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Review

Application of pulsed electric field in non-thermal processing of milk

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Abstract

Pulsed electric field (PEF) is an innovative non-thermal technology which could be used as an alternative to the traditional thermal process to inactivate the microorganisms and enzymes in liquid foods such as milk. Compared to thermal processing, the PEF process is considered more energy efficient as the microbial or enzymatic inactivation is achieved at ambient or mild temperatures by the application of short bursts of high intensity electric fields to liquid food flowing between two electrodes. Extensive international research has been conducted since the 1990s on the development of PEF in the food industry. This article reviews the recent findings on the application of PEF technology in milk processing, the mechanisms and factors affecting microbial and enzymatic inactivation by PEF treatment and the application of PEF in combination with antimicrobials.

Keywords: Pulsed electric field (PEF), nonthermal processing, microbial inactivation, enzymatic inactivation

Introduction

Pulsed electric field (PEF) is largely a non-thermal process that is able to inactivate microorganisms and enzymes to some degree in liquid food such as milk and fruit juice and is reported to have minimum adverse effects on the sensory attributes of these products. The PEF process is considered to be energy efficient since the microbial inactivation is achieved at ambient or moderately elevated temperatures by the application of short bursts of high intensity electric fields to liquid food flowing between two electrodes. A large flux of electrical current in only short bursts flows through the food materials (e.g. milk or fruit juice), which are electrical conductors due to the presence of electrical charge carriers [1, 2]. Bendicho [3] in a review article and Barbosa-Canovas [4] in a chapter of their book have provided a history of PEF technology development and its application in non-thermal processing of liquid food. A comprehensive article has also been published by Gaudreau [5]

on a commercial PEF system and its components and application. However, in our review the main emphasis is on the most recent developments on dairy applications of the PEF technology.

An Overview of PEF Technology

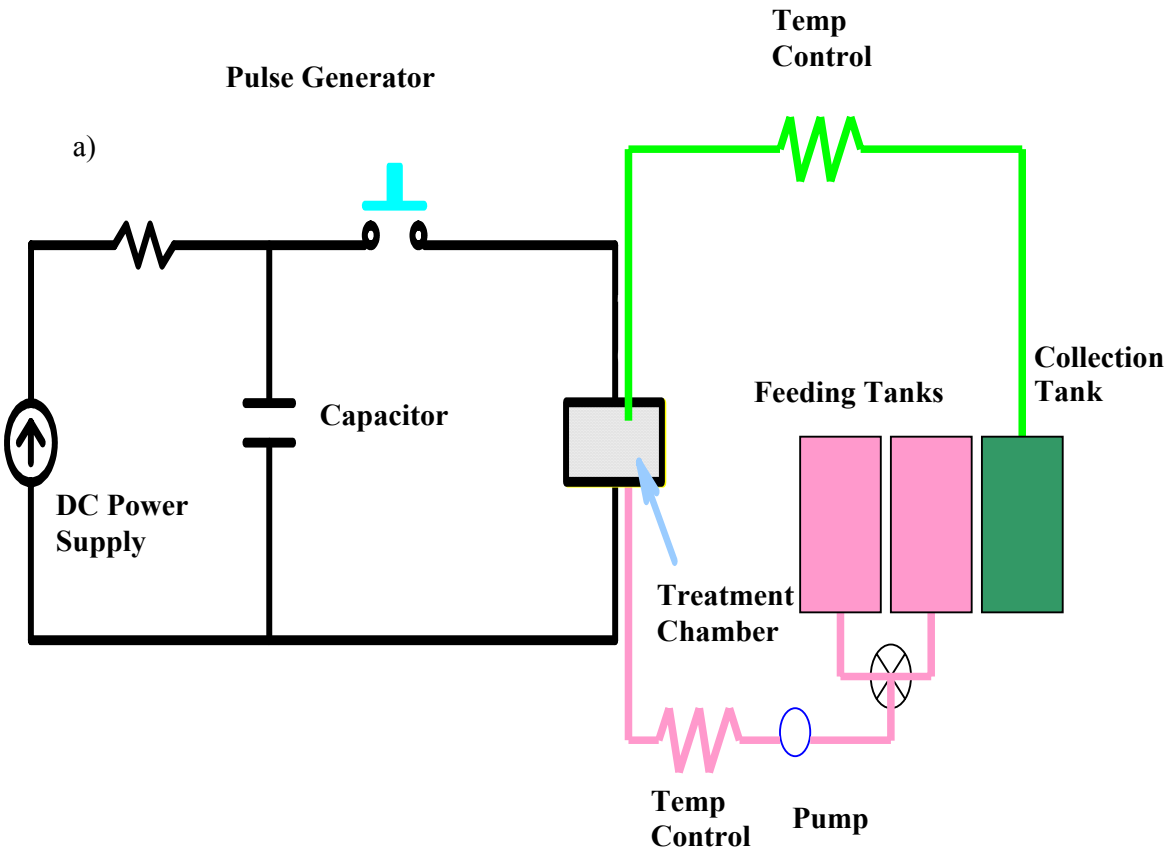
Thermal processes used for food preservation can alter the nutritional and sensory qualities of the food [6]. High-temperature short-time (HTST) pasteurisation has been effectively used for decades as a method of choice to extend the shelf life of milk and to inactivate its pathogenic bacteria [7]. However, it can affect the organoleptic and nutritional properties of milk to varying degrees [8]. In addition, the quest for energy conservation by the manufacturers along with the increasing consumer demand for fresh-like quality food has given rise to the development of non-thermal food preservation processes including ionizing radiation, high-intensity light pulses, high pressure, electric or magnetic fields, antimicrobial chemicals, polycationic polymers, lytic enzymes, as well as pulsed electric field [6, 9, 10].

PEF system

A typical PEF system consists of the following components: a high-voltage power supply, a pulse generator, a number of energy storage capacitors, a treatment chamber (either static or continuous) that houses the electrodes, a pump to pass the liquid food through the treatment chamber (if the system is continuous), cooling and heating baths, measurement devices (voltage, current and temperature), and a central process unit to control operations (Figure 1a & b). The treatment chambers are separated from each other by insulators, which may be ceramic or polymer. Each chamber has two electrodes through which the liquid flows and is exposed to field intensity.

Electrodes

The electrodes are placed inside the treatment chamber at various positions through which the flowing liquid is exposed to the field intensity. The electrodes are made from electro-chemically inert materials such as carbon, stainless steel, titanium, gold, platinum or metal oxides and need to be replaced at least every 100 h of operation due to chemical erosion caused by the flowing liquids and deposits formation on their surface [1]. Kitajima [11] developed a textile electrode made from a combination of polyester fibre, tungsten wire and titanium wire which is reported to be resistant to chemical erosion. Figure 2 shows the positioning of electrodes inside the treatment chambers. The gap between the electrodes determines the intensity of electric field and the average electric field strength (E) is calculated by dividing the peak voltage (kV) by the gap distance (in cm) between the electrodes (d) [1].



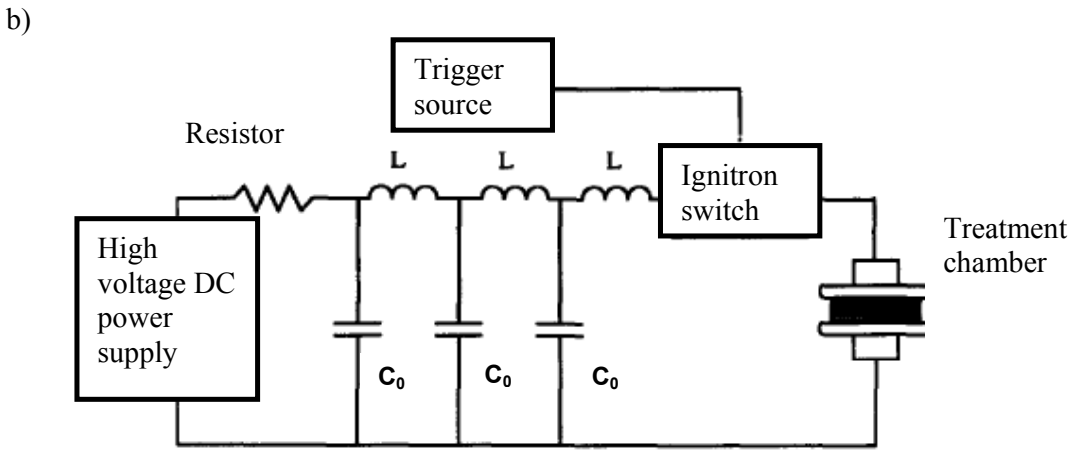


Figure 1 a): Schematic Diagram of a Pulsed Electric Field System; b) Layout of a square pulse generator using a pulse-forming network of 3 capacitors (C_0) and 3 inductors (L) units [1].

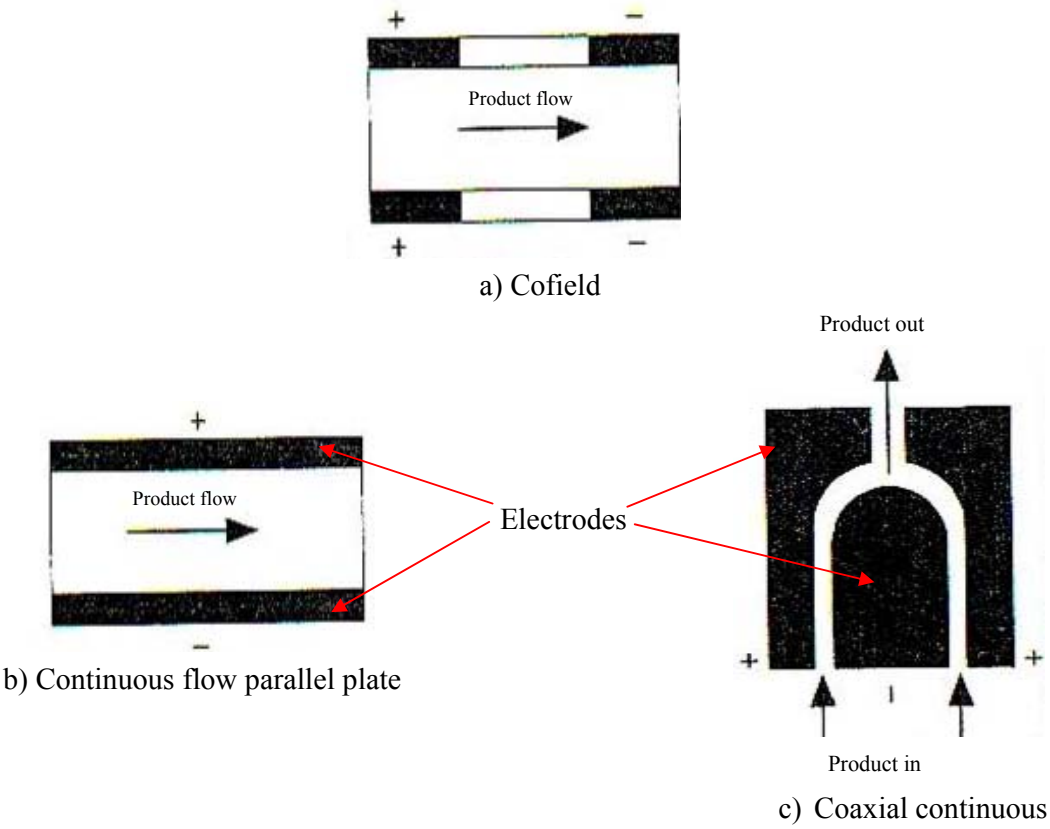


Figure 2: Diagrammatic representation of the PEF treatment chambers [12].

