CHAPTER 17

Pulsed Electric Field Treatment of Food and Product Safety Assurance

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Pulsed electric field (PEF) treatment of food may be applied for various reasons. One is the inactivation of microorganisms at temperatures below those significantly adversely affecting product characteristics such as color and flavor. Before applying any new technology in practice, there must be clear evidence that after processing the product is safe. Consequently, work is needed to prove that relevant food poisoning microorganisms are inactivated. If PEF is intended to replace pasteurization, it must be realized that although both PEF and heat treatments may inactivate microbial cells, the mechanisms of inactivation are likely to differ. While traditional pasteurization will also reduce the activity of many enzymes to a large extent, PEF is unlikely to have a similar effect. It must be shown that the lack of inactivation of certain enzymes does not have undesirable consequences for the safety of the product.

PEF is also likely to cause electrochemical changes, but these may be so small that product safety is not affected. Again, evidence is needed to verify this assumption. Some results of microbiological and toxicological investigations will be presented as well.

It is claimed that pulsed electric fields (PEF) inactivate microorganisms without affecting the quality of the food. If bacteria are changed to the extent that they die, why would nothing happen to the product itself?

Before applying PEF to foods, we have to *know,* and not merely assume, whether the final product is microbiologically and chemically safe. Although we know that PEF can destroy *Staphylococci, Yersinia,* and many other pathogenic and spoilage microorganisms, we also know from our own experiments, as well as from the literature, that there is a wide spread in the PEF sensitivity of microorganisms. The environment of the microorganisms also plays an important role.

For example, pH has a very significant effect: the lower the pH, the stronger the effect (Wouters et al., submitted). The number of microorganisms investigated so far is limited, and there might be PEF resistant ones. Even if that is not the case, whether microorganisms can develop such resistance by adapting to electric pulses should be investigated. There might be survivors with a different, more electrically resistive cell membrane, or a cell membrane that is physically stronger and does not fall apart so easily.

As explained by other researchers, cells may be porated by PEF. Originally, PEF was used to do just that. Porated cells may exchange DNA, and properties may be exchanged between microorganisms. It needs to be clear that the exchange of DNA or RNA between biological cells cannot result in adverse effects.

Damage to the microbial cell membrane may not necessarily be the most important cause of inactivation of the microorganism by PEF. Our own results have shown that under similar conditions, gram-negative microorganisms can be as resistant to PEF as gram-positive microorganisms. If PEF treated microorganisms are observed by microscope, one can see cells with a visibly destructed cell membrane, but most cells seem intact while not being viable any longer. Other causes of inactivation may be changes in transport proteins embedded in the membrane or damage to nucleic acid. More likely, however, all possible damage happen simultaneously, to various extents, depending on the immediate environment of the cell. These changes do not occur selectively to the microbial cell, but also potentially to eukaryotic cells. Destruction of the wall of both prokaryotic and eukaryotic cells may result in the release of enzymes in the product that otherwise would not be released. The enzyme activity may subsequently change the product.

Foods and food ingredients treated by a process not currently used in food production fall within the scope of the European Regulation on Novel Foods, if the process gives rise to significant changes in the composition, the structure, or the level of undesirable substances, and these changes affect the nutritional value or metabolism. A comparison has to be made between products processed conventionally and products produced with the novel process. If analyses show differences, then the influence on nutrition and metabolism has to be addressed. If the changes brought about by the novel process are significant (toxicologically and/or nutritionally), then a risk assessment must result in a decision on the safety status of the food. This decision will have to be endorsed by regulatory bodies.

If cells, that by traditional processing remain intact, burst as a result of PEF treatment, are the released cellular components safe for the consumer? This needs careful investigation. As is known, it takes just 30 micrograms of purified peanut protein to cause the death of a sensitized adult. The potential risk for anaphylactic shock due to allergy may be estimated by calculating how much otherwise contained microbial protein may be released into food. If a product contains a million bacteria per gram, it is noticeably spoiled, and one would not eat it. The dry weight of a million bacteria is approximately 0.1 μg. Assuming

that 50% of the dry weight is protein, if all protein were released into the product it would contain 0.05 μg of bacterial protein per gram. A 100 gram portion then would contain 5 μg bacterial protein mixture. Although this does not prove the safety of a product containing such an amount of bacterial protein, the amount is significantly lower than the potentially deadly quantity of peanut protein.

Applying electrical pulses requires electrodes in contact with the food. If the electrodes are made from metal, corrosion may result in contamination of the food. Metals are acceptable in food, often desirable, but there are safety limits. The maximum concentration of heavy metals in food in many countries is 0.1 mg per kg. Again, the potential for serious risk may be calculated. As the speed of corrosion will strongly depend on the composition of the product in contact with the electrode, the rate of corrosion cannot be predicted. The dissolution of material from the electrodes, however, can be observed and easily quantified by determining the changes in dimension, or by weighing. For example, consider an extreme case: a PEF process unit producing 1000 kg per hr produces in three months time (91 days of 8 hr), 728,000 kg of product. If in that time an electrode with a diameter of 50 mm and a length of 10 mm loses 1 mm of its radius, the volume loss is 1600 mm^3. At a density of 9000 kg per m^3 (valid for copper and nickel), this equals 14,400 mg. Thus, the concentration in the product would be 144,000 mg metal/728,000 kg product, or ~0.02 mg per kg, far below the acceptable concentration. This amount would never seriously contribute to the required daily intake of iron, for example. It is unlikely that the corrosion rate would be as high as assumed, but a requirement could be to replace an electrode after a certain period of usage.

It has been suggested that the chance that products are changed electrochemically is very small, and that the only thing that may happen is the release of minute amounts of hydrogen and oxygen (nanograms per m^3 product) on the electrodes.

