16 Food Quality and Safety Issues during Pulsed Electric Field Processing

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16.1 INTRODUCTION

The increasing consumer demand for fresh-like and safe products is a limiting constraint on selection of food processing technologies. The well-established thermal-based methods are available and provide high degree of microbial safety. However, they also degrade product quality. Therefore, there is currently intense search for nonthermal technologies as alternatives or complementary to thermal processes. Although nonthermal technologies such as high electric field pulses have been demonstrated to have some advantages over the conventional thermal technologies, they have only recently started gaining recognition in the food industry.

Application of these nonthermal technologies offers interesting opportunities for mildly processed safe products with preserved sensory and nutritional qualities. However, the technologies are still mostly in the development stages. This chapter presents some recent advances in application of high-voltage, pulsed-electric fields (PEFs) in food processing with respect to quality and safety issues. Some of the critical parameters of PEF processing are identified and described. Potential applications of PEF in processing for food safety and quality are discussed.

Thermal processing has been used in the food industry for several decades with various degrees of success to inactivate pathogenic microbial load in food products. Heat effectively destroys microorganisms. However, it is the balancing act of preserving food quality and maintaining safety that makes thermal processing not very attractive for some products. The current trend in consumers' preference is for fresh-like, minimally processed, and high-quality products. Emerging technologies for nonthermal (or minimal thermal) processing include high-pressure processing, UV light irradiation, magnetic fields, electron irradiation, ozonation, and PEFs. Interest in these technologies has arisen from the desire to overcome the problems of traditional thermal processing. The technologies enable unique modes of energy transfer to foods and target biological cells to achieve inactivation or modification without significantly heating the products. These methods have their strengths and weaknesses depending on process objectives and the type of food to be processed. Among the various emerging technologies, ultrahigh hydrostatic pressure (UHP), PEF, and UV light methods seem to be the most promising. Studies are ongoing to improve understanding of these technologies. This chapter provides an up-to-date description of the PEF technology, review of developments, trends, and applications of the emerging technology with emphasis on food quality and process kinetics.

PEF processing involves the application of externally generated electric field across a food product with the intent of inactivating pathogenic microorganisms, modifying enzymes, intensifying some processes, or achieving some specific transformation in the product. The technology has long been used for cell hybridization and electrofusion in genetic engineering and biotechnology. Its application is based on the transformation or rupture of cells under a sufficiently high external electric field, resulting in increased permeability and electrical conductivity of the cellular material. This effect named dielectric breakdown (Zimmermann et al. 1976) or electroplasmolysis (McLellan et al. 1991) can be explained by two main factors: (1) electroporation, that is electroinduced formation and growth of pores in biomembranes as a result of their polarization; (2) denaturation of cell membranes as a result of their

ohmic heating caused by the electric resistance of membranes which is typically much higher than that of cell sap content. Beside these, the physiological impact and electroosmotic effect may also influence electroplasmolysis efficiency (Weaver and Chizmadzhev 1996).

PEF technologies have been demonstrated to be a viable alternative to high temperature inactivation of microbial load in liquid foods such as fruit juices and milk (Knorr et al. 1994, Barbosa-Cánovas et al. 1999). The majority of research effort on PEF has been on liquid food pasteurization. However, these technologies have also been shown to be applicable for microstructural modification of vegetable, fish, and meat tissues (Wu and Pitts 1999, Angersbach et al. 2000, Gudmundsson and Mafsteinsson 2001), for intensification of juice yield and for increasing product quality in juice production (Bazhal 2001), for processing of vegetable raw materials (Papchenko et al. 1988a), and for winemaking and sugar production (Gulyi et al. 1994). PEF treatment significantly enhances certain food processing unit operations such as pressing (Bazhal and Vorobiev 2000), diffusion (Jemai 1997), osmotic dehydration (Rastogi et al. 1999), and drying (Ade-Omowaye et al. 2000). These processes generally impact quality of foods. Besides, PEF is known to impact some unique quality attributes to food products that may not be possible with other technologies.

PEF processing involves a short burst of high-voltage application to a food placed between two electrodes (Qin et al. 1995). When high electric voltage is applied, a large flux of electric current flows through food materials, which may act as electrical conductors due to the presence of electrical charge carriers such as large concentration of ions (Barbosa-Cánovas et al. 1999). In general, a PEF system consists of a high-voltage power source, an energy storage capacitor bank, a charging current limiting resistor, a switch to discharge energy from the capacitor across the food, and a treatment chamber. The bank of capacitors is charged by a direct current power source obtained from amplified and rectified regular alternative current main source. An electrical switch is used to discharge energy (instantaneously in millionth of a second) stored in the capacitor storage bank across the food held in the treatment chamber. Apart from those major components, some adjunct parts are also necessary. In case of continuous systems a pump is used to convey the food through the treatment chamber. A chamber cooling system may be used to diminish the ohmic heating effect and control food temperature during treatment. High-voltage and high-current probes are used to measure the voltage and current delivered to the chamber (Barbosa-Cánovas et al. 1999, Amiali et al. 2004, 2006b, Floury et al. 2005). Figure 16.1 shows a basic PEF treatment unit, while Figure 16.2 presents different chamber designs.

The type of electrical field waveform applied is one of the important descriptive characteristics of a PEF treatment system. The exponentially decaying or square waves are among the most common waveforms used. To generate an exponentially decaying voltage wave, a DC power supply charges the bank of capacitors that are connected in series with a charging resistor. When a trigger signal is applied, the charge stored in the capacitor flows through the food in the treatment chamber. Exponential waveforms are easier to generate from the generator point of view. Generation of square waveform generally requires a pulse-forming network (PFN)

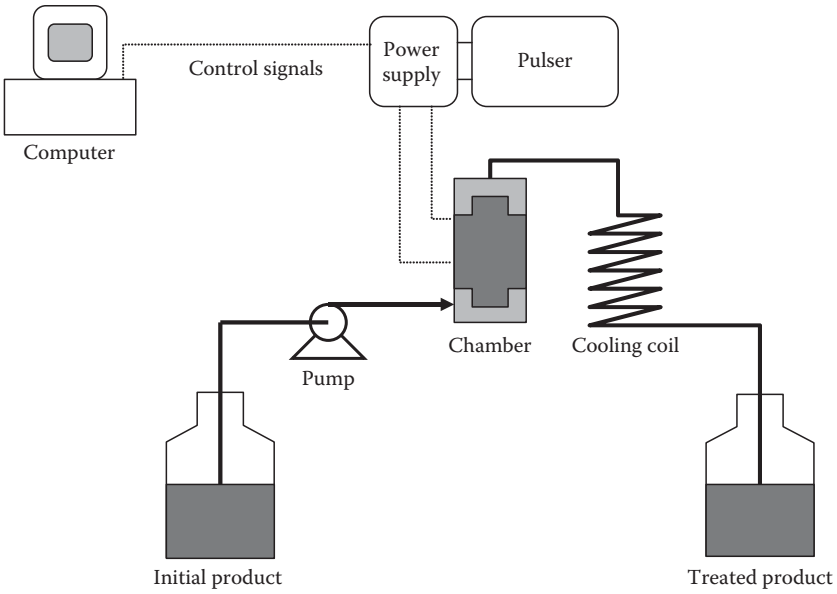

FIGURE 16.1 Schematic diagram of a PEF operation.

consisting of an array of capacitors and inductors. It is more challenging to design a square waveform system compared to an exponential waveform system. However, square waveforms may be more lethal and energy efficient than exponentially decaying pulses (Zhang et al. 1995a, Evrendilek et al. 2005, Amiali et al. 2006b). In order to produce effective square waveform using a PFN, the resistance of the food must be matched with the impedance of the PFN. Therefore, it is important to determine the resistance of the food in order to treat it properly.

The discharging switch also plays a critical role in the efficiency of the PEF system. The type of switch used will determine how fast it can perform and how much current and voltage it can withstand. In increasing order of service life, suitable switches for PEF systems include ignitrons, spark gaps, trigatrons, thyratrons, and semiconductors. Solid-state semiconductor switches are considered as the future of high-power switching. They present better performance and are easier to handle, require fewer components, allow faster switching times, and are more economically sound (Gongóra-Nieto et al. 2002).

16.2 APPLICATION OF PEF IN THE FOOD INDUSTRY

There is a growing interest in the application of PEF in food processing (Barbosa-Cánovas et al. 1999, Dutreux et al. 2000, Fleischman et al. 2004, Floury et al. 2005, Huang et al. 2006, Sobrino-Lopez et al. 2006). Generally, applications of PEF in food processing have been directed at two main categories namely microbial inactivation and preservation of liquid foods, as well as enhancement of mass transfer and texture in solids and liquids.

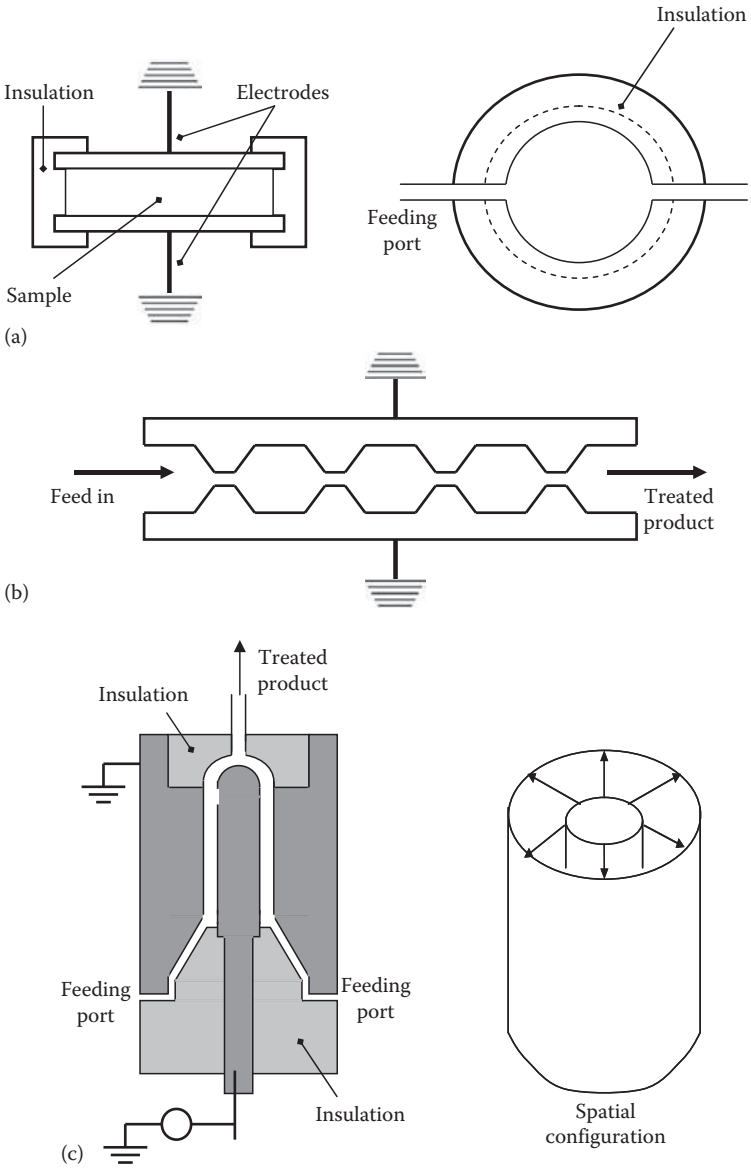

FIGURE 16.2 Designs of treatment chambers for PEF equipment: (a) static chamber, (b) side view of a basic continuous design, and (c) coaxial chamber.