Treatment chambers

Over several decades, static or continuous treatment chambers have evolved and various researchers have designed and custom-made different types of chambers for various products [13]. Figure 3 shows a classification of the static and continuous-flow treatment chambers for pulsed electric field treatment. Dunn and Pearlman [14] designed a cross-field chamber where the electric field is perpendicular to fluid flow, while Martin-Belloso [15] designed a parallel-plate chamber. However, the cofield chambers in which two stainless steel tubes are separated by an insulator and the electric field and the food flow concurrently, are more commonly used (Figure 2a). The cofield designs are more reliable since the chance of electrode erosion is reduced. Furthermore, the chance of local bubble formation that may result in partial discharges is also reduced in this chamber [16].

A static chamber designed by Barbosa-Cánovas [17] consisted of 2 round-edged, disk-shaped stainless steel electrodes with an effective electrode surface area of 27 cm^2 and Polysulfone or Plexiglass insulation material. The gap between electrodes could be adjusted to either 0.95 or 0.5 cm and the chamber could deliver field strengths up to 70 kV/cm and was cooled by water at pre-selected temperatures through jackets built into the chamber.

In continuous flow PEF treatment chambers the liquid food is pumped through pulsing electrodes and is therefore more suitable for large-scale operations. A continuous flow co-field PEF chamber was developed by Yin [18] in which the electric fields were enhanced by using conical insulators. In this chamber the voltage across the treatment zone was almost equal to the supplied voltage (Figure 2b).

A coaxial chamber is basically composed of an inner cylinder surrounded by an outer annular cylindrical electrode that allows food to flow between them (Figure 2c). This treatment chamber has been successfully used in the inactivation of pathogenic and non-pathogenic bacteria, moulds, yeasts and enzymes present in liquid food such as fruit juice, milk and liquid egg pulp [19].

Treatment chambers of a few millimetres in diameter or length are used for laboratory research, whereas pilot scale PEF treatment chambers reach a typical size of a few centimetres. A comprehensive description of treatment chambers geometry and application has been given by Fox [16]. Aspect ratio of the treatment chambers (the ratio of length to diameter of cylindrical chamber) should be larger than 1.5 to guarantee a uniform treatment of all passing fluids [20, 21].

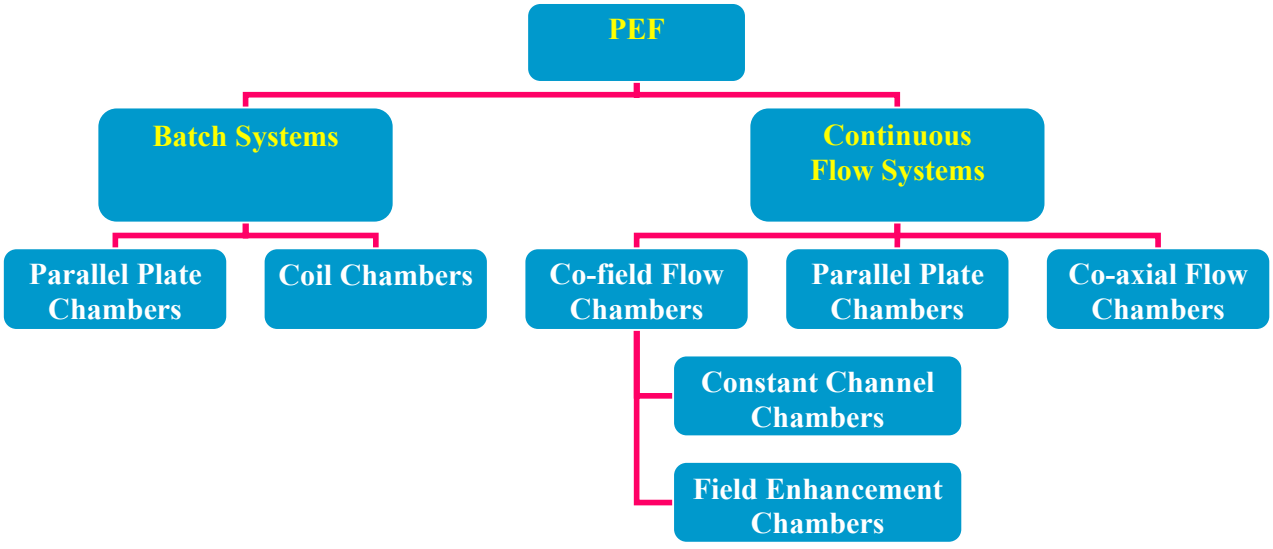


Figure 3: Classification of the static and continuous-flow treatment chambers for pulsed electric field treatment.

Pulse generator

A pulse generator is made up of a high voltage power supply, capacitors, inductors, rectifying circuits and discharge switches and is capable of producing various types of wave shapes such as square or exponential. The capacitors store the energy and release it into the food through an electric discharge switch which turns on and off rapidly. The combination of various capacitors provides for greater storage of energy (Figure 1b). Many devices may be used as the discharge switch including a mercury ignitron spark-gap, a gas spark gap, a thyratron, a magnetic switch or a mechanical rotary switch [1]. A PEF system can be designed as either bipolar (+ and – voltage pulses) or monopolar (all + or all – pulses) [5].

Mechanism of Microbial Inactivation by PEF: Electroporation and Electrical Breakdown

The mechanism underlying the inactivation of microorganisms by PEF is yet to be fully understood and knowledge of the microbial inactivation mechanism is essential in order to design and develop more efficient PEF equipment and define conditions for effective inactivation of microorganisms in food products [22, 23, 24]. According to Sale and Hamilton [25] membrane damage is the direct cause of cell inactivation. The inactivation of microorganisms is related mainly to the changes in the cell membrane and its electromechanical instability [26, 27].

Two mechanisms have been proposed for the mode of PEF action on microbial membrane: electroporation and electrical breakdown. However, both mechanisms are in fact referring to a phenomenon starting by electroporation resulting in electrical breakdown by which the cell wall is perforated and cytoplasm contents leak out resulting in cell death. The electroporation theory suggests that the main effect of an electric field on microbial cells is to increase the membrane permeability due to membrane compression and poration and cell inactivation results from osmotic imbalance across the cell membrane [28]. Figure 4 shows the mechanism of electrical breakdown and cell poration which was initially proposed by Zimmermann[29]. He suggested that the membrane can be considered as a capacitor filled with a dielectric medium.

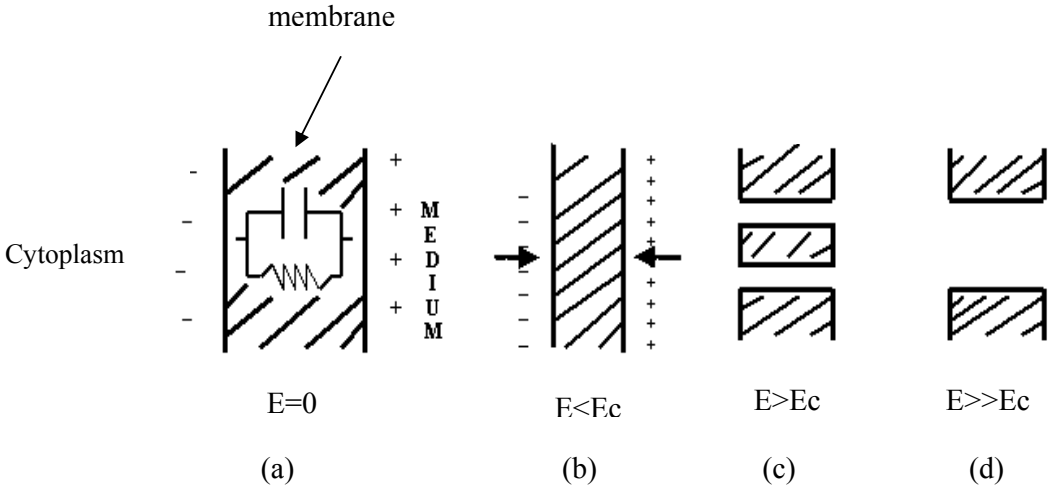


Figure 4: Schematic diagram of reversible and irreversible breakdown of a microbial cell indicating compression by electroporation when exposed to electric field. The membrane acts as a capacitor and is represented by hatched area. Ec is the critical electric field (a) Intact cell membrane; (b) membrane compression; (c) pore formation with reversible breakdown; (d) irreversible breakdown with large pores formation [29].

Based on this theory, when the transmembrane potential is exposed to a higher external field intensity this results in membrane damage. According to Chen and Lee [30] the membrane of a biological cell insulates the shell from cytoplasm while the electrical conductivity of the cytoplasm is 6 to 8 times greater than conductivity of the membrane. When the cell is exposed to an electric field, the free charges generated on the membrane surface are attracted to each other due to the difference in the signs (- and +) which causes a compression pressure resulting in a decrease in membrane thickness (Fig. 4b). Increasing the field intensity leads to more accumulation of surface charges, resulting in a higher electromechanical stress and reversible breakdown of membrane (Fig. 4c) [29, 31]. The membrane thickness decreases by increasing the field intensity which eventually results in an irreversible breakdown through creating larger pores in the membrane (Fig. 4d). If the area of the pores in relation to the membrane surface becomes larger, an irreversible breakdown occurs in the membrane leading to the total destruction of the cell [29]. In large cells the induced potential is greater which makes them more vulnerable to damage compared to smaller cells [30].

Osmotic imbalance is a theory through which the electroporation and electrical breakdown has been described (Figure 5). The cell exposed to an external electric field is "electroporated" through the leakage of ions and small molecules and thus the membrane becomes permeable to water that causes swelling and eventual rupture (electrical breakdown) and lysis of the cell. Therefore, based on the above observations it could be concluded that the inactivation of cells follows a sequence of a primary electroporation with small pores on the cell membrane followed by a secondary electroporation with larger pores which finally causes electrical breakdown and cell lysis. Large pores are obtained by increasing the intensity of the electric field and pulse duration or reducing the ionic strength of the medium [32].

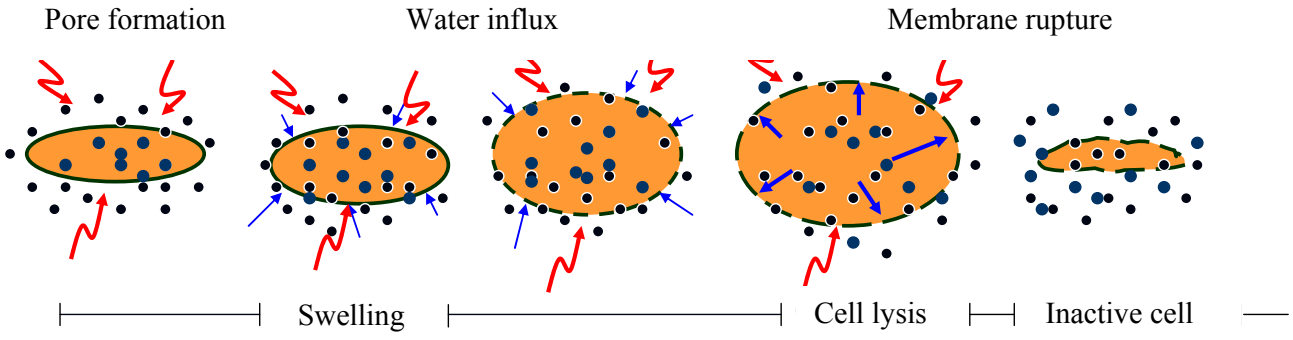


Figure 5: Stages of electroporation in a cell membrane through osmosis (Redrawn from [28]). The red arrows show the field intensity and blue dots are water molecules.

