CHAPTER 4

Enzymatic Inactivation by Pulsed Electric Fields: A Review

H. W. YEOM
Q. H. ZHANG

High voltage pulsed electric field (PEF) systems have been studied as one of the most promising nonthermal food preservation methods. In terms of the microbial inactivation mechanism by PEF, extensive research has been done and the membrane rupture theory is now commonly accepted. However, for the inactivation effect of PEF on enzymes, there are very few reports, and it is still a controversial subject. The comparison of PEF research requires caution in considering different PEF systems that may affect enzyme activity. Generally speaking, a PEF system consists of several parts including a high voltage and pulse generator, a treatment chamber, and a fluid handling system (Table 4.1). The enzyme suspension medium is also important due to the effect this medium may have on enzyme activity under a high voltage pulsed electric field.

Gilliland and Speck (1967) found that lactic dehydrogenase, trypsin, and proteinases of *Bacillus subtilis* were inactivated by electrohydraulic discharge, a submerged high voltage spark system. No residual activity was observed in lactate dehydrogenase solution after 10 discharges at 10 kV using an electrode gap of 0.125 in. Trypsin was also significantly inactivated after 40 discharges. Protease activity of *B. subtilis* cells showed a 60% reduction after 40 discharges. Electrohydraulic treated buffer had no effect on the enzyme activity. Oxidation of free sulfhydryl groups and ferrous sulfate was observed. Oxidation of key components, based on the formation of free radicals in the electrohydraulic discharge system, was proposed as the enzyme inactivation mechanism.

Hamilton and Sale (1967) measured several enzyme activities in cell extracts after d.c.-pulse treatment of intact cell suspensions. The field strength was limited to less than 30 kV/cm by the electrical breakdown of air above the sample. NADH dehydrogenase, succinic dehydrogenase, and hexokinase of

TABLE 4.1. Pulsed Electric Field System.

High voltage and pulse generator	• Electric field strength (kV/cm) • Pulse duration time (μsec or msec) • Frequency (Hz) • Wave form (square or exponential decay)
Treatment chamber	• Electrode material • Electrode gap distance • Electrode configuration • Air contact or not
Fluid handling system	• Continuous or batch process • Flow rate (mL/s) • Cooling system (temperature control)
Enzyme suspension medium	• Water • Buffer • Simulated food • Natural food

E. coli 8196 were not significantly inhibited after d.c.-pulse treatment of the cells at 20–25 kV/cm. The acetylcholinesterase activity of bovine erythrocytes was not reduced after pulse treatment of the erythrocytes. Cellular compartments such as the cell wall might function as a barrier protecting intracellular enzymes against the electric field. Activities of lipase and α-amylase treated at voltages up to 30 kV/cm were not inhibited (data not shown).

Castro (1994) observed a 65% reduction of alkaline phosphatase activity in simulated milk ultrafiltrate at an electric field strength of 22 kV/cm using the static chamber of a commercial electroporation system. The temperature of the milk in the cuvette was measured by inserting a thermocouple immediately after 70 pulses. The temperature increased to 44°C due to Joule heating. Polyacrylamide gel electrophoresis of alkaline phosphatase treated with 70 pulses at 20.8 kV/cm revealed a single protein band identical to the untreated protein, demonstrating that PEF does not cause the hydrolysis of alkaline phosphatase. Polarization of alkaline phosphatase leading to aggregation of the enzyme was proposed as the enzyme inactivation mechanism of PEF.

Vega et al. (1995a) reported a 90% reduction of plasmin (milk alkaline protease) activity in a simulated milk ultrafiltrate at 30 and 45 kV/cm after 50 pulses. The sample temperature was controlled by using heat exchangers on the electrodes, and measured by using a thermocouple attached to the surface of the heat exchanger. Processing temperature was maintained at 15°C, demonstrating that the inactivation of plasmin with a pulsed electric field was nonthermal. The authors suggested that there is a synergistic effect between selected factors (e.g., the number of pulses, electric field, and temperature) in the inactivation of plasmin. The activity of PEF-treated plasmin was not restored after 24 hr of storage at 4°C. This result indicated that permanent inactivation of plasmin could be

achieved by PEF. The inactivation mechanism of plasmin was explained by the changes in protein charge and configuration due to the electrostatic nature of plasmin. Vega et al. (1995b) also applied a pulsed electric field of 20 and 35 kV/cm to protease from *Pseudomonas fluorescens* using a pilot plant pulser. Approximately a 70% reduction of protease activity was observed at 35 kV/cm after 20 pulses. The authors found that the reduction of enzyme activity was related to both electric field strength and number of pulses.

Grahl and Markl (1996) studied the effect of PEF on raw milk enzymes such as lipase, lactoperoxidase, and alkaline phosphatase. At 21.5 kV/cm and high energy input ($Q = 400$ kJ/L), a 65% reduction of lipase activity and a 25% reduction of peroxidase activity were observed. The effect of PEF on alkaline phosphatase in raw milk was negligible (less than 5% reduction). The higher fat content of the milk gave a better protection effect against electrical field pulses.