Large portion of work on PEF has been focused on reducing microbial load in liquid or semisolid foods in order to extend their shelf life and assure their safety. The products that have been mostly studied include milk (Dunn and Pearlman 1987, Grahl and Märkl 1996, Sensoy et al. 1997, Reina et al. 1998, Dutreux et al. 2000, Fleischman et al. 2004, Evrendilek et al. 2005), apple juice (Vega-Mercado et al.

1997, Ortega-Rivas et al. 1998, Zárate-Rodríguez et al. 2000, Charles-Rodríguez et al. 2007), orange juice (Zhang et al. 1997, Yeom et al. 2000, Jia et al. 1999), and liquid egg (Jeantet et al. 1999, 2004, Amiali et al. 2004, 2006a,b, Hermawan et al. 2004). These studies and others have reported successful PEF-inactivation of pathogenic and food spoilage microorganisms as well as selected enzymes, resulting in better retention of flavors and nutrients and fresher taste compared to heat pasteurized products (Barbosa-Cánovas et al. 1999, Ho and Mittal 2000, Barsotti et al. 2002, Bendicho et al. 2002, Van Loey et al. 2002, Espachs-Barroso et al. 2003, Sepúlveda-Ahumada et al. 2005a,b, Sobrino-Lopez et al. 2006).

Another area that is showing a great potential is applying PEF on plant tissues as a pretreatment to enhance subsequent processes such as juice extraction (Bazhal and Vorobiev 2000, Eshtiaghi and Knorr 2002) and dehydration (Angersbach and Knorr 1997, Rastogi et al. 1999, Ade-Omowaye et al. 2000, Taiwo et al. 2002, Lebovka et al. 2007).

16.3 MECHANISM OF MICROBIAL INACTIVATION BY PEF

PEF treatment causes electroporation (generation of pores) of the cell membrane, leading consequently to microbial destruction and inactivation (Tsong 1991, Knorr et al. 1994, Ho and Mittal 1996, Pothakamury et al. 1996, García et al. 2007). Although it is still unclear whether the pore formation occurs in the lipid or the protein matrices, it is believed that electric fields induce structural changes in the membranes of microbial cells based on the transmembrane potential, electromechanical compression, and the osmotic imbalance theories (Zimmermann 1986, Barbosa-Cánovas et al. 1999, Gongóra-Nieto et al. 2002, Ohshima and Sato 2004).

16.3.1 Transmembrane Potential

The membrane in a biological cell acts as an insulator to the cytoplasm, whose electrical conductivity is six to eight orders of magnitude greater than that of the membrane (Chen and Lee 1994). The cell membrane can be regarded as a capacitor filled with a low dielectric constant material ($\varepsilon \approx 2$). When a certain electric field is applied to the cell suspension, the ions inside the cell move along the field until the free charges are accumulated at both membrane surfaces. This accumulation of charge increases the electromechanical stress or transmembrane potential (V_t), to a value that is much greater than the applied electric field (Zimmermann 1986). The V_t gives rise to a pressure that causes the membrane thickness to decrease. A further increase in the electric field intensity reaching a critical transmembrane potential (V_c) leads to a reversible membrane breakdown (pore formation). When the size and number of the pore became larger compared to the membrane surface, irreversible breakdown occurs (Zimmermann 1986, Chen and Lee 1994, Sepúlveda-Ahumada et al. 2005a,b). For a given PEF treatment conditions, the induced potential across the cell membrane is proportional to the size of the microorganism.

16.3.2 Electromechanical Compression

Naturally, the charges on the capacitor plates of the biological cell membrane attract each other. This causes a thinning of the membrane provided that the membrane is compressible (Ho and Mittal 1996). The membrane thickness attained at a given membrane potential is determined by the equilibrium between the electric compression forces and the resulting electric restoring forces. With increasing membrane potential, a critical membrane thickness is reached at which an electric compressive force changed more rapidly than the generated electric restoring forces. The membrane becomes unstable and may be broken through. The emerging pores fill up the internal and external solution, both of which are highly conducting. The resulting increase in the electrical permeability of the membrane leads to a very rapid discharge of the membrane capacitor. An increase in the intensity of the external field will lead first to membrane breakdown at the poles of the cells. The required field strength for this transmembrane breakdown is in the range of 1–20 kV/cm depending on cell radius. The breakdown voltage itself is of the order of 1 V depending on temperature, field duration, and so on. At higher field strength, the breakdown voltage is reached for other membrane sites (Coster and Zimmerman 1975, Ohshima and Sato 2004).

16.3.3 Osmotic Imbalance

It is believed that the cause of membrane rupture is due to the osmotic imbalance generated by the leakage of ions and small molecules induced by the PEF treatment (Kinosita and Tsong 1977). Due to the osmotic pressure of the cytoplasmic content, the cell begins to swell and the pores gradually shrink. When the volume of the cell reaches 155% of its normal volume, rupture of the cell membrane and lysis of the cell occur (Tsong 1990).

Vega-Mercado (1996) further confirmed the osmotic imbalance theory. The author investigated pH, ionic strength effect, and PEF combined effect on *Escherichia coli* inactivation and found that the inactivation of microorganisms is caused mainly by an increase in their membrane permeability due to compression and poration. More than 2 log reductions in plate counts are observed when both pH and electric field are modified: pH from 6.8 to 5.7 and electric field from 20 to 55 kV/cm. Similar results are obtained when the ionic strength is reduced from 168 to 28 mM. The authors concluded that the electric field and ionic strength are more likely related to the poration rate and physical damage of the cell membranes, while pH is more likely related to changes in the cytoplasmic conditions due to the osmotic imbalance caused by the poration.

16.4 FACTORS AFFECTING PEF EFFECTIVENESS

Major factors that affect PEF effectiveness during food processing can be grouped as process factors (electric field intensity, pulse type, treatment time, and treatment temperature), product factors (pH, ionic strength, electric conductivity, and constituents of foods), and microbial factors (type, concentration, and growth stage of microorganisms).

16.4.1 Process Factors

16.4.1.1 Electric Field Intensity

Electric field intensity is one of the main factors that influence the microbial inactivation (Dunn 1996). It is defined as electric potential difference V between two given points in space divided by the distance d between them:

$$E = \frac{V}{d} \tag{16.1}$$

To achieve microbial inactivation the applied electric field needs to be greater than the critical electric field for a particular microorganism (Castro et al. 1993). The electric field should be evenly distributed in the treatment chamber in order to achieve an efficient treatment. An electric field of 16 kV/cm or greater is usually sufficient to reduce the viability of Gram-negative bacteria by 4–5 log cycles and Gram-positive bacteria by 3–4 log cycles. In general, the electric field required to inactivate microorganisms in foods is in the range of 12–45 kV/cm. However, some studies have reported that electric fields of up to 90 kV/cm could be applied to food under a continuous treatment conditions (Zhang et al. 1994a, Dunn 1996, Liang et al. 2002). The fact that microbial inactivation increases with increasing applied electric field (EF) strength is consistent with the electroporation theory, in which the induced potential difference across the cell membrane is proportional to the applied electric field.

The most accepted model showing the relationship between the survival ratio ($S = N/N_0$) of microorganisms and the field strength E was proposed by Hülsheger et al. (1981) and is represented by the relationship

$$\ln(S) = -b_E(E - E_c) \tag{16.2}$$

where

S is the microbial survival rate in fraction given as the ratio of microbial count after treatment
N is microbial count before treatment N_0
b_E is the regression coefficient (cm/kV)
E is the applied electric field
E_c is the critical electric field obtained by the extrapolated value of E for 100% survival

Grahl and Märkl (1996) found that the critical value of electric field E_c was a function of cell size; the bigger the size of a cell, the lower the E_c. They attributed this phenomenon to the transmembrane potential experienced by the cell, which is proportional to the cell size. Also, Hülsheger et al. (1983) found that E_c for Gram-negative bacteria was lower than that of Gram-positive bacteria, which may be explained by the smaller resistance of the former.

16.4.1.2 Treatment Time and Frequency

Apart from electric field intensity, treatment time and pulse frequency are important factors. PEF treatment time is calculated by multiplying the pulse number by the

pulse duration. An increase in any of these variables increases microbial inactivation (Sale and Hamilton 1967). Sepúlveda-Ahumada (2003) proposed that electric field pulses between 1 and 5 μs produced the best results for microbial inactivation. Martín-Belloso et al. (1997) found that pulse width influenced microbial reduction by affecting E_c. Longer widths decreased E_c, resulting, therefore, in higher inactivation. An increase in pulse duration may, however, result also in an undesirable food temperature increase and promotion of electrolytic reactions and electrode position at the electrode surfaces (Zhang et al. 1995a).

Normally, the inactivation of microorganisms increases with an increase in the pulse number, up to a certain number (Hülsheger et al. 1983). Grahl and Märkl (1996) reported that the log reduction of *E. coli* in UHT milk increased from 1 to 4 with the pulse number increasing from 5 to 20 at less than 45°C and 22.4 kV/cm of electric field intensity. Zhang et al. (1994b) also reported that the log reduction of *E. coli* in skim milk increased from 1 to 4 with the pulse number increasing from 16 to 64 at 15°C and 40 kV/cm. Liu et al. (1997) found that microbial inactivation was usually achieved during the first several pulses, additional pulses display a lesser lethality. Zhang et al. (1994a) also noticed that the inactivation of *Saccharomyces cerevisiae* by PEF in apple juice reached saturation up to 10 pulses at an electric field of 25 kV/cm. Elez-Martinez and Martin-Belloso (2007) evaluated the effects of PEF processing conditions on vitamin C and antioxidant capacity of orange juice. The treatments were performed at 25 kV/cm and 400 μs with square bipolar pulses of 4 μs and pulse frequency from 50 to 450 Hz. The retention of vitamin C in orange juice and gazpacho increased with a decrease of pulse frequency.