Pure-Pulse uses a system where a high voltage pulse is followed by a low voltage pulse of reversed polarity, such that the two charges are equal and the electrochemical effects are nullified. Whether relevant electrochemical reactions are slow enough for such a system to work should be investigated.

Others have suggested that the effect of PEF on microorganisms is at least partly the result of a secondary process, in particular the production of chlorine compounds. This could be so, as almost all food contains sodium chloride. Hülsheger and Niemann (1980) found some effect with *E. coli* (Figure 17.1 and 17.2). If starting with 10^6 *E. coli* cells, there is a higher inactivation rate in the presence of *Clostridium*. Starting with 10^8 cells, however, no effect is shown. Possibly, the amount of hypochlorite produced reacts with organic material that is present more abundantly when 100 times as many microorganisms are present. Experiments of Wouters (personal communication) did not show any influence of chloride ions on the inactivation rate of *Listeria* (Figure 17.3). A possible explanation of the difference in findings may be that Wouters used 10 times shorter pulses.

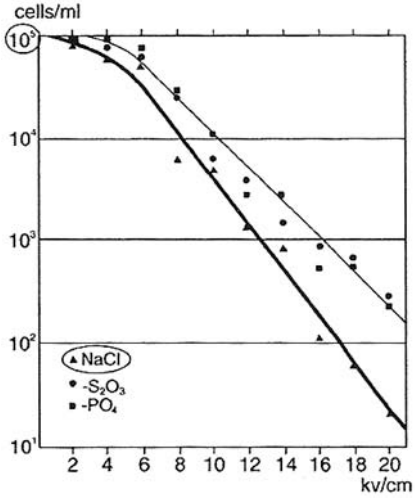

Figure 17.1 Relation between surviving cells and electric field for different electrolyte suspensions with 10^5 cells/mL (Hülsheger and Niemann, 1980).

Because of the legal requirements, we have asked RIKILT-DLO, a Dutch governmental food research institute, to investigate whether there are differences between PEF-treated and heat-treated tomato puree. RIKILT used high resolution one- and two-dimensional proton NMR fingerprinting. Some of the results are summarized in Table 17.1. The main differences were in the concentration

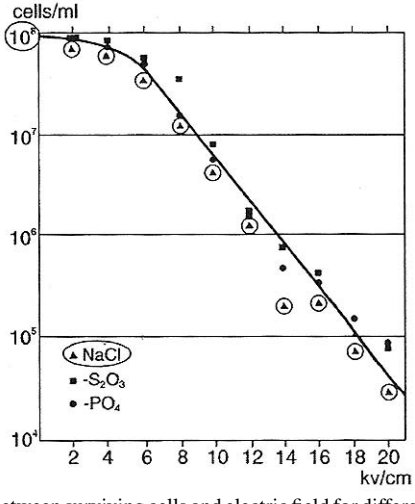

Figure 17.2 Relation between surviving cells and electric field for different electrolyte suspensions with 10^8 cells/mL (Hülsheger and Niemann, 1980).

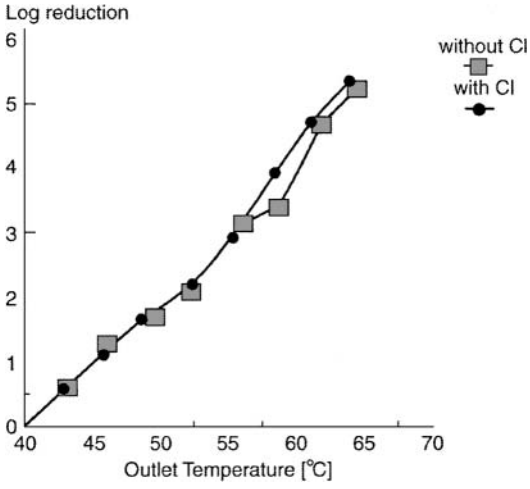

Figure 17.3 Effect of chlorine ions on inactivation of *Listeria innocua* NCTC 11289 in phosphate buffer (pH 5; conductivity 7.8 mS/cm at 40°C inlet temperature; field strength 2.5V/μm) (Wouter et al.).

of amino acids. This is not surprising, given that PEF does not inactivate enzymes, including proteases. The differences in sugar-like compounds are not as easy to explain. In total, RIKILT studied about 2000 peaks, and about 600 of those were different. A few hundred were significantly different. The question

TABLE 17.1. Comparison between Some Compounds in Tomato Purees Treated with Heat or with PEF (Based on High Resolution One- and Two-Dimensional Proton-NMR Fingerprinting by RIKILT-DLO, Wageningen, The Netherlands).

Compound	Concentration in PEF Treated Puree*
Water soluble compounds	
Glucose	−
Oligosaccharides	−−
Tyrosine	+
Valine, leucine, isoleucine	++
Sugar-like compounds	++
Fat soluble compounds	
Aromatic compounds	−
Sugar-like compounds	−/−−
Ribose-like compounds	++
Non-tomatine glycoalkaloids	+
Tomatine glycoalkaloids	−−
Indole type compounds	−

*Concentration in PEF treated product: − slightly lower, −− much lower, + slightly higher, ++ much higher.

to be addressed is whether the observed differences are significant from the toxicological and/or nutritional point of view.

In conclusion, PEF potentially is an attractive process, but before application much research is still needed.

REFERENCES

Hülsheger, H. and Niemann, E. G. 1980. Lethal effects of high voltage pulses on *E. coli* k12. *Radiat. Environ. Biophys.* 18:281–288.

Wouters, P. C., Dutreux, N., Smelt, J. P. P. M., and Lelieveld, H. L. M. Effects of pulsed electric fields on inactivation kinetics of *Listeria innocua*. Appl. Env. Microb. 65(12):5364–5371.