Ho et al. (1997) reported the effects of high voltage pulses on activities of eight enzymes. Lipase, glucose oxidase, and heat stable α-amylase exhibited a large reduction in activity from 75% to 85%; peroxidase and polyphenol oxidase also showed a 30% to 40% reduction. However, alkaline phosphatase showed only a 5% reduction under high voltage pulses. Lysozyme and pepsin were activated at around 50 kV/cm. No change in temperature or pH was observed, indicating that any change in enzyme activity was due to electric field pulses. The authors proposed that the secondary and tertiary structure of the enzyme might play an important role in the inactivation of enzymes based on the results showing different degrees of inactivation according to the enzyme type. In comparison with Castro's results (1994) regarding the inactivation of alkaline phosphatase, the authors suggested that pulse width and pulse waveform may be more important than the electric field strength in the reduction of enzyme activity.

Yeom et al. (1999) conducted experiments with papain protease suspended in a 1 mM EDTA solution. After reaction with reducing agents, activated papain was treated with a pulsed electric field in a continuous system with up to 50 kV/cm of electric field strength at 10°C. A linear relation between residual activity and electric field strength was observed. Further inactivation of papain, separated from activators, was observed within 24 hours of storage after PEF treatment. No significant thermal or chemical effect was found. The loss of α-helical structure, which is related to the papain activity, was observed. The protein structural change by PEF was proposed as one of enzymatic inactivation mechanism.

SUMMARY

As shown in Table 4.2, enzymes have a different sensitivity to PEF treatment. According to the literature, the factors that influence PEF enzymatic inactivation

TABLE 4.2. Summary of PEF-Enzyme Research.

Reference	PEF System	Results
Gilliland and Speck (1967)	• Electrohydraulic discharge • Aluminum core electrode • Electrode gap: 0.125 inch • 0.3 mM KH_2PO_4 solution or microorganism suspension • No temperature control	• *Lactic dehydrogenase:* At 31.5 kV/cm after 10 discharges, 100% reduction • *Trypsin:* At 31.5 kV/cm after 40 discharges, 50% reduction • *Proteinases of* B. subtilis *strain A:* At 31.5 kV/cm after 40 discharges, 60% reduction
Hamilton and Sale (1967)	• Rectangular direct current (d.c.) • Pulse length: 20 μsec • Frequency: 1 Hz • Carbon electrode • Polyethylene spacer with air contact • Temperature control by circulation of water at room temperature	• *NADH dehydrogenase, succinic dehydrogenase and hexokinase* in the extract of pulse treated *E. Coli* 8196: At 20–25 kV/cm, no significant inhibition • *Acetylcholinesterase* in pulse treated bovine erythrocytes, no inhibition • *Lipase and α-amylase* solution: At up to 30 kV/cm, no inhibition
Castro (1994)	• Gene electroporator (Kodak) • Exponential decay wave form • Frequency: 1/15 Hz • Pulse duration time: 0.74 msec or 0.4 msec • Disposable cuvette with air contact • Electrode gap: 0.1 cm • No temperature control	• *Alkaline phosphatase:* —In simulated milk ultrafiltrate: At 22 kV/cm after 70 pulses, 65% reduction —In raw milk and 2% milk: At 18.8 kV/cm after 70 pulses, 59% reduction
Vega et al. (1995a)	• Frequency: 0.1 Hz • Pulse duration time: 2 μsec • A continuous flow chamber • Two parallel stainless steel electrodes and a polysulfone spacer • Closed-loop system • Volume: 15 mL • Flow rate: 45 mL/min • Temperature control: cooling water circulation (15°C)	• *Plasmin* in SMUF (simulated milk ultrafiltrate): At 30 and 45 kV/cm after 50 pulses, 90% reduction
Vega et al. (1995b)	• Pilot plant pulser	• *Proteases from* Pseudomonas fluorescens: At 20 kV/cm, 10 pulses, 25% reduction At 20 kV/cm, 20 pulses,