16.4.1.3 Pulse Shape and Polarity

Exponential decaying and square wave pulses are the two commonly used pulse shapes. Other waveforms such as bipolar, instant charge reversal, or oscillatory pulses have been used depending on the circuit design. An exponential decay voltage wave is a unidirectional voltage that rises rapidly to a maximum value and decays slowly to zero. Therefore, food is subjected to the peak voltage for short period of time. Hence, exponential decay pulses have a long tail with a low electric field, during which excess heat is generated in the food without an antimicrobial effect (Zhang et al. 1995a). Oscillatory decay pulses are the least efficient as they prevent the cell from being continuously exposed to high intensity electric field for an extended period of time, thus preventing the cell membrane from irreversible breakdown over a large area (Jeyamkondan et al. 1999).

The square waveform may be generated by using a PFN consisting of an array of capacitors and inductors or by using long coaxial cable and solid-state switch devices. The disadvantage of using high-voltage square waves lies in trying to match the load resistance of the food with the characteristic impedance of the transmission line. By matching the impedances, a higher energy transfer to the treatment chamber can be obtained. Zhang et al. (1994a) reported 60% more inactivation of *S. cerevisiae* when using square pulses than exponentially decaying pulses.

Although results in literature are not conclusive, bipolar pulses are more lethal than monopolar pulses (square or exponential decay) because bipolar pulses cause the alternating changes in the movement of charged molecules which lead to extra stress in the cell membrane and enhance its electric breakdown (Qin et al. 1994,

Barbosa-Cánovas et al. 1999, Evrendilek and Zhang 2005). Bipolar pulses also offer the advantages of minimum energy utilization, reduced deposition of solids on the electrode surface, and decreased food electrolysis. These advantages were tested by Qin et al. (1994) on *Bacillus subtilis* and Evrendilek and Zhang (2005) on *E. coli* O157:H7 in skim milk.

Ho et al. (1995) proposed instant reversal pulses where the charge is partially positive at first and partially negative immediately thereafter. The inactivation effect of an instant reversal pulse is believed to be due to a significant alternating stress on microbial cell which causes structural fatigue. Amiali et al. (2006b) used instant reversal square wave pulses and found this kind of waveforms to be more efficient than others in terms of egg product pasteurization, since it combines instant reversal charge and square waveform pulses.

16.4.1.4 Treatment Temperature

Treatment temperature influences the effectiveness of PEF on cellular materials. Since PEF treatment normally increases the product temperature (largely due to ohmic heating components), a cooling device is sometimes used to maintain the temperature at levels that maintain the nutritional, sensory, or functional properties of products. On the other hand, application of PEF at mild temperatures tends to enhance the microbial inactivation. Dunn and Pearlman (1987) found that a combination of PEF and heat was more efficient than conventional heat treatment alone. A higher level of inactivation was obtained using a combination of 55°C temperature and PEF to treat milk. Dunn (1996) obtained a 6 log reduction of *Listeria innocua* inoculated in milk after few seconds at 55°C accompanied with PEF. Increasing treatment temperature from 7°C to 20°C significantly increased PEF inactivation of *E. coli* in simulated milk ultrafiltrate (SMUF). However, additional increase in temperature from 20°C to 33°C did not result in any further increase in PEF inactivation (Zhang et al. 1995b). Reina et al. (1998) reported a higher inactivation rate of *L. monocytogenes* in milk with a temperature increase from 25°C to 50°C. At 30°C and 30 kV/cm, a 3.5 log reduction of *L. monocytogenes* was obtained after 600 µs of treatment, whereas at 50°C more than 4 log reductions were obtained. Sepúlveda-Ahumada et al. (2005a) observed a marked increase of PEF inactivation at 55°C on *L. innocua* suspended in a buffer. The electric field intensity and number of pulses were applied in the range of 31–40 kV/cm and 5–35 pulses. These authors found synergy between thermal and PEF treatment. It was suggested that the marked increase of PEF inactivation effectiveness at 55°C may be due to the occurrence of phase transition on the cell membrane of *L. innocua* at this temperature, since it is possible that a thinning of the bacterial membrane would render bacterial cells more susceptible to disruption by electric fields (Jayaram et al. 1992). Ravishankar et al. (2002) also investigated *E. coli* O157:H7 at electric field strength (15–30 kV/cm), pulse number (1–20), and temperature (5°C–65°C) using a static chamber and gellan gum gel as a suspension medium. The authors found that thermal energy began taking effect at 55°C. At this temperature, a 1 log reduction was attributable to thermal energy. Above this temperature, all reductions were attributed entirely

to thermal energy. The authors suggested that there was no synergy between the concurring thermal and PEF energies.

Bazhal et al. (2006) investigated the combined effect of heat treatment with PEF on the inactivation of *E. coli* O157:H7 in liquid whole egg. The electric field strength was varied from 9 to 15 kV/cm and the treatment temperatures were 50°C, 55°C, or 60°C. At 60°C, a 2 log reduction of *E. coli* O157:H7 was obtained using thermal treatment alone, while a combination of heat and PEF resulted in a 4 log reduction. These results indicated a synergy between temperature and electric field.

Increase in the rate of inactivation with temperature is attributed to reduced transmembrane breakdown potentials at higher temperatures (Zimmermann 1986). Stanley (1991) proposed that phospholipid molecules in cell membrane undergo temperature-related transitions, changing from a firm gel-like structure to a less-ordered liquid crystal phase at higher temperature, thus reducing the mechanical resistance of the cell membrane. Another deduction proposed by Schwan (1957) was that the higher lethal effect of PEF combined with heat might be due to the increase in electrical conductivity of the medium, making it similar to electrolytic conduction (Barbosa-Cánovas et al. 1999, Sepúlveda-Ahumada et al. 2005a).

16.4.2 Operation Mode

In general, continuous processes for liquids reached higher inactivation rates than those in batch mode. Martín et al. (1997) reported that in order to achieve a 2 log reduction of *E. coli* inoculated in milk by a batch mode, 64 pulses and 35 kV/cm were needed, while for a continuous mode, 25 pulses and 25 kV/cm were enough. Operation of PEF in continuous mode for solids is much more challenging.

16.4.3 Product Factors

16.4.3.1 pH and Ionic Strength

Vega-Mercado (1996) studied the effect of pH and ionic strength of SMUF medium during PEF treatment. The authors reported that the lower the pH and ionic strength, the higher the inactivation rate. When the ionic strength decreased from 168 to 28 mM, the inactivation ratio increased from no detectable to 2.5 log cycles. Also, when the pH reduced from 6.8 to 5.7, the inactivation ratio increased from 1.5 to 2.2 log cycles. The PEF treatment and ionic strength were responsible for electroporation and compression of the cell membrane, whereas the pH of the medium affected the cytoplasm when the electroporation was complete. Alvarez et al. (2000) also studied the influence of pH of treatment medium on the inactivation of *Salmonella senftenberg* by PEF treatment. The authors found that at the same electric conductivity, inactivation of *S. senftenberg* was greater at neutral (7.0) than acidic pH (3.8).

16.4.3.2 Electrical Conductivity

The electrical conductivity of a medium (σ, s/m), which is defined as the ability to conduct electric current, is an important variable in PEF treatment:

$$\sigma = \frac{d}{RA} = \frac{1}{\rho} \tag{16.3}$$

where

σ is the electrical conductivity of medium (s/m)
R is the resistance of the medium (Ω)
A is the electrode surface area (m^2)
d (m) and ρ are the gap between electrodes and the resistivity, respectively

The electrical conductivity of a medium depends on treatment temperature as defined by

$$\sigma = \alpha T + \beta \tag{16.4}$$

where α and β are constants depending on the composition and concentration of the medium.

At constant temperature conditions, foods with high electrical conductivities (low resistivity) exhibit smaller electric fields across the treatment chamber and therefore are difficult to be treated with PEF process. An increase in electrical conductivity increases the ionic strength of the food, resulting in a decrease in the inactivation rate. Furthermore, an increase in the difference between the electrical conductivity of a medium and microbial cytoplasm weakens the membrane structure due to an increased flow ionic substance across the membrane (Jayaram et al. 1992).

Alvarez et al. (2000) studied the influence of conductivity of treatment medium on the inactivation of *S. senftenberg* by PEF treatment. The authors found that at constant input voltage, electric field strength obtained in the treatment chamber depended on medium conductivity. At the same electric field strength, conductivity did not influence *S. senftenberg* inactivation.

16.4.3.3 Composition

Food components such as fat and protein may influence the effect of PEF on the food product. These effects may be related to the capacity of some substances to shield microorganisms from applied field, or the ability of some chemical species to stabilize or prevent ion migration.

Martín et al. (1997) found that inactivation of *E. coli* in milk was more limited than in a buffer solution, because of the presence of milk proteins. Grahl and Märkl (1996) subjected different media (milk with 1.5% and 3.5% fat, solutions of sodium-alginate) inoculated with *E. coli* and other microorganisms to PEF. The treatment conditions are 5–15 kV/cm, 1–22 Hz, and the temperatures did not exceed 45°C–50°C. The authors noticed that the fat particles of milk seemed to protect the bacteria against electric pulses. Picart et al. (2002) also claimed that whole milk with a higher fat content (3.6%) appeared to reduce *L. innocua* inactivation compared to skim milk at temperatures between 25°C and 45°C, a pulse repeat frequency of 1.1 Hz and electric intensity of 29 kV/cm.

There is currently no agreement on the possible influence of fat content on PEF inactivation. Reina et al. (1998) compared the effect of PEF treatment under 25°C at 30 kV/cm and frequency of 1700 Hz in milk with different fat content. The authors inoculated *L. monocytogenes* into skim milk, 2% fat milk, and whole milk, and evaluated the effects of the fat content on the inactivation rates; no differences were observed among the results. Manas et al. (2001) used 33% emulsified fat cream to test fat effect on the inactivation of *E. coli* by PEF treatment. The treatment was conducted under 34 kV/cm with a pulse frequency of 1.1 Hz and temperatures less than 30°C. The result was that the emulsified lipids do not appear to protect against microbial inactivation by electric pulses. Sobrino-Lopez et al. (2006) also claimed that fat content of the milk did not modify the resistance of *Staphylococcus aureus* to a PEF treatment. Three types of milk (whole, 1.5% and skim) were treated under 25°C, 30–35 kV/cm, and frequency of 100 Hz.

