.” And a business student pictured at the back of the room comments, “Things?! I don’t want to make things. I want to make money!” The student sitting next to him replies, “Listen, let’s sue the business school. We could make some money that way.” Overcoming the obstacle of the unwilling decider means paying special attention to including all of the elements of making the business case for the adoption of new food safety technology, as well as communicating the science behind the benefits of adoption.

3. Leverage Your Knowledge for Success

Given all these hurdles, it may seem that it is better not to try to sell science and technology to deciders. It often seems like the more decision power one possesses, the less one’s understanding of technology and the harder you must work to convince them. The lives of decision makers would likely be easier if science were less complicated, but many are not sure whether they can really do without. Therefore, you may get the opportunity to educate and innovate, which you should be careful not to miss. Just make certain that they get all relevant information and be sure that you know what is relevant—to the deciders. This includes a well-presented summary of the business case and a list of the internal and external

stakeholders who support the proposal. Present credible data to show that you know what you are talking about, and discuss the benefits and drawbacks of implementing the technology. Focus on advantages of the new technology, not on the drawbacks of traditional technologies. (Deciders already know the traditional technologies do work!) Make certain they understand what you present. Finally, there are two very basic rules that are useful to anyone making a proposal for change: (1) listen to understand, and (2) speak to be understood. Johan Cruyff, a famous Dutch football player (well, of my time), once said, “If I had wanted you to understand it, I would have explained it better.” Ultimately, if you want to successfully cross the finish line with a winning proposal, you’ll find that making a plausible business case for change rests on your ability to “explain it better.”

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Chapter 35

Transferring Emerging Food Technologies into the Market Place

Anthos Yannakou

1. Introduction

India and China's rapid growth and increasing investment in science and technology means developed and developing countries need to increase investment in research and innovation to compete globally (Wolff, 2005). The United States, for example, is expected to decline from 35 to 18% of the world's research by 2030, while China will increase from 11 to 18% of the total world's research (Science, 2006).

Innovation requires the sustainable implementation of incremental as well as emerging and radical technologies, which will help the food industry differentiate and produce value-added products without compromising taste, convenience, safety, quality, and cost in order to gain a competitive advantage.

Emerging food processing technologies are an important research component of many leading research institutes throughout the world. Research is not only focusing on traditional processes, such as pasteurization, but is also shifting to nontraditional processing (Mermelstein, 2002). Food Science Australia is a research institute that invests significantly in emerging food processes, and its Innovative Foods Centre has capability in a number of technologies such as high-pressure processing, pulsed electric field, and ultrasonics.

However, although the food industry recognizes the importance of emerging food-processing technologies, successful uptake is difficult. The challenge is to sustainably transfer emerging technologies in an industry where these are (often) seen as disruptive, risky, and difficult to implement when faced with day-to-day competitive pressures in a mature and low-margin food industry.

Obstacles to effective technology transfer are not only in the "receiving" companies, but also in research institutes. New technologies are rarely one-for-one replacements of existing technologies and usually cut across more than one application. Many research institutes look to applications that optimize the technology, want to put their technology into the largest market opportunity (frequently the best place to insert the technology is in niche market applications), and think in terms of how the technology works while the marketplace thinks in terms of functional needs (Technical Insights 1999).

Effective strategies and tactics are available to assist in sustainably transferring emerging food technologies into the marketplace. If this is done successfully, it will help rejuvenate the generally mature food industry, and move onto a new "S-Curve" (Levitt, 1965). Companies generally evolve through a lifecycle from an introductory phase to growth, maturity, and decline. Many food companies are in a mature phase, and a "business as usual" approach will normally lead to decline with increasing cost pressures, commoditization of products, limited innovation, and reduced market share. Mature food companies should look for a new growth phase to

reinvigorate their business, and this can be accomplished through effectively investing in innovation, including emerging processing technologies.

2. Strategies

In thinking through approaches to transfer innovative food processing technologies to the market place, a number of issues need to be considered. What marketing and selling approaches will be most effective with food companies? Once a food company decides to introduce the innovation, how will the final consumer be persuaded to accept products from the innovation?

A set of criteria and guidelines are needed to establish which emerging technologies will be successful, and which will not (Burgelman et al., 2004). For example, does a product or service incorporating the new technology provide enhanced effectiveness in the marketplace serving the final user? Does the operation reduce the cost of delivering the product or service? If at least one of these questions is positive, the probability of successful transfer of an emerging technology is increased. For example, if an emerging technology produces a product with an extended shelf life at a lower cost to traditional methods, it is likely to be in demand by the food industry.

A useful method to decide on strategic approaches to successfully transfer emerging technologies is to start off by understanding possible applications and possible customer groups. A number of strategies are then available (Friar and Balachandra, 1999) as shown in Table 35.1.

- **Substitution:** a new technology replaces an existing technology and will be used in the same application by existing customers. The replacing

technology needs to have a distinct advantage in areas such as cost reduction; an example is the use of continuous separation technologies rather than batch separation technology to make the isolation of bioactive ingredients more economical. A move to continuous flow technology would result in a greater throughput and improved consistency of product.

- **Diffusion:** a new technology expands the customer base. An example of diffusion is the microencapsulation technology, MicroMAX[®], which allowed the incorporation of Omega-3 oils into a wider range of products. This wider range of products made the supplement available to a more diverse customer base, which could then purchase bread and other items which included the supplement.
- **Expansion:** new applications are made possible by the emerging technology, and there is a need to understand the latent and unsatisfied needs of the customer. High-pressure processing has provided food producers with the opportunity to produce foods with an extended shelf life while preserving the fresh-like sensory characteristics.
- **Creation:** using emerging technologies to develop new applications for new customer segments is complex and difficult due to technological and market uncertainties and risks. An example is the industrialization and commercialization of “traditional” foods by indigenous populations for a mass consumer market using emerging and newly developed technology (as was done in the 1950s in South Africa with Sorghum Beer).

The substitution and diffusion strategies are incremental and evolutionary with limited risk and are easier to implement through a demonstration of value creation such as a cost–benefit analysis. Expansion and creation strategies are radical and risky (but potentially with major returns) and require the partnership and education of the customer. In analyzing customer and application intelligence to decide on an appropriate strategy, it is also critical to consult with customers to ensure a realistic and aligned approach.

The evidence on successful commercialization of new technologies, involving the management of

Table 35.1. Strategies for transferring emerging technologies

		Customer Group	
		Existing	New
Technology	Existing	Substitution	Diffusion
Application	New	Expansion	Creation

both technical and market uncertainty, confirms this strategic approach (Chesbrough, 2006).

When a new technology addresses an existing market with known customers, the benefits are largely understood through previous market experiences. This is not the case when market uncertainty exists, as it is difficult to know where to focus the technology development activity. This uncertainty is compounded when commercializing a new technology requires the resolution of both technical and market uncertainty. The most effective way to develop new technologies in new markets is to explore a variety of possibilities and seek rapid feedback at as low a cost as practicable, design tests that are close to the eventual market so that early success with the test is highly correlated with later market success, and instead of detailed planning, instigate some initial pilots and then react quickly to the new information obtained (Chesbrough, 2006).

It is all very well to adopt the appropriate strategic approach, but the proposed and targeted customer and potential adopter also needs to be persuaded of the merits of committing to the innovative technology. "Many companies today are faced with large, complex selling situations. They sell expensive equipment that affects many parts of a customer's company and they work on sales that may take many years to consummate. These major sales need special handling; they are more complex than smaller transactions, their potential profit is larger, and they have a more lasting effect on both buyer and seller" (Shapiro and Posner, 2006). Selling innovative food processes are generally business-to-business, and examples include one-of-a-kind purchases or licensing. They are characterized by a longer selling cycle time, and require larger and riskier decisions by the purchaser. Research into selling complex and large products and services suggest that the traditional selling approaches are less effective (Rackham, 1988; Shapiro and Posner 2006). The approach requires a strategic selling approach that is meticulously planned, is a total process, requires coordination of the buyer and seller, and identifies the customer's needs and relates the company's products to those needs. The objective is to develop longer term account relationships, stressing the long-term benefits of the account

relationship to the customer, and developing trust and credibility. The critical stage, and where the majority of time needs to be spent, is on investigating the customer's needs, and exploring how the product adds value and meets their needs compared to alternative solutions. "Buyer-seller relationships can play a strategic role in the formation of an emerging industry that results initially from technological advancement. Active management of the buyer-seller relationship when an emerging technology enters the marketplace can affect the adoption of the technology by individual firms, contribute to the overall diffusion rate, assist in identifying and addressing a potential buyer's technological need and determine the competitive positioning of the product and the supplier." (Loftus and Meyers, 1994).

A critical issue in ensuring the sustainability of innovative food processing technology is to ensure consumer acceptance of products from the new technology. For example, in the case of new functional foods, due consideration must be given to providing consumers with information about how novel functional foods are produced, as well as their potential health benefits. "In general, consumers are becoming more cynical about technological innovation that they perceive is only conducted to serve the interests of producers and manufacturers, particularly in cases where they have wider concerns about technology impact. The introduction of functional foods will not automatically be successful without the simultaneous introduction of information that is of use to consumers in making informed choices about purchase and inclusion in their diets." (Frewer et al., 2003)

3. Tactics

There are a number of generic tactical approaches that could be implemented within these strategies to help the transfer of emerging technologies.

3.1. Focus in Areas where "Barriers-to-Entry" Are Lowest

Transferring emerging technologies is complex, and it makes sense to start in areas where the probability

of success is the greatest, and where the “barriers-to-entry” are lowest.

Appropriate actions include the following:

- Identify established food companies that view innovation as being critical for their competitiveness. These companies can deal with the duality of short- and long-term actions, invest in a portfolio of incremental and radical research programs, have developed a culture that is flexible and open to change and new ideas, and views collaboration as important.
- Focus on SMEs (particularly those that are entrepreneurial and can absorb and use technology to develop new market-niches), start-up companies and ventures.
- When customers have an opportunity to consider a new product or service, they tend to react in different ways. Innovators (2.5% of the total) are technology enthusiasts, influence change, and tend to be gatekeepers and important communicators. Early adopters (13.5%) are visionaries and opinion leaders, who try out new ideas in a careful way because they believe that the new capability will lead to competitive advantage. The early majority (34%) are thoughtful and pragmatic people and they accept change quicker than the average, particularly if there is a proven track record. The late majority (34%) are skeptical people, and will use new ideas and products only when the majority use them. Laggards (16%) stick to the “old ways,” are critical of new ideas, and will only accept the new technology if it becomes mainstream (from the Rogers Innovation Adoption Curve). When marketing to companies, an ideal start is with the innovators and early adopters. The following guidelines are suggested when marketing to innovators. The product must be an innovation. It must be functional, but not necessarily perfect, and draft manuals are acceptable. It needs to provide opportunities for the customer to talk directly to the developers of the new technology and the innovators need to help educate the visionaries in the company. When marketing to early adopters sell them the “dream” but clearly define the scope and deliverables. Relate the technology to their specific objectives, have them reference other visionaries who have bought (they are the best salesmen), and ensure a reasonable price with implementation completed quickly and on time. Ideally, the early adopters should be satisfied customers who can then act as references for the pragmatists. This process is complex, and it is suggested that efforts are focused on a single bridgehead of customers in a mainstream market segment, such as the horticultural or tropical fruit market for value-added products manufactured via high-pressure-processing (Burgelman et al., 2004).
- Use a substitution or diffusion strategy where possible, as this is less disruptive for food companies to implement.
- Work with empowered and skilled champions in customer companies. Champions are needed to steer the new technology across the “valley of death” that separates discovery from commercialization. The champion must ideally recognize the commercial application, communicate to senior colleagues through completing a business case, and driving through on implementation of the technology project (Markham, 2002).

3.2. Obtain and Analyze Relevant Intelligence

The potential market for emerging technologies is wide, so efforts should be focused in the most promising areas.

A number of techniques are available to help managers make more effective decisions in complex environments by preparing for a wide range of uncertainty and by counteracting typical biases. These techniques can be used in strategic planning for innovative and high-tech industries, where “[d]isruptive technologies do not fit easily within existing managerial mindsets, and their development trajectories are notoriously hard to predict. They can cause major market disruption and rapid loss of market position to incumbent firms.” (Drew, 2006)

A typical process to ensure an organization focuses its efforts on the most effective areas is as follows (Ringland, 2003): forecast the course of events for the available technology into the future with a

set of scenarios that are “logically consistent but distinctly different views of what the future might be”; “scenario planning is especially appropriate to analyze technological innovations” (Drew, 2006); and explore these possible future societies and how they react to these new products or services. For example, in the case of nonthermal food processing technologies (say, using high-pressure processing to produce a new fruit-based product), scenarios of alternative futures could be developed, and the potential markets for the new products explored in each. The market will be affected by factors such as growth in disposable income and consumer demands for convenience and health.

Technology trends both in and outside of the food industry need to be monitored, including other industries. For example, high pressure originated from ceramics, steel, and super alloy production, and radio-frequency from textiles, paper drying, and plastics welding (Barbosa-Canovas 2005).

Market trends and research need to be monitored to develop potential applications for the emerging technology which meet future consumer expectations. There is a general recognition that consumers (particularly in the developed world) will have increased needs for food products which provide value, taste good, are nutritious, are convenient, and “clean green,” safe, and “natural” (Sloan, 2005).

3.3. Build Sustainability in Customer Companies

If emerging food-processing technologies are to be sustainably implemented, companies need to develop the ability to accept and successfully commercialize innovations. The competency set required includes (O'Connor and Ayers, 2005) discovery, through the creation of opportunities such as research and licensing technologies; incubation, through evolving opportunities into business propositions; and acceleration, by ramping up the innovation to a successful business. In addition, companies need to have an external orientation and keep in touch with scientists working on long-term innovative processing research, build a learning capability, and maintain

flexibility to introduce radical innovations as they become relevant (Day, 2000).

An example is a multinational food company funding innovative food-processing research in a major research institute, and applying ongoing improvements to its traditional product range through new processing techniques that give a competitive advantage in taste and shelf life.

Organizations transferring innovative food technologies can assist receiving customers through training, seminars, workshops, and knowledge-transfer activities indicating the benefits, applications, and track record of the technology. The organization can help develop the customer's competence in managing technology and innovation more effectively. It can help move the technology from the innovators and early adapters into the mainstream by, for example, providing the early adopters with prototypes and working with the innovating company to define the form and function of the new technology.

3.4. Facilitate R&D and Innovation in a Manner which Reduces Cost, Uncertainty, and Risk

Many emerging food-processing technologies are being developed in (partially) publicly funded research institutes in many parts of the world, and these, together with governments, provide assistance in helping to transfer these technologies to the food industry. Examples include the following:

- Government-supported funding schemes, such as the Food Innovation Grants (FIG) within the National Food Industry Strategy Ltd in Australia. This scheme has provided 56 Australian food manufacturers with \$AU45 million from FIG (matched by a further \$AU58 million by industry) in grants from 2003 to c035+bib+0016. The results have been encouraging, with 58% of recipients successfully commercializing a new technology.
- Pilot plant facilities exist for research, such as The Innovative Foods Centre within Food Science Australia. The Centre was established with the assistance of the Victorian Government and has been highly successful in its research programs and in

attracting investment from relevant industries. The Centre is, or has been, involved in 30 research collaborations since 2001, of which 7 involved overseas parties and 20 involved industry participants; 6 patent applications have already been filed, and 5 commercialization are currently being implemented. All centre activities are project-based and cover the full spectrum like strategic research, applied research, development work, and scale up, demonstration, technology transfer, and trouble shooting.

- Certain research institutes (such as Campden and Chorleywood Food Research Association Group, and Leatherhead Food International in the UK) provide membership schemes for precompetitive research involvement by food companies in a number of areas, including innovative food processing.

4. Conclusions

The food industry needs to invest in innovation to differentiate and be internationally competitive, and this could include introducing emerging food processes. This investment will assist food companies in the mature phase of the company lifecycle to rejuvenate and move onto a new growth phase.

Emerging food processing technologies have the potential to contribute to the growth of the food industry as the technologies are developed to improve existing process efficiencies and create new opportunities. It is crucial, however, to ensure that these technologies are introduced in a sustainable way.

Although transferring emerging technologies to the food industry in a sustainable way is complex, a number of strategies and tactics are available to assist this process. These include deciding on a marketing and selling approach, communicating with consumers about the new technology, and employing tactics such as starting where the probability of success is highest.

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Chapter 36

New Tools for Microbiological Risk Assessment, Risk Management, and Process Validation Methodology

Cynthia M. Stewart, Martin B. Cole, Dallas G. Hoover, and Larry Keener

1. Introduction

Novel processing technologies offer alternatives to traditional heat processing as a food safety control measure. They may also provide the opportunity to develop innovative new products that have the convenience of a thermally processed food, in terms of shelf life, but that are closer to the fresh product in terms of flavor, texture, and nutritional qualities. More recently, the role of novel processes, such as high-pressure processing, in the safe delivery of heat labile, functional, or nutritional ingredients in foods is being realized. If the potential of these technologies is to be brought to fruition, it is crucial that they are developed and commercialized in a manner that does not compromise, but preferably enhances, product safety.

The International Commission on Microbiological Specifications for Foods (ICMSF) has responded to the need for a scientifically based management system for determining equivalency of control measures for safe food production. ICMSF has proposed a scheme for the management of microbial hazards for foods, which includes the concept of Food Safety Objectives (FSOs). The continued successful commercialization of novel technologies can be facilitated by the use of new risk management tools, such as those developed by the ICMSF, and will also be

dependent on the development of multidisciplinary coordinated programs to assess the efficacy of the new processes and facilitate the successful development and commercial implementation of these technologies.

The validation of any food safety control measure involves the documentation and demonstration that the measure is properly designed to provide the level of hazard control necessary to protect public health. The stringency of the required validation exercise depends on a number of factors, including the severity of the hazard, intended use of the product, and the previous experience with the process and/or product. For example, in the case of the sterilization of low-acid canned foods, the hazard of *Clostridium botulinum* is indeed considered a severe hazard, and hence the required validation steps will need to be the most stringent especially if the new scheduled process is not simply based on the delivery of heat to the product. An approach to process validation, specifically focused on validation of new processing technologies, is discussed in this chapter.

2. New Tools for Microbiological Risk Assessment and Risk Management

Illnesses caused by food-borne pathogenic microorganisms are a major world wide public health issue; reducing and/or preventing these types of illnesses is a major goal of societies (ICMSF, 2005). The importance of different food-borne diseases varies between countries, depending on the foods consumed,

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food processing, preparation, handling and storage methods used, and the sensitivity of the population to these microorganisms. While the total elimination of food-borne disease remains an unattainable goal, both government public health managers and industry are committed to reducing the number of illnesses due to contaminated food (ICMSF, 2005). Although all countries aim to reduce food-borne illness, most have not explicitly stated to what degree they would like to do so in their countries, and they also have different opinions on how they wish to balance costs, including not only financial costs but also considerations of culture, eating habits, etc., with the reduction of illness (ICMSF, 2005). With regards to the food supply, the primary role of national governments is to protect its consumers, with a secondary role of facilitating trade. Traditionally, managing food safety has been done by governments setting prescriptive standards, which provide explicit protection for consumers. For example, regulations may include a specific kill step to minimize the hazard or require end point testing of products; this approach to food safety management has been increasingly constraining to industrial innovation. More recently, new outcome-based approaches, which are risk assessment and scientific evidence based, with a focus on food safety outcomes are being implemented. This approach allows for increased flexibility for innovation by the food industry, while still ensuring the safety of the food supply.

International trade agreements include both the “Sanitary and Phytosanitary (SPS) Agreement” and the “Technical Barriers to Trade (TBT) Agreement,” both of the World Trade Organization (WTO). The SPS agreement has been signed by more than 100 countries and states “whilst a country has the sovereign right to decide on the degree of protection it wishes for its citizens, it must provide, if required, the scientific evidence on which this level of protection rests.” The TBT agreement also requires that a country must not ask for a higher degree of food safety for imported foods than it does for goods produced in its own country (ICMSF, 2005). The criteria used by a country to determine whether a food should be considered safe should be clearly conveyed to the exporting country and should be scientifically justifi-

able (ICMSF, 2001). In order to achieve this, the term “acceptable level of protection” (ALOP) has been used, which is defined as “the level of protection deemed appropriate by the Member (country) establishing a sanitary or phytosanitary measure to protect human, animal, or plant life or health within its territory” (ICMSF, 2002). ALOP is the level of risk that a society is willing to accept; however, most countries have set goals to lower the incidence of food-borne disease and therefore may set limits for future ALOPs. For example, the current level of listeriosis could be 6 cases per million people per year and a country may want to reduce this to 3 per million people per year (ICMSF, 2005).

3. Good Manufacturing Practices, HACCP, and Integrated Food Safety Management Systems

In the early 1970s, the concept of Hazard Analysis Critical Control Point (HACCP) was developed to address the shortcomings and lack of food safety assurance provided by traditional inspection and sampling/testing of lots (ICMSF, 2005). The goal of HACCP is to focus on the hazards of a particular food commodity that are reasonably likely to impact public health if not controlled and to design food products, processing, commercialization, preparation, and use conditions that control those hazards. To be successful, HACCP needs to be built on good practices such as good agricultural practices (GAPs) for control of raw materials, good manufacturing practices (GMPs), and good hygienic practices (GHPs), all of which can be viewed as basic sanitary conditions and practices that must be maintained to produce safe foods, including support activities such as raw material selection, labelling, and coding (Stewart et al., 2002). GMPs form the basis on which HACCP programs are based. HACCP has provided great advancements in the production of safe foods; however, it is specific to a given processing plant and does not directly link the effectiveness of such measures to an expected level of health protection, for example, a reduction in the number of food-borne illnesses occurring in a country.

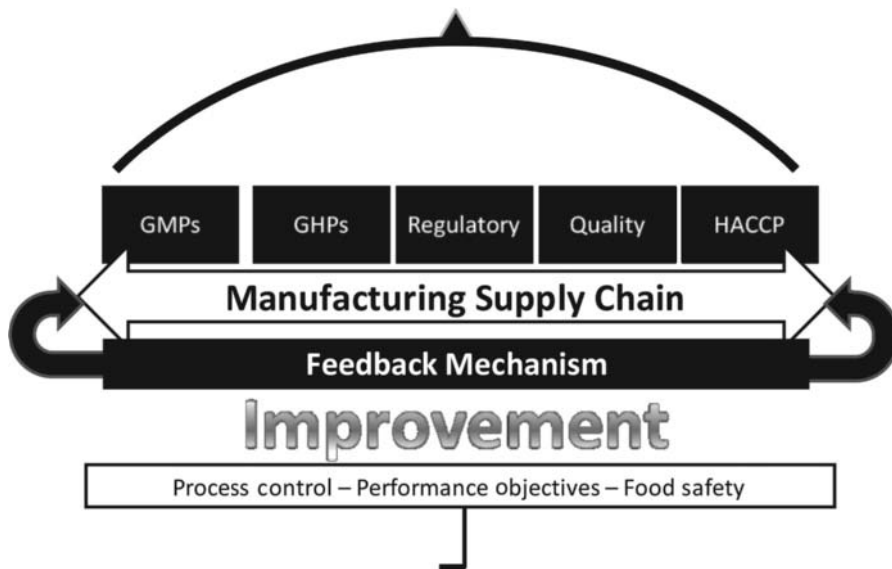


Figure 36.1. Food safety umbrella depicting the elements of an integrated food safety management system, with improvements in performance objectives and food safety as outcomes (Keener, 1999).

While a government often expresses public health goals relative to the incidence of disease, this does not provide food processors, producers, handlers, retailers, or trade partners with information about what specifically they need to do in order to help achieve these societal goals (ICMSF, 2005). In order for these goals to be practically met by the food manufacturers, etc., the targets of food safety set by governments need to be translated into parameters that can be used by food processors to manufacture food and parameters that can be assessed by government agencies. FSOs are intended to form the link from public health-based goals to suitable control measures and allows for the equivalence of control measures to be determined. GMPs, GAPs, GHPs, and HACCP (FDA, 2009) remain essential for food safety management systems to achieve FSOs or performance objectives (POs). Keener (1999) has advocated and developed an integrated food safety system that focuses on the collective risk reducing contributions of the subordinating elements to the manufacturing supply chain in delivering enhanced product safety (Figure 36.1). This integrated approach to food safety management is also reflected in the require-

ments of ISO standard 22000-2005 that were issued in September 2005 (ISO, 2005).

The following new food safety management terms will be used throughout the remainder of this chapter (Codex Committee Food Hygiene ALINORM 04/27/13; Appendix III):

- **FSO:** The maximum frequency and/or concentration of a hazard in a food *at the time of consumption* that provides or contributes to the appropriate level of protection (ALOP). Deciding if and when to use and FSO is the responsibility of governments and should only be used in situations where they will have an impact on public health. Therefore, it is unnecessary to establish FSOs for all foods.
- **PO:** The maximum frequency and/or concentration of a hazard in a food *at a specified step in the food chain before the time of consumption* that provides or contributes to an FSO or ALOP, as applicable. This concept is useful particularly when the FSO is likely to be very low, or “absent in a serving of the food at the point of consumption.” For example, for a processor of ingredients

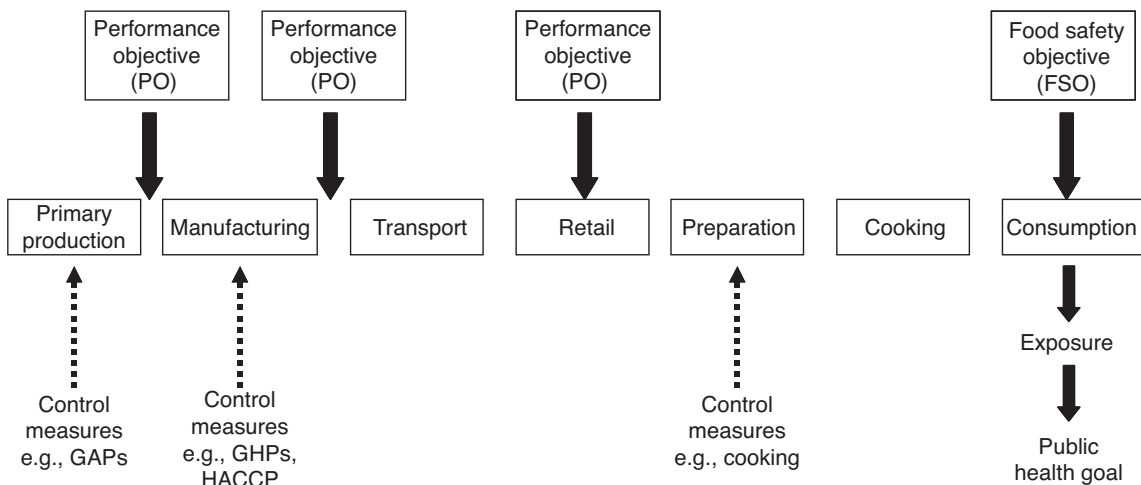


Figure 36.2. Model food chain indicating the position of a food safety objective and derived performance objectives (ICMSF, 2005).

or foods that require cooking prior to consumption, this level may be difficult to be used as a guideline in the factory setting. Therefore, it is often needed to set a level that must be met at earlier steps in the food chain, and this level is called the PO (ICMSF, 2005; Figure 36.2).

4. Performance, Process, and Product Criteria

When designing and controlling food operations, it is necessary to consider likely pathogen contamination, methods of destruction, and factors governing microbial growth, survival, and possible recontamination. Consideration must be given to the subsequent conditions to which the food is likely to be exposed, including further processing and potential abuse (time, temperature, cross-contamination) during storage, distribution, and preparation for use. The ability of those in control of foods at each stage in the food chain to prevent, eliminate, or reduce food safety hazards varies with the type of food and the effectiveness of available technology.

- A *performance criterion (PC)* is defined as “The effect in frequency and/or concentration of a hazard in a food that must be achieved by the applica-

tion of one or more control measures to provide or contribute to a PO or FSO” (CCFH, 2004). When establishing performance criteria, account must be taken of the initial levels of the hazard and changes of the hazard during production, processing, distribution, storage, preparation, and use. An example of a performance criterion is a 6-log₁₀ reduction of salmonellae when cooking ground beef.

- *Process criteria* are the control parameters (e.g., time, temperature, pH, water activity) at a step, or combination of steps, that can be applied to achieve a performance criterion. For example, the control parameters for milk pasteurization in the United States are 71.7°C for 15 seconds. This combination of temperature and time will assure the destruction of *Coxiella burnetii*, as well as other nonspore-forming pathogens that are known to occur in raw milk.
- *Product criteria* consist of parameters that are used to prevent unacceptable multiplication of microorganisms in foods. Microbial growth is dependent on the composition and environment of the food. Consequently, pH, water activity, temperature, gas atmosphere, packaging barrier properties, etc. have an influence on the safety of particular foods. For example, it may be necessary for a food to have a certain pH (e.g., pH 4.6 or below) or *a_w*

(e.g., 0.86 or below) to ensure that it will meet an FSO for a pathogen, for which growth in the product must be limited (e.g., *Clostridium botulinum* or *Staphylococcus aureus*).