Castro [10] further explained electroporation as a phenomenon in which the high voltage electric field temporarily destabilizes the lipid bilayer and proteins of cell membranes. The plasma membranes thus become permeable to small molecules that cause swelling and eventual rupture of the membrane. Vega-Mercado [33] proposed that the main effect of the electric field on bacterial cells was to increase membrane permeability due to membrane compression and poration. Kinosita and Tsong [31] demonstrated that an electric field of 2.2 kV/cm induced pores of *ca.* 1 nm in diameter in human erythrocytes. They suggested a two-step mechanism for pore formation in which the initial perforation is a response to an electrical potential greater than the Ec (critical field intensity) followed by a time-dependent expansion of the pore size. Large pores are obtained by increasing the intensity of the electric field and pulse duration, or by reducing the ionic strength of the medium as shown in Figures 4 and 5.

Pothakamury [34] treated a suspension of *Staphylococcus aureus* in simulated milk ultrafiltrate (SMUF) by 64 pulses of 20, 30 and 40 kV/cm and scanning electron microscopic (SEM) examination showed rough surfaces and small pores in the membrane which led to the leakage of cellular contents. This finding was confirmed by Aronsson [35] who studied *Escherichia coli, Listeria innocua, Leuconostoc mesenteroides* and *Saccharomyces cerevisiae* by means of SEM examination and found a clear difference between untreated and PEF-treated cells (25-35 kV/cm, 20-40 pulses of 2-4 μs).

Parameters Affecting Microbial Inactivation in PEF Treatment

Various parameters affect microbial inactivation by PEF treatment including the type of microorganisms, field intensity, pulse wave shape, conductivity of the medium, the pH, treatment temperature, treatment time (flow rate and pulse frequency) and energy input.

Types of microorganisms

Gram-positive bacteria are more resistant to PEF treatment than Gram-negative ones [36] as they are covered in a thick multilayered peptidoglycan layer. Yeasts are more sensitive to electric fields than bacteria due to their larger size, although at low electric fields they were found by Sale and Hamilton [25] and Qin [37] to be more resistant than Gram-negative bacteria. Some of the early publications on microbial inactivation by PEF treatment are compiled by Barbosa-Canovas [17] and Barbosa-Canovas and Zhang [38].

Growth stage of microorganisms is another factor to be considered in PEF treatment. In general, logarithmic phase cells are more sensitive to stress than lag and stationary phase cells. Microbial growth in logarithmic phase is characterised by a high proportion of cells undergoing division, during which the cell membrane is more susceptible to the applied electric fields [36]. Gaskova [39] reported that the inactivation effects of PEF in the logarithmic phase was 30% greater than in stationary phase.

Field intensity

The intensity of the electric field applied to a medium (e.g. milk or fruit juice) is one of the main factors influencing microbial inactivation [40]. When the cells are exposed to an external electric field with sufficient strength, the field induces the accumulation of electric charges at the non-conductive microbial membranes [41]. Heinz [42] showed that the critical external field strength is highly dependent on the cell size as well as the field orientation. Smaller cells require higher field strength for inactivation. The orientation of the rod-shaped cells along or across the electric field also affects the required field intensity [32]. According

to Toepfl [41], *Listeria innocua* requires a minimum of 15 kV/cm to become inactivated while the larger cells of *S. cerevisiae* are affected at field strength as low as 2-4 kV/cm.

Pulse wave shape

Exponentially decaying and square wave pulses (in bipolar or monopolar form) (Figure 6) are the most common wave shapes used in PEF systems. An exponentially decaying wave is a unidirectional voltage that rises rapidly to a maximum value and decays slowly to zero. Exponential decaying pulses have a long tail with a low electric field, during which excess heat is generated in the food without bactericidal effects.

Square wave pulses maintain a peak voltage above the critical field strength for a longer period of time and are more energy efficient and more lethal than exponentially decaying pulses. However, exponentially decaying pulses are easier to generate and change in direction than square pulses [1]. Bipolar pulses are often reported to be more lethal than monopolar pulses as it is believed that the reversal in the orientation or polarity of the electric field results in a corresponding change in the direction of charged molecules on microbial cell membrane leading to further damage [43, 44]. Bipolar pulses have other advantages such as minimum energy utilisation, reduced deposition of solids on the electrode surface and decreased food electrolysis [17]. However, Beveridge [45] applied 1500 monopolar (2 µs duration) and bipolar (1 µs duration) pulses of 30 kV/cm to *Escherichia coli*, *Listeria monocytogenes* and *Bacillus cereus* suspended in peptone water with various peptone concentrations and hence conductivities and found that monopolar pulses reduced the number of *E. coli* by 3 logs while the reduction resulting from bipolar pulses was less than 1.5 logs. For *L. monocytogenes* the inactivation rates with mono- and bipolar pulses were 1.5 and 0.5 logs, respectively and for *B. cereus* the inactivation rate was about 5 logs for both waves.

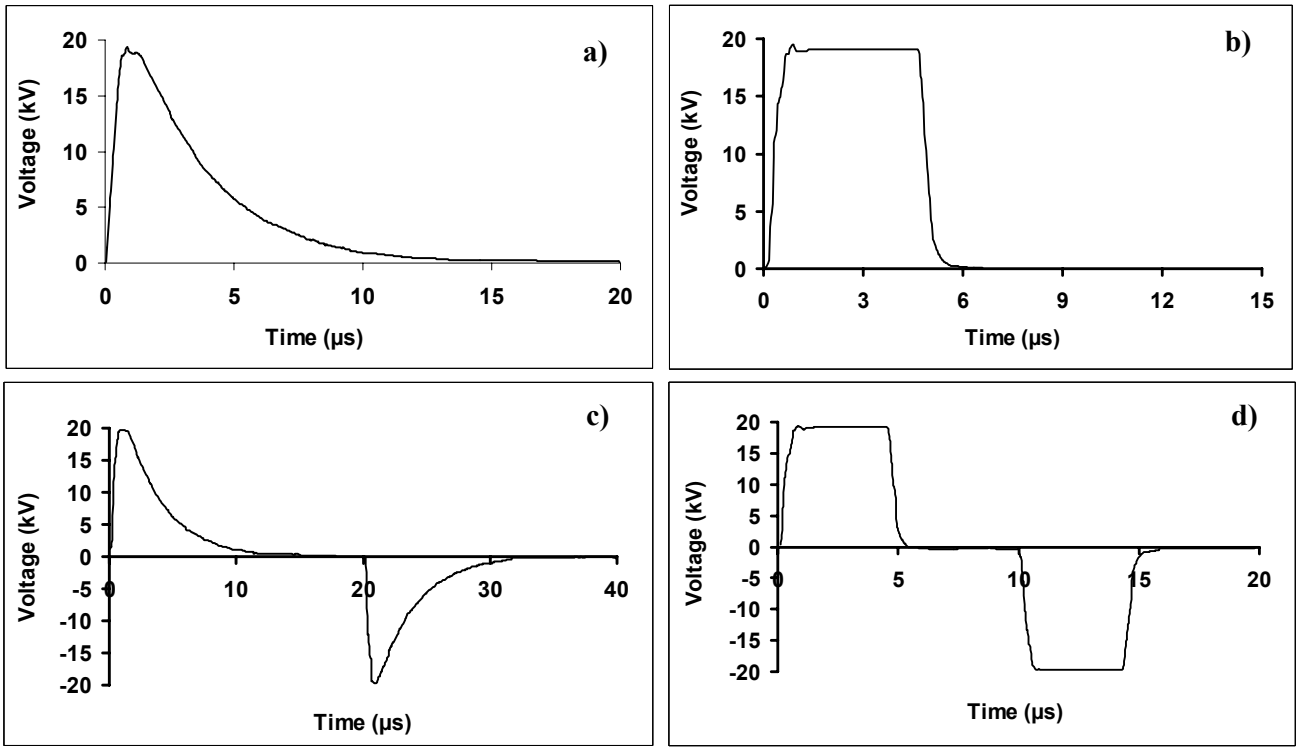


Figure 6: Pulse waveshapes commonly used in PEF technology: (a) Exponentially decaying pulse, (b) Square pulse, (c) Bipolar exponential, (d) Bipolar square, (e) Instant charge reversal [4].

Treatment time and energy input

Treatment time *t* is an important factor in inactivation processes and is defined as the product of number of pulses by the pulse width (μs) and can be achieved in PEF by either changing the flow rate or the pulse frequency. It is calculated as: $t = N_p \times N_c \times W_p$ where N_p is the number of pulses, N_c is the number of treatment chambers and W_p is the pulse width. The number of pulses is calculated from the following formula:

$N_p = T_r$ (residence time, s in each chamber) $\times f$ (pulse frequency, Hz). $T_r = V/F$ where V is the volume of each chamber (mL) and F is the flow rate (mL/s) [46]

Energy input Q into the food for square pulses is calculated as: $Q = vIP_w/V$. Where v and I are the voltage and current of the square pulses, respectively. For exponential pulse waves Q is calculated from: $Q = V_o^2C_oN_p/2V$ *or* $V_o^2t/2RV$ where C_o is the capacitance of the energy storage capacitor, V_o is the initial charge voltage and R is the effective resistance in the circuit [1].

Treatment temperature

Temperature is an inseparable part of PEF treatment since when the electric field is applied to the liquid flowing between the electrodes its temperature rises. Therefore, proper cooling of the product (by using cooling water bath) between the treatment chambers is necessary to avoid heat damage and to limit microbial inactivation only to the PEF effect [33]. Some researchers have shown that PEF treatments at moderate temperatures (50 to 60°C) exhibit additive effects on the inactivation of microorganisms and their spores [14, 47, 48, 49, 50]. In a study conducted by Jayaram [51] the temperature increase from 24 to 60°C enhanced the inactivation of *L. brevis* significantly (by about 6 logs) in a phosphate buffer solution. Dunn and Pearlman [14] observed an increase in the inactivation of *S. dublin* in milk from 1 to 4 logs when increasing the temperature from 40 to 50°C.

Effect of pH

Vega-Mercado [52] found that the PEF-inactivation of *E. coli* in SMUF was greater at pH 5.7 than at pH 6.8. Liu [53] demonstrated that field intensity and benzoic or sorbic acid at pH 3.4 had a synergistic effect on inactivation of *E. coli* O157:H7. The control sample contained 7.19 log CFU/mL bacteria before treatment which was decreased to 4.26 using benzoic acid and to 4.86 log CFU/mL in the presence of sorbic acid after treatment at 25°C with 12.5 kV/cm. Presence of benzoic or sorbic acid (1000 ppm) in the suspending medium at pH 3.4 without field intensity decreased the count by 1.9-2.5 and 0.6-1.1 logs, respectively. Wouters [54] also showed that the PEF treatments of *L. innocua* in phosphate buffer were more effective when the pH of the buffer was lowered from 6.8 to 5.0. However, since the above acids are preservatives, it is possible that the inactivation levels achieved were due to not only the combined effect of pH and PEF treatment but also the antibacterial effects of the acids.