16.5 MICROBIAL INACTIVATION KINETICS

When microorganisms are treated with heat the logarithm of cell population decreases, linearly, with the treatment time for constant treatment intensity. Alternative food processing technologies are also believed to inactivate microorganisms logarithmically. Dose–response models are derived from kinetic data to predict efficiency of variables of alternative processes. For the case of PEF, such models are based on sigmoid inactivation plots, as the one shown in Figure 16.3. As previously stated, the lethal effect of PEF has been described as a function of field intensity, treatment time (pulse duration and number of pulses), and a model constant determined by the microorganism and its physiological status. Microbial inactivation in liquid medium has been reported to follow the first-order kinetics (Hülsheger et al. 1981, Grahl and Märkl 1996) as follows:

$$\log S = B_E (E - E_c) \tag{16.5}$$

where

S is the microbial survival rate
B_E is an electric field constant obtained as the coefficient of regression of the straight survival curves
E is applied electric field
E_c is critical value of electric field below which there will be no inactivation (that is 100% survival)

Treatment time can be calculated as product of number of pulses and pulse width. Inactivation kinetics in terms of treatment time has been given as (Hülsheger et al. 1981, Grahl and Märkl 1996)

$$\log S = B_t \frac{t}{t_c} \tag{16.6}$$

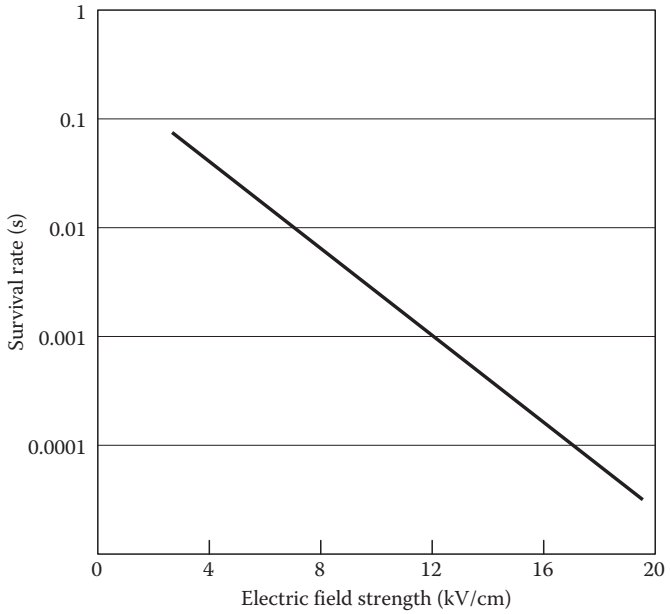

FIGURE 16.3 Typical curve of inactivation rate of microorganisms by PEF treatment.

where

B_t is electric field constant or coefficient of the regression of the straight survival curves

t is treatment time

t_c is the critical time below which there will be no inactivation

Combining Equations 16.5 and 16.6 yields

$$S = \left(\frac{t}{t_c}\right)^{\frac{E-E_c}{k}} \tag{16.7}$$

where k is a constant factor as can be expressed as follows:

$$k = \frac{E - E_c}{B_{t(E)}} = \frac{\log\left(\frac{t}{t_c}\right)}{B_{E(t)}} \tag{16.8}$$

If logarithms to base 10 on both sides of Equation 16.7 are taken, the left-hand side of such equation would represent the inactivation ratio or log reduction, which refers to 90% reduction in the initial microorganism's population (Figure 16.3). The transformed equation would also indicate that inactivation ratio depends linearly on the

applied field strength and logarithmically on the treatment time. Although electric field strength would represent a more pronounced effect than treatment time, both are important elements. Apart from electric field strength and treatment time, some other variables such as pulse characteristics can also influence the microbial inactivation ratio and reaction kinetics in PEF processing. In a particular process, a high-voltage field will be discharged to a food material placed between two electrodes, and it will be released in pulsed form. Operating field strengths range from 10 to 70 kV/cm, while duration of the pulses can vary from μs to ms range. The frequency of the pulses can be as low as a single one, or as high as 2000 pulses per second.

Considering the kinetic equations, smaller values of E_c or t_c and higher values of B_E and B_t indicate greater susceptibility of the particular microorganism to PEF processing. The above first-order kinetics may be simplistic for most PEF applications. Tailing phenomena in microbial inactivation kinetics have been observed (Sensoy et al. 1997). A two-phase kinetic model may be required to adequately describe PEF inactivation kinetics. Some other more complicated inactivation kinetic models have been reported (Anderson et al. 1996, Raso et al. 2000). The search for the most robust kinetic model for PEF inactivation of microorganisms is currently ongoing. Bacteria cells are generally more resistant to PEF inactivation than yeast cells. However, spores are most resistant to PEF. Vegetative cells at the logarithmic growth stage are more susceptible to PEF inactivation. Increasing the conductivity of medium generally decreases effectiveness of PEF for microbial inactivation since the higher the conductivity of a medium, the lower the achievable peak electric field at a constant input energy.

16.6 QUALITY ASPECTS OF PEF PROCESSING

PEF treatment may influence physical and chemical properties of products. The nature and extent of PEF influence on quality changes are still being actively discussed. Barsotti et al. (2002) indicated that PEF treatment of model emulsions and liquid dairy cream may result in dispersal of oil droplets and dissociation of fat globule aggregates. Qin et al. (1995) reported no apparent change in the physical and chemical attributes of PEF processed milk. PEF treatment of various liquid foods including apple juice, orange juice, and milk has not shown any significant physicochemical changes (Jia et al. 1999, Charles-Rodríguez et al. 2007, Shin et al. 2007). PEF processed cranberry juice and chocolate milk retained its physical and chemical characteristics (Evrendilek et al. 2001). There was a slight decrease in vitamin C content in PEF-treated orange juice compared to heat-treated orange juice (Zhang et al. 1997).

Gallardo-Reyes et al. (2008) conducted a comparative study of orange juice pasteurized by ultrahigh temperature (UHT) (processing at 110°C, 120°C, and 130°C for 2 and 4 s) and PEF (20 and 25 kV/cm for 2 ms). The authors concluded that although there was no difference in pH and soluble solids obtained with both treatments and freshly squeezed control samples, the color of PEF-treated sample was closer to the control. There have been attempts to improve juice extraction from plants and fruit products using PEF (Bazhal and Vorobiev 2000, Schilling et al. 2007). PEF may enhance the extraction of higher amounts of valuable compounds into the extraction

juice resulting in high quality. Torregrosa et al. (2006) reported higher vitamin A contents of orange–carrot juices, compared to untreated control samples, when the product was treated at 25 kV/cm. Apple juice contains several phenolic compounds including chlorogenic acid (5-CQA), catechins, procyanidins, quercetin glycosides, and phloridzin. Schilling et al. (2007) monitored the phenolic compositions of juices obtained from PEF-treated mash and untreated samples. The PEF treatments were at the field intensities of 1, 3, and 5 kV/cm. There was no significant difference between treated and untreated samples. However, enzymatic maceration of the apple mash resulted in a marked increase in quercetin glycosides. The authors also reported that there is no difference in the antioxidative capacity of the apples juices. It was then postulated that the fact that the antioxidative potential was not affected indicates that radical formation has not taken place during PEF treatment.

Table 16.1 shows the influence of PEF treatment on the quality of juice expressed from apple and sugar beet. Data on the Table 16.1 were obtained from Lazarenko et al. (1977), Bazhal and Vorobiev (2000), and Bazhal (2001). Bazhal and Vorobiev (2000) reported that juice from the samples treated at E = 800 V/cm was lighter (absorbance = 0.02) than juice expressed from apple tissue pulsed at E = 150 V/cm (absorbance = 0.07). The significant change in juice color may be attributed to the inhibition of polyphenoloxidase by electric fields (Giner et al. 2001). Lazarenko et al. (1977) suggested that electric field can break the chains of pectin molecules resulting in deceased pectin concentrations and thus reducing the kinematic viscosity of the extracted juices. Lower viscosity improves juice filtration. The reduction in transmittance of juice after electroplasmolysis indicates a reduction in suspended particle contents because of improved tissue filtration properties resulting from the additional pores formed in the cell walls after PEF treatment. PEF treatment of sugar beet resulted in increased purity of the extracted juice compared to the traditional processing by thermal plasmolysis. Despite lower temperature leaching for

TABLE 16.1
Qualitative Parameters of Apple Juice from Control and PEF-Treated Samples

	Apple Juice		Sugar Beet Juice	
Parameter	**PEF**	**Control**	**PEF**	**Control**
Density (kg/m^3)	1059.4	1057.7	1.0392	1.038
Brix	13.8	13.1	9.8	9.5
pH	3.91	3.84	5.6	5.65
Pectin (mg/L)	290	517		
Kinematic viscosity (10^{-6} m^2/s)	5.917	6.747	2.284	2.595
Absorbance (wavelength of 520 nm)				
Filtered	0.02	0.39		
Nonfiltered	0.03	1.18		
Transmittance	0.67	0.33		

PEF-treated samples, sucrose loss in pulp decreased from 0.62% (thermally treated beet) to 0.57% (Knorr et al. 2001). It is uncertain if all necessary chemical analysis has been performed to fully ascertain the effect of PEF on quality of processed foods. However, the clear consensus is that liquid food products generally retain their fresh-like quality after PEF treatment.

Solid food products undergo significant changes when treated with PEF. Changes in electrical conductivity of the treated vegetable samples indicated increasing cell permeability (Lebovka et al. 2000, 2001). Table 16.2 shows that diffusion coefficient of sugar from the beetroot increases from 0.68×10^{-9} up to 1.2×10^{-9} m²/s after PEF treatment (Gulyi et al. 1994, Jemai 1997). Elastic modulus of sugar beet

TABLE 16.2
Some Vegetable Tissues Properties Estimated for Untreated (Control) and PEF Pulsed Samples

	Value of Parameter				
Parameter	Control	PEF Treatment	Material	Operation	Reference
Electrical conductivity (S/m)	0.003–0.007	0.035–0.070	Apple	Conductivity measurement	Lebovka et al. (2001)
Electrical conductivity (S/m)	0.03	0.41	Carrot	Conductivity measurement	Rastogi et al. (1999)
Electrical conductivity (S/m)	0.06	0.53	Potato	Conductivity measurement	Knorr and Angersbach (1998)
Porosity (%)	67	75	Apple	Using of penetrometer	Bazhal et al. (2003b)
Water diffusion coefficient (m^2/s)	0.98×10^{-9}	1.55×10^{-9}	Carrot	Osmotic dehydration	Rastogi et al. (1999)
Sugar diffusion coefficient (m^2/s)	0.68×10^{-9}	1.2×10^{-9}	Sugar beet	Leaching	Gulyi et al. (1994)
Mass transfer coefficient (kg/m^2 s)	0.043	0.058	Paprika	Drying	Ade-Omowaye et al. (2002)
Constant drying rate (kg/m^2 s)	9.68×10^{-4}	13.02×10^{-4}	Paprika	Drying	Ade-Omowaye et al. (2002)
Heat transfer coefficient (W/m^2 s)	73.13	98.36	Paprika	Drying	Ade-Omowaye et al. (2002)
Elastic modulus (MPa)	12.5	6.5	Sugar beet	Compression test	Matvienko (1996)
Elastic modulus (MPa)	1.53	0.32	Apple	Compression test	Bazhal et al. (2003b)
Failure stress (MPa)	1.26	0.53	Apple	Compression test	Bazhal et al. (2003b)

decreased after PEF treatment (Bazhal 2001). The microstructure of salmon and chicken changed considerably due to PEF treatment as the muscle cells decreased in size and gaping occurred (Gudmundsson and Mafsteinsson 2001). Electric field treatment generally affects biological cell membranes whereas heating destructs the cell walls (Calderón-Miranda et al. 1999). There is a potential of inducing rheological changes in a product as a result of PEF treatment. This phenomenon depends on the type of product involved and requires detailed investigations.

