FOOD PRESERVATION BY PULSED ELECTRIC FIELDS AND SELECTED ANTIMICROBIALS

By

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AND SELECTED ANTIMICROBIALS

Abstract

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Today's consumers prefer foods with convenience, variety, adequate shelf-life, reasonable cost and environmental soundness, promoting the interest in nonthermal food processing technologies. Pulsed electric fields (PEF) technology exhibits potential especially for fruit juice preservation by providing safety while retaining nutritional and sensory quality. Moreover, combination of PEF with antimicrobials appears to be even more promising. This dissertation explores the possibility of increasing PEF efficiency by using this technology in combination with selected antimicrobials to preserve apple cider.

Chapter 1 introduces an extensive literature review on the fundamental aspects of PEF technology from a historical and technical point of view. Chapter 2 reviews the food preservation applications of PEF with emphasis on low and high acid liquid food products.

Chapter 3 presents the effect of PEF in combination with sodium benzoate, potassium sorbate, cinnamic acid and hydrogen peroxide on microbial inactivation of *E. coli* (ATCC

11775). Selected antimicrobials at two concentrations (500 and 1000 ppm) are combined with PEF for selected electric field intensities (25-40 kV/cm) and number of pulses (5, 12, 20). The extent of synergistic microbial inactivation achieved by each combination is compared to the PEF alone treatment.

Chapter 4 explores the applicability of four mathematical models (Bigelow, Hülsheger, Peleg and Weibull) to predict the microbial response of *E.coli* (ATCC 11775) in apple cider treated by PEF and selected antimicrobials. The advantages and limitations of each model based on the accuracy factors are evaluated.

Chapter 5 investigates some of the engineering aspects contributing to PEF system efficiency. The effects of electrical components, energy density and fluid flow rate on the microbial inactivation are studied.

Chapter 6 evaluates the combined effect of PEF and selected antimicrobials on shelf-life of apple cider. Microbiological and chemical changes such as yeast and total bacteria growth, color, pH, titratable acidity and polyphenoloxidase (PPO) activity in apple cider during refrigerated and room temperature storage are monitored.

Chapter 7 concludes the overall results for the use of PEF in combination with selected antimicrobials as a promising alternative to thermal pasteurization for fruit juices with extended shelf-life and improved quality. Specific areas of interest that require further research are mentioned and recommended as future work.

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CHAPTER ONE

Pulsed Electric Fields Processing of Foods: An Overview

Bilge Altunakar and Gustavo V. Barbosa-Cánovas

1. Introduction

The ever increasing trend towards nutritionally qualified foods has challenged food technology to produce fresh-like foods by replacing thermal treatments with alternative methods of preservation. Thermal processing is a major technology that has been commonly used in the food industry to increase shelf life and maintain food safety with low processing costs (Knorr et al., 1994). To qualify as an alternative method, a new technology should have significant impact on quality while at the same time maintain the cost of technology within feasibility limits. In recent years, several technologies have been investigated that have the capability of inactivating microorganisms at lower temperatures than typically used in conventional heat treatments (Lado and Yousef, 2002). Therefore, nonthermal methods correspond to the expectations for minimally processed foods of fresh quality, which have higher nutritional value because of color and flavor retention. Among all emerging nonthermal technologies, high intensity pulsed electric fields (PEF) is one of the most appealing technologies due to its short treatment times and reduced heating effects with respect to other technologies. High intensity pulse electric fields is highly appreciated as a nonthermal food preservation technology that involves the discharge of high voltage electric short pulses through the food product.

With the use of electric fields, PEF technology enables inactivation of vegetative cells of bacteria and yeasts in various foods. As bacterial spores are resistant to pulsed electric fields, applications of this technology mainly focus on food-borne pathogens and spoilage microorganisms, especially for acidic food products. In addition to the volumetric effect of PEF technology in controlling the microbiological safety of foods in a fast and homogenous manner, successful application provides extended shelf life without the use of heat to preserve the sensory and nutritional value of foods. PEF technology has the potential to economically and efficiently improve energy usage, besides the advantage of providing microbiologically safe and minimally processed foods. Successful application of PEF technology suggests an alternative substitute for conventional thermal processing of liquid food products such as fruit juices, milk, and liquid egg (Mertens and Knorr, 1992; Hodgins et al., 2002; Bendicho et al., 2002a).

This chapter gives an overview of the basics of pulsed electric field processing of foods. Evolution of technology and certain factors involved are summarized with emphasis on a general review of PEF technology for food processing applications.

2. Historical Evolution and Chronological Developments in PEF Processing

There are several ways to use an electrical source for food pasteurization, in the form of ohmic heating, microwave heating, and high intensity pulsed electric fields (HIPEF). Among these, ohmic heating is one of the earliest methods. Ohmic heating relies on the use of heat generated when an electric current passes through the food, and has already been approved for viscous and particulate products, especially for aseptic processing. Application of electric

fields to preserve foods first appeared with the Electro-pure method for pasteurization of milk. In this early process, heat generated by an alternating electrical current (220 to 4200V) was used as a method of thermal sterilization in which heat flowed through the milk. The electrical chamber consisted of a vertical rectangular tube with opposing walls of carbon electrodes insulated with heavy glass. In this process, 100 gallons of preheated raw milk at 52°C was heated to 70°C and held for 15 seconds per hour. Successful results were obtained using the Electro-pure method in the inactivation of *Mycobacterium tuberculosis* and *Escherichia coli.* The method's efficiency in inactivating some bacterial populations resistant to other thermal pasteurization methods generated the use of electric current itself (Palaniappan et al., 1990). However, due to the low capacity of the system and the lack of proper equipment for temperature adjustment and control, the technology did not draw much attention or become commercially popular at that time (Getchell, 1935).

By the 1940s, electric fields were used in the food processing process for purposes other than inactivation of microorganisms. Flaumenbaum (1968) successfully used pulsed electric fields in a process that increases the permeability of plant tissues, facilitating subsequent extraction of cellular fluid. Today many applications of PEF focus on increasing the efficiency of juice extraction from fruits by using PEF as pretreatment (Heinz and Knorr, 2001). The electro hydrolytic method was introduced in the 1950s to inactivate microorganisms suspended in liquid systems. The inactivation was achieved through shock waves generated by an electrical arc, which also caused formation of highly reactive free radicals from the chemical components in foods. This method enabled the use of pulsed electric discharges at different energy levels to inactivate *E. coli, Streptococcus feacalis, Bacillus subtilis, Streptococcus*

cremoris, and *Micrococcus radiodurans* suspended in sterile distilled water (Gilliland and Speck, 1967).

A variety of pulsed electric field equipment and methods was described by Doevenspeck in 1960 (Germany), including a study addressing the nonthermal effects of pulsed electric fields on microbes. The study concentrated on the interaction of pulsed electric fields and microbial cell walls. Sale and Hamilton (1976, 1968) conducted a systematic study to assess the nonthermal bactericidal effects induced by electric fields and reported the lethal effects of homogeneous electric fields on bacteria, which included *E. coli, Staphylococcus aureus, Sarcina lutea, Bacillus subtilis, Bacillus cereus,* and *Candida utilis.* Along with this study, a series of papers were published on the use of electrical field technology, which concluded that bacterial cells exposed to direct electrical fields lose their membrane properties, leading to cellular death. The killing effect of PEF was mentioned as being independent of current density, thus the bacterial destruction was due to nonthermal effects. Sale and Hamilton are considered pioneers in the field and most of their findings are still being used today to assess the critical process parameters for effective PEF inactivation. They emphasized that electric field strength, pulse duration, and morphological parameters of the target microorganisms are the main factors involved in PEF application (Sale and Hamilton, 1968).

Once the basic principles and potential of PEF technology were understood, researchers in the area of genetic engineering and medical science began exploring the action mechanism at a cellular level. Zimmermann et al. (1974) introduced the dielectric rupture theory using a method developed to promote cell fusion, which would provide control over the permeability

of cell membrane in localized zones. This process then referred as electrical breakdown or reversible electroporation is still being used to explain the action mechanism of electric fields on cells. Several studies followed the work of Zimmerman on the action mechanism of electrical pulses, combining the theory of membrane disruption by electric fields with the previous work on process parameters, sensitivity of microorganisms, and inactivation mechanisms (Kinosita and Tsong, 1977; Sugar and Neumann, 1984; Tsong, 1990; Ho and Mittal, 1996). By the 1980s, the sensitivity of different kinds of microorganisms to pulsed electric fields was studied by a group led by Hulsheger, in which the earliest mathematical expressions were derived to describe inactivation kinetics as a function of electric field strength and treatment time (Hulsheger and Neumann, 1980; Hülsheger, 1981; Hülsheger et al., 1983).

In the 1980s, interest in PEF technology suddenly increased among scientific communities in the commercialization of PEF applications. Exploration of PEF technology was complicated without the collaborative work of researchers from different fields of study. Multidisciplinary research groups, including microbiologists, food scientists, and electrical engineers supported by food companies, began investigating the unknowns of pulsed electric fields, which eventually became known as an emerging innovative nonthermal technology for food processing applications (Table 1).

These investigations were followed by several patents on PEF, mainly focused on the microbial inactivation, tissue response to electric fields, enzyme inactivation, engineering aspects, inactivation kinetics, modeling, and scale-up (Table 2).

One of the first industrial applications of PEF technology was the ESTERIL process, developed by Krupp Mashinentechnik (Hamburg, Germany) in the late 1980s for electrical sterilization and pasteurization of pumpable electrically conductive media (Sitzman, 1990). Successful results were reported from these early applications (Hamburg University in conjunction with Krupp) and thus PEF technology proved to be a promising treatment for fluid foods such as orange juice and milk. In a later study, a microbial reduction of 4D was reported for *Lactobacillus brevis* inoculated milk when treated with 20 pulses of 20 μs at 20 kV/cm, *S. cerevisiae* inoculated orange juice treated with 5 pulses of 20 μs at 4.7 kV/cm, and *E. coli* inoculated sodium alginate treated with 5 pulses of 20 μs at 14 kV/cm (Grahl et al., 1992). The ELCRACK process was also developed by Krupp Mashinentechnik (Hamburg, Germany), the disruption of vegetable and animal cell membranes subjected to pulsed electric fields. The process consisted of exposing the slurry of comminuted fish or meat to high intensity electric pulses, which caused disruption of the cell membranes and subsequent release of fat from the cells during the separation step (Sitzman, 1995). PurePulse Technologies, a subsidiary of Maxwell Laboratories (San Diego, CA), developed CoolPure® pulsed electric field processing systems in 1995 for antimicrobial treatment of liquids and pumpable foods. That same year, the U.S. Food and Drug Administration (FDA) released a "letter of no objection" for the use of pulsed electric fields, thus approving industrial application of PurePulse Technologies. In 1996, PEF treatment on liquid eggs was also approved by the FDA with certain conditions to be accepted. Regulations required active and continuous communication between the FDA and production facility during development of this novel process within specified safety margins. Recognition by regulatory agencies enabled further implementations with an increasing interest in the technology.

From 2000 to the present, great advances have been achieved towards commercialization of PEF applications in food processing. However, translating the technical parameters into affordable and effective PEF systems within legal regulations is not easy. Currently, commercial PEF systems are available that include both bench-top and industrial systems, as those provided by PurePulse Technologies, Inc. and Thomson-CSF, besides many different lab-scale and pilot-scale PEF systems. The use of solid-state high voltage pulsed power systems, developed by Diversified Inc., has provided reliability and process consistency in PEF processing when used in a laboratory scaled up to commercial food processing applications. The present challenge is to increase treatment capacity with the use of feasible high power systems, by optimizing the overall PEF system design in light of critical process parameters.

3. Present Status of PEF Technology and Applications

The extent of improvement in a food processing company achieved by an emerging technology generally reflects the interest in that technology by the food industry. The method of high intensity pulse electric fields used to inactivate microorganisms has been under research for nearly 45 years, initiated with the first patent received by Doevenspeck in 1960. During this time span the technology has proven to be most effective in the inactivation of vegetative bacteria, yeasts, and molds, while bacterial spores are much more tolerant (Qin et al., 1996). Many successful steps have been taken in the design of system components and inactivation mechanism for different species. However, some technical issues still remained unsolved. Inactivation kinetics and the effect of PEF on spores are some of the most discussed issues in recent studies. Methods applied to thermal processing technologies by

plotting logs of the numbers of survivors against log or treatment time, or number of pulses, have been used to explain inactivation kinetics neglecting the deviations from linearity for these plots (Zhang et al., 1995b). The lethal effects of PEF processing on spores are still being explored by means of effective delivery of pulses into the relatively non-conductive and dehydrated cytoplasm of spores, which was proposed as the main resistance mechanism (Hamilton and Sale, 1967). Additionally, the synergistic effect of PEF technology in combination with other mild preservation methods is one of the research interests popular in recent years. The use of antimicrobials as nisin or other bacteriocins has been proposed as having lethal effects on electroporation (Kalchayanand et al., 1994).

Considering the effectiveness of PEF treatment on liquid products, such as milk, fruit juices, liquid egg, and any other pumpable food products, extensive research has been done to implement the process at an industrial level. Flavor freshness, economic feasibility, improvements in functional and textural attributes and extended shelf life are some of the main points of interest besides achievement of microbiological safety of food products (Dunn, 2001). Among all liquid products, PEF technology has been most widely studied with apple juice, orange juice, milk, liquid egg, and brine solutions (Qin et al., 1995).

The rapid increase in the market segment of fruit juices has led manufacturers to seek ways to overcome the thermal effects of processing while increasing the shelf life of juices (Hodgins et al., 2002; Sadler et al., 1992). Application of PEF is especially promising for the citrus industry, which is concerned with the spoilage microorganisms and resultant production of off-flavor compounds such as lactic acid bacteria (Hendrix and Red, 1995).

PEF processing has been successful in a variety of fruit juices with low viscosity and electrical conductivity such as orange, apple, and cranberry juice. Recent studies reported more than a 3-log reduction in orange juice (Qin et al., 1998) and apple juice (Evrendilek et al., 2000), and a 2-log reduction in cranberry juice (Jin and Zhang, 1999). *Lactobacillus brevis* inoculated in citrus juice was studied by Elez-Martinez et al. (2005), achieving 5.8 log reductions when processed at 35 kV/cm for 1000 μs using bipolar pulses. A 6.2-log reduction was achieved in grape juice at 51°C when 20 pulses of 80 kV/cm were applied with nisin addition (Wu et al., 2005). Additionally, the color change in fruit juices (subject to prolonged storage) was reportedly less in juices treated by PEF, as in a recent study of PEF treated orange juice stored at 4°C for 112 days; there was less browning than thermally pasteurized juice, which was attributed to conversion of ascorbic acid to furfural (Yeom et al., 2000). Retention of carotene in orange-carrot juice and color in apple juice were also reported, respectively (Esteve et al., 2001; Evrendilek et al., 2000).

Studies conducted on the effects of PEF on dairy products such as skim milk, whole milk, and yogurt compromise a major section of PEF applications (Alvarez and Ji, 2003). Milk is very susceptible to both spoilage and pathogenic microorganisms requiring the application of thermal pasteurization under current regulations, which ensures safety but generally results in a cooked flavor (Wirjantoro and Lewis, 1997). Dunn and Pearlman (1987) were the first researchers to conduct a challenge test prior to shelf life study on homogenized milk inoculated with *Salmonella dublin* and *E. coli* treated with 36.7 kV/cm and 40 pulses for 25 min. PEF treated milk was reported to contain 5-log and 3-log reductions in bacterial populations for *S. dublin* and *E. coli*, respectively, when compared to untreated milk (Dunn,

1996). Another study by Qin et al. (1995b) confirmed the retention of the physical, chemical, and sensory attributes of PEF treated milk with application of 40 kV/cm of 6 pulses after 2 weeks of refrigerated storage. A significant inactivation of *Saphylococcus aureus* inoculated skim milk treated by PEF was also reported (Evrendilek et al., 2004).

In addition to inactivation of microorganisms and enzymatic treatments, the application of PEF provides highly effective pre-treatment for foods and food ingredients in an ecological and gentle way (Guderjan et al., 2005). The ability of pulsed electric fields to destroy cell membranes by removing the cellular turgor component of the texture and exerting an estimable influence on the viscoelestic properties of the plant tissue has been used in several applications (Lebovka et al., 2003). Beginning with Flaumenbaum (1968), an electrical treatment of apple mash, known as electro-plasmolysis, achieved an increase in juice yield by 10-12 %. Further yield enhancement was achieved with treatment on carrot juice (Knorr et al., 1994), red beet juice (Bouzrara and Vorobiev, 2003), and grape and sugar beet juice (Estiagi and Knorr, 2002). The effect of combined pressure-PEF treatment resulted in permeabilization of the cell matrices, improving mass transfer across the cell membranes (Angersbach and Knorr, 1999). As a result, the treatment enhances the solid-liquid expression of different biological tissues and increases the juice yield (Vorobiev et al., 2004; Wang and Sastry, 2002). The application involves the usage of electric fields at 5-10 kV/cm for 0.1-10 microseconds at room temperature, which could be a good alternative against traditional methods of plant tissue treatment (Lebovka et al., 2005).

PEF technology has recently been used in alternative applications including drying enhancement, enzyme activity modification, preservation of solid and semi-solid food products, and waste water treatment, besides pre-treatment applications for improvement of metabolite extraction. The ability of PEF to increase permeabilization means it can be successfully used to enhance mass and heat transfer to assist drying of plant tissues. Studies conducted on different plant tissues such as potato tissue (Agersbach and Knorr, 1997), coconut (Ade-Omowaye et al., 2000), carrots (Rastogi et al., 1999), mango (Tedjo et al., 2002), and apple slices (Ade-Omowaye et al., 2002) reported increased yield of water removal by 20-30% when exposed to low intensity electric fields. Pre-treating red peppers at the PEF-enhanced initial drying rate indicated that combining PEF and partial osmotic dehydration in solutions before air drying may offer good potential in enhancing mass transfer rates and preserving the color quality of red peppers (Ade-Omowaye et al., 2003). As stated before, PEF technology commonly focuses on processing pumpable and homogenous liquid foods free of particles and air. In the case of solid foods the mixture is mixed with air, contributing to the low electrical conductivity of the product, hence, not limiting the maximum applicable electric field intensity. Some studies conducted on model foods (Zhang et al., 1994), viscous foods such as yogurt and rice pudding (Ratanatwirong et al., 2001), or particulate foods such as pea soup with plastic beads (Dutreux et al., 2000) reported successful results for PEF applications on solid or semi-solid foods.

4. Fundamental Aspects of Pulsed Electric Fields Treatment

Thermal processing is traditionally accomplished by subjecting the food to a temperature range of 60 to 130°C for a few seconds to minutes, involving a large amount of energy transfer to the food (Jay, 1992). Although the target of the energy is to destroy microorganisms for preservation of food, many unwanted reactions are enhanced that lead to undesirable changes, including the loss of the nutritional and organoleptic quality of food (Alwazeer et al., 2003). Alternative technologies for inactivating microorganisms without reliance on heat are not new concepts, their development for use as food preservation treatments still receives considerable attention in only recent years. Nonthermal food processing technologies, with the use of ambient or near-ambient temperatures, may provide an alternative to thermal technologies by means of improving safety while maintaining product quality and economic feasibility.

Several nonthermal processing technologies were proposed based on the same basic principle of keeping food below temperatures normally used in thermal processing. This would retain the nutritional quality of food including vitamins, minerals, and essential flavors while consuming less energy than thermal processing. High hydrostatic pressure, oscillating magnetic fields, intense light pulses, irradiation, the use of chemicals and biochemicals, high intensity pulse electric fields, and the hurdle concept were all recognized as emerging nonthermal technologies in recent years (Barbosa-Canovas et al., 1999). Each of the nonthermal technologies has specific applications in terms of the types of foods that can be processed. Among these, pulsed electric fields (PEF) is one of the most promising nonthermal processing methods for inactivation of microorganisms, with the potential of

being an alternative for pasteurization of liquid foods. Comparable to pasteurization, yet without the thermal component, PEF has the potential to pasteurize several foods via exposure to high voltage short pulses maintained at temperatures below 30-40°C. The basic definition of PEF technology relies on the use of high intensity pulsed electric fields (10-80 kV/cm) for cell membrane disruption where induced electric fields perforate microbial membranes by electroporation, a biotechnology process used to promote bacterial DNA interchange. Induction of membrane potentials exceeding a threshold value often result in cell damage and death (Zimmerman, 1986).

PEF technology is based on a pulsing power delivered to the product placed between a set of electrodes confining the treatment gap in a PEF chamber. The equipment consists of a high voltage pulse generator and a treatment chamber with a suitable fluid handling system and necessary monitoring and controlling devices (Figure 1). Food product is placed in the treatment chamber, either in a static or continuous design, where two electrodes are separated with a nonconductive material to avoid electrical flow from one to the other. Generated high voltage electrical pulses are applied to the electrodes, which then conduct the high intensity electrical pulse to the product placed between the two electrodes. The food product experiences a force per unit charge, the so-called electric field, which is responsible for the irreversible cell membrane breakdown in microorganisms (Zimmermann and Benz, 1980). Inactivation of microorganisms exposed to high voltage PEF is related to the electromechanical instability of the cell membrane, while the dose of the application is adjusted by means of electric field intensity, number of pulses, and treatment time.

4.1. System Components

The high intensity pulsed electric field processing system consists of a high voltage source, capacitor bank, switch, and treatment chamber. Generation of pulsed electric fields requires a discharge of electrical energy within a short period of time. This is accomplished by the pulse-forming network (PFN), an electrical circuit consisting of one or more power supplies with the ability to charge voltages (up to 60kV), switches (ignitron, thyratron, tetrode, spark gap, semiconductors), capacitors (0.1-10 μF), inductors (30 μH), resistors (2Ω -10MΩ), and treatment chambers (Gongora-Nieto et al., 2002).

4.1.1. Power Supply

High voltage pulses are supplied to the system via a high voltage pulse generator at required intensity, shape, and duration. The high voltage power supply for the system can either be an ordinary source of direct current (DC) or a capacitor charging power supply with high frequency AC inputs that provide a command charge with higher repetitive rates than the DC power supply (Zhang et al., 1996). The simplest PFN is an RC (resistance-capacitance) circuit in which a power supply charges a capacitor that can deliver its stored energy to a resistive load (treatment chamber) in microseconds, by activation of a switch (Gongora-Nieto et al., 2002). Total power of the system is limited by the number of cycles a capacitor can be charged and discharged in a given time. The electrical resistance of the charging resistor and the number and size of the capacitors determine the power required to charge the capacitor, wherein a smaller capacitor will require less time and power to be charged than a larger one. The capacitance Co (F) of the energy storage capacitor is given by Eq. (1):

$$C_0 = \frac{\tau}{R} = \frac{\tau \sigma A}{d} \quad (1)$$

where τ (sec) is the pulse duration, R (Ω) is the electrical resistance, σ (S/m) is the conductivity of the food, d (m) is the treatment gap between electrodes, and A (m^2) is the area of the electrode surface. The energy stored in a capacitor is defined by the mathematical expression:

$$Q = 0.5 C_0 V^2 \quad (2)$$

where Q is the stored energy, C_0 is the capacitance, and V is the charge voltage.

The second component of the PFN is the high voltage switching device needed to instantaneously discharge the stored energy through the PFN circuit. The switch plays an important role in the efficiency of the PEF system, and it is selected based on its ability to operate at a high voltage and repetition rate. There are two main groups of switches currently available: ON switches and ON/OFF switches. ON switches provide full discharging of the capacitor but can only be turned off when discharging is completed. ON switches can handle high voltages with relatively lower cost compared to ON/OFF switches, however, the short life and low repetition rate are some disadvantages to be considered for selection. The Ignitron, Gas Spark Gap, Trigatron, and Thyratron are some of the examples from this group. ON/OFF type switches have been developed in recent years that provide control over the pulse generation process with partial or complete discharge of the capacitors. Improvements on switches, mainly on semiconductor solid-state switches, have resulted in longer life spans

and better performance. The Gate turn off (GTO) thyristor, the insulated gate bipolar transistor (IGBT), and the symmetrical gate commutated thyristor (SGCT) are some examples from this group (EPRI and Army, 1997; Barsotti et al., 1999; Barbosa-Cánovas et al., 1999; Gongora Nieto et al., 2002; Sepulveda and Barbosa-Cánovas, 2005).

For a pulse-forming network system, the relative electrical value of each component determines the shape of the pulse. In a capacitance-resistance circuit, the pulse generated is exponentially decaying (Figure 2) where the voltage across the treatment chamber as a function of time is defined as:

$$V(t) = V_0 e^{-t/\tau} \tag{3}$$

where V_0 is the voltage charged in the capacitor of the PFN, t is the pulse duration time, and τ=RC is the time constant where in a RC circuit pulse duration equals approximately five time constants (Cogdell, 1999). Considering the exponential decaying behavior of the delivered energy, τ can be adopted as the effective pulse width, calculated as the time required for the input voltage to decay to 1/e (37%) of its maximum value (Zhang et al., 1995a; Grahl and Mark, 1996; Barsotti et al., 1999; Gongora-Nieto et al., 2002). More complex PFN systems can provide square pulses, bipolar pulses, and instantaneously reversal pulses, as illustrated in Figure 2.

4.1.2. Treatment Chamber

One of the most important and complicated components in the processing system is the treatment chamber. PEF investigators studying inactivation and preservation effects have been highly inventive in treatment chamber design (Figure 3). Several different designs have been developed through the years for this key component, wherein high voltage delivered by the power supply is applied to the product located between a pair of electrodes. The basic idea of the treatment chamber is to keep the treated product inside during pulsing, although the uniformity of the process is highly dependent on the characteristic design of the treatment chamber. When the strength of applied electric fields exceeds the electric field strength of the food product treated in the chamber, breakdown of food occurs as a spark. Known as the dielectric breakdown of food, this is one of the most important concepts to be considered in treatment chamber design. Dielectric breakdown of the food is generally characterized as causing damage on the electrode surfaces in the form of pits, a result of arching and increased pressure, leading to treatment chamber explosions and evolution of gas bubbles. Intrinsic electrical resistance, electric field homogeneity, and reduction and generation of enhanced field areas are some other important design criteria for a successful design in terms of energy consumption and low product heating (Sepulveda and Barbosa-Cánovas, 2005). Treatment chambers are mainly grouped together to operate in either a batch or continuous manner; batch systems are generally found in early designs for handling of static volumes of solid or semi-solid foods. Sale and Hamilton (1967), Dunn and Pearlmann (1987), and Mizuno and Hori (1991) have shown some examples of static chamber designs. Those design served as a basis for the evolution of continuous chambers that would provide advantageous

pumping for efficient use in industrial applications. The concentric cylinder, concentric cone, and co-field treatment chambers are some examples of successful continuous flow chambers.