Once the FSO is set, determination of several factors in achieving the FSO must be made. When establishing performance criteria, consideration must be given to the initial level of a hazard and changes in the hazard during production and processing, distribution, storage, preparation, and use. A performance criterion can be defined by the equation:

$$H_0 - \sum_i^m R_i + \sum_j^n I_j \leq \text{FSO}$$

The formula is frequently simplified to

$$H_0 - \Sigma R + \Sigma I \leq \text{FSO}$$

Here, FSO is the food safety objective, H_0 is the initial level of the hazard, ΣR is the total (cumulative) reduction of the hazard, and ΣI is the total (cumulative) increase of the hazard; FSO, H_0 , R , and I are expressed in $\log_{10}$ units.

It should be recognized that the parameters that may be used in the above equation are point estimates, whereas in practice, they will have a distribution of values associated with them. If data exist for the variance associated with the different parameters, then the underlying probability distributions may be established using an approach similar to that in risk assessment.

Control measures can be put into place to manage each part of the process and generally fall into three categories (ICMSF, 2002):

Controlling initial levels of a hazard (H_0)

- Avoiding food with a history of contamination or toxicity (e.g., raw milk, raw molluscan shellfish harvested under certain conditions)
- Selecting ingredients (e.g., pasteurized liquid eggs or milk)
- Using microbiological testing and criteria to reject unacceptable ingredients or products

Reducing the level of a hazard (ΣI)

- Destroying pathogens (e.g., freezing to kill certain parasites, disinfectants, pasteurization, irradiation)
- Removing pathogens (e.g., washing, ultrafiltration, centrifugation)

Preventing an increase of the hazard (ΣR)

- Preventing contamination (e.g., adopting GHPs that minimize contamination during slaughter, separating raw from cooked ready-to-eat foods, implementing employee practices that minimize product contamination, using aseptic filling techniques)
- Preventing growth of pathogens (e.g., chilling and holding temperatures, pH, a_w , preservatives)

Microbiological criteria need to be accompanied by information including the nature of the food product, the sampling plan, methods of examination, and the microbiological limits to be met (ICMSF, 2005). Traditional microbiological criteria are designed to be used for testing a lot or shipment of food for acceptance or rejection. In contrast, the FSO and PO are maximum levels and do not specify the details needed for testing (ICMSF, 2005). However, microbiological criteria can be based on POs in certain instances where testing of foods for a specific microorganism can be an effective means for their verification. While there are several approaches that can be used (e.g., lot testing, process control testing), they all compare the results against a predetermined limit. The ICMSF (2002) has provided guidance on the establishment of microbiological criteria.

The responsibility of marketing food that is not harmful to consumers when used as intended is the responsibility of the various food businesses along the food production chain; this responsibility does not change with the introduction of the FSO and PO concepts. When establishing FSOs, it should be evaluated to determine if it is technically achievable through the application of GAPs, GMPs, GHPs, and HACCP (Stewart et al., 2002). Once it is determined that the FSO is achievable, performance and process/product criteria are established, and the process is then implemented through GMPs and HACCP. If

the proposed FSO is not technically feasible, then modifications of the product, the process, if technically possible, and/or the FSO may be necessary. If no technically achievable solutions can be found, and the risk is too great, then it may be necessary to ban the product and/or the process. For example, the banning of high-risk tissues (spinal cord, root ganglia, tonsils) of beef to be sold for human consumption due to the inability to detect and/or eliminate bovine spongiform encephalopathy. In addition, as new information regarding a particular hazard or product emerges, FSOs may be modified.

Since the FSO is the maximum level of a hazard at the point of consumption, this level is often low and therefore measurement is often difficult (ICMSF, 2005). Compliance with POs at earlier steps in the food chain is a way of ensuring that the FSO will be met. While microbiological testing can be used, in most cases validation of control measures, verification of the results of monitoring the critical control points, as well as auditing good practices and HACCP systems will provide reliable evidence that POs, and thus FSOs, will be met. The next section of this chapter will discuss process validation and verification in more detail.

5. Process Validation Methodology

5.1. Introduction

Food safety, while a much discussed subject, is an elusive concept that is frequently a source of much consternation for regulatory authorities and food processors. Assessing the public health (safety) status of a food is a risk-based activity and a conundrum. That is, what is an acceptable level of risk and for who is that level appropriate? With this as a backdrop, consider the following definition of food safety: “*Food safety is the biological, chemical or physical status of a food that will permit its consumption without incurring excessive risk of injury, morbidity or mortality*” (Keener, 2005). Logically, then, it follows that judging food safety is judging acceptability of risks; a normative, qualitative, or frequently a political activity. At the end of the day, irrespective of the considerations or scenario, methods will be required for properly assessing and validating the fact that the ac-

cepted and established levels of risk, corresponding with safe food, are being achieved.

A new food process that imparts an intended function of product safety assurance requires validation. According to the US Food & Drug Administration, process *validation* is the deliberate, science-based act of “establishing documented evidence which provides a high degree of assurance that a specific process will consistently produce a product meeting its predetermined specifications, and quality attributes” (FDA, 1987), including product safety. Process *verification* is “confirmation by examination and provision of objective evidence that specified requirements have been fulfilled.” The distinctions between validation and verification are subtle and are frequently a source of confusion. In short, verification subordinates validation. That is, verification assumes that the subject process or procedure has previously been validated. Moreover, verifying that the critical attributes of a process have been achieved may be the assigned responsibility of properly trained machine operator or technician; by contrast, however, process validation should always require the skills of a multidisciplinary and cross-functional team, including a process authority (FDA, 1997).

Nonthermal food processing technologies that do not have a history of commercial application in the modern marketplace require validation to ensure the safety of their products. This position is supported by both European Union (EU) and US legislation relating to novel foods. For example, the Novel Foods Regulations of the EU (NFR, 1997) would require validating the safety of foods or food ingredients that have not been widely used as human food in the European community before May 15, 1997. Foods derived from novel processing technologies, including nonthermal methods, are subject to these requirements. Analogous requirements for the prescriptive demonstration of product safety have been codified by the US Food and Drug Administration (FDA, 1997). See Chapter 37 for a more detailed description.

To ensure safety, one asks if the outputs of the manufacturing processes are verifiable; that is, can proof of process performance be documented (Figure 36.3)? If the answer is “yes,” data can be collected

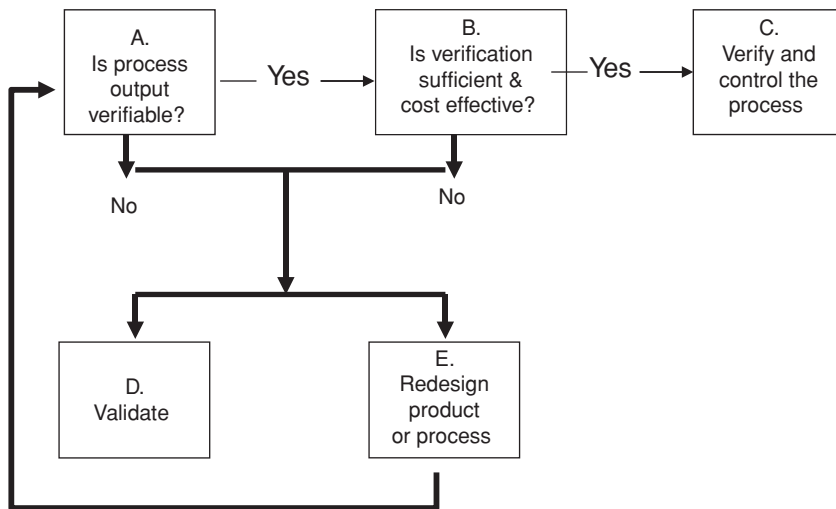


Figure 36.3. Process validation decision tree.

to verify that the process can be reliably controlled and determined sufficient and cost-effective. If its output cannot be verified, then the process must be redesigned for purposes of validation (Hojo, 2004). The worst case scenario in process validation would be disregard or a lack of knowledge of a set of conditions encompassing upper and lower processing limits and circumstances, including those within standard operating procedures, which pose the greatest chance of process or product failure when compared to ideal conditions. Such conditions do not necessarily induce product or process failure (FDA, 1987). The model (Hojo, 2004) shown in Figure 36.3 can be a useful tool in deciding on whether or not a process needs to be validated.

6. Process Validation Methods

Process validation is necessary to make certain that safety, quality, and effectiveness have been designed and are built into the product produced by the process. In other words, process validation is a product- and process-specific activity. A faulty manufacturing process can be relied upon to produce an equally faulty or defective product. In terms of preserving and protecting public health, it is crucial that all aspects of the manufacturing process are understood

and that all anomalous conditions are either assigned a cause or eliminated prior to offering such products into the marketplace.

While samples can be taken and analyzed during the process for purposes of monitoring (verifying) process-critical parameters that correlate with safety and quality, not every single product produced in the process can be inspected or tested. Therefore, each step of the manufacturing process must be controlled in order to maximize the probability that the finished product meets all safety, quality, and design specifications (FDA, 1987). This is analogous to a HACCP approach.

Examples of processes that require validation are those that fit into the categories as a sterilization process, an aseptic fill process/sterile package-sealing process, a heat-treatment involving shelf life extension of perishable products, a critical monitoring system (e.g., the detection/prevention of prions in meat), and a novel food-processing technique. The last type of process is the subject focus of this paper.

Validation methods can be classified as concurrent, retrospective, or prospective based on the timing between process validation and release of the product (FDA, 1987):

- *Concurrent validation* is conducted at the same time manufacturing is taking place. This type of

validation can be considered the approach carrying the greatest risk. Concurrent validation is useful for validating line-extensions or other situations involving relatively minor changes to the manufacturing process, equipment, or methods for calibrating process-critical measuring devices. Concurrent validation, if properly conducted, requires that all data are analyzed and a cause is assigned to all anomalies, can be a cost-effective approach to process validation.

- *Retrospective validation* is the validation of a process for a product already in distribution and based upon accumulated production, testing, and control data. Retrospective validation, in most instances, is expensive and will likely involve decisions to withdraw or recall products from the marketplace. Typically, this method is employed as part of a scheme used to assign root cause to a process failure, where there is reason to question the public health status of the finished product. To be effective, retrospective validation will require impeccable records keeping (i.e., traceability of ingredients), including process control data, outputs from monitoring activities, and of corrective actions taken in response to process deviations.
- *Prospective validation* is conducted prior to the distribution of either a new product or a product made under a revised manufacturing process where the revisions may affect the characteristics (risks) of the product. To best ensure product safety, prospective validation is obviously the preferred method.

Process validation, irrespective of the selected method, generally involves the following elements: (1) equipment installation qualification, (2) product performance qualification, and (3) process capability qualification.

Equipment installation qualification establishes confidence that process equipment and ancillary systems, as they are installed, are capable of consistently operating within established limits and tolerances (FDA, 1987). Equipment installation, for example, might consider the following: room ventilation or other HVAC elements, utilities (electrical, water, natural gas), pump ratings, meter tolerances,

tank capacities, heater ratings, or personnel traffic patterns. For installation qualification, examples of applicable elements of validation protocol are the equipment design. This information includes the materials of construction and description of sanitation and maintenance of the equipment. The installation conditions (e.g., wiring diagram, utilities requirements) are included in the protocol. Additional elements are equipment calibration, maintenance and reliability specifications, safety features, the software and programming parameters, all vendor documentation, environmental conditions recommended for installation (e.g., temperature, humidity, dust level limits), and necessary on-hand spare parts.

Product performance qualification establishes confidence through appropriate testing that the finished product produced by a specified process meets all release requirements for functionality and safety. The product performance qualifications concern the inherent properties of the food product. The relevant information for the product includes the organoleptic or finished product specifications, pH, water activity, conductivity, redox potential, titratable acidity, viscosity, moisture, solids, °Brix (refractive index), particle-to-liquid distribution ratio, levels of microorganisms (e.g., pathogens, surrogates, or spoilage agents), and the chemical residue profile (e.g., indicators and toxins).

The third element of validation is *process performance qualification* that establishes confidence that the process is effective, capable, and reproducible. Process performance qualification is typically based on a statistical analysis of the measures provided to control the process. Resulting from this analysis are the upper and lower control limits of the process. Certainly, the control limits must show the process capable of consistently performing at a level of normal variation that will preclude the production of defective or unsafe products. The process performance qualifications, depending on the product and process, may represent a longer list of items that require consideration. Information includes the human factors impacting the process, such as training requirements for workers and possible ergonomic issues. The *process operating procedures* are required, preferably in specific detail, along with process change control

procedures. Software parameters and results of screening experiments to establish the key process parameters are also required. Also necessary are the control limits (i.e., time, temperature, conductivity, pressure, flow rates, electrical field density, and line speed), short-term stability and capability (e.g., control charts and other monitoring devices), raw material and packaging specifications (e.g., input variation), and material-handling specifications (e.g., temperature, time, shelf-life, storage conditions).

7. Approach to Process Validation

The step-wise approach to validation is as follows:

- Form and train a multifunctional team in validation procedures, a “Process Validation Team”
- Develop a “Master Validation Plan”
- Develop “Process Validation Protocols”
- Initiate trials according to Protocol
- Analyze data and report findings

7.1. Process Validation Team

In order to properly validate a food process, it is necessary to compose a team of individuals that can represent the expertise to accurately evaluate the process as one that can be reliably controlled. A complete team to validate a nonthermal food process can comprise the areas of food physics and chemistry, microbiology, mechanical engineering, electrical engineering, process engineering, food safety regulations, food packaging, employee safety, sanitation and hygiene, quality assurance, product development, food chemistry, and toxicology. A process validation team is multifunctional and should include a process authority, especially if the process is intended to produce acidified or low-acid shelf-stable products (FDA, 1997). See Chapter 37 for further details on regulatory requirements.

7.2. Master Validation Plan

The master validation plan is an important first step in the validation process and serves as a statement of purpose or intent. The plan provides a summary of

the major activities that are to occur during the validation process. The master plan does not articulate the minute details of execution. These are presented in the validation protocols. The master plan is used to gain support and consensus of both the validation team and external reviewers. Developing a functioning master validation plan will usually involve five parts:

1. *Approach*: As defined earlier, the approach is either prospective, concurrent, or retrospective based on the timing of the actual validation related to the release of the product.
2. *Methods*: The methodologies used in a master validation plan would generally involve detailed descriptions of challenge tests, count reduction studies, inactivation modeling, component-swapping studies, analyses of failure modes and effects, response surface studies, and other designed experiments.
3. *Specific requirements*: These specifications define the process and the product with delineation of the expected outcomes.
4. *Seek peer review*: To make the examination as thorough and complete as possible, outside evaluation by independent experts is advised.
5. *Involve regulatory authorities*: Efficient use of time and resources dictate that regulatory agencies be an intimate part of the validation plan. Information should be actively sought from such agencies as the FDA and USDA to avoid costly mistakes. See Chapter 37 for more details on regulatory requirements.

7.3. Develop Process Validation Protocols

The protocols are a written plan stating how validation will be conducted and includes testing parameters, product characteristics, production equipment, analytical methods, methods of calibration, and decision points on what constitutes acceptable test results. The validation protocols delineate the minute details of materials and methods for executing the validation process.

These procedures determine and specify what to verify, how to verify, and how many times to

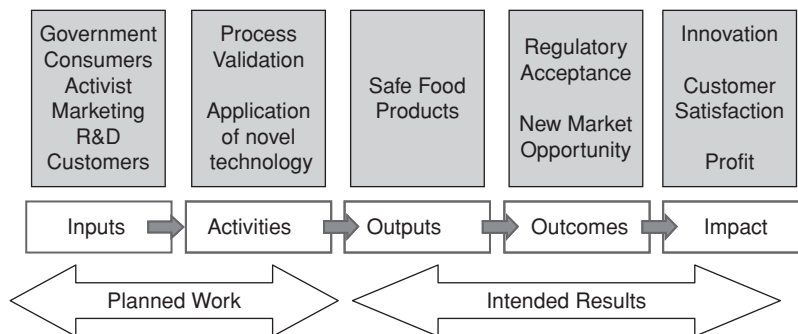


Figure 36.4. Hypothetical manufacturing process, after W.K. Kellogg Foundation model, showing the relationship between process inputs and expected output or intended results.

verify for statistical significance, as well as the procedures to define the acceptance/rejection criteria and the documentation that will be required for validation.

8. Variation and Validation

Variation is an inherent and unavoidable feature of most phenomena man-made and otherwise. All processes will be subject to variation in performance; no two outputs will ever be *exactly* the same. The complex manufacturing processes used in the production of human food products are highly susceptible to variation. Extremes in the performance of these processes may give rise to the production of unsafe and dangerous products.

The Logic Model (W.K. Kellogg Foundation, 2004) for a hypothetical manufacturing process (Figure 36.4) emphasizes the relationship between input variation and variation that will manifest as output in the finished product. The Kellogg model implies and lends support to a long-held principle espoused by Deming (1993): “If the process isn’t right, the product won’t be right.” Recall that process validation is a process- and product-specific activity. Excessive variation in the process will transmit faults or corresponding variation to the finished product. Again, process *validation* is the deliberate, science-based act of “establishing documented evidence which provides a high degree of assurance that a specific process will consistently produce a product

meeting its predetermined specifications, and quality attributes” (FDA, 1987) including product safety. Deming also told that good science dictated the testing and inspecting the manufacturing processes rather than finished products. In this regard, the planned work to validate the process is crucial to result in outputs and outcomes that are represented in safe food products and regulatory approval.

Deming compared stable processes to unstable processes by defining the capability of a process to succeed by its ability to consistently fall within upper and lower specification or control limits (Figures 36.5 and 36.6). Deming noted two kinds of mistakes: a *bad* mistake is when one reacts to an outcome as if it came from a unique or special cause, when in actuality it came from a common cause that will probably occur again, and a *catastrophic* mistake is

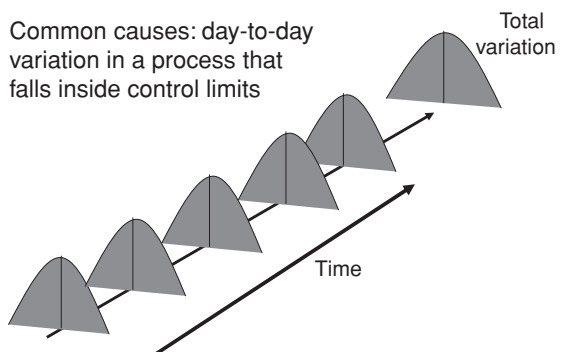


Figure 36.5. Stable process. (After Deming, 1993).

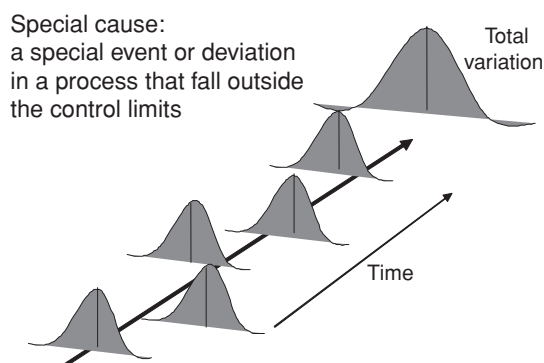


Figure 36.6. Unstable process (After Deming, 1993).

where one reacts to an outcome as if it came from a common cause, when it actually came from a special cause. A contemporary analogy is the disaster of the space shuttle Columbia. A compelling quote from that failure was published by the *Wall Street Journal* (7/23/03). “At fault, we are told, is a culture that failed to see the shuttle as an ‘experimental’ vehicle, needing constant and comprehensive monitoring, like a controlled experiment. Every particle of data ought to be collated and examined, all mysteries solved, anomalies explained and defective parts replaced, before the next flight goes up.” This puts great emphasis on a process that can measure defects and defect opportunities. A defect is an event that does not meet specification for critical product characteristics, and a defect opportunity is the point at which the critical characteristic is either met or missed.

Specification limits are necessary for defining the boundaries in which we want a process to perform. Anything beyond the lower and upper specification limits (LSL and USL) are defects. Specification limits are goal posts for determining process defects. Appropriate action (e.g., inspect, adjust, modify, rework, remove) is required when a defect occurs outside the LSL or USL. One needs to consider that specifications are often incorrect or inadequate and that specification limits are not control limits. Specification limits are what we want from the process or a statement of how we would like the process to perform. Process control limits by contrast are the real mathematical measure of what a process is capable

of delivering. Specification limits are frequently arbitrary and written so that they exceed the capability of the process, resulting in the inevitable production of defects.

9. Summary

New preservation technologies such as high-pressure processing, ultra violet light, and pulsed electric fields offer advantages in meeting consumer demands of freshness, convenience, and safety. There is no single process that will allow the high-quality production of every food product while ensuring safety; all of these processes as well as thermal processing have their own set of limitations and advantages. Food safety management systems based on FSOs and POs provide greater flexibility in how food operators can control hazards. Confidence in the safety of a food depends on the ability of the food industry to control variability in initial numbers of the hazard (H_0), in processes that reduce the hazard (ΣR), and in controlling the increase of the hazard (ΣI) throughout the food chain. Variability must be considered during process validation to ensure safety but also to avoid over-processing. In the future, FSOs and POs will become increasingly important to achieve national public health goals and to determine equivalency of safety for foods in international trade (Stewart et al., 2007).

Successful food safety management is fundamentally about prediction. That is, given a body of data emanating from manufacturing process, one should be able to predict the stability of the process and therefore opine with confidence about the public health status of the food manufactured using those processes. Validation, establishing with great confidence that a specific set of processes will reliably result in the production of products that meet predetermined specification, is a crucial first step in enabling the food safety manager the ability to predict the outcome of a process. It is generally accepted by regulatory officials and food safety professionals that testing and inspecting finished products does not provide confidence or a scientific basis for issuing proclamations about the public health status of those foods. This has resulted in recent years in the

development and adoption by industry of the HACCP system for food safety management. The undergirding of the HACCP system is built on principles of process validation.

Validating novel preservation methods can take a number of forms; however, the elements enumerated here provide a good overview of the essential elements necessary for demonstrating the effectiveness of a novel process. Prospective validation is best-suited for evaluating novel processes. This approach, combined with the risk management strategies outlined in this chapter, will allow for the continued successful commercialization of novel processing technologies that meet the needs of the consumers while not only ensuring, but in some cases improving, public health.

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Chapter 37

Regulations and Alternative Food-Processing Technologies

Stephen H. Spinak and John W. Larkin

Producing food involves a multitude of critical processing considerations and management decisions, the most important of which should be the public health safety of the finished food item. We do not have to look too far to see the importance of this mind-set. Past public health risks associated with fresh spinach, raw almonds, ground beef, and refrigerated carrot juice are just some of the different food items that have resulted in significant changes in the way the food industry processes and handles these food products. The development of food-processing operations must be proactive, not reactive to public health risks. If a company waits until it has to deal with damage control from a health risk, the costs associated with rebuilding the commodity group are often more than small and sometimes medium-sized companies can tolerate. Since food regulations are designed to prevent public health hazards from developing, it makes a lot of sense for a food company to place minimization of potential health risks at the same level of importance as that of economic sustainability.

Governmental regulations of the food industry include local, state, and federal health departments. Finding your way through the different levels of food safety regulations can be at times like solving a maze puzzle. Looney et al. (2001) comment that a food technologist of today needs to be not only a scientist, but also a lawyer and a regulator. They also point out

that understanding of the regulatory environment is complicated at the federal level since there are “no less than 12 separate agencies that oversee aspects of food safety.” It is outside the scope of this chapter to try to bring clarity to the confusing compilation of federal regulatory oversight. Instead, we will look at the scientific principles that need to be incorporated into the developmental phase of alternative processing technologies that are going to be used in the production of food items.

If we look at the reasons for much of today’s regulatory oversight, we find that there has been an outbreak condition that has precipitated the desire to prevent it from happening again. The outcome of this type of behavior has resulted in a strong “command-and-control” type of regulatory environment. This includes much of the existing food-processing regulatory structure within the Food and Drug Administration (FDA), including the Pasteurized Milk Ordinance (PMO), low-acid canned food regulations (21 Code of Federal Register (CFR) parts 108, 113, 114), and infant formula quality control regulations (21 CFR parts 106 and 107), to name just a few. This “command-and-control” oversight has benefited the food industry and the consumer. However, in each case, the regulatory oversight was developed around an existing food-processing industry. When dealing with alternative processing technologies, the developed food-processing industry may not exist. Thus, the focus on alternative technologies needs to be on the use of risk assessment and management to properly establish food-processing operations that will prevent future health hazards.

Nonthermal Processing Technologies for Food, Edited by
Zhang, Barbosa-Cánovas, Balasubramaniam, Dunne, Farkas, and Yuan.
This chapter remains with the U.S. Government.

By elevating food safety concerns to the same level of industrial oversight as that of economic viability of the food product, doors are opened to the food-processing community for being more creative of how it addresses food safety concerns versus waiting for the federal agencies to develop “command-and-control” regulatory restrictions. The International Commission for the Microbiological Specifications of Foods (ICMSF, 2006) has published a discussion on the “Food Safety Objective” (FSO) concept for the development of a food-processing operation. As defined by the ICMSF, FSO is “The maximum frequency and/or concentration of a hazard in a food at the time of consumption that provides or contributes to the appropriate level of protection (ALOP).” The FSO concept looks at the traditional performance objectives associated with many food-processing operations, but also includes the concept of targeting the overall impact of the food process so that the final food product has an acceptable level of safety to the consumer. (see Stewart et al., this volume). Identifying a targeted FSO to a food-processing operation opens doors to the selection of numerous potential alternative processing steps that can be used to design the process. The processor has the option to implement any number of different performance objectives that together deliver the overall food safety objective.

The FDA desires to promote innovation by encouraging the food-processing industry to adopt process establishment procedures that are based on the food safety objective concept. Thus, the question then becomes what is FDA looking for with regard to their evaluation of emerging technologies when using the FSO concept? Obviously, the first issue would be the safety of the end product. This would involve an evaluation of what the process treatment dose should be targeting (i.e., organism(s) of concern) to assure a safe process. There might be different treatment targets dependent on the intended food product being processed and the product’s expected shelf life and storage conditions. Processors are going to need to have a good understanding of the ecology of microorganisms and how they interact in the food matrix. Can postprocessing distribution and usage cause changes in the food product that would

allow organisms not identified in the process design to cause changes in the food so that a new organism of concern becomes the focus of the safety of the food item? Also, there might be a need to deal with chemical issues, whether the compounds are formed during the production of the food product or as a result of potential chemical migrations between foods and the package/process. The labeling of the food product might need to be addressed dependent on the type of process being used; this is especially important for any process that incorporates irradiation technology, which is regulated by food additive rules in the Code of Federal Regulations. Lastly, there is going to be a need to be able to validate the delivery of the process that is used to create the final end product so that appropriate control and monitoring measures can be implemented to ensure that every single food package produced is safe.

1. Organism of Concern

Without a doubt, one of the first items that must be addressed with any emerging food process is its effectiveness when dealing with traditional pathogens. Traditional shelf-stable thermal processes for low-acid foods have almost exclusively focused on the spore-forming pathogen *C. botulinum*. For thermal pasteurization of grade A milk products, the target organism has been *Coxiella burnetti*. However, when dealing with emerging alternative processing technologies we need to take a step back and look at all potential pathogens. We do not know *a priori* what the organism of concern is for a specific process/food product. The scope of consideration might include spore-forming and vegetative pathogens, viruses, and parasites. We cannot assume that what was true for thermal processing is going to be true for an alternative process. This does mean that a microbial screening process most likely will need to be conducted for an alternative technology and that this screening will need to be conducted to be able to demonstrate that all of the potential food-borne pathogenic risks have been addressed.

When dealing with the development of a pasteurization process not currently defined by regulation section 403(h)(3)(B)(i) of the FD&C Act (see

below) requires four elements to be characteristic of a process before the product can be labeled as pasteurized: (1) reasonable assurance it is able “to achieve destruction or elimination in the food of the most resistant microorganisms of public health significance that are likely to occur in the food,” (2) a treatment that is “at least as protective of the public health as a process or treatment” already specified by regulation, (3) “effective for a period that is at least as long as the shelf life of the food when stored under normal and moderate abuse conditions,” and (4) reviewed and accepted by the FDA as safe.

Food, Drug and Cosmetics Act. Sec 403.

A food shall be deemed to be misbranded. . .

(h) Representation as to standards of quality and fill of container. If it purports to be or is represented as—

...

(3) a food that is pasteurized unless—

- A. such food has been subjected to a safe process or treatment that is prescribed as pasteurization for such food in a regulation promulgated under this Act;
- B. (i) such food has been subjected to a safe process or treatment that—
 - (I) is reasonably certain to achieve destruction or elimination in the food of the most resistant microorganisms of public health significance that are likely to occur in the food;
 - (II) is at least as protective of the public health as a process or treatment described in subparagraph (A);
 - (III) is effective for a period that is at least as long as the shelf life of the food when stored under normal and moderate abuse conditions; and
 - (IV) is the subject of a notification to the Secretary, including effectiveness data regarding the process or treatment; and
- (ii) at least 120 days have passed after the date of receipt of such notification by the Secretary without the Secretary making a determination that the process or treatment involved has not been shown to meet the requirements of subclauses (I) through (III) of clause (i).