Conductivity and ionic strength of the medium

The electric conductivity of a medium (σ, Siemens/m or mS/cm) is an important parameter in PEF treatment. In food with high electrical conductivities, electric fields with smaller peaks are generated across the treatment chamber which do not have the required killing effects [55]. An increase in ionic strength of a liquid leads to increased conductivity, resulting in a decreased microbial inactivation level. Furthermore, large conductivity difference between the medium and microbial cytoplasm weakens the membrane structure due to an increased flow of ionic materials across the membrane. Thus, the inactivation level of microorganisms increases with decreasing medium conductivity [56].

Different media have been used in PEF studies such as distilled water. Simpson [57] found that using different media can affect the resistance of microorganisms treated with PEF. For instance, *Salmonella Typhimurium* (CRA 1005) was reported more sensitive than *Listeria monocytogenes* (NCTC 11994) to PEF treatment in distilled water (10, 15 and 20 kV/cm), 10 mM Tris–maleate buffer, pH 7.4 (15 kV/cm) and model beef broth (0.75%, w/v; 15 kV/cm). Therefore, the medium and its properties such as pH and conductivity influence the efficacy of PEF treatment [58].

Several research groups have reported that by decreasing the conductivity of the treatment media it is possible to increase the inactivation level of *L. brevis*, *E. coli*, *S. cerevisiae*, *S. dublin* and *L. innocua* [51, 54, 56, 59, 60]. However, Alvarez [61] reported that conductivity did not influence the inactivation of *S. Senftenberg* in citrate-phosphate buffer with pH of 7 while Gaskova [39] found that the inactivation of *S. cerevisiae* was inversely related to the medium conductivity. The differences in reported results arise from the different PEF parameters employed, using various media with different chemical properties and conductivities.

The effectiveness of PEF treatment may be affected by the type of cations present in the medium. Hülsheger [62] treated *E. coli* K12 suspended in different electrolyte solutions of identical conductivities at pH 6-8 with 10 pulses at field intensity of 12 kV/cm and demonstrated that bivalent cations such as Mg^{+2} or Ca^{+2} had a protective effect on membranes by reducing the sensitivity of treated cells, thus resulting in reduced PEF damage. However, no such protective effects were found with monovalent cations Na^{+} and K^{+}.

Microbial Studies on PEF-treated Milk and SMUF

The bulk of research activities reported in the literature have focused on the impact of PEF treatment on microbial and enzymatic inactivation in milk or SMUF. The SMUF is a salt solution with composition similar to milk ultrafiltrate. It was proposed by Jeness and Koops [63] and is now widely used in dairy-related research [64]. The level of microbial inactivation has been found to be mainly dependent on the electric field strength, number of pulses applied during the process and treatment time [19, 33, 64, 65].

Various studies published on treatment of milk by PEF have proven this technology as an effective method for the inactivation of moulds, yeasts and vegetative bacterial cells. The microorganisms inactivated by PEF belong to the major G+ and G- bacteria [1, 2, 36, 43, 53, 56, 60, 66, 67, 68, 69, 70]. Various researchers have reported 1 to 6 logs inactivation of different strains of *E. coli* (pathogenic and non-pathogenic) in milk (UHT, skim, whole, partially skim), egg pulp, pea soup, apple juice, SMUF, 0.1% NaCl saline, phosphate buffer (pH 7.0) and sodium alginate [2, 25, 40, 53, 66, 71, 72, 73, 74].

Dutreux [75] PEF treated *E. coli* and *L. innocua* suspended in pasteurised skim milk and in phosphate buffer (with similar pH and conductivities) with inlet and outlet temperatures of 17°C and 37°C, a flow rate of 0.5 L/min, frequency of 3 Hz and field intensity of 41 kV/cm. The number of surviving organisms was determined after the application of 0, 3, 10, 20, 35 and 60 pulses (pulse width unknown). Transmission and scanning electron microscopy was used to examine *E. coli* cells subjected to 60 pulses. Changes in the cytoplasm were observed and the cell surface appeared rough. The cells' outer membranes were partially destroyed allowing leakage of the cytoplasm and changes in the cytoplasm were observed.

Rowan [47] investigated the influence of treatment temperature and PEF intensity on the viability of *Mycobacterium paratuberculosis* cells suspended in 0.1% (w/v) peptone water and in sterilised cow's milk. The viability was assessed through direct viable counts and transmission electron microscopy (TEM). Treatment at 50°C with 2,500 pulses of 5 Hz and 30 kV/cm reduced the number of viable *M. paratuberculosis* cells by approximately 5.3 logs in 0.1% peptone water and 5.9 logs in cow's milk, while PEF treatment at 5°C reduced the cells only by 1.6 logs. Heating alone at 50°C for 25 min or at 72°C for 25 s resulted in 0.01 and 2.4 logs reduction, respectively. Thus, under the conditions tested, PEF treatment at 50°C was found to be more effective than thermal pasteurisation for the inactivation of *M. paratuberculosis*.

Evrendilek and Zhang [76] investigated the effects of pulse polarity and "pulse delaying time" (the time elapsed between two consecutive crests passing a given point) on the inactivation of *E. coli* O157:H7 in apple juice and skim milk treated at field strengths of 31 and 24 kV/cm, respectively. Various pulse delaying times of 3 to 1430 μs were applied to both products. The pH and electrical conductivity for apple juice were 3.7±0.24 and 2.3 mS/cm and for skim milk 6.7±0.65 and 6.2±3.4 mS/cm, respectively. The average temperatures of apple juice before and after PEF treatment were 9±1°C and 29±2°C, and for skim milk 7±2°C and 30±3°C, respectively. A significant difference was observed in *E. coli* O157:H7 numbers in skim milk between mono (1.27 logs) and bipolar (1.96 logs) pulses, but not in apple juice (2.6 and 2.63 logs, respectively) at the pulse delaying time of 20 μs. The differences in the inactivation level can be attributed to the difference in pH and ionic composition of skim milk and apple juice.

Fernandez-Molina et al. [77, 78, 79] investigated the shelf life of various PEF-treated skim milks at room temperature or conventional heating at 60 or 65°C for 21 s as well as the combination of PEF treatment and heat or organic acids (acetic or propionic acids) on total number of aerobic bacteria (including *Pseudomonas fluorescens*) and the shelf life of skim milk. In all three studies, PEF treatment with exponential decaying pulses, field intensities of 30 to 50 kV/cm, pulse frequency of 4 Hz and treatment temperature of 40 to 65ºC in combination with organic acids had a greater effect on inactivation of microorganisms than PEF alone or combined with mild temperature. They concluded that to achieve a higher level of microbial inactivation in milk, PEF can be used in combination with heat or organic acids.

In an attempt to further extend the shelf-life of HTST-pasteurised milks by PEF treatment Sepulveda [80] subjected the pasteurised milk to PEF treatment immediately after pasteurisation and after 8 days storage at 4°C using field intensity of 35 kV/cm and 2 pulses of 2.3 μs duration each. The final temperature was 65°C with a residence time of less then 10 s. It was shown that the application of PEF immediately after pasteurisation could extend the shelf life of milk up to 60 days at 4°C, while PEF processing after 8 day storage resulted in a longer shelf life of 78 days due to further eradication of enteric and psychrotrophic bacteria by PEF.

The effects of a combination of PEF with heat treatment on inactivation of *Salmonella enteritidis* in skim milk was evaluated by Floury [81]. The selected field intensity was 47 kV/cm with a pulse frequency of 60 Hz, temperature of 62°C for 19 s, flow rate of 83 mL/min and pulse width of 500 ηs. The treatment at ambient temperature resulted in 1.2 logs reduction while the combination of heat (62ºC) and PEF nearly doubled the reduction to 2.3 logs. They concluded that the lethality of the combined treatments was mostly "additive" rather than synergistic.

Alkhafaji and Farid [74] achieved up to 6.6 logs reduction in the numbers of *E. coli* (ATCC 25922) suspended in SMUF, by applying square bipolar pulses of 1.7 μs and pulse frequency of 200 Hz, with treatment time of 100 to 900 μs and field intensity of 37 and 43 kV/cm at a flow rate of 2.5 mL/s and final temperature of <38°C using a custom-made "multi-pass treatment chamber", which included several treatment chambers set on a wooden platform. They concluded that the microbial inactivation depends on electric field strength and that a higher flow rate leads to uniform treatment.

Shamsi [50] investigated the effects of PEF treatments of raw skim milk at field intensities of 25-37 kV/cm, pulse frequency of 200 Hz and final product temperatures of 15°C or 60ºC on the inactivation of total microflora, *Pseudomonads* and *Enterobacteriaceae*. At 15ºC, PEF treatments of 28, 31, 43 and 37 kV/cm resulted in < 1 log reduction in total microflora and *Pseudomonads* count, while the *Enterobacteriaceae* count reduced by ≥ 2.1 logs to below the detection limit of 1 CFU/mL. When the temperature was set to 60°C the PEF treatments at 25, 29, 31 and 35 kV/cm resulted in up to 2.4 logs reduction in total microflora and at least 5.9 and 2.1 logs reduction in *Pseudomonads* and *Enterobacteriaceae* counts, respectively. An additive effect was observed between the field intensity and heat on the inactivation of the total microflora in raw skim milk at 60°C, while without PEF treatment the reduction at 60°C of total microflora, *Pseudomonads* and *Enterobacteriaceae* were 2.3, 2.4 and 1.6 logs, respectively.

Craven [49] achieved >5 log reduction in the number of inoculated *Ps. fluorescence* in UHT milk inoculated after treatment at 31 kV/cm, flow rate of 60 mL/min, treatment time of 19.6 μs, pulse width of 2 μs and pulse frequency of 200 Hz, with an outlet temperature of 55°C. The non-PEF treatment control had only 0.2 log inactivation. Compared with the non-PEF control milk, the shelf life of the PEF-treated milk at 4°C was extended by at least 8 days to 13 and 11 days for inoculation levels of 10^3 and 10^5 CFU/mL, respectively.

In all the above studies, PEF has been shown to be partially effective in microbial inactivation, however, to achieve a higher inactivation it may be necessary to combine the PEF treatment with other factors such as heat. Table 1 lists a summary of reports published over the last decade on the effects of PEF treatment on microorganisms in milk and SMUF and the PEF system operation.

Table 1: Microbial inactivation in milk and simulated milk ultrafiltrate using pulsed electric field treatment.