The evolution of treatment chambers began with static chambers consisting of U-shaped polyethylene spacers composed of carbon electrodes supported on brass blocks (Sale and Hamilton, 1967). The electrode area and amount of food that could be treated were regulated by using different spacers. The maximum electric field that the chamber could handle was limited to 30 kV/cm due to the electrical breakdown of air above the food. Dunn and Pearlmann (1987) continued to improve designs with a chamber consisting of two stainless steel electrodes separated by a cylindrical nylon spacer. This chamber was one of the earliest designs incorporating parallel plate geometry using flat electrode surfaces separated by an insulated spacer. Liquid foods were introduced through a small aperture located in one of the electrodes, which could also be used for temperature measurement during processing. The limitations experienced with this chamber geometry were mainly due to surface tracking on the fluid, resulting in arching. Thus, conical geometry naturally evolved offering the advantage of ease in eliminating bubbles (Zhang et al., 1995b). Two round-edged, disc-shaped stainless-steel electrodes were polished to mirror surfaces, and polysulfone or Plexiglas was used as insulation material. Cooling was maintained by circulating water at pre-selected temperatures through jackets located in the electrodes. This chamber was completely sealed and thus different from other geometries, so to prevent possible sparking or high pressure development, a pressure release device was included within the treatment chamber.

The earliest attempts at continuous treatment chamber design were by Dunn and Pearlmann (1987) in their use of different geometries based on the same principle of circulating food through a closed system. The electrodes were separated from the food by ion conductive membranes made of sulfonated polystyrene, while an electrolyte was used to facilitate electrical conduction between electrodes and ion permeable membranes. New geometry designed for static operation was modified by adding baffled flow channels inside for operation as a continuous chamber (Zhang et al., 1995b; Qin et al., 1996). The concept of enhanced electric fields in the treatment zone was a milestone for treatment chamber designs. The design was composed of a continuous treatment chamber with coaxial conical electrodes and introduced by Bushnell et al. (1993), later followed by the continuous treatment chamber with parallel electrodes coaxial cylindrical electrodes (Qin et al., 1997). Among all designs to date, coaxial and co-field arrangements are currently favored over most designs. The coaxial arrangement has inner and outer cylindrical electrodes, shaped to minimize the electrical enhancements with uniform fluid flow. In a simple coaxial chamber design, the electric field intensity is not uniform and changes with change in location, as shown in the following equation (Zhang et al., 1995b):

$$E = V /(r \ln(R_2 / R_1)) \tag{4}$$

where r is the radius of electric field measurements and R_2 and R_1 are the radii of the outer and inner electrodes, respectively. The advantageousness of coaxial configurations in providing a well defined electric field distribution is based on the idea of predicting and controlling non-uniformity of electrical field distribution. Co-field designs introduced by Yin

et al. (1997) comsisted of two hollow electrodes separated by an insulator providing a tube for product flow. Based on the same principles for coaxial arrangement, co-field designs enabled handling a higher load resistance, allowing the pulser to operate at lower currents in the treatment chamber when compared with coaxial design (Dunn, 2001). Several research teams have proposed a number of different geometries for chambers, such as the glass coil static (Lubicki and Jarayam, 1997), needle-plate, and ring-cylinder continuous treatment chambers (Sato et al., 2001). Limitations and the applicability of each arrangement have shaped new routes towards innovative designs.

Operation and performance of the PEF system are generally controlled by a central computer connected to the high voltage pulse generator. The computer controls the voltage and pulsing frequency in addition to operation of pumps. Data logs of temperature at different points, including flow rate, voltage, current, and power curves of applied pulses, are also recorded using appropriate probes and an oscilloscope card fed into the central computer (Sepulveda and Barbosa-Cánovas, 2005).

5. Effectiveness of PEF Treatment

Sufficient microbial reduction by PEF has already been confirmed, however, the degree of inactivation strongly depends on several constraints. The lethality factors contributing to the effectiveness of pulsed electric field technology can be grouped as technical, biological, and media factors. Each group of determinant factors is related to type of equipment, processing parameters, target microorganism, and type and condition of media used.

5.1. Technological Factors

Electric field intensity has been identified as the most relevant factor affecting microbial inactivation by PEF treatment. Electric field intensity in combination with total treatment time mainly contributes to the extent of membrane disruption (Hamilton and Sale, 1967). Critical electrical field intensity must be reached for any effect to take place, and with a transmembrane potential exceeding a threshold value of around 1 V, exponential disruption is often observed resulting in cell damage and death (Zimmerman, 1986; Hulsheger et al., 1981).

A good understanding of the electrical principles behind PEF technology is essential for a comprehensive analysis of the PEF system. The electrical field concept, introduced by Faraday, explains the electrical field force acting between two charges. When unit positive charge q located at a certain point within the electric field is generated in the treatment gap (E_r), it experiences force F identified by position vector r (Blatt, 1989). The electrical field per unit charge is then defined as shown in Eq. (5):

$$E_r = \frac{F_{qr}}{q} \tag{5}$$

The electrical potential difference (V) between voltage across two points, separated by a non-conductive material, results in generation of an electric field between these points, with an electrical intensity (E) directly proportional to the magnitude of potential difference (V) and inversely proportional to the distance (d) between points, as given in Eq. (6).

$$E = \frac{V}{d} \tag{6}$$

High intensity pulse electric fields can be applied in the form of exponential decay, square, wave or oscillatory-either monopolar (only positive pulses) or bipolar (alternating positive and negative pulses). Square and exponential decay pulse shapes are the two most commonly used applications due to their effectiveness to inactivate microorganisms. Square pulses are reportedly superior to exponential decay pulses since they ensure a 60% higher inactivation rate for *S. cerevisiae* and consume 15% less energy with fewer than 20 pulses applied at 12 kV/cm (Zhang et al., 1994a). The superiority of square pulses over exponential decay pulses is due to the uniformity of electric field intensity during each pulse (Figure 4). When maximum voltage is applied in the case of square pulses, the electric field intensity remains constant for that pulse duration. However, in the case of exponential decay, the voltage exceeds beyond an effective voltage while exponentially increasing to a peak and decreasing to zero, and therefore has no bactericidal effect, thus contributing to the heating effect and considered to be waste (Qin et al., 1994; Pothakamury et al., 1996).

Treatment time for a PEF application is defined as a function of pulse width and number of pulses. In RC circuits, the total pulse duration equals approximately (5τ) five time constants (Cogdell, 1999). Considering the exponential decaying behavior of the delivered energy, τ can be adopted as the effective pulse width, calculated as the time required for the input voltage to decay to 1/e (37%) of its maximum value (Zhang et al., 1995b; Grahl and Mark, 1996; Barsotti et al., 1999; Gongora-Nieto et al., 2002). In general, PEF treatments are

applied in the form of short pulses to avoid excessive heating or undesirable electrolytic reactions (Sepulveda and Barbosa-Cánovas, 2005).

5.2. Biological Factors

Biological factors that include the individual characteristics of target microorganisms and their physiological and growth states are determinant factors affecting PEF application. The susceptibility of a microorganism to PEF inactivation is highly related to the intrinsic parameters of the microorganism such as size, shape, species or growth state. Generally, gram-positive vegetative cells are more resistant to PEF than gram-negative bacteria, while yeasts show a higher sensitivity than bacteria. Induction of electric fields into cell membranes is greater when larger cells are exposed to PEF treatment (Sale and Hamilton, 1967; Hulsheger et al., 1983; Zhang et al., 1994). Most of the studies on yeast activation have been conducted on *S. cerevisiae*. Among bacterial species, yeasts were reported to be more susceptible to PEF inactivation than bacterial cells, probably due to their larger size, while gram-negative species are more resistant than gram-positive species (Jacob et al., 1981; Lubicki and Jarayam, 1997; Hulseger et al., 1983). Initially, the extensive research by Sale and Hamilton (1967) stated that neither the growth phase nor the temperature of the medium affects the inactivation mechanism. However, the higher sensitivity of microorganisms in the logarithmic phase, as compared to ones in the stationary phase, was observed in later years (Hulsheger, 1983). *E. coli and S. cerevisiae* are two of the most studied microorganisms in the area of PEF processing. Extensive studies have been concentrated on several species including gram-negative pathogens *Salmonella dublin, S. typhimurium*, and *E. coli* O157:H7; gram-positive microorganisms *S. aureus* and *L. monocytogenes*; spore-forming pathogens *B.*

cereus; and non-pathogenic flora like *P. flourescens*. In general, the effectiveness of PEF treatment is highly dependent on the individual characteristics of biological systems, which may deviate from general resistance guidelines.

In comparison with the extensive research devoted to the destruction of microorganisms by PEF, there are few reports on the inactivation of enzymes by PEF (Yeom et al., 1999). Conventionally, enzymes in foods are inactivated by thermal processing (Ho et al., 1997). Studies to explore enzyme inactivation by PEF first began with Gilliland and Speck (1967), however the reported studies were not consistent concerning the potential effectiveness of PEF treatment on enzymes. In recent years, Vega-Mercado et al. (1995; 2001) reported a 90% reduction of plasmin in addition to 60% and 80% reductions of maximum proteolytic activity of protease extracted from *Pseudomonas flourescens* dispersed in skim milk and tyriptic soy broth, respectively. In a study conducted by Ho et al. (1997), several enzymes dispersed in buffer solutions were assayed. For eight different enzymes, a 70-80% activity reduction was observed for lipase, glucose oxidase, and α-amylase, while the activity reduction was 30-40% for peroxidase and polyphenol oxidase and 5% for alkaline phosphatase. Lysozyme and pepsin were further reported to increase their activity after PEF treatment in this study. Giner et al. (2000) reported the inactivation of pectin methyl esterase (PME) from tomato. Another microbial enzyme studied (suspended in SMUF) is lipase from *P. flourescens* (Bendicho et al., 2002b), for which an inactivation of 62% was reported in batch mode and 13% in the continuous flow process. Enzyme inactivation is generally explained as due to the effect of high intensity pulse electric fields (in the form of denaturation) to change the magnitude of forces acting in native structures, such as

hydrophobic interactions, hydrogen bonding, ion pairing, or molecular structure. It has also been proposed that electric fields influence the conformational state of proteins, therefore contributing to enzyme denaturation (Tsong and Astunian, 1986). Compared to the number of studies reported for enzyme inactivation by PEF, little information is available on the mechanism of inactivation, which may be due to the lack of analysis of enzyme structural data (Yeom et al., 1999).

Most of the research focuses on the inactivation of vegetative cells of bacteria, while only a few reports are available on the inactivation of spores describing a limited effect of PEF. *Bacillus cereus* spores were mostly resistant (approximately 1 log reduction) to a mild PEF treatment at electric field strength of 20 kV/cm and 10.4 pulses in a study conducted on apple juice (Cserhalmi et al., 2002). Another study conducted by Pagan et al. (1998) found that *Bacillus cereus* spores were not affected by PEF treatment of 60 kV/cm for 75 pulses at room temperature. On the other hand, Marquez et al. (1997) reported 3.42 and 5 log reductions of *Bacillus subtilis* and *Bacillus cereus* spores, respectively, with PEF treatment of 50 kV/cm for 50 pulses at 25°C in salt solution. Additionally, mold condispores were reported to be sensitive to PEF in fruit juices whereas *Neosartorya fischeri* ascospores were resistant to PEF treatments (Raso et al., 1998). The variation in reported results could be explained as the influence of spore resistance by factors other than the process parameters, such as preparation of the spores or media factors.

5.3. Media Factors

Beginning with the detailed research of Sale and Hamilton (1967), most of the inactivation studies on the effectiveness of PEF treatment were accomplished using model systems that included distilled water, deionized water, phosphate buffer, and simulated milk ultrafiltrate (SMUF). The physical and chemical characteristics of food products are known to strongly influence the effectiveness of microbial inactivation during PEF application (Wouters et al., 2001), thus the challenge experienced using real food systems was due to the important role of the media's chemical and physical characteristics. These factors most likely influence the recovery of injured microbial cells and their subsequent growth following PEF exposure, since the presence of food components, such as fats and proteins, has reportedly had a preventive effect on microorganisms against PEF treatment (Grahl and Markl, 1996; Martin et al., 1997; Ho et al., 1995). On the contrary, a possible repair process might be delayed by unfavorable growth conditions (Aronsson et al., 2004). Similar to the intrinsic parameters of microorganisms, treated media has its own intrinsic factors such as conductivity, resistivity, dielectric properties, ionic strength, pH, and composition. Each of these parameters influences the PEF treatment either alone or in combination.

Conductivity and ionic strength are closely related. Generally, lowering the electrical conductivity of treatment media reduces the temperature and applied power and therefore increases the electric field intensity and effectiveness of PEF application. This lowering of electrical conductivity should be at an optimal level to ensure establishment of the transmembrane potential (Hulsheger et al., 1981; Jarayam et al., 1993; Vega-Mercado et al., 1996; Sepulveda and Barbosa-Cánovas, 2005). The mobility of the ions in the solution is

increased with increasing temperature, which in turn increases the conductivity (Heinz et al., 2002).

Temperature is one factor that has been proposed to correlate with microbial inactivation. Although PEF application is strictly a nonthermal processing technology, the synergistic effect of temperature on foods (due to changes in the properties of cell membranes) becomes greater when foods are subjected to high intensity pulse electric fields (Jarayam et al., 1997). The temperature of the medium in which cells are suspended has a significant influence in determining the membrane fluidity properties. At low temperatures, the phospholipids are closely packed into a rigid gel structure, while at high temperatures, they are less ordered and the membrane has a liquid-crystalline structure. This phase transition from gel to liquid is dependent on temperature and can affect the physical stability of the cell membrane (Stanley, 1991). The synergistic effect of mild temperatures (below 65°C) in combination with PEF provided promising results as an alternate technology to pasteurization (Zhang et al., 1995b; Sepulveda et al., 2002; Aronsson et al., 2001; Jarayam et al., 1992).

The influence of pH and water activity (a_w) on microbial growth was documented by Jay (1992). It was found that changes in acid content and a_w of a food dramatically affect the type of microorganism growing in that particular food, however, the relationship between a_w, pH, and pulsed electric fields was not fully explored. In earlier studies, pH was reported to have no effect on the inactivation of microorganisms (Sale and Hamilton, 1967; Hulseger et al., 1981). But more recently, the important effect of pH on inactivation kinetics was demonstrated (Vega-Mercado et al., 1996) with a study on *E. coli,* where inactivation was

greater at pH 5.7 compared to pH 6.8. In a study by Wouters et al. (1999), microbial reduction was 3.4 log units greater for *L. innocua* at pH 4.0 than at pH 6.0. Another study conducted on mold ascospores in fruit juices (Raso et al., 1998) demonstrated even higher inactivation rates with lower pH values. On the contrary, the resistance of *Salmonella senftenberg* was reportedly higher with PEF treatment at pH 3.8 than at pH 7.0 (Alvarez et al., 2000).

5.4. Inactivation Kinetics and Modeling

The use of first order kinetics to describe the relationship between inactivation and electric field strength, or treatment time, is common in most inactivation studies. The significant reduction in the inactivation rate at a given electric field when treatment times are long, so-called the tailing effect, has been mentioned by several researchers in recent years (Peleg, 1995; Cole, 1999). Considering the fact that not all microorganisms show identical resistance to inactivation, to the applied stress, it is not surprising to observe a spectrum of resistance. The tailing behavior under less stressful treatments was reported to be more obvious, compared to highly stressful conditions in which microorganisms die close together. The need for models describing the death kinetics with PEF treatment has been responded to by several researchers (Peleg, 1995; Sensoy et al., 1997; Alvarez at al., 2003; Rodrigo et al., 2003; Wouters et al., 2001). Hulsheger and Niemann (1980) proposed the first model for PEF inactivation of microorganisms based on the dependence of the survival ratio S (N/N_0) on the electric field intensity E, according to the following expression:

$$\ln(S) = -b_E(E - E_c) \qquad (7)$$

where b_E is the regression coefficient, E is the applied electric field, and E_c is the critical electric field for the condition of 100% survival. The slope of straight survival curves is expressed as regression coefficient and the critical electric field (E_c) was reported to be a function of cell size and pulse width. Another version of this model was introduced by Hulsheger et al. (1981) relating the microbial survival fraction (S) with PEF treatment time (t), as in the following equation:

$$\ln(S) = -b_t \ln(\frac{t}{t_c}) \tag{8}$$

where b_t is the regression coefficient, t is the treatment time, and t_c is the critical treatment time obtained for t when survival fraction is 100%. Rearrangement of these models enabled derivation of kinetic constants for cited microorganisms (Hulsheger, 1983). The susceptibility of a microorganism to inactivation was correlated to kinetic constants where a small value for kinetic constant indicates a wide span in the inactivation rate curve.

Peleg (1995) proposed a second model describing the sigmoid shape of the survival curves for PEF treatment. The model basically represents the percentage of surviving organisms as a function of the electric fields and applied number of pulses. In this model, the steepness of the sigmoid shape is represented by a critical electrical field intensity corresponding to 50% survival (E_d) and a kinetic constant as a function of number of pulses (K_n), as given in the following equation:

$$S = \frac{1}{1 + e^{\frac{E - E_d}{K}}} \tag{9}$$

where a small value for K indicates a wide span in the inactivation rate curve and lower sensitivity to PEF, and a small value for E_d indicates less resistance to PEF treatment.

6. Future Aspects and Economic Analysis of PEF Processing

Today's consumers expectations that food products provide convenience, variety, adequate shelf life and caloric content, reasonable cost, and environmental soundness make the modifications of existing technologies and the adoption of novel processing technologies essential. Industrial food producers in competitive markets increasingly have to consider novel processing technologies in order to increase food safety and the ability to offer better products to consumers. As stated earlier in this chapter, the potential of PEF technology to replace traditional processing methods has further inspired researchers from both academia and industry to explore the unknowns of pulsed electric fields, while achieving improvements in current techniques. Possible factors related to the effectiveness of PEF technology have already been scanned and further research is being conducted to reach a complete understanding and to minimize drawbacks. At this point, in order to prove the applicability of PEF technology for practical use, laboratory outcomes must achieve a certain level of optimization for the process to gain importance.

6.1. Combination Studies

A major trend in the application of inhibitory techniques is to employ new combinations of techniques that deliver effective preservation without the extreme use of any single technique

(Leistner, 2000). Even though nonthermal inactivation is claimed in PEF processing, the inactivation effect of PEF on microbial flora and the shelf life extension of refrigerated products can be increased by applying PEF in combination with other stress factors, such as the presence of antimicrobial compounds like nisin and organic acids, increased water activity, pH, and mild heat.

Coupling of electric field treatments with moderate temperatures (50 to 60°C) is one of the most appealing combinations studied so far due to the synergistic effect on the inactivation of microorganisms, where at constant electric field strength, inactivation increases with rise in temperature. Temperatures used in combinations with PEF are held below those used in the pasteurization and sufficient cooling is provided to ensure proper food temperatures. Sensoy et al. (1997) achieved nearly a 2-log cycle increase in the inactivation of *Salmonella dublin*, by increasing the process temperature from 10 to 50°C with 100 μs PEF treatment at 25 kV/cm. Reina et al. (1998) reported the same results with *Listeria monocytogenes* inoculated in whole milk and treated with 600 μs PEF treatment at 30 kV/cm, where 3.5 and 4 log reductions were achieved at 10 and 50°C, respectively. Other microorganisms such as *Salmonella enteridis, E. coli*, and *Lactobacillus brevis* have shown similar effects. The use of mild sub-lethal thermal conditions in combination with PEF has proven to enhance the effectiveness of treatment, however the mechanism behind inactivation has not been determined yet. Schwan (1957) proposed the hypothesis that reduction in the charging time of bacterial membranes due to increased electrical conductivity of the media caused by increased temperatures was a factor. Slightly higher temperatures were suggested to change the phase state of cell membranes (Jarayam et al., 1992) or to weaken the cell membrane,

thus favoring destabilization and cell death (Pothakamury et al., 1996; Vega-Mercado et al., 1996).

In the presence of antimicrobial agents affecting the microbial membrane, such as nisin or organic acids, an increased inactivation in response to PEF was observed (Pol et al., 2000). The exposure of *L. innocua* to nisin after PEF treatment has an additive effect on the inactivation of microorganisms compared to PEF alone (Calderon-Miranda et al., 1999b; Terebiznik et al., 2000). Even though the mechanism behind this synergistic effect has not been fully understood, PEF treatment was suggested to facilitate the incorporation of nisin into the cytoplasmic membrane, resulting in formation of larger pores on the microbial membrane (Pol et al., 2000). As stated as a media factor before, lowered pH is one of the substantial hurdles enhancing the efficacy of other antimicrobial processes. PEF inactivation of *E. coli* O157:H7 in a 10% glycerol solution was enhanced synergistically by lowering pH from 6.4 to 3.4 using sorbic and benzoic acids (Liu et al., 1997). In a study conducted by Evrendilek and Zhang (2001), lowering pH of *E. coli* O157:H7 to 3.6 before PEF treatment resulted in higher inactivation rate compared to pH of 5.2 or 7.0. Similar to the nisin effect, 5% added ethanol increased the efficiency of PEF treatment on vegetative *Bacillus subtilis* cells due to the ability of ethanol to alter fluidity of microbial membranes (Heinz and Knorr, 2000). Acidification of the medium with hydrochloric acid, on the other hand, did not show extra inactivation of raw milk's microflora when compared to PEF alone (Smith et al., 2002). Considering the different effects of lowered pH on PEF, much more research is required for commercial adaptation (Yousef, 2001).

Combining high pressure treatment of 200 MPa for 10 min with PEF treatment to inactivate vegetative *Bacillus subtilis* cells increased specific pulse energy. For this process, kinetic changes in membrane components such as phase transition of lipids or proteins suggested these factors contributed to the level of inactivation by PEF (Heinz and Knorr, 2000).

6.2. Drawbacks and Limitations

A lot of research in the field of food engineering has focused on new preservation technologies, but very few of these methods have been implemented by the food industry until now due to their limitations and drawbacks. Besides numerous possibilities and the advantages of PEF technology there are some shortcomings that future research should address. The main limitations of this technology are scaling up of the system; bubble formation leading to electrical breakdown of the treated product concerning safety issues; particulate foods; availability of commercial units; and resistance of some microbial species including bacterial spores.

The major concern for commercialization of PEF technology, considering competition in the market, is the initial investment cost. Generation of high voltage pulses with sufficient peak power is the main limitation in processing large quantities of fluid economically. Industrial PEF equipment used to be rare and expensive, having a limited capacity of around 1,800 Lh^{-1} (Mittal et al., 2000), however with proper equipment, PEF is an energy efficient process compared to thermal pasteurization. The price of PEF equipment from bench-top to pilot plant models ranges from 40,000 to $500,000, and operating cost is estimated around $0.02 per liter (Gongora-Nieto et al., 2002). Despite high capital investments, operation costs

proved to be lower than traditional thermal processing technologies (Barbosa-Cánovas et al., 1999). With recent developments, commercial-scale PEF systems processing between 1,000 and 5,000 liters of liquid foods per hour are available, which would add only 0.03 to $0.07/L to final food costs (OSU, 2005).

The application of the technology is restricted to food products with low electrical conductivity capable of withstanding high electric fields. PEF technology has demonstrated to be applicable for pumpable foods while not much information exists on solid and particulate foods. PEF treated products are therefore limited to some extent in order to provide uniformity of electric field distribution within the treatment chamber (Qin et al., 1995).

The inactivation mechanism of PEF is assumed to be due to hyperpolarization and subsequent dielectric breakdown. However, in some cases, this high intensity short time pulsing can generate electrochemical reaction products with bactericidal properties, depending on the medium composition. Therefore, the model medium for inactivation studies should be conducted in properly chosen systems to exclude indirect inactivation by electrochemical reaction products. In the case of treatment of foods, more attention is required to design treatment conditions that minimize electrochemical changes in order to avoid loss in product quality and safety (Kristien et al., 2004).

As a final perspective, one of the major hurdles to commercialization is the lack of experimental standardization between PEF studies to date, which in effect disables the

development of a common language for parameters and measures. Numerous critical process factors, diversity of experimental conditions, and a wide range of equipments complicate the process of comparing the effectiveness of treatments, hence limiting the conclusions drawn on the influences of different factors on microbial inactivation (Wouters et al., 2001). In general, evaluation of PEF treatment delivery has relied on estimations of treatment time and the electric field experienced by a food product while confined within a treatment chamber. However, experts in this technology agree on the experimental inconsistencies due to the lack of methods that can accurately measure the treatment delivery (CFSAN-FDA, 2000). Additionally, energy density or energy per pulse is rarely reported in most of the studies except for ones mentioned qualitatively correlated with the inactivation level (Evrendilek et al., 2000; Giner et al., 2000; Heinz et al., 1999; Zhang et al., 1994a). In fact, low energy of certain pulse waveforms and overall energy consumption of the PEF treatment are very attractive to the food industry due to the possibility of lower costs compared to most other technologies (Ho et al., 1997; Gongora-Nieto et al., 2004).

The fundamental aspects of PEF technology, both from a historical and technological point of view, were reviewed in this chapter. Continued research on the use of pulsed electric fields for food preservation combined with development of more effective and affordable processing systems would help to overcome hurdles and make commercial processing available in the near future.

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Table 1. International groups working on PEF (Barbosa-Cánovas et al., 1999).

