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Pulsed Electric Fields in Food Preservation

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33.1 Introduction

There are many different ways of applying electric energy for food pasteurization. These include ohmic heating [1–3], microwave heating [4–6], low electric field stimulation [7,8], high-voltage arc discharge [9–12], and high-intensity pulsed electric field (PEF) application [13–15]. Ohmic heating is one of the earliest forms of electricity applied to food pasteurization [1]. This method relies on the heat generated in food products when an electric current is passed through them. Getchell [2] described the ohmic heating method in milk pasteurization. A 220 V, 15 kW alternating current supply was applied to milk through carbon electrodes in an electrical heating chamber. The milk was heated to and held at 70°C for about 15 s. It has been reported that ohmic heating is suitable for viscous products and foods containing particles, and this method is considered to be a promising technique for the aseptic processing of foods [3].

Microwave heating has been extensively applied everyday in households and the food industry [4]. Many food materials possess very low values of static conductivity. However, when they are subjected to microwave fields, they exhibit very high values of alternating field conductivity and consume

considerable energy [5]. The heat generated by microwaves is used for heating processes. Studies on microbial inactivation using microwave energy have concluded that microbial death is caused solely by thermal mechanisms [6].

Low electric field stimulation has been explored as a method of bacterial control of meat. In electrical stimulation of meat, an electric field of 5–10 V/cm is applied as alternating current (ac) pulses to the sample through electrodes fixed at opposite ends of the long axis of the muscle [7]. Recently, a very low field (0.4 V/cm) has been applied in a 6-L treatment medium in search of an easy, safe, and practical method to eliminate bacteria for food processing purposes. Several species of bacteria in saline solution were inactivated [8]. Salt solutions and their concentrations play a very important role in this method [48].

Inactivation of microorganisms and enzymes contained in food products by electric discharge began in the 1920s with the Electropure process for milk [16], which consisted of passing an electric current through carbon electrodes and heating milk to 70°C to inactivate *Mycobacterium tuberculosis* and *Escherichia coli.* Beattie and Lewis [17] demonstrated a lethal effect of electrical discharges on microorganisms when the applied voltage used to treat food was increased to 3000–4000 V. The electrohydraulic treatment was introduced in the 1950s to inactivate microorganisms suspended in liquid foods. The inactivation of microorganisms was attributed to a shock wave generated by an electric arc that prompted the formation of highly reactive free radicals from chemical species in food [14]. Gilliland and Speck [18] applied pulsed electric discharges at different energy levels for the inactivation of *E. coli*, *Streptococcus faecalis*, *Bacillus subtilis*, *Streptococcus cremoris*, and *Micrococcus radiodurans* suspended in sterile distilled water as well as for trypsin and a protease from *B. subtilis* [47].

Sale and Hamilton [19] demonstrated the nonthermal lethal effect of homogeneous electric fields on bacteria such as *E. coli*, *Staphylococcus aureus*, *Micrococcus lysodeikticus*, *Sarcina lutea*, *B. subtilis*, *B. cereus*, *B. megaterium*, *Clostridium weichii*, and yeasts such as *Saccharomyces cerevisiae* and *Candida utilis.* In general, an increase in the electric field intensity and number of pulses led to an increase in the inactivation of microorganisms (Figure 33.1 and Table 33.1). Other factors that influence

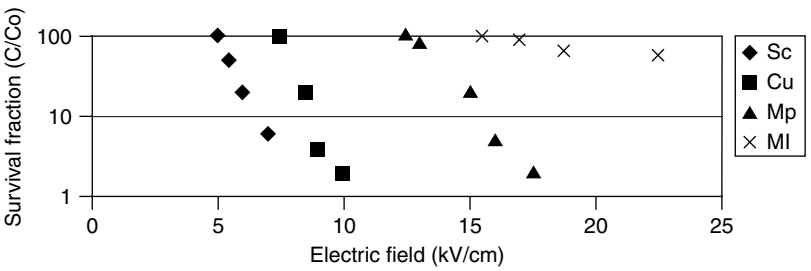

FIGURE 33.1 Relationship between survival fraction and electric field strength (1020 μs pulses). Sc: *Saccharomyces cerevisiae*; Cu: *Candida utilis*; Mp: Motile pseudomonad; MI: *Micrococcus lysodeikticus*. (Adapted from W. A. Hamilton and A. J. H. Sale, *Biochem. Biophys. Acta* 148: 789 (1967).)

TABLE 33.1

Activity of *Staphylococcus aureus* after PEF Treatment

Electric Field (kV/cm)	Survivors (%)	Protoplasts Not Lysed
0.00	100	100
9.25	100	100
14.25	35	43
19.50	0.9	16
24.00	0.3	3
27.50	0.6	2

Source: Adapted from W. A. Hamilton and A. J. H. Sale, *Biochem. Biophys. Acta* 148: 789 (1967).

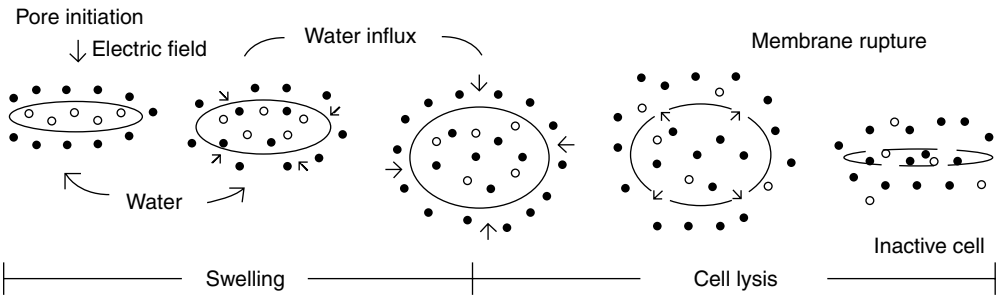

FIGURE 33.2 Mechanism of cell inactivation. (Adapted from T. Y. Tsong, *Biochim. Bioeng.* 24: 271, 1990.)

microbial inactivation by PEFs are the treatment temperature, pH, ionic strength, and conductivity of the medium containing the microorganisms [9,20–26].

Formation of pores on cell membranes by high-intensity PEFs (HIPEFs) is not entirely understood. Zimmermann et al. [27], applying the dielectric rupture theory, concluded that membrane rupture is caused by an induced transmembrane potential approximately 1 V larger than the natural potential of the cell membrane. The reversible or irreversible rupture (or electroporation) of a cell membrane depends on factors such as intensity of the electric field, number of pulses, and duration of the pulses [28–31]. The plasma membranes of cells become permeable to small molecules after being exposed to an electric field; permeation then causes swelling and the eventual rupture of the cell membrane (Figure 33.2).

In September 1996, the U.S. Food and Drug Administration (FDA), based in Washington, DC, released a "letter of no objection" for the use of PEFs to treat liquid eggs. To meet the FDA requirements [32] in filing a new and a novel process, it is necessary to (a) establish an active and continuous dialog with the FDA during process development, (b) meet with the FDA to describe the process, (c) invite the FDA to a site visit (pilot and production facility), and (d) draft and provide the FDA with an outline of the proposed filing.

The objective of the FDA is to conduct a scientific evaluation of the process to determine if the aseptically produced product poses a potential public health hazard, and if all the critical factors necessary to render the product commercially sterile are monitored and controlled. The filing information of the new process must contain

1. Equipment design: a description of the system, control mechanisms used, and fail safe procedures
2. Product specifications: a full description of the product, including physical/chemical aspects, critical factors, and influence of processing on the critical factors
3. Process design: a complete description of the critical/processing conditions used in the manufacture of the product
4. Validation: a physical demonstration of the accuracy, reliability, and safety of the process

In the area of PEFs, there are many possible project-development designs related to (a) unknown destruction kinetics of microbial pathogens (e.g., *Clostridium botulinum*), (b) identification of proper indicator organisms, (c) uniformly delivered treatment, (d) impact of processing conditions (e.g., temperature, pH, moisture, and lipid content), (e) identification/monitoring of critical factors (e.g., surface and intensity), and (f) food additives.

33.2 Engineering Aspects of PEFs

The concept of pulsed power is simple: electric energy at low power levels is collected over an extended period and stored in a capacitor. That same energy can then be discharged almost instantaneously at very high levels of power. The generation of PEFs requires two major devices: a pulsed power supply and a treatment chamber, which converts the pulsed voltage into PEFs.

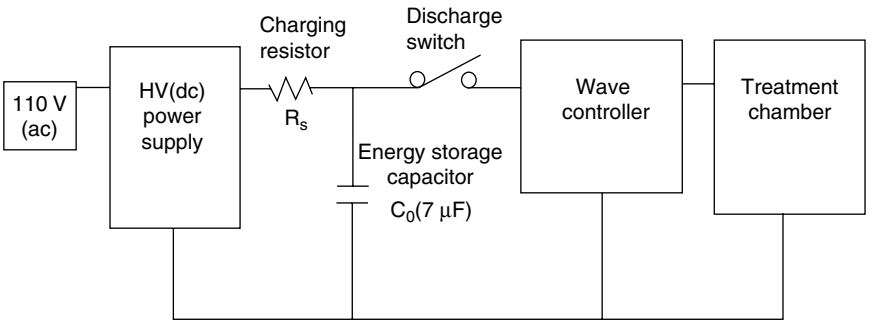

FIGURE 33.3 Major components of commercial electroporator GeneZapper.

33.2.1 Bench-Top Unit

A commercial electroporator (e.g., GeneZapper, IBI-Kodak Company, Rochester, NY) may be used as a bench-top pulsed power supply. This unit provides a maximum of 2.5 kV pulses. The instrument consists of a capacitor (7 μF), charge and discharge switches, and a wave controller. The wave controller may be connected to the electroporator to improve the discharge pattern. Treatment cuvettes with a 0.1-cm electrode gap and l00 μL volume may be used for PEF treatments, which give a maximum intensity of approximately 25 kV/cm. Appropriate voltage and current monitors should be attached to the GeneZapper to measure the PEF treatments. Figure 33.3 illustrates the major components of the GeneZapper. This bench-top unit provides a convenient method for determining the inactivation kinetics for selected microorganisms.

33.2.2 Lab-Scale Pulser

Exponential decay electric pulses could be generated by discharging a capacitor into a chamber containing the food (Figures 33.4 through 33.6). Current designs for power supplies are able to provide up to 40 kV. Capacitors of 5 μF are used to store the electric energy that is discharged across metal electrodes, creating the electric field used to inactivate microorganisms and enzymes. A mercury ignitron spark gap may be used as the discharge switch. This type of unit may be employed for inactivation studies in a continuous mode. Pulsed voltage across the treatment chamber may be monitored by a resistance voltage divider. Electric current may be monitored by a Rogowski coil connected to a passive integrator. Both voltage and current waveforms may be monitored using a digital oscilloscope.

33.2.3 Treatment Chambers

A static PEF treatment chamber consists of two electrodes held in position by insulating materials that also form an enclosure containing food materials. Uniform electric fields can be achieved by parallel plate electrodes with a gap sufficiently smaller than the electrode surface dimension. Disk-shaped, round-edged electrodes can minimize electric field enhancement and reduce the possibility of dielectric breakdown of fluid foods. A continuous flow-through treatment chamber (Figure 33.7) was developed at Washington State University (WSU) to test the flow-through concept using low flow rates. The chamber consisted of two electrodes, a spacer, and two lids. Each electrode was made of stainless steel, whereas the spacer and lids were made of polysulfone. A flow channel was provided between the two electrodes to eliminate dead corners as well as to ensure uniform treatment.

The operating conditions for the parallel plate continuous chamber were as follows: chamber volume 20 or 8 cm^3 electrode gap 0.95 or 0.51 cm; PEF intensity 35 or 70 kV/cm; pulse width 2–15 μs; pulse rate 1 Hz; and food flow rate 1200 or 600 cm^3/min. Cooling of the chamber was accomplished by circulating water at a selected temperature through jackets built into the two stainless steel electrodes. It should also be pointed out that a completely sealed treatment chamber is dangerous. When the test fluid experiences a spark, high pressure develops rapidly and the chamber may break apart.

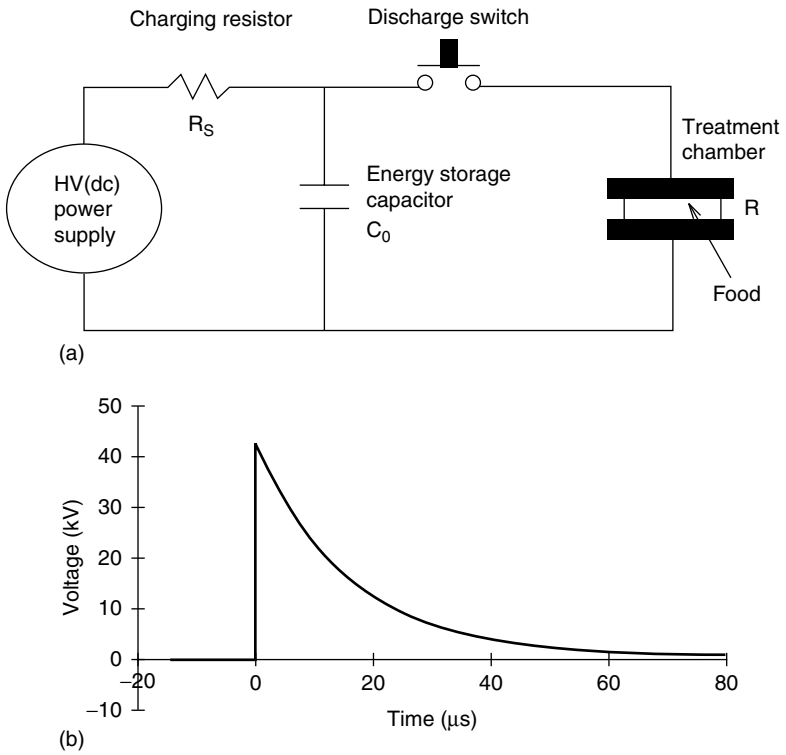

FIGURE 33.4 (a) A simplified circuit for producing exponential decay pulses and (b) a voltage trace across the treatment chamber.