Microorganisms	Treatment medium	E[b] (kV/cm)	T[c] (°C)	Log reduction	PEF Unit	Treatment Chamber	Wave shape	PF[j]/Pw[k]	References
E. coli	SMUF[a]	36 & 60	40	6.0 & 9.0	Laboratory size (WSU[d] prototype)	Continuous	Exponentially decaying	40 µs	[82]
L. monocytogenes	Milk	25 & 35	25 50	1-4.0	Bench scale continuous (OSU[e]-4A)	Cofield flow	Monopolar square	1,700 Hz	[69]
L. innocua	Skim milk	30, 40 & 50	22 28 34	1.7, 2.0 & 2.5	Pilot plant (Physics International[f])	Continuous	Exponentially decaying	3.5 Hz	[8]
E. coli *L. innocua*	Skim milk	41	37	2.3-4.0 & 0.7-3.9	Continuous	Continuous	Not reported	10 - 63 Hz	[75]
L. innocua	Whole milk	29	35.5	1.13	Batch (Centralp-Enertronic[g])	Batch	Exponentially decaying	1.1-100 Hz	[83]
P. fluorescens	SMUF	16.4 & 37.3	50 90	Various	Batch (University of Lleida[h]) & Continuous (WSU)	Static	Exponentially decaying	2 – 3.5 Hz	[64]
L. lactis	Skim milk	35	52	3.0	Bench scale continuous (OSU-3C)	Cofield flow	Bipolar square	500 Hz	[84]
L. monocytogenes	Skim milk	15 &30	<50 55	1- 4.5	CoolPure Pulser[i]	Static	Monopolar square	5-50 Hz	[85]
E. coli O157:H7	Skim milk	24	30	2.0, 1.27 & 1.88	Bench scale continuous (OSU-4A)	Cofield flow	Bipolar & monopolar square	2.8 µs	[76]
Pseudomonads	UHT milk		15 40 45 55	3.1	Lab scale continuous (OSU-4A)	Cofield flow	Monopolar	200 Hz	[49]
Enterobacteriaceae				2.4					
Pseudomonads	Skim milk	25-37	15 & 60	5.9	Lab scale continuous (OSU-4A)	Cofield flow	Monopolar	200 Hz	[50]
Total microflora				2.1					

[a]simulated milk ultrafiltrate; [b]field intensity; [c]temperature; [d] Washington State University; [e]Ohio State University; [f]Physics International, 2700 Merced St, San Leandro, CA, USA; [g]Centralp-Enertronic, F-38070, St. Quentin Fallavier, France; [h]University of Lleida, E-25001 Lleida, Catalonia, Spain; PurePulse Technologies, San Diego, CA, USA; [j]pulse frequency; [k]pulse width

PEF in Combination with Antimicrobials

A number of researchers have demonstrated that the microbial inactivation in milk by PEF treatment can be enhanced by the combined use of low levels of food grade antimicrobials or other hurdle technologies [86]. Among the most widely investigated antimicrobials are nisin and lysozyme. Nisin, a peptide bacteriocin from *Lactococcus lactis* is one of the most commonly used food grade antimicrobials. Lysozyme and plant extracts have been used in combination with PEF treatment [58]. Pol [87] subjected vegetative cells of *B. cereus* to either low doses of nisin (0.06 μg/mL, equivalent to 2.4 IU/mL), mild PEF treatment (16.7 kV/cm, 50 pulses each of 2 μs duration), or the combination of nisin and PEF treatment. The latter treatment resulted in 1.8 logs extra reduction in *B. cereus* numbers than the sum of the reductions obtained from the individual treatments, indicating a synergistic effect.

Calderon-Miranda [88] combined PEF treatment with nisin addition to inactivate *Listeria innocua* in skim milk. The selected field intensities (and temperatures) were 30 (22^{o}C), 40 (28^{o}C) and 50 (34^{o}C) kV/cm and the number of pulses applied were 10.6, 21.3 and 32, respectively. The sensitization exhibited by PEF treated *L. innocua* to nisin was assessed for 10 or 100 IU nisin/mL. *Listeria innocua* count was reduced to 2, 2.7 and 3.4 logs after exposure to the field intensities of 30, 40 and 50 kV/cm in presence of 10 IU nisin/mL while at 100 IU nisin/mL under the same PEF treatment conditions the reduction increased to 2.5, 3 and 3.8 logs. The increase in microbial reduction was attributed to the additive effect of nisin on PEF treatment.

Effects of PEF on Enzymes in Milk or SMUF

The impact of PEF on enzymatic inactivation is a matter of controversy since in several cases a high level of inactivation has been reported while in other cases no effect has been observed [64, 89, 90]. The mechanism of enzyme inactivation by PEF is unclear [91, 92], but it is believed to be due to unfolding, denaturation and breakdown of covalent bonds and oxidation-reduction reactions caused by intense electric fields in the protein structure [93].

Not all researchers appear to have taken into account the temperature effects in PEF treatment which is an important variable in enzyme inactivation. The effects of PEF treatment on activities of various milk enzymes including alkaline phosphatase (AlP), lipases, lactoperoxidase (LPX) and proteases (e.g. plasmin) in milk or SMUF has been reported by several researchers [64, 94, 95].

According to Ho [96] enzyme inactivation requires a more severe PEF treatment than that needed for inactivating microorganisms. The higher the electric field intensity and temperature, the greater reduction in enzyme activity is achievable. Various researchers have reported large variations in the inactivation for different enzymes in milk and SMUF by PEF treatment [97]. Table 2 lists a summary of reports on the effects of PEF treatment on enzymes in milk and SMUF.

Grahl and Märkl [59] treated raw milk by applying exponentially decaying pulses of 21.5 kV/cm in a batch PEF system with a gap of 0.5 cm between electrodes and reported that LPX and AlP "did not show any noticeable inactivation" although lipase was inactivated by 60% at energy input of Q > 200 kJ/L. Van Loey [89] reported that almost 100% of the LPX activity in raw milk was retained after applying 100 pulses of 5 μs at a field intensity of 19 kV/cm and pulse frequency of 1 Hz and that even after energy input of 500 kJ/L no loss of LPX activity was observed.

Table 2: Enzyme inactivation in milk and simulated skim milk ultrafiltrate by pulsed electric field treatment.

Enzymes	Treatment medium	E[d] (kV/cm)	T[e] (°C)	Inactivation (%)	Treatment Chamber	Wave shape	PF[h]/Pw[i]	References
[a]AlP	Skim milk	21.8	43.9	65	N/R[g]	Exponentially decaying	70 pulses of 400 µs	[98]
Plasmin	SMUF[c]	15-45	60 and 80	90	Continuous with parallel electrodes	N/R	0.1 Hz	[95]
AlP, LPX[b] lipase	Whole milk	21.5	<50	Trace Trace 60	Batch	Exponentially decaying	22 Hz	[59]
AlP LPX	Whole milk	19	70	74 0	Batch	Monopolar square	1 Hz	[89]
Lipase *(P. fluorescens)*	SMUF	16.4 -27.4	<34	62.1	Batch & coaxial continuous	Exponentially decaying	2-3.5 Hz	[3]
Protease (*B. subtilis*)	SMUF/ Skim milk	19.7-35.5	<46	62.7-81	Batch & coaxial continuous	Monopolar square	67, 89 and 111 Hz	[99]
AlP	Skim milk	25-37	15 & 60	inactivation	Continuous cofield	Monopolar square	200 Hz, 19.2 µs	[50]
Protease (*B. subtilis*)	Skim milk	19.7-37.3	34 and 40	Activation-inactivation[f]	Batch & coaxial continuous	Monopolar square	22 pulses of 67 Hz and 80 pulses of 4 µs at 0.1 Hz	[100]
	SMUF	16.4-27.4	34 and 40	Activation-inactivation[f]	Batch & continuous coaxial	Monopolar square	22 pulses of 67 Hz and 80 pulses of 4 µs at 0.1 Hz	

[a]Alkaline phosphatase; [b]lactoperoxidase; [c]simulated skim milk ultrafiltrate; [d]field intensity; [e]treatment temperature; [f]depending on the frequency of treatment; [g]not reported; [h]pulse frequency; [i]pulse width

They also evaluated the susceptibility of AlP in raw milk to PEF treatment of 200 pulses of 40 μs at 10 kV/cm at a maximum temperature of 70°C and achieved 74% inactivation. They attributed the inactivation of AlP solely to PEF treatment, however, it is obvious that high temperature might have played a role in the inactivation process. Castro [98] reported that 59-65% of AlP was inactivated in SMUF, skim milk, low-fat milk (2%) and whole milk after PEF treatment at 18.8 - 22 kV/cm.

The effects of PEF and heat treatment on an extracellular lipase from *Pseudomonas fluorescens* suspended in SMUF have been studied by Bendicho [64]. The treatment chambers used were of parallel and co-axial configurations for batch and continuous flow modes, respectively. Samples were treated with 80 pulses at field intensities of 16 to 37 kV/cm. Batch-mode PEF equipment was used to expose SMUF to 80 pulses at 27.4 kV/cm (unknown treatment time) which resulted in 62.1% drop in lipase activity. However, when SMUF was exposed to PEF treatments of 80 pulses at 37.3 kV/cm and 3.5 Hz in the continuous flow mode, an inactivation rate of only 13% was achieved. The treatment temperature never exceeded 34˚C. The greater "unexpected" inactivation in batch mode was attributed to the higher energy level input (505 kJ/L) compared to continues mode (424 kJ/L) despite the fact that the field intensity in the former was higher. As a comparison, HTST and LTLT pasteurisation of samples inactivated only 5 and 20% of lipase, respectively.

Shamsi [50] investigated the effects of PEF treatments at field intensities of 25-37 kV/cm at final PEF treatment temperatures of 15°C and 60ºC on the inactivation of AlP in raw skim milk. At 15ºC, field intensities of 28, 31, 34 and 37 kV/cm resulted in 24, 25, 31 and 42% drop in AlP activity, while treatment at 60°C at field intensities of 25, 29, 31 and 35 kV/cm resulted in 29, 42, 56 and 67% inactivation. The difference in field intensities was due to the change in milk conductivity at 30 or 60ºC. The inactivation of AlP at 60°C without PEF treatment was only 22%. It was concluded that PEF was effective for the inactivation of AlP and that temperature had an additive effect on PEF treatments.

Effects of PEF Treatment on the Functionality of Milk Proteins and Fat Globules

There are very few studies on the effects of PEF on functionality of fat and proteins in milk as most of the studies have focused on microbial and enzymatic inactivation. However, since the protein and fat functionality affect the yield and physical characteristics of the products made from milk, it is of great importance to expand research in this area. For example, thermal or non-thermal treatment of cheese milk can directly or indirectly affect the final physical and sensory properties of cheese.

Shamsi [101] investigated the effects of PEF treatment on the rheological and textural properties of rennet induced curds of bovine milk. Raw skim milk was treated using a laboratory scale pulsed electric field (PEF) system at 35 and 38 kV/cm with monopolar pulses at final temperatures of 30 or 60°C, respectively. The flow rate was 60 mL/min resulting in a total pulse treatment time of 19.2 μs and total specific pulsing energy input of 154 kJ/L. Rennet was added to the milk samples and incubated at 32°C for 60 min. Elastic or storage modulus (G') and viscous or loss modulus (G'') of gels were determined using a rheometer, while the gel firmness was measured by a Texture Analyser. Gels made from untreated 30°C control skim milk showed the highest G' (40 Pa), G'' (14 Pa) and firmness (69 g) followed by the gels of 60°C control milk (G'=32 Pa, G″=11 Pa, firmness= 65 g). The PEF treatment at 38 kV/cm at 30°C decreased the G', G'' and firmness to 24 Pa, 9 Pa and 55 g while at 35 kV/cm and 60°C resulted in G', G'' and firmness of 26 Pa, 10 Pa and 58 g,

respectively. Pasteurisation of milk at 63°C for 30 min reduced the *G′*, *G″* and firmness to 11 Pa, 4 Pa and 36 g, while those at 72°C for 15 s resulted in a *G′*, *G″* and firmness of 22 Pa, 8 Pa and 40 g, respectively. High heat-treated (97°C for 10 min) skim milk formed no gel at all. The PEF treatment reduced rheological attributes and firmness of the rennet induced gels, however, the extent of these reductions was much less pronounced than those caused by thermal pasteurisation treatments. Casein micelle size was not affected by PEF treatment but high heat treatment of milk resulted in a significant increase (p<0.05) in micelle size. The rennet coagulation time (RCT) was increased by PEF treatment, however, compared to HTST pasteurisation it was shorter.