Institution	Country
Catholic University of Leuven	Belgium
University of Guelph	Canada
AGIR	France
University of Bordeaux	France
University of Montpellier	France
CPC Europe	Germany
Technical University of Hamburg	Germany
Technical University of Berlin	Germany
ICE Tech	Iceland
ATO-DLO	The Netherlands
Unilever Research Vlaardingen	The Netherlands
University of Aberdeen	Scotland
University of Lleida	Spain
University of Zaragoza	Spain
Tetra Pak	Sweden
University of Lund	Sweden
Nestle	Switzerland
Campden and Chorleywood Food Research Assoc.	UK
Natick Laboratories	USA
National center of Food Safety and Technology	USA
Ohio State University	USA
PurePulse Technologies	USA
University of Wyoming	USA
Washington State University	USA

Table 2. Patents on Pulsed Electric Field Technology (Barbosa-Cánovas et al., 2005)

Reference	Patent
Held and Chauhan (2002)	Destruction of waste-activated sludge
Morshuis et al. (2002)	Device and method for pumpable food products
De Jong and Van Heesch (2002)	Pulse electric field treatment system
Lelieveld and Volanschi (2001)	Method and apparatus for preserving food
Zhang and Qin (2001)	High voltage pulse generator
Mastwijk and Bartels (2001)	Integrated modular design for treatment chamber
Bushnell et al. (2000)	A serial electrode treatment cell for pumpable food
Mittal et al. (2000)	Method and apparatus for electrically treating foodstuff
Addeo (2000)	Use of PEF coupled with rotational retorting
Bushnell et al. (2000)	Uniform product flow in a PEF treatment chamber
Qin et al. (1998)	Continuous flow electrical treatment chamber
Hayden (1998)	Method of inactivation in liquids
Yin et al. (1997)	High voltage PEF chambers for liquid products
Qin et al. (1997)	Continuous flow electrical treatment chamber
Zhang et al. (1996)	Batch mode food treatment using PEF
Bushnell et al. (1996)	Process for inactivation of microorganisms with PEF
Bushnell et al. (1995a,1995b)	Prevention of electrode fouling, and electrochemical effects in PEF systems
Bushnell et al. (1993)	PEF systems for extension of shelf life
Bushnell et al. (1991)	PEF systems for extension of shelf life
Dunn et al. (1991)	Methods for preservation by PEF
Doevenspeck (1991)	Electric impulse method and device for treatment
Dunn et al. (1989)	Methods for preservation with PEF
Dunn and Pearlmann (1989)	Device for shelf life extension for liquid foods
Dunn and Pearlmann (1987)	Methods for shelf life extension of liquid foods

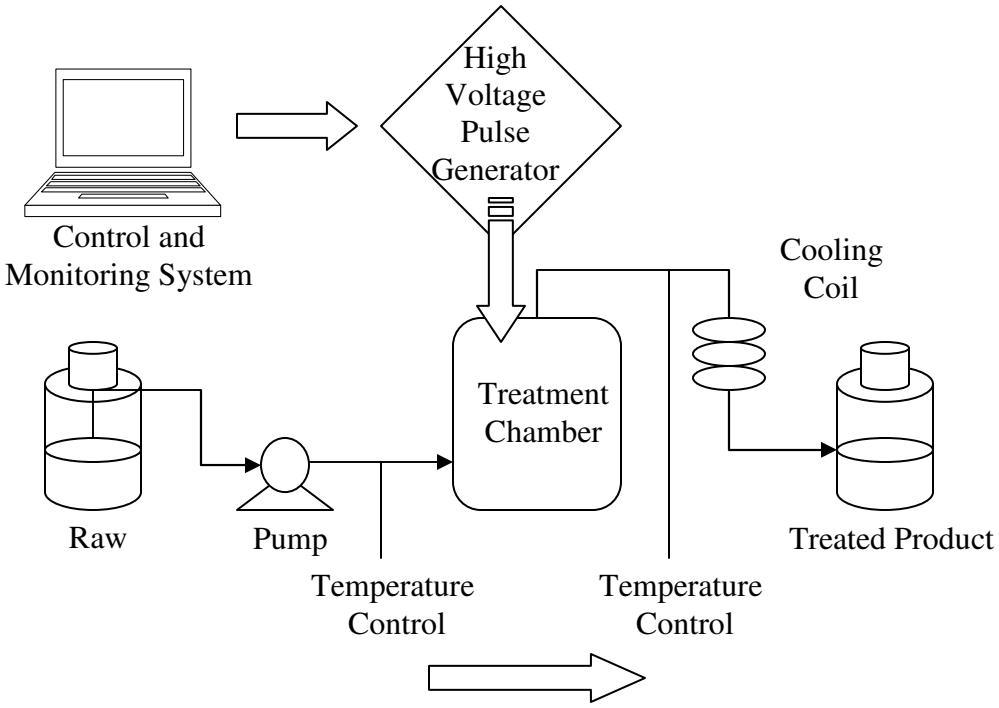
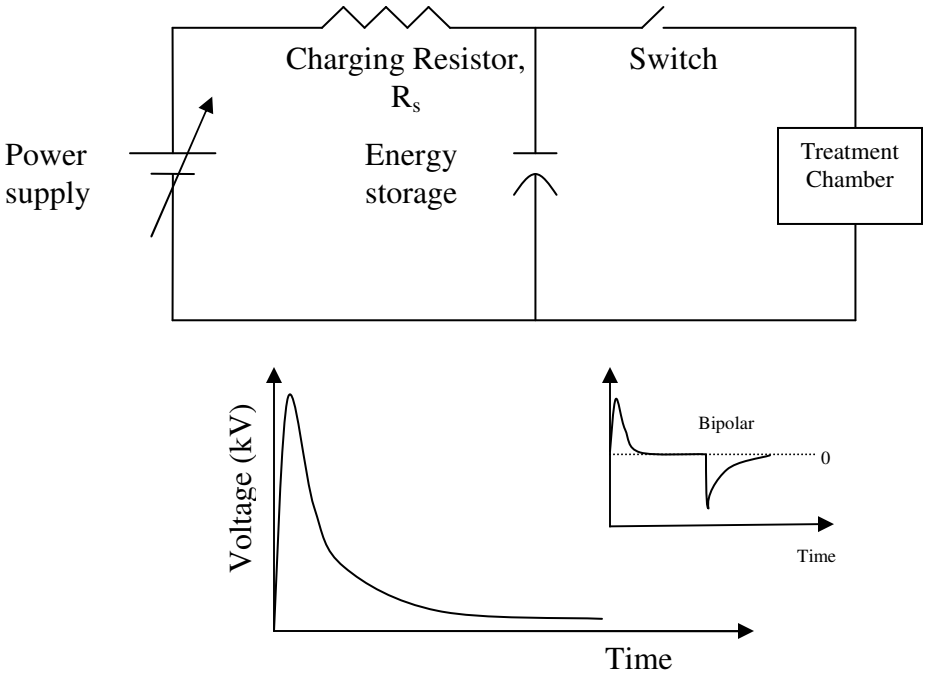
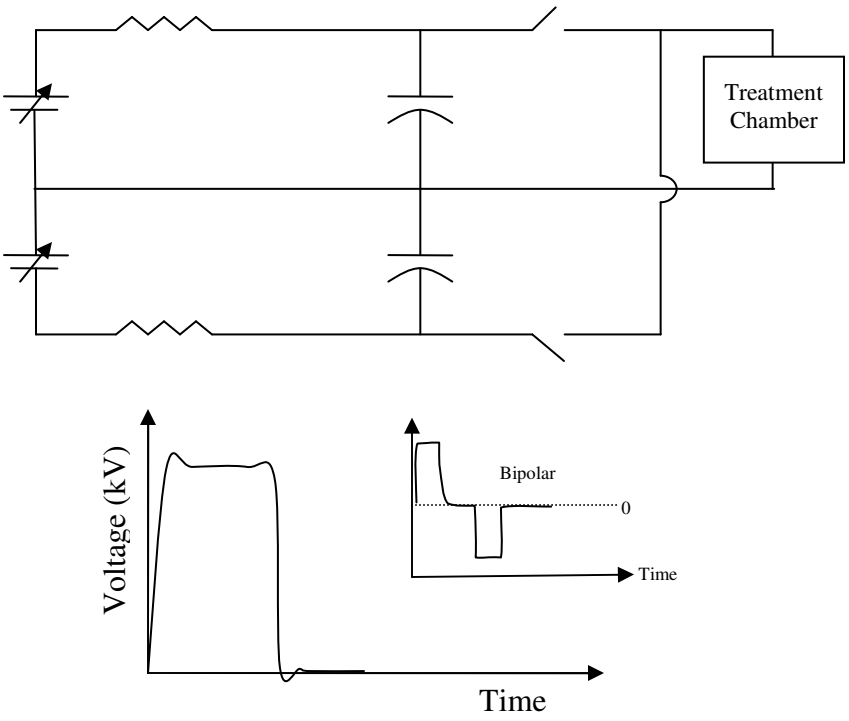


Figure 1. Flow chart of a PEF food processing system with basic components.



a. Monopolar exponential decaying circuit and possible waveform



b. Monopolar square circuit and possible waveform

Figure 2. Commonly used pulse wave shapes and the generic electrical circuits.

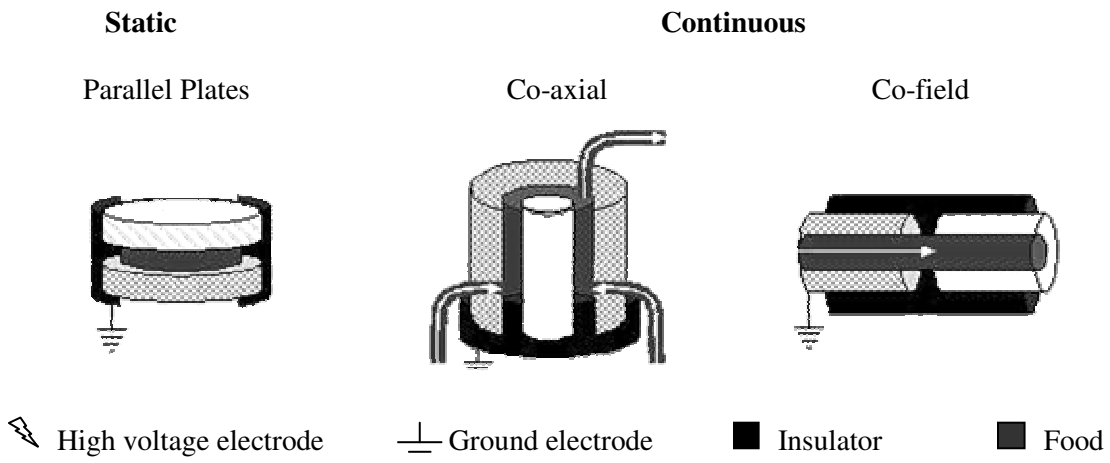


Figure 3. Schematic configurations of the three most used PEF treatment chambers.

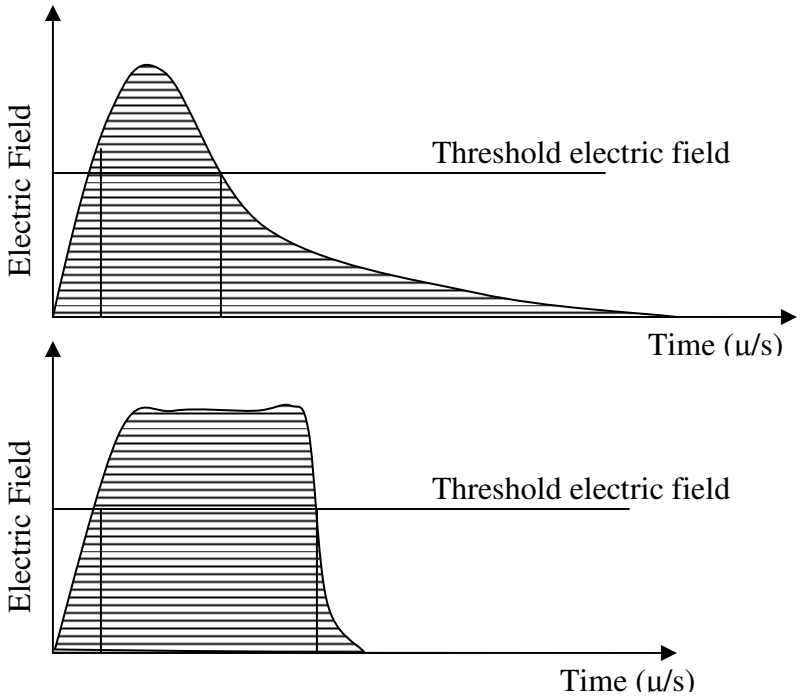


Figure 4. Effective energy of decaying and square pulses (Góngora-Nieto et al., 2002).

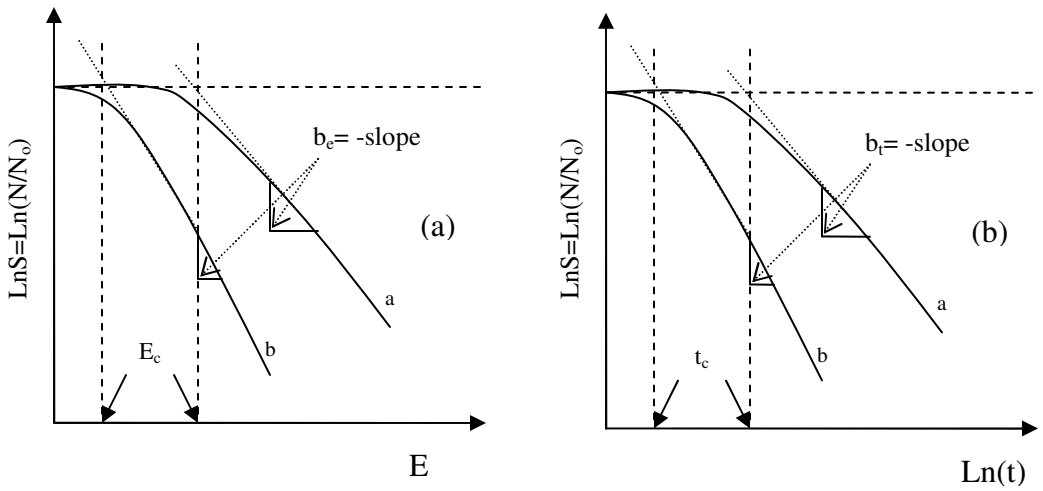


Figure 5. Dependence of microbial survival fraction on the (a) electric field and (b) treatment time. Curves, a, correspond to resistant microorganisms and curves, b, to sensitive microorganisms S, survival fraction; N, microbial count; E, electric field; b, kinetic constant; t, time. Subscripts: 0, initial; c, critical; t, time; e, electric field.

CHAPTER TWO

Applications of Pulsed Electric Fields for Food Preservation

Bilge Altunakar, Subba Rao Gurram, Gustavo V. Barbosa-Cánovas

1. Introduction

Interest in pulsed electric fields (PEF) application for food processing is increasing due to the growing demand of consumers for healthy, fresh, minimally processed, and nutritious food products with long shelf life. Although heat pasteurization is still the leading technology for food preservation, development of alternative methods to eliminate quality loss are highly encouraged by the food industry. PEF is an innovative nonthermal technology with the potential to supplement or replace heat pasteurization methods for certain applications. The use of high intensity pulsed electric fields in the form of short pulses allows lower processing temperatures compared to conventional methods, while inactivating both spoilage and pathogenic microorganisms with a minimum loss of volatile compounds and color during processing and storage (Barbosa-Cánovas et al., 1999). In the previous chapter, an introduction to the PEF technology has been reviewed and this chapter aims to provide the applications of PEF technology for preservation of food commodities. Before presenting the historical highlights of commercialization of PEF technology, it is useful to mention the consecutive stages visited in determining the feasibility of PEF pasteurization process for any food product.

Applicability of PEF technology for any food product involves a series of stages related to the processed products' microbial safety, quality attributes, shelf life, consumer acceptance and cost. Microbial challenge study is the beginning step visited to evaluate whether the

technology is capable of meeting the target food safety limit or not. The amount of treatment and the optimum treatment conditions are established once the inactivation studies are conducted and process variables determined accordingly (i.e. maximum electric field intensity, number of pulses). The target of microbial challenge studies are generally selected among the native microbial populations in raw products. For some foods, yeasts and molds are the point of interest, while for other food commodities certain bacteria are responsible for the deterioration reactions. Examination of quality factors in terms of a food's microbial, chemical and physical characteristics is the second stage, which begins after the effective amount of treatment for the target microorganism is assessed. The third step is the evaluation of sensory attributes prior to and during shelf-life studies. The major claim of PEF treatment is to protect food from the destructive effects of thermal processing while fulfilling microbial safety and feasibility concerns. Sensory scores reflect the consumer acceptability of a treated product; therefore they judge the acceptability and success of the product in the market.

The majority of these studies are conducted with pilot plant scale equipment where it is somewhat easier to handle, maintain, and control. However, applicability of a new process cannot be completed without scale-up studies. Vast amounts of research in the field of food engineering have concentrated on new preservation technologies but very few studies have been implemented by the food industry until now. The reason is the challenge in scaling up the system from pilot to industrial production plant. Considering the highly competitive market, the initial investment, operation, maintenance, and costs of production are the major points of interest to be verified for commercialization. Industrial PEF equipment used to be

rare and expensive, having a limited capacity of around 1800 l/h (Mittal et al., 2000), however with proper equipment energy savings is greater than in thermal pasteurization.

Application of PEF technology for food preservation purposes gained considerable attention in the last decades. The ability of PEF to eliminate the deleterious effects of thermal processes on foods without altering nutritional and organoleptic qualities enabled a variety of applications in food processing and bioprocessing since the 1960s (Doevenspeck, 1960; Sale and Hamilton, 1967; Flaumenbaum, 1968). Beginning with a brief review on historical milestones using electricity in food preservation, in this chapter, we will discuss current food preservation applications of PEF technology for different food commodities, including process evolution, shelf life studies and future developments.

2. Historical Overview

Food processing by electrical methods for inactivation of microorganisms and enzymes in foods began as early as the 1920s. The earliest attempt was conducted using the Electro-pure method in Liverpool (England), with the application of an alternating electrical current (220 to 4200V) for milk pasteurization (Beattie and Lewis, 1925). In this early process, preheated raw milk was subjected to continuous electrical current and thermally pasteurized with the generated heat at 70°C for 15 seconds. *Mycobacterium tuberculosis* and *Escherichia coli* were successfully inactivated with this method. Furthermore, the efficiency of the process on inactivating some bacterial populations resistant to other thermal pasteurization methods brought up the idea to use electric current itself (Palaniappan et al., 1990). However, the low processing capacity and the lack of proper equipment for temperature control were the major

drawbacks, which restricted the technology from becoming commercially popular (Getchell, 1935).

By the 1940s, pulsed electric fields were being used for other than food preservation purposes such as altering permeability of plant tissues. In the following years (1950s), the electro-hydraulic method was first introduced for inactivation of microorganisms suspended in liquid media. Shock waves generated by an electrical arc passed through the system, which also caused formation of highly reactive free radicals from the chemical components in foods. This method enabled the use of pulsed electric fields at different energy levels to inactivate *E. coli, Streptococcus feacalis, Bacillus subtilis, Streptococcus cremoris,* and *Micrococcus radiodurans* suspended in sterile distilled water (Gilliland and Speck, 1967).

Finally by the mid-1960s, a variety of pulsed electric fields equipment and methods were reported by Doevenspeck (Germany). Their extensive study emphasized the nonthermal effects of pulsed electric fields on microorganisms besides the interaction of pulsed electric fields and microbial cell walls. Sale and Hamilton (1967, 1968) were the pioneering scientists that focused on the nonthermal bactericidal effects induced by pulsed electric fields. In their systematic study, they reported the lethal effects of homogeneous pulsed electric fields on bacteria such as *E.coli, Staphylococcus aureus, Sarcina lutea, Bacillus subtilis, Bacillus cereus,* and *Candida utilis.* These researchers demonstrated that when bacterial cells are exposed to pulsed electric fields, direct current pulses cause a loss in the semi-permeability properties of the cell membranes, and that permanent loss of these properties leads to cellular death. Another important result from their study concerned the

relevant factors involved with the PEF process. They concluded that the effect of PEF is not due to heat generation or electrolysis but is independent of the current density and energy input, moreover, the electric fields strength, pulse duration, and size and shape of the microbes are the most relevant factors affecting inactivation (Knorr et al., 1994). A series of papers published about this study and most of their findings are still current today (Sale and Hamilton, 1968).

Zimmermann et al. (1974) first introduced the dielectric rupture theory using a method developed to promote cell fusion, which would provide control over the permeability of cell membrane in localized zones. This process, referred to as electrical breakdown or reversible electroporation, is still used today to explain the mechanism of action of pulsed electric fields. Following Zimmerman's work several studies reported combining membrane disruption theory by pulsed electric fields with previous work on process parameters, sensitivity of microorganisms, and inactivation mechanisms (Kinosita and Tsong, 1977; Sugar and Neumann, 1984; Tsong, 1990; Ho and Mittal, 1996). By the 1980s, the sensitivity of different kinds of microorganisms to pulsed electric fields was studied by a group led by Hülsheger, in which the earliest mathematical expressions were derived to describe inactivation kinetics as a function of electric field strength and treatment time (Hülsheger and Neumann, 1980; Hülsheger et al., 1981; Hülsheger et al., 1983).

In the 1980s, interest in PEF technology suddenly increased among scientific communities for the commercialization of PEF applications. Investigations by multidisciplinary research groups, including microbiologists, food scientists, and electrical engineers supported by food

companies, who eventually started researching the unknowns of PEF technology, enabled PEF to become an emerging innovative nonthermal technology. Beginning in 1987, the number of research groups studying PEF technology, parallel to the number of patents filed for specific chamber and equipment designs, increased rapidly all around the world.

In 1990, Krupp Maschinentechnik GmbH in Hamburg, Germany, developed the ELTRACK process for electrical cracking of vegetable and animal cells, as well as the ESTERIL process for electrical sterilization/pasteurization of pumpable liquid media (Sitzmann, 1990). A collaborative study by Krupp and University of Hamburg reported successful preliminary results for PEF pasteurization of orange juice and milk (Grahl et al., 1992) (Figure 1).

Following this initiative step, PurePulse Technologies, a subsidiary of Maxwell Laboratories (San Diego, CA), achieved the first regulatory effort to implement PEF at industrial level with the Cool Pure pulsed electric field process. In 1995, the Food and Drug Administration (FDA) expressed no objection for the use of this process for microbial treatment of liquids and pumpable foods. Pure Pulse Technologies owns several patents that include pulse forming network (PFN) components, chamber characteristics, and specific switch adjustments to preserve fluid foods treated with high-voltage pulses (Dunn and Pearlman, 1987). Among all research groups, Washington State University (WSU), Ohio State University (OSU) and several European groups have led the development of the technology with their comprehensive programs for PEF preservation of foods, including patents for specific chamber and equipment designs. The earliest design constructed by the WSU group was a modified version of an electroporator used to treat inoculated skim milk ultra-filtrate

(SMUF), with electric field strength of 20 kV/cm (Pothakamury et al., 1995). A volume-temperature controlled static chamber was designed afterwards with 12.5 and 25 ml temperature controlled static chambers and a high frequency pulser (10 Hz) capable of treating liquid foods in the continuous mode. 9 log cycles reduction of microbial population (*E.coli*) in SMUF were reported using 65 pulses with 70 kV/cm peak electric field (Martin et al., 1994; Zhang et al., 1995a; Qin et al., 1995; Vega-Mercado et al., 1997). Similarly, a 7 log cycle microbial reduction was achieved with liquid whole eggs in the range of 30-40 kV/cm (Ma et al., 1998). Currently, the system designed by the WSU group consists of a coaxial treatment chamber design connected to a pulse-forming power unit capable of delivering a peak voltage of 40 kV (Qin et al., 1997). The OSU group implemented an integrated pilot plant system with aseptic packaging and patented the co-field treatment chamber configuration (Figure 2) with a pulse-forming network (PFN) capable of delivering higher energy rates for microbial inactivation (Zhang et al., 1997).

In 2000, Diversified Technologies, Inc. (Bedford) built and installed the first commercial scale Pulsed Electric Field System (60 kV bipolar pulser) at Ohio State University's Department of Food Technology. Diversified Technologies, Inc. (DTI) offers several smaller research and development units, as well as commercial units that can run up to 20,000 l/hr. Most recently in 2005, the first commercial PEF equipment implemented in the U.S. to process fruit juices was used by Genesis Company in collaboration with DTI and the OSU group (Figure 2). Key findings of research groups working on PEF all over the world contribute to the commercialization of this novel nonthermal technology.

3. Preservation of Foods with Pulsed Electric Fields

A typical PEF system consists of a high-voltage pulse generator, treatment chamber, fluid handling system, and control and monitoring devices. The configuration and characteristic of each component vary among different research groups and equipment models (Figure 3).

The basic principle of the PEF technology is the application of short pulses of high electric fields with duration of microseconds and intensity in the order of 20-80 kV/cm. The processing time is calculated by multiplying the number of pulses times with effective pulse duration. The process is based on pulsed electrical currents delivered to a product placed between a set of electrodes; the distance between electrodes is termed as the treatment gap of the PEF chamber. The food product is placed in the treatment chamber, either in a static or continuous design, where two electrodes are connected together with a nonconductive material to avoid electrical flow from one to the other. Generated high voltage electrical pulses are applied to the electrodes, which then conduct high intensity electrical pulses to the product placed between the two electrodes. The food product experiences a force per unit charge, the so-called electric field, which is responsible for the irreversible cell membrane breakdown in target microorganisms (Zimmermann and Benz, 1980). The effectiveness of PEF against bacteria relies on the intensity of the applied electric field to electroporate the microorganisms. Electroporation results in loss of integrity, inactivation of proteins associated with resulting channels, leakage of cellular contents, and finally to cell death (Sizer & Balasubramaniam, 1999).

Significant PEF inactivation has been shown on a broad variety of vegetative microorganisms including spoilage as well as pathogenic strains. Flavor freshness, economic feasibility, improvements in functional and textural attributes and extended shelf life are also some of the main points of interest, besides the achievement of microbiological safety (Dunn, 2001). In mid-1900, pulsed electric fields at different energy levels were successfully applied, inactivating *E.coli, Streptococcus faecalis, Bacillus subtilis, Streptococcus cremoris*, and *Micrococcus radiodurans* suspended in sterile distilled water, as well as inactivating tyrpsin and a protease from *Bacillus subtilis*. Today, extensive research is going on for the inactivation of important spoilage (yeasts, molds, pseudomonas spp.) and pathogenic flora (*E.coli* O157:H7, *Listeria spp*. and *Salmonella spp*.) suspended in real food products such as juices, milk, and liquid eggs. Liquid products are highly conducive to PEF processing, thus the technology is mostly applied to popular juice products (e.g. apple and orange) as well as dairy products (e.g. milk, liquid egg, and brine solutions) (Qin et al., 1995). Overall, the different applications of PEF technology for preservation purposes can be classified based on the acidity of treated foods (fruit products) and non-acidity of foods (milk and dairy products).

3.1. High Acid Products

The effectiveness of PEF treatment for a liquid product depends on the product's physical properties, which includes electrical conductivity, density, and viscosity. The conductivity (or resistivity) of the media is related to the material's capacity to conduct electric current, therefore influencing the maximum electric field achievable for a given input power and maximum temperature rise. Low conductivity (higher resistivity) requires lower power inputs

and results in lower temperature rises. Products with low electrical conductivity and viscosity, and high density, will be the easiest and most energy efficient to process using PEF (Ruhlman et al., 2001). The most studied pumpable food commodities, based on their conductivities (in Siemens per meter) below 60ºC from lower to higher, are beer (0.1-0.25 S/m), fruit juices (0.1-0.3 S/m), milk products (0.4-1 S/m), and liquid egg (0.5-1 S/m). As a result, the effectiveness of PEF on pumpable liquid products with lower conductivity (e.g. fruit juices) was the main focus of research being conducted on commercialization of PEF technology at the industrial level.

Concerns over the safety of unpasteurized juices led the FDA to issue juice HACCP regulations that required a 5-log reduction in pertinent pathogen populations in juices being processed. As a result, some juice producers adopted the method of pasteurization to accomplish the minimum 5-log reduction in pathogens. However, many producers, especially small operations, do not utilize pasteurization because of perceived adverse effects on product quality and acceptability. The rapid increase in the market segment of fruit juices has led manufacturers to seek ways to overcome the thermal effects of processing besides increasing their shelf life (Hodgins et al., 2002; Sadler et al., 1992). At this point, PEF application has extensively been explored for improving juice safety while maintaining product quality and economic feasibility.