16.6.1 PEF Effects on Milk and Cheese Quality

In recent years, with the demand of high-quality milk and milk products, more and more researchers have focused on studies of loss of sensory and physicochemical characteristics in milk and milk products following treatment with pulse electric field (Qin et al. 1995, Dunn 1996, Bendicho et al. 1999, Evrendilek et al. 2001, Li et al. 2003, Michalac et al. 2003, Sepúlveda-Ahumada 2003, Sampedro et al. 2005, Shin et al. 2007, Yeom et al. 2007, Shamsi et al. 2008). As for the PEF effect on cheese process and quality, limited research work was found (Sepúlveda-Ahumada et al. 2000). Dunn (1996) reported that milk treated with PEF (E = 20–80 kV/cm) suffered less flavor degradation. The author proposed the possibility of manufacturing dairy products such as cheese, butter, and ice cream using PEF-treated milk although no detailed information was given in his report. Qin et al. (1995) carried out a study of shelf life, physicochemical properties, and sensory attributes of milk with 2% milk fat, treated with 40 kV/cm of electric field and of 6–7 pulses. No physicochemical or sensory changes were observed after treatment, in comparison with a sample treated with thermal pasteurization. Bendicho et al. (1999) studied the destruction of riboflavin, thiamine (water soluble), and tocopherol (liposoluble) in milk by treatment with PEF (E = 16–33 kV/cm; N = 100 pulses). The vitamin concentrations before and after treatment were determined by HPLC. The authors observed no destruction of vitamins by treatment with pulses. Michalac et al. (2003) studied the variation in color, pH, proteins, moisture, and particle size of UHT skim milk subjected to treatment with PEF (E = 35 kV/cm; W = 3 μs; and time = 90 μs). The authors saw no differences in the parameters studied (color, pH, proteins, moisture, and particle size) before and after treatment.

Sepúlveda-Ahumada et al. (2000) compared the textural properties and sensory attributes of Cheddar cheese made with heat-treated milk, PEF-treated milk (E = 35 kV/cm; N = 30 pulses), and untreated milk. In the hardness and springness study, the cheeses made from milk pasteurized by any method were harder than those made from untreated milk. In the sensory evaluation, the panelists also found differences between the cheeses made from untreated milk and milk treated by PEF or heat. Regardless of the differences, the authors still considered using PEF-treated milk to obtain cheese as a feasible option in order to improve the product quality.

Yeom et al. (2007) studied a commercial, plain, low-fat yogurt mixed with fruit jelly and syrup. They observed the changes in physical attributes (pH, color, and Brix) and sensory attributes during storage at 4°C after treatment with PEF (electric field = 30 kV/cm; treatment time = 32 μs) and heat (T = 65°C; time = 30 s). The sensory

evaluation indicated that there were no changes between the control samples and the treated samples. There was also no variation in the color, pH, and Brix.

Evrendilek et al. (2001) studied color, pH, Brix, and conductivity at 4°C, 22°C, and 37°C in milk with chocolate using treatment with PEF (E = 35 kV/cm; W = 1.4 μs; time = 45 μs), and PEF + heat (112°C and 105°C, 33 s). They compared the results with a control sample not treated by PEF or heat. Measurement of the a, b, and L parameters at 4°C revealed that the treatments of PEF at 105°C and PEF at 112°C did not cause changes in color. Sepúlveda-Ahumada (2003) treated HTST pasteurized milk with electric field of 35 kV/cm and 2.3 μs of pulse width, at a temperature of 65°C for less than 10 s. PEF treatments were applied either immediately after thermal pasteurization to produce an extended shelf life product, or 8 days after thermal pasteurization to simulate processing after bulk shipping. Application of PEF immediately after HTST pasteurization extended the shelf life of milk to 60 days, while PEF-processing after 8 days caused a shelf life extension of 78 days, both were proving to be successful strategies to extend the shelf life of milk.

Li et al. (2003) investigated the effects of PEFs and thermal processing on the stability of bovine immunoglobulin G (IgG) in enriched soymilk. PEF at 41 kV/cm for 54 μs caused a 5.3 log reduction of natural microbial flora, with no significant change in bovine IgG activity. Analysis using circular dichroism spectrometry revealed no detectable changes in the secondary structure or the thermal stability of secondary structure of IgG after the PEF treatment (Li et al. 2005). However, in an experiment investigating the effect of temperature on the stability of IgG during PEF treatment (30 kV/cm, 54 μs), up to 20% of IgG was inactivated when the temperature was increased to 41°C (Li et al. 2003).

Shin et al. (2007) applied PEFs with square wave pulse to whole milk inoculated with *E. coli*, *Pseudomonas fluorescens*, and *Bacillus stearothermophilus*. The samples were exposed to 30–60 kV/cm electric field intensity with 1 μs pulse width and 26–210 μs treatment time in a continuous PEF treatment system. Eight log reductions were obtained for *E. coli* and *P. fluorescens* and 3 logs reduced for *B. stearothermophilus* under 210 μs treatment time, 60 kV/cm pulse intensity at 50°C. There was no significant change in pH and titration acidity of milk samples after PEF treatment.

Shamsi et al. (2008) determined the effects of PEF treatments on the inactivation of alkaline phosphatase (ALP), total plate count (TPC), *Pseudomonas*, and *Enterobacteriaceae* counts in raw skim milk at field intensities of 25–37 kV/cm and final PEF treatment temperatures of 15°C and 60°C. At 15°C, PEF treatments of 28–37 kV/cm resulted in 24%–42% inactivation in ALP activity and <1 log reduction in TPC and *Pseudomonas* count, whereas the *Enterobacteriaceae* count was reduced by at least 2.1 log units to below the detection limit of 1 CFU/mL. PEF treatments of 25–35 kV/cm at 60°C resulted in 29%–67% inactivation in ALP activity and up to 2.4 log reduction in TPC, while the *Pseudomonas* and *Enterobacteriaceae* counts were reduced by at least 5.9 and 2.1 logs, respectively, to below the detection limit of 1 CFU/mL. Kinetic studies suggested that the effect of field intensity on ALP inactivation at the final PEF treatment temperature of 60°C was more than twice that at 15°C. A combined effect was observed between field intensity and temperature in the inactivation of both ALP enzyme and the natural microbial flora in raw

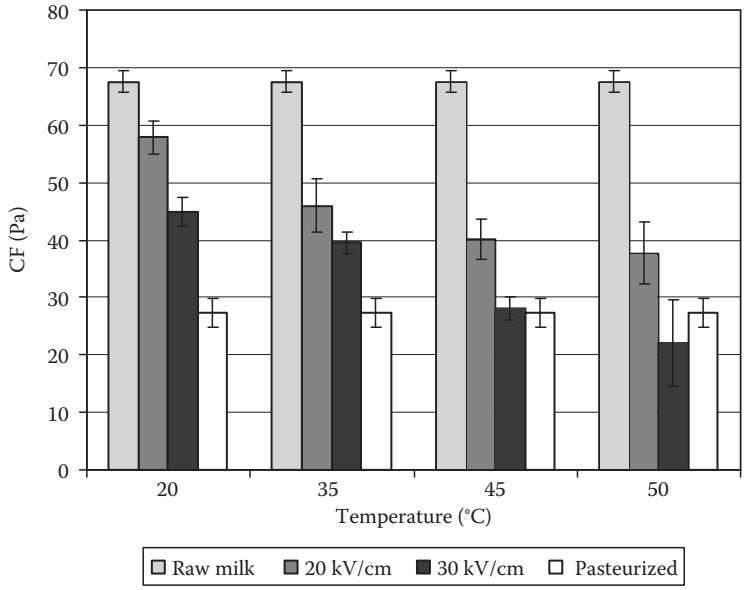

FIGURE 16.4 Effect of electric field intensity and temperature on CF. The applied pulse width is 2 μs, pulse frequency is 2 Hz, and pulse number is 120.

skim milk. The results of this study suggest that PEF as a nonthermal process can be employed for the treatment of raw milk in mild temperature to achieve adequate safety and shelf life while preserving the heat-sensitive enzymes, nutrients, and bioactive compounds.

Figure 16.4 shows that electric field intensity *E* and treatment temperature significantly affected rennet coagulation properties of milk in terms of curd firmness (CF) (Yu et al. 2008). Increasing both *E* and temperature decreased CF. Raw milk coagulum showed the highest CF (67.5 Pa), while pasteurized milk gave the lowest CF value (27.4 Pa) and PEF-treated milk presented values in between. For PEF-treated samples, all the CF values obtained at 20 kV/cm were significantly higher than the CF values for pasteurized milk samples. Also, most CF data obtained under 30 kV/cm were significantly higher than the CF values for pasteurized milk samples, except those treated at 50°C. The result implied that treating milk with PEF and mild temperature impacts less changes in terms of milk coagulation properties compared to heat pasteurized milk. This result is consistent with the finding of Dunn (1996) who studied the PEF-treated raw milk (E = 20–80 kV/cm; pulse width = 1–10 μs) and concluded that no significant physicochemical changes were observed within similar experimental range. Qin et al. (1995) also reported similar result for 2% fat milk (treated using 40 kV/cm and 6–7 pulses) in comparison with a sample treated with thermal pasteurization.

The Yu et al. (2008) data showed that applying electric field of 30 kV/cm combined temperature of 50°C may lead to similar coagulating effect as thermal pasteurization ($p > 0.05$). Fox et al. (2000) showed that heat treatment of milk at temperatures above 65°C adversely affects its rennet coagulability. Thus, for the purpose of cheese production, the treatment temperature under higher electrical intensity (30 kV/cm)

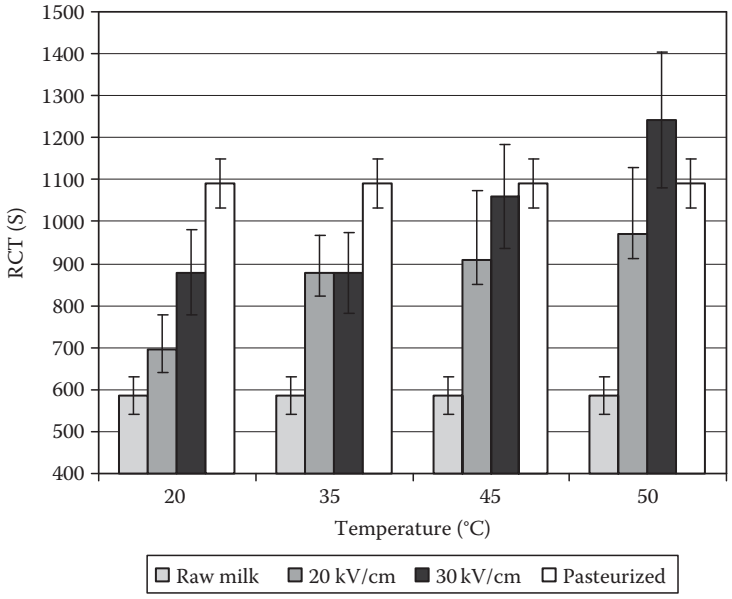

FIGURE 16.5 Effect of electric field intensity and temperature on CF (RCT). The applied pulse width is 2 μs, pulse frequency is 2 Hz, and pulse number is 120.

and longer pulses (120 pulse numbers) may not exceed 50°C in order to obtain desirable curd formation.