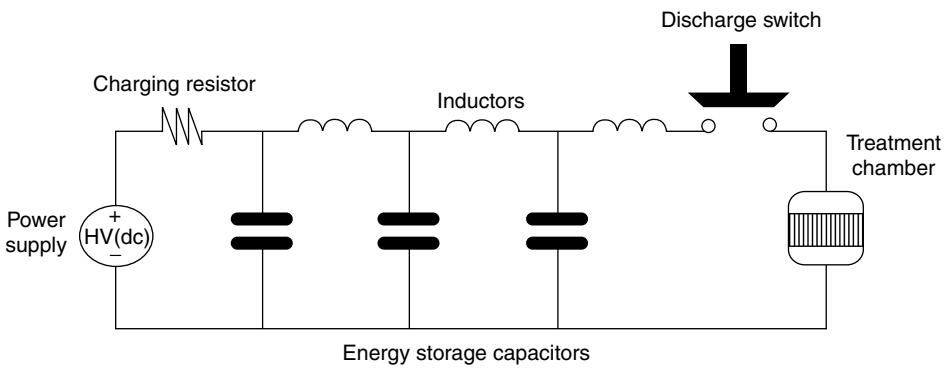

FIGURE 33.5 Typical pulser configuration for high-intensity pulser electric fields.

A pressure-release device must be included in the treatment chamber design to ensure safety of the operation.

A coaxial treatment chamber (Figure 33.8) with a uniform field distribution along the fluid path was designed at WSU. The fluid is fed into the chamber through the bottom region and treated product exits at the top of the chamber. The protruded surface, located at the outer-grounded electrode, enhances and makes the electric field uniform within the treatment region while it reduces the field intensity in other regions of the fluid path. Cooling fluid is circulated to control the temperature between the inner high-voltage electrode and the outer-grounded electrode. The gap in the coaxial electrode or the liquid food thickness along the direction of the electric field can be selected by changing the diameter of the inner electrode.

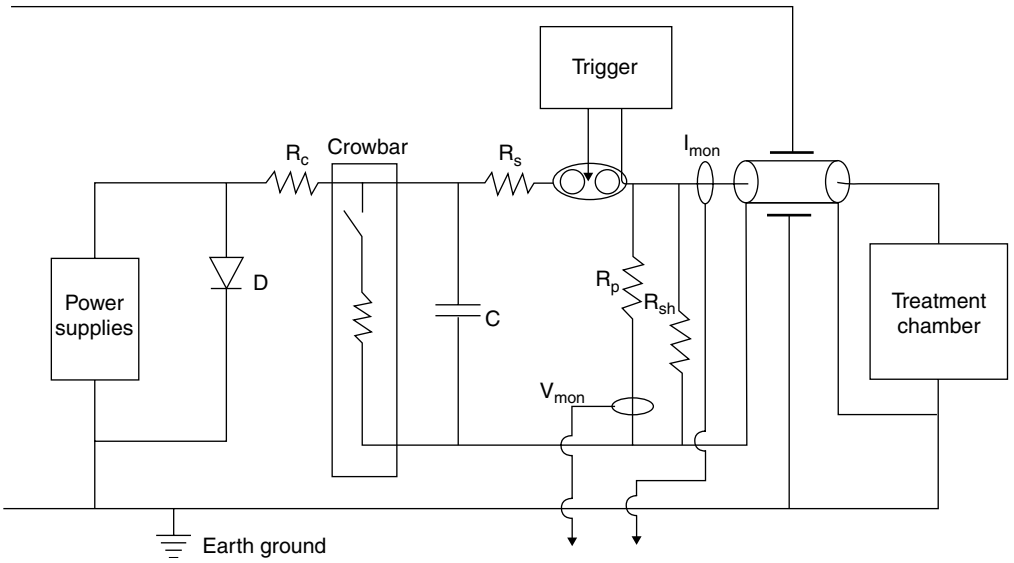

FIGURE 33.6 Current setup of the PEF facility at Washington State University. The pulser has a 16 kJ/s charging power supply, 40 kV peak charging voltage, and 10 Hz pulse repetition rate. C: storage capacitor; D: power supply protection diode; R_c: charging resistor; R_s: series resistor; R_{sh}: shunt resistor; R_p: voltage-measuring resistor; I_{mon}: current monitor; V_{mon}: voltage monitor.

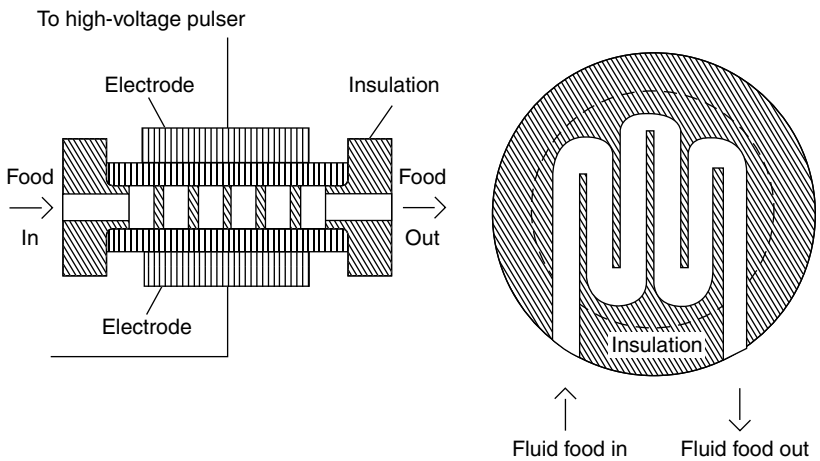

FIGURE 33.7 Schematic drawing of a flow-through treatment chamber. Fluid inside the chamber is baffled to avoid dead spots.

33.2.4 PEF Process Design

33.2.4.1 HACCP Principles and PEF Technology

The PEF process is summarized in Figure 33.9. The key operations are the receiving of raw materials, PEF treatment, aseptic packaging operation, and finished product storage and distribution. The following analysis [33] is based upon the seven principles of hazard analysis and critical control points (HACCP).

33.2.4.1.1 Hazard Assessment

Microbial hazards are the main concern throughout the PEF operation. Raw materials contain spoilage microbes and pathogens that may spoil the ingredient or raw material or may be harmful to the consumer. Storage facilities for raw materials may increase the risk of microbial contamination from soil

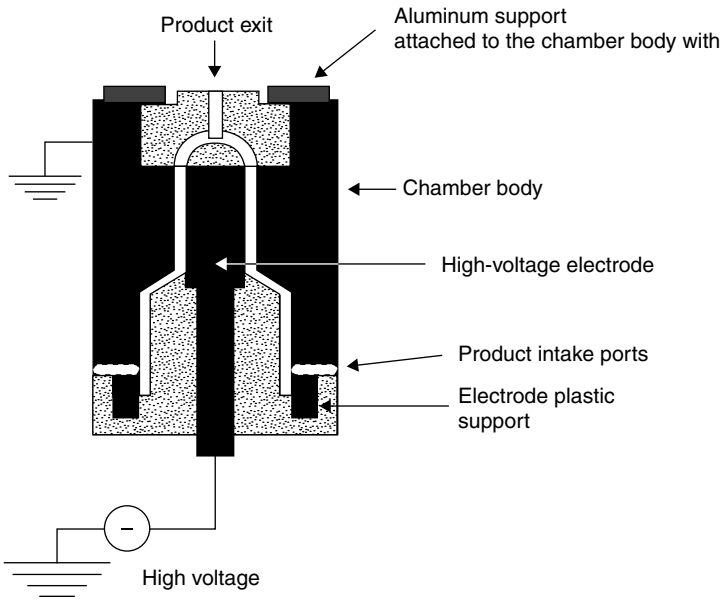

FIGURE 33.8 Schematic of the REF continuous treatment chamber.

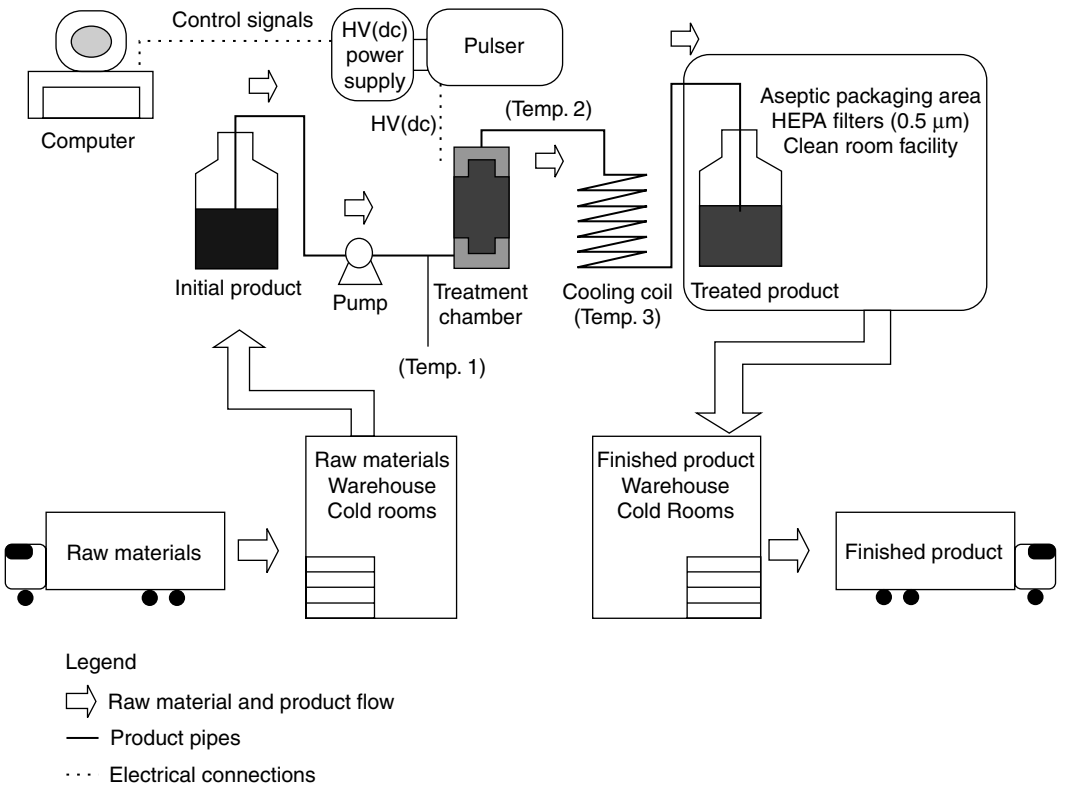

FIGURE 33.9 PEF unit operations layout.

and water deposits. The cleanliness of processing equipment plays a key role in preventing microbial contamination, thus the multiple assembly parts must always be properly sanitized. Inappropriate aseptic packaging operations and storage conditions may result in spoilage of the product.

The chemical hazards to consider are the presence of antibiotic and pesticide residuals on raw materials, electrically induced chemical reactions, and excessive detergent–sanitizer residues from processing and packaging equipment. Physical hazards include foreign matter in raw materials (e.g., stones, rubber, plastic, metal, and eggshells), metal particles from the treatment chamber after a spark, and plastic or rubber pieces from seals.

The final risk classification may be defined in terms of the product (milk, apple juice, eggs, soups, etc.). Six microbiological hazard characteristics, as well as chemical and physical hazard characteristics, are defined by the National Advisory Committee on the Microbiological Criteria for Foods (NACMCF) and will be used to classify PEF products. In general, the final hazard classification should occur between risk categories IV and VI as defined by the NACMCF.

33.2.4.1.2 Critical Control Points: Determination, Limits, Procedures, and Corrective Actions

The following critical control points (CCPs) should be selected to ensure the safety of PEF products: receiving and storage section, PEF treatment section, and aseptic packaging section. The main factors considered and monitored for each CCP are handling and processing time, temperature of material, and cleanliness of equipment and utensils. The treatment conditions (electric field intensity, pulsing rate, input voltage, input current, and chamber temperature) should be monitored and recorded on a continuous basis. Uniform PEF treatment requires the design and construction of a pulser that accomplishes variable pulsing rates, charging rates, voltage settings, pulse widths, and pulse shapes. Pulser components such as power source, computerized controls, triggering mechanism, overloads, dummy loads, and treatment chamber should comply with defined specifications and characteristics such as maximum operating temperature, maximum voltage and current outputs, and reliability (mean time between failures, yields, etc.). The reliability of the pulser may be measured in terms of number of pulses with correct energy level per unit of time as well as total pulses per unit of time. Monitoring devices may include oscilloscopes for voltage and current measures, and pulse counters.

Standard operating procedures (SOPs) should be in place to define aspects such as reception, storage, and preparation of raw materials, to ensure proper handling and reduce the risk of contamination. The pulsing and packaging units must have procedures to specify the assembly and disassembly of the machinery. Cleaning specifications such as frequency and type of detergents and sanitizers to be used should be established to prevent contamination between products. The operational parameters for PEF treatments must be specified for each food product based upon its microbial risk, initial microbial counts, physical and chemical characteristics (e.g., pH, ionic strength, and composition), and the maximum time to complete the processing of each food (i.e., time from initial discharge of raw materials to the end of the packaging operation). Alternative procedures must define the corrective actions associated with deviations from process specifications or CCP limits. Quality assurance procedures must be developed for the approval or rejection of PEF-treated products based on the CCP limits and corrective actions.

33.2.4.1.3 Record Keeping

Record keeping is a key aspect not only in a PEF operation, but also in any successful manufacturing operation. The status of raw materials, process and packaging sequence, as well as storage and shipping procedures, must be reflected in the batch or lot documents. Proper design of the documents is an important and difficult task because the documents must provide enough space for critical measurements without confusing the operator.