For high acid products with pH below 4.6, PEF application is especially promising due to concerns over spoilage microorganisms leading to the production of off-flavor compounds such as lactic acid bacteria (Hendrix and Red, 1995). PEF processing (25-75 kV/cm) for only

a few microseconds has been successful in a variety of fruit juices having low viscosity and electrical conductivity such as orange, apple, and cranberry juice (Sizer & Balasubramaniam, 1999). Studies carried out so far have shown adequate inactivation levels after very short treatment periods at low temperatures for a number of spoilage and pathogenic microorganisms, molds, and yeasts, such as *Saccharomyces cerevisiae* (Grahl et al., 1992; Grahl and Mark, 1996; McDonald et al., 2000), *Escherichia coli* (McDonald et al., 2000; Rodrigo et al., 2003), and *Lactobacillus plantarum* (Rodrigo et al., 2000). Minimal quality and nutritional changes in fruit juices were also reported by several research groups (Table 1, 2) (Zhang et al., 1996; Yeom et al., 2000; Min et al., 2002a). The effect of PEF was also studied in apple juice (*Escherichia coli, Saccharomyces cerevisiae, Zygosaccharomyces bailii, Byssoclayms fulva, Byssoclayms nivea, Neosartorya fischeri),* orange juice (*S. cerevisiae, Listeria innocua, E. coli*, *Leuconostoc mesenteroides, B. fulva, B. nivea, N. fischeri, Z. bailii*), peach juice (*L. brevis, L. innocua*), grape, pineapple and cranberry juices (*B. fulva, Z. bailii, B. nivea, N, ficheri*), and mixed orange-carrot juice (*Lactobacillus plantarum*) (Espachs-Barroso et al., 2003). Additionally, the color change in fruit juices (subject to prolonged storage) was reportedly less in juices treated by PEF; in a recent study on PEF-treated orange juice stored at 4°C for 112 days, there was less browning compared to thermally pasteurized juice, which was attributed to conversion of ascorbic acid to furfural (Yeom et al., 2000). Retention of carotene in orange-carrot juice and color in apple juice were also reported, respectively (Esteve et al., 2001; Evrendilek et al., 2000).

3.1.1. Apple Juice (Apple cider)

Apple juice has been produced and consumed in most apple-producing regions of the United States for years. It is a traditional fall beverage in many parts of the country (as is apple cider, a similar product processed differently). But recent advancement in production and marketing has made year-round consumption of this beverage possible, and it is often available in areas of the country where apples are not traditionally grown. Much of the appeal of apple juice is due to its fresh apple flavor and aroma as well as its full-bodied texture. Pasteurization is believed by many to adversely affect the flavor and aroma. Juice producers have traditionally relied upon the acidity of juice and refrigerated storage for preservation of the product. More recently, preservatives such as potassium sorbate and sodium benzoate have been employed. However, outbreaks of *E. coli* O157:H7 and *Salmonella spp.* food borne illness associated with consumption of contaminated, unpasteurized apple juice have caused much concern about the safety of the product as it is currently produced (Besser et al., 1993; Steele et al., 1982).

Qin et al. (1994, 1995) reported some of the earliest studies of the effect of PEF pasteurization on the quality attributes and shelf life of apple juice together with other liquid foods. In his early PEF study (1994), apple juice treated with 12 kV/cm and 20 exponential decay pulses inactivated 4 log cycles of *S. cerevisiae.* In his later study (1995), both reconstituted and freshly prepared apple juices were stored at 4-6ºC and subjected to 10 pulses with an electric field intensity of 50 kV/cm and pulse duration of 2 µs. The initial process temperature was kept at 8.5ºC and the maximum temperature reached during processing was 45ºC. These PEF treated apple juices were aseptically packaged and stored

for shelf life studies. The results together with other liquid foods treated are summarized in Table 3.

Harrison et al. (1996) observed that PEF treatment at 40 kV/cm reduced the number of *S. cerevisiae* inoculated in apple juice by 3 log cycles. Likewise, Vega-Mercado et al. (1997) reported that the shelf life of apple juice treated with 16 pulses and stored at room temperature (22-25ºC) for as long as eight weeks showed no apparent change in physicochemical and sensory properties. Further study by Evrendilek et al. (2000) showed that PEF treatment at 35 kV/cm for 94 µs total treatment time significantly extended the shelf life of apple juice (and apple cider) while no change was measured in ascorbic acid content. Recently, Cserhalmi et al. (2002) reported 4 log inactivation of *S. cerevisiae* in apple juice with treatment at 20 kV/cm and 10.4 square wave pulses. A comprehensive study by Heinz et al. (2002) reported inactivation of different microorganisms in apple juice (pH 3.4) with PEF treatment at 34 kV/cm, initial temperature 55ºC, and specific energy input 40 kJ/kg, as 6.2, 6.5, 4.3, and 4.9 log reductions for *E. coli, R. rubra*, *A. niger*, and *L. rhamnosus*, respectively.

3.1.2. Orange Juice

Loss of vitamin C, change in color, and destruction of fresh flavor are the main disadvantages in thermal treatment of citrus beverages. The main cause of juice rejection is the butter-milk off-flavor and off-odor of citrus juices (Kimball et al., 1999). These adverse characteristics are due to a compound called diacetyl, which is produced by the growth of lactic acid bacteria (*Lactobacillus* and *Leuconostoc*); *Lactobacillus brevis* is among the most important citrus juice spoilage microorganisms (Parish, 1988; Hendrix and Red, 1995).

Spoilage microorganisms can be inactivated by thermal treatment but due to irreversible loss of fresh juice flavor and reduction of nutrients, and undesirable browning reactions as a result, the citrus industry has become increasingly interested in PEF technology (Giner et al., 2000). The earliest PEF studies on commercial, freshly squeezed, high pulp orange juice were conducted by Dunn and Pearlman (1987), who applied 35 exponentially decaying pulses with 33-35 kV/cm electric field intensities. The native microbial flora of the juice consisting of a mixture of yeasts, molds and bacteria was inactivated by 5 logs and the shelf life was extended by more than one week. They reported that the PEF treated orange juice was acceptable after 10 days while untreated juice was unacceptable after four days. Zhang et al. (1997) then evaluated the flavor of PEF treated orange juice in terms of D-limonene and ethyl butyrate loss, which was evaluated by comparing PEF treated orange juice at 35 kV/cm and two different treatment times (240 µs and 480 µs) with heat pasteurized orange juice (91ºC, 30 sec), using freshly squeezed orange juice as the control. The loss in content of D-limonene and ethyl butyrate was 16.7 and 1.2% in PEF treated juice whereas heat pasteurization resulted in a content reduction of 44.9 and 21.5%, respectively. In the same study, shelf life of PEF treated orange juice was also evaluated under different PEF conditions. The results are summarized in Table 4.

Similarly, Qiu et al. (1998) reported 4.2 log reductions for yeast and bacterial flora in orange juice with treatment at 60 kV/cm exponential decaying pulses and time of 0.85 µs. This study also reported that the use of square wave pulses (360 days) were more effective for shelf life extension compared to exponential decay pulses (210 days).

Inactivation of other microorganisms in orange juice, such as 2.5 log reductions of *L. plantarum* with PEF treatment at 28.6-35.9 kV/cm and 10-50 µs (Rodrigo et al., 2000) and 5-6 log reductions of *E.coli, L. innocua,* and *L. mesenteroides* at 30 kV/cm, 2 µs and 54ºC (McDonald et al., 2000), also support the successful application of PEF treatment for orange juice pasteurization. Yeom et al. (2000) reported 7 log reductions of yeast and molds by using continuous, square monopolar co-field chambers at 20-35 kV/cm electric field intensity and 39-59 µs total treatment time.

Recent inactivation and shelf life studies found that shelf life can be successfully extended to 112 days at 4, 22 and 37ºC. Ayhan et al. (2001) studied the shelf life of PEF treated (35 kV/cm and 59 µs) single strength orange juice and reported that the use of a glove box sanitized with hydrogen peroxide and germicidal UV light provided 112 days of shelf life at 4 and 22ºC. Min et al. (2002a), using the same packaging system, obtained 5-6 log reductions of microorganisms and 16 weeks of shelf life at 4ºC after 40 kV/cm and 97 µs PEF treatment. Elez-Martinez et al. (2005) studied *Lactobacillus brevis* inoculated in citrus juice and achieved 5.8 log reductions with PEF treatment at 35 kV/cm for 1000 µs using bipolar pulses. A 6.2-log reduction was achieved in grape juice at 51°C when 20 pulses at 80 kV/cm were applied with nisin addition (Wu et al., 2005).

In addition to the excellent intrinsic sensory and nutritive characteristics of orange juice, blended juices such as orange and carrot juice were also studied; natural microflora was inactivated by 4 logs after PEF treatment at 40 kV/cm and 130 µs (Rodrigo et al., 2003). The incorporation of carrot juice is a valuable contribution to the health of the consumer due to

the combination of high vitamin content in oranges and high carotenoid content in carrots. Torregrosa et al. (2005) compared the vitamin A content in orange-carrot juice after thermal and PEF pasteurization and reported that the PEF treated juice blend was higher in vitamin A after 25-30 kV/cm treatment.

3.1.3. Cranberry Juice

Cranberry is the third most favorite juice flavor in the United States, after orange and apple (Holmes and Starr, 1993). Besides being a popular flavor, cranberry products have an attractive color due to anthocyanin pigments. However anthocyanin containing products are susceptible to color deterioration during processing and storage, which results in anthocyanin degradation and brown pigment formation (Skrede et al., 1992). Jin and Zhang (1999) studied the effect of PEF treatment on cranberry juice and reported that PEF application, 40 kV/cm for 150 μs, by retarding the growth of molds and yeasts, extended shelf life of cranberry juice compared to thermally pasteurized juice samples. They also concluded that the anthocyanin content in PEF treated samples were unchanged while thermally pasteurized samples were degraded.

3.2. Low Acid Products with Limited Shelf-life

Traditional and typical preservation techniques for low acid foods, with a pH above 4.6, include pasteurization combined with refrigeration, freezing, dehydration, and retort processing, among other. Severe heat treatment is required to maintain the safety and proper shelf life of these products, which include milk, cheese, eggs, meats, vegetables and formulated products such as soups, baked products and entrees, all of which are closely

monitored by regulatory agencies due to their potential for growth of many pathogens (i.e. bacteria that pose health hazards). In most cases, the product's fresh flavor, vitamin content, and some physicochemical characteristics are degraded due to the deleterious effects of heat application. Therefore, PEF technology is a promising nonthermal alternative to thermal treatment.

3.2.1. Milk

Milk was the first food product used in early microbial challenge studies investigating the effects of pulsed electric fields. As mentioned in the historical highlights earlier in this chapter, inactivation of bacteria and enzymes in food products using electric discharges started in the 1920s with the ElectroPure processes, which pasteurized milk (Fetterman, 1928) by direct passage of an alternating current through milk (Getchell, 1935). Dunn and Pearlman (1987) worked with homogenized and pasteurized milk inoculated with *Salmonella dublin* and treated each milk sample with 40 pulses of 36.7 kV/cm over a 25 min time period. Pathogenic bacteria were not detected in milk chilled after treatment and stored at 7-9^{o}C for 8 days. After 8 days, the untreated milk samples showed a microbial load of 10^{7} per ml, whereas the treated milk samples showed only 400 per ml. Dunn and Pearlman also observed a 3D reduction in milk inoculated with *E. coli* when 23 pulses at 28.6 kV/cm for 100 μs were applied. Grahl et al. (1992) reported a 4.6 D microbial reduction for *Lactobacillus brevis* inoculated in milk treated with 20 pulses for 20 μs at 22 kV/cm.

Qin et al (1995) used preprocessed 2% fat milk, applying a multi-step PEF treatment with two steps of 7 pulses each and one step of 6 pulses. The PEF processed milk was then

aseptically filled into flexible film bags. The microbial and chemical analysis after PEF treatment and shelf life studies were carried out on the milk stored at 4^{o}C. No apparent changes in physical and chemical characteristics were observed in the milk stored for 14 days. Sensory analysis showed that no significant difference observed between the heat pasteurized and PEF-pasteurized milk. Pothakamury et al. (1995) reported 4 to 5 log cycles (4-5D) of *E. coli* inoculated in SMUF (simulated milk ultra filtrate) using high voltage pulsed electric fields with 50-60 pulses at a peak electric field strength of 16 kV/cm and pulse duration of 200-300 μs. The authors demonstrated that the extent of inactivation increases when the applied electric field strength is increased (Pothakamury et. al 1995). The survival fraction is equal to the ratio of the number of bacterial cells per ml after PEF treatment to the number of cells per ml in the untreated control, SMUF. With the increase in electric field strength more energy is supplied to the cell suspension, therefore more inactivation is observed. In addition to increasing the electric field strength, a reversing polarity treatment can be included to increase the rate of microbial inactivation. Also, by introducing square wave pulses as compared to exponentially decaying pulses, the extent of microbial inactivation can be increased. Calderon-Miranda et al. (1999) reported 1.7, 2, and 2.5 log cycle reductions of *Listeria innocua* when 32 pulses were applied at electric field intensity of 30, 40, and 50 kV/cm. The inactivation of *L. innocua* accomplished by PEF-Nisin treatment was 2.5, 3, and 3.8 log cycle reductions for 2 pulses at electric field intensity of 30, 40, and 50 kV/cm, respectively.

Calderon-Miranda et al. (1999b) studied the combination of PEF and nisin to control microorganisms in milk. The main objective of this study was to inactivate *L. innocua*

suspended in skim milk by PEF and to determine the sensitivity of *L. innocua* to nisin after PEF application. Electric field intensities of 30, 40, and 50 kV/cm were applied at 10.6, 21.3, and 32 pulses, respectively. The exposure of *L. innocua* to nisin after PEF had an additive effect on the inactivation of microorganisms. The exposure of milk to 10 IU nisin/ml after 32 pulses reduced the *L. innocua* by 2, 2.7, and 3.4 U at electric field intensity of 30, 40, and 50 kV/cm, respectively. The highest inactivation of *L. innocua* obtained with PEF was 2.4 U for an electric field intensity of 50 kV/cm at 32 pulses. A synergistic effect on the inactivation of *L. innocua* was observed as the electric field intensity, number of pulses, and nisin concentration was increased. The proposed model for inactivation of *L. innocua* by PEF with nisin proved to be adequate in predicting the extent of inactivation. It is also a viable alternate method for preservation of skim milk.

Qin et al. (1998) observed a significant reduction of 6 to 9 log cycles in SMUF inoculated with *E.coli* when 50 pulses of 60 kV/cm and 80 pulses of 70 kV/cm were applied respectively. In the case of *Staphylococcus aureus*, a reduction of 5 log cycles was observed when subjected to 40 pulses at 60 kV/cm. Fernandez-Molina et al. (2001) reported 2.6-2.7 log cycle reductions for *Listeria innocua* ($1.2x10^9$) and *Pseudomonas fluorescens* (3.8×10^7) inoculated in pasteurized skim milk and subjected to PEF up to 200 μs at 50 kV/cm. Raso et al. (1999) observed a 4-log cycle reduction when raw milk inoculated with *Staphylococcus aureus* was subjected to PEF treatment.

Besides several theories proposed for the inactivation mechanism of vegetative bacterial cells subjected to PEF, scanning electron microscopy (SEM) and transmission electron

microscopy (TEM) are the two commonly used methods to observe the effects of the process on the structure of microorganisms. Among these theories, using advanced microscopic methodologies, Pothakamury et al. (1997) proposed cell membrane electroporation and consequently microorganism destruction. Calderon-Miranda et al. (1999) also reported pore formation on *L. innocua* at electric field strength of 40 kV/cm in skim milk and stated that the inactivation of *L. innocua* was a consequence of cell membrane rupture and loss of membrane functionality. *L. innocua* cells treated by PEF-Nisin at an electric field intensity of 30 kV/cm exhibited an increase in the cell wall surface roughness and lack of cytoplasm. The authors also observed cell wall and cell membrane rupture when exposed to an electric field intensity of 40 kV/cm. In addition, extracellular material was visible and attributed to cytoplasmic leakage. The thickness of the cell wall of *L. innocua* exposed to 40 kV/cm was also reduced slightly compared to the untreated cells. Pothakamury et al. (1997) also reported similar results for *Staphylococcus aureus* treated by PEF with electric field intensity of 20 kV/cm and 64 pulses. When PEF with an electric field intensity of 50 kV/cm was applied to *L. innocua*, visible damage to the cell membrane was observed. Picart et al. (2002) reported that inactivation of *Listeria innocua* suspended in skim milk occurred due to collapsed and/or segmented bacteria. All such results on microbial inactivation clearly show that PEF is an adequate alternative to thermal pasteurization, and capable of achieving 3 to 6 log cycles of destruction for most vegetative pathogenic species (Manas and Vercet, 2006).

3.2.2. Milk for Cheese Production

Cheese, a very nutritious, healthy food due to its protein content, is a leading dairy product worldwide. Some of the earliest studies on milk for cheese manufacture using PEF

technology were conducted by Dunn and Pearlman (1987) and Dunn (1995). Further study by Dunn (1995) indicated less flavor degradation and no physical or chemical changes in enzyme activity, starter growth, rennet clotting yield, cheese production, calcium distribution, casein structure, and fat or protein integrity in raw milk after 20-80 kV/cm PEF treatment. Based on the tested attributes he suggested the use of PEF treated milk to promote the manufacture of dairy products such as cheese, butter, and ice cream with fresh flavor.

Sepúlveda-Ahumada et al. (2000) studied the textural properties and sensory attributes of Cheddar cheese made with PEF (35 kV/cm; 30 pulses) treated milk, raw milk and thermally processed milk (LTLT: 63^{o}C for 30 min, HTST: 72^{o}C for 15 sec). They made twelve 1.5 kg cheeses, vacuum packed in polyethylene bags, and stored at 4◦C for 5 weeks. It was observed that heat treatments were more effective in microbial inactivation than PEF treatment and explained that it could be due to the high protein content in milk. The authors also observed that total coliform count in all the pasteurization processes (including PEF) obtained the same final counts, which were close to zero; they argued that the difference in the inactivation by PEF of the two types of bacteria may be due to differences in the cell membrane. The presence of media components such as fat and proteins has been related to a protective effect on microorganisms against PEF, and the inactivation of microorganisms is complex foods represent a major challenge when compared to simple suspension systems. Some researchers claim that some substances shield microorganisms from the applied electric field (i.e. microorganisms suspend in substances with high electrical permittivity within a nonhomogeneous product) or stabilize or prevent ion migration (Toepfl, 2006).

Texture is an important characteristic in cheese, the assessment of which consumers express their acceptability and preference (Jack et al., 1993). Sepúlveda-Ahumada et al. (2000) reported that Cheddar cheese made from milk pasteurized by LTLT was more adhesive compared to other treatments (HTST, PEF) and that no significant differences were observed among other cheeses. In the cohesiveness study, the cheeses made from thermally treated milk had greater cohesiveness that those made from PEF-treated milk, although the differences were very small. It has been shown that cohesiveness increases when temperature increases. The cheeses made from milk pasteurized by any method were harder than those made from untreated milk. In the case of springiness, cheeses made from pasteurized milk obtained the highest values. The differences in cheese behavior in adhesiveness and cohesiveness, compared with hardness and springiness was attributed to cheese maturation. In the sensory evaluation, the panelists did not find differences between cheeses made from untreated milk and milk treated by HTST, but did find differences between those cheeses and the ones made from milk treated by PEF or LTLT. The differences were perceived more in terms of aroma than texture. Nonetheless, it was suggested that milk pasteurized by pulsed electric fields to obtain cheese is a feasible option.

3.2.3. Egg and Egg Products

Thermal processing of liquid whole egg (LWE) above 57^{o}C is a major concern of egg producers and consumers due to the detrimental changes in the physical and functional properties of egg products (Cunningham, 1986; Baker, 1990; Delves-Broughton, 1992). The bacteria *Pseudomonas fluorescens*, can grow during refrigerated storage, causing storage problems and thus becoming a threat to the quality and shelf life of liquid egg products. Heat

pasteurization is effective for these types of flora, but due to the high protein content of LWE, protein coagulation may occur. Nonthermal processing methods may offer effective solutions for this problem.

Egg products have high electrical conductivity (low electrical resistivity) and generate a low electric field peak across the treatment chamber during PEF treatment (Barbosa-Cánovas et al., 1999). Liquid whole eggs have a complex composition with rich protein content (12.8–13.4%) and fat presenting obstacles for PEF processing. Additionally, the presence of fat diminishes the effect of PEF on microorganisms, performing a protective role by absorbing the active radicals and ions resulting from discharges (Sampedro et al., 2006). The high viscosity of liquid egg products can be a further problem in the application of PEF. Several studies have reported successful PEF inactivation of pathogenic and food spoilage microorganisms as well as selected enzymes, resulting in better retention of nutrients and flavors, with a fresher taste, compared to heat pasteurized products (Barsotti et al., 2002; Bazhal et al., 2006; Ho and Mittal, 2000; Jeyamkondan et al., 1999; Rasgoti, 2003; Van Loey et al., 2002).

Martín-Belloso et al. (1997) reported a 6D reduction in liquid egg inoculated with *E. Coli* and treated with an electric field of 25.8 kV/cm at 100 pulses of 4 μs, at 37^{o}C. Jeantet et al. (2004) reported 4 log reductions of *Salmonella enteritidis* in model solution with application of 3 to 80 kV/cm at pulse width 50 ns at 3 μs. Most recently, Amiali et al. (2007) observed 1.4, 2.3, and 3.7 log reductions of *E coli* or *Salmonella enteritidis* in liquid egg yolk with 20 kV/cm at 210 μs and processing temperatures 20, 30 and 40^{o}C respectively; they also

reported 1.9, 2.3, and 4.9 log reductions with 30 kV/cm, keeping the other parameters constant. The authors finally reported a maximum of 5 log reductions for both *E. coli* or *Salmonella enteritidis* using PEF treatment of 30 kV/cm at 40^{o}C. However, the residual inoculum (approximately 3x10^{3} CFU/ml) in yolk could reach levels of public health concern, particularly under mild to moderate temperature abuse conditions. Therefore PEF processing must be used under strict refrigeration conditions to ensure the safety of processed egg products.

In a study by Hermawan et al. (2004) from the OSU group, fresh whole eggs were consecutively cracked in a clean environment, blended in a sterilized household blender at low speed for 30 s, filtered through four layers of sterile cheesecloth, homogenized with a hand-held laboratory homogenizer at high speed for 2 min, and finally filtered twice through four layers of sterile cheesecloth into a sterile 1-L glass capped bottle. *S. enteritidis* (1 ml) was added to 10 ml of de-shelled blended whole eggs at room temperature and kept at 4^{o}C for 48 h, which was then added to 200 ml of LWE at room temperature. The OSU-3C PEF pulse generator was used for this study, with PEF treatment conditions at 1.2 ml flow rate, 200 pulses/s frequency, 2.12 μs pulse duration, 25 kV/cm electric field strength, 13-14 A current, and 250 μs total treatment time. The inoculated samples were then subjected to different treatments: Heat at 55^{o}C, PEF +55^{o}C, 55^{o}C + PEF, and 60^{o}C for 3.5 min. Microbial counts of *S. enteritidis* were conducted in triplicate using TSA incubated at 37^{o}C for 48 h. Shelf life studies were also conducted by storing the experimental samples at 4^{o}C for 70 days. Density and apparent viscosity were measured using a Cannon-Fenske viscometer, size 200. The color values L, a, and b were measured using a Hunter L-a-b UltraScan

Colorimeter. Results showed 4.3 log cycle reductions in liquid whole eggs inoculated with *S. enteritidis,* treated at 25 kV/cm, 250 μs, and 55°C, with no significant changes observed in viscosity, color, pH, and Brix, but presenting significantly longer shelf life (over 60 days) when stored at 4°C compared to heat treated samples.

Some studies have focused on the effects of PEF on gel formation, structural changes in proteins, and other properties in whole eggs and egg products (Table 5). A study by Fernández-Díaz et al. (2000) compared prepared gels from fresh egg white in terms of mechanical properties (rigidity, elasticity index, relaxation time, and water holding capacity); no significant difference between the treated and untreated gels was observed. The authors concluded that partial protein unfolding or enhanced SH ionization and the extent of SH reactivity increased with the energy dissipated, through conversion to the S^- form (3.7 of the four groups), making them more reactive. Pérez (2002) observed two steps in the gelation process, using dynamic rheology during PEF application: 1) the gelation of conalbumin and lysozyme and, 2) the gelation of ovalbumin. Pérez also reported that the gel texture and microstructure were affected by PEF application, with less hardness and adhesiveness as the number of pulses increased. Pérez and Pilosof (2004) analyzed the possible alterations that pulses might cause in protein structure, denaturation temperatures, enthalpy changes, and related functional properties. Table 5 gives a summary of results and other references related to the effects of PEF on egg white gel formation, structural changes and other properties of whole egg and egg products.

Qin et al. (1995) focused on changes in the physicochemical properties of whole eggs and egg-derived products after treatment with pulses. These authors specifically studied the change in color, viscosity and sensory attributes of fresh liquid egg samples with the addition of citric acid (0.15% w/v), treated by pulses in three steps under a temperature maintained below 50ºC to avoid coagulation. After the treatment, samples were packaged aseptically and stored at refrigeration temperature for up to 4 weeks. After pulses were applied, the authors observed a decrease in viscosity and an increase in color, in terms of β-carotene; however in an acceptance test the sensory panel did not observe any differences between scrambled eggs prepared from PEF-treated egg and fresh egg. Góngora-Nieto et al. (2001) also did not observed any color change in fresh liquid egg in PEF treated samples. Hermawan et al. (2004) also reported no changes in viscosity, electrical conductivity, Brix, pH and color when liquid whole egg was treated in combination with PEF (25 kV/cm) and temperature (55ºC), as compared to non-treated liquid whole egg samples. Amiali et al. (2007) reported that as the processing temperature of the egg derivatives increased so did the electric conductivity, however the specific energy and energy applied per pulse decreased. The liquid egg products showed the highest conductivity values (0.22 to 1.1S/m) when compared with fruit juices (0.13–0.63 S/m). The energy transferred to the products increased or decreased depending on the treatment cell load resistance. It was also concluded that the cell treatment resistance must be adequately matched to the PEF transmission line load in order to transfer the maximum electrical energy to the food product. Further studies are necessary to optimize the energy delivered by PEF for the processing of liquid whole eggs. Overall the PEF technology could be a promising technology for improving the quality and safety of liquid whole eggs and its derivatives.