Figure 16.5 shows that treatment temperature and electric field intensity *E* also significantly affected rennet coagulation time (RCT) (Yu et al. 2008). Higher *E* and temperature led to longer RCT. RCT is a good index of the gelation potential of milk. A low RCT usually shows potentially good gel formation and high gel strength. Raw milk obviously obtained the lowest RCT (586.2 s). Pasteurized milk obtained highest RCT (1091.0 s) while PEF-treated samples got the medium RCT values.

The values of RCT obtained using an electric field intensity of 20 kV/cm and temperatures from 18°C to 50°C were significantly lower than that of heat pasteurized samples. However, at a 30 kV/cm electric field intensity, in order to keep the RCT value lower than that of heat pasteurized milk, the treatment temperature could not exceed 45°C. The RCT results again confirm that minimal changes were impacted to the milk product when appropriate PEF intensities and temperatures were used for processing. Evrendilek et al. (2001) studied a yogurt drink prepared using combined PEF (E = 30 kV/cm; treatment time = 32 μs) and heat (60°C, 32 s). The authors reported no significant differences between the control sample and the treated samples in terms of color, soluble solids, and pH. However, when milk was subjected to long-duration pulses (Perez and Pilosof 2004), or high-intensity electric fields (45–55 kV/cm) as described by Floury et al. (2005), the structure of milk protein was apparently modified.

Perez and Pilosof (2004) attributed the effects of PEF on milk proteins as due to polarization of the protein molecule, dissociation of noncovalently linked protein

subunits involved in quaternary structure, and changes in the protein conformation so that buried hydrophobic amino acids or sulfydryl groups are exposed. If the duration of the electric pulse is high enough, hydrophobic interactions or covalent bonds may occur, forming aggregates. Similarly, Floury et al. (2005) explained the effect of PEF on milk protein as due to the modification of the apparent charge after exposure to intense electric fields and then modification of ionic interactions between the proteins.

The modification of milk protein structure may lead to changes in milk functional properties such as coagulation, foaming, and emulsifying. Different authors have reported varying levels of electric field strengths and temperatures beyond which changes in milk properties will occur. Coagulation properties of raw milk may be better preserved by using lower electric field strength (≤30 kV/cm) and temperature (≤50°C) combinations.

16.6.2 Effects of PEF on Color and Texture of Fruits and Vegetables

Very limited information is available in the literature on the effect of PEF on color and texture of vegetables and fruits. Knorr and Angersbach (1998) reported an increase of the enzyme polyphenoloxiase (PPO) from potato-cultured cells. The results were explained as due to cell membrane rupture and the subsequent decompartmentalization of the enzymes. The authors reported that the application of PEF beyond the optimum conditions (15–30 pulses at electric field strengths between 1.5 and 3.0 kV/cm, with a pulse width of 500 μs) resulted in the enzymatic browning of potato-cultured cells. Arevalo (2003) observed an increased color change reaction rate (up to 2.5 times) after subjecting apple slices to an electric field in the range from 0.75 to 1.5 kV/cm. Up to 60 pulses of 100 μs width square wave was applied. The compressive strength of apple tissue was reduced by 21%–47% as a result of the application of electric field pulses (Figure 16.6). In another study Arevalo (2003), using 0.75 and 1.5 kV/cm field intensity and 5, 30, or 120 pulses of 100, 200, and 300 μs pulse, the impact of PEF on color changes rate of potato slices also increased but the effect of pulse width did not produce any significant effect. The maximum compressive strength of potato was not affected by the PEF treatment. Thus there may be a saturation point beyond which the effect of PEF treatment is no longer relevant. Galindo et al. (2008) also studied the effect of PEF application on the potato tissues. The authors observed the effect on diffusion of fluorescent dye FM1-43 through the cell wall. The electric field strength was varied from 30 to 500 V/cm with 1 ms rectangular pulse. The result showed a slower diffusion of FM1-43 in the electropulsed tissue when compared with the untreated sample. They also suggested that electric field decreased the cell wall permeability. This response was mimicked by exogenous H_2O_2 and blocked by sodium azide, an inhibitor of production of H_2O_2 by peroxidase.

In a study on dehydration properties of carrots, Rastogi et al. (1999) reported changes in the textural characteristics of samples that were subjected to PEF pretreatments. The study showed the loss of turgor pressure and a softening of the tissue due to the damage induced by PEF pretreatments. It was also reported that further softening was very limited with increasing electric field above 1.09 kV/cm. Taiwo

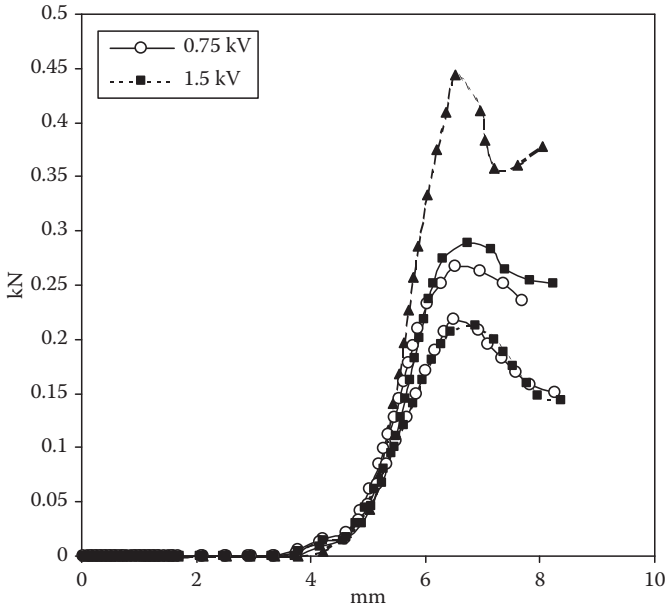

FIGURE 16.6 Maximum compressive strength force (kN) in apple slices. *n* represents the number of pulses.

et al. (2001) studied the effect of different pretreatments on texture and color changes of apple slices. The PEF applied as a pretreatment in the study consisted of 20 pulses of 800 µs with an electric field strength of 1.4 kV/cm. The authors reported decay in compressive strength of the apple tissue and a browning reaction (therefore a subsequent decrease in lightness values) due to application of PEF. The *L* color parameter was measured directly after application of PEF pretreatment before samples were osmotically dehydrated. In a subsequent study, Taiwo et al. (2002) compared quality characteristics of apple slices subjected to PEF and osmotically rehydrated. The authors studied color and textural changes of the apple slices after rehydration. Results showed that PEF-treated samples presented higher deformation force which was explained by the fact PEF-treated samples retained more solids. Results also showed that longer rehydration times and higher temperatures of the immersion fluid produced darker products. No conclusive results were obtained on the effect of PEF on the color of rehydrated samples. Bazhal et al. (2003a,b) studied the influence of PEF treatment on morphological changes in apple tissues. The later were subjected up to 60 pulses of 1 kV/cm electric field strength at 1 Hz pulse frequency and 300 µs pulse duration. The authors reported an increase of porosity from 63% to 69.4% after electroplasmolysis (Figure 16.7). The sizes of the induced pores were smaller compared to the pores of untreated samples and were comparable with the tissue cell wall thickness. The overall average mean size of the PEF-induced pores was 5.86 µm, which is lower than 7.81 µm obtained for untreated samples. In addition, by determining electrical conductivity, disintegration index, and failure stress of apple samples, a linear dependency was observed between failure stress and degree of

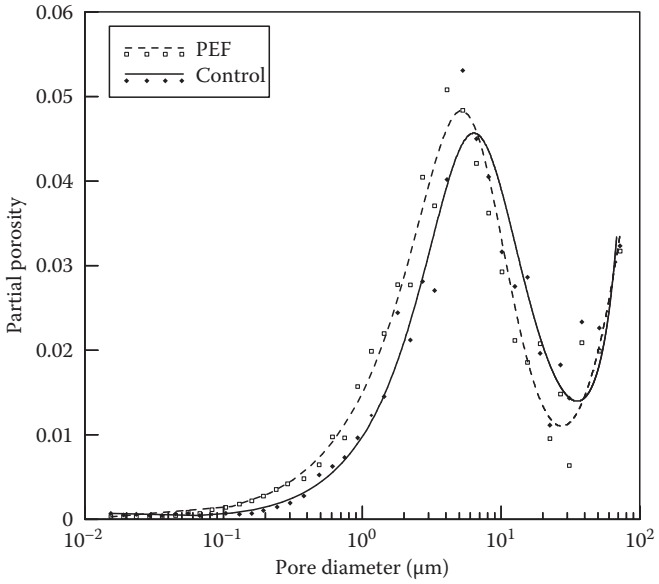

FIGURE 16.7 Distribution of pores in untreated and PEF-treated apple samples with conductive disintegration index in the range of 0.9–1 (n = 60 pulses, E = 1000 V/cm, t_i = 300 μs, and f = 1 Hz). Points represent averaged data from three determinations. Lines were obtained with least square polynomial fitting of experimental data.

electroplasmolysis. They concluded that electroplasmolysis affects not only plasmalemma membrane but also cell wall integrity of samples. Also, the failure stress decreased with intensification of electrical treatment.

Other authors have focused on inactivation of the enzymes that are responsible for the browning reaction observed in various fruits and vegetables (Ho et al. 1997, Giner et al. 2001, Giner et al. 2002, Aguiló-Aguyo et al. 2008). PEF treatments were applied on the enzyme extracts of apple, pear, tomato, and mushrooms. The strength of the electric field ranged from 2.4 to 35 kV/cm with 20 μs pulse width. Though the range of PEF parameters used in the studies differs from the PEF parameters used for the cell membrane permeabilization of plant tissues, valuable information can be drawn from the results.