33.2.4.2 Hazard and Operability Study (HAZOP) Principles and PEF Technology

The main concern of individuals working in a PEF facility is the voltage intensity, which reaches the kilovolt range. A typical pulser configuration is presented in Figure 33.10. A high-voltage power supply is selected to charge the capacitor (eventually more than one) and a discharge switch releases the stored electric energy from the capacitor through the product in the form of an electric field. The power supply, capacitor, and treatment chamber must be confined in a restricted access area with interlocked gates.

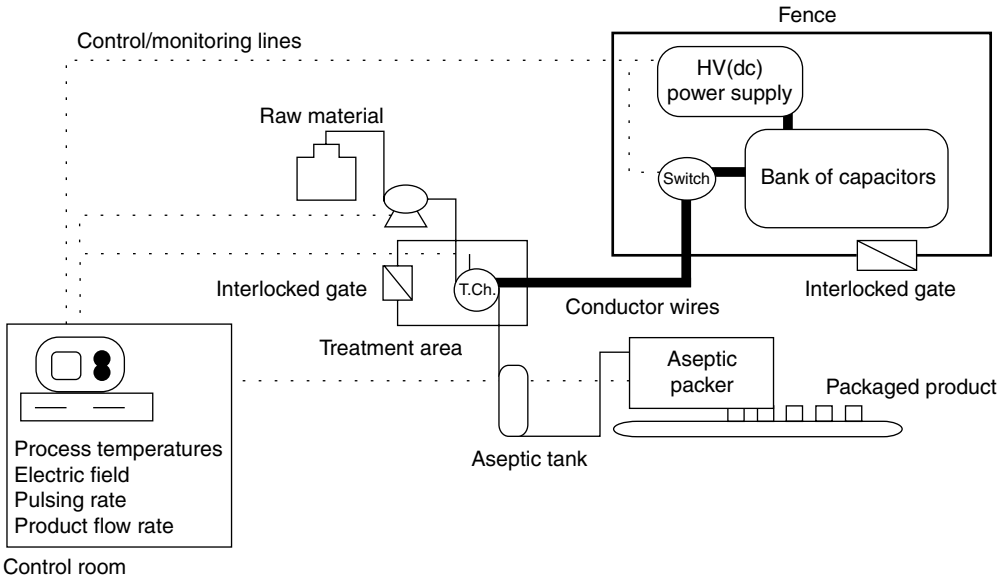

FIGURE 33.10 Schematic diagram of a PEF equipment configuration.

The gates will turn off the pulser if they are opened while the power supply is on. Emergency switches must be accessible in case of a process failure. Also, discharging bars must be provided to discharge the elements in the circuit before maintenance or inspection of the unit occurs. To prevent the leakage of high voltage through any fluid (food or refrigerant) in contact with the treatment chamber, all connections to the chamber will be isolated and the pipes carrying materials to or from the chamber are connected to ground.

Electrical and mechanical devices such as pumps, computers, and packaging machines must be protected using safeguards. Proper warning signs must be in place regarding the safety hazards (high-voltage and high-intensity electric field) in the processing area. The information related to the operation and maintenance procedures must be contained in standard operating procedures (SOPs). The personnel involved in the PEF operation must be trained and instructed in these SOPs.

The selection of appropriate detergents and sanitizers must comply with the FDA and USDA/FSIS regulations or those of equivalent organizations in other countries. Proper protection devices such as face masks or goggles, aprons, boots, and gloves must be used by employees while applying and removing the cleaning solutions. A complete procedure must be in place to define what kind, when, where, and how to use the cleaning and sanitizing solutions. Proper record keeping is required to avoid contamination of the products with detergent or sanitizer solutions. A complete layout of the facility, including details about location of utilities, location of equipment, and emergency exits, must be available. Changes in the configuration of the facility must be reflected in the layout.

33.2.5 Currently Used PEF Technology

PurePulse Technologies Co., a subsidiary of Maxwell Laboratories in San Diego, CA, owns three U.S. patents to preserve fluid foods such as dairy products, fruit juices, and fluid eggs by treatment with high-intensity electric discharges from about 5 to 100 kV/cm with flat-topped exponentially decaying pulse shapes. Pulse duration is controlled to prevent electrical breakdown of the food product; the typical duration is between 1 and 100 μs with repetition rates between 0.1 and 100 Hz [11,34]. The patents describe both a batch and continuous processing system and recommend that HIPEF treatments be applied to preheated liquid foods, which enhance microbial inactivation and shelf-life stability.

Dunn and Pearlman [11] reported more than five logarithmic cycles of microbial count reduction (5D reduction) of naturally occurring microorganisms in orange juice after 35 pulses of 100 μs at a voltage intensity of 33.6–35.7 kV/cm and a process temperature of 42°C–65°C. The shelf life of orange juice was increased from 3 days to 1 week with no significant change in odor or taste. A 3D reduction of *E. coli*

(ATCC-10536) inoculated in homogenized and pasteurized milk exposed to 23 pulses of 100 μs at 28.6–42.8 kV/cm was also reported. When a similar test run was carried out using milk seeded with *Salmonella dublin* prior to treatment with 36.7 kV/cm and 40 pulses of 100 μs at 63°C, no *Salmonella* and only 20 cfu/mL of milk bacteria was found. These results may suggest that deactivation from the PEF treatment process is selective and that *S. dublin* are preferentially deactivated over the milk bacteria. Yogurt inoculated with *Streptococcus thermophilus*, *Lactobacillus bulgaricus*, and *Saccharomyces cerevisiae* was treated with 20–100 μs pulses at 23–38 kV/cm at a process temperature of 63°C, resulting in a 2D reduction of the lactic acid bacteria and *S. cerevisiae* [11].

The *ELSTERIL* process, developed by Krupp Maschinentechnik GmbH (Hamburg, Germany) in the late 1980s and early 1990s, is used for the sterilization and pasteurization of liquid and electrically conductive media [13,35,36]. Krupp Maschinentechnik GmbH, in association with the University of Hamburg, reported microbial inactivation when PEF was applied to fluid foods such as orange juice and milk [36]. A microbial inactivation exceeding 4D has been found for *Lactobacillus brevis* inoculated in milk and treated with 20 pulses of 20 ps at 20 kV/cm, *S. cerevisiae* inoculated in orange juice and treated with 5 pulses of 20 μs at 4.7 kV/cm, and *E. coli* inoculated in sodium alginate and treated with 5 pulses of 20 μs at 14 kV/cm [35,36]. However, no inactivation of the endospores of *B. cereus* or the ascospores of *Bacillus nivea* was reported [36]. A substantial reduction in ascorbic acid and lipase activity was observed in milk treated with the *ESTERIL* process [36]. The taste of milk and orange juice did not significantly change after the electric field treatments [36].

The disruption of cell membranes to release fat from animal cells was conducted using a process called *ELCRACK* (Krupp Maschinentechnik GmbH, Hamburg, Germany). The *ELCRACK* process consists of the exposure of a slurry of comminuted fish or slaughterhouse offal to high-intensity electric pulses that break down cells, leading to increased fat recovery during the separation step after it is pumped through one or more treatment chambers [35]. Washington State University has a patent for the design and development of a static PEF chamber and has filed another for the design and development of a continuous PEF chamber intended for processing liquid foods with PEF treatments [37–40].

33.3 Applications of PEF in Food Processing

The application of PEF as a food processing tool is gaining popularity, since it represents a nonthermal alternative to conventional pasteurization and sterilization methods. The PEF approach, which does not involve the use of added preservatives, is expected to be more appealing to consumers who are skeptical about the use of chemicals in foods. Furthermore, the PEF treatment, being a nonthermal process, may also have no significant detrimental effect on heat-labile components present in foods such as vitamins. The major disadvantage of PEF operation is the initial investment. A pilot plant-size pulser may cost around $250,000. Other units for industrial use are available at prices that range from $450,000 to $2,000,000.

33.3.1 Inactivation of Microorganisms

Raw and reconstituted apple juice, peach juice, skim milk, beaten eggs, and pea soup exposed to PEFs of 25–45 kV/cm were treated using the chamber designed at Washington State University. *E. coli* inoculated in skim milk and exposed to 60 pulses of 2 μs width at 45 kV/cm and 35°C was reduced by 2D [25]. A reduction of 6D was observed in liquid egg inoculated with *E. coli* and treated with an electric field of 25.8 kV/cm and 100 pulses of 4 μs at 37°C [41]. *E. coli* and *B. subtilis* inoculated in pea soup and exposed to PEFs of 25–33 kV/cm (10–30 pulses of 2 μs) provided a limited inactivation ($<$1.5D) when the process temperature of pea soup was below 53°C, while microbial inactivation was 4.4D with process temperatures between 53°C and 55°C [26].

33.3.1.1 Simulated Milk Ultrafiltrate (SMUF)

The inactivation of *E. coli* varied as a function of the electric field intensity, number of pulses, and pH. Low-field intensity (20 kV/cm) resulted in insignificant inactivation of microorganisms independent of temperature and pH ($p > 0.05$). Meanwhile, inactivation of *E. coli* increased with an increase in the

number of pulses and an increase in the electric field from 40 to 55 kV/cm. The inactivation was more significant at pH 5.69 than at pH 6.82 ($p < 0.05$) (Figures 33.11 and 33.12). The temperature effect (10°C or 15°C) on the inactivation for these experiments was not statistically significant ($p > 0.05$). Table 33.2 summarizes the inactivation results after eight pulses for each of the experimental conditions.

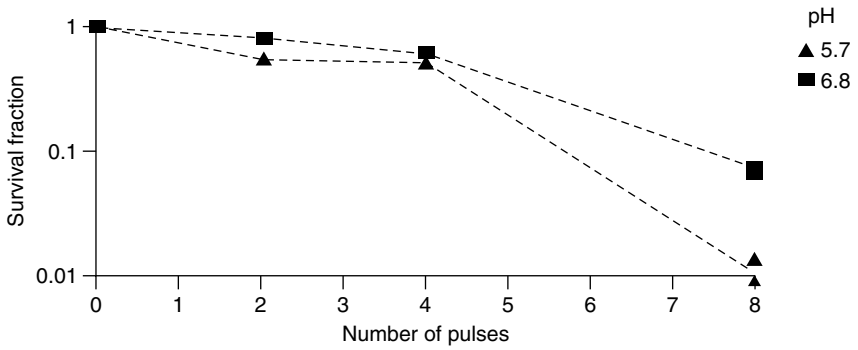

FIGURE 33.11 Inactivation of *E. coli* suspended in SMUF, using 40 kV/cm at 10°C, two samples per each experimental condition. (From H. Vega-Mercado et al., *Food Res. Int.* 29(2): 117, 1996.)

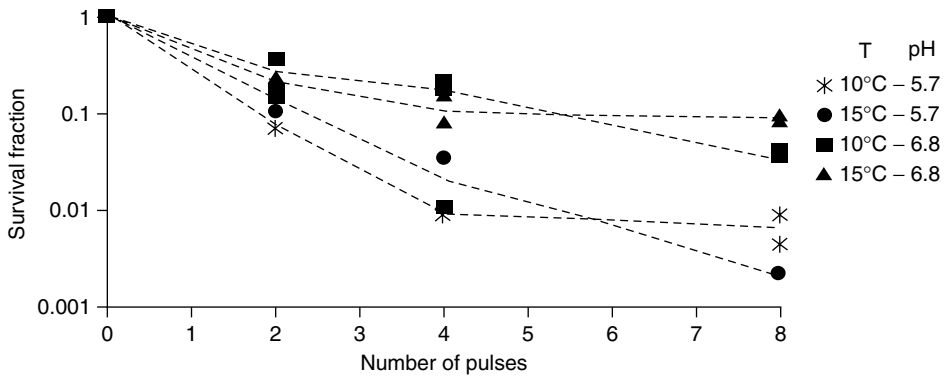

FIGURE 33.12 Inactivation of *E. coli* suspended in SMUF, using 55 kV/cm, two samples per each experimental condition. (From H. Vega-Mercado et al., *Food Res. Int.* 29(2): 117, 1996.)

TABLE 33.2

Effect of Processing Parameters on the Inactivation of *E. coli* Suspended in SMUF after Eight Pulses

		Number of Log Cycle Reduction	
Description	**pH**	**10°C**	**15°C**
20 kV/cm	5.7	0.00[a]	0.20[a]
	6.8	0.00[a]	0.06[a]
	5.7	1.95[b]	1.85[b]
	6.8	1.16[c]	1.00[c]
	5.7	2.22[d]	2.56[d]
	6.8	1.45[e]	1.10[c]

Note: Log cycle reduction data with similar superscripts are not significantly different at $\alpha = 0.05$, two samples per each experimental condition.
Source: H. Vega-Mercado et al., *Food Res. Int.* 29(2): 117 (1996).

The role of pH in the survival of microorganisms is related to the ability of the organisms to maintain the cytoplasm pH near neutrality [42]. Membrane permeability increases due to formation of pores in the cell wall during PEF treatment [30] and the rate of transport of hydrogen ions may also increase due to the osmotic imbalance around the cell. Thus, a reduction in cytoplasm pH may be observed because a higher number of hydrogen ions are available than at a neutral pH. The change in pH within the cell may induce chemical modifications in fundamental compounds such as DNA or ATP, as discussed by Wiggins [43] and Dolowy [44]. Also, oxidation and reduction reactions such as those proposed by Gilliland and Speck [18] may occur within the cell structure induced by the PEF treatment.

The ionic strength of the solution also plays an important role in the inactivation of *E. coli.* An increase in the ionic strength increases the electron mobility through the solution, resulting in a decrease in the inactivation rate. The reduced inactivation rate in high-ionic-strength solutions can be explained by the stability of the cell membrane when exposed to a medium with several ions [30]. The effect of ionic strength can be observed in Figure 33.13, where a difference of 2.5 log cycles was obtained between the 0.168 and 0.028 M solutions.