4. Hurdle Concept and Combination Studies

Today the food industry is making use of a combination of factors to achieve food preservation. The simultaneous combination of preservative factors becomes a hurdle to the microorganism, producing shelf stable and safe food products. The use of selected hurdles in a food may provide an additive effect on microbial inactivation, and a synergistic effect can be observed when the hurdles take up different targets in the microbial cell (Leistner, 2000). PEF can be used in food preservation as a single preservative factor in combination with other hurdles such as antimicrobials.

Application of PEF at mild temperatures has been suggested as a way to enhance the effectiveness of PEF as a preservation method (Hülsheger et al., 1981; Dunn and Pearlman, 1987). In most PEF studies, temperature effect is reported to be a synergistic factor and has been used to extend the shelf life of pumpable food products like milk and fruit juices (Jayaram et al., 1992). The use of sub-lethal thermal conditions in combination with PEF could potentially facilitate the application of effective treatments without the need for severe PEF conditions. The effect of mild temperature on the effectiveness of PEF has not been fully determined but there are several strong theories proposed on the matter. The reduction in charging time of bacterial membranes due to increased electrical conductivity of the media under higher temperatures (Schwan, 1957); changes in the phase state of cell membranes (Jarayam et al., 1992); and the reduced membrane break-down potential (Kinosita et al., 1977) are some of the most relevant theories proposed so far. In a study conducted by the WSU group, the bactericidal effectiveness of PEF applied on *Listeria innocua* suspended in McIllvaine buffer was investigated, and a significant increase in effectiveness of PEF over

55ºC was reported (Sepulveda et al., 2005). Likewise, Evrendilek et al. (2000) reported the effect of combining PEF and heat (60°C for 30 s) in processing fresh apple cider and reconstituted apple juice inoculated with *E. coli* O157:H7. The PEF system improved the microbial shelf life of apple cider, and during storage the retention of vitamin C content in the reconstituted apple cider was 100 and 95% at 4°C and 22°C, respectively. Supported by many other studies conducted, the synergistic effect of mild temperatures on PEF has been reported.

Similarly, the use of antimicrobials in combination with PEF has been more effective in inactivating microbial flora and extending the shelf life of refrigerated products than PEF treatment alone. PEF stresses the microbial cell, making it more sensitive to antimicrobials such as nisin and organic acids (Kalchayanand et al., 1994). Synergism with the use of antimicrobials is already reported to have a significant effect on inactivation kinetics of PEF due to the facilitated entry of undissociated acids into bacterial cells (Liu et al., 1997). With the use of antimicrobials in combination of PEF, a population of *E. coli* treated at 12.5 kV/cm in a solution containing 1000 ppm of benzoic acid, with pH of 2.4, was reduced by 4 log cycles. Likewise, exposure of microorganisms like *Listeria innocua* to PEF induces a stress and may cause sub-lethal injury to the cells or increase their sensitivity to nisin. The effect of nisin on the cytoplasmic membrane of gram-positive cells is enhanced by its combination with PEF (Calderón-Miranda et al., 1999a,b).

Bazhal et al. (2006) investigated the inactivation of *Escherichia coli* O157:H7 in liquid whole egg using thermal and pulsed electric field batch treatments, both alone and in

combination. Electric field intensities of 9 to 15 kV/cm and a threshold temperature of 50°C were used for thermal inactivation. PEF enhanced the inactivation of *E. coli* when the sample temperature was higher than the thermal threshold temperature. The maximum inactivation of *E. coli* obtained using thermal treatment alone was approximately 2 logs. However, combined heat and PEF treatments resulted in up to 4 log reductions. The results indicated a synergistic effect when PEF and temperature were applied to inactivate *E. coli* in liquid whole egg; the higher the PEF intensity and treatment temperature, the more the inactivation.

5. Final Remarks

The vast amount of literature on microbial inactivation by PEF under a broad range of treatment conditions (Barbosa-Cánovas et al., 1999; Wouters et al., 2001; Heinz et al., 2002) has already agreed on the effectiveness of PEF processing. These successful applications for inactivation of yeasts, molds and their sporulated forms, and some highly acid-resistant vegetative bacteria, have proved to be alternatives for conventional thermal processing. Future investment in PEF technology is highly recommended based on the current results of pilot plant studies, and the first commercial application of this novel technology, preservation of fruit juices, has already begun. The appealing characteristics of nonthermally processed products in the market will definitely enhance the application of PEF. However, similar to other novel technologies, it is worth mentioning that PEF technology is still in the early stages of commercialization and that a more in depth examination is required to fully understand the mechanisms at play. Standardization of protocols and enabling of communication between research groups are also needed.

The food preservation applications of PEF technology were reviewed in this chapter from historical and technological points of view. Continued research on the use of pulsed electric fields for food preservation, along with development of more effective and affordable processing systems, would help overcome the hurdles blocking its use and make commercial PEF processing available in the near future.

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Table 1. Inactivation by PEF of *Z. bailii* vegetative cells and ascospores in fruit juices (Raso et al., 1998).

Fruit juices	Electric conductivity (μs/cm)	Peak voltage (kV)	Electric field (kV/cm)	Pulse width (μs)	Inactivated vegetative cells (log_{10})	Inactivated ascospores (log_{10})
Apple	$2.5x10^3$	19.4	32.3	2.5	4.8	3.6
Orange	$3.6x10^3$	20.6	34.3	2.0	4.7	3.8
Grape	$2.7x10^3$	21	35	2.3	5	3.5
Cranberry	$1.1x10^3$	21.9	36.5	3.3	4.6	4.2
Pineapple	$3.1x10^3$	19.8	33	2.2	4.3	3.5

Table 2. Inactivation by PEF of mold *N. fischeri* ascospores and *B. fulva* conidiospores in fruit juices (Raso et al., 1998).

Fruit juices	pH	Electrical conductivity (μs/cm)	Peak voltage (kV)	Electric field (kV/cm)	Pulse width (μs)	Inactivated *B. fulva* conidiospores (log_{10})	Inactivated *N. fischeri* ascospores (log_{10})
Apple	4.1	$2.5x10^3$	19.4	32.3	2.5	3.5	-
Orange	3.9	$3.6x10^3$	20.6	34.3	2.0	3.7	0.1
Grape	3.9	$2.7x10^3$	21	35	2.3	5.5	0.4
Cranberry	3.0	$1.1x10^3$	21.9	36.5	3.3	5.9	0.1
Pineapple	3.5	$3.1x10^3$	19.8	36.5	2.2	4.8	-
Tomato	4.1	$4.8x10^3$	18	30	2.0	3	-

Table 3. PEF processing of selected liquid foods (Qin et al., 1995).

Food	Apple juice from concentrate	Fresh apple juice	Raw skim milk	Beaten eggs	Green pea soup
Peak electric field (kV/cm)	50	50	40	35	35
Pulse duration	2	2	2	2	2
Pulse number	10	16	20	10	32
Initial temperature(ºC)	8.15±1.5	8.15±1.5	10±1.5	8.15±1.5	22±2
Maximum treatment temperature(ºC)	45±5	45±5	50±4	45±5	53±2
Storage temperature (ºC)	22-25	4-6	4-6	4-6	4-6
Shelf-life (days)	28	21	14	28	10

Table 4. Results of past studies on PEF application on orange juice (Adapted from Rodrigo et al., 2005).

Source	Juice	Study	Results	Conditions
Dunn and Pearlmann, 1987	Fresh orange juice	Microbial flora and shelf life	5D, 7 days shelf life extension	Exponential 33-35 /cm, 1-100 µs, 35 pulses
Grahl et al., 1992	Orange juice	*S. cerevisiae*	5D	67.5 kV/cm, 5 pulses
Dunn, 1995	Fresh orange juice	Microbial flora	Inactivation	CoolPure system, 20-80 kV/cm
Zhang et al., 1996	Orange juice	Microbial, enzyme, shelf life, flavor	6D, 6 weeks RT, 80% flavor retention	OSU system, 35 kV/cm, 200 µs
Qui et al., 1998	Reconstituted orange juice	Yeast and bacterial flora	4.2 D	Square wave, 35 kV/cm, 60 µs
Rodrigo et al., 2000	Pasteurized blended orange-carrot juice	*L. plantarum*	2.5 D	WSU system, exponential, 29-36 kV/cm, 480 µs
McDonald et al., 2000	Pasteurized orange juice	*E. coli, L. innocua, L. mesenteroides*	5-6 D	Coaxial chamber, 30 kV/cm 2 µs
Yeom et al., 2000	Fresh orange	Total aerobic plate count	7D	Continuous, square monopolar, 20-35 kV/cm, 59 µs
Esteve et al., 2001	Fresh orange-carrot blend	Carotene, cryptoxanthin	No change	Continuous, square bipolar, co-field chamber, 25-40 kV/cm, 59 µs
Min et al., 2002a	Fresh orange juice	Microbial flora, PME, color, flavor, texture	5-6 D, 16 weeks shelf life at 4˚C	OSU, continuous commercial scale, 40 kV/cm, 97 µs
Rodrigo et al., 2003	Pasteurized orange juice/carrot juice	*E. coli*	More inactivation with higher orange juice content	OSU, continuous, square bipolar, co-field, 25-40 kV/cm, 40-340 µs

Table 5. Results of past studies on the effect of PEF application on gel formation, structural changes, and other properties in whole egg and egg products (Sampedro et al., 2006).

Source	Type of Sample	Type of Study	Equipment Conditions	Results
Ma et al. (1998)	Sponge cake made with treated egg and untreated egg	Physical attributes and sensory evaluation	Continuous, co-axial chamber, 5 steps, E=48 kV/cm, N=20 pulses, W=2 µs *Laboratory-Size Prototype, WSU*	No differences between density and whiteness Strength of cake made with egg treated with PEF greater than Untreated sample Sensory panel found no differences
Fernández-Díaz et al. (2000)	Ovalbumin solution 2% and fresh egg white	Analysis of reactive sulphhydryl groups, UV absorbance of aromatic amino acids, thermal gelation, texture of gels and water-holding capacity	Discontinuous, exponential, 0.7–2.3 J/pulse*ml, 100 Hz, V=5.7 ml, Ø=0.38 cm, T<29°C, E=27–33 kV/cm, W=0.3µs, (20 nF) and 0.9µs (80 nF), N=50–400 pulses in series of 20 or 50 at 1.1 Hz	Increase in SH reactivity. No changes in polarity and conformation of tyrosine and tryptophan Slight reduction in gelling properties
Pérez (2002)	Egg	Dynamics of gelation and gel properties	Discontinuous, E=12.5 kV/cm, 3–10 pulses *Gene Pulser II Electroporation System*	Gelation T first point was increased and gelation T second point was decreased with PEF PEF affected hardness and adhesiveness
Pérez and Pilosof (2004)	β-lactoglobulin solution and fresh egg white	Changes in protein structure and functional properties	E=12.5 kV/cm, 1-20 pulses *Electroporation System*	High denaturation in both, low aggregation in β-lg, high T and low gelling time

Where,

E- Electric field intensity

N- Number of pulses

W- Pulse width (duration of pulse)

Ø – Diameter of treatment chamber

V- Volume of chamber

Figure 1. A 5 kw pilot PEF system.

Figure 2. OSU 60 kV, 750 A bipolar PEF system (source: Diversified Technologies).

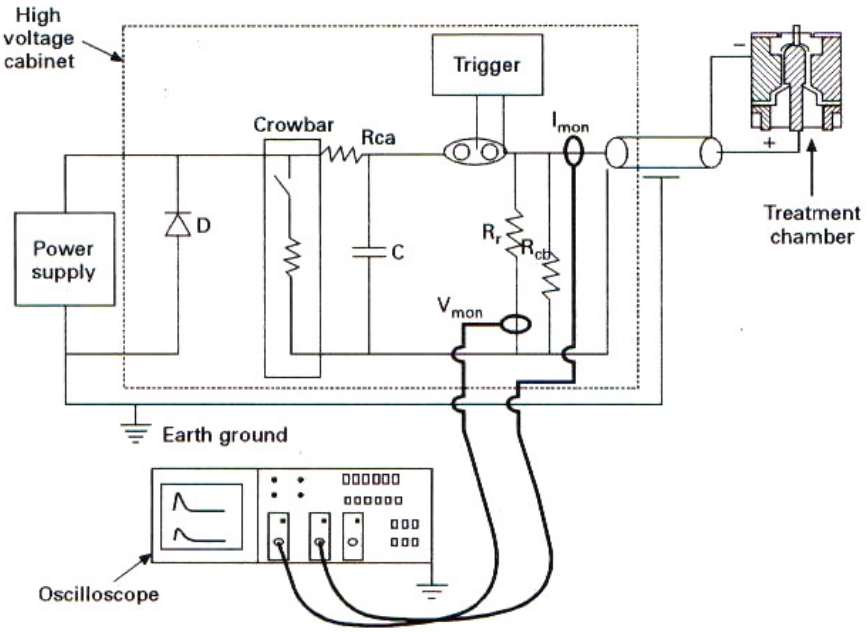

Figure 3**.** High voltage PEF measurement system. *D*, diode; *R*, resistor.

(Ch, charging; Sh, shunt; P, second shunt); *C*, capacitor; *V*, voltage; *I*, current.

CHAPTER THREE

Synergistic Inactivation of *Escherichia coli* in Apple Cider by Pulsed Electric Fields and Selected Antimicrobials

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Abstract

The effect of pulsed electric fields (PEF) in combination with four selected antimicrobials, sodium benzoate, potassium sorbate, cinnamic acid and hydrogen peroxide at two concentrations (500 ppm and 1000 ppm) on microbial inactivation of *E. coli* (ATCC 11775) was investigated. Inoculated apple cider was treated with 20, 12, and 5 pulses of 2.5 μs pulse width at peak electric fields of 40, 35, 30, and 25 kV/cm. PEF treatment in the absence of antimicrobials reduced the microbial load by 5.0 log cycles (CFU/ml), where the addition of cinnamic acid, hydrogen peroxide, sodium benzoate and potassium sorbate prior to PEF increased the lethality to 7.0, 7.5, 7.3 and 6.0 log cycles, respectively, for the maximum electric field strength used (40 kV/cm). The extent of synergistic microbial inactivation was significantly affected by the electric field strength and the number of pulses. The concentration and the type of antimicrobials were more effective at lower electric field strengths and shorter process time. Overall, the combination of PEF and all selected antimicrobials ensured a 5 log-cycle reduction of *E. coli* (ATCC 11775) cells in fresh apple cider for electric fields greater than 35 kV/cm at 20 pulses, demonstrating that the treatment successfully meets Food and Drug Administration (FDA) requirements for juice pasteurization.

1. Introduction

Pulsed electric fields (PEF) is one of the most promising technologies among nonthermal methods of food preservation for offering alternative to traditional processing methods for liquid foods in retention of color, flavor and nutrient (Barbosa-Cánovas et al., 1999). Besides enabling fresh-like quality attributes, PEF technology has been extensively studied for its success on inactivation of undesirable microorganisms in liquid food products without the need for high processing temperatures. The proven effectiveness of PEF in processing pumpable food products such as fruit juices, beverages, milk, sauces, and liquid eggs favored commercialization of this emerging technology (Qin et al., 1995).

Application of PEF is attained by delivering high-voltage pulses to a product placed between two conductive electrodes within a treatment chamber, and the product experience a force per unit charge, the electric field, which is responsible for the irreversible electrical cell membrane breakdown of microorganisms (Zimmerman and Benz, 1980). The pore-forming feature of pulsed electric fields induces a potential across the membrane of a living cell suspended in the electric field, when this potential exceeds a threshold potential of 1 volt, the cell lysis occurs (Sale and Hamilton, 1968). The key parameters for bacterial inactivation are electric field strength, pulse duration, treatment time and temperature of the food material; however, the optimum combination of these parameters depends on the bacterial species (Palaniappan et al., 1990).

The inactivation effect of PEF on microbial flora and the shelf-life extension of foods can be increased by combining PEF with other stress factors. The simultaneous combination of

preservative factors becomes a hurdle to the microorganism, which may provide additive effect on microbial inactivation, and synergistic effect can be observed when the hurdles take up different targets of the microbial cell (Leistner, 1992). The use of antimicrobials in combination with PEF has been more effective in inactivating microbial flora and extending the shelf-life of refrigerated products than PEF treatment alone due to the increased sensitivity of cells to antimicrobials by electric fields (Kalchayanand et al., 1994).

Antimicrobials or chemical preservatives have been defined by Food and Drug Administration (FDA, 1979) as "any chemical preservative when added to food tends to destroy or inhibit the growth of harmful bacteria". The mode of action of antimicrobials can either be by reaction with cell membrane causing increased permeability and loss of cellular constituents, inactivation of essential enzymes or destruction of functional inactivation of genetic material (Davidson and Branen, 1981). Benzoic acid, sorbic acid and their water soluble salts are organic compounds that are used widely as food preservatives. The antimicrobial activity of these acids is related to the transfer of their undissociated forms into the microbial cell (Booth, 1985). Since PEF increases the uptake of exogenous molecules by bacterial cells, the entry of undissociated antimicrobial molecules may be facilitated and thus their bacterial action is increased (Salmond et al., 1984).

Apple cider has been produced and consumed in most of the apple producing regions of the United States for many years. Demand for apple cider is due to its fresh apple flavor and aroma as well as its full-bodied texture where apple cider differentiates from apple juice due to lack of the pasteurization and clarification steps during processing. Cider producers have

traditionally relied upon the acidity of cider and refrigerated storage for preservation of the product. However, outbreaks of *E. coli* O157:H7 associated with consumption of contaminated, unpasteurized apple cider have caused much concern about the safety of the product as it is currently produced. Outbreaks of the food borne illness caused by *E. coli* O157:H7 have been associated with consumption of contaminated apple cider (Besser et al., 1993; Steel et al., 1982). Concerns over the safety of unpasteurized juices have led the FDA to issue juice HACCP regulations that require 5-log reduction in populations of the pertinent pathogen in the juice being processed. Majority of cider producers have adopted pasteurization as a means of accomplishing a minimum 5-log reduction in pathogens since pasteurization is arguably the best approach for the elimination of *E. coli* O157:H7 from cider. However, pasteurization is believed to adversely affect the sensory characteristics responsible for the appeal of fresh cider. Cider producers, food regulatory agencies, and other researchers are seeking alternate means of assuring the safety of apple cider. At this point, alternate processing techniques are being explored for improving juice safety while maintaining product quality and economic feasibility. PEF has shown potential success for pathogen reduction in fruit juices where the combination of PEF with chemical preservatives appears to be more promising. Several studies reported increased inactivation response to PEF in the presence of antimicrobial agents (Pol et al., 2000) and the additive effect of PEF on the inactivation of microorganisms compared to PEF alone (Calderón-Miranda et al., 1999b; Terebiznik et al., 2000). However there are few studies comparing the effectiveness of antimicrobials from different origins and their antimicrobial activities under different levels of dominant PEF parameters.

The objective of this study was to evaluate the effectiveness of pulsed electric fields in combination with selected antimicrobials: potassium sorbate, sodium benzoate, hydrogen peroxide, and cinnamic acid, on inactivation of *E. coli* (ATCC 11775). A nonpathogenic surrogate, *E.coli* (ATCC 11775), that would not pose health hazard in the pilot plant area and whose behavior closely resembled that of the *E. coli* O157:H7 was used in the study (Eblen et al., 2005). Gradual treatment levels of electric field intensity, pulse number (treatment time) and antimicrobial concentration were tested to determine the optimum process parameters that provide a minimum 5-log reduction of target bacteria in fresh apple cider.

2. Materials and Methods

The effect of combination of PEF and four selected antimicrobials in two different concentrations was studied at selected electric field intensities and treatment times to determine the effect of these processing parameters on bacterial inactivation, and was compared with samples that are PEF treated only. Fresh apple cider was obtained from Tree Top Inc. (Selah, WA) and stored at -20°C for the entire period of the experiments. Prior to experimentation, required amount of juice was thawed and diluted with water to obtain Brix° value of commercial apple juices (12.6°Brix). Selected concentration of each antimicrobial was added to the apple cider 30 minutes prior to PEF process. PEF alone with 40 kV/cm electric field for 50 μs was used as the control treatment. For the first part of the study, the effect of each selected antimicrobial alone (without PEF) on microbial inactivation was also measured by applying 1000 ppm of each antimicrobial to the inoculated apple cider at room temperature for 45 minutes.

2.1. Selected Antimicrobials

Two levels, 500 ppm and 1000 ppm of each antimicrobial, sodium benzoate (NaB), cinnamic acid (CA) (Sigma-Aldrich Inc., St. Louis, MO, 63103), potassium sorbate (KS) and hydrogen peroxide (HP) (Mallinckrodt Baker Inc., Phillipsburg, NJ) were used in the experiments. The levels of antimicrobials concentrations were adjusted in view of their maximum regulatory permitted level and their average use in commercial food products (Cords and Dychdala, 1993; Conner, 1993; Sofos, 1989).

Sodium benzoate (MW:144.1) (C_6H_5COONa) is the water soluble salt of benzoic acid and known to be the first chemical preservative approved by FDA (Sofos, 1989). As a common property for weak organic acids and their salts, the antimicrobial activity of benzoates is higher at lower pH, thus, they are most suitable for foods and beverages that naturally are in a pH range below 4.5 or that can be brought into this range by acidification. As a food preservative, the main advantage of benzoates is low price, ease of incorporation into products and lack of color (Figure 1a) (Sofos, 1989).

Potassium sorbate (MW=150.22) ($C_6H_7O_2K$) is the potassium salt of sorbic acid and its more water soluble than the acid form. In recent years, potassium sorbate is widely used through out the world as preservatives for various foods, animal feeds, pharmaceuticals, cosmetics and other industrial applications. Potassium sorbate has the lowest allergenic potential among all food preservatives with natural taste and low cost (Figure 1b) (Sofos, 1989).

Cinnamic acid (MW=148.16) (3 phenyl-2-propenoic acid, $C_6H_5CH=CHCOOH$) is a conjugated α, β-unsaturated aromatic fatty acid (phenylacrylic acid), widely distributed in plants and used in fragrances and flavoring agents (Hoskins 1984). This acid occurs naturally in cranberries, prunes, cinnamon and cloves, and in plant parts used as spices (Beuchat and Golden 1989). It has low toxicity with an acceptable daily intake (ADI) of 1.25 mg kg^{-1}, and is approved for food use by the FDA (Kirk and Othmer 1993). Cinnamic acid is slightly soluble in water and known to be highly lipophylic (Figure 1c) (Conner, 1993).

Hydrogen peroxide (MW=34.01) is classified as generally recognized as safe for use in food products as a bleaching agent, oxidizing and reducing agent, and antimicrobial agent with safe decomposition products: oxygen and water. Antimicrobial action of hydrogen peroxide, reported by Brock et al. (1984), is due to the production of powerful reaction products in food systems, such as singlet or superoxide oxygen, rather than as an antimicrobial molecule. Gould and Dring (1975) theorized the antimicrobial and sporicidal activity of hydrogen peroxide on the oxidation of sulfhyrly groups and double bonds in proteins, lipids, and surface membranes. The compound has been reported to be active against a wide spectrum of organisms including bacteria, yeast, mold, virus and spore-forming organisms when used concentrations of 0.001-0.1 % however has only been approved by FDA for cheese and milk processing so far (Figure 1d) (Cords and Dychdala, 1993).

2.2. Buffer solution

When performing in-flow PEF treatment, the cross contamination of lines carrying treated and untreated product was avoided by pre-equilibrating the system with sterile Mc Ilvaine

buffer matching the conductivity of apple cider. After reaching the desired pulsing frequency with stable outlet temperature, the inlet line to the system was switched from the sterile buffer to the apple cider without an interruption in pulsing. Electrical conductivity of the buffer solution was adjusted by diluting 1 part of buffer with 15 parts of deionized water, reaching a final conductivity of 0.23 S/m at 20^{o}C to resemble the electrical conductivity of apple cider. One liter of Mc Ilvaine (citric-phosphate) buffer (pH 7, 4^{o}C) was prepared by mixing 178 mL of 0.1 M citric acid solution (21.01 g$C_6H_8O_7 \cdot H_2O$ L^{-1}) and 822 mL of 0.2 M Na_2HPO_4 (28.40 g Na_2HPO_4 L^{-1}) (Perrin and Dempsey, 1974).

2.3. Microbiology

Two milliliters of frozen *E.coli* (ATCC 11775) were obtained from stock at -21°C (1ml of microorganisms plus 1 ml of sterile glycerol). Thawed *E.coli* (ATCC 11775) was then added to 100 ml nutrient broth (Difco, Kansas MO) for growth on a rotary platform shaker (MSB-3322A-I, GS Blue Electric, Blue Island, IL) at 30°C and 225 rpm until reaching the early stationary phase (27 hours). Optical density was evaluated at 25°C by a spectrophotometer (Hewlett-Packard, Palo Alto, CA) at 540 nm to determine the early stationary phase where the microbial population reaches to 10^9 CFU/ml. 100 ml of the inoculum were transferred into 10 liters of apple cider to obtain an initial bacterial concentration of approximately 10^7 CFU/ml. Bacterial concentration in treated and untreated samples was determined by the pour-plate method. Serial decimal dilutions in sterile 0.1% peptone (Difco, Kansas MO) water were plated on Eosin Methylene Blue (EMB) Agar. All samples were plated in duplicate and incubated at 35±0.5°C for 48 h in a controlled temperature chamber. After incubation, colonies were quantified following standard procedure (AOAC, 1995).

Inactivation effectiveness was quantified by determining the logarithmic difference between the numbers of bacteria before and after treatment, and expressed as logarithmic cycle's reduction. Each study was conducted in triplicate using independent prepared batches of buffer and fresh bacteria for every replicate.

2.4. Pulsed Electric Fields Treatment

A pilot plant PEF system (Physics International, San Leandro, CA), consisting of a continuous concentric cylindrical treatment chamber with 0.6 cm gap and 25 mL effective treatment volume (Qin et al., 1995) was used for all treatments (Figure 2). 5, 12, and 20 exponentially decaying pulses, corresponding to 12.5, 30 and 50 microseconds, respectively, with peak electric field intensities of 25, 30, 35, 40 kV/cm and 2.5±0.1 μs pulse width were delivered to the treatment chamber by discharging a 0.5 μF capacitor. The pulse width approximated by the electrical circuit's time constant, time taken for the peak voltage to decay exponentially to 37% i.e., 1/e of its maximum value, and the treatment time, t was calculated by:

$$t = \tau * n \tag{1}$$

where τ is the effective time constant measured as 37% of the maximum peak voltage, and n is the number of pulses applied (Góngora-Nieto et al., 2004). Chilled apple cider (4ºC) was pumped at a flow rate of 72 l/h and the outlet temperature did not exceed 60ºC. Inlet and outlet temperatures, pulse shape, voltage, current, and energy flow were monitored throughout the process. Electrical parameters were monitored with a digital oscilloscope

(Hewlett-Packard 54530A, Colorado Springs, CO) connected to the treatment chamber through high voltage probes. The average electric field intensity was calculated by:

$$E_{CO} = \frac{V}{r \ln\left(\frac{R_1}{R_2}\right)} \quad (2)$$

where E_{CO} (kV/cm) is the electric field strength for coaxial electrodes, V (kV) is the average peak voltage, r (cm) is the radius at which the electric field is measured, and R_1 and R_2 are the radii of inner and outer electrodes (Barbosa-Cánovas et al., 1999).