For purposes of paragraph (3), a determination by the Secretary that a process or treatment has not been shown

to meet the requirements of subclauses (I) through (III) of subparagraph (B)(i) shall constitute final agency action under such subclauses.

When identifying the microorganism of public health significance, two issues that must be addressed, as identified above, are the resistance of pathogenic organisms to the process and the reasonable likelihood of the organisms being in the food. This means that an ultraviolet light-based process may target one organism of concern for apple juice and a different organism of concern for pretreatment of packaging material for extended shelf life perishable foods because different organisms may contaminate the products.

The FDA (FDA, 2004) *Juice HACCP Hazards and Controls Guidance, First Edition*, examines how a processor might identify what the “pertinent microorganism” (V.C.1.1) is for a process.

One way to identify the pertinent microorganism for your juice is to consider whether there have been any illness outbreaks associated with this type of juice, and what microorganisms have caused the outbreaks. If certain pathogens have been demonstrated, that is, through outbreaks, to be potential contaminants in certain juices, then the pertinent microorganism for your process typically should be one of these pathogens.

The Juice HACCP hazard guide also points out that there are going to be a number of products that have not had a significant outbreak history for a specific organism of concern and, in those cases and possibly even in those that have had a history, organisms that are ubiquitous in nature need to be considered. For example, for a low-acid juice product that is refrigerated, the process developer should consider as part of the hazard analysis both nonproteolytic and proteolytic *Clostridium botulinum* because of the ubiquitous nature of *C. botulinum* spores.

Along with the identification of the pathogen of concern, a processor should identify an appropriate nonpathogenic organism that can be used to demonstrate the effectiveness of the process for validation and verification purposes within the processing environment. This surrogate organism will need to behave in a way that will allow its reduction to be

correlated to that of the pathogen of concern. The surrogate should be resistant enough to the process to be measurable after intervention doses are applied.

Identification of an appropriate surrogate organism(s) and the pathogen of concern can be complicated by multieffect processing systems (i.e., surface treatment/washing followed by high-pressure processing). For multieffect processing systems where the technologies for each effect differ widely in mechanisms of reductions of the pathogen of concern, the processor may need to evaluate the safety of the process using more than one organism of concern. This type of problem may also be at issue when both a processing method and a food additive (bacteriocins) are used to render the product safe. FDA continues to be interested in the potential application of bacteriocins, but some food additives may need to undergo a premarket approval evaluation by FDA before they can be incorporated into the process.

2. Food Additive/Food Contact Concerns

The FDA and International Food Information Council (FDA and IFIC, 2010) defines a food additive as

In its broadest sense, a food additive is any substance added to food. Legally, the term refers to “any substance the intended use of which results or may reasonably be expected to result—directly or indirectly—in its becoming a component or otherwise affecting the characteristics of any food.” This definition includes any substance used in the production, processing, treatment, packaging, transportation or storage of food. The purpose of the legal definition, however, is to impose a premarket approval requirement. Therefore, this definition excludes ingredients whose use is generally recognized as safe (where government approval is not needed), those ingredients approved for use by FDA or the US Department of Agriculture prior to the food additives provisions of law, and color additives and pesticides where other legal premarket approval requirements apply.

The FDA and IFIC (2010) further breaks food additives down into “direct” and “indirect”:

Direct food additives are those that are added to a food for a specific purpose in that food. For example, xanthan gum—used in salad dressings, chocolate milk, bakery fillings, puddings and other foods to add texture—is a direct additive. Most direct additives are identified on the ingredient label of foods.

Indirect food additives are those that become part of the food in trace amounts due to its packaging, storage or other handling. For instance, minute amounts of packaging substances may find their way into foods during storage. Food packaging manufacturers must prove to the US Food and Drug Administration (FDA) that all materials coming in contact with food are safe before they are permitted for use in such a manner.

If an alternative process incorporates either a direct or an indirect additive into the finished product, the processor may need to file a food additive petition or a premarket notification with the FDA if the additive has not been already approved for that application. It is always advisable for a processor to consult with the FDA when dealing with direct and indirect additive issues to determine if the process may require either a food additive petition or a premarket notification.

When ultraviolet light was first being considered as an alternative processing technology for treatment of 100% juice products, not only did the processor need to be in compliance with the FDA Juice HACCP rule (21 CFR part 120), but at that time a food additive petition had to be filed (Federal Register, 1999) with FDA to request for a modification of the regulation (21 CFR part 179.39) to allow for juice to be processed by ultraviolet light. Subsequent to the petition, the FDA amended 21 CFR part 179.39 in November of 2000 (Federal Register, 2000b) to allow for the treatment of juice products with ultraviolet light if the process operated at conditions sufficient to have a Reynolds number in excess of 2,200. It should be pointed out here that the FDA does not determine all of the processing conditions that make a process safe with regards to direct or indirect additives. What the Agency does do is evaluate if there is sufficient evidence supplied by the processor to determine if the process specified in the petition/notification is safe. When a company wants to operate outside of those conditions

previously approved, they need to submit a new petition/notification. In the case of ultraviolet light, there may be conditions where processes having Reynolds numbers lower than 2,200 may be safe, but since the FDA has not received any petition/notifications to this effect, the regulation does not now allow for its application.

3. Labeling Issues of Concern

There are a number of different elements of a process/product that are addressed by FDA regulations with regard to the labeling of a food product. The main points of discussion for this section will be labeling components that are specific to the processing technology. Probably, the number one question concerning labeling for alternative processing technologies has been the question of whether or not the product can be labeled as “pasteurized.” Second to the question of pasteurization is the desire to label the product as fresh or “fresh like.” This section will not address questions with regard to nutritional labeling concerns. As is the case for direct/indirect additives, the best thing a food processor can do when dealing with health-based claims and nutritional labeling is to request clarification from the FDA.

For a long time, pasteurization was considered by the FDA as a process that could only take place if heat was involved in the delivery of the process. In 2002, a modification of the FD&C Act took place where pasteurization was better defined (see section 403(h) of the FD&C Act above). Shortly after the change in the FD&C Act, the FDA and the Food Safety Inspection Service (FSIS) of the United States Department of Agriculture (USDA) requested that the National Advisory Committee on Microbiological Criteria for Foods (NACMCF) determine the “requisite scientific parameters for establishing the equivalence of alternative methods of pasteurization” (NACMCF, 2006).

The report issued by the NACMCF defines pasteurization as

Any process, treatment, or combination thereof, that is applied to food to reduce the most resistant microorganism(s) of public health significance to a level that is

not likely to present a public health risk under normal conditions of distribution and storage.

The report discusses each of the elements within the definition of pasteurization. The NACMCF committee recognizes that the main goal of pasteurization is the reduction/removal of the organisms of public health concern but that it may also have a significant effect on the overall shelf life of the food item. They also recognized that the reduction/removal of the organism(s) of concern can be accomplished by any number of different mechanisms; organisms can be removed physically from the food product or rendered inactive by some treatment dose. The report recommends that a FSO approach should be used when establishing the public health level of risk that the final food product should have.

When a food processor is dealing with any alternative process technology that is delivering a pasteurization-type result (which can also include sterilization processes), even if they are not going to be labeling the product as pasteurized, they should consult this report because it covers most of the process development concerns that need to be addressed when developing a safe food process. The committee was given five issues for which recommendations were to be delivered back to the FDA and USDA/FSIS. Expressed as questions, these issues were

1. “What are the scientific criteria that should be used to determine if a process is equivalent to pasteurization?”
2. “What, if any, further research is needed to determine criteria?”
3. “What is the most resistant microorganism of public health significance for each process?”
4. “What data need to be acquired to scientifically validate and verify the adequacy of a proposed technology? How much data would be considered adequate? To what degree can models and published literature be relied upon as contributing to validation?”
5. “What biological hazards might be created as a consequence of the pasteurization treatment?”

When an alternative process is used to produce a food product, it often results, by design, in a quality more characteristic of the unprocessed product than that created by the traditional processing method. Thus, numerous times the food industry has indicated a desire to label the alternative processed food item as “fresh.” FDA has defined the conditions where a food item can be considered “fresh” within title 21 CFR 101.95:

(a) The term “fresh,” when used on the label or in labeling of a food in a manner that suggests or implies that the food is unprocessed, means that the food is in its raw state and has not been frozen or subjected to any form of thermal processing or any other form of preservation, except as provided in paragraph (c) of this section.

Current regulations allow for very few treatments to a food item that would still enable the product to be labeled as fresh. On July 21, 2000 (Federal Register, 2000a), the FDA hosted a meeting to discuss the use of the term “fresh” for alternative technologies. Numerous comments were received from both the open meeting and submissions to the Docket. FDA also hosted a number of consumer focus groups on the use of the term “fresh.” At this writing, the FDA had not modified the definition of “fresh” within 21 CFR 101.95 from that which existed before the request for comments.

4. Process Validation Concerns

The NACMCF report recommends seven steps for process validation:

1. “Conduct a hazard analysis to identify the microorganism(s) of public health concern for the food.”
2. “Determine the most resistant pathogen of public health concern that is likely to survive the process.”
3. “Consider the level of inactivation needed. Ideally, this would involve determining the initial cell numbers and normal variation in concentration that occurs before pasteurization.”
4. “Assess the impact of the food matrix on pathogen survival.”
5. “Validate the efficacy of the pasteurization process.”
6. “Define the critical limits needed during processing to meet the performance standard.”
7. “Define the specific equipment and operating parameters for the proposed pasteurization process. This may include developing specific GMPs in addition to the HACCP system.”

These are excellent tasks that should be conducted when validating a process. There is only one item that a processor might consider adding to this list, :

8. Steps to be used when recovering from a process deviation.

Steps 1–3 are to a large degree part-and-parcel with the processor’s understanding of the organism of concern. As an understanding is developed for what the organism of concern should be for a given food/process, the processor should have a good assessment of the initial load of the organism of concern.

There are two current approaches to setting the target dose of a process. Traditionally, process treatments have been established by targeting a level of reduction/elimination of the organisms of concern. A good example is the 5 log reduction target used for the pathogen of concern in 100% juice pasteurization processes. A log reduction target by its nature is not a process designed to generate a specific level of safety; instead, it is designed around a potential risk. It assumes that the initial load is not going to exceed a potential maximum and that to deliver the level of safety that is considered necessary a specified performance standard (log reduction) is required. The other approach would be to specify the final level of safety that is necessary for the food/process and treat the product to guarantee that level of safety. The FSO concept is directed toward this type of approach. This latter approach would then make the initial load of the organism of concern a critical control point and would allow the processor to take advantage of a controlled system designed to deliver ingredients to a process low in the organism of concern. In either case, a risk assessment of the food/process will allow for a knowledgeable safety target.

Regardless of whether you are dealing with a pasteurization or sterilization process, there is no industry consensus of how to best go about setting the level of safety of the process. Many are under the misunderstanding that sterilization is based on a 12 log reduction of *Clostridium botulinum*; in fact, food regulations define commercial sterility as the process that eliminates the growth of spores or vegetative organisms in the finished product. This means that the safety of a sterilization process can be designed with either elimination or prevention in mind. The process can take advantage of both the food matrix that could prevent growth and the process treatment that could inactivate the spores. The food industry has not tended to adopt or promote any FSO type standard by which a product would be considered sterile. Pflug, in 1987, (Pflug, 1987) proposed an end point process establishment procedure for commercial sterilization of food where he recommended a target probability of 10^{-9} CFU/unit for *Clostridium botulinum*, 10^{-6} CFU/unit for spoilage mesophilic organisms, and/or 10^{-2} CFU/unit for thermophilic organisms. The pharmaceutical industry has, for a long time, promoted and used as a standard 10^{-6} CFU/unit for sterilization of drugs and devices (FDA, 1994). When prevention is not taken into account, for many foods, the food industry has adopted a 5–7 log reduction of *Clostridium sporogenes* PA3679 as an acceptable performance standard for thermally treated commercially sterile food. Because of the greater heat resistance of the *Clostridium sporogenes* PA3679 spores to heat inactivation, this works out to be an equivalent of 5–7 minutes at 121.1°C, which translates into a 20–28 log reduction of *C. botulinum*. Note, for some food products, higher log reductions of PA3679 are needed in order to render the product commercially sterile.

In general, performance standards for pasteurization processes have varied. For example, pasteurization of 100% juice products uses a 5 log reduction of the organism of concern, the treatment of raw almonds uses a 4–5 log reduction of *Salmonella*, and pasteurization of liquid eggs has used a 8.75 log reduction of *Salmonella* as performance standards (NACMCF, 2006). There is no agreed FSO level for the establishment of pasteurization processes. How-

ever, a generalization could be made based on the potential initial load of vegetative pathogens and the currently used performance standards that a level of safety in the range of 10^{-3} CFU/unit has been used.

The importance of step 4 cannot be overstated. The food matrix can have a number of effects on the delivery of a process. One way to look at the importance of the food matrix is to look at the attributes of the food as “attenuators.” This can include pH, water activity, salt content, antimicrobial agents, etc. Attenuators can have an effect on both the growth conditions of the pathogens and the destruction kinetics associated with the reduction of the organism. Also, attenuators can change in their influence during the process. The influence of attenuators can be measured by actual biological validation of the process or by way of models that accurately characterize the inhibition and reduction of the organism of concern. The simplest procedure to understand the effect of attenuators might be to conduct biological validation of the process.

For step 5, when conducting the actual validation of a sterilization/pasteurization process, the question to be answered is can the process deliver the necessary reduction to every food unit every time? The expectation is that at this point in the process, the organism of concern and the target treatment dose is well accepted; the focus is on the process and its operational performance. Does the process deliver a uniform treatment between food units with respect to temperature, pressure, concentration, time, x-ray or UV doses absorbed, etc. (i.e., the processing parameters that affect the treatment dose)? Many times models can be developed where the effect of the process on the microorganism’s viability can be estimated. However, the processor needs to remember that biological hazards do not know that they are required to always act according to a model. Unless there is a clear understanding of the ability of a model to describe the operational conditions of a process and how it affects the associated reduction in the organism of concern, the processor needs to consider conducting physical biological validation. After years of investigation, today’s canning industry relies heavily on modeling of the thermal reduction of the organism of concern. One of these models was developed by

Ball in 1923 (Ball, 1923). In the technical report that Ball used to publish his model, he reported on many of the potential pitfalls the model has and posed the following interesting question:

Mention of influences which introduce error into the calculations described in this paper has been so frequent that the reader no doubt feels inclined now to ask "What is the use? Of what value is it? Can any work which contains so many inherent sources of error have any practical value?"

Ball understood that immediate dependence on his model for the establishment of a thermal canning operation was not advisable. Since the publication of Ball's model, numerous manuscripts have been published discussing the conditions under which the model should be used, resulting in an excellent understanding of when and when not to use the model.

Validation of a process must take into consideration process variability and how to conduct the experiments so that this variability is accounted for; often this involves a validation study operating the process just under its established critical control points. Conversely, the results from a validation study may be used to set the critical control points of a process by using the confidence limits calculated from the study. Often, because of the originality of the process, validation studies are conducted on new equipment where it, most likely, is operating at its maximum potential. Care needs to be used to make sure that a false sense of adequacy is not received on the validation of new equipment. The processor needs to consider the establishment of appropriate verification tests on the process so that the process can be tested over a period of operational conditions (time, shift, cleanliness, and maintenance) so that a history of adequacy can be developed for the process. The importance of a proper change control, verification, and maintenance program must be part of the Good Manufacturing Practices (GMPs) that are developed as part of the final steps to the overall establishment of a process.

Incorporated with the setting of the critical control points of the process is making sure that the critical control points can be measured and recorded. Sometimes, it is difficult to measure a critical fac-

tor on a continuous basis. In this case, the processor needs to determine how best to record the key steps of the process so that there is sufficient evidence that the correct processing conditions were maintained during the manufacturing of the product.

The last step that was added here to those recommended by the NACMCF committee for the validation of a processing system is not necessarily a required element, but it is important for the overall operation of the process. Deviations of a process will occur; the need is to make sure that there is sufficient understanding of the process to be able to appropriately institute the correct procedures to be used to address the deviation. The two approaches in dealing with a deviation are (1) segregate and hold those products that were not treated according to the established process, evaluating them to see if the produced product is safe from potential public health risks, and (2) adjust the processing conditions while the treatment is being delivered in such a way as to add additional dose to the product so that the final product has an equivalent treatment as that which was specified by the process design.

In either case, the processor needs to understand how changes in the process will affect the delivery of the needed treatment dose. This understanding of the process usually comes during the process development and validation steps. Without this additional understanding of the process, the 4 options for the processor when dealing with deviations are (1) conduct the additional necessary experiments to determine if the changes in the process experienced during the deviation still results in an equivalently safe product (2) fully reprocess, (3) divert the product to an alternate treatment technology (e.g., such as to a retort process) and (4) destroy the product.

By conducting the necessary experiments during the process development and validation steps so that the processor has a thorough understanding of the process over a range of processing conditions, the processor will be able to establish appropriate GMP procedures to deal with a deviation and, potentially, to actually specify some processing adjustments that could be used by the equipment operator to deal with a deviation while it is occurring. When considering the establishment of operator adjustments to the

process so that it delivers an equivalent process, the processor must set maximum and minimum changes in the process that can still allow for the delivery of an equivalent process. For example, for a UV process that has a loss of intensity of the lamp during a long period of use, the operator can slow the product flow rate so that the desired treatment is maintained, but there is a minimum flow rate that the operator cannot go below and still maintain a Reynolds number above 2,200 (as required by the food additive regulations).

5. Responsibilities

When a new process, or for that matter any food-processing operation, is being developed, there are going to be a large number of different people and companies involved. This includes equipment manufacturers, process authorities, co-packagers, and product manufacturers. Expertise of the process can be distributed across all of those involved or predominantly reside in just one of the parties. Regardless of who is involved with the development of a process, the safety of the finished product is the responsibility of the food product manufacturer. The food product manufacturer must be able to demonstrate that the process as designed was delivered to each and every food unit and that they are safe. This includes the ability of the processor to be able to determine if changes to the process (ingredient changes, equipment modifications, maintenance, and process adjustments) affect the safety of the finished product.

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Chapter 38

Future Prospects for Nonthermal Processing Technologies—Linking Products with Technologies

C. Patrick Dunne

1. Introduction

The US Army Soldier Research and Development Center in Natick, Massachusetts, focuses on everything for the individual soldier, from the helmets to the boots, to the clothing, and it is the home of the Department of Defense Combat Feeding Program. There are over 120 specialists with expertise on food and food service equipment for the military, and there is an affiliated group that conducts military nutrition research as part of the army medical R&D command. For over 15 years, we have conducted research in-house and have sponsored collaborative research with academia and industry in the study of advanced food-processing technologies, with the main goal of improving shelf-stable combat rations for military use. This review will draw upon this goal-oriented research and development program in respect to nonthermal processing of various foods. At the end of the day, the goal is to have something one can hold in their hand and put in their mouth that tastes much better than what is currently out there. There is a record of innovation coming out of Natick that bears noting. They have pioneered applications of freeze drying and food irradiation since the 1960s and 1970s. In the 1970s, retort pouch technology was developed as the basis of current individual combat rations, the Meal, Ready-to-Eat, or MRE. The

first MREs were produced and issued in 1980 and version 30 is now being produced with 24 different menus. Some lessons have been learned over the past 15 years on how to transfer new advances in technology from the pilot plant to industry and what it takes to get such technology established in the marketplace. It was Natick who pioneered the retort pouch and got the clearances for the packaging material, established the thermal processes, and demonstrated the quality and utility benefits of the flatter profile flexible polymeric pouches. However, in the United States, it took almost 20 some years, during which the military was using the retort pouch, to get retort pouches really flying off the shelves in the commercial market as colorful pouches containing tuna and salmon products.

Figure 38.1 shows a progression for combat ration development where a focus in advanced food technology is now coupled with advanced understanding of nutrition and physiology. One key requirement that really drives Natick to be a technology innovator and technology center is a 3-year shelf life requirement at a nominal 80°F, which is far above normal industry requirements and also demands spending much effort in both packaging and processing technology. Right now, Natick is working on foods to eat-out-of-hand for new rations, which also offer soldiers some optimization of human performance under stress with items such as caffeinated chewing gum. These products are very popular now in places like Iraq and Afghanistan, and active packaging systems are being looked at to further reduce the logistics burden of shipping all around the world, and then ensuring

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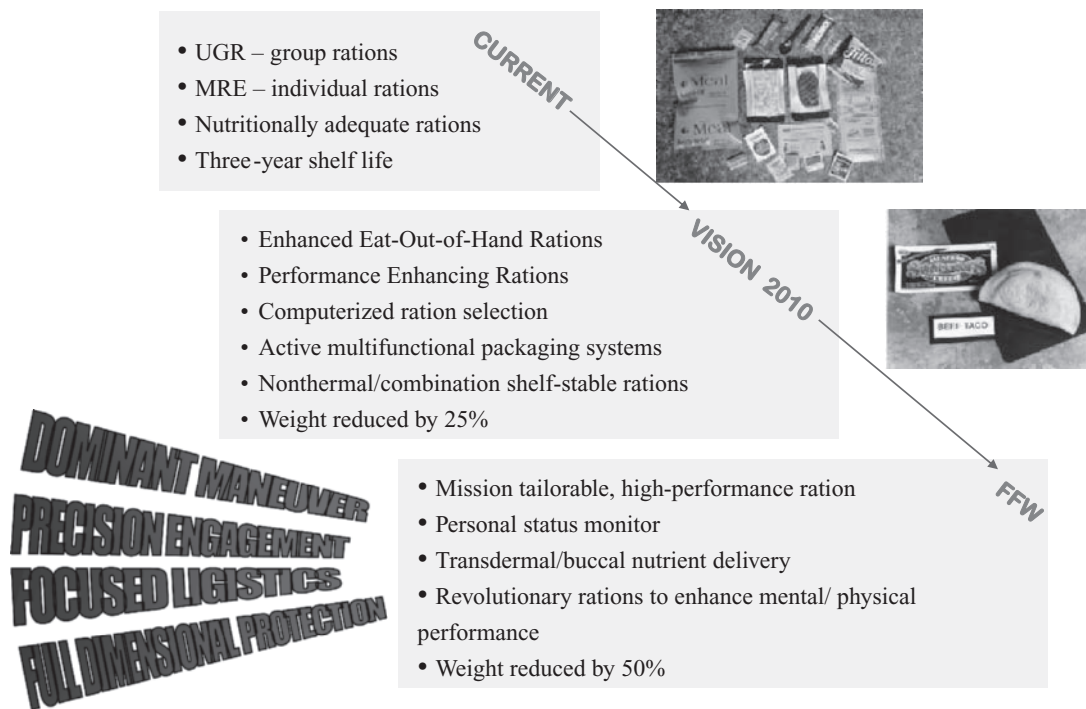


Figure 38.1. Progression of the combat ration R & D process.

when they are in the hands of the Warfighter, the rations are easy to carry and even eat on the move. Natick is also trying to reduce the waste of such products; there are 17 or 18 different items in this MRE. For heating, there is now a little bag with a chemical heater contained in each MRE to heat up the entrée component in 10 or 15 minutes, by activating the heater with a little water. But if time needed to heat the food is limited, some shelf-stable sandwiches are provided that last a couple years and still taste good. In the future, military rations will be even more tailored to the individual, with more of this “star wars” type food. For example, Natick presently has a team of medical people working on a personal status monitor. The goal initially was to just find out if someone was alive or dead by monitoring their heart rate. Now, they are looking at measuring energy expenditures, and maybe someday a red light on the monitor will mean it is time to eat a carbohydrate-rich energy bar, indicating the person’s glucose is low or it is time for a “slug of water” because body water (i.e., free

water available) is low. That possibility is not too far away, and along that vein, Natick has been looking at things like transdermal or buccal delivery, which is a fancy name for what’s happening in chewing gum. The idea is that caffeine in gum goes into the blood stream a whole lot faster because it does not have to go through the digestive tract. Researchers are now conducting pharmacokinetics studies on these items for advanced nutrient delivery. In short, Natick is first trying to increase the physical performance of soldiers, and second, reduce the amount of food stuff carried around.

To achieve these goals, it is important to look at all the emerging processes for stabilizing food—see Figure 38.2 for a type of technology shopping list. Natick wants a home for each of these technologies in one of their ration commodity areas; they have commodity groups, a group ration team, and an individual ration group, with people working on assault rations used for short periods of time and of high intensity effort where resupply is limited. Soldiers also

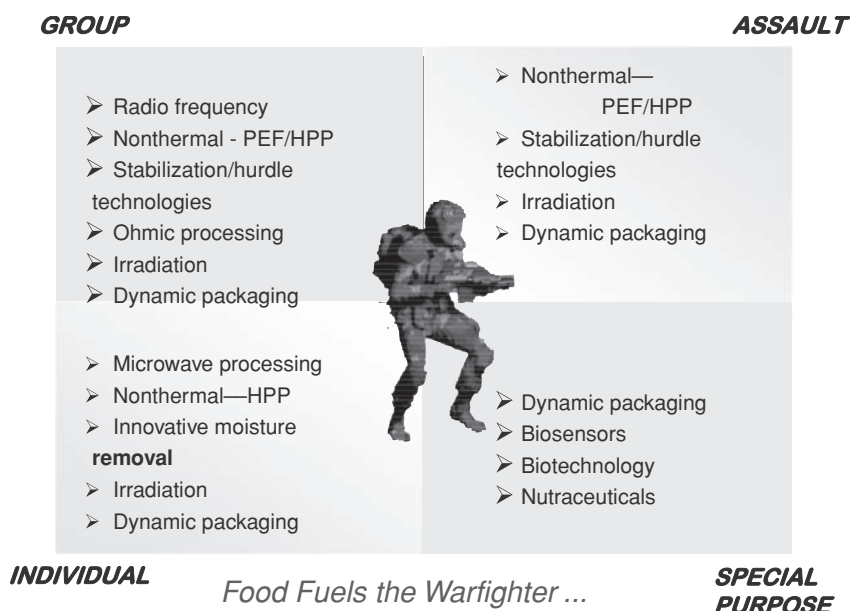


Figure 38.2. The four combat ration platforms with supporting technologies.

must be able to carry everything on their back. The backpacker rations, which can be bought commercially by consumers at camping stores, rely on freeze drying technology, which was first exploited by the military. However, Natick is looking at the next generation of assault rations, which are just going into production and rely on hurdle and intermediate moisture technologies. These rations can be eaten right away without adding water as demanded for freeze dried foods. It is here that some of the new nonthermal processing technologies are going to be a part of the assembly of advanced processing and packaging technologies. The advantages of these novel processing technologies are listed in Table 38.1.

Along the way, some of these technologies have emerged into commercial practice. A new definition of pasteurization has emerged that includes nonthermal approaches in addition to classic thermal processes (Nat Comm. Microbial safety of foods), resulting in products with minimal chemical and physical changes. Nonthermal methodologies have been shown to achieve a 5–6 log reduction of the pathogen(s) of most concern in a particular food, but, of course, a thermal component enters when the aim

is to sterilize the product by inactivating microbial spores of both pathogens and spoilage organisms. This has resulted in a combination process (i.e., a combination of hurdles) of nonthermal and thermal technologies.

Again, a desired outcome for implementation of new processing technologies is improved overall nutrient content of the processed foods that approaches that of freshly prepared foods. This processed foodstuff does have to be packaged appropriately to

Table 38.1. Advantages of nonthermal preservation technologies

- “Fresher” taste and texture
- Pasteurization with minimal chemical and physical changes (nonthermal)
- Possible sterilization by combination processes
- Note: Military shelf life—6 months at 38°C and 3 years at 27°C
- Improved nutrient content
- Maintain higher quality of extended shelf life
- New product categories
- Possible integration to improve classic food-processing unit operations

maintain stated nutrient content over the life of the product. Something people forget about is the Nutrition Labeling and Education Act (NLEA). The numbers on the food label describing nutrient content are supposed to be for the duration of the product. Thus, manufacturers tend to overfortify a product when using traditional thermal processes to account for losses due to processing. Later in this chapter, the advantages of nonthermal processes are illustrated in more detail with examples from different product categories. Here, there is real opportunity to define new product categories that are not really possible with current thermal processing technologies, especially within the functional foods area, and even in the organic foods area. One underappreciated niche for commercialization of novel technologies is what Dietrich Knorr and colleagues in Germany have demonstrated by integrating some of these processes to improve some of the classical food-processing operations, such as improving dehydration or recovery of solids by an initial poration step with electric field pulses (Chapter 13, this volume.)

2. Overview of Novel Processing Technologies

As part of a decision matrix for “when and where” the best applications of new processing technologies may be found, an overview of their capabilities and constraints is presented in Table 38.2.

In this type of R&D, all researchers have potential clients that will want to know why there is money being spent on such processes; they may ask what is it going to do for us? Realistically, one process alone is not going to process every food. So, to give some balance to this overview, one should also consider the advances made in more novel thermal volumetric heating technologies (shown at the top of Table 38.2); these include ohmic heating, microwave, and radio frequency heating. But the one question that keeps coming back is how to measure the dose of energy delivered to the food; related to this issue are the number of constraints that may vary from one technology to another.