The growth stage of *E. coli* affected the effectiveness of PEF treatments (36 kV/cm at 7°C, two and four pulses). Cells in the logarithmic phase were most sensitive to the electric field treatments compared to cells in the stationary and lag phase (Figure 33.14) as reported by Pothakamury et al. [45]. Figures 33.15 and 33.16 present the effect of temperature on the log-cycle reduction of *E. coli* using exponentially decaying pulses and square wave pulses of 35 kV/cm. The rate of inactivation increases with an increase in the temperature. Coster and Zimmermann [46] suggested synergistic effects of high-intensity electric fields with moderate temperatures. The rate of inactivation increased when square wave pulses were used compared to exponentially decaying pulses. Similar results were reported for *S. aureus* when exposed to PEF at 9 and 16 kV/cm and *L. delbrueckii* and *B. subtilis* when exposed to 9, 12, and 16 kV/cm. Figures 33.17 through 33.19 present the reported results by Pothakamury et al. for *S. aureus*, *L. delbrueckii*, and *B. subtilis* suspended in SMUF.

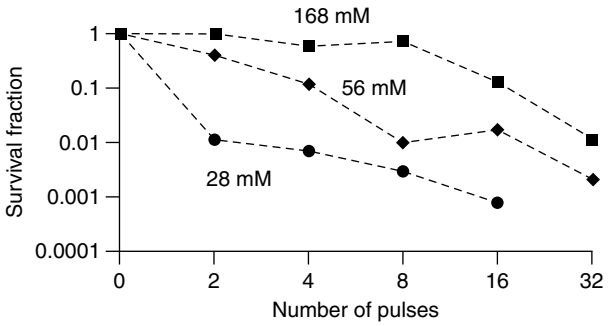

FIGURE 33.13 Effect of ionic strength on the inactivation of *E. coli* suspended in SMUF, at 40 kV/cm and 10°C, two samples per each experimental conditions. (From H. Vega-Mercado et al., *Food Res. Int.* 29(2): 117, 1996.)

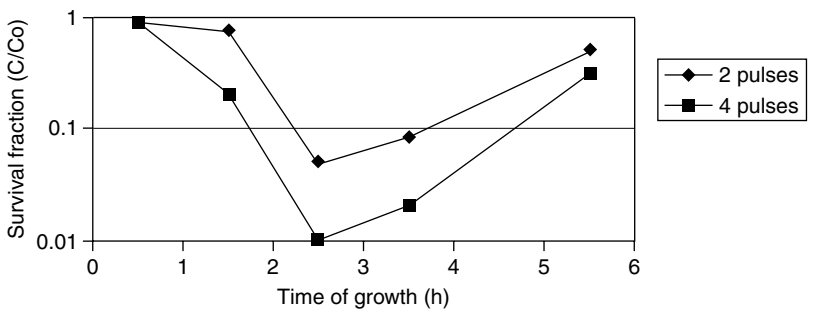

FIGURE 33.14 Effect of growth stage on the PEF inactivation of *E. coli* suspended in SMUF. (From U. R. Pothakamury et al., *Food. Res. Int.* 28(2): 167, 1995.)

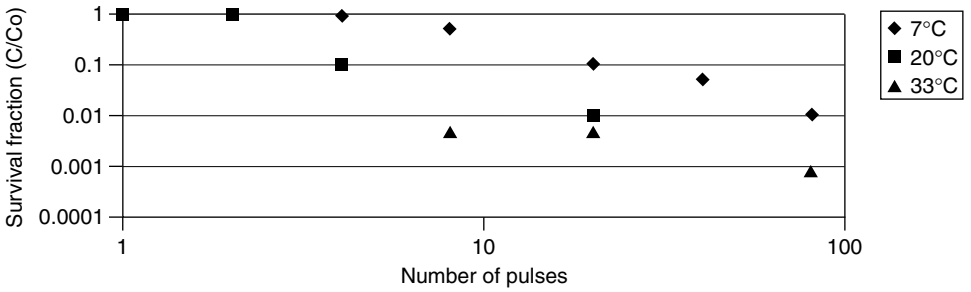

FIGURE 33.15 Effect of temperature on PEF inactivation of *E. coli* suspended in SMUF, using exponential decay pulses. (From U. R. Pothakamury et al., *Food. Res. Int.* 28(2): 167, 1995.)

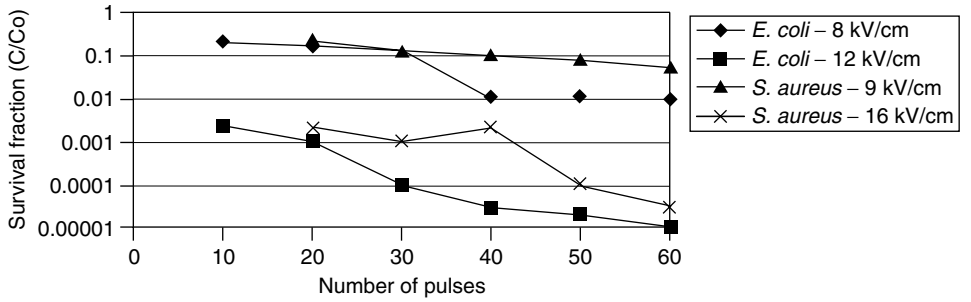

FIGURE 33.16 Effect of temperature on PEF inactivation of *E. coli* suspended in SMUF, using square wave pulses. (From U. R. Pothakamury et al., *Food. Res. Int.* 28(2): 167, 1995.)

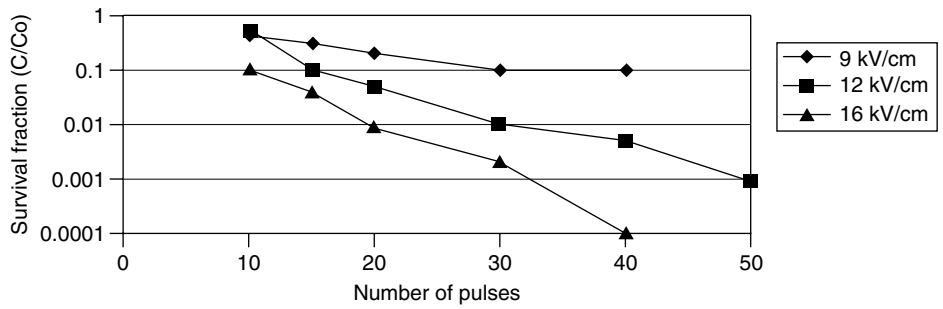

FIGURE 33.17 Inactivation of *E. coli* and *S. aureus* in SMUF by REF. Simplified circuit for exponential decay pulse generation and voltage trace of an exponential decay pulse. (From U. R. Pothakamury et al., *Span. J. Food Sci. Technol.* 35(1): 101, 1995.)

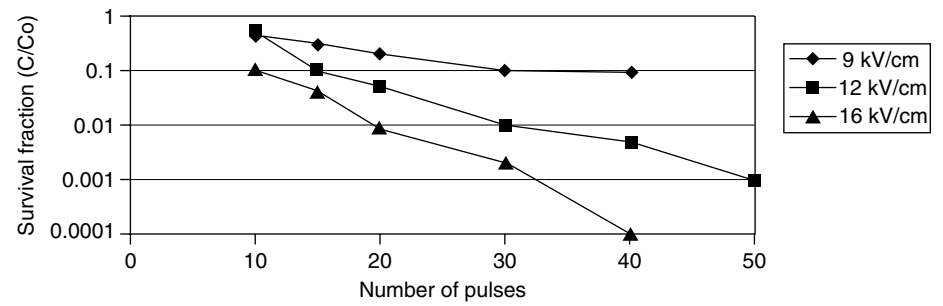

FIGURE 33.18 Inactivation of *L. delbrueckii* suspended in SMUF. (From U. R. Pothakamury et al., *Span. J. Food Sci. Technol.* 35(1): 101, 1995.)

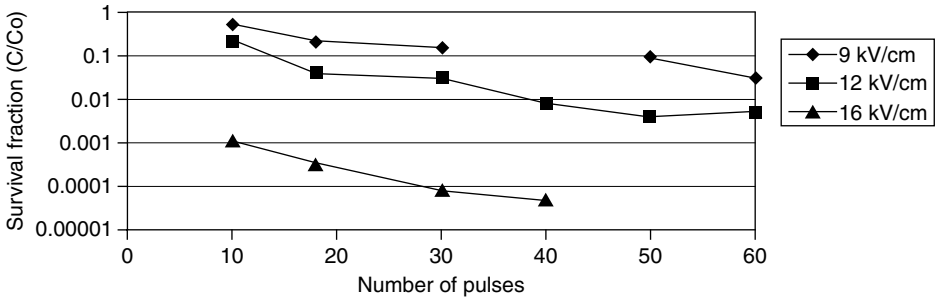

FIGURE 33.19 Inactivation of *B. subtilis* suspended in SMUF. (From U. R. Pothakamury et al., *Span. J. Food Sci. Technol.* 35(1): 101, 1995.)

TABLE 33.3

Inactivation of an *E. coli–B. subtilis* Mixture Suspended in Pea Soup Using PEF

		28 kV/cm		30 kV/cm	
Flow Rate Frequency	**Number of Pulses**	**Process Temperature**	**Log Reduction (D)**	**Process Temperature**	**Log Reduction (D)**
0.5 L/min	15	43	0.7	55	2.3
4.3 Hz	30	39	1.6	55	4.0
0.7 L/min	15	41	0.7	53	4.4
6.7 Hz	30	41	0.7	55	4.8
0.75 L/min	10	32	0.8	41	1.1
4.3 Hz	20	31	1.0	42	1.0

Source: H. Vega-Mercado et al., *J. Food Proc. Pres.* 20(6): 501 (1996).

33.3.1.2 Pea Soup

PEF inactivation of *E. coli* and *B. subtilis* suspended in pea soup depends on the electric field intensity, number of pulses, pulsing rate, and flow rate [49] (Table 33.3). The maximum bulk temperature of the peak soup achieved during the PEF treatment was 55°C and is a function of both flow rate and pulsing rate. PEF treatments with a bulk temperature below 53°C resulted in limited microbial inactivation (<1.64D). Microbial inactivation dependence on process temperature may be explained by changes in the sensitivity of the microorganisms to PEF when the temperature exceeds 53°C. Thermal inactivation of microorganisms was avoided by cooling treated pea soup to 20°C. Thermal inactivation of *E. coli* requires up to 10 min at 61°C when suspended in bouillon [50].

PEF inactivation of *B. subtilis* and *E. coli* decreased almost 2D when the microorganisms were mixed together in pea soup. Figures 33.20 through 33.22 summarize the inactivation of *E. coli*, *B. subtilis*, and the mixture of organisms suspended in pea soup and exposed to selected treatment conditions [49]. There is a significant difference in the inactivation levels ($p < 0.05$) between *E. coli* alone and *E. coli* mixed with *B. subtilis*. PEF inactivation of *E. coli* alone reached 6.5D after 30 pulses at 30 kV/cm and flow rate of 0.5 L/mm, while an inactivation of 4.0D was observed when *E. coli* was mixed with *B. subtilis*. *B. subtilis* alone had 5.0D when exposed to 33 kV/cm at 4.3 Hz and 0.5 L/mm, while only 2.0D were observed when mixed with *E. coli* and exposed to 20 pulses at 30 kV/cm, 4.3 Hz and 0.75 L/mm or 3.5D after 30 pulses. The results for the inactivation of *E. coli* and *B. subtilis* using PEF demonstrate the feasibility of the technology for preservation of foods containing suspended particles and gelatinized starch.

33.3.1.3 Liquid Eggs

High-intensity PEF (26 kV/cm) treatment in continuous flow systems (continuous recirculation and simple pass) inactivates *E. coli* inoculated in liquid egg 6D with a peak processing temperature of 37.2 ± 1.5°C

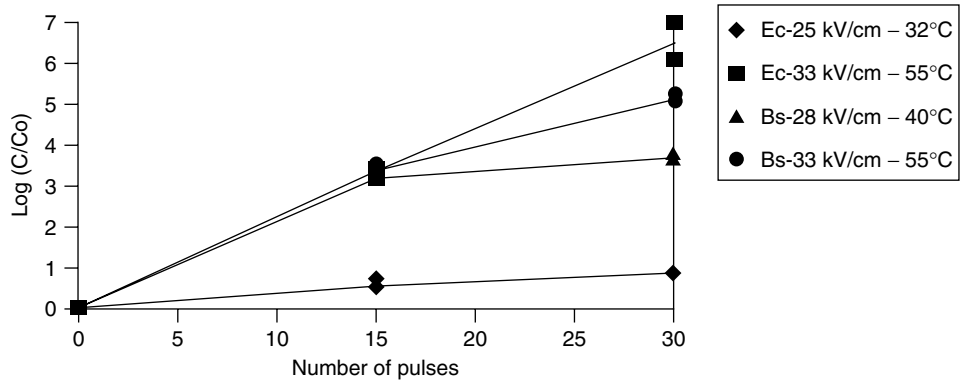

FIGURE 33.20 Inactivation of microorganisms suspended in pea soup using PEF at 0.5 L/min and 4.3 Hz (Ec: *E. coli*; Bs: *B. subtilis*). (From H. Vega-Mercado et al., *J. Food Proc. Pres.* 20(6): 501, 1996.)

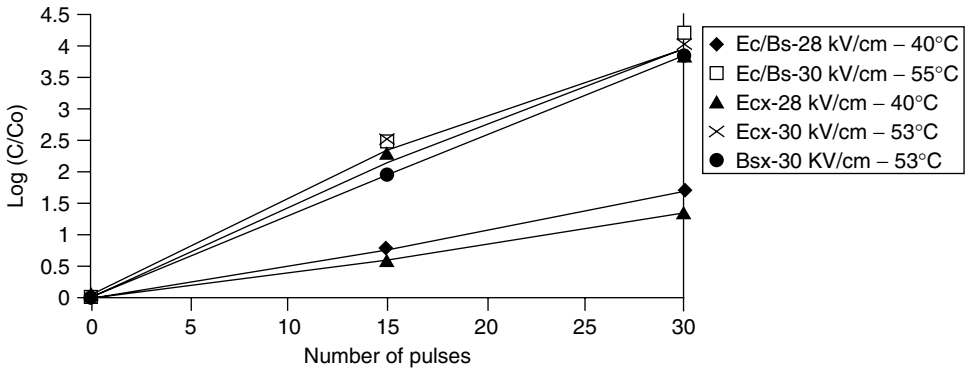

FIGURE 33.21 Inactivation of mixture of microorganisms suspended in pea soup using PEF at 0.5 L/min and 4.3 Hz (Ec: *E. coli*; Bs: *B. subtilis*; Ec/Bs: the overall inactivation for the mixture of microorganisms; Ecx: the inactivation of *E. coli* in the mixtures; Bsx: inactivation of *B. subtilis* in the mixture). (From H. Vega-Mercado et al., *J. Food Proc. Pres.* 20(6): 501, 1996.)