16.6.3 Effects of PEF on Quality of Fruit Juices

Fruit juices are perceived as nutritive, healthy and, even, functional contributors to the human diet. The general consumer seems to imply that intake of some valuable nutrients, such as vitamin C, can be guaranteed by drinking any type of fruit juice on a regular basis. Consumers tend to choose any particular fruit juice due to its unique combination of sensory attributes, such as color, aroma, and flavor. Orange juice is said to be the most consumed juice worldwide while apple juice runs, apparently, in a stable second position. Tropical fruit juices are also in high demand, but tend to be more related to its use in mixtures of the cocktail type. Some other fruit juicy

products, like peach or mango nectar, may also be considered healthy and nutritive, but their availability is more limited globally, due to the smaller massive scale of the fruits used as their raw materials.

In statistics terms, citrus juices are the most popular fruit juices with more than 50% of the international commercialization volume of juices (Varnam and Sutherland 1999). Within citrus juices, orange juice represents an approximate 60% of all Western Europe consumption of juices and juice-based drinks and a similar amount (60%) of all fruit juice sales in the United States (Parker 2006b). Apple juice, on the other hand, represents roughly a 60% of the global production of orange juice (Parker 2006a).

According to the figures presented above, orange and apple juice would be considered the most important type of fruit juices and investment should be likely to occur in order to promote research and technology transfer to expand their markets. There might be, therefore, a vested interest in research groups and fruit growers to team up and try to obtain orange and apple juices of the highest possible quality to compete and gain consumer acceptance globally. Nutritive and sensory quality should be considered paramount in all technology transfer efforts, since fruit juices are the type of products in which the consumer associates freshness with quality. Research and development is also important and needs to be promoted for other type of juices, but the priority will be inevitably higher in the more demanded products.

Pasteurization of fruit juices has been traditionally performed by thermal processing either as a low temperature–long time (LTLT) treatment or a high temperature–short time (HTST) technique. UHT has been also employed for treatment of fruit juices. HTST pasteurization is a continuous process that shows several advantages over batch pasteurization, which is the conventional way of applying the LTLT method. In HTST, fresh juice flows through a holding tube or flow arrangement in which it is heated at a given temperature for a specific time. UHT processing, also known as aseptic processing, involves the production of a sterile product by rapid heating to high temperatures followed by a short holding time and ending with a rapid cooling. The processed product is filled into a presterilized container within a sterile environment, to provide a prolonged shelf life.

HTST pasteurization is, possibly, the most widely employed technique for heat treatment of orange and apple juices. In terms of industrial scale, HTST is normally carried out in a plate heat exchanger, which represents the advantage of having the stages of preheating, heating, holding, and cooling in a single passage. Commercially, orange juice is pasteurized by HTST at 90°C–95°C for 15–30 s (Braddock 1999), while apple juice is processed at 77°C–88°C for a 25–30 s by the same method (Moyer and Aitken 1980). Aseptic processing has gained popularity as a thermal pasteurization technique for both juices. Temperature and holding time in this case could be as much as 138°C for at least 2 s for both types of juice (Lewis 1993). Aseptic processing produces shelf-stable juices and other products with shelf lives as high as 8 months without refrigeration (Ellis 1982). There is, however, a typical cooked flavor detected in aseptically processed juices.

Thermal pasteurization is quite efficient for preventing microbial spoilage of fruit juices but the applied heat may also cause undesirable biochemical and nutritious changes, which may affect overall quality of the final product. Orange juice can undergo quality degradation due to microbiological and enzymatic activities and

chemical reactions (Chen et al. 1993). Spoilage microorganisms and native enzymes can be inactivated by thermal treatment, but thermal treatment causes the irreversible loss of fresh juice flavor (Braddock 1999) as well as a reduction of nutrients and the initiation of undesirable browning reactions in the juices (Chen et al. 1993). Thermal pasteurization of fruit juices can also cause undesirable changes, such as loss of vitamin content and color changes due to browning, mainly triggered by enzymatic reactions (Yeom et al. 2000).

With increasing demand to obtain processed foods with better attributes than have been available to date, food researchers have pursued the discovery and development of improved preservation processes with minimal impact on the fresh taste, texture, and nutritional value of food products. As previously discussed, fruit juices may present a series of undesirable effects when processed by conventional, thermal methods of pasteurization. Alternatives to traditional treatment, which do not involve direct heat, have been investigated in order to obtain fruit juices safe for consumption, but with sensory attributes resembling the fresh product. The first attempt to improve processed fruit juice quality could be considered, in fact, the variation of the temperature–time relationship in aseptic processing. As previously mentioned, such variations gave rise to a new generation of fruit juices with extended shelf life, which represents an obvious advantage of course, but some sensory impairment still remained due to the heat involved in the treatment. This is what could be considered as a "third generation" of processing alternatives, which seek to eliminate heat completely in pasteurization of fruit juices and other fluid foods such as milk and dairy products. PEF has already been successfully applied in treatment of fruit juices; the main developments are presented below.

16.6.3.1 Orange Juice

The flavor of orange is due to more than 200 chemical compounds (Maarse 1991), and is comprised of hydrocarbons, aldehydes, esters, ketones, and alcohols. Limonene is the most important flavor compound in quantity, although not in quality (Siezer et al. 1988). It has been reported (Ahmed et al. 1978) that acetaldehyde, citral, ethyl butyrate, limonene, linalool, octanal, and α-pinene are the major contributors to orange juice flavor. The development of off-flavors in orange juice has been attributed to the degradation of limonene to α-terpineol and other compounds (Tatum et al. 1975).

Thermal processing is conventionally used to pasteurize orange juice, but also reduces nutritional and flavor qualities and produces undesirable off-flavor compounds (Ekasari et al. 1986). The citrus industry has been exploring alternative methods with minimal heat treatment to increase markets by improving quality, and PEF may be one of such alternatives. The case for orange juice and its possible treatment with PEF, as a function of quality and stability, has been extensively studied at Ohio State University (Jia et al. 1999, Yeom et al. 2000). Effects of PEF (35 kV/cm for 59 μs) on the quality of orange juice were investigated. The PEF-treated juice was compared with juice pasteurized by heat at 94.6°C for 30 s. PEF pasteurization prevented the growth of microorganisms at 4°C, 22°C, and 37°C for 112 days and inactivated 88% of activity of the enzyme pectin methyl esterase. The juice treated by PEF retained greater amounts of vitamin C and some representative flavor compounds, than the juice pasteurized by heat during storage at 4°C. The PEF

pasteurized orange juice also presented lower browning index than the thermally processed juice. Pertaining sweetness, expressed as Brix, and pH, no significant difference was observed for any of the pasteurization methods.

In terms of specific flavor compounds, it was found that 40% of decanal was lost by heat treatment at 90°C for 3 min while no loss was observed by PEF treatment at 30 kV/cm, either at 240 or 480 μs (30). Octanal showed a loss of 9.9% for the heat treatment and 0% for any of the two PEF treatments. Some compounds suffered losses for the PEF treatments, but always in less proportion than the heat pasteurized juice. For example, 5.1% and 9.7% of ethyl butyrate were lost for the 240 and 480 μs treatments, respectively, but 22.4% was lost in the thermal process. The loss of these volatile compounds in orange juice treated by PEF was attributed to the vacuum degassing system of the PEF unit (Jia et al. 1999).

The advantage of PEF compared with thermal processing was also observed in nutritive aspects. PEF-treated orange juice retained a significantly higher content of ascorbic acid than heat pasteurized juice during storage at 4°C (Yeom et al. 2000). Although more research needs to be completed before considering PEF as the sole treatment to retain completely all flavor and color components of orange juice, it can be stated that PEF pasteurized juice retains more flavor and shows less browning than conventionally pasteurized juice. Under certain conditions, PEF-treated orange juice retains ascorbic acid better than heat-treated juice. All these findings are important and may prove invaluable for the adaptation of PEF as a real alternative for orange juice pasteurization.

16.6.3.2 Apple Juice

As stated earlier, apple juice is a popular beverage worldwide, which is perceived as a wholesome and nutritious product. Overall quality of apple juice is an important factor to consider in processing, since some attributes such as aroma, color, and flavor are appreciated by the final consumer and are associated with freshness and authenticity. Flavor components in apple juice are numerous, so flavor identification may be considered more complex than the correspondent to orange juice due to the aromatic nature of apples. Eight odor-active volatiles have been, however, identified as the most important contributors for the aroma–flavor authenticity of apple juice (Cunningham et al. 1986).

Some research on the application of PEF to pasteurize apple juice has been carried out. The use of PEF has achieved satisfactory microbial inactivation in several applications and has proved to be energy efficient also. An investigation of PEF inactivation has demonstrated that, to achieve a 7 log reduction in survivability of *S. cerevisiae* in apple juice, PEF utilized less than 10% of the electric energy for heat treatment (Qin et al. 1994). It has been also reported (Mittal 1998) that a PEF low energy pulser with an instant-charge-reversal pulse waveform was successfully used in apple cider treatment. The consumed energy was as low as 5.76 J/mL at 20°C, compared with the 50 J/mL normally required in conventional thermal processing. It has been reported that 6 log reductions in survivability of the indigenous aerobic bacteria of apple juice were obtained using PEF at 50 kV/cm. PEF has been compared directly with HTST in pasteurization of apple juice (Charles-Rodríguez et al. 2007) finding that PEF was efficient in microbial inactivation, as well as in preserving

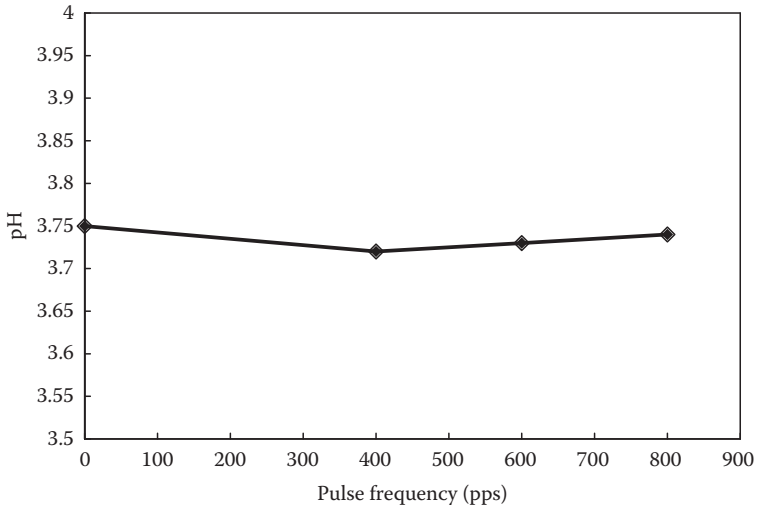

FIGURE 16.8 pH as a function of pulse frequency for treatment at 36 kV/cm in PEF-pasteurized apple juice. (Adapted from Charles-Rodríguez, A.V. et al., *Food Bioprod. Process*., 85C, 93, 2007.)