Floury [102] found that the PEF treatments at the field intensities of 45 or 55 kV/cm with pulse widths of 500 and 250 ηs (square monopolar pulses) respectively, decreased the coagulation time. At a total treatment time of 2.1-3.5 μs, a significant drop in casein micelle size was observed while the viscosity of milk decreased and the coagulation properties were enhanced.

Wüst [103] assessed the physical attributes of cottage cheese made from PEF-treated skim milk. The treatment was conducted by applying bipolar square pulses of 2 μs at field intensities of 25 and 28 kV/cm with pulse frequencies of 200 and 400 Hz and flow rate of 120 mL/min at a treatment temperature of <45°C. It was found that increasing the field strength decreased the strength of the cottage cheese gel and marginally increased the yield of cottage cheese compared to cheeses made from raw or pasteurised skim milk. The "raw milk" odour was also removed from the samples treated at a frequency of 400 Hz.

Sepulveda [104] compared the quality attributes of cheddar cheese made from PEF-treated and pasteurised milks (LTLT and HTST). The pulsing rate was set at 3.3 Hz and 30 exponentially-decaying pulses of 35 kV/cm. Cooling devices were used to keep the milk temperature below 30^{o}C. The raw milk cheddar cheese was used as the control and textural and sensory attributes were determined in all samples. In the PEF-treated milk cheese, hardness and springiness increased compared to the control cheeses while adhesiveness and cohesiveness remained unchanged.

Modelling of Microbial and Enzymatic Inactivation

The development of mathematical expressions to define and quantify the effects of processing parameters on treatment effectiveness is an important task. Mathematical models can be used to gain insight into possible mechanism of action or to predict the microbial concentration and shelf life of processed products [105].

There are several models [106, 107, 108] to explain mathematically the relationship between microbial or enzymatic inactivation with field intensity or treatment time, among which the Hülsheger [62] model is the most reported one. This model relates the microbial survival fraction S with PEF treatment time t, as $ln\ N/N_0$ *or* $S = -b_E(E-E_c)$, where N_0 and N are the microbial population before and after PEF treatment, b_E is the regression coefficient and E_c is extrapolated critical value for field intensity. The model was based on the assumed linear relationship between the log survival fraction and field strength as well as a linear relation between fraction of survivors and treatment time. The $ln\ N/N_0$ corresponds to the natural log of the survival fraction and yields a straight line when plotted against treatment time in semilog charts.

The Hülsheger model has also been adapted by some researchers [46] as $ln(A/A_0) = -b_E(E-E_C)$ to describe the enzymatic inactivation after PEF treatments where A_0 and A are the enzyme activity before and after PEF treatment. Optimum processing conditions should be established to obtain the maximum inactivation level with the minimum heating effect.

Conclusion

PEF technology can be considered as a potential alternative to traditional thermal pasteurisation of milk with the advantages of minimising sensory and nutritional damage, thus providing fresh-like products. However, more investigation is needed to understand the mechanism of PEF effects and to achieve a maximum level of enzymatic and microbial inactivation in order to make PEF technology applicable in the dairy industry. Most PEF systems used for treatment of dairy or non-dairy products have been limited to bench top or pilot scale systems.

There are currently various research groups in Australia, Belgium, Canada, China, France, Germany, Iceland, Japan, the Netherlands, New Zealand, Scotland, Spain, Sweden, Switzerland, Taiwan, the United Kingdom and the USA working on different industrial applications of PEF. In 1995 CoolPure^R PEF process developed by PurePulse Technologies (4241, Ponderosa Ave., San Diego, CA, 92123, USA) was approved by the FDA for treatment of pumpable food. This was the first regulatory effort to introduce PEF process in the food industry [109]. Also Genesis Juice Cooperative (325, West Third Suites B, Eugene, Oregon 97401, USA) distributed its PEF-treated fruit juices in 2005 in seven all-organic juices: apple, apple-strawberry, carrot, carrot-celery-beet, herbal tonic, strawberry lemonade and ginger lemonade [110]. However, the company stopped using PEF in 2006 for unknown reasons and replaced it with high pressure technology. A PEF pilot plant facility is also operating at Ohio State University's Food Science and Technology Department. Diversified Technologies Inc. (35 Wiggins Ave., Bedord MA 01730, USA) has manufactured the pilot and commercial PEF systems for waste stream treatment costing between US$250,000 to 500,000 with different powers and process volumes. However, no industrial scale PEF plant has so far been established to "pasteurise" milk for public consumption.

The PEF technology has some shortcomings, which must be taken into account in future research. For instance, the presence of air bubbles may lead to non-uniform treatment as well as operational and safety problems [111]. Presently, the PEF application is restricted to liquid food products that can withstand high electric fields. The particle size of the liquid food in both static and flow treatment modes could cause system malfunction. The maximum particle size in the liquid must be smaller than the gap between the electrodes in the chamber in order to maintain a uniform processing operation and there should be no clumping of particles [2]. In addition, there are still technical hurdles to overcome in achieving successful application of PEF technology at an industrial scale such as designing treatment chambers with maximum output and efficiency, preventing ohmic heating (which can adversely affect heat-sensitive products), need for highly specific electrical pulsing equipment and switches able to handle high voltages and minimising the electrolysis between the electrodes and product [16]. A need is also felt for an indicator of PEF-treatment adequacy of milk similar to AlP which is used as a quality assurance index for milk.

Despite these shortcomings, PEF has been shown to be effective in eliminating pathogens and spoilage organisms and capable of producing safe fresh-like food. Thus it seems to be a promising alternative for traditional thermal methods in cold pasteurising of liquid food.

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References

1. Zhang, Q.H., Barbosa-Canovas, G.V. and Swanson, B.G. (1995). Engineering aspects of pulsed electric fields pasteurisation, **Journal of Food Engineering,** Vol. 25, pp. 261-281.

2. Barbosa-Canovas, G.V., Pierson, M.D., Zhang, Q.H. and Schaffner, D.W. (2000). Pulsed Electric Fields. Special Supplement: Kinetics of Microbial Inactivation for Alternative Food Processing Technologies, **Journal of Food Science**, Vol. 65, pp. 65-79.

3. Bendicho, S., Barbosa-Canovas, G.V. and Martin, O. (2002). Milk Processing by High Intensity Pulsed Electric Fields. Review Paper, **Trends in Food Science and Technology,** Vol. 13, pp. 195-204.

4. Barbosa-Canovas, G.V. and Sepulveda, D. (2005). Present status and the future of PEF technology. In *Novel Food Processing Technologies.* (Eds. G.V. Barbosa-Canovas, M.S. Tapia, M.P. Cano). CRC Press, pp. 1-44.

5. Gaudreau, M., Hawkey, T., Petry, J. and Kempkes, M. (2008). Pulsed electric field processing for food and waste streams, **Food Australia**, Vol. 60 (7), pp. 323-325.

6. Mertens, B. and Knorr, D. (1992). Developments of nonthermal processes of food preservation, **Food Technology**, Vol. 5, pp. 124-133.

7. Steele, J. (2000). History, trends and extent of pasteurisation, **Journal of the American Veterinary Medical Association,** Vol. 217, pp. 175-178.

8. Calderon-Miranda, M.L., Barbosa-Canovas, G.V. and Swanson, B.G. (1999). Inactivation of *Listeria innocua* in skim milk by pulsed electric fields and nisin, **International Journal of Food Microbiology**, Vol. 51, pp. 19-30.

9. Pothakamury, U.R., Barbosa-Canovas, G.V. and Swanson, B.G. (1993). Magnetic-field inactivation of microorganisms and generation of biological changes, **Food Technology**, Vol. 47, pp. 85-93.

10. Castro, A., Barbosa-Canovas, G.V. and Swanson, B.G. (1993). Microbial inactivation of foods by pulsed electric fields, **Journal of Food Processing and Preservation,** Vol. 17, pp. 47-73.

11. Kitajima, N., Ueda, K., Ohshima, T. and Sato, M. (2007). Development of Textile Electrode for Microbial Inactivation with Pulsed Electric Field, **Textile Research Journal**, Vol. 77, pp. 528-534.

12. Takhistov, P. (2006). Pulsed Electric Field in Food Processing and Preservation Food, **Science and Technology**, Vol. 149, pp. 126-131.

13. United States Patent 4764473, Chamber for the treatment of cells in an electrical field, http://www.freepatentsonline.com/4764473.html.

14. Dunn, J.E. and Pearlman, J.S. (1987). Methods and apparatus for extending the shelf-life of fluid food products. Maxwell Laboratories, Inc. U.S. Patent 4,695,472.

15. Martin-Belloso, O., Qin, B.L., Chang, F.J., Barbosa-Canovas, G.V. and Swanson, B.G. (1997). Inactivation of *Escherichia coli* in skim milk by high intensity pulsed electric fields, **Journal of Food Process Engineering**, Vol. 20, pp. 317-336.

16. Fox, M.B., Esveld, D.C. and Boom, R.M. (2007). Conceptual design of a mass parallelized PEF microreactor, **Trends in Food Science and Technology**, Vol. 18, pp. 484-491.

17. Barbosa-Canovas, G.V., Gongora-Nieto, M.M., Pothkamury, U.R. and Swanson, B.G. (1998). Preservation of foods with pulsed electric fields, Academic Press, CA, USA. pp. 1-19 ISBN 0-12-078149-2.

18. Yin, Y., Zhang, Q.H. and Sastry, S.H. (1997). High voltage pulsed electric field treatment chambers for the preservation of liquid food products. U.S. Patent 5,690,978.

19. Martin-Belloso, O., Vega-Mercado, H., Qin, B.L., Chang, F.J., Barbossa-Canovas, G.V. and Swanson, B.G. (1997). Inactivation of *Escherichia coli* suspended in liquid egg using pulsed electric fields, **Journal of Food Processing and Preservation**, Vol. 21, pp. 193-208.

20. Fiala, A., Wouters, P.C., van den Bosch, H.F.M. and Creyghton, Y.L.M. (2001). Coupled electrical-fluid model of pulsed electric field treatment in a model food system, **Innovative Food Science and Emerging Technology**, Vol. 2, pp. 229-238.

21. Morren, J. Roodenburg, B. and de Haan, S.W.H. (2003). Electrochemical reactions and electrode corrosion in pulsed electric field treatment chambers, **Innovative Food Science and Emerging Technology**, Vol. 4, pp. 285-295.

22. Martin-Belloso, O., Bendicho, S., Elez-Martinez, P. and Barbosa-Canovas, G.V. (2005). Does high-intensity pulsed electric fields induce changes in enzymatic activity, protein conformation, and vitamin and flavour stability? In *Novel Food*

Processing Technologies. Ed. Barbosa-Canovas, G.V., Tapia, M.S. and Cano, M.P. pp. 87-103, CRC Press, Florida.

23. Martin-Belloso, O. and Elez-Martinez, P. (2005). Food Safety aspects of pulsed electric field. In *Emerging Technologies for Food Processing*. Ed. Da-Wen Sun, Elsevier Academic Press, London, pp. 184-217.

24. Weaver, J.C. and Chizmadzhev, Y.A. (1996). Theory of electroporation: **A review: Bioelectrochemistry and Bioenergetics**, Vol. 41, pp. 135-160.