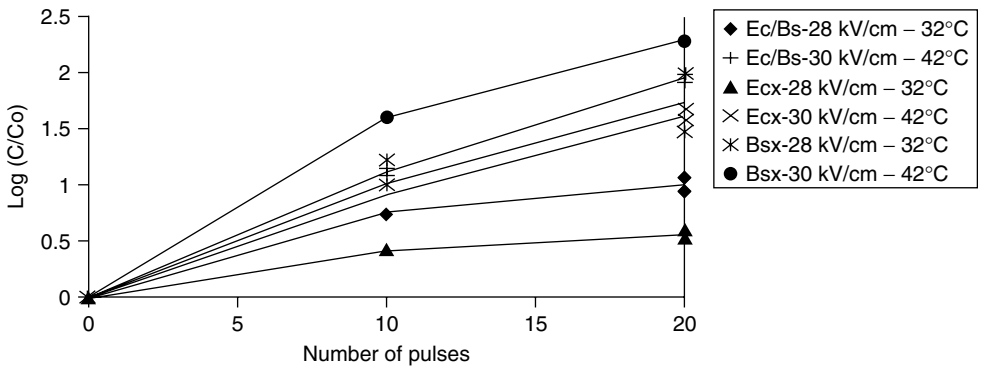

FIGURE 33.22 Inactivation of mixture of microorganisms suspended in pea soup using PEF at 0.75 L/min and 4.3 Hz (Ec: *E. coli*; Bs: *B. subtilis*; Ec/Bs: the overall inactivation for the mixture of microorganisms; Ecx: the inactivation of *E. coli* in the mixture; Bsx: the inactivation of *B. subtilis* in the mixture). (From H. Vega-Mercado et al., *J. Food Proc. Pres.* 20(6): 501, 1996.)

(Table 33.4 and Figures 33.23 and 33.24). PEF treatments with 4 μs pulses were more effective than 2 μs pulses (Figures 33.25 and 33.26), which may be explained by the amount of energy applied to the liquid egg [41]. Figure 33.27 illustrates the effect of energy input in the inactivation of *E. coli*, with energy input (in Joules) calculated as follows:

$$\frac{\text{Energy}}{\text{Pulse}} = 0.5CV^2$$

where C is the capacitance, 0.5 μF for 2 μs pulses and 1.0 μF for 4 μs pulses, and V the measured potential across the treatment chamber (15.6 kV). The total energy input (in Joules) after n pulses is calculated by:

$$\text{Total energy} = n^* \text{ energy/pulse}$$

The survival fraction of *E. coli* in liquid egg is reduced almost 6D with 12,000 J applied in pulses of 4 μs (Figure 33.26). Grahl et al. [36] nearly reached 5D by exposing *E. coli* suspended in sodium alginate to an electric field of 14 kV/cm with five pulses of 20 μs. Zhang et al. [51] observed a 6D reduction in

TABLE 33.4

Treatment Conditions for Liquid Egg Exposed to PEF

	Operating Conditions	
Description	**Treatment 1**	**Treatment 2**
Pulse duration (μs)	2	4
Capacitance (μF)	0.5	1
Input voltage (kV)	40	30
Input flow rate (L/min)	0.5	0.5
Input pulse rate (Hz)	1.25, 2.5	1.25, 2.5
Peak voltage (kV)	15.5	15.5
Peak current (kA)	8.0	8.0
Electric field intensity (kV/cm)	26	26
Pulse energy (J)	60	120
Maximum temperature (°C)	37	37

Source: O. Martin et al., *J. Food Proc. Pres.* 21: 193 (1997).

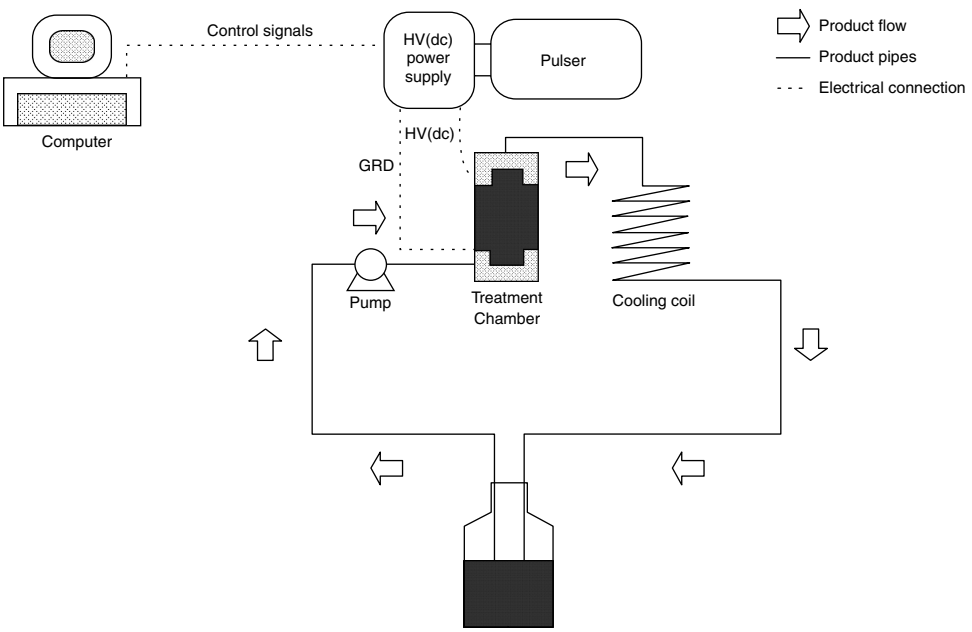

FIGURE 33.23 Continuous recirculation PEF operation.

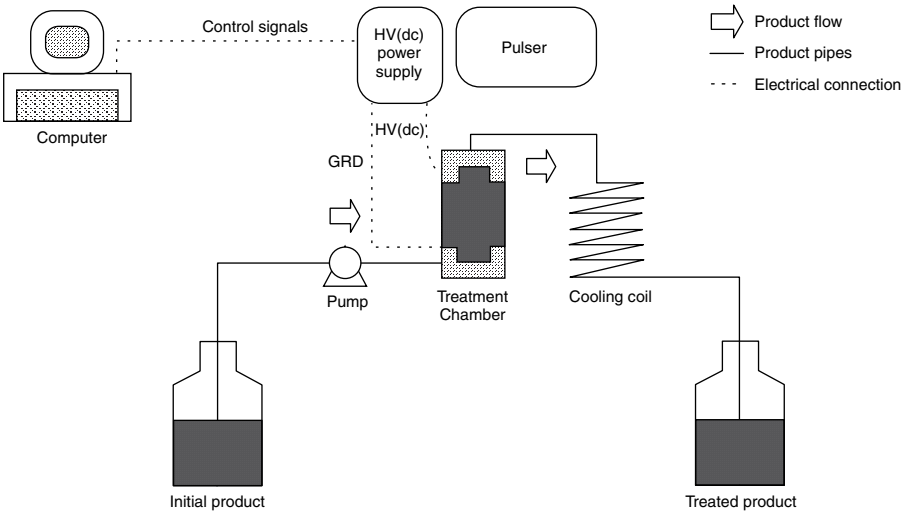

FIGURE 33.24 Single-pass PEF operation.

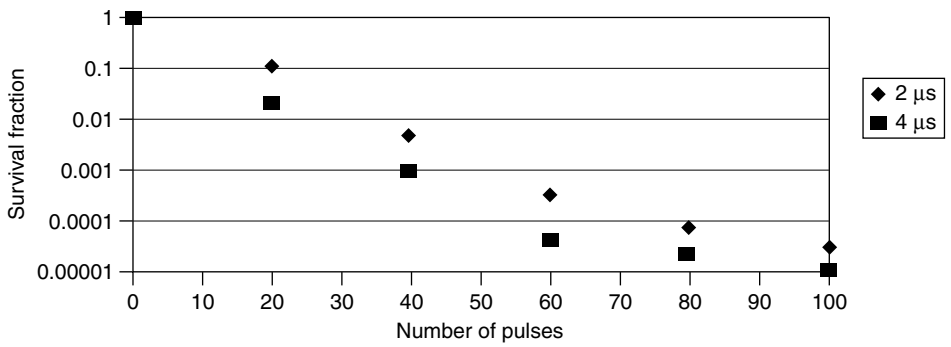

FIGURE 33.25 *E. coli* in liquid egg after PEF treatment at 26 kV/cm and 37°C in a continuous recirculation system. (From O. Martin et al., *J. Food Proc. Pres.* 21: 193, 1997.)

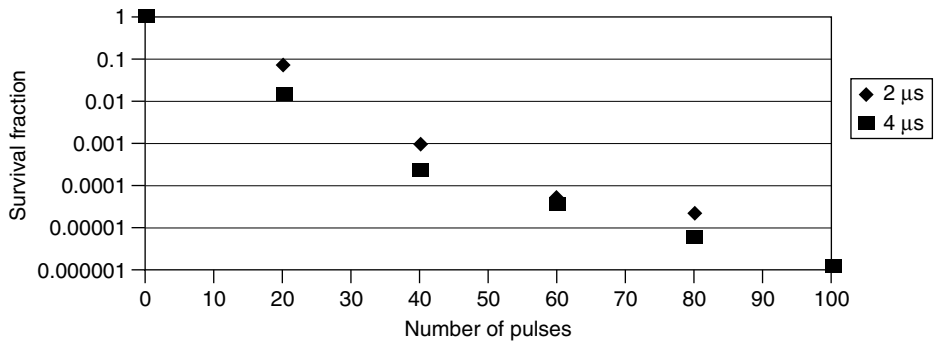

FIGURE 33.26 *E. coli* in liquid egg after PEF treatment at 26 kV/cm and 37°C in a stepwise system. (From O. Martin et al., *J. Food Proc. Pres.* 21: 193, 1997.)

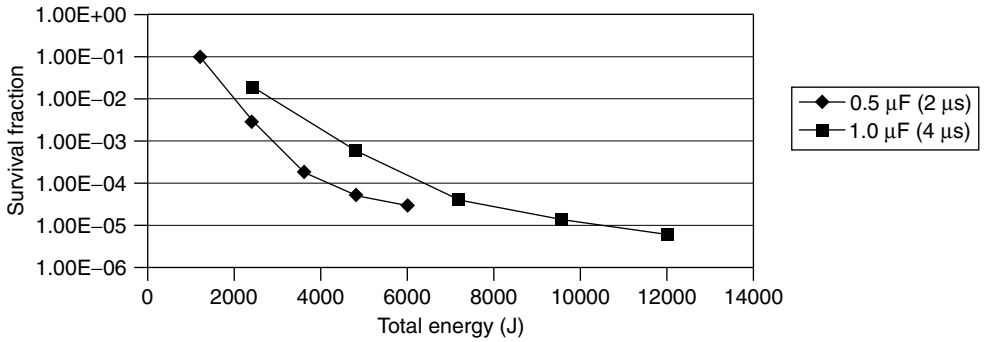

FIGURE 33.27 *E. coli* in liquid egg after PEF treatment at 26 kV/cm and 37°C as a function of input energy. (From O. Martin et al., *J. Food Proc. Pres.* 21: 193, 1997.)

E. coli suspended in potato dextrose agar and exposed to 64 pulses of 40 kV/cm at 15°C and a 9D reduction using 70 kV/cm and *E. coli* suspended in SMUF [52].

Proteins, an important nutrient for microbial growth, diminished the effectiveness of the PEF treatment [18,53]. The inactivation of microorganisms using PEF is more difficult in food materials than in buffer solutions [51]. In general, the bactericidal effect of PEF is inversely proportional to the ionic strength and increases with electric resistivity [5,26]. The electric resistance of liquid egg (1.9 Ω) is low compared to other foods and makes necessary the exposure of liquid egg to a large number (100) of pulses.

There was no significant difference ($p > 0.05$) in the effectiveness of PEF treatment when the pulse rate varied from 1.25 to 2.50 Hz, as the inactivation of *E. coli* in liquid egg was at least 4D if the number of pulses and pulse width remained constant. There was also no significant difference ($p > 0.05$) between the inactivation of *E. coli* using continuous recirculation or stepwise treatments.

33.3.1.4 Apple Juice

Commercial apple juice ultrafiltrated and exposed to different PEF treatments showed no changes in pH, acidity, vitamin C, glucose, fructose, and sucrose content [54] as summarized in Table 33.5. The inactivation of *S. cerevisiae* suspended in apple juice is affected by the intensity of the electric field, treatment time, and number of pulses [55,56]. Figure 33.28 illustrates the microbial count of *S. cerevisiae* as a function of peak field intensity when two pulses were used and the selected field intensities were 13, 22, 35, and 50 kV/cm. The rate of inactivation increases with an increase in field intensity [56]. Microbial inactivation is a function of the number of pulses as illustrated in Figure 33.29. An inactivation of 6D is reported after 10 pulses of 35 kV/cm at 22°C–34°C. The shelf life of PEF-treated apple juice increases over 3 weeks when stored at either 4°C or 25°C as illustrated in Figure 33.30.