TABLE 4.3. *Continued.*

		50% reduction At 35 kV/cm, 10 pulses, 60% reduction At 35 kV/cm, 20 pulses, 70% reduction
Grahl and Märkl (1996)	• High-voltage generator with 5–15 kV d.c. voltage • Frequency: 1–22 Hz • Batch vessel BV-25 with air contact • Two plain parallel carbon electrodes • Electrode gap: 0.5 cm • Electrode area: 50 cm^2 • Number of pulses: 1–20 • No temperature control	• Enzymes in raw milk: At 21.5 kV/cm and high energy input ($Q = 400$ kJ/L) *Lipase,* 65% reduction *Peroxidase,* 25% reduction *Alkaline phosphatase,* <5%
Ho et al. (1997)	• High voltage pulse generator ($\leq$30 kV d.c.) with instant charge reversal • Exponential decay wave form • Circular shape and batch treatment chamber $\leq$148 mL •Two circular and parallel stainless steel electrodes • Electrode gap: 0.3 cm • Horizontally positioned chamber to avoid electrical sparking • No cooling system • Pulse duration time: 2 μsec • Frequency: 0.5 Hz • Electrode distance: 0.3 cm • Number of pulses: 30 • Enzyme in buffer solution or deionized water	• *Peroxidase:* At 73.3. kV/cm, 30% reduction • *Alkaline phosphatase:* At 83.3 kV/cm, 5% reduction • α-*Amylase:* At 80 kV/cm, 85% reduction • *Lipase:* At 88 kV/cm, 85% reduction • *Lysozyme:* At 13.3 kV/cm, 60% reduction At 50 kV/cm, 10% reduction • *Glucose oxidase:* At 50 kV/cm, 75% reduction • *Polyphenol oxidase:* At 50 kV/cm, 40% reduction • *Pepsin:* At 40 kV/cm, 150% increase
Yeom et al. (1999)	• PEF bench-scale processing unit • Stainless steel electrode • Electrode gap: 0.2 cm • Co-field flow, tubular chamber • Frequency: 1500 Hz • Pulse duration time: 4 μsec • Square wave and monopolar pulse • Temperature control: 10°C • Number of pulses: up to 500 • Enzyme in 1 mM EDTA solution	• *Papain:* —With activators (L-Cys and DTT): At 50 kV/cm, 50% reduction —Without activators: At 50 kV/cm, 90% reduction after 24 hr storage at 4°C

can be summarized as follows:

(1) Electric parameters
   - electric field strength (Vega et al., 1995a, 1995b; Yeom et al., 1999)
   - total treatment time or number of pulses (Vega et al., 1995a, 1995b; Yeom et al., 1999)
   - pulse duration time and pulse width (Ho et al., 1997)

(2) Enzymatic structure
   - key components such as active site (Gilliland and Speck, 1967)
   - secondary structure (Ho et al., 1997; Yeom et al., 1999)
   - tertiary structure (Ho et al., 1997)

(3) Treatment temperature (Vega et al., 1995a)

(4) Suspension medium (Grahl and Märkl, 1996)

PEF treatment does not cause any significant increase of temperature when a short pulse duration time is applied to a solution with low electric conductivity. Therefore, thermal inactivation of enzymes due to Joule heating can be excluded from the PEF enzymatic inactivation mechanism. When a cooling system is used, the nonthermal effect of PEF on enzyme activity is obvious (Vega et al., 1995a; Yeom et al., 1999). The pH of the enzyme solution is not changed after PEF treatment (Ho et al., 1997; Yeom et al., 1999). Therefore, reduction of enzyme activity by pH change is not the cause of PEF enzymatic inactivation. Conformational change of enzymes has been suggested as the PEF enzymatic inactivation mechanism by several researchers (Vega et al., 1995a; Ho et al., 1997; Yeom et al., 1999). To elucidate the PEF enzymatic inactivation mechanism, more information about the structure and function of enzymes under high voltage pulsed electric fields is necessary.

REFERENCES

Castro, A. J. 1994. Pulsed electric field modification of activity and denaturation of alkaline phosphatase. Ph.D. Dissertation. Washington State University.

Gilliland, S. E. and Speck, M. L. 1967. Mechanism of the bactericidal action produced by electrohydraulic shock. *Appl. Microbiol.* 15(5):1038.

Grahl, T. and Märkl, H. 1996. Killing of microorganisms by pulsed electric fields. *Appl. Microbiol. Biotechnol.* 45:148.

Hamilton, W. A. and Sale, A. J. 1967. Effects of high electric fields on microorganisms. *Biochimica et Biophysica Acta*. 148:789.

Ho, S. Y., Mittal, G. S., and Cross, J. D. 1997. Effects of high field electric pulses on the activity of selected enzymes. *J. of Food Eng.* 31:69.

Vega, H. M., Powers, J. R., Barbosa-Cánovas, G. V., and Swanson, B. G. 1995a. Plasmin inactivation with pulsed electric fields. *J. Food Sci.* 60(5):1143.

Vega, H. M., Powers, J. R., Barbosa-Cánovas, G. V., Swanson, B. G., and Luedecke, L. 1995b. Inactivation of a protease from *Pseudomonas fluorescens* M3/6 using high voltage pulsed electric fields. *IFT 1995. Annual Meeting*. Book of Abstracts, paper no. 89-3. p. 267.

Yeom, H. W., Zhang, Q. H., and Dunne, C. P. 1999. Inactivation of papain by pulsed electric fields in a continuous system. *Food Chem.* 67:53.