In any flow or continuous system, the residence time as well as heat gradients must be determined

Table 38.2. Capabilities and constraints of novel food-processing technologies

Process	Capability	Constraint
Ohmic heating	Flow system for particulates; can formulate to heat solids before liquid phase	Residence time/heat gradients
Microwave	Rapid heating of prepackaged solids	Uniformity/depth of penetration; incompatible with foil layer
RF	Rapid heating of packaged solids	Uniformity and compositional dependence
High pressure	Instant transmission in fluids and solids; treat any food in flexible containers	Does not inactivate spores except at high temp; equipment capital cost
Pulsed electric field	Pumpable products	High conductivity a problem

and be under control for a reliable process. In microwave processes, uniformity and depth of penetration are the main determinants of the size and type of food packages that can be processed. One “show-stopper” is that application to packages used by the military with Al foil barriers cannot be penetrated by microwaves. However, with a similar foil barrier lid stock for polymeric 6 lb retortable trays, used in group rations, a demonstration system at Washington State University was able to use RF heating; the foil may have been another antenna for the RF processor. It did appear that there is a stronger compositional dependence in the RF process compared to microwave processes. But what RF heating does offer in its ability to heat up with more penetration depth than that of the microwave region, at 915 or 2,450 MHz. There will be some trade-offs and added constraints in the application of RF or MW technology to real foods, and that’s why Natick’s collaborators and contractors are exploring this technology’s feasibility for a wide variety of foods.

A prime example of a decrease in a constraint is how much high-pressure processing has advanced with close to 10 years use of high-pressure

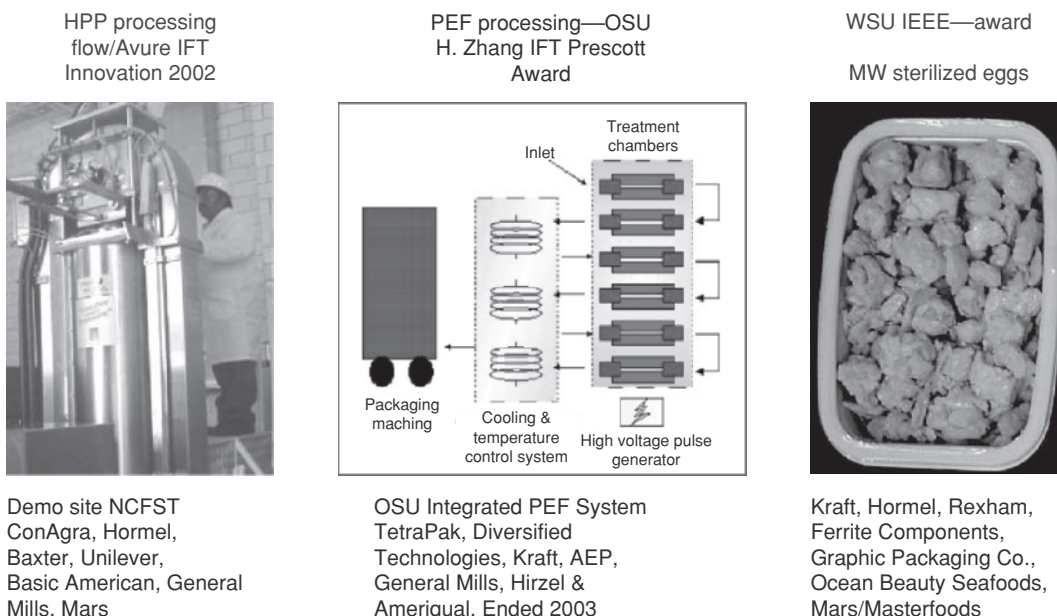


Figure 38.3. Dual use program to develop new food-processing technologies.

pasteurization. Equipment reliability in high-pressure processing used to be an issue, but with experience gained by equipment manufacturers in a number of installations for high-pressure pasteurization, the issue seems to have been resolved. Producers such as Fressurized Foods (formerly Avomex) now pressure process tons of guacamole per day. The capital cost of high pressure equipment is an issue, but the idea of treating almost any food in flexible containers, which is also very scalable to larger containers or larger pasteurization high-pressure vessels, offers a lot of opportunity to the food industry.

As for pulsed electric field (PEF) processing, the technology is only applicable to pumpable products. Researchers have addressed certain issues, such as the limits on viscosity in PEF continuous flow processing, which can be partially overcome by increasing the process operating temperature. High conductivity is another problem with PEF, as well as a limit in size of particulates in the fluids to some degree. Part of the problem is how much money can be allocated for PEF equipment. The constraint is that in order to obtain a large enough gap (greater than 1 cm

diameter), a large power supply capable of delivering a needed critical electric field above 20 kV/cm. is required. In addition, there are other major cost drivers that depend on the nature of the electrical properties of the food being processed.

Figure 38.3 shows an approach using governmental, academic, and industrial participants in consortia to address the barriers to commercialization of selected novel food-processing technologies. It takes a team with varied expertise to move a new technology from the laboratory to the pilot plant, where it can be refined and then scaled up for production and implementation. Fortunately, a new mode of government for conducting business was applied to new food-processing technologies, called Dual Use Science and Technology (DUST) partnership, emerged in 1999 just after some initial feasibility studies of novel processing technologies had been successfully completed. Here, a new contracting model came into use, wherein the government put up a share of Natick's talents and research efforts and some funding. But because Natick also wanted someone to actually make use of the emerging technologies, they invited

industrial and/or academic partners to buy in and support them in a joint enterprise, to work together and share the development cost of such technologies and to combine talents. This was started in 1999 as a congressional mandate for a portion of the military science and technology budget, called “dual-use”, which meant that both military and civilian enterprises could both benefit after the technology was put into practice. The program was modeled based on the National Bureau of Standards Advanced Technology program; the DoD tried to make it an attractive venture by giving up some rights to data to the industrial consortium members in order to attract participants who did not normally do business with the military.

In Figure 38.3, a lot of familiar names in the food-processing industry are listed that joined one of the DUST consortia Natick sponsored. Time-wise, the first consortium was the PEF consortium started at Ohio State in 1999 with Howard Zhang as principal investigator. Although that project officially concluded in 2003, there was a 2-year wait for the first industrial adoption of PEF technology by a small organic fruit juice processor, Genesis Juice (Eugene, Oregon). The next consortium for high-pressure sterilization of low-acid foods was with Flow Interna-

tional, which spun off the firm Avure Technologies for high-pressure food-processing equipment manufacture with Ed Ting as principal investigator and was started in 2000; however, it really took until 2008 to reach the prime goal with the filing of a pressure-assisted thermal sterilization of mashed potatoes accepted by FDA in February 2009. The modified Avure 35 liters pilot scale unit pictured in Figure 38.3 was used at the National Center for Food Science and Technology for this project. Now, bigger industrial units for pasteurization are 200–300 liters capacity units. The third DUST consortium started at Washington State University for demonstration of microwave sterilization of low-acid foods in 2001; it recently filed a semicontinuous process for sterilization of mashed potatoes in a single-serve polymeric tray in 2009, which was accepted by FDA.

As Figure 38.4 shows, there are a number of potential technologies available for stabilizing or pasteurizing different food commodities, but in Figure 38.5, there are a more limited number of sterilization processes for developing improved shelf-stable products. There are challenges with many of the commodities, even in having a safe extended shelf life as minimally processed refrigerated products, let alone

Product type	Current processes	Future processes
Dairy products	Thermal	HPP/PEF
Fruits & veges	Chemical washes	Ozone/irradiation/pulsed light
Fruit juices	Thermal/HPP	HPP/PEF/UV/pulsed light
Eggs	Thermal	Irradiation/ozone/PEF
Baked goods	MAP	Hurdle processes
Complex foods	MAP	Hurdle processes/irradiation?
Meats	Irradiation/HPP	Shockwave
Seafood	Chemical washes/HPP	HPP/irradiation

Figure 38.4. Novel processing technologies for pasteurization.

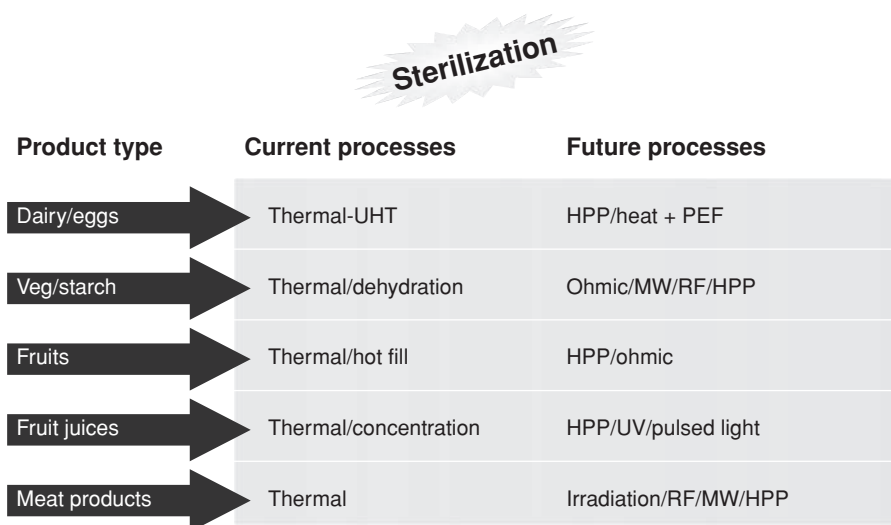


Figure 38.5. Novel processing technologies for food sterilization.

a next generation of commercially sterile shelf-stable products. As far as selecting the correct technology for specific food products, this subject will be considered throughout rest of this chapter. There is a need for honest brokers to assess the fit of a technology to specific products. Thus, this book will attempt to give background information needed to assess the potential of many technologies and will provide a set of criteria and strategies with some case studies.

Figure 38.4 shows all the common or current process used in the middle column. Most consumers should know that raw milk is thermally processed for pasteurized milk. Looking into the future, clearly the pasteurized milk ordinances will need to be followed, so one of Natick's research approaches is to add a nonthermal process to a thermal one. For example, adding PEF to a thermal process may extend the shelf life, at both refrigerated and nonrefrigerated temperatures even up to shelf life the equivalent of UHT milk. However, this milk may not have the cooked flavor found in UHT milk. Current issues with fresh fruits and vegetables deal more with safety than shelf life extension. Right now, chemical washes followed by special modified atmosphere packaging are used, which also prolongs freshness somewhat. Other processes are certainly coming along,

with some applications being demonstrated at a fairly high level, for example with ozone or chlorine dioxide. Physical preservation techniques being studied do have some limitations. For pulsed light, the problem is that it only treats surfaces, whereas irradiation faces regulatory barriers that have been crossed recently, but consumer acceptance remains an issue. The consumer market will largely determine what stays on the potential technology list; what it takes to move to the current product application category is demonstrated by HPP in processing fruit juices, ready-to-eat meats, and seafood because of the quality delivered in addition to safety assurance and shelf life extension. Price points will guide certain adoptions of new technologies. For example, pasteurized or frozen concentrate orange juice has really become a commodity, with tankers coming from Brazil to supplement US domestic production. Unless PEF machines can be installed in Brazil, the market is not going to get the fresh quality that would support a higher price. Another avenue is to build a benefit image, such as with pomegranate juice, to sell the antioxidant benefits and health links of such products. These emerging "healthy" foods offer opportunities to look at new technology solutions to improve their safety, add shelf life, and thus expand distribution.

The shrinking shelf space today in grocery stores of canned goods demonstrates that there are opportunities to provide what consumers now want. People now want “crunchy” veggies, not a soggy product. Surprisingly, HPP can produce a crunchy product for a many foods, fruits in particular. Shelf-stable beef products have been around for years, for example, the Dinty Moore beef stew remembered by most former boy scouts. There are now possible, better ways to produce shelf-stable beef products. If a beef steak that tastes fresh off the grill is desired, one must fly in the NASA space shuttle. There astronauts can eat irradiation-sterilized meat entrees. Natick prepares fajitas and other items as well, which are packed and shipped off to a contract irradiator for processing prior to going on the shuttle. NASA also has an exemption from the FDA for the use of sterilization doses of irradiation used in space feeding systems.

3. Criteria for New Technology Implementation

It takes a multidisciplinary team effort to move a novel food-processing technology from the laboratory bench to even a limited scale production. To guide this effort involving microbiologists, food technologists, chemists and biochemists, as well as sensory and consumer scientists, together with engineers and inventors who are offering a new technology to improve food preservation, a set of criteria can be used to help establish metrics and benchmarks and to guide process development, as follows:

1. Microbiological safety and stability
 - Commercial sterility—10 days at 25°C (acid foods) or 35°C (low-acid foods)
 - Aerobic plate count—injury/recovery assessment included
 - Anaerobic plate count
 - Yeast and mold counts
 - Psychrophilic count and special indicators, for example, *Listeria* in dairy foods and ready-to-eat meats, *Salmonella* in eggs, *E. coli* in fruit juices
 - Determine lethality of process with indicator microorganism(s)—may use inoculated pack study.
 - Absence of pathogens or spoilage organisms after extended incubation to estimate possible shelf life
2. Sensory acceptance
 - Consumer acceptance—9-point hedonics with suitable reference benchmarks; Natick uses either fresh or freshly prepared cooked items for “high-end goal” and commercial or military shelf-stable items of same type, to mark starting baseline.
 - Specific attribute trained panels—shelf life monitoring. Sometimes accelerated shelf life testing at high storage temperatures is done to anticipate problems.
3. Chemical/physical/biochemical stability
 - Color and texture by instrumental methods
 - pH and enzymatic activity
 - Sugar/organic acids/aldehydes profiles done by HPLC
 - Reaction products, for example, pyroglutamate from glutamine, furals from sugars or ascorbic acid, can also be determined by HPLC
 - Nutrient content/nutritional analysis
 - Phytochemicals content, for example, total phenolics content by colorimetric method

In researching a new food preservation process to engineer and optimize the technology, the engineer must start working together with the microbiologist to target an indicator organism and target kill level (e.g., at least 10^5 of the population of 10^7 inoculation in simple media or buffer, then in food matrices). There have been advances in the last 10 years, catalyzed by the Nonthermal Processing Division of the Institute of Food Technologists, where microbiologists interacting with engineers in research of novel nonthermal processes have seen new things in this area of study. Processes like high hydrostatic pressure or PEF do not necessarily kill microorganisms, but a large fraction may be injured, such as to either die off over time or recover in that particular media. Hence, treating and counting the survivors is no longer the main tactic used; one must learn more

about the stress responses and how to control them, perhaps by modifying treatment conditions, while becoming more adept in understanding the mechanisms of microbial inactivation by the technology being employed. There are special indicators for certain foods as seen in the previous list of criteria, which must be taken care of before a technology can be scaled up. To determine the lethality of the process, the indicator organisms are needed, and probably some inoculated pack studies must be done. Until suitable nonpathogenic surrogate organisms are identified, some testing with pathogens likely found in the food of interest should be conducted. Focus totally on pathogen inactivation should be avoided, because if the processed food spoils, a company brand name or a particular technology application will not have a good future. Therefore, in the process development stages, it is important to make sure that some of the common spoilage organisms are handled as well.

After the microbiologist and engineers have at least scoped out the feasibility of a new food-processing technology, it is time to bring in the food technologists to apply the process to selected foods. Previous chapters in this book by Christine Bruhn and Armand Cardello highlighted the need to involve sensory scientists and representatives of consumer populations early in the development of a novel technology, once the safety and stability of the process have been assured. There are two key questions to ask: does the product taste good, and is it appealing? Natick actually runs consumer panels for internal development projects; they also team up with others in grants and consortia that are looking at new food-processing technologies; Natick's mission in this enterprise is to assure the acceptance and actual shelf life potential of foods. One example of this type of team effort was recently published for a high-pressure-treated ranch dressing (Waite et al., 2009). For shelf life assessment, Natick uses attribute panels with 12–15 trained panel members. It is a kind of failure-mode analysis, or one way of looking at it, it allows seeing what might well change with time. The panels are subjected to a number of prospective military items that have been processed with advanced technology processes; consumer pan-

els with a target of 36–40 at a sitting are selected randomly from a much larger population. Natick's goal for sensory panels in assessing foods processed by new technologies is to help technologists and engineers make decisions in the optimization of a process and formulation and further to find out the possible value added. Also, it is important to make sure benchmark products are used. For example, when assessing a new high-pressure-treated product, Natick in this case would use a canned or thermally processed food of the same type as well as a freshly prepared one and measure where the new technology product fits into that spectrum.

It is also important to employ appropriate physical or chemical methods listed in the list of criteria as added stability indicators to help evaluate the main quality degradation mechanisms at work in prototype processed food items. Some of the simplest tools are very appropriate for looking at a processed product's stability over time at different temperatures. A simple pH meter is a useful tool because when things start to change either chemically, or as a result of microbial action, pH is likely to change. For more information on the causes of pH changes, the individual organic acids can be looked at with HPLC method, HPLC analysis also may be used to look at changes in sugars in foods as well as some reaction products that increase over time in storage. For example, the amino acid glutamine degrades with heat, eliminating ammonia, which in turn leaves a cyclic compound called pyroglutamate. This effect is interesting because it could potentially be used as either a process damage or storage indicator for tomatoes, potatoes, and even dairy products (Waite et al., 2009). In stored processed fruit products, it has been found that both ascorbic acid and the sugars might degrade to hydroxymethylfurfural in either the process or during high-temperature storage (Kluter et al., 1996). Here, a cautionary note for use of non-thermal processes in pasteurization of fruit juices is that unlike the more familiar thermal methods, certain residual enzymes such as polyphenol oxidase may remain in the product, affecting the color and flavor of the product during storage. But the real thrust in marketing fruit and vegetable products today is in the phytochemical content or in providing some kind

Table 38.3. Characteristics of processing technologies

Processing Factors	Thermal	High Pressure	Pulsed Electric Fields
Inactivation indicator	Time/temp.	Pressure/time/temp.	Pulse energy, frequency, number-and duration
Batch or continuous	Either	Batch/semi cont.	Continuous
Process in package	Yes	Yes (flexible pkg.)	No
Continuous processing with aseptic pkg. later	Yes—limited by particulates	Demonstrated acid foods	Yes—limited by particulates
Chemical changes in foods	Many	Little or none	Limited
Physical changes in food	Many	Some—gelation	Limited—membranes
Inactivation of food enzymes	Yes—most	Varies	Limited

of indicator of a food's antioxidant potential that indicates the value added to nutrition, and then whether the novel process in question preserves more of activity in food than the traditional thermal process.

4. Applications of Selected Nonthermal Technologies in Food Processing

Table 38.3 provides an overview of two of the most studied nonthermal processing technologies, high-pressure and PEF, compared to the long practiced use of thermal processing in pressurized high-temperature sterilization retorts. As a general rule, all processes require data to ensure food safety, based on measuring the doses of key physical parameters and determining if sufficient energy is delivered to reach the desired state of inactivation as targeted for key microorganisms in a given food. In classic thermal processing, one must know where the cold spot or minimally processed zone is within the array of food packages in the processing equipment, that is a steam retort. In developing a new retort process, monitoring of temperature and time during a process cycle is conducted via thermocouples imbedded in selected packages; thus-the process is controlled by monitoring temperature in the retort media. The added dimension of pressure in establishing initial microbial inactivation targets and process monitoring implies an increased level of difficulty because measurements must be conducted inside a vessel under very high pressures of 60,000 psi or more. A PEF food processor offers added complexity in mea-

suring, as very fast transient pulses are in the kilovolt range, so now a storage oscilloscope or transient recorder is needed instead of (and in addition to) a simpler temperature recording device. Although PEF and high-pressure processes are considered nonthermal, it needs to be recognized that there is still a thermal component associated with each one, and the intrinsic and external thermal aspects of these technologies determine many of the effects on both the food and the microorganisms in food. For instance, there is some compression heating of food packages and the water surrounding the food in a hydraulic press, and significant joule heating of foods that is dependent on their conductivity in the PEF process. However, the exposure to high temperatures that causes many physical and chemical changes in cooked or thermally processed foods is much shorter and usually at lower end temperatures, so HPP and PEF can have minimal effects on the chemistry of processed foods. High pressure can affect the macromolecules in foods, altering texture by gelation of starches or proteins. Most high-pressure processing on a commercial scale is carried out in batches of prepackaged foods, as are familiar canned foods coming out of industrial retorts. PEF in particular lends itself to continuous processing, where fluid foods are passed over or through electrodes and then packaged postprocessing in aseptic containers. Aseptic thermal processing can also be done on a continuous basis, but it has been mostly limited to nonparticulate foods. These new processes do not inactivate all the key native food enzymes typically inactivated in classic thermal processing, so residual

- Solid-state DTI pulser
- Tetra Pak aseptic unit
- Benco aseptic packaging
- Up to 50 kV/cm
- Typical 30 to 90 μ s treatment
- 500 to 2,000 L/h
- Viscous limits, particles to 1/8"
- Temperature control
- Boron carbide electrodes



DTI & Genesis Juice IFT Industrial Achievement
Award 2007 Unit now at USDA ERRC

Figure 38.6. Production scale PEF system.

enzyme action may be a determinant of the shelf life of HPP or PEF-treated foods.

4.1. Pulsed Electric Fields

Active research in applications of PEF has been ongoing for close to 20 years, and earlier chapters in this book capture many of the technical accomplishments. Here, the focus will be on applications that have pushed this technology to beyond the processing of fruit juices to other naturally acidic foods such as applesauce or fruit flavored yogurts. Equipment has matured over time and now a large PEF unit based on solid-state electronics exists, as shown in Figure 38.6. This unit was originally set up at Ohio State University under a DUST Partnership led by Ohio State University and Army Natick Soldier R and D Center (Natick), with a high capacity. One of the industrial partners, Tetra-Pak, donated the integrated fluid handling system that allowed (in the same flow system) comparing a thermal treatment to a PEF treatment, or a sequential PEF or thermal treatment in either order. Packaging was conducted with an in-line Benco aseptic cup packaging machine. This technology evolved from a smaller laboratory unit integrated in a pilot plant, used to

produce prototype PEF stabilized products (Dunne and Kluter, 2001). On a smaller scale, demonstration products can be prepared by packaging in glass bottles, using glove boxes or other clean filling systems matched to the flow of the PEF system's delivery. Because there is direct contact between PEF equipment and the food materials, food engineers need to work with chemists to determine what may be leaking off of the electrodes into the food, under the guidance of food additive regulations. Pure Pulse, one of Natick's key contractors from the 1990s, did receive a FDA letter of no objection for a graphite/carbon electrode, but the newer units will have electrodes made of boron carbide or stainless steel, or even titanium, which was used in the initial PEF commercial system operated by Genesis Juice of Eugene, Oregon (2006–2007).

Among the several products evaluated jointly by Ohio State University and Natick to demonstrate the benefits of PEF processing, one real winner was Fuji applesauce (Figure 38.7). Fuji apples are nice and sweet and you do not need added sugar to make applesauce. The sensory ratings were done by Natick using a 9-point hedonic or quality scale, where 5 was neutral—"neither like nor dislike" and 7 was "like very much". It is always interesting when a

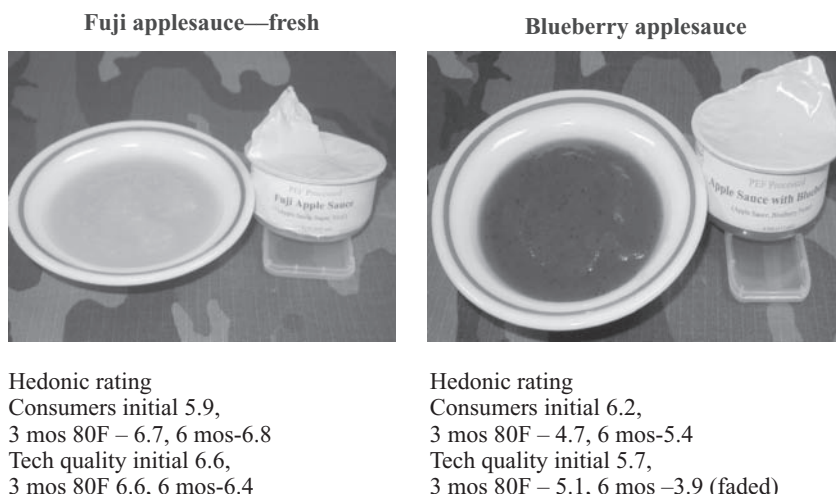


Figure 38.7. PEF products—applesauce.

new process and product are tested on consumers because unexpected results do happen. For Fuji applesauce, consumer scores initially averaged 5.9. The technical attribute quality panel rated the applesauce higher initially and commented that it tasted really fresh with some crunchiness. Then, after storage at 80°F, the product softened up and became what consumers were used to buying and the score went up one point. The applesauce maintained that level for 12 months, and then started to go down because it had turned a little brown. The change in color is evidence of a chemical reaction, with ascorbic acid and sugars in this case undergoing browning reactions, although conversely, the fresh-like flavor was retained. Blueberry applesauce was also tried as a way of adding phytonutrient antioxidants, but the blueberry pigment was not very stable in the product and sensory scores declined with time in storage (Jin et al., 2009). In this way, every product studied is a learning experience; products are stored for a while to observe the chemistry that happens, and diagnosis is conducted to determine the causes of problems observed. For blueberry applesauce, the reactions of anthocyanins with ascorbic acid and oxygen were the main concern, and residual enzymes may also be involved. Another issue was that both the cups and lid stock for the applesauces were not of high-

barrier packaging materials, and over time in storage oxidation of susceptible constituents is likely. Other analyses showed that ascorbate in PEF juices went to 0 in 6 months because too much oxygen was leaking through the packaging.

A number of shelf life studies with PEF-treated fruit-containing yogurt-based puddings were also conducted by Ohio State University and Natick. For instance, blueberry and other flavored yogurts were demonstrated to have good shelf lives after PEF processing. In the case of strawberry products, it was decided to improve fresh flavor impact using IQF (individually quick frozen) strawberries instead of strawberry preserves, but PEF treatment did not improve the flavor or overall score of the product. In addition, PEF did not improve the stability of the product either, as there was growth of some spores to spoil the product. These and other related studies involve steps needed to optimize a process, starting with the formulation and iterations of some stability tests. For instance, the blueberry color and flavor in the yogurt drink held up pretty well in overall quality during storage, much better than the blueberry in the applesauce. Strawberry puddings (top of Figure 38.8) were also tested in a pilot plant PEF system at OSU and lasted a good long while as well; the process was based on a combination of heat and



Hedonic rating 6.1(6.3 OSU)- initial;
6.5 – 3mos 4C; 6.4 - 6 mos. 4C;
5.3 – 24 mos. 4C

Hedonic rating
Initial – 4.5,
3 mos 80F – 2.6



Blueberry yogurt drink



Hedonic rating
Initial – 5.3, 3mos 80F – 4.1

**Strawberry yogurt drink -
IQF strawberries, no added
color**

Figure 38.8. PEF yogurt products.

PEF. These puddings were not really deemed to be flavored yogurts since the live organism counts demanded for yogurts (about a 10^6 level) did not exist. As for the environmental effects on the sensitivity of PEF treatment in general, lowering the pH will help, such as in juices or fermented dairy products, which are naturally low in pH. Also, higher temperatures, especially above the threshold for microbial membrane transitions (approximately 40°C), really make PEF much more effective than thermal pasteurization alone.

The big news in 2007 was the initial commercial application of PEF pioneered by Genesis Juice of Eugene, Oregon. There was a huge regulatory incentive in their case, because as a small organic juice processor, they had experienced some problems complying with the juice HACCP requirements. Genesis Juice went to the local extension agent at Oregon State, who explained there is a new technology called PEF for juice pasteurization and were then referred to Ohio State University. Genesis Juice then actually

had in their production plant a loaner PEF machine from Ohio State university, that is, the same pilot plant PEF unit resulting from 5 years of research by Dr. Howard Zhang's group and supported by Natick. The unit had a throughput of 200 Liters an hour. Thus, Genesis Juice packed a variety of organic juices in glass bottles of different sizes in this unit, and in the beginning shipped products primarily in the state of Oregon, but later shipped to other locations. Figure 38.9 shows a larger bottle of Genesis apple juice plus two thermally processed products bought at a Massachusetts supermarket as benchmarks for sensory and shelf life studies conducted at Natick. One was refrigerated, and the other was in the shelf-stable organic foods section. One interesting fact noted on the labels of smaller size Genesis Juice products, found in refrigerated display cases, was the labeling "processed by this novel technology" and listing of the US patent number granted to Ohio State University for PEF electrodes in a flow system. Table 38.4 displays data on the Natick study done in 2008



Figure 38.9. PEF apple juice.

comparing the raw unprocessed juice, which was shipped in raw form to observe the chemistry of the juice as affected by PEF treatment; Natick then monitored refrigerated storage at 40°F. A one and a half log reduction in aerobics was reported after one-month shelf life. Initial yeast and mold counts were below detection levels in unit; at 1-month point of storage mold growth was detected. The target refrigerated shelf life for the organic juices was one month. Sensory scores from a trained panel were above 6 on a 9-point quality scale for 3 weeks. The comparable reference products held in the same storage conditions was about the same range, but the thermally processed organic juice rated much lower, at 4.9. Panelists found that PEF juice had a fresher flavor. Natick then determined total phenolics in the

juice as a measure of the level of phytochemical antioxidants. There was some reduction after the PEF process. The organic acid HPLC profiles showed essentially zero ascorbic acid in apple juices from Genesis Juice. Some apple juice products on the market have added ascorbic acid as a processing or shelf life aid. There was not much effect from PEF processing on the sugar profile, as would be the case with conventional heat treatment. The sucrose level dropped somewhat, but the main sugar in the apple juice was fructose, as found in the apple itself. Genesis Juice was awarded the Industrial Innovation Award by the Institute of Food Technologists in 2008, but later that year closed down operations in Oregon based on lack of funds needed to support a wider distribution of their products to build a market.