33.3.1.5 Skim Milk

33.3.1.5.1 Treatment in a Static Chamber System

PEF treatment inactivates *E. coli* in skim milk at 15°C. The principal parameters influencing the microbial inactivation are the applied electric field intensity and treatment time, which can be expressed by the number of pulses (n) when the width of each pulse is fixed [15]. The *E. coli* survival fraction decreases when milk is treated with an increasing number of pulses at a constant field intensity (Figure 33.31). The rate of inactivation of *E. coli* increases with an increase in the electric field intensity at a constant number of pulses (Figure 33.32). Less than 1 log reduction in *E. coli* population was observed for PEF treatments of 20, 25, and 30 kV/cm and 64 pulses at 15°C. However, PEF treatments at 45 kV/cm, 64 pulses, and 15°C lead to a nearly 3 log cycle reduction [57]. The reported results are consistent with those of Dunn and Pearlman [11], but these authors mentioned that the treatment temperature increased up to 43°C.

Similar *E. coli* inactivation was obtained with 20 kV/cm PEF in saline solution [58]. Hulsheger et al. [21] reduced the population of 4 log cycles by applying 20 kV/cm PEF for *E. coli* inoculated in phosphate buffer, and Grahl et al. [36] reached a nearly 5 log cycle reduction by treating *E. coli*

TABLE 33.5

Apple Juice Chemical Properties before and after PEF

Sample	pH	Acidity (Malic Acid)	Vitamin C (mg/100 g)	Glucose	Fructose	Sucrose
Control	4.10 ± 0.02	2.63 ± 0.02	1.15 ± 0.01	2.91 ± 0.33	4.95 ± 0.64	2.18 ± 0.25
PEF-T1	4.36 ± 0.03	2.67 ± 0.02	1.02 ± 0.02	2.87 ± 0.06	4.96 ± 0.11	2.25 ± 0.06
REF-T2	4.18 ± 0.01	2.75 ± 0.07	1.12 ± 0.00	3.01 ± 0.34	5.08 ± 0.67	2.21 ± 0.31
REF-T3	4.09 ± 0.01	2.63 ± 0.02	1.02 ± 0.00	2.90 ± 0.09	4.89 ± 0.13	2.13 ± 0.06
REF-T4	4.23 ± 0.01	2.61 ± 0.00	1.15 ± 0.24	2.57 ± 0.25	4.33 ± 0.47	2.43 ± 0.13

Note: The data presented are average values of two experiments each carried out in duplicate.
Source: M. V. Simpson et al., Influence of PEF on the composition of apple juice, Internal Report, Washington State University, Pullman, WA, 1995.

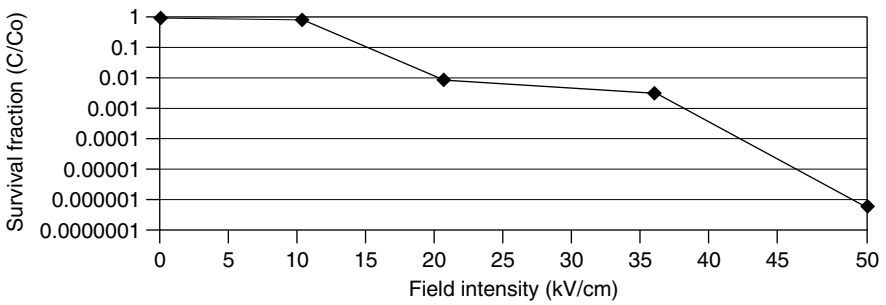

FIGURE 33.28 Survival fraction of *S. cerevisiae* as a function of peak field intensity when two 2.5 μs pulses were applied. (From B. L. Qin et al., *Proceedings of IEEE/IAS Meeting*, Orlando, FL, October 1995.)

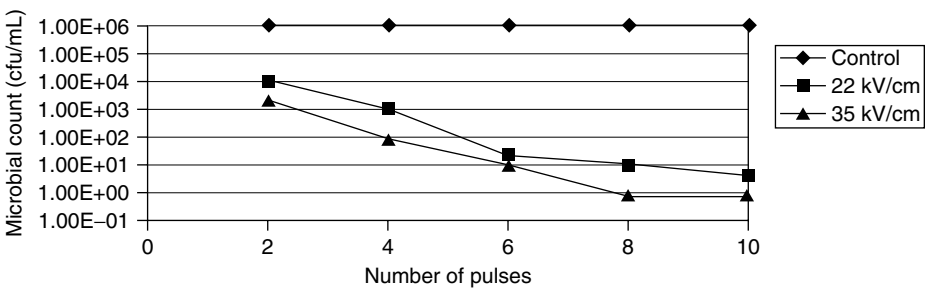

FIGURE 33.29 Microbiological count of *S. cerevisiae* in apple juice as a function of the number of 2.5 μs pulses. (From B. L. Qin et al., *Proceedings of IEEE/IAS Meeting*, Orlando, FL, October 1995.)

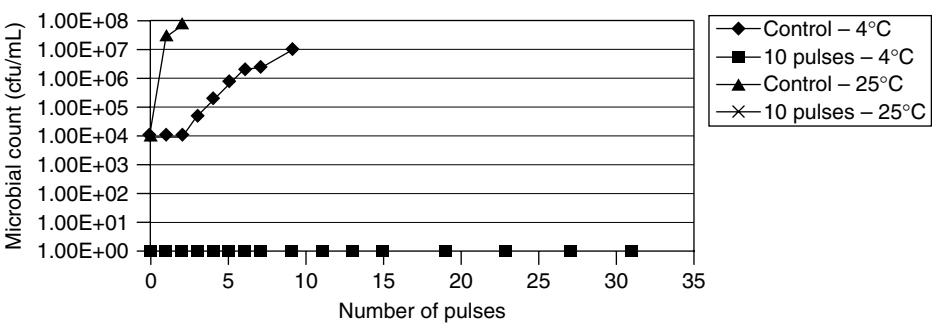

FIGURE 33.30 Shelf life of apple juice after PEF treatment of 10 2.5 μs pulses at 36 kV/cm. (From B. L. Qin et al., *Proceedings of IEEE/IAS Meeting*, Orlando, FL, October 1995.)

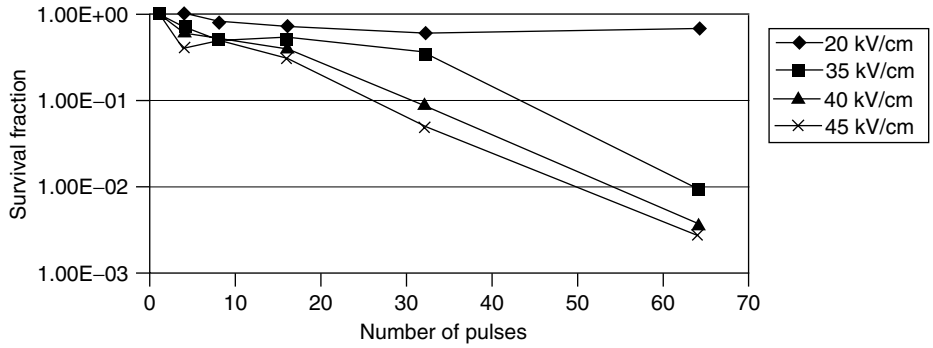

FIGURE 33.31 Inactivation of *E. coli* in skim milk at 15°C in a static chamber at several field intensities. (From O. Martin et al., *J. Food. Proc. Eng.*, 20: 317–336, 1997.)

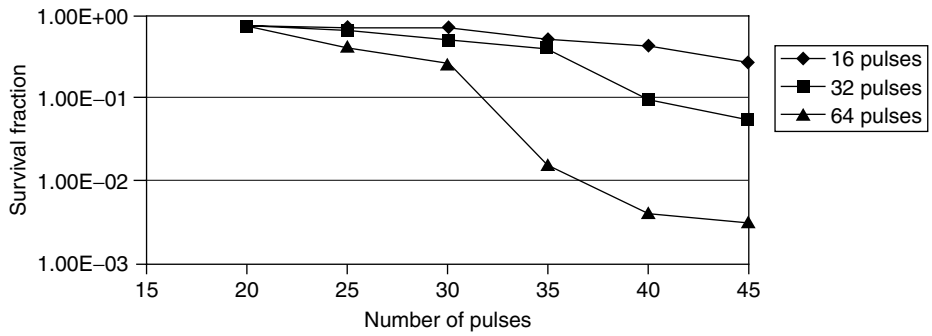

FIGURE 33.32 Inactivation of *E. coli* in skim milk at 15°C in a static chamber with different number of pulses. (From O. Martin et al., *J. Food. Proc. Eng.*, 20: 317–336, 1997.)

TABLE 33.6

Kinetics Constant of Hulsheger's Model for *E. coli* Inactivation in Skim Milk by PEF[a]

Electric Field Intensity (kV/cm)	Number of Pulses (n)	n_{min}	E_c (kV/cm)	K (kV/cm)	R^2
35	<64	15.2	—	5.6	0.829
40	<64	13.0	—	6.1	0.958
45	<64	11.0	—	8.0	0.985
<45	16	—	18.7	2.9	0.833
<45	32	—	20.4	3.9	0.861
<45	64	—	19.9	2.7	0.924

Note: R^2=correlation coefficient for regression analysis (p=0.05).

[a] Treatment in astatic chamber.

Source: O. Martin et al., *J. Food Proc. Eng.*, 20: 317–336, 1997.

suspended in sodium alginate solution with 26 kV/cm PEF. The inactivation of *E. coli* in potato dextrose agar by applying 64 pulses of 40 kV/cm at 15°C resulted in a 6 log cycle reduction. Notice that PEF inactivation kinetics in semisolid products are different from the PEF inactivation kinetics in fluids because *E. coli* cells are fixed in a gel matrix, which increases uniformity of inactivation [51]. Inactivation of *E. coli* in skim milk by PEF treatment in a static chamber satisfied Hulsheger's model (Table 33.6) because the destruction of this microorganism in skim milk followed a first-order kinetic for both the electric field intensity and the number of pulses.

Martin et al. [57] reported that the minimum number of pulses (n_{min}) necessary to inactivate the microorganisms in skim milk at 45 kV/cm using a static chamber is 11 and 15 pulses at 35 kV/cm, respectively. The critical electric field (E_c) is 19.9 kV/cm with 64 pulses at 45 kV/cm, which is higher than the value reported by Grahl et al [36] for *E. coli* suspended in sodium alginate solution (14 kV/cm). Zhang et al. [51] calculated 17.5 kV/cm E_c for *E. coli* in semisolid model foods.

It is more difficult to reduce the survival fraction of microorganisms present in skim milk than in buffer solutions and model foods because the composition of skim milk is complex (i.e., high protein content 33–40 g/L) [59]. These substances diminish the lethal effect of PEF in microorganisms because they absorb free radicals and ions, which are active in the breakdown of cells [18,53]. Moreover, the inactivation of bacteria by PEF is a function of solution resistance, which is inversely proportional to ionic strength. Survival fractions decrease when medium resistance increases and ionic strength decreases [26,60]. The measured resistivity of skim milk is 310 Ω cm and that of buffer solutions is even higher. Since dilution of milk increases the resistivity and decreases protein concentration, the effectiveness of PEF treatment is improved. The inactivation rate of *E. coli* suspended in skim milk/water (1:2.3) and exposed to 40 kV/cm in a static chamber at 15°C is higher than when less diluted skim milk (1:1) is used (Figure 33.33).

33.3.1.5.2 Treatment in a Continuous System

PEF treatment in a continuous flow chamber also inactivates *E. coli* inoculated in skim milk. An increase in field intensity or number of pulses produces greater bacterial inactivation (Figures 33.34 and 33.35) and microorganism death follows first-order kinetics with both field intensity and number of pulses

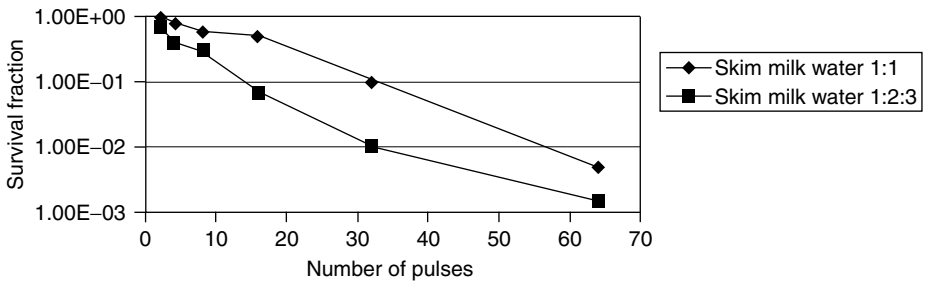

FIGURE 33.33 Effect of skim milk dilution in the inactivation of *E. coli* by 35 kV/cm PEF treatment in a static chamber at 15°C. (From O. Martin et al., *J. Food. Proc. Eng.*, 20: 317–336, 1997.)

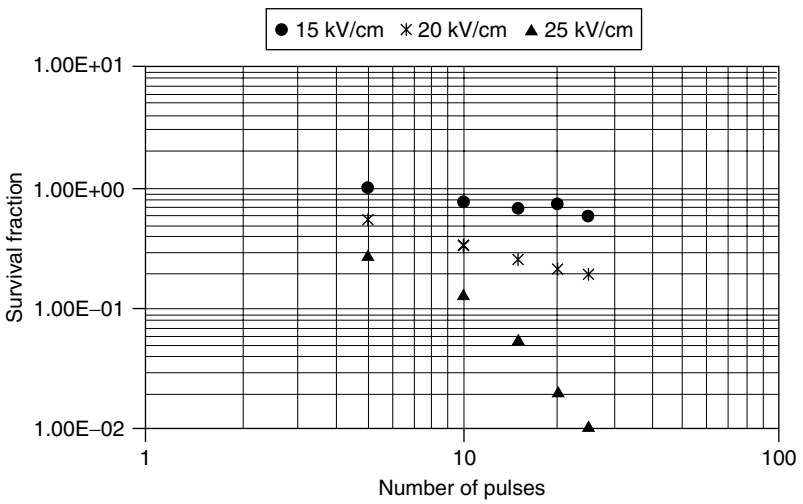

FIGURE 33.34 Inactivation of *E. coli* in skim milk at 15°C in a continuous chamber at different intensities. (From O. Martin et al., *J. Food. Proc. Eng.*, 20: 317–336, 1997.)