25. Sale, A.J.H. and Hamilton, W.A. (1967). Effects of high electric fields on microorganisms I. Killing of bacteria and yeast, **Biochimica et biophysica acta**, Vol. 163, pp. 34-43.

26. Jacob, H.E., Foster, W. and Berg, H. (1981). Microbial implication of electric field effects. II. Inactivation of yeasts cells and repair of their cell envelope, **Zeitschrift für Allgemeine Mikrobiologie**, Vol. 21, pp. 225-233.

27. Coster, H. G. L. and Zimmermann, U. (1975). The mechanisms of electrical breakdown in the membrane of *Valonia utricularis*, **Journal of Membrane Biology,** Vol. 22, pp. 73-90.

28. Tsong, T.Y. (1990). Reviews on electroporation of cell membranes and some related phenomena, **Bioelectrochemistry and Bioenergetics**, Vol. 24, pp. 271.

29. Zimmermann, U. (1986). Electrical breakdown, electropermeabilization and electrofusion, **Reviews of Physiology, Biochemistry Pharmacology**, Vol. 105, pp. 175-256.

30. Chen, W. and Lee, R.C. (1994). Altered ion channel conductance and ionic selectivity induced by large imposed membrane potential pulse, **Biophysical Journal**, Vol. 67, pp. 603-612.

31. Kinosita, K.J. and Tsong, T.Y. (1977). Voltage induced pore formation and haemolysis erythrocytes, **Biochimica et biophysica acta**, Vol. 471, pp. 227-242.

32. Schoenbach, K.H., Peterkin, F.E., Alden, R.W. and Beebe, S.J. (1997). The effect of pulsed electric fields on biological cells: Experiments and applications, **IEEE Transactions on Plasma Science**, Vol. 25, pp. 284-292.

33. Vega-Mercado, H., Martin-Belloso, O., Chang, F.J., Barbosa-Cánovas, G.V. and Swanson, B.G. (1996). Inactivation of *Escherichia coli* and *Bacillus subtilis* suspended in pea soup using pulsed electric fields, **Journal of Food Processing and Preservation,** Vol. 20, pp. 501-510.

34. Pothakamury, U.R., Barbosa-Canovas, G.V., Swanson, B.G. and Spence, K.D. (1997). Ultrastructural changes in *Staphylococcus aureus* treated with pulsed electric fields, **Food Science and Technology International**, Vol. 3, pp. 113-121.

35. Aronsson, K., Lindgren, M., Johansson, B.R. and Ulf, R. (2001). Inactivation of microorganisms using pulsed electric fields: the influence of process parameters on *E. coli, L. innocua, L. mesenteroides and S. cerevisiae*, **Innovative Food Science and Emerging Technology**, Vol. 2, pp. 41-54.

36. Hülsheger, H., Potel, J. and Niemann, E.G. (1983). Electric field effects on bacteria and yeast cells, **Radiation and Environmental Biophysics**, Vol. 22, pp. 149-162.

37. Qin, B.L., Pothakamury, U.R., Vega-Mercado, H., Martin, O., Barbosa-Cánovas, G.V. and Swanson, B.G. (1995). Food pasteurisation using high-intensity pulsed electric fields, **Food Technology**, Vol. 49, pp. 55-60.

38. Barbosa-Canovas G.V. and Zhang Q.H. (2001). Pulsed Electric Fields in Food Processing Fundamental Aspects and Applications. (Eds. G. Barbosa-Canovas and Q.H. Zhang). Technomic Publishing Co. Inc.,

39. Gaskova, D., Sigler, K.J., Anderova, B. and Plasek, J. (1996). Effect of high-voltage electric pulses on yeast cells: Factors influencing the killing efficiency, **Bioelectrochemistry and Bioenergetics**, Vol. 39, pp. 195-202.

40. Hülsheger, H. and Niemann, E.G. (1980). Lethal effects of high-voltage pulses on *E. coli* K12, **Radiation and Environmental Biophysics**, Vol. 18, pp. 281-288.

41. Toepfl, S., Heinz, V. and Knorr, D. (2005). Overview of Pulsed Electric Field Processing for Food. In *Emerging Technologies for Food Processing*. (Ed. Da-Wen Sun). London: Elsevier Academic Press, pp. 69-99.

42. Heinz, V., Alvarez, I., Angersbach, A. and Knorr, D. (2002). Preservation of liquid foods by high intensity electric fields-basic concepts for process design, **Trends in Food Science and Technology,** Vol. 12, pp. 103-111.

43. Ho, S.Y., Mittal, G.S., Cross, J.D. and Griffiths, M.W. (1995). Inactivation of *Pseudomonas fluorescens* by high voltage electric pulses, **Journal of Food Science,** Vol. 60, pp. 1337-1343.

44. Qin, B.L., Zhang, Q., Barbosa-Cánovas, G.V., Swanson, B.G. and Pedrow, P.D. (1994). Inactivation of microorganisms by pulsed electric fields with different voltage waveforms, **IEEE Transactions on Dielectrics and Insulation**, Vol. 1, pp. 1047-1057.

45. Beveridge, J.R., MacGregor, S.J., Marsili, L., Anderson, J.G., Rowan, N.J. and Farish, O. (2002). Comparison of the Effectiveness of Biphase and Monophase Rectangular Pulses for the Inactivation of Microorganisms Using Pulsed Electric Fields, **IEEE Transactions on Plasma Science**, Vol. 30, pp. 1525-1531, August.

46. Min, S., Min, S.K., and Zhang, Q.H. (2003). Inactivation kinetics of tomato juice lipoxygenase by pulsed electric fields, **Journal of Food Science**, Vol. 68, pp. 1995-2001.

47. Rowan, J.N., MacGregor, S.J., Anderson, J.G., Cameron, D. and Farish, O. (2001). Inactivation of *Mycobacterium paratuberculosis* by pulsed electric fields, **Applied and Environmental Microbiology**, Vol. 6, pp. 2833-2836.

48. Spilimbergo, S., Dehghani, F., Bertucco, A. and Foster, N. R. (2003). Inactivation of Bacteria and Spores by Pulsed Electric Field and High Pressure CO_2 at Low Temperature, **Biotechnology and Bioengineering**, Vol. 82, pp. 118-124.

49. Craven H. M., Swiergon, P., Ng, S., Midgely, J., Versteeg, C., Coventry, M. J. and Wan, J. (2008). Evaluation of pulsed electric field and minimal heat treatments for microbial inactivation of pseudomonads in milk and enhancement of milk shelf-life, **Innovative Food Science and Emerging Technology**, Vol. 9, pp. 211-217.

50. Shamsi, K., Versteeg, C., Sherkat, F. and Wan, J. (2008). Alkaline phosphatase and microbial inactivation by pulsed electric field in bovine milk, **Innovative Food Science and Emerging Technology**, Vol. 9, pp. 217-223.

51. Jayaram, S., Castle, G.S.P. and Margaritis, A. (1993). The effect of high field DC pulse and liquid medium conductivity on survivability of *L. brevis*, **Applied Microbiology and Biotechnology**, Vol. 40, pp. 117-122.

52. Vega-Mercado, H., Pothakamury, U.R., Chang, F.J., Barbosa-Cánovas, G.V. and Swanson, B.G. (1996). Inactivation of *Escherichia coli* by combining pH, ionic strength and pulsed electric fields hurdles, **Food Research International**, Vol. 29, pp. 117-121.

53. Liu, X., Yousef, A.E. and Chism, G.W. (1997). Inactivation of *Escherichia coli* O157:H7 by the combination of organic acids and pulsed electric fields, **Journal of Food Safety**, Vol. 16, pp. 287-299.

54. Wouters, P.C., Dutreux, N., Smelt, J.P.P.M., Lelieveld, H.L.M. (1999). Effects of pulsed electric fields on inactivation kinetics of *Listeria innocua*, **Applied and Environmental Microbiology,** Vol. 65, pp. 5364-5371.

55. Barbosa-Canovas, G.V., Gongora-Nieto, M.M., Pothkamury, U.R. and Swanson, B.G. (1999). *Preservation of Foods with Pulsed Electric Fields.* pp. 1-19. CA: Academic Press.

56. Jayaram, S., Castle, G.S.P. and Margaritis, A. (1992). Kinetics of sterilization of Lactobacillus brevis cells by the application of high voltage pulses, **Biotechnology and Bioengineering**, Vol. 40, pp. 1412-1420.

57. Simpson, R.K., Whittington R., Earnshaw, R.G. and Russell, N.J. (1999). Pulsed high electric field causes 'all or nothing' membrane damage in *Listeria monocytogenes* and *Salmonella typhimurium*, but membrane H^+-ATPase is not a primary target, **International Journal of Food Microbiology**, Vol. 48, pp. 1–10.

58. Mittal, G.S. and Griffiths, M.W. (2005). Pulsed Electric Field Processing of Liquid Foods and Beverages. In *Emerging Technologies for Food Processing*. (Ed. Da-Wen Sun) London: Elsevier Academic Press, pp. 99-139.

59. Grahl, T. and Märkl, H. (1996). Killing of microorganisms by pulsed electric fields, **Applied Microbiology and Biotechnology**, Vol. 45 (1/2), pp. 148-157.

60. Sensoy, I., Zhang, Q.H. and Sastry, S.K. (1997). Inactivation kinetics of *Salmonella dublin* by pulsed electric fields, **Journal of Food Process Engineering,** Vol. 20, pp. 367-381.

61. Álvarez, J. Raso, A. Palop and F.J. Sala (2000). Influence of different factors on the inactivation of *Salmonella* Senftenberg by pulsed electric fields, **International Journal of Food Microbiology**, Vol. 55, pp. 143–146.

62. Hülsheger, H., Potel, J. and Niemann, E.G. (1981). Killing of bacteria with electric pulses of high fields strength, **Radiation and Environmental Biophysics**, Vol. 20, pp. 53-65.

63. Jeness, R. and Koops, J. (1962). Preparation and properties of a salt solution which simulates milk ultrafiltrate, **Netherlands Milk and Dairy Journal**, Vol. 16, pp. 153-164.

64. Bendicho, S., Estela, C., Fernandez-Molina, J.J., Barbosa-Canovas, G.V. and Martin, O. (2002). Effects of high intensity pulsed electric fields and thermal treatments on a lipase from *Pseudomonas fluorescens*, **Journal of Dairy Science**, Vol. 85, pp. 19-27.

65. Qin, B.L., Chang, F.J., Barbosa-Cánovas, G.V. and Swanson, B.G. (1995). Nonthermal inactivation of *S. cerevisiae* in apple juice using pulsed electric fields, **Lebensmittel Wissenschaft and Technologie**, Vol. 28 (6), pp. 564-568.

66. Zhang, Q.H., Monsalve-Gonzalez, A., Qin, B.L., Barbosa-Cánovas, G.V. and Swanson, B.G. (1994). Inactivation of *S. cerevisiae* in apple juice by square-wave and exponential-decay pulsed electric fields, **Journal of Food Process Engineering**, Vol. 17, pp. 469-478.

67. Pothakamury, U.R., Vega-Mercado, H., Zhang, Q.H., Barbosa-Canovas, G.V. and Swanson, B.G. (1996). Effect of growth stage and processing temperature on the inactivation of *E. coli* by pulsed electric fields, **Journal of Food Protection**, Vol. 59, pp. 1167-1171.