Table 38.4. Effects of PEF process on organic apple juice from Genesis Corp., Eugene, Oregon

Product	Malic Acid mg/100 mL	APC	Sucrose	Glucose g/100 g	Fructose
Fresh raw juice	224	1.14×10^4	7.0	5.6	8.9
PEF treated—initial	182	4.87×10^2	5.5	4.5	8.3
Sensory scores—9-point quality scale technical panel					
PEF tested 2/28 (held at 40°F)		6.6			
3/13		6.5			
3/21		6.4			
Total phenolics untreated juice—34.9 mg/L gallic acid equivalent initial PEF juice 2/28–21.1 mg/L					



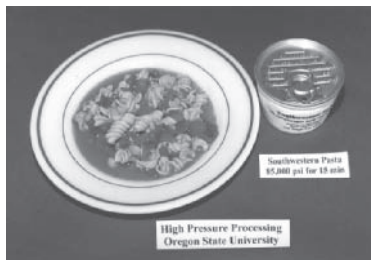
Figure 38.10. Commercial HPP food.

4.2. High-Pressure Processing

high-pressure processing is now gaining wide acceptance in the marketplace for a wide variety of refrigerated foods, as can be seen in Figure 38.10. Benefits of the use of high pressures to inactivate target pathogenic microorganisms are the extension of the shelf life of refrigerated products and the production of high-quality products with minimal use of food additives that consumers will buy. Because the Army is primarily interested in improving the quality of shelf-stable items for use in packaged combat rations, Natick started funding investigations of high-pressure processing in the early 1990s at Oregon State University in partnership with the University of Delaware; together, they studied acidified foods under high pressure without extra added heat, and the effort was led by some of the pioneers in high-pressure processing, notably Dan Farkas and Dallas Hoover (Hoover et al., 1989) who also had Marsha Walker as a product developer. They developed a nice family of very tasty products, such as the Southwestern pasta shown in the top left of Figure 38.11. The tomatoes are very bright with a good flavor essence that was maintained over two years in storage at room temperature. Another product

developed showing the application of high-pressure processing to high-quality shelf-stable products was Spanish rice with green peppers; the peppers were crunchy and maintained that quality in the product, which was packed and processed right into single-serve thermoformed cups. The failure mode in these room temperature shelf life products was corrosion of the aluminum lidstock, not deterioration of the food. Marsha Walker later joined Avomex, now Fressurized Foods; as vice president of research and development, she is responsible for one of the major success stories in the growth of a novel technology application in the food industry. Their initial breakthrough product was a guacamole produced from freshly harvested avocados; this high-pressurized product has a shelf life supportive of national distribution.

The next step in high-pressure processing was to explore the use of heat combined with high pressure to inactivate bacterial spores. A DUST consortium was started in 2000 sponsored by Natick and Avure Technologies of Kent, Washington; the project was centered at the National Center for Food Safety and Technology of the Illinois Institute of Technology, with industrial partners (listed in Figure 38.11), to demonstrate production of shelf-stable



Project objective:
Optimize and validate
High-pressure
processing to ensure
safety and nutritional
value of fresh-like
rations and commercial
items

*Partners—Hormel,
Unilever, Basic
American Foods,
ConAgra, Baxter,
NCFST, Gen. Mills,
Mars/Masterfoods*

Precursor - Oregon
State Univ. Acidified Products

*Demonstration
Contract 1999-2001*

Avure

2002 IFT Industry
Achievement Award

2005 FLC Tech.
Transfer Award
Shared by C. P.
Dunne of Natick



Figure 38.11. high-pressure processing of low-acid food FY00-08 dUST.

low-acid foods. The essential concept was to make use of the physical properties in compression heating to achieve a rapid sterilization cycle that could improve product quality—faster and better than that possible in a commercial retort. Figure 38.12 outlines a pressure–temperature cycle for a pressure-assisted thermal sterilization process as put into practice by the DUST consortium. A 35 Liter pilot size high-

pressure system (bottom right, Figure 38.11) was modified by Avure Technologies to operate reliably at final temperatures of at least 121°C, as demanded for commercial sterilization by thermal means. The product in flexible containers was preheated to 90°C, which as pressure was applied in the closed vessel with a 2-minute come up time reached an end temperature of >121°C.

The first solid line in Figure 38.13 is an example of what happens in high-pressure sterilization, showing a longer hold time of 5 minutes (instead of 3 minutes) for mashed potatoes, because for product quality reasons, the maximum hold temperature was below 121°C with an egg product. This pressure-accelerated thermal process is only a fifth of the total time the traditional retort cycle would normally be. Other high-pressure sterilization products have been evaluated by Evan Turek and the Kraft people (Lau and Turek, 2007). They showed the importance of pretreatment such as using a salt-containing marinade or slight poaching in brine so that proteins do not toughen up under the HPP process. The resulting flavor of the product was something more like poached salmon than a retorted flavor, which at Natick is called a “retorted note,” not tinny but

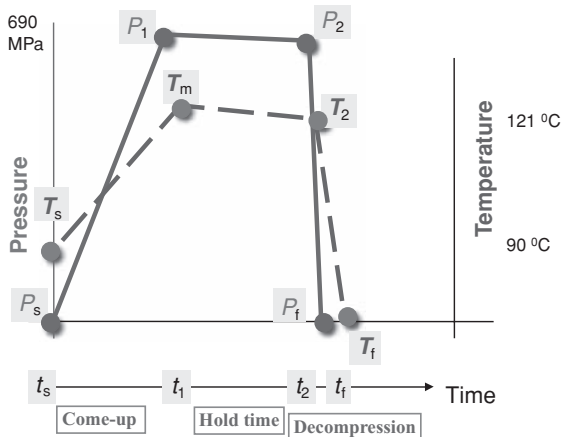


Figure 38.12. PATS pressure/temperature cycle.

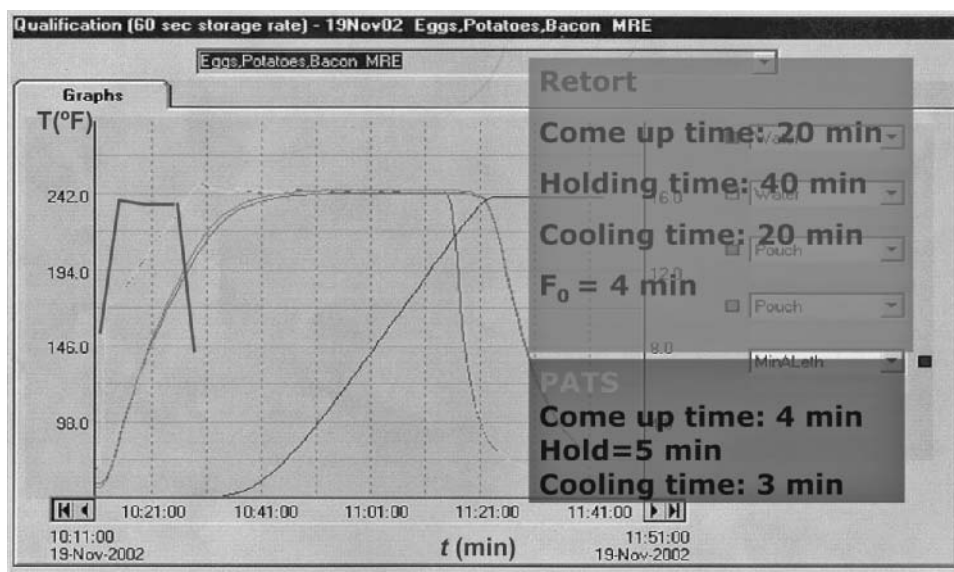


Figure 38.13. Process temperature profile: retort versus PATS.

a sort of metallic note, or a sharp note; this is not the case in high-pressure sterilization systems with minimal thermal processing. To some degree, similar results can be achieved with microwave processing. But again, pretreatment and formulation are important steps in making the best possible final processed product.

In summary some of the things we have learned about high-pressure processing are listed below: (1) Increasing the initial temperature for high-pressure treatment will increase effectiveness for vegetative bacteria reduction; optimal treatments may exceed 40°C under pressurization, and there is often a significant fraction of injured bacteria. (2) Bacterial spores are not easily inactivated by high pressure; therefore, shelf-stable products can be obtained only with acid foods unless the process is run at elevated temperatures, such as in the PATS process. (3) Sensory studies conducted at Natick and other centers have shown that food properties are much more fresh-like than thermally processed products. (4) High-protein foods are subject to texture changes by HPP; however, in some cases, pretreatment and formulation can minimize the effects. Process and product optimization can then

proceed once safety has been demonstrated. Figure 38.14 shows a consumer market emergence matrix for high-pressure processing developed by one of the major high-pressure equipment providers, Avure Technologies. That market is currently limited to pasteurized foods held under refrigeration. The successful products with a higher market share have certain food safety risks that are mitigated by high-pressure processing and have sensory properties superior to products from more traditional processes that may rely on additives to achieve food safety and shelf life targets. The technology barriers to move from pasteurization to sterilization are formidable and may not be achieved in all food categories. There is obvious added benefit to having extended shelf life of foods in the military, which has a logistic chain that, for example, on short notice must move “a city” of 200,000 people or more. The military demands this kind of extended shelf life to draw upon reserves in deployments, but whether it is really needed in the consumer world is not yet clear. Nevertheless, the strategy of combining a nonthermal processing technology with temperature is important in addressing the first targets for sterilization with starch-based potato and pasta items where quality benefits may

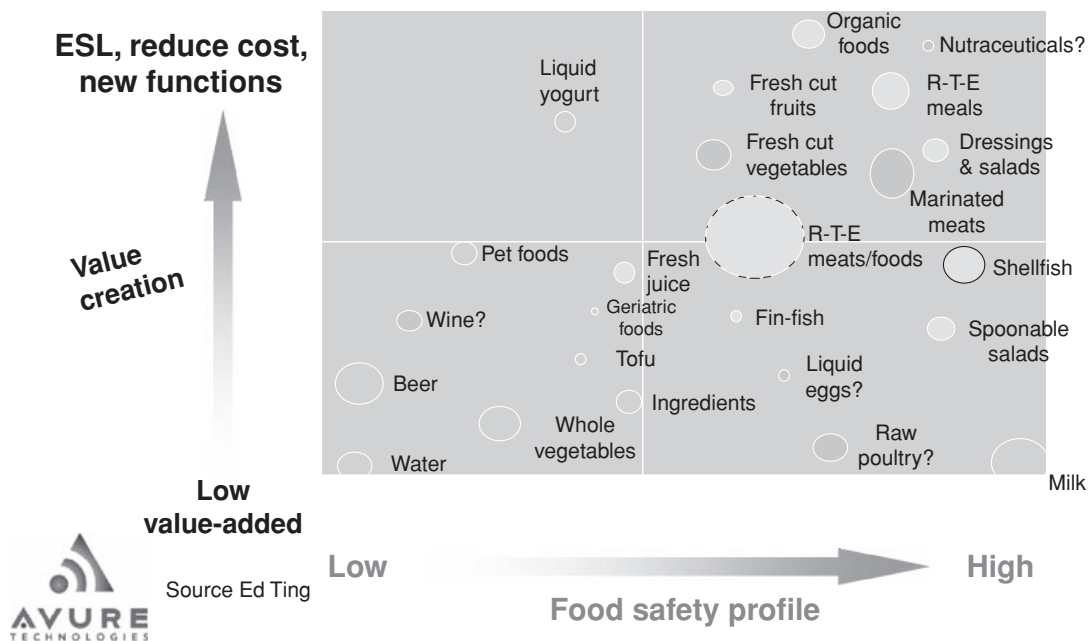


Figure 38.14. HPP market segmentation matrix.

lead to products with dual military and civilian use. To meet shelf life and quality requirements for MREs and similar shelf-stable packaged low-acid foods, a Pressure Assisted Thermal Sterilization (PATs) process was developed and tested. Mashed potato packaged in a retort pouch was picked as the first product for FDA filing in September 2008 and accepted in February 2009. The main motivation is that the current military MRE mashed potato is not terribly appetizing, tastes somewhat browned, and also looks brown. The replacement PATs product had much higher sensory panel scores from consumers and a trained technical panel.

A list of opportunity areas for high-pressure processing follows:

- Ready-to-eat or heat and serve meat/meal items
- Seafood—self-shucking bonus
- Desserts, puddings, and dairy items
- Sauces and savory foods
- Potatoes and other starches, especially for breakfast—first target for sterilization
- Whole muscle meats, partially precooked

- New market categories, for example, avocado products and other “fresh” items with extended shelf life
- Functional foods

There is growing demand for ready to heat and serve meals, including meat items. Evidence of the consumer trend is the growth in the deli counter in the supermarket, and a shift in allocation of shelf space to feature convenience products. High-pressure technology can integrate putting the sauce into the meat with pressure marination, which can be done with whole-muscle meats, partially precooked to tenderize the final product. However, pasteurization of uncooked red meats with high pressure is not really viable because pressure will denature the muscle pigment myoglobin and cause a red to brown color shift. Chicken and other fresh poultry meats treated with pressure do not have such major changes in appearance after high-pressure treatment. An emerging market category for the use of high pressure and nonthermal technologies is in the category of functional foods, where these processes have less effect

on micronutrient content, especially bioactive phytochemicals when compared to thermal processes of fruit and vegetables (see Chapter 33, this volume). As more is learned on the effect of pressure processing on the structure of food macromolecules, polysaccharides and proteins in particular, food technologists may learn how to exploit the changes in new classes of “designer foods.” For example, one might retard starch retrogradation somewhat with the pressure treatment.

5. Summary and a Look into the Future

Without dwelling on many of details of new processing technologies that are covered in previous chapters in this book, there are several issues surrounding new processing technologies as they move in stages from laboratory research to pilot plant development projects and then into commercial production and marketing. One set of issues deals with process novelty. Here, industry must weigh quality benefit versus the cost of switching to a new technology for processing foods. Consumer acceptance will be the key as seen in Chapters 31 and 32 in this volume. Here, the focus may be to sell convenience and quality including nutritional value, and the feature of fresh-like character of processed foods with extended shelf lives will have some issues with using a “Fresh” statement on the label under current FDA guidelines expressed in the Code of Federal Regulations. Safety of the new products must be given. The previous chapter by Spinak and Larkin (see Chapter 37, this volume) discusses regulations as applied to alternative food-processing technologies. Research and development teams exploring applications of new technologies to classes of food they are interested in seeking regulatory approval will have to gain familiarity with the regulations most applicable to their chosen technology approaches. Regulatory barriers to introduction of new refrigerated extended shelf life products will be different and lower compared to shelf-stable products. Then, within the shelf-stable food category to replace traditional canning technology guidelines for acid or acidified foods come under Code of Federal Regulations (CFR) 21 Chapter 114, and the traditional low-acid

canned food regulations of 21 CFR 113 govern the applications of any process to produce shelf-stable products with pH of 4.7 or above. Here, the process must be designed to inactivate bacterial spores and to control the pathogen of most concern, *Clostridium botulinum*. The emerging new processes do put a certain demand on the regulatory agencies, and one key effort in this regard was produced by a contract between the FDA and the IFT, to review what was known about the kinetics of microbial inactivation of alternative food-processing technologies to provide a science base to aid regulatory decisions. A report of the study was published in a special issue at the end of the year 2000 in the *Journal of Food Science* (Anon, 2000), and it should still be on the FDA website at <http://vm.cfsan.fda.gov/~comm/ift-toc.html>. This volume hopes to cover some of the 10-year period of research since this review was published. It is clear that the principles of Good Manufacturing Practices (GMP) and Hazard Analysis and Critical Control Points (HACCP) should be used in the development and testing phases of novel processes to guide their implementation. Successful research in this area of emerging food processes requires interdisciplinary teams of engineers, microbiologists, food scientists and technologists, chemists, and sensory scientists with a vision to provide consumers with better safe processed foods. In the end state, the products need to be more fresh-like. Whether they can be labeled fresh-like or fresh is a whole other issue. The impact can be seen in certain products that are market successes now, such as high-pressure-treated guacamole or ready-to-eat meat products. The volatile aroma retention is a key benefit, and the visual aspect is also enhanced.

On the basis of more than 15 years of experience in R&D efforts for variety of novel processes for pasteurization of foods, I came up with a decision tree for possible technology choices, which is shown in Figure 38.15. Certain guidelines should be kept in mind during the research feasibility stages. There are goals to be set for determining if a uniform and reliable process can meet goals for level of microbial reduction that should be targeted for the processing of that food in question. The R&D team needs to assess importance of compositional

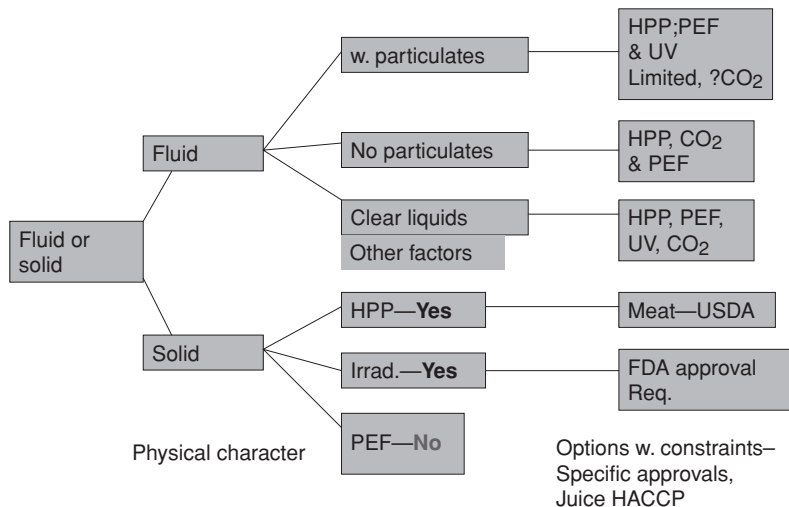


Figure 38.15. My original decision tree—physical pasteurization methods.

dependence of the food's microbial population, including effects of the food matrix on the recovery of injured microbes after some type of nonthermal processing. For fluid systems, such as fruit juices, the presence of particulates as well as the optical density will place some limits of the technology that can be applied to reach a target inactivation level, such as a 10^5 reduction of the pathogen of most concern in that product. Juice pasteurization in continuous flow systems has been demonstrated in systems using high pressure, PEF, dense phase CO_2 , and ultraviolet light (UV) in specially designed fluid reactor systems. And for certain technologies that work in fluids, there are problems if there are particulates in them. Because particulates may harbor microorganisms, the energy applied to inactivate must penetrate the particulates. UV light would have problems with shielding, and large particulates have a practical size limit based on the electrode geometry in continuous flow PEF systems of one half a centimeter or less. If the food needing to be pasteurized is a solid, the nonthermal options are more limited when the need is to go beyond reduction of microbial populations on the surfaces. High pressure and ionizing radiation have both been demonstrated to be effective in a variety of foods, and much attention has

been given to meat items because of the concerns with *Escherichia coli*, *Salmonella*, and *Listeria*. Any treatment of meat items must have approval of the USDA, and the current food laws demand that ionizing radiation be treated as a food additive with a separate petition to demonstrate both safety and efficacy of the treatment of each different item to be accepted by the FDA.

Some common themes have emerged in the areas of safety and process assurance that have to be dealt with to move the technology from the laboratory into industrial practice. One of the first needs, expanding on the first line of Table 38.3, is the dosimetry to measure the energy applied to and absorbed by the foods to cause a reduction in microbial populations. In the classic mode of thermal pasteurization or sterilization, the keys to this inactivating dose are temperature to measure absorbed heat and the time of that high temperature exposure. Typically, a thermocouple or actual thermometer connected to a time-based recorder is used to monitor a thermal process. Inactivation of organisms by high pressure requires a determination of the pressure reached as well as the holding time at that pressure and the temperature. Bigger challenges in dosimetry are found in flow versus batch systems to measure the

energy absorbed by the food during the process, where the residence time distribution in the treatment zone must be known in the flow process. For complex physical systems using PEF, the energy comes in short microsecond bursts of intensity, so knowing energy absorbed in terms dictated by Ohms law means that voltage and current as well as pulse frequency and duration will have to be captured by a rapidly recording oscilloscope. A second need is to determine process uniformity, or in cases of known nonuniformity to know where the equivalent of a cold spot is and to establish a process sufficient to inactivate the microorganisms in that “cold spot” to the target level. IR or some other microelectronic device may be needed to address this issue. Since physiologists now can give people a pill-sized monitor to swallow and give a radio report of the body’s internal core temperature, we would hope that our food technology suppliers would be able to do the same for us who produce foods in a number of manners where the process safety is determined by time and temperature. It may be unrealistic to have real time signaling because the signal may have to be sent out through metal walls. But there are miniaturized devices, such as RFIDs with smart clocks and temperature sensors in them. And in the future, some of those will be rugged and small enough to be appropriate to adopt for either in-vessel or in-package process monitoring. The third need is to develop some understanding of and modeling of the microbial inactivation by the given treatment of each novel processing technology. Environmental factors especially the pH and available water or water activity as well as the viscosity of products in continuous flow systems will affect the novel processes in manners similar to effects on thermal processes. Models will also be needed along with confirmatory measures to understand the energy distribution of foods being processed. There are likely to be a variety of interactions involving some chemistry of the foods being processed as well as the biochemistry of the microbial populations in the foods. One advantage of nonthermal processes, such as PEF or high pressure is that chemical changes resulting in changes in food color, taste, texture, or aromas are lesser than those of a thermal process

of similar effectiveness in microbial control. Some recent publications offer comparisons of effects of different processes on either macromolecules (Li et al., 2006) or important nutrients in foods (Sanchez-Moreno et al., 2009). We are now at a point where some of the novel technologies described in this book and now adopted and put in to practice in the marketplace. Successful products will all have optimized formulations and preprocessing steps. The other key feature of the successful product is a proper packaging system to protect the quality of the foods within. Today’s competitive industry still has to look at the costs and quality tradeoffs and overall benefits while assuring customers of the products safety. As shown in Figure 38.14, which represents a market distribution possibly 5 years ago, the largest circles are those offering both convenience and quality advantage of high-pressure processing. Getting the right packaging may be overlooked in the early stages of process/product optimization, but it is a necessity to get the quality to be maintained for the target shelf life of the product. high-pressure processing serves as an example because of wider adoption than most of the novel nonthermal processing technologies. It offers some major benefits, such as the ability to treat foods in any flexible container. But we do have to deal with certain limitations, such as the need for minimal head space to avoid a big air gap difficult to pressurize. And one major issue with some of high barrier packaging material is that the laminations have issues with its sensitivity to pressure and temperature, and those get even greater at higher temperatures that will be required to produce shelf-stable foods (Koutchma et al., in press). Before any product is released to the marketplace, the developer must run some shelf life tests with proper packaging.

As researchers try to move from pasteurization and shelf life extension of refrigerated foods to the development of new shelf-stable products with non-thermal technologies, the burden of proof of safety and stability gets much greater. Now, bacterial spores are the target in addition to vegetative organisms. We need to understand more about spore physiology, and those researchers investigating a novel technology must learn more about inactivation and germination

of bacterial spores (Setlow, 2007). We are now hopeful that combinations of certain natural preservatives with nonthermal processes will offer improved microbial control (Chapters 27 and 28, this volume). One other major research need is to develop better equipment, not only to do the processes but to monitor the processes. There are inherent difficulties to be overcome in adapting current temperature monitoring devices to function in high-electrical fields on inside a high-pressure vessel. One must believe that the advances in the quality and safety of foods the new processing technologies offer will stimulate the scientific and engineering advances needed for the growth of new food industrial systems in the current global marketplace.

Acknowledgments

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Section VI

Appendices: Fact Sheets

Appendix 1

High Pressure Processing

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Extension FactSheet

FSE-1-04

Food Science and Technology, 2015 Fyffe Road, Columbus, OH 43210-1007

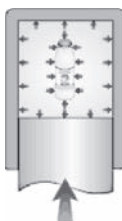
High Pressure Processing

Fact Sheet for Food Processors

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1. Why high pressure processing?

High Pressure Processing (HPP) is a method of food processing where food is subjected to elevated pressures (up to 87,000 pounds per square inch or approximately 6,000 atmospheres), with or without the addition of heat, to achieve microbial inactivation or to alter the food attributes in order to achieve consumer-desired qualities. Pressure inactivates most vegetative bacteria at pressures above 60,000 pounds per square inch. HPP retains food quality, maintains natural freshness, and extends microbiological shelf life. The process is also known as high hydrostatic pressure processing (HHP) and ultra high-pressure processing (UHP).



2. How does this technology benefit consumers?

High pressure processing causes minimal changes in the 'fresh' characteristics of foods by eliminating thermal degradation. Compared to thermal processing, HPP results in foods with fresher taste, and better appearance, texture and nutrition. High pressure processing can be conducted at ambient or refrigerated temperatures, thereby eliminating thermally induced cooked off-flavors. The technology is especially beneficial for heat-sensitive products.

3. How does HPP work?

Most processed foods today are heat treated to kill bacteria, which often diminishes product quality. High pressure processing provides an alternative means of killing bacteria that can cause spoilage or food-borne disease without a loss of sensory quality or nutrients.

In a typical HPP process, the product is packaged in a flexible container (usually a pouch or plastic bottle) and is loaded into a high pressure chamber filled with a pressure-transmitting (hydraulic) fluid. The hydraulic fluid (normally water) in the chamber is pressurized with a pump, and this pressure is transmitted through the package into the food itself. Pressure is applied for a specific time, usually 3 to 5 minutes. The processed product is then removed and stored/distributed in the conventional manner. Because the pressure is transmitted uniformly (in all directions simultaneously), food retains its shape, even at extreme pressures. And because no heat is needed, the sensory characteristics of the food are retained without compromising microbial safety.

4. Can HPP be used for processing all foods?

Like any other processing method, HPP cannot be universally applied to all types of foods. HPP can be used to process both liquid and solid foods. Foods with a high acid content are particularly good candidates for HPP technology. At the moment, HPP is being used in the United States, Europe, and Japan on a select variety of high-value foods either to extend shelf life or to improve food safety. Some products that are commercially produced using HPP are cooked ready-to-eat meats, avocado products (guacamole), tomato salsa, applesauce, orange juice, and oysters.

HPP cannot yet be used to make shelf-stable versions of low-acid products such as vegetables, milk, or soups because of the inability of this process to destroy spores without added heat. However, it can be used to extend the refrigerated shelf life of these products and to eliminate the risk of various food-borne pathogens such as *Escherichia coli*, *Salmonella* and *Listeria*. Another limitation is that the food must contain water and not have internal air pockets. Food materials containing entrapped air such as strawberries or marshmallows would be crushed under high pressure treatment, and dry solids do not have sufficient moisture to make HPP effective for microbial destruction.

5. Will the process damage the food product?

During HPP, pressure is uniformly applied around and throughout the food product. For example, a grape placed between fingers can be easily squeezed and broken; this is because the pressure is not applied evenly from all sides simultaneously. On the other hand, if the same grape is squeezed from all sides simultaneously, it will not be crushed. This can be demonstrated by placing a grape inside a soda bottle filled with water. By squeezing the bottle, you pressurize the water inside as well as the grape. Yet the grape is not damaged, no matter how hard you squeeze. In the same way, foods processed by high pressure will not be damaged by the applied pressure.



6. What is the shelf life of an HPP processed product?

In general, HPP can provide shelf lives similar to thermal pasteurization. Pressure pasteurization kills vegetative bacteria and, unless the product is acidic, it requires refrigerated storage. For foods where thermal pasteurization is not an option (due to flavor, texture or color changes) HPP can extend the shelf life by two to three fold over a non-pasteurized counterpart, and improve food safety. As commercial products are developed, shelf life can be established based on microbiological and sensory testing.

7. Are HPP products commercially available?

Yes. High pressure processed products are commercially available in the United States, European, and Japanese retail markets. Examples of high-pressure processed products commercially available in the United States include fruit smoothies, guacamole, ready meals with meat and vegetables, oysters, ham,



chicken strips, fruit juices, and salsa. Low acid, shelf-stable products such as soups are not commercially available yet because of the limitations in killing spores with HPP. This is a topic of current research.