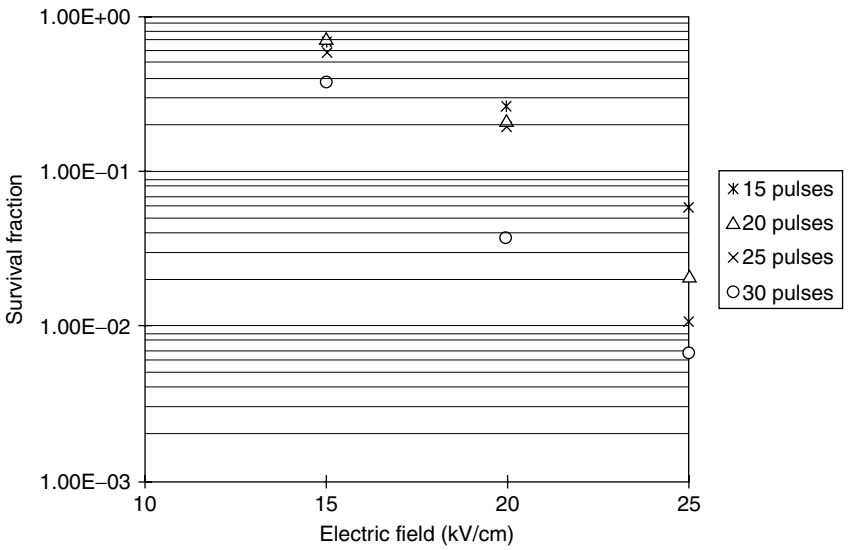

FIGURE 33.35 Inactivation of *E. coli* in skim milk at 15°C in a continuous chamber with different number of 1.8 ms pulses. (From O. Martin et al., *J. Food. Proc. Eng.*, 20: 317–336, 1997.)

TABLE 33.7

Kinetics Constant of Hulsheger's Model for *E. coli* Inactivation in Skim Milk by PEF[a]

Electric Field Intensity (kV/cm)	Number of Pulses (n)	n_{min}	E_c (kV/cm)	K (kV/cm)	R^2
15	<30	5.4	—	3.9	0.918
20	<30	1.9	—	9.5	0.997
25	<30	2.7	—	5.8	0.955
<30	15	—	13.82	4.3	0.985
<30	20	—	14.62	2.2	0.968
<30	25	—	14.44	2.2	0.938
<30	30	—	12.34	3.5	0.992

Note: R^2=correlation coefficient for regression analysis (p=0.05).

[a] Treatment in astatic chamber.

Source: O. Martin et al., *J. Food. Proc. Eng.*, 20: 317–336, 1997.

(Table 33.7). The E_c when PEF treatment was carried out in a continuous system at 30 kV/cm maximum electric field intensity was between 12.34 and 14.62 kV/cm, and n_{min} ranged from 1.9 to 5.4 pulses. These values were lower than those obtained in the same treated product using the static system.

In general, PEF treatment in continuous systems is more effective in terms of microorganism inactivation than in static systems due to the treatment uniformity being greater. Moreover, in this study even though both chambers are of the parallel plate type, the treatment volume in a static chamber is higher (14.5 mL) than the continuous flow chamber (8 mL). Therefore, the energy density (defined as energy divided by volume) is higher in continuous systems.

The effectiveness of PEF treatment also depends on pulse duration, which increases the *E. coli* inactivation because the energy applied in each pulse is higher. Applying 25 pulses of 0.7μs each at 25 kV/cm in a continuous flow chamber reduces the survival fraction of *E. coli* inoculated in skim milk less than 1 log cycle, but a treatment in the same chamber with the same number of pulses and field intensity and a 1.8 μs duration pulse reduces the survival fraction by more than 2 log cycles (Figure 33.36).

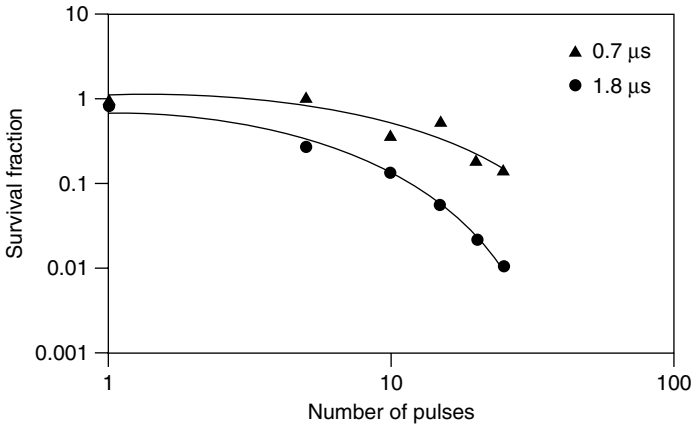

FIGURE 33.36 Effect of pulse duration in the inactivation of *E. coli* in skim milk at 15°C by 25 kV/cm PEF treatment in a continuous chamber. (From O. Martin et al., *J. Food. Proc. Eng.*, 20: 317–336, 1997.)

33.3.2 Denaturation of Proteins

33.3.2.1 Alkaline Phosphatase

The activity of alkaline phosphatase (ALP) in pasteurized milk products has public health significance, since the presence of active ALP indicates inadequate pasteurization or cross-contamination with raw milk [61]. In fresh raw milk, ALP is present in association with the membrane of fat globules; in skim milk it is in the form of lipoprotein particles.

The inactivation of ALP by PEF is a function of the field intensity, the fat content of the milk, and the concentration of ALP. The activity of ALP decreases with an increase in field intensity [61]. A reduction of 43%–59% in ALP activity is reported when the enzyme is suspended in 2% milk and exposed to 70 pulses of 0.40–0.45 ms at 14.8–18.8 kV/cm (Figure 33.37). Seventy pulses of 0.74 ms of a field strength of 22 kV/cm applied to 2 mg/mi ALP in SMUF reduced the ALP activity by 65% (Figure 33.38). The activity of ALP dissolved in UHT-pasteurized 2 and 4% milk was reduced by 59% when exposed to 70 pulses of 0.40 ms at 18.8 kV/cm, while a 65% reduction was observed in nonfat milk as illustrated in Figure 33.39. ALP suspended in milk (1 mL of raw milk in 100 mL of 2% milk) using 13.2 kV/cm and 43.9°C after 70 pulses showed a reduction of 96% in activity, whereas heat treatment at 43.9°C for 17.5 min showed only a 30% reduction (Figure 33.40). Castro [61] demonstrated a reduction in initial velocity of fluoroyellow production of ALP as a function of number of pulses, as illustrated in Figure 33.41. Castro also found that PEF-treated ALP is more susceptible to trypsin proteolysis (70 pulses of 0.78 ms at 22.3 kV/cm), as illustrated in Figure 33.42. The inactivation of ALP is attributed to conformational changes induced by PEF [31,61].

33.3.2.2 Plasmin and a Protease from Pseudomonas fluorescens M3/6

The proteolytic enzyme plasmin and a protease from *Pseudomonas fluorescens* M3/6 were also inactivated using PEFs. A 90% inactivation of plasmin activity was observed during 30 and 45 kV/cm, 10–50 pulses of 2 µs duration, and a process temperature of 10°C and 15°C [61] as presented in Figures 33.43 and 33.44. Meanwhile, 80% inactivation was found for a protease extracted from *P. fluorescens* when dispersed in Triptych soy broth and exposed to 20 pulses of 2 µs at 11–18 kV/cm and 20°C–24°C. A 60% inactivation was detected when inoculated in sterilized skim milk and exposed to 98 pulses of 2 µs at 15 kV/cm and 50°C (Figure 33.45); no inactivation was detected when inoculated in a sterilized casein–Tris buffer and exposed to a PEF treatment similar to that for skim milk. The decreased effectiveness of PEF in the inactivation of the protease in skim milk and the casein–Tris

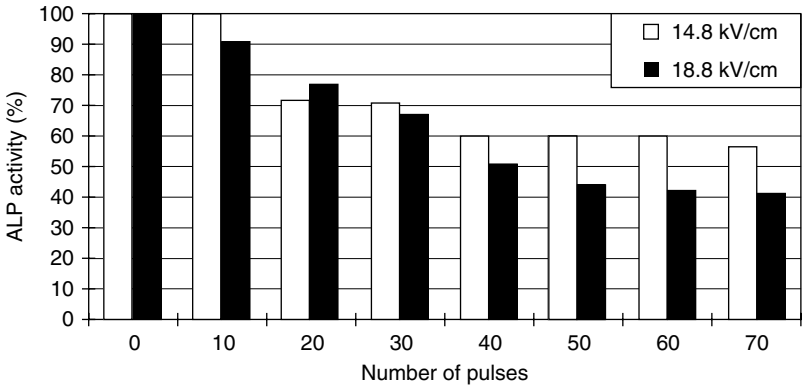

FIGURE 33.37 PEF inactivation of ALP diluted in UHT pasteurized 2% milk. (From A. J. Castro, Ph.D. thesis, Washington State University, Pullman, WA, 1994.)

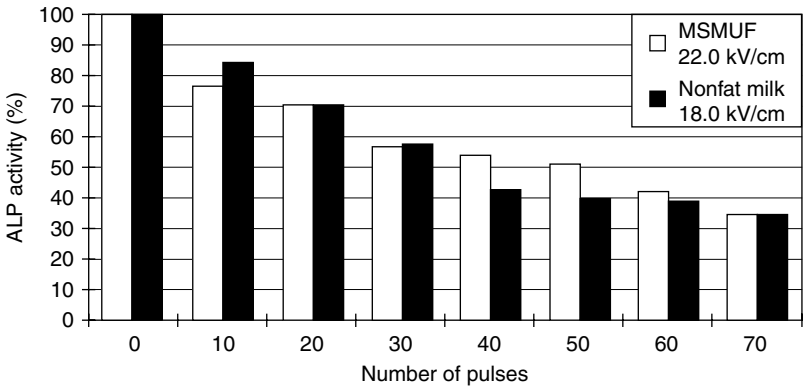

FIGURE 33.38 PEF inactivation of ALP diluted in MSMUF or nonfat milk. (From A. J. Castro, Ph.D. thesis, Washington State University, Pullman, WA, 1994.)

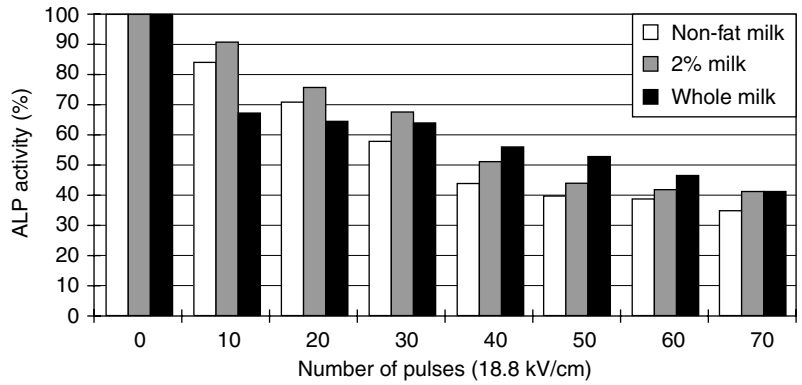

FIGURE 33.39 PEF inactivation of ALP diluted in UHT pasteurized nonfat, 2% and whole milk. (From A. J. Castro, Ph.D. thesis, Washington State University, Pullman, WA, 1994.)

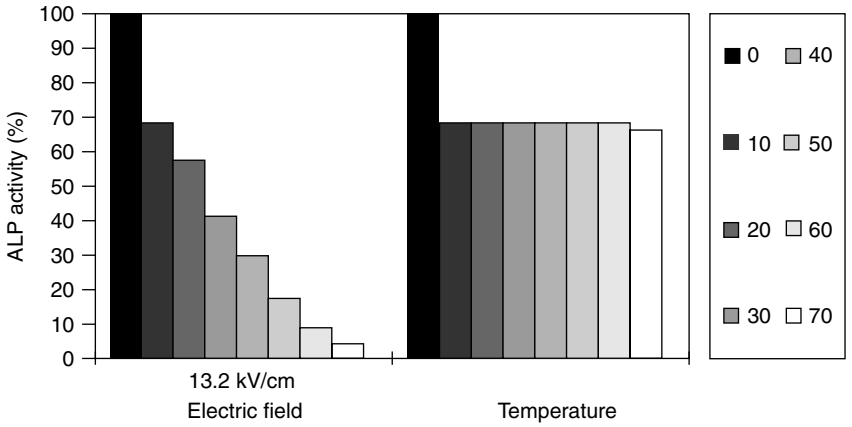

FIGURE 33.40 Inactivation of alkaline phosphatase by PEF or heating at 44°C for 17.5 min. (From A. J. Castro, Ph.D. thesis, Washington State University, Pullman, WA, 1994.)

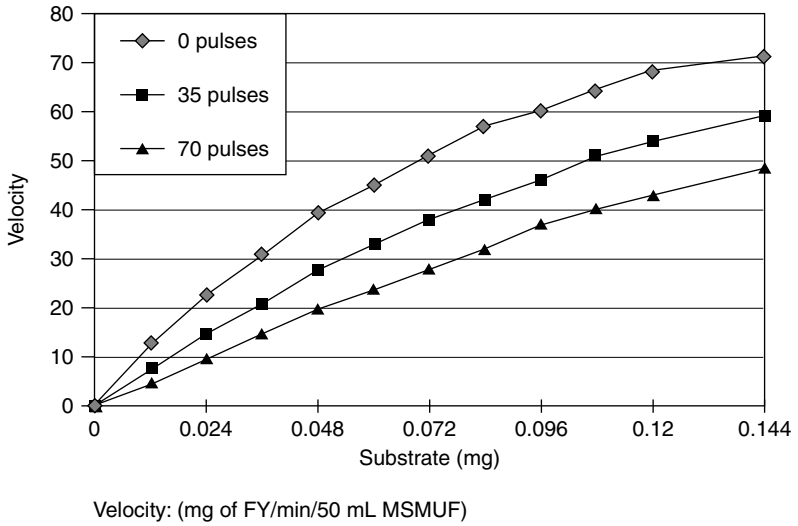

FIGURE 33.41 Initial velocity of fluoroyellow (FY) producing reaction of ALP in MSMUF treated with 0.78 ms pulses of 22.3 kV/cm.