2.5. Statistical Analysis

Each PEF treatment was performed in triplicate and the data obtained was further subdivided into independent sets defined by different combinations of electric field intensity, number of applied pulses, and level of antimicrobials. A one-way ANOVA was conducted using the statistical analysis software (SAS Institute, Cary, NC) and least significant difference (LSD) multiple comparison test (P<0.05) was used to determine the significant difference within experimental conditions and antimicrobials in terms of bacterial inactivation.

3. Results and Discussion

Inactivation of *E.coli* (ATCC 11775) after PEF treatment of 25, 30, 35, 40 kV/cm maximum electric field intensities for 12.5, 30 and 50 µs, combined with two concentrations of sodium benzoate, potassium sorbate, cinnamic acid and hydrogen peroxide are given in Table 1. The PEF treatment at the maximum electric field strength used (40kV/cm and 50 µs), in the

absence of antimicrobials, reduced the microbial load by 5.0 log cycles (CFU/ml), where the addition of cinnamic acid, hydrogen peroxide, sodium benzoate and potassium sorbate prior to PEF increased the lethality to 7.0, 7.5, 7.3 and 6.0 log cycles. The effectiveness of PEF in microbial inactivation has already been proven in vast number of studies. The focus of this study was to establish the effectiveness of selected antimicrobials under the influence of key PEF variables. If the proper antimicrobial could be selected based on their activity in combination with optimum PEF conditions, it is possible to assure legal microbial reduction requirements without over processing the product.

3.1. Synergistic Effect of Antimicrobials

Antimicrobials tested in this study showed synergistic responses to microbial inactivation when combined with different levels of PEF treatment factors (Figure 3). Bacterial inactivation of antimicrobials alone was only 1 log cycle and not significantly different from non-treated (fresh apple cider) sample. PEF treatment in the absence of antimicrobials (control) resulted in 5 log cycles of inactivation where combination of PEF and antimicrobials achieved 7 log cycles of inactivation and was significantly different from the control treatments. The difference in the inactivation activities of tested antimicrobials highly depends on their mode of action during the PEF process.

Among all, cinnamic acid, a naturally occurring phenolic antimicrobial which includes an aromatic ring with hydroxyl groups in its structure, showed the most effective inactivation regardless of the electric field strength applied (Figure 4c). The mechanism of phenolic compounds for inactivating microorganisms has not been fully explained; however, it is

generally attributed to their effects on cellular membranes (Davidson, 1993). Their interference with the cell membrane permeability which causes leakage of cellular components and inhibition of active transport of nutrients were reported (Eklund, 1985). The reported mode of action of cinnamic acid on cellular membranes approved the results obtained in this study since the influence of electric fields on bacterial membranes was maximized when combined with cinnamic acid. Additionally, for being phenolic in nature, the presence of terpenes in cinnamic acid structure might be effective on impairment of enzymes including those involved in energy production and structural component synthesis (Trainter and Grenis, 2001). Compared to other antimicrobials used in this study, the longer side chain of cinnamic acid may also have caused the superior effectiveness of this antimicrobial, since phenolic compounds with longer side chain length known to have more antimicrobial activity (Davidson and Branen, 1981).

The effectiveness of potassium sorbate and sodium benzoate were significant when combined with highest electric field intensities (35 and 40 kV/cm) (Figures 4a and 4b). The antimicrobial response of these two compounds was similar due to the common elements in mechanism of action of organic acids. The activity of organic acids is highly pH dependent and the undissociated form of the acid is primarily responsible for antimicrobial activity (Davidson, 1993) thus, the use of organic acid antimicrobials is generally limited to acidic foods with lower pKa values (Table 2) (Doores, 1993). Different from the antimicrobial activity of the cinnamic acid on bacterial membranes, in their undissociated form, organic acids penetrate into the cell through lipid bilayer of the membrane. Once inside the cell, the acid dissociates, because the cell interior has a higher pH than the exterior. Protons generated

from intracellular dissociation of the organic acid acidify the cytoplasm and extruded to the exterior by consuming energy, which will eventually deplete the cellular energy (Hunter and Segel, 1973). Taking into account that sodium benzoate was the first antimicrobial approved by FDA and potassium sorbate was the easiest to be used in processing due to its high solubility, these two antimicrobials provide broad application possibilities in combination with PEF for the fruit juice industry.

The antimicrobial effect of hydrogen peroxide was significant with higher electric fields (35 and 40 kV/cm) (Figure 4d). The origin and mechanism of action of hydrogen peroxide is different from other antimicrobials tested in this study. Among several different theories proposed to explain the mechanism involved in the cell inactivation by hydrogen peroxide, according to Brock et al. (1984) the decomposition products of hydrogen peroxide such as singlet or superoxide oxygen are responsible for the bacterial inactivation. Superoxide oxygen for being a higher energy form of oxygen is extremely reactive against microbial cells, yet it is also generated via specific enzyme systems that naturally occur in milk, honey, and living cells such as lactoperoxidase and myeloperoxidase enzyme systems (Fridovic, 1975). In another theory proposed by Gould and Dring (1962), the antimicrobial activity of hydrogen peroxide is due to the oxidation of sulfhydrly groups and double bonds in proteins, lipids, and surface membranes. Considering both theories on inactivation mechanism of hydrogen mechanism, based on the inactivation data during PEF process, the lethal effect of decomposition products seemed to be coupled by the destructive effect on cell membranes, providing an effective treatment combination. If required legal approvals provided, the use of

this antimicrobial in combination with PEF could be beneficial when the initial load of the product is high.

3.2. Effect of Electric Field Intensity

The extent of antimicrobial effectiveness was highly dependent on the PEF variables applied where, in general, inactivation of the *E.coli* (ATCC 11775) by electrical pulses in combination with antimicrobials was enhanced by increased electric field strength, treatment time (number of pulses) and self-generated temperature. Figure 5 represents the inactivation of *E.coli* (ATCC 11775) after exposure to peak electric field intensities of 25, 30, 35 and 40 kV/cm for 50 microseconds (20 pulses) in combination with selected antimicrobials. The use of higher electric field intensity resulted in significant increase in microbial inactivation rate. Beyond the critical electric field intensity (approximately 30 kV/cm) selected antimicrobials did not show significant difference from each other, however all antimicrobials were significantly different from PEF-alone treatment (40kV/cm). Microbial reduction in the absence of antimicrobials was 5 log cycles while the log reduction reached 7 logs with the use of antimicrobials in combination with PEF treatment. Microbiological results were in accordance with the literature values reported by Liu et al., (1997) where the use of 1000 ppm of benzoic acid in combination with PEF treatment (12.5 kV/cm) resulted 4 log cycles of inactivation in the *E. coli* population.

Below critical electric field, inactivation of cinnamic acid added sample was significantly different from the inactivation achieved by other antimicrobials. The influence of electric fields on permeability of cell membranes facilitates the entry of antimicrobials into the

microbial cell where they dissociate and contribute to microbial inactivation (Hunter and Segel, 1973). Thus, beyond critical electric field intensity where the microbial inactivation curve is steeper (more inactivation), the antimicrobial activity of organic acids (potassium sorbate and sodium benzoate) is higher. However, in the case of cinnamic acid, for which the mode of action is generally attributed the effects on cellular membranes (Davidson, 1993) where the influence on cell membrane permeability causes leakage of cellular components (Eklund, 1985), the extent of antimicrobial activity was not significantly affected by the strength of the electric fields (Figure 5).

3.3. Effect of Treatment Time (number of pulses)

Inactivation plots against treatment time (Figure 4a-4c) and electric field intensity (Figure 5) when 1000 ppm of selected antimicrobials is used in combination with PEF were given where PEF-alone treatment at 40 kV/cm was used as control. The electric field strength and treatment time (number of pulses) are the two most important variables contributing to the effectiveness of the PEF process and self generated temperature increases when these two variables are increased (Sale and Hamilton, 1967; Zhang et al., 1994). Analysis of the data showed that the inactivation effectiveness markedly increased when higher electric field intensity and longer treatment time were applied regardless of the antimicrobial used. As we know that phospholipid molecules in cell membranes undergo temperature related phase transition, changing from a packed gel-like structure at low temperatures to a looser liquid-crystalline phase at higher temperatures, reducing the thickness and mechanical resistance of cell membranes (Stanley, 1991), hence coupling of PEF with mild heat treatment enhanced microbial inactivation (Sepulveda et al., 2005). The marked increase in microbial inactivation

may be indicative of the occurrence of such phase transition, and change in membrane fluidity under extreme PEF conditions and elevated temperatures where thinning of the bacterial membrane would render bacterial cells more susceptible to disruption by both electric fields and antimicrobial activity, as indicated by the dielectric breakdown theory (Zimmermann and Benz, 1980).

PEF technology is considered a nonthermal preservation technology in view of the fact that electric field itself is responsible for microbial inactivation, not the temperature. However, processing temperature is one of the most relevant process parameters in PEF surpassed in importance by electric field intensity and treatment time. The increased effectiveness of PEF at higher temperatures is explained by the dependence of rheological properties of microbial membranes as explained above. Unless the boundaries of the commonly considered non-lethal thermal conditions (up to and around 60˚C for less than few seconds) are surpassed during the PEF process, nonthermal effects govern the PEF preservation process with thermal enhancement (Barsotti and Cheftel, 1999; Dunn, 2001).

3.4. Effect of Antimicrobial Concentration

Figures 6a and 6b represents effect of antimicrobial concentration on microbial inactivation under different treatment times. The effect of antimicrobial concentration was significant only for cinnamic acid when the treatment time is lower (30 microseconds) with an electric field intensity of 40 kV/cm, suggesting that the effect of treatment time has greater effect on inactivation compared to the antimicrobial concentration. As explained above, when longer treatment times together with high electrical voltages are applied, self-generated heat assists

inactivation. Parallel to findings in this study, PEF treatment at moderate temperatures (50-60ºC) enhanced the inactivation of microorganisms due to weakening of bacterial cell membranes and leading to cell death (Pothakamury et al., 1996).

3.5. Effect of Conductivity and Ionic Strength

Electrical conductivity of ionic solutions is temperature dependent and have increasing trend as a function of temperature (Figure 7a). When the antimicrobial concentration in the ionic solution is high leading to stronger ionic strength, the dependence of temperature was also increased and could be observed with a steeper slope (Figure 7b). Strong temperature dependency of ionic solutions is generally explained by their kinetic state where an increase in temperature increases the mobility of ions throughout the solution, causing an increase in the conductivity (Heinz et al., 2002).

Evaluating the change of electrical properties during PEF process is useful to understand the differences between the activities of antimicrobials tested in this study since electrical conductivity and ionic strength are the two important characteristics of treated products affecting the biological changes during the PEF process (Barbosa-Cánovas et al., 1999). In addition to their mode of action on bacterial cells, the effectiveness of cinnamic acid and hydrogen peroxide during PEF process might be due to their lower conductivities, hence potassium sorbate and sodium benzoate have the highest conductivities at 30˚C (Table 2) and within a temperature range of 15-35˚C their conductivities were significantly higher than cinnamic acid and hydrogen peroxide. The higher the conductivity of the food product treated in the treatment chamber, the lower the electrical resistance to the flowing current.

This in turn, reduces the proportion of applied charging voltage in the form of electric field strength between the electrodes of the treatment chamber. The conductivity of the media, which is related to the material's capacity to conduct electric current, will influence the maximum electric field achievable for a given input power and the maximum temperature rise during processing. Lowering the conductivity (high resistivity) reduces the temperature rise and applied power, thus increasing the electric field intensity and overall microbial effectiveness (Vega-Mercado et al., 1996).

4. Conclusions

Antimicrobials tested in this study showed significant synergistic response to microbial inactivation when combined with different levels of PEF treatment factors. Inactivation of *E.coli* (ATCC 11775) markedly increased when higher electric field intensity and longer treatment time were used regardless of the antimicrobial type and concentration. Among the four selected antimicrobials, cinnamic acid was the most effective antimicrobial in terms of bacterial inactivation, followed by hydrogen peroxide, potassium sorbate and sodium benzoate. Potassium sorbate and sodium benzoate did not significantly differ from each other for bacterial inactivation; however, both have significant synergistic effect when combined with intense PEF treatments. The difference between antimicrobial activities was mainly due to their mechanism of action on bacterial cells while the electrical properties of selected antimicrobials might also have contributed to the lethality.

The focus of this research was to explore the synergistic inactivation of a microorganism of major concern for the juice industry, *E.coli* O157:H7, by combining the killing effect of

several convenient antimicrobials and optimized PEF conditions. Novel to the majority of research in this area, contribution of selected antimicrobials were compared based on their possible mode of action as well as their electrical properties. If the proper antimicrobial could be selected based on microbial activity in combination with optimum PEF conditions, it is possible to assure legal microbial reduction requirements without over processing the product. Considering solubility, regulatory issues and ease of processing, potassium sorbate and sodium benzoate are recommended in combination with PEF treatment for increased inactivation of *E.coli* (ATCC 11775) in apple cider. This research has shown that the combination of PEF and all selected antimicrobials successfully meet FDA requirements for juice pasteurization by ensuring a 5 log-cycle reduction of *E. coli* (ATCC 11775) cells in fresh apple cider for electric fields greater than 35 kV/cm, at 20 pulses. Further research on the sensory properties of apple cider treated with PEF in the presence of selected antimicrobials is recommended to evaluate the consumer acceptance. Cinnamic acid derivatives in the form of soluble salts, as in the case of organic acids and their salts, could also be studied as a substitute which offers ease of processing and pasteurized juice safety in PEF treated apple cider.

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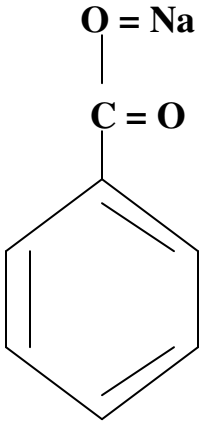
Table 1. Inactivation (log CFU/ml) of *E.coli* (ATCC 11775) in fresh apple cider treated with PEF and selected antimicrobials (PEF-only treatment was used as the control).

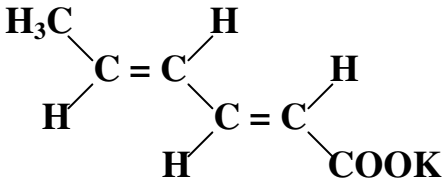
Mean inactivated E. coli (ATCC 11775) population (log CFU/ml)										
E. Field (kV/cm)	*Treatment time (μs)*	*Control*	*Sodium Benzoate*		*Potassium Sorbate*		*Cinnamic Acid*		*Hydrogen Peroxide*	
		0	500	1000	500	1000	500	1000	500	1000
40	50.0	-5.0±0.3	-6.8±0.3	-6.8±0.5	-7.3±0.3	-7.2±0.5	-7.0±0.0	-7.0±0.0	-7.3±0.0	-7.3±0.0
	30.0	-3.0±0.2	-3.8±0.4	-4.0±0.7	-3.0±0.2	-3.2±0.5	-5.6±0.3	-6.7±0.3	-4.3±0.2	-4.6±0.2
	12.5	-2.0±0.2	-2.2±0.2	-2.2±0.3	-1.9±0.4	-2.1±0.5	-3.9±0.3	-5.0±0.2	-2.6±0.1	-2.7±0.3
35	50.0	-4.9±0.3	-5.5±0.3	-5.8±0.9	-7.3±0.3	-7.2±0.1	-6.8±0.1	-7.0±0.1	-6.6±0.4	-7.3±0.2
	30.0	-2.4±0.4	-2.9±0.2	-2.8±0.4	-2.3±0.1	-2.5±0.2	-5.3±0.2	-6.2±0.3	-3.6±0.2	-4.1±0.3
	12.5	-1.2±0.3	-1.8±0.1	-1.4±0.4	-1.0±0.2	-1.3±0.3	-3.7±0.2	-4.5±0.1	-2.4±0.5	-2.5±0.2
30	50.0	-3.2±0.2	-3.4±0.4	-4.2±0.1	-3.8±0.2	-4.0±0.3	-5.6±0.3	-6.0±0.1	-5.3±0.3	-2.7±0.3
	30.0	-1.9±0.3	-2.5±0.2	-2.3±0.3	-1.8±0.2	-1.9±0.5	-4.6±0.2	-5.4±0.2	-2.6±0.3	-1.9±0.4
	12.5	-1.4±0.5	-1.4±0.1	-1.2±0.2	-0.7±0.3	-0.9±0.5	-3.5±0.5	-4.3±0.3	-1.4±0.5	-1.1±0.5
25	50.0	-1.5±0.2	-2.0±0.3	-1.6±0.3	-0.7±0.2	-1.5±0.1	-3.6±0.5	-5.7±0.2	-1.0±0.2	-1.4±0.2
	30.0	-1.0±0.3	-1.5±0.4	-1.1±0.3	-0.4±0.3	-0.6±0.3	-2.9±0.5	-5.1±0.2	-0.6±0.3	-0.9±0.1
	12.5	-0.6±0.2	-0.6±0.3	-0.7±0.2	-0.2±0.5	-0.4±0.5	-2.2±0.5	-4.0±0.2	-0.4±0.5	-0.5±0.1

Table 2. Conductivity (S/m) and pKa values for 1000 ppm antimicrobial added apple cider (30^0C).

	Conductivity (S/m)	pKa
Cinnamic acid	0.273	3.89
Hydrogen peroxide	0.284	11.65
Sodium benzoate	0.330	4.20
Potassium sorbate	0.348	4.76



(a)



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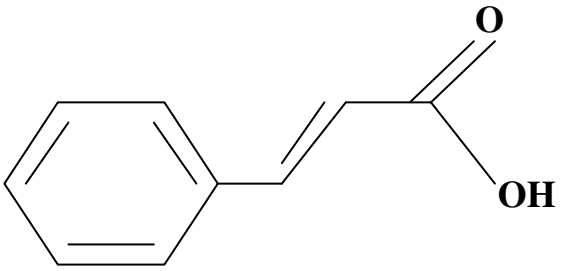

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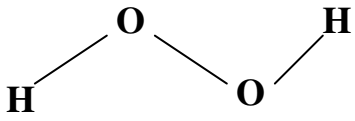


(d)

Figure1. Molecular configuration of selected antimicrobials: (a) sodium benzoate, (b) potassium sorbate, (c) cinnamic acid, (d) hydrogen peroxide.

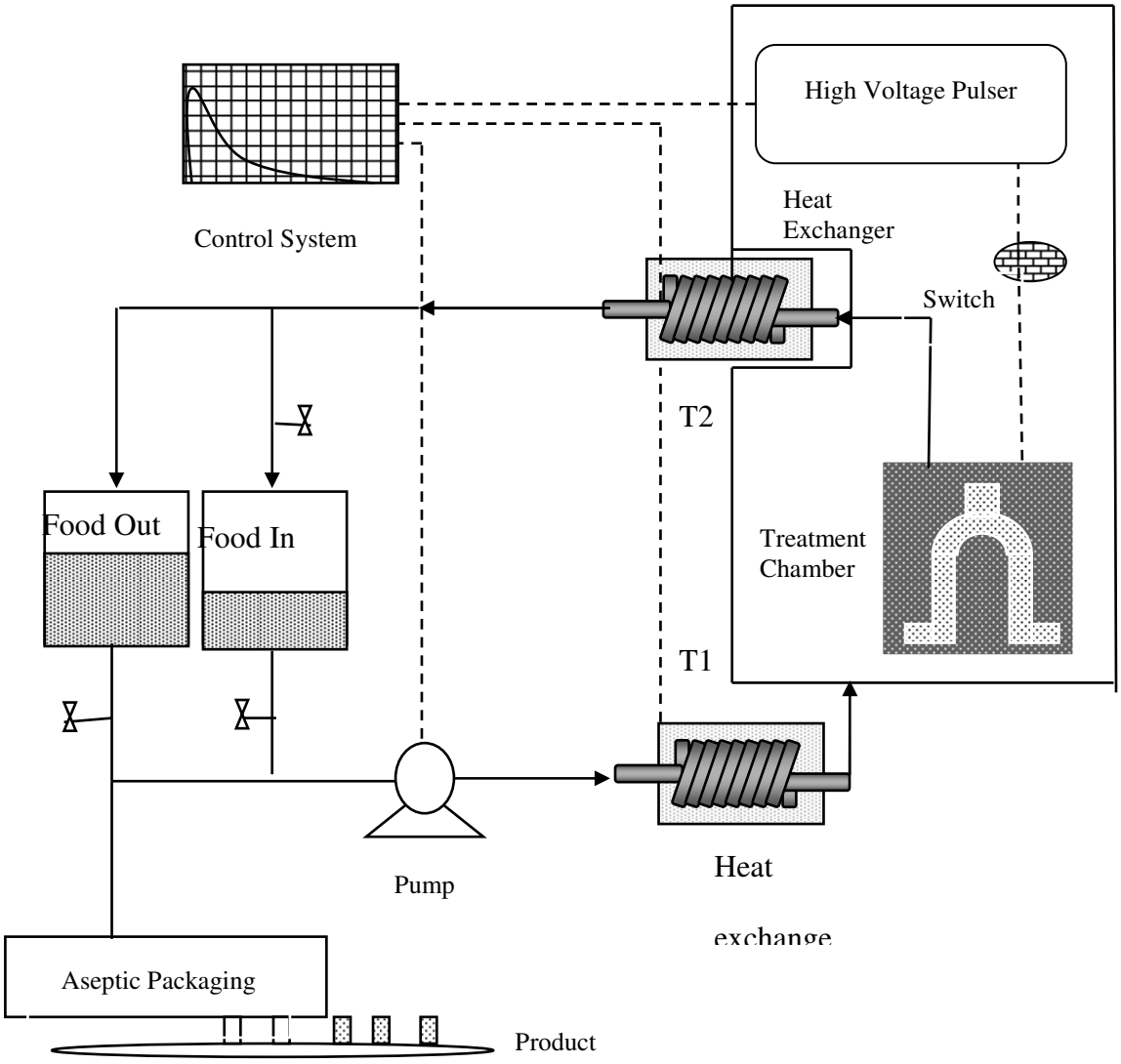


Figure 2. Schematic representation of a continuous pulsed electric field system.

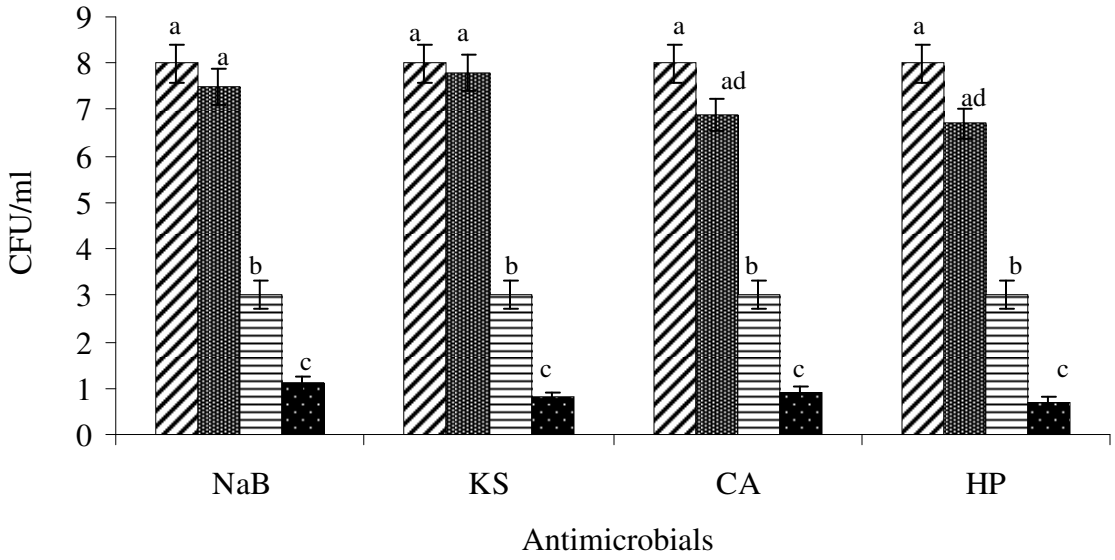


Figure 3. Relative effects of treatments on microbial inactivation, columns represent the survivors after different treatments: (▨) no treatment (untreated fresh apple cider), (▩) 1000 ppm antimicrobial only at 25˚C for 45 minutes (no PEF), (▤) PEF only (40kV/cm and 50 µs), (■) combination treatment of PEF (40kV/cm and 50 µs) and selected antimicrobials.

(NaB: Sodium benzoate, KS: Potassium sorbate, CA: Cinnamic acid, HP: Hydrogen peroxide)

Error bars represent standard deviation for each data point.

Different letters indicate the significant difference (P<0.05)

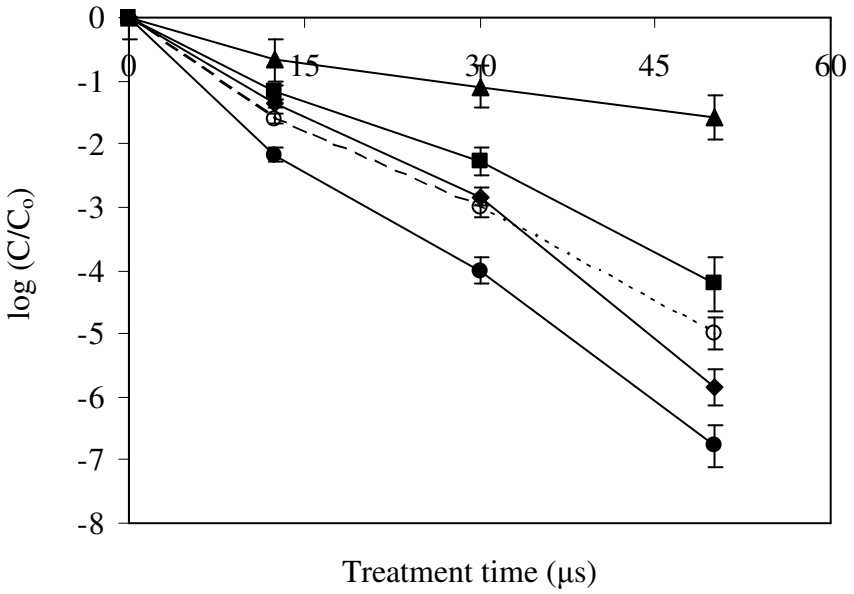


Figure 4a. Survival of *E.coli* (ATCC 11775) in fresh apple cider after PEF treatment in combination with 1000 ppm sodium benzoate, with peak electric field intensities of 40, 35, 30 and 25 kV/cm for 12.5, 30 and 50 microseconds. (○) PEF only (40 kV/cm); (●) 40 kV/cm; (♦) 35 kV/cm; (■) 30 kV/cm; (▲) 25 kV/cm.