8. What functional properties does HPP impart to food products?

It is generally known that high pressure has very little effect on low molecular weight compounds such as flavor compounds,

vitamins, and pigments compared to thermal processes. Accordingly, the quality of HPP pasteurized food is very similar to that of fresh food products and the quality degradation is influenced more by subsequent storage and distribution rather than the pressure treatment. Pressure also provides a unique opportunity to create and control novel food textures in protein-based or starch-based foods. In some cases, pressure can be used to form protein gels and increase viscosity without using heat.

9. How are HPP processed foods stored?

HPP products currently marketed worldwide are primarily distributed refrigerated. In some cases this is necessary for safety (to prevent the growth of spores in low-acid foods). For acid foods, refrigeration is not a necessity for microbial stability, but is used to preserve flavor quality for extended periods of time.

10. Is commercial scale equipment available?

Yes. In the United States, Avure Technologies (Kent, WA) sells commercial size (215-liter capacity) batch type HPP equipment.



A 215-liter batch system has the capacity to produce about 10 million pounds of food per year.

The company also makes semi-continuous systems for the processing of clear liquids such as juices. While commercial pressure vessels have the pressure limit of 100,000 pounds per square inch, research machines can go up to 150,000 pounds per square inch. Pilot/lab scale HPP research systems are made by several vendors in the United States (see the OSU HPP website for more details).

11. Is HPP equipment safe to operate?

High pressure equipment design is a mature technology and has its origin in the chemical processing industry. Most high-pressure vessels are manufactured under guidelines established by the American Society of Mechanical Engineers (ASME) boiler and pressure vessel codes. Processors should also ensure that the vessels are manufactured, installed, tested, and operated according to relevant state regulations. With a little training, food plant personnel can learn to safely operate the equipment.

12. How economical is HPP processing?

A commercial scale, high-pressure vessel costs between \$500,000 to \$2.5 million dollars depending upon equipment capacity and extent of automation. As a new processing technology

with a limited market, pressure-processed products may cost 3 to 10 cents per pound more to produce than thermally processed products. With two 215-liter HPP units operating under typical food processing conditions, a throughput of approximately 20 million pounds per year is achievable. High throughput is accomplished by using multiple pressure vessels. Factory production rates beyond 40 million pounds per year are now in operation. As demand for HPP equipment grows, capital cost and operating cost will continue to decrease. Consumers benefit from the increased shelf-life, quality, and availability of value-added products and new types of foods that are impossible to make using thermal processing methods.

13. What regulatory approval is required for commercializing an HPP processed product?

HPP does not present any unique issues for food processors concerning regulatory matters or labeling. The requirements are

similar to traditional thermal pasteurization or sterilization in the United States, where the Food and Drug Administration (FDA) and the Department of Agriculture (USDA) are responsible for evaluating and monitoring the safety of HPP processed foods.

14. Are facilities available for product development before venturing into HPP processing?

There are a number of research facilities throughout the United States and Europe where food processors can evaluate HPP technology. In Ohio, facilities are available at The Ohio State University in the Department of Food Science and Technology and the Department of Food, Agricultural and Biological Engineering. Food processors are invited to take advantage of the expertise of OSU faculty members and facilities to conduct confidential product evaluations for food safety, quality, and shelf life, and to obtain guidance on product development. The resources at OSU can be accessed for a nominal fee.

For additional information, contact:

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College of Food, Agricultural and Environmental Sciences,
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Visit OSU HPP technology web page:

<http://grad.fst.ohio-state.edu/hpp/>

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Appendix 2

Pulsed Electric Field Processing

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Extension FactSheet

FSE-2-05

Food Science and Technology, 2015 Fyffe Road, Columbus, OH 43210-1007

Pulsed Electric Field Processing Fact Sheet for Food Processors

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1. What is pulsed electric field processing?

Pulsed electric field (PEF) processing is a non-thermal method of food preservation that uses short bursts of electricity for microbial inactivation and causes minimal or no detrimental effect on food quality attributes. PEF can be used for processing liquid and semi-liquid food products.

2. How does this technology benefit consumers?

PEF processing offers high quality fresh-like liquid foods with excellent flavor, nutritional value, and shelf-life. Since it preserves foods without using heat, foods treated this way retain their fresh aroma, taste, and appearance.

3. How does PEF work?

PEF processing involves treating foods placed between electrodes by high voltage pulses in the order of 20–80 kV (usually for a couple of microseconds). The applied high voltage results in an electric field that causes microbial inactivation. The electric field may be applied in the form of exponentially decaying, square wave, bipolar, or

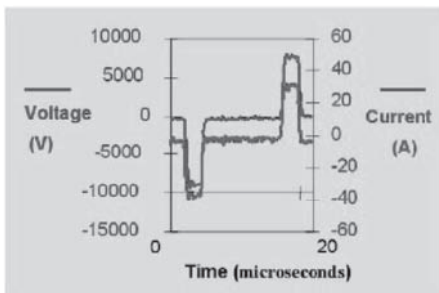
oscillatory pulses and at ambient, sub-ambient, or slightly above-ambient temperature. After the treatment, the food is packaged aseptically and stored under refrigeration.

4. How does PEF inactivate microorganisms?

PEF treatment has lethal effects on various vegetative bacteria, mold, and yeast. Efficacy of spore inactivation by PEF in combination with heat or other hurdles is a subject of current research. A series of short, high-voltage pulses breaks the cell membranes of vegetative microorganisms in liquid media by expanding existing pores (electroporation) or creating new ones. Pore formation is reversible or irreversible depending on factors such as the electric field intensity, the pulse duration, and number of pulses. The membranes of PEF-treated cells become permeable to small molecules; permeation causes swelling and eventual rupture of the cell membrane.

5. What types of foods benefit from PEF treatment?

Application of PEF technology has been successfully demonstrated for the pasteurization of foods such as juices, milk, yogurt, soups, and liquid eggs. Application of PEF processing is restricted to food products with no air bubbles and with low electrical conductivity. The maximum particle size in the liquid must be smaller than the gap of the treatment region in the chamber in order to ensure proper treatment. PEF is a continuous processing method, which is not suitable for solid food products that are not pumpable. PEF is also applied to enhance extraction of sugars and other cellular content from plant cells, such as sugar beets. PEF also found application in reducing the solid volume (sludge) of wastewater.



6. What is the shelf-life of a PEF processed product?

In general, the shelf-life of PEF-treated and thermally pasteurized foods is comparable. PEF pasteurization kills microorganisms and inactivates some enzymes and, unless the product is acidic, it requires refrigerated storage. For heat-sensitive liquid foods where thermal pasteurization is not an option (due to flavor, texture, or color changes), PEF treatment would be advantageous.



7. How are PEF processed foods stored?

PEF pasteurized products currently are stored refrigerated. In some cases (for example, milk), this is necessary for safety (to prevent the growth of spores in low-acid foods). For acid foods, refrigeration is not necessary for microbial stability, but is used to preserve flavor quality for extended periods of time.

8. Is commercial scale equipment available?

Yes. In the United States, the first commercial scale continuous PEF system is installed at The Ohio State University's Department of Food Science and Technology. This PEF system is part of a new food treatment system assembled by a DoD sponsored, University directed industry consortium. Diversified Technologies Inc., Bedford, MA, builds commercial PEF systems of processing volumes ranging from 500 to 2,000 liters per hour, with The Ohio State University supplying the PEF treatment chambers.

9. Is PEF equipment safe for the environment?

Yes. This process uses ordinary electricity. The facility meets electrical safety standards and no harmful environmental by-products are produced.

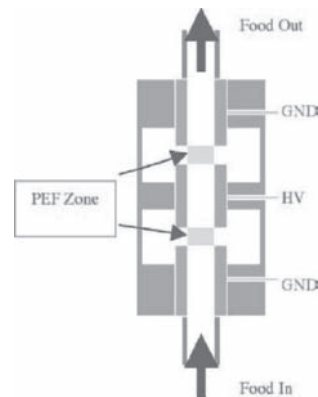
10. What is an integrated continuous PEF Processing System?

An integrated PEF system consists of a fluid handling unit, high voltage pulse generator, PEF treatment chambers,

and packaging machine. The fluid handling unit delivers stable, uniform flow with sterilize-in-place (SIP) and clean-in-place (CIP) functions. The pulse generator supplies high voltage pulses into foods flowing through PEF treatment chambers. Treated foods are packaged continuously.

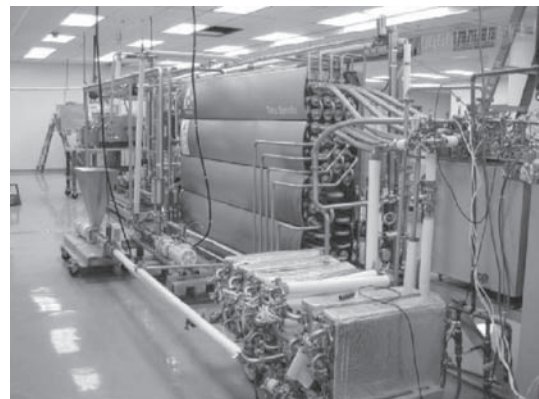
11. What is a PEF treatment chamber?

A PEF treatment chamber consists of at least two electrodes and insulation that forms a volume, i.e., PEF treatment zone, where the foods receive pulses. The electrodes are made of inert materials, such as titanium.



12. How economical is PEF processing?

PEF is an energy efficient process compared to thermal pasteurization. The PEF processing would add only \$0.03–\$0.07/L to final food costs. A commercial-scale PEF system can process between 1,000 and 5,000 liters of liquid foods per hour and this equipment is scalable. Generation of high voltage pulses having sufficient peak power (typically megawatts) is the limitation in processing



large quantities of fluid economically. The emergence of solid-state pulsed power systems, which can be arbitrarily sized by combining switch modules in series and parallel, removes this limitation.

13. What regulatory approval is required for commercializing a PEF processed product?

Currently, regulatory requirements are evolving, but will likely involve the development of Hazard Analysis Critical Control Point (HACCP) plan for most juices and beverages. A current USDA project will address these very points.

14. Are facilities available for product development before venturing into PEF processing?

An industrial scale-up PEF pilot plant facility is available at The Ohio State University in the Department of Food Science and Technology. Food processors are invited to take advantage of the expertise of OSU faculty and staff, and facilities to conduct confidential product evaluations for food safety, quality, and shelf-life, and to obtain guidance on product development. A portable pilot scale PEF processing system is also available for customer-site evaluation. The resources at OSU can be accessed for a nominal fee.



For additional information, contact:

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Visit the OSU PEF technology web page at: <http://fst.osu.edu/pef>

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Appendix 3

Ozone

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FACT SHEET

OZONE

Fact Sheet for Agri-Food Processors

1. Why Ozone Processing of Foods?

On June 26, 2001, the U.S. FDA approved ozone, a gas that is strong oxidizing agent capable of acting as a disinfectant¹, as an Antimicrobial Agent for direct contact with foods of all types. This material can control microorganisms of all types without producing halogenated by-products. Ozone has been used since the early 1900s for controlling microorganisms in portable waters, and its uses have been extended to the treatment of waters used for swimming pools, bottled waters, cooling towers, soft drinks, washing of food processing and handling equipment, etc. Applied in the gas phase, ozone also has found applications in odor and mold control and the storage and packaging of harvested agricultural products and processed foods. Current effective applications for ozone in food processing plants include process water treatment for reuse, for processing and storage of foods, and for packaging of foods. When applied, ozone decomposes, returning to the oxygen from which it was made, leaving no chemical residues from the antimicrobial agent itself on the food products.

¹For this Fact Sheet, the term “disinfectant” will be used as a generic term for any product capable of killing or reducing the number of microorganisms on food products as well as on food preparation surfaces. The specific terms “antimicrobial agent”, “sanitizer”, and “sterilizer” will be used whenever referencing the numbers of microorganisms removed from a food or food preparation surface.

2. How Does Ozone Treatment Benefit Consumers?

The primary benefit of ozone in food processing is its ability to control microorganisms of all types, including storage microorganisms. Consequently, the shelf life of many food products can be increased, sometimes by simply washing the foods in water containing ozone (fresh cut salad mixtures, apples for candying, strawberries, blueberries, etc.). Addition of ozone does not add potentially toxic residues to the food products which it contacts. However, because ozone is simultaneously a strong disinfectant as well as a strong oxidant, close attention must be paid during initial testing to guard against the indiscriminate overuse of ozone, which can cause oxidative damage to the food product(s) being treated.

Additional benefits of ozone include less storage of chemicals and pesticides (alternatives to ozone) on-site, lower amounts of chemical residues passed into our ecosystem, and less chloramines in processing plant air for workers to breathe (when chlorine chemicals are used).

3. How Does Ozone Technology Benefit Food Processors?

Since ozone is not classified as a pesticide chemical (it is unstable and cannot be produced at a

central manufacturing facility, packaged, stored, and shipped), its use on foods allows microorganism control without the use of chemical pesticides. If chlorine is being used for microorganism control, the use of ozone allows the use of less chlorine – sometimes the total elimination of chlorine – while achieving the same target for microorganism control as previously attained with chlorine. Less chlorine means less equipment maintenance as well as fewer halogenated reaction products. In turn, this can result in cost savings for the user. See the IOA Pan American Group web site for examples of User Success Reports (www.io3a.org).

4. What Is Ozone?

Ozone, O_3 , is a gas – the same ozone gas that is generated constantly in the Earth's stratosphere when the Sun's high-energy ultraviolet rays first encounter oxygen that makes up our atmosphere. Some oxygen molecules (O_2) are ruptured, forming single oxygen species that are quite reactive. When such active oxygen species encounter other oxygen molecules, the two species combine to form the very strongly reactive ozone molecule, O_3 .

Gaseous ozone is partially soluble in water – its solubility increases as the water temperature decreases. The molecule itself is a very strong oxidizing agent, and this oxidizing power makes it a very strong disinfectant. Ozone's disinfecting action involves the chemical oxidation of cell membranes of microorganisms. When cell walls are ruptured by oxidation, the interiors of the cells are exposed and the cells are killed. When the cells are killed in this manner, they usually do not regrow or mutate.

Viruses are chemical in structure, not living organisms. When ozone attacks viruses, it does so by rupturing key chemical bonds in their DNA structures. Given sufficient ozone for sufficient time, viruses also are destroyed (inactivated).

Ozone can be applied either as a gas or in aqueous solution. Microbial oxidation and subsequently disinfection can occur in both phases. Reactions of all types occur much more slowly in the gas phase, particularly when the air to which ozone gas is applied

is dry. As the relative humidity increases, reaction rates of ozone increase rapidly.

In aqueous solution, reactions of ozone are rapid, and many ozone reaction rates (including disinfection) increase with increasing temperature and pH. The increase in rate of disinfection with increasing pH is opposite to the disinfection performance of aqueous solutions of chlorine, in which the rate of disinfection decreases dramatically as pH increases.

Ozone, being unstable, quickly reverts to the molecular oxygen from which it was made. This reaction occurs more rapidly in water, and more slowly in the gas phase.

5. How Is Ozone Applied And Controlled?

5.1. For Treating Water

Food processing plant water can come from various sources – from a municipality, from a well, or from a river, lake or stream. If the water has not been treated, then ozone can be of great assistance. The necessary application rates must be determined according to the impurities present in the influent water. For surface waters ozone can aid the action of flocculating clays as well as chemical compounds. Filtration can be aided by oxidation of dissolved organic compounds, thus increasing filter run times to backwashing. Ozone can serve as a disinfectant as the treated water enters the food processing plant.

Ozone is applied in large scale drinking water plants by means of porous diffusers placed strategically in large concrete contacting tanks. In smaller plants, ozone usually is applied via injectors. Control of ozone addition is by means of appropriate analytical instrumentation that measures ozone levels either in the liquid or gas phases – sometimes in both phases. Application and control of ozone to water is similar regardless of the specific use of the treated water.

5.2. For Process Water Recycle and Reuse

For proper treatment of process waters for reuse, attention must be paid to the materials now contained

by the process waters. The process water to be recycled and reuse should be treated by appropriate procedures, which can include one or more of the following treatment steps: flocculating agents (as necessary to assist removal of organic contaminants and colloidal materials), sedimentation, dissolved air flotation (DAF), filtration, treatment (with ozone) prior to recycling, etc. A significant advantage of ozone is that it saturates the water being treated with oxygen, giving the ozone-treated water a higher optical transmittance.

5.3. For Wastewater Treatment and Disposal

Wastewaters from food processing plants usually contain high levels of organic contaminants. These usually are easily biodegradable and as such are amenable to biological wastewater treatment. However, this type of treatment requires rather large land areas and time (~ 30 days or more) for completion. Biological treatment is the least costly method of wastewater treatment, requiring the least amount of added energy from the food processor. When land is not available, or there is insufficient time for bioprocessing, ozone can provide a technology to reduce BOD and COD levels. Ozone has been shown to aid sludge dewatering, thus aiding filtration. Biotreated effluents can be disinfected with ozone, thus avoiding disinfection with chlorine, and also avoiding the formation of chlorinated organics that pass into the environment and enter the food chain.

5.4. For Washing Foods – Whole or Cut

For this application, water must be very clean and free of organisms. Usually this is not an application for recycled water. However, because of the oxidative properties of ozone, many organic contaminants of food wash waters are destroyed by ozone, allowing the wash water to be used for a longer period of time before disposal. Strickland Foods, for example, washes their fresh cut salad mixes with water containing ozone, and then sends that water to recycle. Meanwhile, the ozone-washed salad mixes then are washed with chlorinated water. Prior to in-

stalling ozone, chlorine was the sole disinfectant at this facility. Since installing ozone, both water use and chlorine use have dropped considerably, and the plant wastewater effluent volume also has decreased considerably, resulting in significant cost savings.

5.5. For Storage of Raw Food Products

Based on prior research, water for this application is treated with ozone to a level appropriate for the specific food. Foods are washed, and then packaged (sometimes allowing exposure to air, others not). Many times, raw food products are stored in an atmosphere containing gaseous ozone. In these cases, it is important to know the concentrations of ozone necessary to protect the food product(s) from damage. Examples: Onion and Potato Storage – see User Success Reports on IOA-PAG Web Site (www.io3a.org).

5.6. For Packaging of Foods

Foods normally are cleaned, then packaged. Chilean grapes, for example, are washed with water containing ozone (to lower contamination effects from *Rhizopus stolonifer*), then packaged in air-breathing plastic bags. Carrots are stored in air-breathing bags in ozone-containing atmospheres. However, when finally packed for distribution, carrots sometimes are packaged in sealed plastic bags. Specific types of packaging are required for specific foods. When packaging is to be in air-tight sealed plastic bags, ozone treatment can be followed by carbon dioxide and/or nitrogen. Ozone disinfects surface microorganisms, then the inert gases flush away any remaining oxygen that may allow growth of detrimental organisms.

5.7. For Treating Process Room Air

If workers are not present in a food processing room (rare circumstances – such as a storage room), ozone can be applied throughout the room air to levels that are effective for their intended purposes, but which may exceed federal government regulations for ozone in air (see Item 10). When workers are

present, room air should be treated with levels of ozone that are below federal regulations, and these may be too low to accomplish their air-treatment objective. In these cases, contaminated air can be removed from the room, treated with appropriate quantities of ozone, excess ozone destroyed, and the cleaned air then returned to the processing room. Another approach is to mount ozone-generating UV lamps in the processing room ceiling. When turned on, these lamps produce lower concentrations of ozone than by the corona discharge technique used for treating large quantities of water. Since ozone gas is slightly heavier than air, it will fall from the ceiling UV bulbs to the floor of the processing room. Depending on the degree of odors present (ex. a garlic processing room) the odorants normally rise upwards, where they can encounter the descending ozone gas and be destroyed. At 6 feet above floor level, there may be no ozone at all (as measured by a wall-mounted ozone monitor pre-set at just below the appropriate OSHA level).

Still another approach is to install an ozone generator in ceiling corners and have each generator fitted with a timer. During times of human occupancy, the ozone generators are turned off. When the plant closes for the night, the timers automatically turn on the ozone generators, and then turn them off an hour or so prior to human occupancy. Gaseous ozone usually dissipates within an hour. To be sure there is no ozone above federal levels when workers return; a fan can be turned on a few minutes before workers return to exhaust the last traces of ozone from the processing room. This approach is not practical for heavy odors, or odors that develop quickly during processing. For mild odors, this procedure is a simple solution. This treatment also can greatly reduce the level of airborne mold in process room air.

Another application for ozone is in controlled atmosphere rooms in which stone fruit or apples typically are stored. These are large rooms used to preserve fruit harvested in summer/fall so they can be sold during winter/spring, after summer stocks are gone and prices are higher. In these storage rooms, negative pressures are used, along with nitrogen gas flooding, cold temperatures (35°F) plus a slight residual of ozone for mold and mildew control.

5.8. For Plant Washdown and Cleaning

Many ozone equipment suppliers offer specially designed “ozone-wash carts” that are portable and produce a pressurized water stream that contains a few parts per million of ozone dissolved in water. The solution is under pressure, thus the aqueous stream can perform the normal water washdown functions. However, because of the presence of dissolved ozone, wherever the aqueous spray contacts a surface, microorganisms on that surface will be attacked by ozone.

A special modification of this application for ozone is the washing of workers’ boots and waterproof aprons, when leaving the processing room for the day. Boots are hung on special racks outside the food processing room, and these are rinsed with ozone-containing water from the portable ozone-water-washer.

Ozone washes can replace Best Management Practice washing procedures that utilize strong sanitizers. They are usually used when a quick washdown is needed during break periods and shift changes. The ozonated water does not adversely affect products left in conveyors and on cutting tables. These same products sprayed with chlorine- and/or peroxide-containing sanitizers would need to be discarded. Ozone washes can clean many food and container surfaces; however, they do not provide residual microbial protection.

6. Can Ozone Be Used For Processing All Foods?

Yes. Ozone is approved as an Antimicrobial Agent for direct contact with all foods. However, attention must be paid to applying only the minimum amounts of ozone necessary to accomplish its intended purpose(s). Excessive application of ozone may damage the foods being treated, and doses that are too low may not provide the needed microbial protection.

7. Can Ozone Damage Food Products?

Yes. Because ozone is a strong oxidizing agent that also acts as a strong disinfectant, it is important prior

to use to determine appropriate ozone application dosages and times of treatment for each intended use. In the fruit and carbonated drink industries, over-treatment with ozone can produce clear and watery-tasting juice products – because all the juice flavor and color is removed.

8. What Is The Shelf-Life Of An Ozone-Treated Product?

The shelf-life of an ozone-treated food product varies with the food and its prior processing. Under proper ozone treatment conditions, usually shelf-life can be increased 15–35%. Because ozone does not provide residual protection, it is necessary that ozone-treated foods be stored in environments that are as clean as practicable at the proper temperatures.

9. What is the Commercial Availability of Ozone?

Ozone as a gas or in solution is not an item of commerce, because of its unstable nature. However, equipment to generate, contact, monitor, and control ozone has been used to treat water commercially for over 100 years. Many other commercial applications of ozone now abound, and equipment to generate ozone in food processing plants is readily available. Two types of ozone generators are available for food processors – corona discharge and ultraviolet radiation (185 nm). Corona discharge is a process by which electrical energy is passed between two closely positioned electrodes, thus creating a continuous silent electrical discharge – which generates ozone in the gas phase. For UV radiation, high energy UV rays pass through surrounding air, generating ozone, but in much smaller amounts and concentrations than can be generated by corona discharge. For small quantities in gas phase applications, ozone generated by UV radiation is effective and at lower costs than by corona discharge ozone generation.

Of interest to food processors is the fact that while there are many commercial suppliers of ozone generating and application/control equipment, there are fewer suppliers of integrated systems. Ozone equipment suppliers are thorough at making dependable

ozonation equipment, and tend to focus on specific niches in the food processing markets. However, many suppliers are not yet so familiar with food processing applications for ozone.

10. What Are The Federal Regulations Concerning Exposure To Ozone in a Food Processing Plant?

Although ozone levels in air are regulated by the U.S. EPA, the U.S. FDA and the OSHA, only the OSHA regulations apply to food processing plants. The OSHA Permissible Exposure Level (PEL) for workers exposed to ozone is 0.10 ppm, time-weighted average over an 8-hour working day, five days per week. OSHA also has established a Short-Term Exposure Level (STEL) of 0.30 ppm, over a 15-minute time period, not to be exceeded more than twice daily.

11. What Functional Properties Does Ozone Impart to Foods?

Ozone is a strong oxidant and as such would be expected to cause alterations in nutrient levels in foods if high concentrations are used for extended periods. However, ozone does not penetrate deeply into foods, and any impact on nutrient content is limited to nutrients on the surfaces of foods. As stated by Dr. John W. Erdman, Jr., in reviewing “Nutrient Impact” (of Ozone on Foods) in the 1997 *EPRI Expert Panel Report: Evaluation of the History and Safety of Ozone in Processing Food for Human Consumption*, “it appears that under properly-controlled preservation conditions, ozone causes only minor losses of nutrient content, lower than some other processes commonly in use.”

12. How Are Ozone-Processed Foods Stored?

Foods processed by ozone are stored in the same manner as normally processed foods. The only difference is that if the use of ozone provides an extension of shelf-life, then perhaps special care in packaging and storage is appropriate, to realize the commercial benefits of that additional shelf-life. A caution also

is warranted to avoid the over-use of ozone when treating. Ozone-treated products should be handled carefully and not abused. They can be recontaminated very easily.

13. Is Ozone Equipment Safe To Operate?

Yes, provided that appropriate ozone monitors and controls are designed into the ozonation system at the beginning and the equipment is operated according to manufacturers' recommendations. The key is to avoid exposure of plant personnel to ozone in excess of the OSHA PEL and STEL regulations. Ozone is not a systemic poison (as is chlorine gas). Nevertheless, ozone is a strong oxidant, and high levels can damage lung tissues if breathed over long periods of time. Modern day ozonation systems for food processing are (or should be) designed so that in the event of an ozone monitor reading an ozone level in excess of the OSHA PEL, an alarm sounds, and the monitor can even stop the flow of electric power to the ozone generator(s), thus ceasing the production of ozone. The odor of ozone at levels near or greater than the STEL is pronounced and noticed quickly by workers.

14. How Economical Is Ozone Processing?

Economics associated with ozone are variable. Ozone generating equipment costs are higher than equipment for some applications of other Antimicrobial Agents. However, process cost benefits from the use of ozone are high. See any of the User Success Reports posted on the IOA web site. The driving force behind each of these USRs is money savings for the ozone user over costs prior to adopting ozone. For example – savings in onion and potato crop losses by preventing the spread of fungal diseases gives the user more product to sell. For a food packaging user, ozone allows better chiller water cleanup and less water use. For a garlic processor, ozone allowed elimination of sodium hypochlorite, with its attendant maintenance and chemicals expenditures, including storage and handling, plus the ever-present safety

hazards. And for all ozone-treated foods reported, a higher quality product is provided than before ozone was employed.

15. What Regulatory Approvals Are Required For An Ozone-Processed Product?

The U.S. FDA has approved ozone as an *Antimicrobial Agent* for Direct Contact With Foods of all types (*Federal Register* 66(123):33829-33830, June 26, 2001.). The USDA also has approved ozone for contact with meats and poultry (Dec. 21, 2001), but requests a case-by-case submission of proposed application of ozone for its permission to use. In the early 1980s, the FDA approved ozone as a *disinfectant* for bottled water and as a *sanitizer* for bottled water processing lines. However, approval for use in bottled water was coupled with the statement that “all other food applications for ozone must be approved by means of a food additive petition.

Approval of ozone as an *Antimicrobial Agent* means that ozone can provide at least two-logs of inactivation of microorganisms. For ozone to be *called* a *sanitizer*, by the FDA it must be capable of providing five-logs of microbial inactivation when applied on specific foods. For ozone to be *approved* by FDA as a *sanitizer* in food processing plants, a Food Additive petition must be filed. Processors are free to declare ozone sanitizer treatment as GRAS (Generally Recognized As Safe) and submit such GRAS Affirmations to the FDA. This approach is not usually looked upon with favor by food processors, although it is often used by the pharmaceutical industry.

In 1999, FDA turned over its responsibilities for “sanitizers” to the U.S. EPA, and EPA refers to ozone as a “sanitizer” for direct contact with foods and for food processing equipment.

If food products are to be treated with ozone (as an Antimicrobial Agent) but are to be marketed and used as neutraceuticals or pharmaceuticals, the processor is advised to contact FDA to determine whether such uses for ozone-treated products are allowed under current FDA drug regulations or whether a special application for approval of use must be submitted.

16. Are Testing Facilities Available for Product Development Before Venturing Into Ozone Processing?

Yes. A potential user of ozone can contact one of several consultants or academic institutions who have experience in treating foods with ozone and can advise whether existing data are sufficient to design an ozone processing system. Most likely the recommendation will be to do some testing at the food processing plant itself. A few suppliers of ozone equipment now have sufficient experience with installations of their equipment at food processing

facilities to be able to advise the prospective ozone user and to conduct a product development program. ClearWater Tech, Purfresh, Praxair, Del Ozone, Pacific Ozone, and Ozonia are among those firms having on-site testing facilities for demonstrating ozone equipment to prospective users.