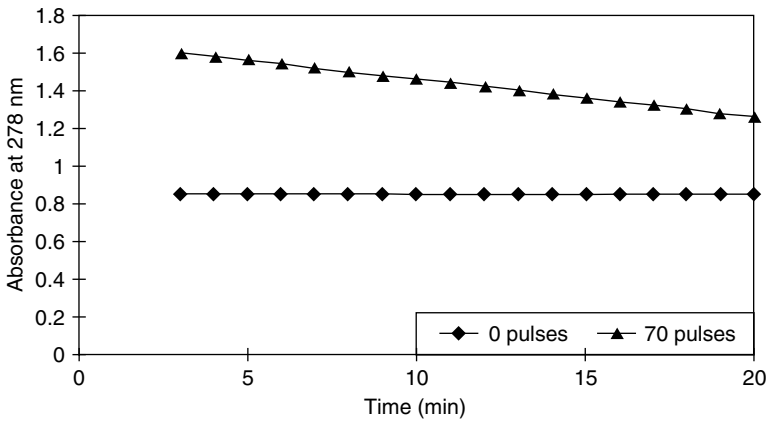

FIGURE 33.42 Trypsin digestion of native and PEF-treated alkaline phosphatase. (From A. J. Castro, Ph.D. thesis, Washington State University, Pullman, WA, 1994.)

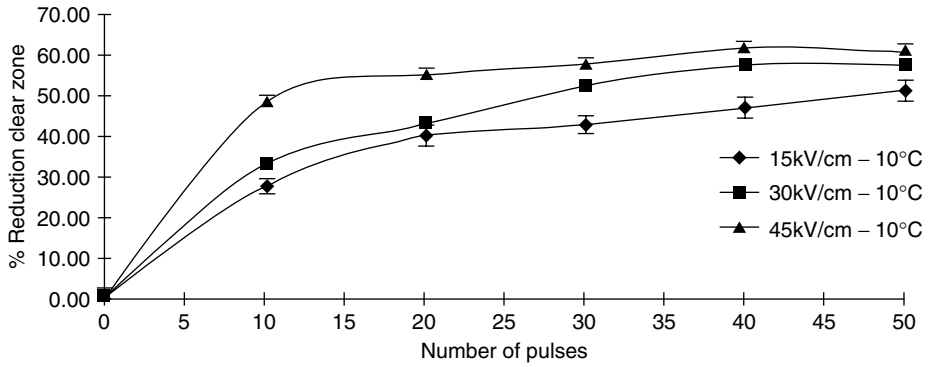

FIGURE 33.43 PEF inactivation of plasmin at 10°C. (From H. Vega-Mercado et al., *J. Food Sci.* 60: 1143, 1995.)

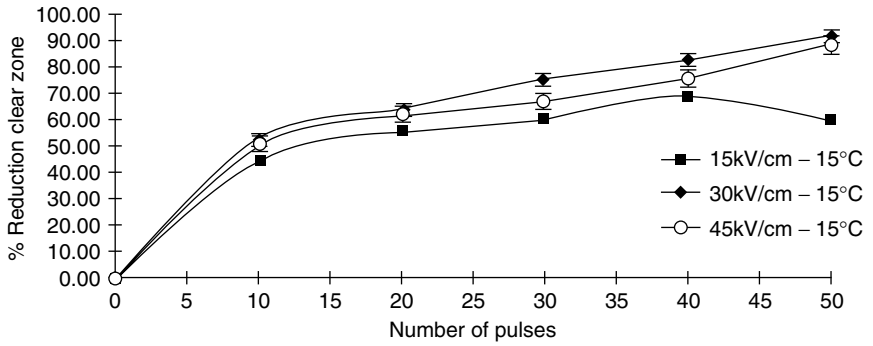

FIGURE 33.44 PEF inactivation of plasmin at 15°C. (From H. Vega-Mercado et al., *J. Food Sci.* 60: 1143, 1995.)

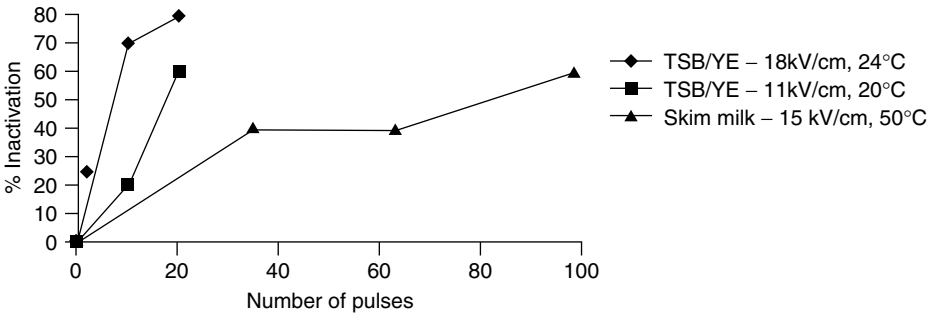

FIGURE 33.45 Inactivation of a protease from *P. fluorescens* M3/6 in Triptych soy broth enriched with yeast extract (TSB/YE, pulsing rate of 0.25 Hz) and skim milk (pulsing rate 2 Hz) using 2 μs pulses. (From H. Vega-Mercado et al., *Proceedings of ICEF7, Seventh International Congress on Engineering and Food*, The Brighton Center, Brighton, UK, 13–17 April, 1997, p. C73.)

buffer may be attributed to a protective role of the substrate (i.e., casein) against conformational changes of the enzyme induced by the electric fields [63].

The susceptibility of casein to proteolysis varies as a function of treatment conditions [63]; a HIPEF treatment of 25 kV/cm at 0.6 Hz and 30°C was found to increase the proteolytic activity in skim milk inoculated with a protease from *P. fluorescens* M3/6. However, 14 or 15 kV/cm at 1 or 2 Hz and 30°C

had no significant effect on the susceptibility of casein in skim milk proteolysis, and no significant change was observed in the susceptibility of casein suspended in a casein–Tris buffer when exposed to treatment conditions similar to those for skim milk [63].

The inactivation of the protease from *P. fluorescens* M3/6 when exposed to PEF does not depend on the presence of calcium in the media containing the protease (Figure 33.46). The inactivation is the same for the three solutions containing 0, 10, or 15 mM calcium. The proteolytic activity of the protease was reduced 30% after exposure to 20 pulses of 700 μs at 6.2 kV/cm and 15°C–20°C [64].

In contrast to PEF, thermal inactivation of the protease suspended in SMUF does vary with calcium content. Heated samples containing either 10 or 15 mM calcium retained 71% of the original activity compared to 12% retention on samples without calcium after 5 min of heating, followed by a steady decrease in activity as a function of the heating time (Figure 33.47). The analysis by HPLC using the hydrophobic interaction column (HIC) of PEF (20 pulses, 15 mM Ca^{2+}) and heat-treated (5 min, 15 mM Ca^{2+}) samples showed differences in the retention time and high peak of the eluted protein when compared to nontreated samples (Table 33.8). EDTA has a significant inhibitory effect on the proteolytic activity of the protease (Figure 33.48). This result is similar to reported data for the protease from *P. fluorescens*. PEF treatment of samples containing EDTA enhanced the inactivation of the protease in SMUF (Figure 33.49).

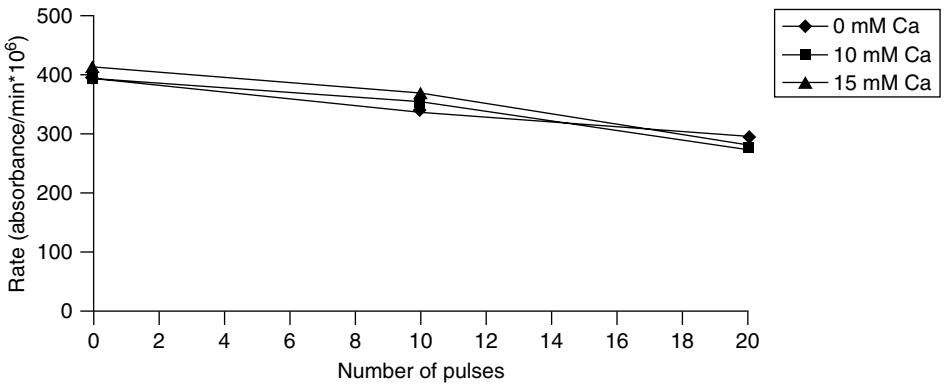

FIGURE 33.46 PEF inactivation of protease from *P. fluorescens* M3/6 at 6.2 kV/cm. (From H. Vega-Mercado, Ph.D. thesis, Washington State University, Pullman, WA, 1996.)

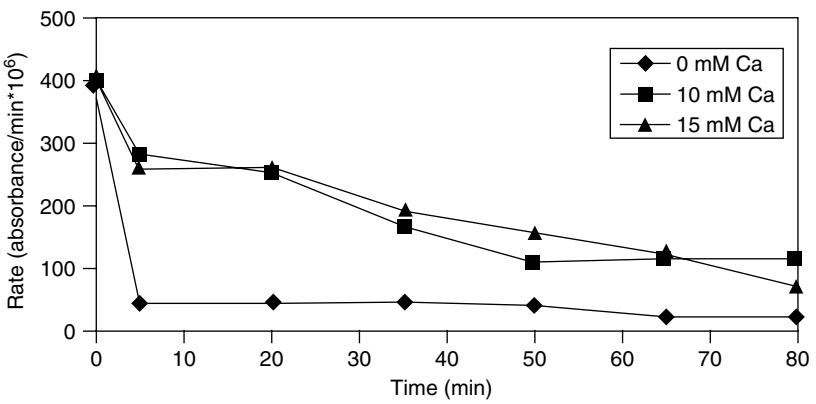

FIGURE 33.47 Thermal inactivation of protease from *P. fluorescens* M3/6. (From H. Vega-Mercado, Ph.D. thesis, Washington State University, Pullman, WA, 1996.)

TABLE 33.8

Hydrophobic Changes of Protease Suspended in SMUF Induced by PEF and Thermal Treatments

Sample	Retention Time (min)	Peak (mm)
Control	6.01	22.9
20 pulses[a]	5.96	25.4
Heat-treated[a]	5.93	20.6

[a] 15 M Ca^{2+}.

Source: H. Vega-Mercado, Ph.D. thesis, Washington State University, Pullman, WA, 1996.

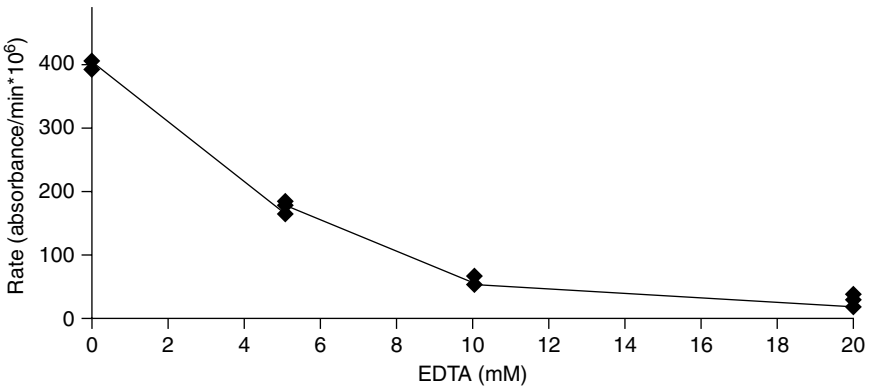

FIGURE 33.48 Inhibitory effect of EDTA on a protease from *P. fluorescens* M3/6. (From H. Vega-Mercado, Ph.D. thesis, Washington State University. Pullman, WA, 1996.)

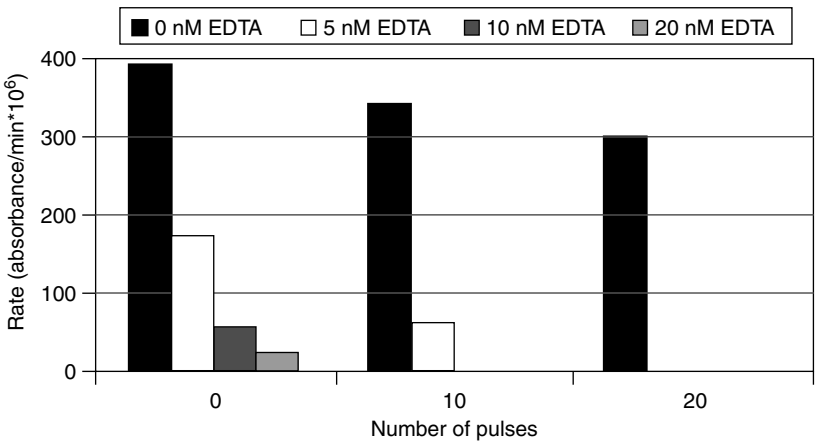

FIGURE 33.49 PEF inactivation of a protease from *P. fluorescens* M#/6 in SMUF with EDTA. (From H. Vega-Mercado, Ph.D. thesis, Washington State University, Pullman, WA, 1996.)

33.4 Final Remarks

The research on PEFs as a nonthermal process needs to include not only the inactivation of microorganisms, but the inactivation of enzymes, retention of vitamins, and the effect of PEF treatments on other food components. The reported inactivation of enzymes, as well as the increased proteolysis of casein

following exposure to PEF, suggests that detailed research is needed in areas other than preservation. PEFs could be utilized as an effective hurdle when used in combination with other preservation factors such as pH and water activity or as a complementary step with mild thermal processes.

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