Error bars represent standard deviation for each data point.

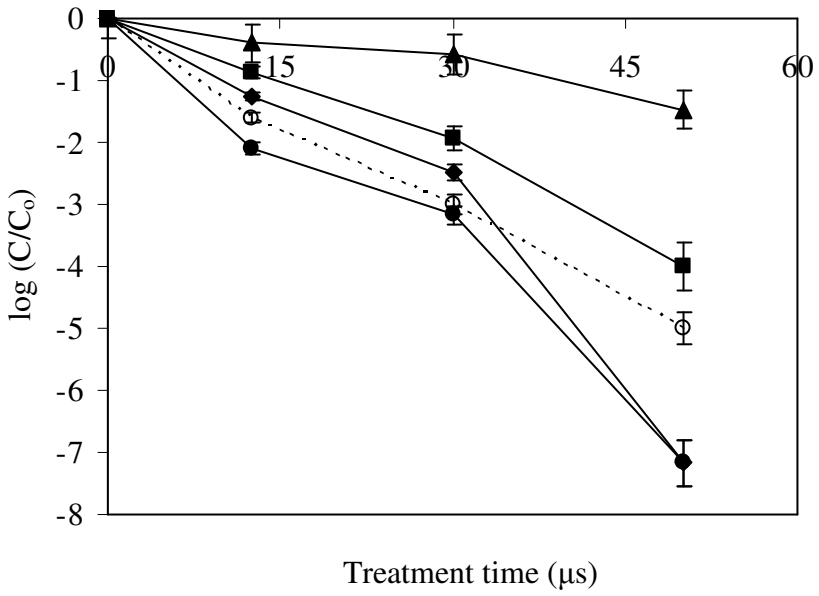


Figure 4b. Survival of *E.coli* (ATCC 11775) in fresh apple cider after PEF treatment in combination with 1000 ppm potassium sorbate, with peak electric field intensities of 40, 35, 30 and 25 kV/cm for 12.5, 30 and 50 microseconds. (○) PEF only (40 kV/cm); (●) 40 kV/cm; (♦) 35 kV/cm; (■) 30 kV/cm; (▲) 25 kV/cm.

Error bars represent standard deviation for each data point.

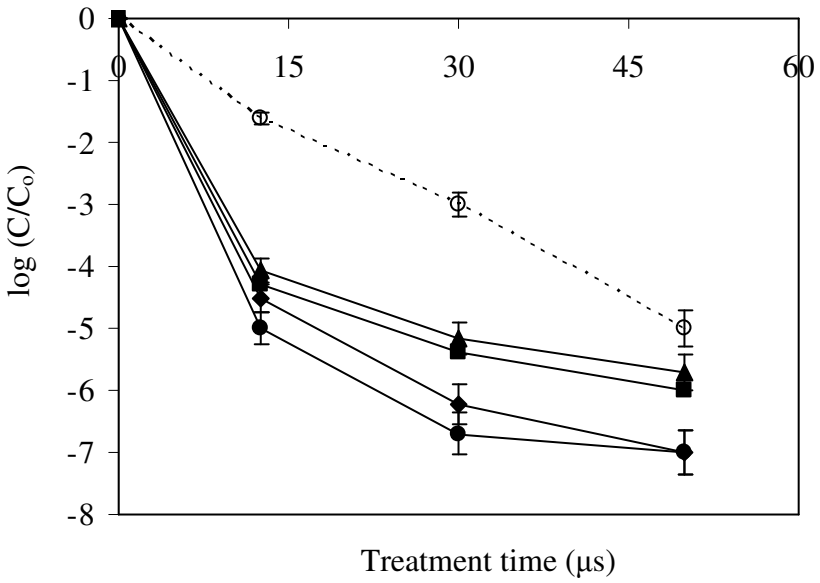


Figure 4c. Survival of *E.coli* (ATCC 11775) in fresh apple cider after PEF treatment in combination with 1000 ppm cinnamic acid, with peak electric field intensities of 40, 35, 30 and 25 kV/cm for 12.5, 30 and 50 microseconds. (○) PEF only (40 kV/cm); (●) 40 kV/cm; (♦) 35 kV/cm; (■) 30 kV/cm; (▲) 25 kV/cm.

Error bars represent standard deviation for each data point.

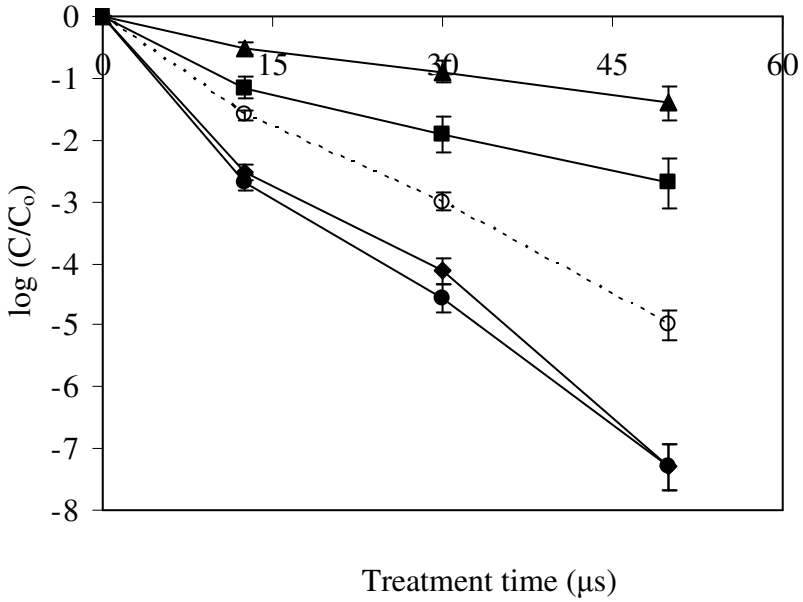


Figure 4d. Survival of *E.coli* (ATCC 11775) in fresh apple cider after PEF treatment in combination with 1000 ppm hydrogen peroxide, with peak electric field intensities of 40, 35, 30 and 25 kV/cm for 12.5, 30 and 50 microseconds. (○) PEF only (40 kV/cm); (●) 40 kV/cm; (♦) 35 kV/cm; (■) 30 kV/cm; (▲) 25 kV/cm.

Error bars represent standard deviation for each data point.

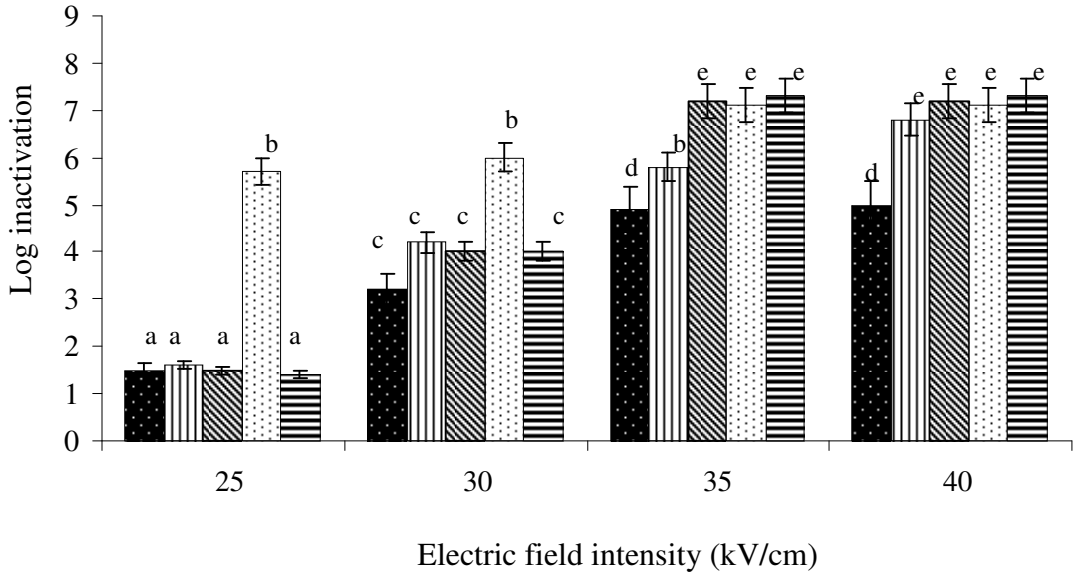


Figure 5. Inactivation of *E.coli* (ATCC 11775) after exposure to PEF with 25, 30, 35, 40 kV/cm and 50 μs (20 pulses) combined with selected antimicrobials (1000 ppm). (▪) PEF only, (▥) sodium benzoate, (▧) potassium sorbate, (▫) cinnamic acid, (▤) hydrogen peroxide.

Error bars represent standard deviation for each data point.

Different letters indicate the significant difference (P<0.05)

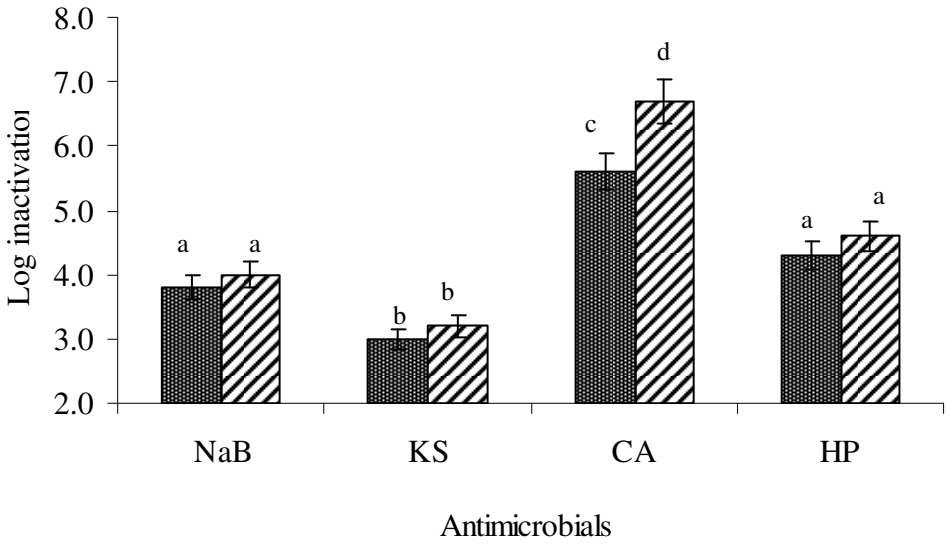


Figure 6a. Inactivation of *E.coli* (ATCC 11775) after PEF at 40 kV/cm and 30 microseconds (12 pulses) for (▩) 500 ppm; (▨) 1000 ppm of antimicrobial concentrations.

(NaB: Sodium benzoate, KS: Potassium sorbate, CA: Cinnamic acid, HP: Hydrogen peroxide)

Error bars represent standard deviation for each data point.

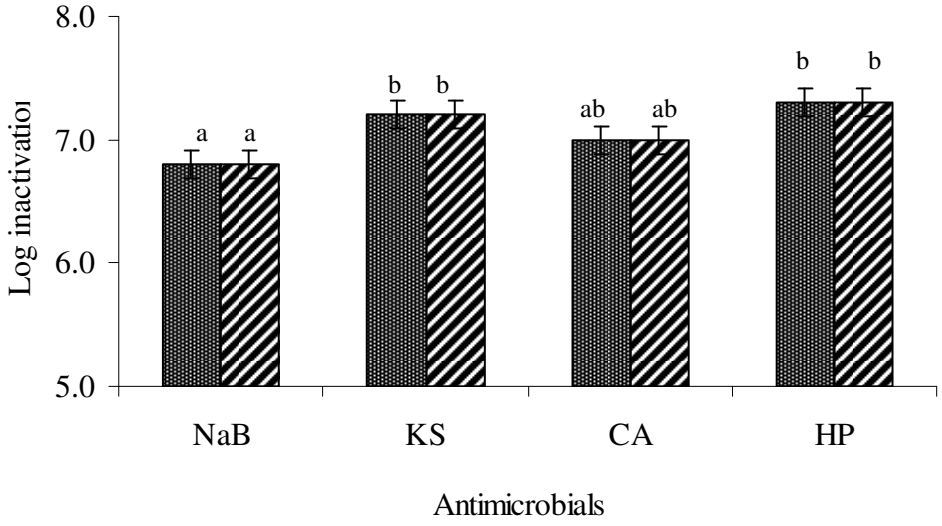
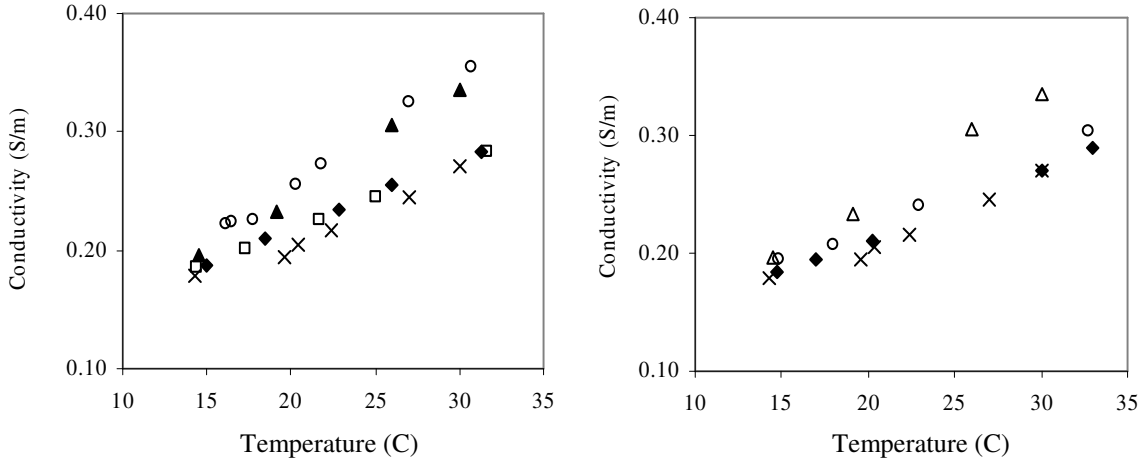


Figure 6b. Inactivation of *E.coli* (ATCC 11775) after PEF at 40 kV/cm and 50 microseconds (20 pulses) for (▩) 500 ppm; (▨) 1000 ppm of antimicrobial concentrations.

(NaB: Sodium benzoate, KS: Potassium sorbate, CA: Cinnamic acid, HP: Hydrogen peroxide)

Error bars represent standard deviation for each data point.



(a) (b)

Figure 7a. Conductivity of apple cider after addition of selected antimicrobials (1000 ppm) (○) potassium sorbate; (▲) sodium benzoate; (♦) hydrogen peroxide; (□) cinnamic acid; (x) apple cider only (no antimicrobials)

Figure 7b. Conductivity of apple cider after addition of different sodium benzoate concentrations: (∆) 1000 ppm**;** (○) 500 ppm; (●) 100 ppm; (x) apple cider only (no antimicrobials).

CHAPTER FOUR

Modeling Microbial Inactivation of *E.coli* (ATCC 11775) after Exposure to Pulsed Electric Fields and Selected Antimicrobials

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Abstract

The inactivation kinetics of *E.coli* (ATCC 11775) in fresh apple cider treated by pulsed electric fields (PEF) in combination with selected antimicrobials was studied. Experimental data were fitted to Bigelow, Hülsheger, Peleg kinetic models and Weibull frequency distribution function. Electric field strength ranged from 25 to 40 kV/cm and the treatment time varied between 12.5 and 50 microseconds. The inactivation of *E.coli* (ATCC 11775) was enhanced with the use of selected antimicrobials (cinnamic acid, hydrogen peroxide, sodium benzoate and potassium sorbate). Traditional concept of D and z values, established for sterilization processes, might be insufficient for validating nonthermal processes when combined methods are involved under intense PEF treatment conditions (higher electric field strength and/or longer treatment time). Inactivation of *E.coli* during PEF in the presence of cinnamic acid resulted in a completely concave upward survival curve where the survival curves for other antimicrobials tested are mostly concave downward at higher electric fields (35-40 kV/cm) and almost linear at lower electric fields (25-30 kV/cm). The use of Weibull distribution is recommended for combination studies where more than one stress factor is involved based on higher correlation coefficients and better accuracy factors (A_f) provided.

1. Introduction

In recent years, several technologies that have the capability of inactivating microorganisms at lower temperatures than the conventional heat treatments have been investigated (Lado and Yousef, 2002). Pulsed electric fields (PEF) is one of these innovative technologies that encompass the discharge of high voltage electric short pulses through a food. It has been demonstrated that PEF can efficiently inactivate vegetative bacterial cells and yeast while retaining nutritional and organoleptic qualities (Barbosa-Cánovas et al., 1999). The technology focuses on acidic products such as fruit juices where spore forming bacteria are not a major concern for spoilage (Ayhan et al., 2001). The effectiveness of PEF can further be increased if applied in combination with antimicrobials for the inactivation of pathogenic and spoilage microorganisms without altering the quality (Altunakar et al., 2007, unpublished data).

As the interest in nonthermal technologies increases, so does the interest in their safety. For any technology aiming microbial inactivation, the interpretation of the survival curves is of high importance. Predictive microbiology, an emerging multidisciplinary area of food science, aims to develop and apply mathematical models that predict the influence of process parameters and environmental variables on microbial inactivation (McDonald and Sun, 1999). Mathematical models predicting the response of pathogens and spoilage microorganisms to any technology is useful to design safe and effective processes since the ability to understand and model the survival of pathogens in foods or during process is critical to the safety of the food supply (McKellar and Lu, 2004).

Primary models are generally utilized to describe survival curves where the changes in microbial population are analyzed with respect to time. For thermal and nonthermal inactivation of microorganisms, four types of survival curves are generally observed: linear curves, curves with shoulder (concave downward), curves with a tail (concave upward), and sigmoidal curves (Xiong et al., 1999). Several complex inactivation curves are shown in Figure 1.

Linear survival curves have been fully employed for thermal technologies to interpret microbial inactivation. D value and the "thermal death time" are supposed to represent the organism's heat resistance; however, different authors claim that this value is not constant since it has an inherent error due to the concavity of the curve and depends on the number of points used for its determination (Peleg and Cole, 2000).

In spite of common application of linear curves, non-linear survival curves were also reported for some bacteria almost 100 years ago (Moat et al., 1971). The possible explanation for non-linear kinetics is summarized into two groups (Stringer et al., 2000). The first group includes factors related to the limitations in experimental design such as variations in heating procedure, use of mixed cultures or populations, clumping, protective effect of dead cells, method of enumeration or poor statistical design which would eventually result in tailing of the survival curves. The second group consists of the factors related to the normal features of the inactivation process such as multiple hit mechanisms as in the case of combination studies, natural distribution of heat sensitivity and heat adaptation. For the second group the expected shape of the curves would be sigmoidal or concave upward (Cerf, 1977).

In general, downward concavity can be interpreted as a reflection of the increasing sensitivity of the organism to accumulated damage. As the applied stress increases throughout the process, organism becomes more sensitive and the inactivation rate increases. In the case of upward concavity, or "tailing", since not all microorganisms show identical resistance to the applied stress, generally a spectrum of resistance is observed. After the ones with lower resistance are eliminated, survivors with progressively increasing resistance remain during the process (Peleg and Penchina, 2000; Peleg and Cole, 2000).

Microbial inactivation studies demonstrated that microbial inactivation by PEF is influenced by several factors among which the field intensity and treatment time are the most important parameters (Wouters et al., 2001). Linear and concave upward types of survival curves are the most commonly observed survival curves of microbial cells during PEF process. The simplest approach assuming at sufficiently high electric field strengths, the microbial inactivation follows the kinetics of a unimolecular reaction (first order kinetics) and a semi-log plot of inactivation data against treatment time (or number of pulses) would yield a straight line. However, significant reduction of inactivation rate at a given electric field when treatment times are long, so-called the tailing effect, has been mentioned by several researchers in recent years (Peleg, 1995; Cole, 1999). The tailing behavior under less stressful treatments was reported to be more obvious, compared to highly stressful conditions in which microorganisms die close together. Depending on the microorganism and treatment conditions, the graphical representation of the logarithm of the number of survivors plotted against the constant electric field intensity varies (Heinz et al., 1999).

PEF technology has been under research for nearly 45 years and its effectiveness in reducing microbial populations has been widely documented (Barbosa-Cánovas et al., 1999). Even though the majority of PEF studies focus on the effectiveness of microbial inactivation studies, the kinetics of inactivation has not been fully explained. Application of PEF in food preservation requires a reliable model that accurately describes the microbial inactivation rate and the model should be able to establish appropriate treatment conditions that can achieve known levels of microbial inactivation, allowing the production of stable and safe foods. For this purpose, quantitative and kinetic data on the inactivation of spoilage and pathogenic microorganisms by PEF are needed. Moreover, besides measuring microbial response to PEF, consequently, unification of the criteria for evaluation of the effectiveness of process conditions are required (Peleg and Cole, 2002).

Obviously, among several different models employed to describe kinetic inactivation data of the PEF process, the model selection should be based on the advantages and limitations of each model. As usual, there exists a tradeoff between complexity and accuracy of models used. On one hand, simple models with fewer parameters (Bigelow) would give insightful estimations for the behavior of the survival curve; however, their use is limited with the intensity of the application. On the other hand, increasing the complexity of the models (Hülsheger, Peleg, Weibull) with more parameters would provide higher precision and flexibility. The mathematical models considered in this study were selected due to their common usage in PEF studies. The applicability of each model for the PEF treatment alone has been reported in literature; however, there is a certain need for in-depth studies to explore the effect of combination studies on microbial response (Rodrigo et al., 2002).

The objectives of this research were to apply, compare and validate predictive models (Bigelow, Hülsheger, Peleg and Weibull distribution) quantifying the influence of PEF with selected antimicrobials: potassium sorbate, sodium benzoate, hydrogen peroxide, and cinnamic acid, on the inactivation of *E.coli* (ATCC 11775). Gradual levels of electric field and treatment time were used to evaluate microbial response to PEF conditions. The concentration of antimicrobials (1000 ppm) was selected in view of their maximum regulatory permitted level in food products (Sofos, 1989) with a nonpathogenic surrogate, *E.coli* (ATCC 11775), that would not pose health hazard in the pilot plant area and whose behavior closely resembled that of the *E. coli* O157:H7 was used (Eblen et al., 2005).

1.1. Bigelow Model

Classically, thermal inactivation during sterilization processes is dynamically described in analogy of the microbial kinetics as a first order decay reaction of the microbial population with respect to time:

$$\frac{dS}{dt} = -kS \tag{1}$$

where S is the survival ratio (N_t/N_0) and k is the rate constant. The model proposed by Bigelow (1921) introduced the TDT (thermal death time) with decimal reduction time (D) and the model in the form of:

$$D_{value} = \frac{t}{\log N_0 - \log N_t} \quad (2)$$

$$\log(S) = -\frac{t}{D} \quad (3)$$

where *S* is the survivor fraction of microorganisms, *D* is the decimal reduction time and mathematically the negative inverse of the survival curve slope, *k,* and *t* is the treatment time. The magnitude of *k* (and hence of D) is a characteristic of the heat resistance of the organism. This approach has been used in defining D (decimal reduction time) and z (dependence of D on temperature) values for thermal treatment and has also been used to describe the linear sections of inactivation curves obtained from nonthermal treatments such as high pressure (Musa and Ramaswamy, 1997) and PEF (Sensoy et al. 1997, Castro et al., 1993).

1.2. Hülsheger Model

Among several approaches that have been proposed to overcome limitations of first order kinetics, Hülsheger et al., (1981) proposed the first mathematical model to describe inactivation of microbial cells by PEF. Hülsheger model assumes a linear relationship between the logarithm of survivor microorganisms and the logarithm of treatment time after a given electric field intensity. The advantage of Hülsheger model is to allow calculating a critical treatment time and critical electric field strength over which treatments start being lethal for the microorganisms with a constant inactivation rate. Since, the critical values are unique for each microorganism, they are useful to analyze the microbial behavior against

different treatment conditions, or combination of treatments. According to this model the inactivation is:

$$LnS = -b_E(E - Ec) \tag{4}$$

$$LnS = -b_t \ln\left(\frac{t}{t_c}\right) \tag{5}$$

where S is the survivor fraction, b_t and b_E are proportionality constants that are dependent on experimental conditions such as treated media and target microorganisms, t is the treatment time, t_c is the critical treatment time, E is the electric field intensity, and E_c is the critical electric field which can be empirically determined by extrapolating a survival curve towards zero inactivation or calculated theoretically. After a certain treatment time and electric field strength, E_c, t_c, could be considered constant and the survivor fraction is expressed as:

$$S(E,t) = \left(\frac{t}{t_c}\right)^{(-(E-E_c)/k)} \tag{6}$$

where E_c and t_c are the threshold values of the treatment time and electric field strength, respectively, and k is constant. The E_c intercept of the regression line that describes between the electric field strength and the natural logarithm of the survivor fraction and the abscissa that corresponds for the 100 % of survivors (Figure 2).

1.3. Peleg Model

Recent developments for modeling microbial inactivation are based on the fact that individuals in a population are not identical and their resistance to a lethal agent could be in various degrees (Cerf, 1977). If we consider the survival curve as a cumulative form of a temporal distribution of lethal events, each microbial cell belonging to the same strain and population is inactivated at a specific time. Therefore, the survival curve is a representation of the distribution of cell resistance (Rodrigo et al., 2001) and can be described by distribution functions (Peleg and Cole, 1998).

Peleg (1995) proposed a sigmoid function based on Fermi equation to describe the relationship between the percentage of survivors and the electric field strength for a given number of pulses. Fermi's function applied to PEF treatment is given by:

$$S(E,n) = \frac{100}{1+\exp\left[\frac{E-V_c(n)}{k(n)}\right]} \tag{7}$$

where S is the percentage of surviving microorganisms, E is the electric field strength, $V_c(n)$ is a critical level of E where the survivor level is 50 %, and $k(n)$ is a parameter related to the steepness of the curve around $V_c(n)$. The model suggests that there are two main regions of the survival curve: the negligible effect region where inactivation rate by electric fields is rather slow or negligible, followed by a region in which the number of survivors falls exponentially with a high inactivation rate (Figure 3). $V_c(n)$ represents the midpoint of this falling region while $k(n)$ indicates the inactivation rate in this region.

1.4. Weibull Distribution Model

Both Hülsheger and Peleg's models are able to describe the microbial inactivation by PEF; however, their potential use to model inactivation data is limited to few log cycles (Sensoy et al., 1997). In the case of higher levels of inactivation where intense PEF conditions or combination of stress factors are involved, these models might yield less accurate estimations. The mathematical model of the cumulative form of the Weibull distribution initially proposed by Peleg and Cole (1998), was modified by Mafart et al., (2002) and lastly in the form proposed by Van Boekel, (2002) gained popularity due to its simplicity and flexibility. This model has been successful to model microbial death by different inactivation methods for its ability to describe concave upward, concave downward and linear survival curves at the same time (Alvarez et al., 2006).