For Additional Information, Contact:

The International Ozone Association, Pan American Group, Scottsdale, AZ; www.io3a.org

R.G. Rice April 9, 2007

Appendix 4

Food Irradiation

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Why should I be interested in irradiated food?

Irradiation improves food safety and quality. Even though the United States food supply has achieved a high level of safety, hazards exist. The Centers for Disease Control and Prevention has estimated that 76 million illnesses, 325,000 hospitalizations, and 5000 deaths occur each year due to foodborne illness. Although all are at risk, children, people over age 55 or 60, diabetics, and those whose immunity is compromised are especially vulnerable.

Irradiation provides extra protection against foodborne illness which is unavailable by any other means. Even when meat, poultry, or eggs are prepared with the most advanced sanitation measures possible, harmful bacteria may be present. Irradiation provides an additional safeguard for the consumer, destroying 99.9% or more of *E. coli* O157:H7, *Salmonella*, *Campylobacter*, *Listeria*, or other harmful bacteria that may be in raw food.

High quality tropical fruits can be shipped to California or other states because irradiation destroys harmful fruit flies, such as the Mediterranean fruit fly, before they become an infestation problem in our state.

Irradiation increases the shelf life of several fresh foods because it slows the ripening of fruit and prevents potatoes and onions from sprouting. Spices and herbs have been fumigated to increase safety. Irradiation can replace chemical fumigation, producing safe, high quality spices and herbs.

The U.S. Food and Drug Administration (FDA) may soon approve the use of irradiation to increase the safety of fresh sprouts because it can destroy harmful bacteria that may be under the sprout seed coat. The FDA may also soon approve irradiation of prepared luncheon meats, and other ready-to-eat foods because the process can increase the safety of such prepackaged foods.

What is food irradiation?

Irradiation exposes food to ionizing energy for a specific length of time, depending on the purpose of the treatment. This treatment complements good manufacturing practices and increases overall food safety.

Food is irradiated in a special processing facility where it is exposed to an electron beam, or X-Ray, generated from electricity or gamma rays produced from cobalt 60. The food is monitored to assure that the exact treatment level is achieved.

Is irradiated food safe?

Yes. Irradiated foods are safe and wholesome. After reviewing hundreds of studies on the effects of irradiation on food safety and quality, scientists from the U.S. Food and Drug Administration, the U.S. Department of Agriculture, and health organizations such as the World Health Organization, the American Medical Association, and the American Dietetic Association have endorsed the safety of irradiated food. To ensure their health, astronauts have eaten irradiated food since the beginning of the space program.

Does irradiation cause chromosome damage, cancer, tumors, or other health problems?

No. The FDA has evaluated irradiation for 40 years and found the process to be safe. Numerous scientific studies conducted world wide clearly confirm that there are no health problems or toxicity concerns associated with irradiation.

Does irradiation make food radioactive?

No. During the irradiation process, food moves through an energy field, but never touches the energy source and does not become radioactive. The amount of energy and type of radiation used to irradiate food is enough to kill foodborne bacteria, but it does not make the food radioactive, just as luggage does not

become radioactive after passing through a security checkpoint at the airport.

Many common items, such as cotton balls, adhesive bandages, baby bottles, and medical supplies are irradiated for safety. None are made radioactive.

Do I handle irradiated food differently than other foods?

No. Handle irradiated food as you would any other perishable food. Irradiation destroys 99.9% or more of harmful bacteria like *E. coli* O157:H7 and *Salmonella*. However, it reduces but does not destroy all spoilage bacteria. Meat and poultry should still be refrigerated to slow the growth of spoilage bacteria and maintain food quality. Irradiation leaves no chemical residue in the food, so irradiated foods could be accidentally contaminated after treatment; therefore, proper handling and preparation should be followed to assure food safety.

If thorough cooking destroys harmful bacteria, what is the advantage of irradiated meat and poultry?

Irradiation destroys harmful bacteria before they come into the kitchen. Eating irradiated foods should reduce foodborne illness that results from accidental cross contamination or cooking at too low a temperature. Food irradiation provides an additional level of protection for consumers.

Is irradiated food still nutritious?

Irradiated food is nutritious and flavorful. Nutritional changes produced by irradiation of food are less or are comparable to those produced when food is cooked or frozen. Thiamin is reduced when pork is irradiated and some vitamin A is reduced when eggs are irradiated, however the difference is so small that it has no effect on the American diet.

Changes in the nutritional value of fruits and vegetables are insignificant. Some irradiated fruits may even be more nutritious and flavorful because irradiated fruits can stay on the tree longer than those treated by other methods that guard against the accidental transport of tropical insects.

Is food irradiation approved by the government?

Food irradiation has been approved by the Food and Drug Administration after a thorough food safety review. Irradiation is the most researched food technology in U.S. history. Scientists with the FDA have evaluated numerous studies that have examined the safety and nutritional value of irradiated food. The U.S. Department of Agriculture has evaluated and approved irradiation of meat and poultry. Food irradiation has been approved by more than 40 countries worldwide and endorsed for safety by the World Health Organization.

How do I know food has been irradiated?

Irradiated food has a distinctive logo:



and the words "Irradiated" on its packaging. Some may also describe the process as "cold pasteurized" or "electronically pasteurized" for better consumer understanding.

Is worker and community safety protected in food irradiation facilities?

Yes. Irradiation facilities are strictly regulated. Facilities using gamma rays must be constructed to withstand earthquakes and other natural disasters without endangering surrounding communities or workers. Electron beam and X-ray facilities must follow the same safeguards used by hospitals. Workers are trained in the safe operation of

irradiation equipment, and their personal safety is protected by a multifaceted protection system within plants. Companies must follow state and local government requirements as well as those issued by the Environmental Protection Agency, the Occupational and Safety Health Administration, and the Department of Transportation.

Who says irradiated food is safe?

U.S. Food and Drug Administration
U.S. Department of Agriculture
American Medical Association
American Dietetic Association
Centers for Disease Control and Prevention
World Health Organization
US Public Health Service
American Public Health Association
California Environmental Health Association
- and many more

Where can I get additional information on food irradiation?

Visit the websites listed or call the Center for Consumer Research at the University of California, Davis, for more information (530-752-2774). At each site, click on "food irradiation" or type "irradiation" in the search box.

<http://amrcatalog.ucdavis.edu>

www.extension.iastate.edu

www.cdc.gov/

www.fda.gov/search.html

www.health.state.mn.us

www.ific.org

(Scroll down to "Questions and Answers About Food Irradiation")

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Author: Christine M. Bruhn, Ph.D.

Cover illustration: Anne Spiller-Kashuba

University of California, Agricultural & Natural Resources Publication 7255.
<http://amrcatalog.ucdavis.edu>

Frequently Asked Questions About

Food Irradiation



The University of California
Division of Agricultural and
Natural Resources

Appendix 5

Irradiation: A Safe Measure for Safer Iceberg Lettuce and Spinach

Reprinted with permission of Food and Drug Administration

Irradiation: A Safe Measure for Safer Iceberg Lettuce and Spinach

On August 22, 2008, the Food and Drug Administration (FDA) published a final rule that allows the use of irradiation to make fresh iceberg lettuce and fresh spinach safer and last longer without spoiling.

Irradiating fresh iceberg lettuce and spinach will help protect consumers from disease-causing bacteria. Infections from bacteria such as *Salmonella* and *Escherichia coli* O157:H7 (*E. coli*) continue to be a public health problem in the United States. Illnesses from these bacteria range from uncomfortable symptoms to life-threatening health problems. Severe illness from *E. coli*, for example, can lead to kidney failure.

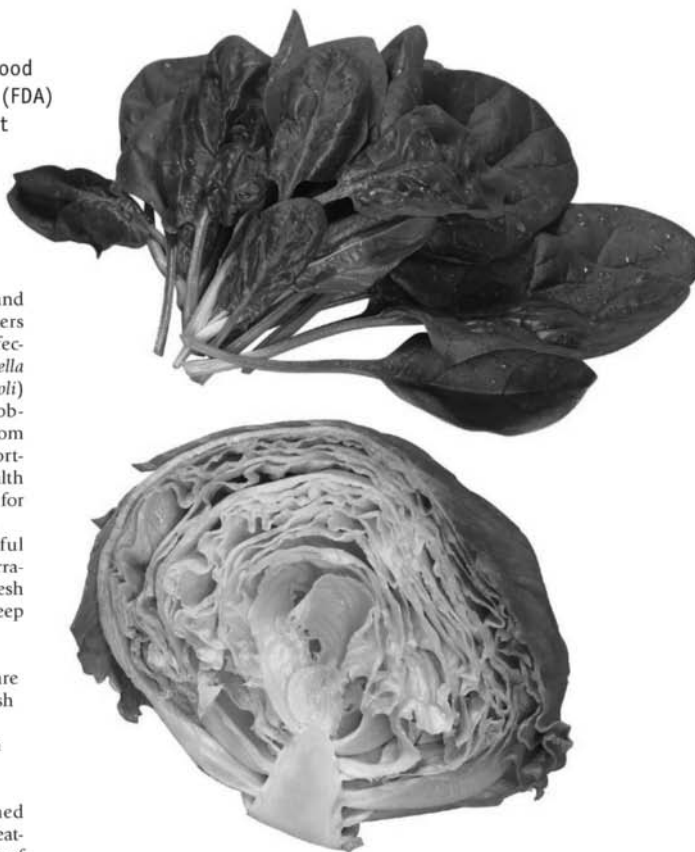
In addition to controlling harmful bacteria and other microorganisms, irradiating fresh iceberg lettuce and fresh spinach will allow the greens to keep longer without spoiling.

The foods affected by the final rule are

- loose, fresh iceberg lettuce and fresh spinach
- bagged iceberg lettuce and spinach

Irradiation and Safety

Irradiation (also sometimes termed "ionizing radiation") is a process of treating products with a measured dose of





FDA requires that foods that have been irradiated bear the “radura” logo along with the statement “Treated with radiation” or “Treated by irradiation.”



radiation. Food irradiation is not new. FDA has conducted irradiation safety evaluations for more than 40 years and has determined the process to be safe for use on a variety of foods.

After studying the safety of irradiating fresh iceberg lettuce and fresh spinach, FDA has determined that these greens, when irradiated under the conditions specified in the final rule, retain their nutrient value and are safe to eat.

FDA considers irradiation a complement to, not a replacement for, proper food-handling practices by producers, processors, and consumers. Irradiation is an additional tool that may be used to reduce the levels of disease-causing microorganisms on fresh iceberg lettuce and fresh spinach.

Irradiation does not take the place of washing. FDA continues to recommend that consumers wash fresh and bagged produce before eating unless the packaging specifically states that the product has been prewashed.

Irradiation of Other Foods

Many foods are already permitted to be irradiated to control bacteria and keep the foods longer without spoiling. For example, FDA approved the irradiation of red meat in 1997 after

reviewing numerous scientific studies conducted worldwide on the effects of irradiation on various meat products. Other examples of foods that may be irradiated to kill microbes include spices, poultry, and molluscan shellfish (such as oysters, clams, mussels, and scallops).

FDA has previously allowed lettuce, spinach, and some other foods to be irradiated to kill insects or to slow spoilage. However, the doses used for these purposes are lower than what is required to kill most disease-causing bacteria.

How Will I Know if My Fresh Iceberg Lettuce or Spinach Has Been Irradiated?

Irradiation of iceberg lettuce and spinach is voluntary on the part of food processors. FDA requires that foods that have been irradiated bear the “radura” logo along with the statement “Treated with radiation” or “Treated by irradiation.”

FDA’s Role in Safe Irradiation of Food

FDA regulates sources of irradiation (the equipment used) for foods as “food additives” that require approval before being allowed on the market.

FDA approves a source of irradiation for use on food only after it has determined that irradiating the food is safe at a maximum dose specified by FDA. The agency continues to evaluate the safe use of irradiation in additional foods. **FDA**

This article appears on FDA’s Consumer Health Information Web page (www.fda.gov/consumer), which features the latest updates on FDA-regulated products. Sign up for free e-mail subscriptions at www.fda.gov/consumer/consumerenews.html.

For More Information

FDA’s Fact Sheet on Irradiation
www.cfsan.fda.gov/~dms/irradlet.html

Appendix 6

Pulsed Light Treatment

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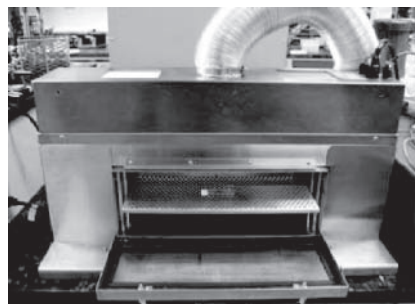
Pulsed Light Treatment

Fact Sheet for Food Processors

Carmen I. Moraru, Ph.D.

Associate Professor

Department of Food Science, Cornell University



1. What is Pulsed Light treatment?

Pulsed Light (PL) technology is an alternative to thermal treatment for killing pathogenic and spoilage microorganisms in foods, including bacteria, yeasts, molds, and viruses. The treatment consists in applying a series of very short, high power pulses of broad spectrum light. Xenon flashlamps emit an optical spectrum that covers a range of wavelengths from the UV cut off of the envelope material (about 180 nm) to IR (around 1100 nm).

2. How is PL treatment dose quantified?

The Pulsed Light treatment dose is quantified by “fluence,” which represents the light exposure of a substrate and is expressed in J/cm². Sometimes the treatment dose is reported as a combination of number of pulses and distance from the light source. When this approach is used, it is critical that the pulse characteristics (width, fluence per pulse) are also reported.

3. How does PL work?

Pulsed Light systems consist of several common components (Figure 1). A high voltage power supply (1) provides electrical power to the storage capacitor (2), which stores electrical energy for the flash lamp (4). The pulse-forming network (3) determines the pulse shape and spectrum characteristics. A trigger signal (5) initiates discharging of the

electrical energy to the flash lamp, which is the key element of a Pulsed Light unit.

The flash lamp is filled with an inert gas, such as xenon, which emits broadband radiation that ranges from UV to NIR. A high-voltage, high-current electrical pulse is applied to the inert gas in the lamp, and the strong collision between electrons and gas molecules cause excitation of the latter, which then emit an intense, very short light pulse (1 μ s to 0.1 s).

The exact mechanisms by which Pulsed Light causes cell death are not yet fully understood, but it is generally accepted that UV plays a critical role in microbial inactivation. The antimicrobial effects of UV light on bacteria are attributed to structural changes in the DNA, as well as abnormal ion flow, increased cell membrane permeability and depolarization of the cell membrane. Some studies also indicated observable injurious effects on yeast cells and mold spores following exposure to Pulsed Light.

4. Does PL treatment cause heating effects?

Depending on the product characteristics, limited heating effects can be noticed. Heating is usually too modest to account for microbial inactivation or to cause structural and sensory changes in the treated products.

5. Can PL be used for processing all foods?

Pulsed Light treatment is able to inactivate pathogenic and spoilage microorganisms in a range

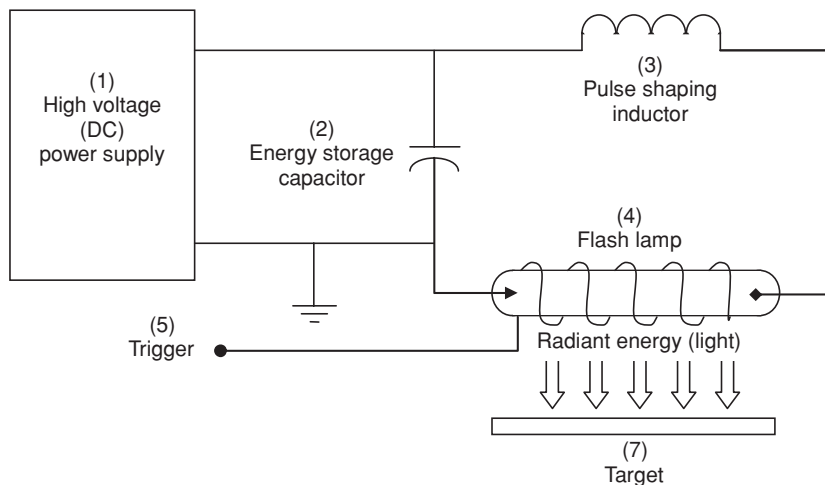


Figure 1. Functional diagram of a Pulsed Light system. Adapted from Xenon Corporation.

of food products and food contact materials. Since the effectiveness of PL is strongly influenced by substrate characteristics, the treatment is most effective on smooth, non-reflecting surfaces or in liquids that are free of suspended particulates, such as clear fruit juices or water. One of the potential applications of Pulsed Light is surface decontamination of fresh fruits and vegetables or ready to eat products such as meats. Treatment of foods packaged in “UV-transparent” packaging materials can be used for a terminal surface decontamination of ready to eat prepackaged foods. For any PL treatment to be fully effective, uniform, 360° exposure of the treated food is critical.

6. Does PL treatment have a negative effect on the quality of treated foods?

At doses within the FDA approved limits, no significant changes of product quality and sensory properties have been observed.

7. Are there PL units available commercially?

There are commercial Pulsed Light systems for both batch and continuous treatments. In the US,

batch configurations are available from the company Xenon Corporation (Wilmington, MA - For over 45 years Xenon Corporation has placed thousands of Pulsed Light systems worldwide on line in multiple markets, providing 24/7 operation). The French company Claranor (Manosque, France) has developed a wide range of Pulsed Light equipment for the food and pharmaceutical industries, including: static equipment (*Tecum*), units for unwrapped or packaged products on flat conveyor belts (*Gratia*), on spool-bars (*Plena*) or in tunnels (*Dominus*), in-line treatment units for caps, pre-formed packaging, films, or jars (*Ventris*), and a reactor for in-line treatment of clear liquids and water (*Maria*).

8. Is PL equipment safe to operate?

It is important that workers are not exposed directly to the high intensity light emitted by the flash light during its operation. In order to prevent this from happening, most PL equipment comes with safety devices that do not allow the operation of the unit while the chamber door is open. For additional protection, it is possible to use commercially available UV absorbing quartz panels.

9. What is the regulatory status of Pulsed Light for use in commercial food applications?

In the United States, FDA has approved the use of Pulsed Light for the decontamination of food or food contact surfaces, provided that the treatment uses a xenon lamp with emission of wavelengths between 200-1000 nm, with a pulse width not exceeding 2 ms and the cumulative level of the treatment not exceeding 12 J/cm² (FDA Code 21CFR179.41).

10. Are any facilities available for developing PL applications before commercialization?

There are several university based research laboratories where food processors can evaluate PL technology, both in the United States and in Europe. The Department of Food Science at Cornell University has the capability of carrying out Pulsed Light research and development work on a fee for service basis.

Appendix 7

Power Ultrasound

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Power Ultrasound

Fact Sheet

Daniela Bermúdez-Aguirre and Gustavo V. Barbosa-Cánovas

Why ultrasound?

Ultrasound technology has shown important advances in food processing in the last few years. This nonthermal technology, which is applied at low frequency (power ultrasound) in combination with heat, has been used successfully to inactivate pathogenic bacteria in a number of liquid foods, satisfying current pasteurization standards. The final characteristics in the quality of these sonicated products is akin to fresh products and sometimes it is even better (i.e. color). The main reason for this technology's effectiveness is based on the cavitation generated by ultrasound in the food from sound waves passing through the medium, which in turn disrupt the cell membranes, generating microbial inactivation. The use of ultrasound in combination with heat allows reducing the processing times considerably for some of the evaluated products and has the potential of energy and economic savings. Ultrasound equipment is easy to operate at lab and pilot plant scale and appears to be an environmentally friendly technology for processing food.

How does ultrasound work?

As already stated, power ultrasound consists of sound waves applied at low frequency (around 16 kHz). When sound waves enter a liquid medium, sound is

transmitted as sinusoidal waves and energy is propagated throughout the system in the form of vibration. This vibration is composed of cycles of compression and expansion moving the media particles, and is a direct result of the sound waves (Knorr et al., 2004). When the energy (i.e. vibration) reaches an optimum level (depending on the characteristics of the medium such as volume, temperature, composition) an important increase of pressure takes place in the medium; this increase generates thousands of bubbles and produces the physical phenomenon called cavitation (Povey and Mason, 1998; McClements, 1995). Cavitation can be transient or stable, a difference that depends on the size of the bubbles produced during cavitation and the speed of bubble growth. Cavitation is responsible for cell disruption, breakdown of microstructures, and production of free radicals in the medium; therefore cavitation can be used for different activities in food processing. As a result, ultrasound has been explored for use in extraction, emulsification, filtration, crystallization, and inactivation of enzymes and microorganisms (Mason, 2003).

Can all foods benefit from ultrasound processing?

At present low frequency ultrasound is only used to process liquid foods, mainly because of the cavitation

phenomenon that takes place in liquid media. Sound waves need to travel through a liquid to produce bubbles for cavitation to occur. Most of low frequency (power) ultrasound has been applied to liquid foods such as juice or milk. Also, power ultrasound is able to extract components from food materials, such as pigments; however, if the material being extracted forms part of a solid matrix, a solution is used in conjunction with ultrasound to extract the components. There are other uses in the food industry for ultrasound at very high frequencies (1 to 10 MHz) that are not processing applications, such as its application in quality assurance for all kinds of foods, even solids. In this case the mechanism of action, i.e. the propagation of waves, is completely different and is considered a nondestructive application. The medium particles oscillate in response to the low energy while exposed to the ultrasonic waves, and simply return to their equilibrium position when the source of ultrasound is removed. This oscillation of particles produces a form of energy that has been associated with the presence of foreign bodies, the presence (or absence) of food components, and detection of quality defects in food.

How ultrasound inactivates microorganisms?

The main mechanism of microbial inactivation is cavitation. Cell membranes are disrupted because of the violent implosion and explosion of bubbles in the medium. There are two types of cavitation responsible for cell inactivation: stable and transient. Stable cavitation generates small bubbles in the medium producing strong eddies and micro-currents; when stable cavitation raises the maximum energy and becomes violent, another physical phenomenon is observed known as micro-streaming. The force generated in stable cavitation is the rubbing of the cell's surface, which mainly damages the cell membrane (Earnshaw, 1995). The second type, transient cavitation, is considered more lethal to microorganisms because of the strong explosions that take place in the medium when bubbles collapse between them. These explosions cause the temperature and pressure to increase dramatically in the medium, creating instantly

hot spots in the medium when pressures raise upward to 100 MPa and temperatures to 5000 K (Earnshaw, 1995). This cavitation is responsible for removing material from the cell surface and for pressure and temperature impacting the cells directly. Other indirect effects of cavitation have been reported concerning cell inactivation, such as the formation of free radicals in the medium with a possible bactericidal effect.

Will the process damage the product?

Up until now, research of ultrasound technology has not revealed any negative effects on treated products. Fruit juices and milk have been the main products sonicated; after successfully achieving pasteurization standards for these products, further research did not show any important changes in nutritional content or physicochemical properties (Ugarte-Romero et al., 2006; Bermúdez-Aguirre et al., 2009). Some changes have been observed in the microstructure of foods after sonication, for example in milk. Cavitation is responsible for the breakdown of fat globules in milk; cavitation has a homogenization effect in milk, conferring whiter color and better stability that can be applied to the processing of other dairy products to achieve better quality. Thus far, only a few studies have been conducted in detail to observe the microstructure changes that may occur in sonicated food products, but so far only positive changes have been observed.

What is the shelf-life of sonicated products?

Data showing that the shelf-life of milk pasteurized by ultrasound can be extended is limited. It has been shown that ultrasound is able to promote and speed up chemical reactions in the medium; however, there is a lack of evidence confirming that ultrasound extends the shelf-life of the product because of these reactions. As for the microbial inactivation observed in sonicated food, which also affects shelf-life, the presence of free radicals such as OH^- and H^+ and others (depending on the kind of food) could be responsible for the delay of bacterial growth in

some foods. So far, most of the sonication studies deal with the inactivation of microorganisms and enzymes, not the storage life of products. One of the few shelf-life studies on sonicated products showed that milk can be pasteurized using thermo-sonication and its storage life doubled with this technology, as compared with conventional pasteurization; also, the degree of spoilage depends on the fat content of the milk (Bermúdez-Aguirre and Barbosa-Cánovas, 2008). However, there is little scientific evidence verifying these facts and more research on liquid foods is needed.

Is the technology commercially available?

While most studies of ultrasound as a food processing and preservation tool are conducted at the lab and pilot plant scale, a number of industrial systems have been installed in food processing plants, with the majority remaining unexposed to the outside world. Commercial ultrasonic equipment is now available for some specific unit operations (i.e. extraction, emulsification, crystallization), not for food preservation, and nearly all are customized and tailored for specific products (Patist and Bates, 2008).

Most research on food preservation has been conducted on microbial and enzymatic inactivation. However, the toxicological and chemical profiles of treated foods have not been explored yet. Further, commercialization of novel products from emerging technologies requires many certification studies and validation of the chemical and microbial quality of such products to ensure safety for consumers. At present, ultrasound is a novel technology that has been explored in the lab with successful results, but it is still under development; current research is encouraging and has a promising future, but equipment manufacturers need to collaborate with food scientists to resolve specific issues related to this technology. For example, the manufacture of sonotrodes is under development using new and more resistant materials to reduce the erosion of this component after long processing times. Systems working under pressure and with cooling jackets are available now

for pilot plant use to increase the effectiveness of the treatment; lab scale units are also available.

Is the equipment safe to operate?

Classic ultrasound equipment consists of three components: electrical power generator, transducer, and emitter. The power generator takes the energy from the electrical source; the transducer converts electrical energy into mechanical energy; the emitter is in charge of delivering sound energy into the medium through radiation of the waves (Povey and Mason, 1998). Ultrasound equipment is safe and easy to operate, even though some of its small devices are noisy during regular operation; the bigger units are built inside special cabinets to cushion the noise and facilitate ease of operation. Smaller units are designed to work with small reactors or treatment chambers using small volumes of liquid (50 to 500 ml); bigger units are able to process large volumes (around a few liters).

Has ultrasound been approved by regulatory agencies?

At this time, ultrasound is one of the newest technologies under research in food engineering and it has not yet been approved by any regulatory agencies. Any novel technology requiring approval by a regulatory agency must satisfy very comprehensive criteria regarding food safety, both from the microbiological and chemical points of view. At present, researchers of ultrasound technology in food engineering are still studying the microbiology, the enzymatic and chemical aspects of sonicated food. This knowledge will allow establishing the criteria for processing some type of foods under specific processing conditions, as well as the compilation of information needed for future submission to regulatory agencies, such as the U.S. Food and Drug Administration (FDA), to achieve approval of this technology for food processing. Future research mostly needs to be conducted on microbial inactivation, enzymatic activity, toxicological aspects, and other food properties.

The FDA regulates all new processing techniques to be used in food engineering very carefully so as to

provide the consumer with safe processing technologies and food products from the microbiological and chemicals points of view.

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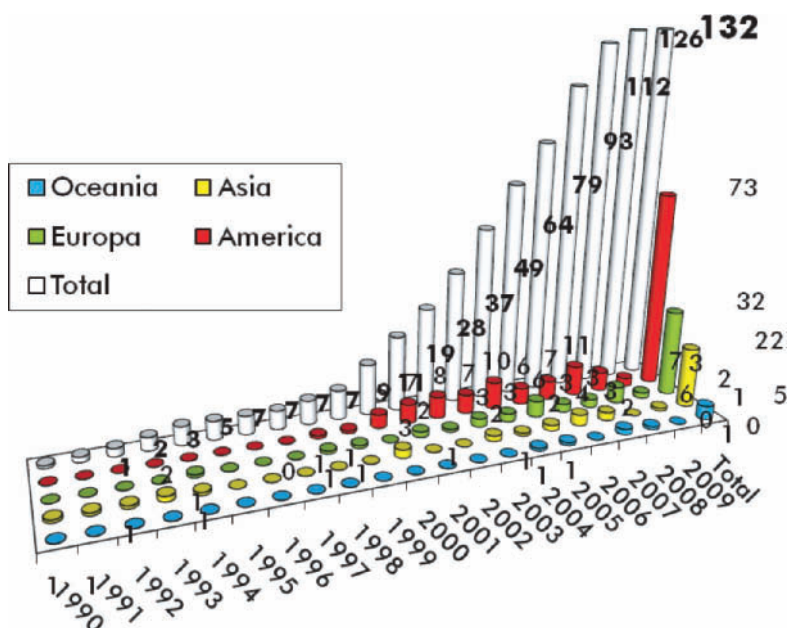


Plate 1. Number of high-pressure equipment units in use around the world as of 2009 (Tonello, 2009).



Plate 2. Typical small pressure vessels using a threaded closure. (Photo courtesy of Pressure Biosciences Inc.)



Plate 3. Wire winding can be used for both the pressure vessel and the yoke to support the end closure force. (Photo courtesy of Avure.)



Plate 4. Pressure vessels, and their associated closure retaining yokes, can be operated at any desired angle of repose from horizontal to vertical. Units are available that tilt to receive product through the top opening, swing to a vertical position to engage a yoke during pressure treatment, then tilt to release treated product from their bottom opening. (Photo courtesy of Elmhurst.)