68. Marquez, V.O., Mittal, G.S. and Griffiths, M.W. (1997). Destruction and inhibition of bacterial spores by high voltage pulsed electric fields, **Journal of Food Science**, Vol. 62, pp. 399-409.

69. Reina, L.D., Jin, Z.T., Yousef, A.E. and Zhang, Q.H. (1998). Inactivation of *Listeria monocytogenes* in milk by pulsed electric fields, **Journal of Food Protection**, Vol. 61, pp. 1203-1206.

70. Evrendilek, G.A., Zhang, Q.H. and Richter, E.R. (1999). Inactivation of *E. coli* O157:H7 and *E. coli* 8739 in apple juice by pulsed electric fields, **Journal of Food Protection**, Vol. 62, pp. 793-796.

71. Matsumoto, Y., Satake, T., Shioji, N. & Sakuma, A. (1991). Inactivation of microorganisms by pulsed high voltage applications. Conference Record of IEEE Industrial Applications Society Annual Meeting, pp. 652-659.

72. Pothakamury, U.R., Monsalve-Gonzalez, A., Barbosa-Cánovas, G.V. and Swanson, B.G. (1995). Inactivation of *Escherichia coli* and *Staphylococcus aureus* in model foods by pulsed electric fields technology, **Food Research International**, Vol. 28 (2), pp. 167-171.

73. Evrendilek, G.A., Jin, Z.T., Ruhlman, K.T., Qiu, X., Zhang, Q.H. and Richter, E.R. (2000). Microbial safety and shelf life of apple juice and cider processed by bench and pilot scale PEF systems, **Innovative Food Science and Emerging Technologies**, Vol. 1(1), pp. 77-86.

74. Alkhafaji, S.R. and Farid, M. (2007). An investigation on pulsed electrics technology using new treatment chamber design, **Innovative Food Science and Emerging Technologies**, Vol. 8, pp. 205-212.

75. Dutreux, N., Notermans, S., Wijtzes, T., Gongora-Nieto, M.M., Barbosa-Canovas, G.V. and Swanson, B.G. (2000). Pulsed electric fields inactivation of attached and free-living *Escherichia coli* and *Listeria innocua* under several conditions, **International Journal of Food Microbiology**, Vol. 54, 91-98.

76. Evrendilek, G.A. and Zhang, Q.H. (2005). Effects of pulse polarity and pulse delaying time on pulsed electric fields-induced pasteurisation of *E. coli* O157:H7, **Journal of Food Engineering**, Vol. 68, pp. 271-276.

77. Fernandez-Molina, J.J., Fernandez-Gutierrez, S.A., Altunakar, B., Bermudez-Aguirre, Swanson, B.G. and Barbosa-Canovas, G.V. (2005). The Combined Effect of Pulsed Electric Fields and Conventional Heating on the Microbial Quality and Shelf-life of Skim Milk, **Journal of Food Processing and Preservation**, Vol**.** 29, pp. 390-406.

78. Fernandez-Molina, J.J., Barbosa-Canovas, G.V. and Swanson, B.G. (2005). Skim Milk Processing by Combining Effect of Pulsed Electric Fields and Thermal Treatments, **Journal of Food Processing and Preservation**, Vol. 29, pp. 291-306.

79. Fernandez-Molina, J.J., Fernandez-Gutierrez, S.A., Altunakar, B., Bermudez-Aguirre, Swanson, B.G. and Barbosa-Canovas, G.V. (2005). Inactivation of *Pseudomonas fluorescens* in Skim Milk by Combinations of Pulsed Electric Fields and Organic Acids, **Journal of Food Protein**, Vol. 68, pp. 1232-1235.

80. Sepulveda, D.R., Gongora-Nieto, M.M., Guerrero, J.A. and Barbosa-Canovas, G.V. (2005). Production of extended-shelf life milk by processing pasteurised milk with pulsed electric fields, **Journal of Food Engineering**, Vol. 67, pp. 81-86.

81. Floury, J., Grosset, N., Lesne, E. and Jeantet, R. (2006). Continuous processing of skim milk by a combination of pulsed electric field and conventional heat treatments: does a synergetic effect on microbial inactivation exist? **Lait**, Vol. 86, pp. 203-211.

82. Qin, B.L., Barbosa-Cánovas, G.V. and Swanson, B.G., Pedrow, P.D. and Olsen, R.G. (1998). Inactivating microorganisms using pulsed electric fields continuous treatment system. **IEEE Transactions on Dielectrics and Electrical Insulation**, Vol. 34, pp. 43-50.

83. Picart, L., Dumay, E. and Cheftel, J.C. (2002). Inactivation of *Listeria innocua* in dairy fluids by pulsed electric fields: influence of electric parameters and food composition. **Innovative Food Science and Emerging Technologies**, Vol. 3, pp. 357-369.

84. Michalac, S., Alvarez, V., Ji, T. and Zhang, Q.H. (2003). Inactivation of selected microorganisms and properties of pulsed electric field processed milk. **Journal of Food Processing and Preservation**, **27**, 137-151.

85. Fleischman, G.J., Ravishankar, S. and Balasubramaniam, V.M. (2004). The inactivation of *Listeria monocytogenes* by pulsed electric fields treatment in a static chamber. **Food Microbiology**, Vol. 21, pp. 91-95.

86. Raso, J., Pagan, R. and Condon, S. (2005). Nonthermal technologies in combination with other preservation factors. In Novel Food Processing Technologies. (Eds. G.V. Barbosa-Canovas, M.S. Tapia, M.P. Cano) CRC Press, pp. 453-475.

87. Pol, I.E, Mastwijk, H.C., Bartels, P.V. and Smid, E.J. (2000). Pulsed-electric field treatment enhances the bactericidal action of nisin against Bacillus cereus. **Applied and Environmental Microbiology**, Vol. 66, pp. 428-430.

88. Calderon-Miranda, M.L., Barbosa-Canovas, G.V. and Swanson, B.G. (1999). Transmission electron microscopy of *Listeria innocua* treated by pulsed electric fields and nisin in skimmed milk, **International Journal of Food Microbiology**, Vol. 51, pp. 31-38.

89. Van Loey, A., Verachtert, B. and Hendrickx, M. (2002). Effects of high electric fields pulses on enzymes, **Trends in Food Science and Technology**, Vol. 12, pp. 94-102.

90. Zhong, K., Wu, J., Wang, Z., Chen, F., Liao, X., Hu, X. and Zhang, Z. (2007). Inactivation kinetics and secondary structural change of PEF-treated POD and PPO. **Food Chemistry**, Vol. 100, pp. 115-123.

91. Giner, J., Gimeno, V., Barbosa-Canovas, G.V. and Martin, O. (2001). Effects of pulsed electric fields processing on apple and pear polyphenoloxidases. **Food Science and Technology International**, Vol. 7, pp. 339-345.

92. Ohshima, T., Tamura, T., Sato, M. (2007). Influence of pulsed electric field on various enzyme activities, **Journal of Electrostatics**, Vol. 65, pp. 156-161.

93. Barsotti, L. and Cheftel, J.C. (1999). Food processing by pulsed electric fields. II. Biological aspects, **Food Reviews International**, Vol. 15, pp. 181-213.

94. Castro, A.J., Swanson, B.G., Barbosa-Canovas, G.V. and Zhang, Q.H. (2001). Pulsed Electric Field Modification of Milk Alkaline Phosphatase. In G. V. Barbosa-Canovas, Q. H. Zhang, *Pulsed Electric Fields in Food Processing-fundamental aspects and applications. Food preservation technology series* Lancaster (PA): Technomic Publishing Co. pp. 65-82.

95. Vega-Mercado, H., Powers, J.R., Barbosa-Canovas, G.V. and Swanson, B.G. (1995). Plasmin inactivation with pulsed electric fields, **Journal of Food Science**, Vol. 60, pp. 1143-1146.

96. Ho, S.Y., Mittal, G.S. and Cross, J.D. (1997). Effects of high field electric pulses on the activity of selected enzymes, **Journal of Food Engineering***,* Vol. 31, pp. 69-84.

97. Martin-Belloso, O., Bendicho, S., Elez-Martinez, P. and Barbosa-Canovas, G.V. (2005). Does high-intensity pulsed electric fields induce changes in enzymatic activity, protein conformation, and vitamin and flavour stability? In *Novel Food Processing Technologies*. (Eds. G.V. Barbosa-Canovas, M.S. Tapia, M.P. Cano) CRC Press, pp. 87-103.

98. Castro, A. (1994). Pulsed electric field modification of activity and denaturation of alkaline phosphatase. PhD dissertation. Washington State University, Pullman, WA.

99. Bendicho, S., Barbosa-Canovas, G.V. and Martin, O. (2003). Reduction of protease activity in simulated milk ultrafiltrate by continuous flow high-intensity pulsed electric field treatments, **Journal of Food Science***,* Vol. 68, pp. 952-957.

100. Bendicho, S., Marselles-Fontanet, A.R., Barbosa-Canovas, G.V. and Martin-Belloso, O. (2005). High intensity pulsed electric fields and heat treatments applied to a protease from *Bacillus subtilis*. A comparison study of multiple systems, **Journal of Food Engineering**, Vol. 69, pp. 317-323.

101. Shamsi, K., Wan, J., Versteeg, C. and Sherkat, F. (2009). Effects of Pulsed Electric Field Treatment on the Rheological and Textural Properties of Rennet-Induced Curds of Bovine Milk. Poster presented at the 4th Innovative Foods Centre Conference, 17-18 September 2008, Brisbane, Australia.

102. Floury, J., Grosset, N., Leconte, N., Pasco, M., Madec, M. and Jeantet, R. (2006). Continuous raw skim milk processing by pulsed electric field at non-lethal temperature: effect on microbial inactivation and functional properties, **Lait**, Vol. 86, pp. 43-57.

103. Wüst, R., Pearce, R., Ortega-Rivas, E. and Sherkat, F. (2004). Pulsed electric fields treatment of milk affects the properties of the cottage cheese gel. 9th International Congress on Engineering and Food, Abst. No 896: 87. 7-11 March, Montpellier, France.

104. Sepulveda, D.R., Ortega-Rivas, E. and Barbosa-Canovas, G.V. (2000). Quality aspects of cheddar cheese obtained with milk pasteurised by pulsed electric fields, **Food and Bioproducts Processing,** Vol. 78(2), pp. 65-71.

105. Lund, D. (1983). Considerations in modelling of food processes, **Food Technology**, Vol. 1, pp. 92-94.

106. Peleg, M. (1995). A model for microbial survival after exposure to pulsed electric fields, **Journal of the Science of Food and Agriculture**, Vol. 67, pp. 93-99.

107. Peleg, M. (1999). On calculating sterility in thermal and nonthermal preservation methods, **Food Reviews International**, Vol. 32, pp. 271-278.

108. Peleg, M. and Cole, M. (1998). Reinterpretation of microbial survival curves, **Critical Reviews in Food Science and Nutrition**, Vol. 38, pp. 353-380.

109. Food and Drug Administration (2000). Kinetics of Microbial Inactivation for Alternative Food Processing Technologies, http://www.cfsan.fda.gov/~comm/ift-arc.html, retrieved from internet on 19.05.2008.

110. Clark, P. (2006). Pulsed electric field processing, **Food Technology**, Vol. 60, pp. 66-67.

111. Sherkat, F. (2006). Advances in Pulsed Electric Field Technology. Oral presentation at the 39th Convention of Australian Institute of Food Science and Technology, 9-12 July, Adelaide Australia.