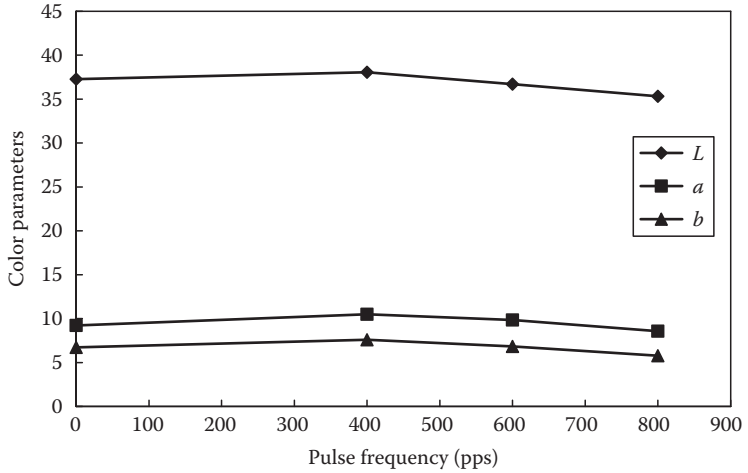

FIGURE 16.9 Color parameters as a function of pulse frequency for treatment at 36 kV/cm in PEF-pasteurized apple juice. (Adapted from Charles-Rodríguez, A.V. et al., *Food Bioprod. Process*., 85C, 93, 2007.)

some quality attributes, such as pH and color (Figures 16.8 and 16.9). HTST, on the other hand, had apparent effects on pH and browning increase.

Comparative studies of PEF and other nonthermal techniques have been also reported. In a particular investigation (Zárate-Rodríguez et al. 2000), PEF and ultrafiltration (UF) were utilized for pasteurization of apple juice. No significant changes were observed in variables such as pH, Brix, and acidity, expressed as malic acid, for the PEF-treated juice and the ultrafiltered one. Color was the quality attribute

that did show change for membrane treatments. The observed trend was for the juice to become darker as a function of applied transmembrane pressure. Similarly to UF treatments, relative color changes were observed but the registered effect was opposite, i.e., the treated juices became paler as a function of applied field strength. Color changes were independent of pulse number but dependent on field strength. The different color ratio perception in UF and PEF-treated juices could be due to haze formation, which may be caused by tannins, proteins, and carbohydrate polysaccharides. It has been reported that proteins, independently and in association with phenols, are responsible for fruit juice turbidity, as well as for postclarification haze and sediment formation (Flores et al. 1988). Haze is formed quite often by interaction of these biochemical compounds in many types of fruit juices. Since enzyme inactivation capability of PEF has been reported (Vega-Mercado et al. 1995) a tannin–protein or carbohydrate–protein bonding would not be possible due to the lack of the protein fraction, and little or no haze formation would be likely to occur in the PEF-treated juice. On the other hand, in the UF pasteurized juice the presence of proteins may have caused an incipient haze formation, because all the interactions mentioned above would be important. In such a case, haze or turbidity might have been registered as a deviation to the darker side of the specific absorbance value considered as the control or reference. The observed browning in the UF processes is considered a quality problem, but there is some evidence that might be controlled by membrane pore size effects (Zárate-Rodríguez et al. 2000).

Direct effects of PEF on volatiles of apple juice, and comparison with a conventional thermal treatment, have been also been investigated (Aguilar-Rosas et al. 2007). PEF and HTST were tested in order to determine decrease in concentration of eight odor responsible volatiles. In general terms, PEF retained better most of the volatile compounds responsible for color and flavor of the apple juice. For example, as shown in Table 16.3, hexanal and hexyl acetate were only lost in 7% and 8.4%, respectively, when using PEF, while they were virtually lost by HTST. Also,

TABLE 16.3
Percentage of Volatiles Losses, Compared with Untreated Sample, in Apple Juice Treated by Two Methods

Compound	Loss Percentage for PEF	Loss Percentage for HTST
Acetic acid	39.792 ± 20.84	100
Hexanal	7.042 ± 9.32	62.348 ± 5.35
Butyl hexanoate	18.108 ± 7.72	36.273 ± 24.86
Ethyl acetate	77.458 ± 29.23	67.126 ± 39.33
Ethyl butyrate	60.190 ± 17.80	88.398 ± 12.46
Methyl butyrate	30.081 ± 31.37	51.200 ± 19.56
Hexyl acetate	8.408 ± 16.12	22.910 ± 21.99
1-hexanal	14.101 ± 7.65	69.307 ± 5.62

Source: Adapted from Aguilar-Rosas, S.F. et al., *J. Food Eng*., 83, 41, 2007.
Note: Differences by a Student *t*-test for independent samples ($p < 0.05$, $n = 3$).

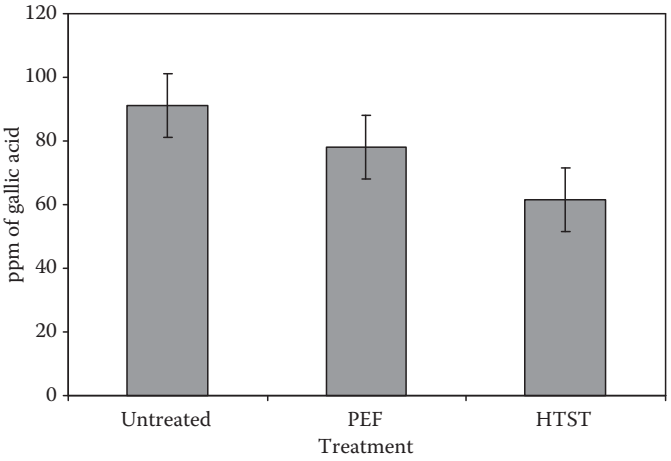

FIGURE 16.10 Effect of treatment method on total phenol compounds of pasteurized apple juice treated by HTST pasteurization and PEF technique. (Adapted from Aguilar-Rosas, S.F. et al., *J. Food Eng.*, 83, 41, 2007.)

important biochemical substances in apple juice, such as phenol compounds, were better retained by PEF than by HTST treatment (Figure 16.10).

16.6.3.3 Other Types of Fruit Juices

Reports on some other types of juices are not abundant in the literature. Peach nectar was pasteurized by PEF (Gutierrez-Becerra et al. 2002). The effects of field strength and pulse frequency were evaluated for reduction in microbial count, as well as for variation in pH and color of the treated juice. An appropriate microbial inactivation was shown, while no significant changes were observed in pH and color. The results obtained suggest that PEF may be used as alternative for peach juice pasteurization, to preserve quality and sensory attributes.

16.7 OPTIMIZATION OF PEF PARAMETERS

As obvious from the preceding discussion, pertinent parameters of PEF from the point of view of processing include pulse characteristics, electric field strength, and treatment time. The pulse characteristic factors include waveform, frequency, width, and number. Also microbial and product characteristics and design of treatment chamber may influence effectiveness of PEF treatment since it determines distribution of electric fields across product. Successful application of PEF for a product depends on rational and innovative optimization of these parameters. PEF applications allow for better control of electric power input and effective permeabilization of cellular membranes without significant temperature elevation (Weaver and Chizmadzhev 1996). In general, the transmembrane voltage u_m induced on the cell membrane due to an external electric field is given as (Zimmermann 1986)

$$u_m \sim \alpha d_c E \cos\theta \tag{16.9}$$

where

d_c is cell diameter
E is electric field strength
θ is the angle between a point on membrane surface and direction of electric field strength
α is a parameter depending on the cell shape ($\alpha = 0.75$ for spherical cell and $\alpha = 1$ for rectangular cell)

The smaller the exposed cells are in the electric field, the higher the field strength required for creating a critical transmembrane potential needed for the cell membrane's plasmolysis. Mean diameters of microorganisms and biological tissue cells are in the ranges of $10\,nm^{-1}$ to $1\,\mu m$ and $10\,\mu m^{-1}$ to 1 mm, respectively (Aguilera and Stanley 1999). Therefore, understandably high electric field pulses with voltages in the range of 20–50 kV/cm are used to kill microorganisms for PEF pasteurization. However, for solid materials such as vegetables tissues, for which cells are usually larger than microbial cells, electroplasmolysis can be obtained at much lower electric field strengths. A number of publications report that electroplasmolysis of vegetable cells may be achieved at moderate electric field pulses with voltages in the range of 0.3–3 kV/cm (Ngadi et al. 2001). Lower electric field pulses in the range of 0.1–0.3 kV/cm have also been reported (Kupchik et al. 1998).

It has been established experimentally that the electrical treatment time needed for electroplasmolysis is inversely proportional to electric field strength; the higher the field strength, the less specific energy consumption needed for achieving the same degree of plasmolysis (Lebovka et al. 2000). In liquids, the extent to which electric field strength can be increased is limited by dielectric breakdown of the products and uncontrolled temperature increase in the products. Therefore, it is vital to balance the need for higher electric field with product response. Different authors have reported different effectiveness of PEF application for various products. There are currently inherent practical difficulties involved in optimizing PEF processing of foods. It is difficult to compare treatment results obtained by different authors using different PEF systems. PEF systems are very expensive and it has not been possible to build systems that allow wide variation of pulse parameters. Therefore, studies have been conducted using equipment with restricted range of parameters.

Two experimental methods have been proposed for determination of the optimal field strength E for PEF treatment of different solid food tissues. One of the methods is based on estimation of the characteristic damage times τ and the total energy consumption factor during treatment τE^2 as a function of electric field strength (Lebovka et al. 2002). The other approach is based on estimation of the maximal change of sample disintegration index caused by the energy input during each pulse (Bazhal et al. 2003a). The optimal E value for electroplasmolysis depends on the type of tissue and is higher for cells with developed secondary cell wall. The overall goal of PEF treatment objective (for instance microbial inactivation in liquid medium vs. textural enhancement in solid medium), the material to be treated and all pulse parameters must be taken into consideration in order to optimize PEF treatment for a product.

16.8 CONCLUSIONS

PEF applications hold several promises for modifying and improving quality and safety of foods. In particular, since it is essentially a nonthermal technology, PEF treatment can be used to minimally process products and preserve their delicate sensory and nutritional qualities. Although impressive progress has been made in understanding the mechanism and influence of PEF processing, much remains to be done. The process parameters need to be optimized for specific products to match equipment used. There is need to develop a common equipment-independent platform for assessing PEF processing.

The use of PEF technology to pasteurize a wide variety of foodstuffs and obtain high-quality, competitive products in world markets can be considered a plausible reality. Scientific validation of pasteurization efficiency has been carried out and the technique renders results comparable with the conventional, thermal way of pasteurizing many food products. Safety is definitely preserved, while quality appears virtually unaltered by use of PEF. The engineering challenge of making equipments commercially viable has also advanced. It can be, therefore, considered that the technology is ready for industrial scale application and that a number of PEF-pasteurized food products will be seen in supermarket shelves in a foreseeable future.

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