Plate 5. Historically, large pressure vessels have been built to operate in the vertical position and are loaded and unloaded through their top opening. (Photo courtesy of Avure.)



Plate 6. A second-generation yoke-based system with a 215 L volume which operates at a pressure of 600 MPa (87,000 psi). It is vertically orientated to minimize floor space. A high power pump-intensifier is located below the loading and unloading area. (Photo courtesy of Avure.)



Plate 7. A third-generation 350 L volume, 600 MPa (87,000 psi) system that is capable of a 2,300 kg (5,000 pounds) per hour throughput. It is easily integrated into a horizontal product delivery subsystem. (Photo courtesy of Avure.)

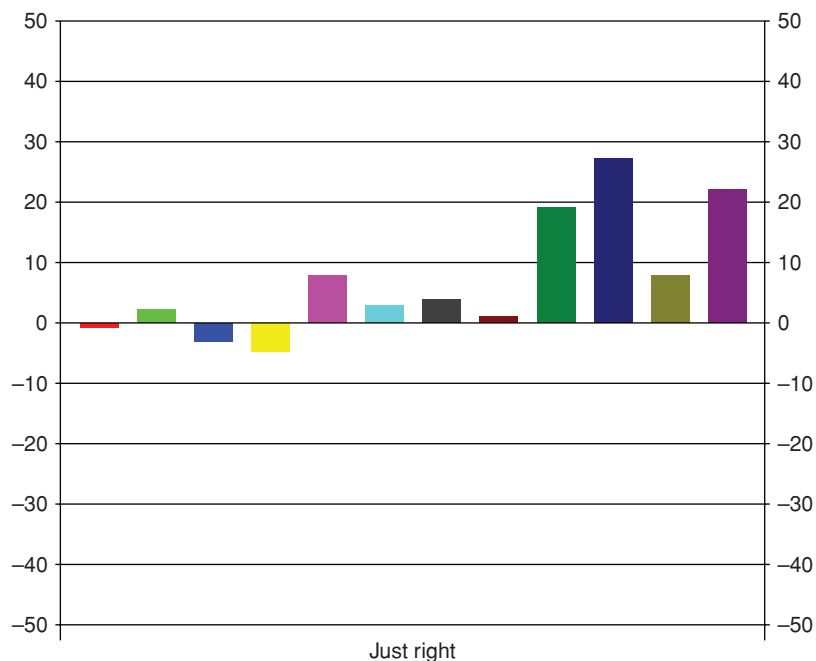


Plate 8. This graph displays the results of the “ripeness” from data acquired from the “Just Right” scale. This could have been generated from naive consumers who were asked to rate their perception of how optimal the color of the fruit was or the data could have been generated from trained panelists who are rating the “ripeness” from specific criterion. The 12 treatments are seen graphically in a way that is intuitive and easy to see relative comparisons.

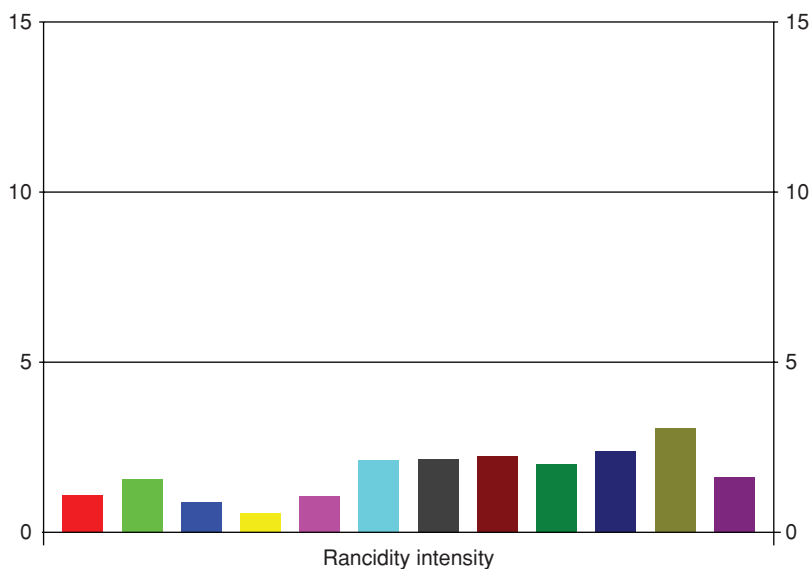


Plate 9. Bar graph results of “Rancidity intensity” for 12 different samples.

Eggs-trained panel quality ratings (1–9 point scale), HPP experimental samples

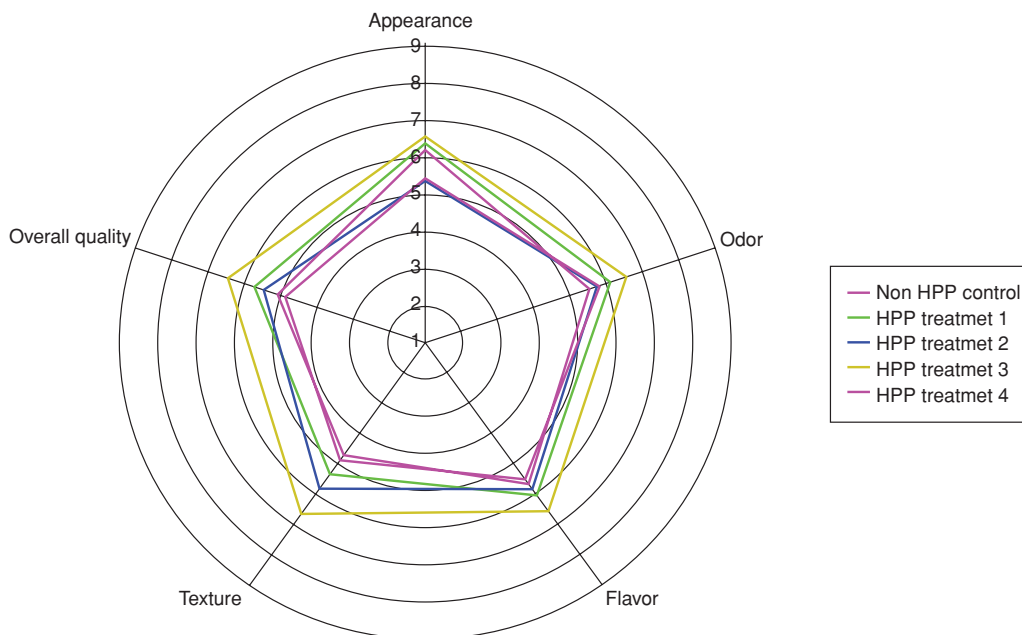


Plate 10. Spider graph display of HPP data from several treatments. The spider graph provides a visual comparison/fingerprint of products overlaying each other. In this graphic, the HPP treatment 3 is clearly the highest rated in all categories. (Note: this graph was derived from a 1- to 9-point quality scale (see Figure 7.3), *not* a 1- to 9-point Hedonic scale (see Figure 7.1)).

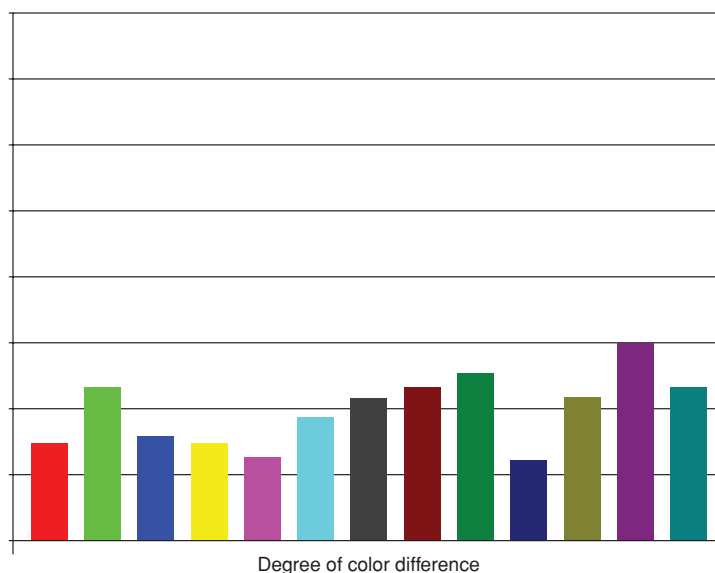


Plate 11. Degree of difference scale and results (treatments vs. control) When scales are different, it is sometimes useful to standardize the rating so that other attributes or characteristics can be viewed on the same graph. Caution should be used when multiple scales are used. Work to assure the scaling of a graph is the same as the scaling that was used to get the rating and to not create misleading information to those viewing graphs. Errors are commonly made in data acquisition and data presentation that can be misleading or confusing when multiple scales are used together to collect data and to present the data. Data Standardized to a 9-point scale—data appropriately presented (number removal simplifies understanding to an intuitive level).

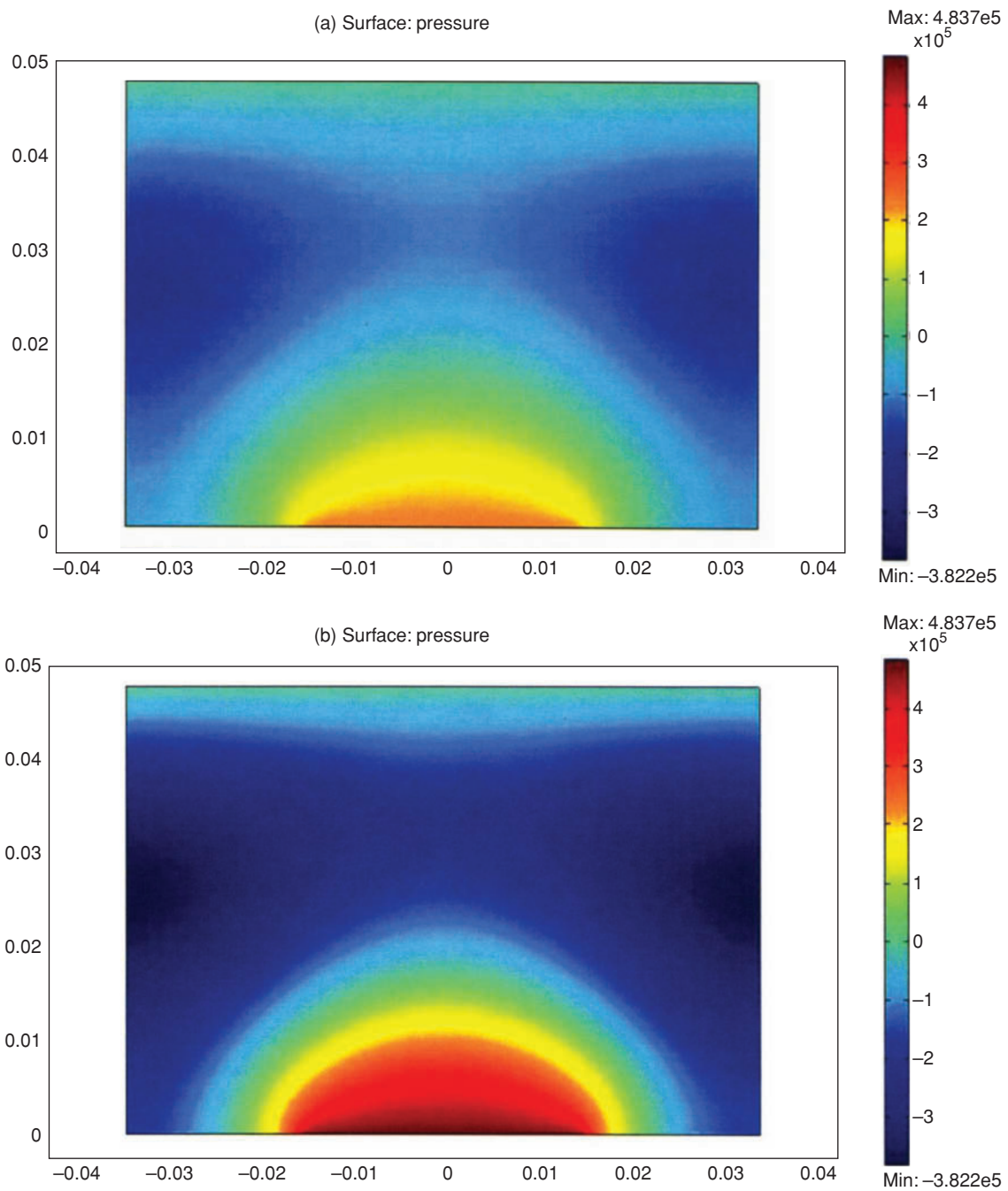


Plate 12. Distribution of energy densities as a function of the distance from the ultrasonic transducer (Sáez et al., 2005).



Plate 13. 16 kW continuous power ultrasound treatment system. (Courtesy of Hielscher USA, Inc.)

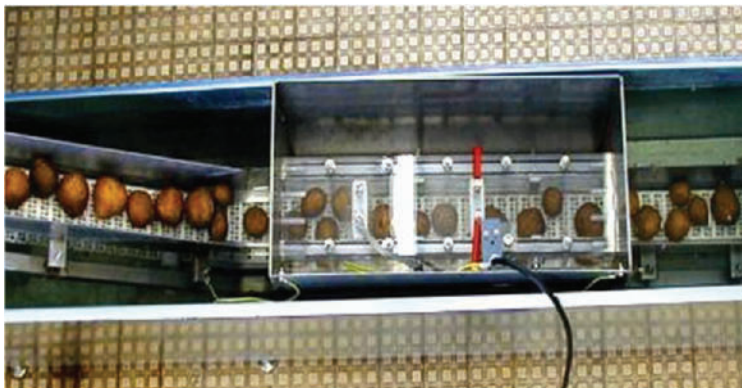


Plate 14. Treatment chamber examples.



Plate 15. 5 kW ELCRACK[®] technical scale system.

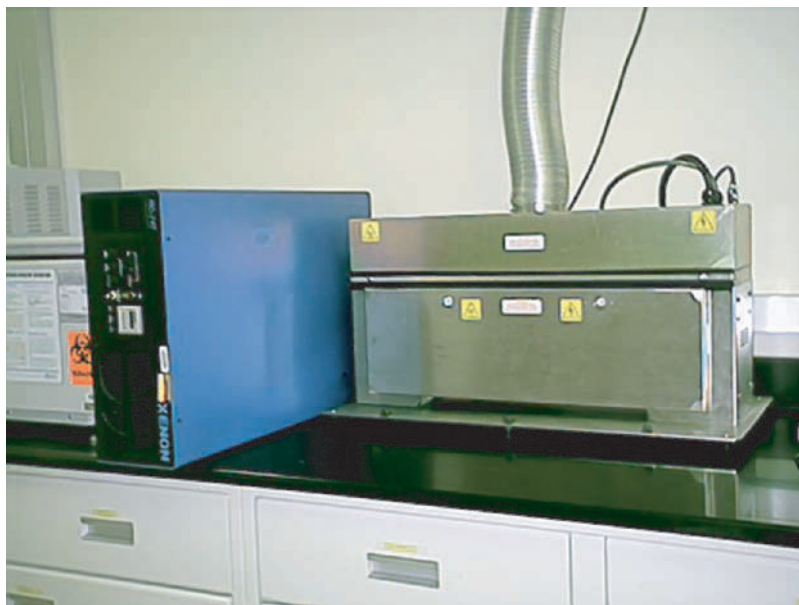


Plate 16. SteriPulse[®]-XL 3000 pulsed UV-light sterilization system (Xenon Corporation[®], Wilmington, MA).

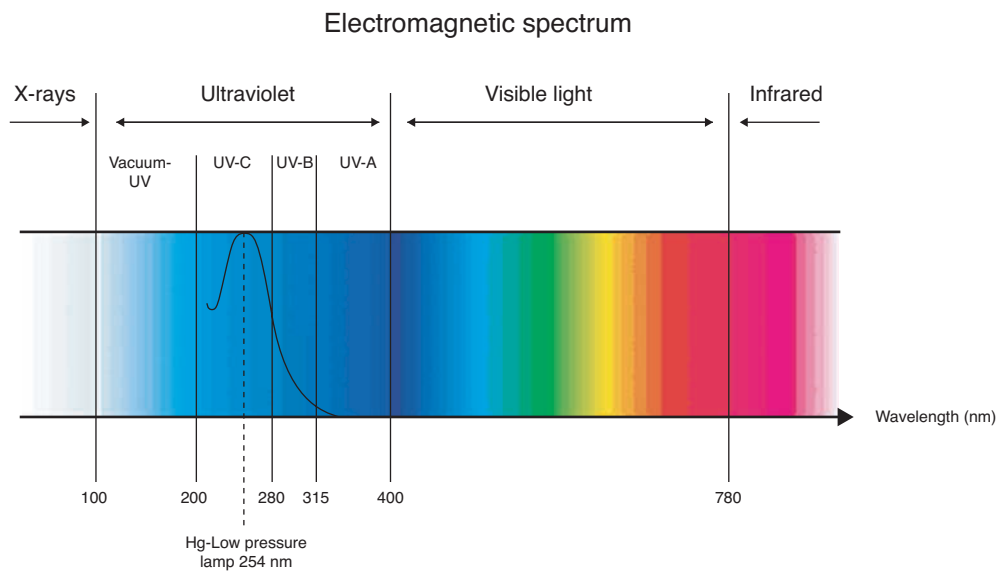


Plate 17. Electromagnetic spectrum. (Adapted from Anonymous, 2007b.)

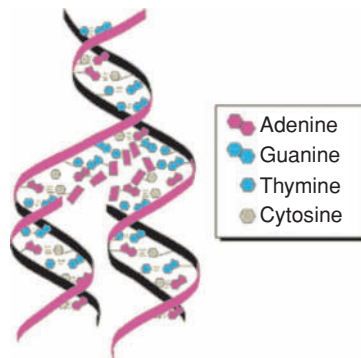


Plate 18. UV-light effect at DNA level. (Adapted from Anonymous, 2007a.)

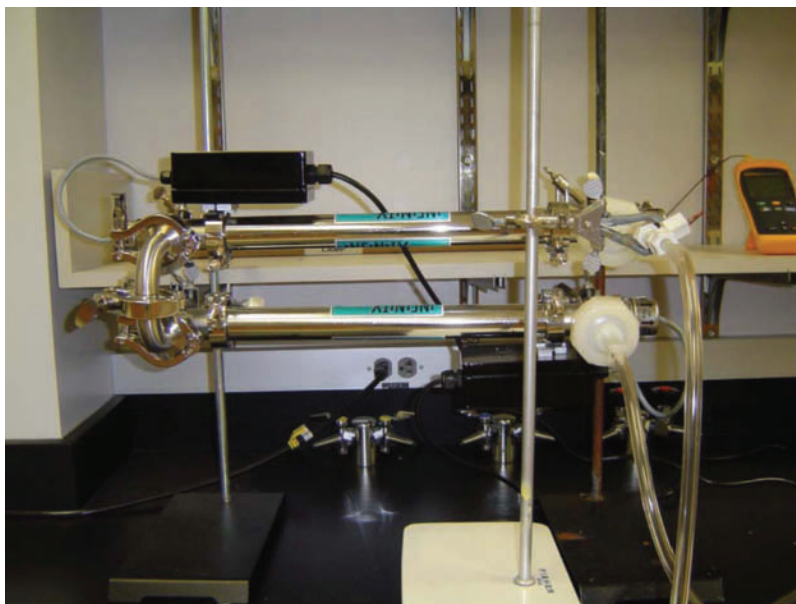


Plate 19. Two UV-C assembled systems by Guerrero-Beltrán, J.A. and Barbosa-Cánovas, G.V. (Washington State University).

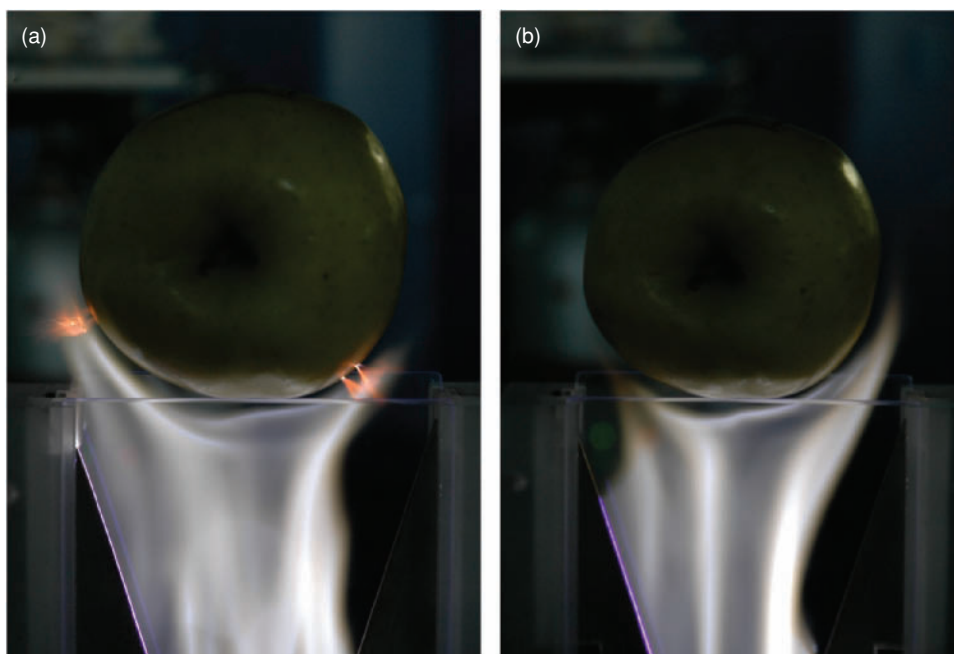


Plate 20. Gliding arc NTP treatment of Golden Delicious apples. NTP produced using air as feed gas (300 L/minute), operating at 10 kV. Treatments pictured are of power levels 450 mA (a) and 190 mA (b). (Image courtesy B.A. Niemira, US Department of Agriculture.)



Plate 21. A good ozone generator is an essential element to the integrated system.

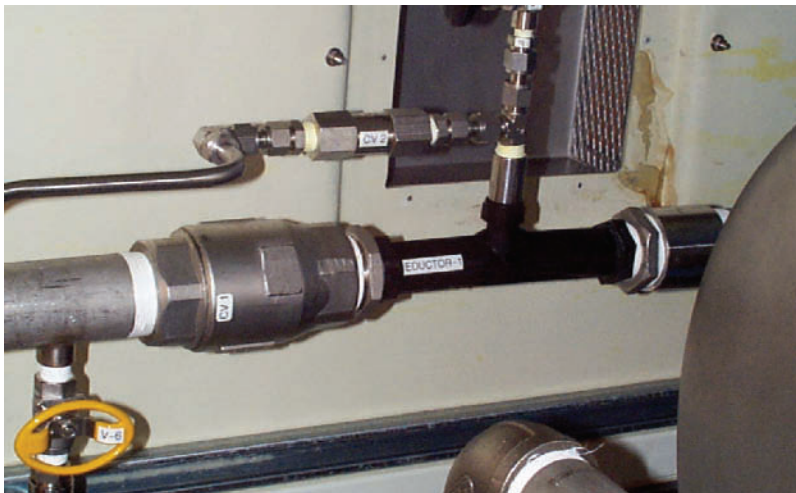


Plate 22. To achieve a good mass transfer of ozone gas into the aqueous system, Venturi injector is one of solutions.

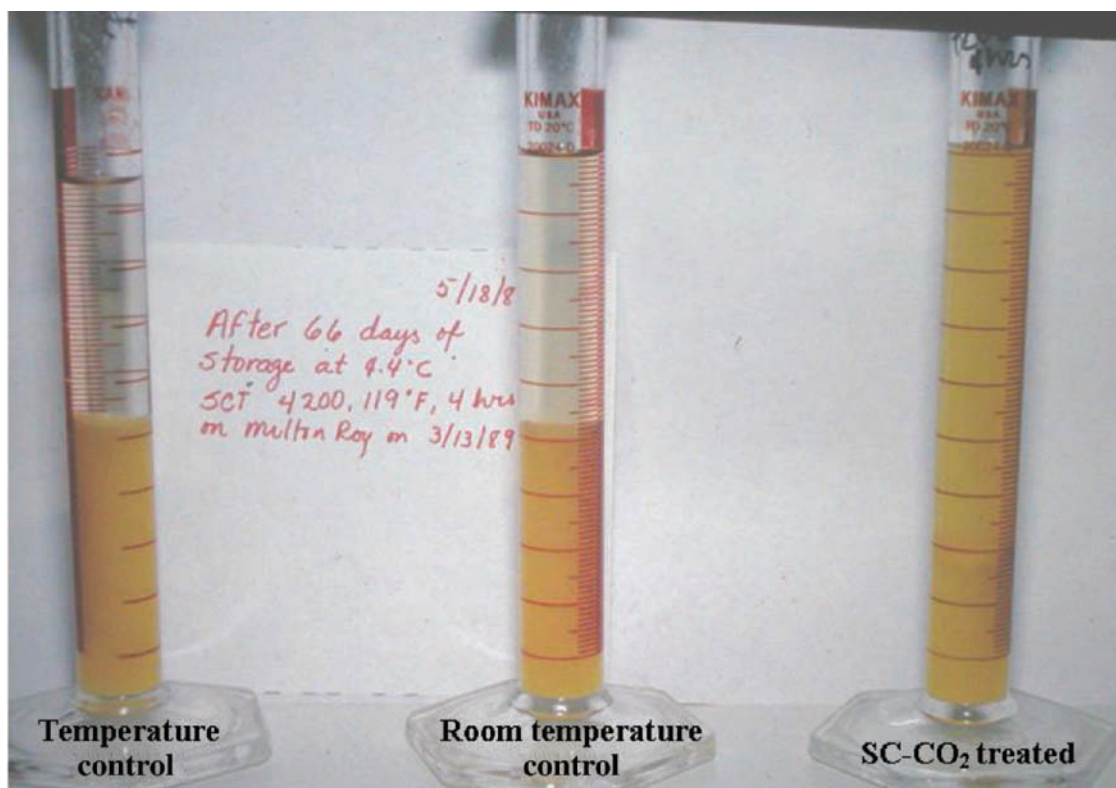
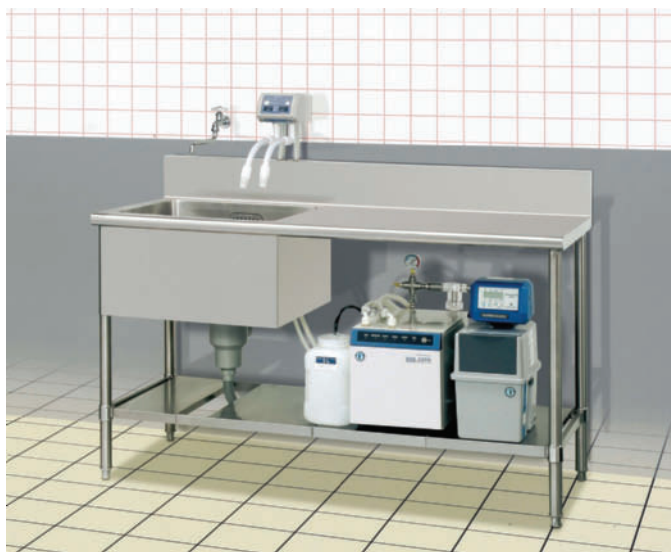


Plate 23. Cloud stability of temperature control, room temperature control, and DPCD treated orange juice samples after 66 days of refrigerated storage at 4.4°C.



(a)



(b)

Plate 24. EO water generators (Hoshizaki Electric Co. Ltd, Japan).



(a)



(b)

Plate 25. Neutralized EO water generator (MIOX Corporation, Albuquerque, NM).

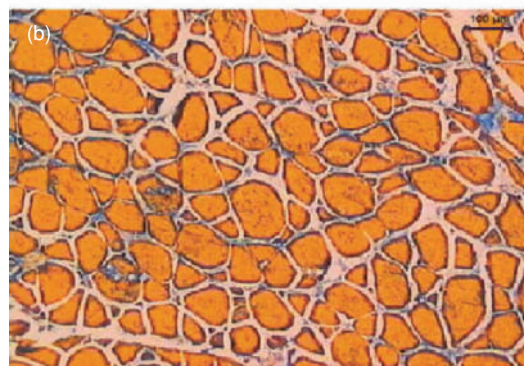
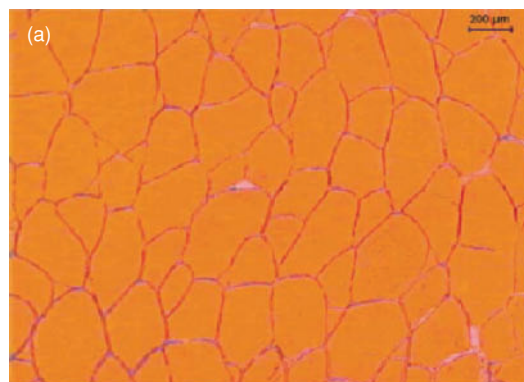


Plate 26. These electronmicroscopic photographs show clearly that PEF affects the structure of salmon cells. There is, however, no evidence that it also changes the chemical composition of the fish. (From Gudmundsson and Hafsteinsson, 2001.)