The use of the Weibull distribution function has been found effective in modeling the curvature of survival curves for *C. botulinum* spores during heat treatments (Peleg and Cole, 1998) and for microorganisms such as *S. typhimurium* or *L. monocytogenes* exposed to lethal agents like chlorine or potassium sorbate (Peleg and Penchina, 2000). The equation for estimating the survival fraction, based on Weibull distribution function, is given by:

$$S(t) = \exp\left[-\left(\frac{t}{n}\right)^{b}\right] \tag{8}$$

where S is the survivor fraction (N/N_o), b and n are constants that depend on the electric field intensity (E), and t is the treatment time. The Weibull model estimates the microbial

inactivation through two parameters unique to each microorganism: the scale parameter (b) and the shape parameter (n). The scale parameter, b, represents a characteristic time necessary to inactivate 0.434 log cycles of the population. The shape parameter, n, represents the form of the curve and accounts for upward concavity of a survival curve (n<1), a linear survival curve (n=1), and downward concavity (n>1) (van Boekel, 2002). Distribution models assume that, not all the cells would die at the same time, therefore values of inactivation time (t_c) would follow a probability density function in the form of:

$$t_c = n\Gamma(1 + b^{-1}) \tag{9}$$

Where Γ is the gamma function and t_c is the mean inactivation time for the process. Both n and mean t_c vary with the intensity of the electric field applied (Rodrigo et al., 2001).

2. Materials and Methods

Fresh apple cider inoculated with 10^7 CFU/ml of *E.coli* (ATCC 11775) was PEF treated in combination with 1000 ppm of selected antimicrobials (sodium benzoate, cinnamic acid, potassium sorbate, and hydrogen peroxide). Levels of PEF factors, electric field intensity (25, 30, 35 and 40 kV/cm) and treatment time (12.5, 30 and 50 microseconds) were used to follow the microbial response against gradual increase of stress factors. Four different inactivation models (Bigelow, Hülsheger, Peleg and Weibull) were used to fit the inactivation data obtained from four different antimicrobial treatments with PEF, as well as the PEF-only treatment (control).

Fresh apple cider was obtained from Tree Top Inc. (Selah, WA) and stored at -20°C until used in the experiments. Prior to experimentation, required amount of juice was thawed and diluted with water to obtain Brix° value of commercial apple juices (12.6°Brix). 1000 ppm of each antimicrobial, sodium benzoate (NaB), cinnamic acid (CA) (Sigma-Aldrich Inc., St. Louis, MO, 63103), potassium sorbate (KS) and hydrogen peroxide (HP) (Mallinckrodt Baker Inc., Phillipsburg, NJ) were added to the apple cider 30 minutes prior to PEF process.

2.1. Microbiology

Two milliliters of *E.coli* (ATCC 11775) was added to 100 ml nutrient broth (Difco, Kansas MO) for growth on a rotary platform shaker (MSB-3322A-I, GS Blue Electric, Blue Island, IL) at 30°C and 225 rpm until reaching the early stationary phase (27 hours). Optical density was evaluated at 25°C in an 8452 A diode array spectrophotometer (Hewlett-Packard, Palo Alto, CA) at 540 nm to determine the early stationary phase until the concentration of the microbial population has reached to 10^9 CFU/ml. 100 ml of the inoculum were transferred into 10 liters of apple cider to obtain an initial bacterial concentration of approximately 10^7 CFU/ml. Bacterial concentration was determined by the pour plate method. Serial decimal dilutions in sterile 0.1% peptone (Difco, Kansas, MO) water were plated on Eosin Methylene Blue (EMB) Agar. All samples were plated in duplicate and incubated at 35±0.5°C for 48 h in a controlled temperature chamber. After incubation, colonies were quantified following standard procedures (AOAC, 1995). Each study was conducted in triplicate using independent prepared batch of fresh bacteria for every replicate.

2.2. Pulsed Electric Fields Treatment

A pilot plant PEF system (Physics International, San Leandro, CA), consisting of a continuous concentric cylindrical treatment chamber with 0.6 cm gap and 25 ml effective treatment volume (Qin et al., 1995) was used for all treatments. 5, 12, and 20 exponentially decaying pulses, corresponding to 12.5, 30 and 50 microseconds, respectively, with peak electric field intensities of 25, 30, 35, 40 kV/cm and 2.5±0.1 μs pulse width were delivered to the treatment chamber by discharging a 0.5 μF capacitor. The pulse width approximated by the electrical circuit's time constant, time taken for the peak voltage to decay exponentially to 37% (i.e. 1/e) of its maximum value, and the treatment time, *t* was calculated by:

$$t = \tau * n \tag{10}$$

where τ is the effective time constant measured as 37% of the maximum peak voltage, and *n* is the number of pulses applied (Góngora-Nieto et al., 2004). Chilled apple cider (4ºC) was pumped at a flow rate of 72 l/h and the outlet temperature did not exceed 60ºC. Inlet and outlet temperatures, pulse shape, voltage, current, and energy flow were monitored throughout the process. Electrical parameters were monitored with a digital oscilloscope (Hewlett-Packard 54530A, Colorado Springs, CO) connected to the treatment chamber through high voltage probes. The average electric field intensity was calculated by:

$$E_{CO} = \frac{V}{r \ln\left(\frac{R_1}{R_2}\right)} \tag{11}$$

where E_{CO} (kV/cm) is the electric field strength for coaxial electrodes, V (kV) is the average peak voltage, r (cm) is the radius at which point electric field is measured, and R_1 and R_2 are the radii of inner and outer electrodes (Barbosa-Cánovas et al., 1999).

2.3. Statistical Analysis

The accuracy factor (A_f) suggested by Ross (1996) was used to evaluate the precision of the analysis and to compare the accuracy of estimation by each model. The A_f is defined as:

$$Af = 10^{\frac{\Sigma\left|\log\left(\frac{pred}{obs}\right)\right|}{n}} \tag{12}$$

where A_f is the accuracy factor, *pred* is the predicted value, *obs* is the observed value, and n is the number of observations. The ratio of predicted to observed means the relation between the survivor fraction predicted by the model and the one obtained experimentally. The A_f value is equal to 1 when there is perfect agreement between predicted and observed values. The model parameters were computed by using the least squares criterion of the Solver function in the Excel 5.0 package (Microsoft, Seattle). A one-way ANOVA was conducted using the statistical analysis software (SAS Institute, Cary, NC) and least significant difference (LSD) multiple comparison test ($P<0.05$) was used to determine the significant difference between treatment conditions.

3. Results and Discussion

Survival curves corresponding to the inactivation of *E.coli* (ATCC 11775) by PEF (40, 35, 30, 25 kV/cm for 12.5, 30 and 50 μs) and selected antimicrobials in apple cider are shown in Figure 4(a-e). The inactivation of *E.coli* was enhanced as the electric field strength and treatment time increased for all antimicrobials tested. Inactivation of *E.coli* during PEF in the presence of cinnamic acid resulted in a completely concave upward survival curve where the survival curves for other antimicrobials tested are mostly concave downward at higher electric fields and almost linear at lower electric fields. The observed difference between the shapes of the survival curves after PEF treatment in the presence of different antimicrobials could be due to different mechanism of inactivation achieved by selected antimicrobials during PEF.

Clearly, among all antimicrobials tested, the presence of cinnamic acid in combination with electric fields significantly influenced the shape of the survival curve. The possible mechanism explaining the effect of cinnamic acid on inactivation curve could be based on the mechanism of action possessed by this compound during PEF. Cinnamic acid is a naturally occurring phenolic antimicrobial consisting of an aromatic ring with hydroxyl groups in its structure. Different from other antimicrobials tested in this study whose lethal effects were mostly related to their intracellular activities, the mechanism of phenolic compounds for inactivating microorganisms is generally attributed to their effects on cellular membranes (Davidson, 1993). Their interference with the cell membrane permeability causes leakage of cellular components leading to cell death (Eklund, 1985). This proposed mechanism of action for cinnamic acid suggests that the effect of cinnamic acid on bacterial

membranes combined with the effect of PEF on the permeability of cell membranes provided the most efficient inactivation compared to the other antimicrobials tested even at the beginning of PEF process while extent of antimicrobial activity was not significantly affected by the strength of electric field, treatment time or temperature.

The undissociated form of the potassium sorbate and sodium benzoate are primarily responsible for their antimicrobial activity (Davidson, 1993) and in their dissociated form, they penetrate the cell membrane. Once inside the cell, the acid dissociates, because the cell interior has a higher pH than the exterior. Protons generated from intracellular dissociation of the organic acid acidify the cytoplasm and extruded to the exterior by consuming energy, which will eventually deplete the cellular energy (Hunter and Segel, 1973). Based on this mechanism of action proposed for potassium sorbate and sodium benzoate, it is no surprise that the rate of inactivation sharply increases for longer treatment times. Based on the temperature development within the system subjected to longer PEF treatment times, the phospholipid molecules in cell membranes undergo temperature related phase transition, changing from a packed gel-like structure to a looser liquid-crystalline, reducing the thickness and mechanical resistance of cell membranes (Stanley, 1991). Hence, the influence of electric fields on permeability of cell membranes facilitates the entry of antimicrobials into the microbial cell where they dissociate and contribute to microbial inactivation (Hunter and Segel, 1973).

The influence of different stress levels of PEF on microbial resistance has been quantified by using several models in literature. Studies exploring the effect of antimicrobials on microbial

response have been limited to certain antimicrobials such as nisin. In this study, the influence of PEF in combination with selected antimicrobials on shape of the survival curves was quantified by analyzing the data with both traditional (Bigelow, Hülsheger) and the evolving (Peleg, Weibull) mathematical models in order to compare the effectiveness of treatment conditions.

3.1. Bigelow Model

Decimal reduction times as a function of electric field strength with first order kinetic constants for *E.coli* (ATCC 11775) are shown in Table 1. For the highest electric field strength achieved (40 kV/cm), D value for the PEF-only control group (10.59 µs) was significantly different from all combination treatments for which D values did not significantly differ from each other (7.82-7.58 µs). However for the lowest electric field strength achieved (25 kV/cm), D value for cinnamic acid combined PEF was 9.72 µs (R^2>0.75) where the value for the remaining treatments were similar to each other.

As the survival curves for all antimicrobials except cinnamic acid indicate that, below a certain electric field strength and treatment time, PEF scarcely affected viability of *E. coli,* indicating that there exists a limit for electric field intensity and treatment time to be defined as critical. Beyond this critical electric field strength or treatment time the inactivation curves observed to be steeper with higher rate of inactivation. Upward concavity was observed in the survival curves for sodium benzoate, potassium sorbate and hydrogen peroxide below critical PEF conditions indicating that during the PEF process the less resistant members of the bacterial population were inactivated faster where the remaining cells are more resistant

to the treatment and inactivated with a slower rate. However beyond critical electric field or treatment time the shape of the curves turned to downward concavity indicating that the lethal effect of the PEF process increased so did the rate of destruction with respect to time.

Results indicate that the traditional concept of D and z values, established for sterilization processes, might be insufficient for validating nonthermal processes when combined methods are involved under intense PEF treatment conditions (higher electric field strength and/or longer treatment time). Bigelow model enables calculation of the inactivation rate by using first order linear kinetics where the estimated decimal reduction time (-2.303/k) is the average slope of data points. Therefore, the possible presence of the upward or downward concavity in the survival curve could not be taken into account and counted as the limitation for this model. For being the simplest model employed among others, based on the values of decimal reduction time calculated by the Bigelow model, a quick comparison of the effectiveness of selected antimicrobials in combination with PEF is possible where the treatment conditions are not extreme (25-30 kV/cm) and the survival curves are close to linear. According to this, D value for all antimicrobials tested (7.20-7.94 µs) were significantly less than the D value for PEF only treatment (10.59 µs) for 40 kV/cm electric field strength. Cinnamic acid treatment in combination with PEF provided the most efficient combination with shortest thermal death time (7.94-9.60 µs) for 40-25 kV/cm, with accuracy factors (1.76-1.84) and the lowest regression coefficient (R^2>0.75) indicating the need for more accurate mathematical models.

3.2. Hülsheger Model

The natural log of the survival fraction as a function of electric field intensity (E) and critical electrical field (E_c) through a proportionality constant (b_E) is calculated for each treatment time. Theoretically derived values of model parameters together with their accuracy factors (A_f) are given in Table 2 and empirical values of E_c extrapolated from observed data are given in Figure 5.

In general, common for all antimicrobials and the control treatment, an increase in treatment time (number of pulses) reduced the value of critical electric field strength (E_c) and increased the proportionality constant (b_E). This result indicated that with the use of longer treatment times, it is possible to reduce the value of threshold electric field intensity beyond which the treatment is significantly lethal. Similarly, the increasing trend for the proportionality constant (b_E) with respect to treatment time pointed the increase in inactivation rate beyond critical electric field as the proportionality constant (b_E) represents the steepness of the inactivation curve around E_c.

Theoretical values of E_c were significantly same (~20 kV/cm) for selected antimicrobials regardless of the treatment time. The value of b_E at the highest treatment time (50 µs) was unique to each antimicrobial where potassium sorbate (11.19) and hydrogen peroxide (10.54) provided the highest inactivation rate. Among all antimicrobials tested, the model was not suitable to describe the experimental data for cinnamic acid probably due to the different mechanism of action possessed by this compound during PEF. The extreme concavity of the

inactivation curve avoided the implementation of this model for cinnamic acid as the extrapolation of the curve pointed negative values for critical electric field strength.

The assumption of Hülsheger model for linearity between the log of the survivor fraction and the electric field strength above a certain critical level (E_c) failed to describe survival curves with concavity. Based on the accuracy factors calculated for each treatment time and electric field combination applied, the accuracy factor deviated from 1 especially when extreme PEF conditions were involved resulting more than 3-4 log cycles inactivation. The failure of the model in the presence of antimicrobials and extreme PEF conditions could be explained by the contribution of other stress effects to the effect of PEF for inactivation. As in the case of antimicrobial combination with PEF, when more than one mechanism of inactivation is involved, the deviation of accuracy factor from 1 is higher (2.23-4.67) compared to PEF only control treatment (1.94). This observation suggests that not all the individuals of bacterial population have the same resistance and lethal effects involved (antimicrobials, electric fields, heat) takes up different targets in the population during the process. Hence, for prolonged treatment times where the effect of thermal component assists antimicrobial activity of sodium benzoate, potassium sorbate and hydrogen peroxide together with the effect of electric fields could not be predicted by Hülsheger model accurately. Similarly, the data obtained for cinnamic acid treatment where the antimicrobial action on cellular membranes coupled with electric fields begin right after the addition of the compound could not be predicted by this model for the same reason.

Compared to the Bigelow model, Hülsheger model was preferable for eliminating the non-linear region at the beginning of the PEF process and taking into account the relatively linear part of the survival curve. Indeed, when there exists only one mechanism of inactivation as in the case of PEF only treatment, Hülsheger model was reported to be accurate describing the survival curves (Zhang et al., 1994b; Martin-Belloso et al., 1997; Sensoy et al, 1997). Therefore, even though the model failed to predict the survival curves in the presence of combined stress factors; for the sake of simplicity, the estimated values of critical electric field and inactivation rate (steepness) provided by this model are both very valuable design tools to predict and compare the lethality of PEF treatment regardless of the conditions.

Both Hülsheger and Bigelow models are based on the mechanistic theory with an assumption of identical resistance to a lethal agent in a microbial population. However the observed survival curves in this study and their varying concavity suggests that, vitalistic models assuming the existence of a distribution of microbial sensitivity to the inactivation process would give better accuracy (Stewart et al., 2002).

3.3. Peleg Model Based on Fermi's Distribution

The applicability of Peleg's model to the survival curves of each antimicrobial and PEF-only treatment are shown in Figure 6. The parameters of Fermi's function are listed in Table 3 along with their accuracy factors. In this table, V_c indicates a level of electric field where survival fraction is 50%, and the parameter k refers to the steepness of the survival curve around V_c. Statistical criteria indicated that the fit was highly satisfactory (R^2>0.98) for selected antimicrobials except the one for cinnamic acid (R^2>0.80).

It was clearly noticeable, and quantifiable in terms of the model's constants that lethality progressively increased as a result of increasing number of pulses. This was primarily expressed in lower critical electrical field intensity, V_c (drop from 25.53 to 19.93 for PEF-only, 23 to 20 for sodium benzoate and 20 to 8.09 for cinnamic acid). The same effect was observed in the decay rate as expressed by the magnitude of k, that it dropped from 8.71 to 3.12 for PEF-only and 8.63 to 2.12 for sodium benzoate and 3.94 to 3.50 for cinnamic acid. This behavior suggests that the difference in V_c values for 12.5, 30 and 50 microseconds might not be significant but inactivation proceeds significantly faster at a higher number of pulses (longer treatment time) as indicated by the parameter k. Inactivation was complete at longer treatment times, so data adjusted well to the tail of the function. In other words, treatment time of the process possessed stronger impact on microbial inactivation compared to the electric field strength. Moreover, the resistance of the population against treatment time was unique for each antimicrobial where the value of critical electric field at which fifty percent of the microbial population was inactivated did not significantly varied among treatments.

The sigmoidal shape of the survival curves proposed by Peleg's model suggests that the system's resistance to the lethal stress is changing throughout the process as indicated by the change of curvature in survival curves. The inactivation kinetics observed in the presence of antimicrobials followed approximately the same pattern except the one for cinnamic acid. For sodium benzoate, potassium sorbate and hydrogen peroxide, at lower electric fields (25-30 kV/cm), slight upward concavity of the survival curves turned to slight downward concavity throughout the process with a steeper incline at higher electric fields (35-40

kV/cm). This result indicated that the lethal effect in the presence of these antimicrobials seemed to be enhanced by subsequent increase in PEF intensity in terms of treatment time due to the lowered functionality of microbial cell membranes under intense PEF and increased treatment temperature.

In the case of cinnamic acid, the extreme upward concavity of the survival curves regardless of the electric field strength and treatment time suggests that the individuals of the microbial population with gradual resistance were exposed to the lethal effect of cinnamic acid coupled with the effect of electric fields at the beginning of the treatment. The majority of the population was inactivated as represented by a sharp decrease in the curve without the need for reaching a critical electric field strength or treatment time. The more resistant members of the population were gradually inactivated with a slower pace throughout the process as the treatment time increased.

The accuracy factors derived for the majority of the antimicrobials tested in this study assured the applicability of the model (1.18-2.5) except cinnamic acid (2.15-2.86); however, a more flexible model that is able to predict the microbial response against all selected antimicrobials would be preferable.

3.4. Weibull Distribution

Parameters of Weibull distribution for *E.coli* (ATCC 11775) are given in Table 4 and Figure 7 shows the survival curves of predicted and observed data of all treatments. For the PEF-only treatment, the shape parameter (n) was close to 1 at higher electric field intensities

representing almost linear behavior (0.96) and the values for cinnamic acid were significantly below 1 at all PEF conditions (0.43-0.65). Shape parameter (n) for hydrogen peroxide (0.60-0.87) was slightly below 1 where sodium benzoate and potassium sorbate were not significantly different from each other.

The values for the shape parameter (n) derived for combination treatments indicated that at higher electric field fields (35-40 kV/cm), where survival curves covered more than 3 log cycles, upward concavity ($n<1$) of the curves could be due to the presence of gradual resistance of individuals in the population. Even though a pure culture of microbial population was used in the study, there might be morphological differences based on the size and the shape of the cells as well as the differences in cell membrane composition within the microbial population could lead to variation of microbial resistance against stress factors. Moreover, the presence of more than one stress factors, being dominant at different stages of the whole treatment could lead to the upward concavity of the survival curve. It has also been suggested that the variation of response within the microbial population might result upward concavity of the survival curve due to local variation of the electric field strength in the treatment chamber (Heinz et al., 2001). Hence, for a cylindrical co-axial treatment chamber, the intensity of the electric field varies with respect to the radial distance between electrodes. Therefore, the lethal effect exerted by the electric fields on microbial population might slightly change according to the location inside the treatment chamber.

The scale parameter (b) of Weibull distribution can be considered as a rate constant representing the scale of the survival curve in the form of cumulative distribution of

microbial inactivation. For a given shape parameter (n), an increase in the value of scale parameter (*b*) indicates that microbial response against the stress factor is widely distributed among the individuals of the population. For this case, the probability density function for microbial resistance is stretched out. On the opposite, a relatively small value of the scale parameter (*b)* indicates that the majority of the population was inactivated at a certain treatment time. This treatment time could be determined by calculating the mean of the distribution function. The scale parameter (*b*), for each antimicrobial tested in this study significantly decreased when the electric field strength increased suggesting that the inactivation was strongly enhanced beyond critical electric field intensity (25 kV/cm). Similar to the results obtained from other models employed, the smallest scale constant was for the cinnamic acid (0.052-0.95) and hydrogen peroxide (1.96-7.17) while scale parameters for potassium sorbate were the highest among all (8.53-13.62). Calculated scale parameters for PEF-only group (7.87-3.98) within a range of electric field intensity (25-40 kV/cm) were also in accordance with literature values where Van Boekel (2002) reported scale parameters for *E.coli* in a range of 22-25 kV/cm as 7.6-4.9 and shape parameters (*n*) for the same range as 0.488-0.483 with R^2>0.97. Advantageous to other models employed in this study, Weibull distribution provided accuracy factors closely approximating to 1 for control (1.09), cinnamic acid (1.16), sodium benzoate (1.05), potassium sorbate (1.21) and hydrogen peroxide (1.06). Similarly, correlation coefficients generally found to be higher suggesting the adequacy of the Weibull distribution function to estimate microbial inactivation for intense or combined PEF factors.

This study aimed to explore the possible advantages and limitations of four mathematical models to predict microbial response against combined effect of antimicrobials of different origin under gradual levels of PEF factors. Among simple models Hülsheger model provided valuable design tools to be used as an insightful and quick comparison for the influence of different antimicrobials on the survival curve with less accuracy. Peleg model, on the other hand, provided better fit for three of the antimicrobials tested. However, for a detailed and complete observation of the microbial response during PEF process in the presence of different antimicrobials, the use of more flexible and accurate models is essentially required. Therefore, the use of Weibull distribution is highly recommended for combination studies where more than one stress factor is involved.

4. Conclusions

The applicability of four commonly used mathematical models (Bigelow, Hülsheger, Peleg and Weibull) to predict microbial inactivation by PEF and selected antimicrobials was tested and compared based on the accuracy factors of models. The Weibull survival function provided the best model to describe the inactivation of *E.coli* (ATCC 11775) by PEF treatment in the presence of selected antimicrobials as verified by accuracy factors closely approximating 1. Inactivation of *E.coli* during PEF in the presence of cinnamic acid resulted in a completely concave upward survival curve where the survival curves for other antimicrobials tested were generally concave downward at higher electric fields (35-40 kV/cm) and almost linear at lower electric fields (25-30 kV/cm). The observed differences between the shapes of the survival curves were due to different mechanism of inactivation achieved by selected antimicrobials during PEF. Being the simplest model used, Bigelow

model was insufficient to describe antimicrobial synergism to PEF. Hülsheger model was also unable to predict the microbial response; however, provided critical electrical fields and treatment times for insightful and quick comparison of processes with less accuracy (2.23-4.76) compared to Peleg and Weibull models. Among two-parameter models, Peleg's model based on Fermi's distribution successfully predicted the microbial response against combined stress factors with accuracy factors (1.18-2.5); however, was limited to certain antimicrobials. The ability of Peleg's model to describe concavities of the survival curves suggested that the system's resistance to stress factors is changing throughout the process and a more flexible model with more fit parameters is required. The use of Weibull distribution model with three parameters enabled the observation of varying microbial response against selected antimicrobials under intense PEF conditions (35-40 kV/cm and 50 microseconds). Weibull distribution model was highly recommended for being the most flexible and accurate model tested in this study.

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Table 1. Decimal reduction time (D_{value}=*-2.303/k*) for *E. coli* (ATCC 11775) in apple cider after exposure to PEF (50 µs) and selected antimicrobials.

(k: inactivation rate, A_f: accuracy factor)

	Charging Voltage (V)	Electric field (kV/cm)	D_{value} (µs)	k (μs^{-1})	A_f
PEF-only	20	25	38.38	0.06	1.39
	25	30	15.35	0.15	1.15
	30	35	10.63	0.22	1.13
	35	40	10.47	0.22	1.22
Cinnamic acid	20	25	9.60	0.24	1.76
	25	30	9.21	0.25	1.80
	30	35	7.68	0.30	1.68
	35	40	7.94	0.29	1.84
Sodium benzoate	20	25	38.38	0.06	1.58
	25	30	12.12	0.19	1.08
	30	35	8.86	0.26	1.07
	35	40	7.68	0.30	1.13
Potassium sorbate	20	25	32.90	0.07	1.17
	25	30	12.79	0.18	1.11
	30	35	7.20	0.32	1.32
	35	40	7.43	0.31	1.29
Hydrogen peroxide	20	25	38.38	0.06	1.23
	25	30	19.19	0.12	1.28
	30	35	7.20	0.32	1.15
	35	40	7.20	0.32	1.21

Table 2. Constants for the model proposed by Hülsheger for inactivation of *E.coli* (ATCC 11775) in apple cider after exposure to PEF and selected antimicrobials.

(E_c: critical electric field intensity, b_F: model constant, A_f: accuracy factor)

	Treatment time (μs)	E_c (kV/cm)	b_E	A_f
PEF-only	50.0	19.29	6.56	1.94
	30.0	19.02	3.59	1.10
	12.5	20.33	2.44	1.18
Sodium benzoate	50.0	16.39	7.28	2.23
	30.0	19.26	5.18	1.35
	12.5	20.00	2.24	1.62
Potassium sorbate	50.0	20.82	11.19	2.70
	30.0	20.95	4.86	1.37
	12.5	25.06	2.33	1.30
Hydrogen peroxide	50.0	20.85	10.54	4.67
	30.0	20.79	6.11	1.77
	12.5	23.87	4.85	1.88
Cinnamic acid		N/A*		

***** *Inactivation data was unable to be described by this mathematical model due to extreme upward concavity of the survival curve for cinnamic acid. .*

Table 3. Constants for the model proposed by Peleg based on Fermi's distribution for inactivation of *E.coli* (ATCC 11775) in apple cider after exposure to PEF and selected antimicrobials.

(V_c: Model constant for critical electric field, k: model constant, A_f: accuracy factor)

	Treatment time(μs)	V_c (kV/cm)	k	A_f
PEF-only	50.0	19.93	3.12	1.51
	30.0	21.12	4.98	1.42
	12.5	25.53	8.71	1.06
Cinnamic acid	50.0	8.09	3.14	2.15
	30.0	12.81	3.50	2.80
	12.5	20.00	3.94	2.86
Sodium benzoate	50.0	20.91	2.12	2.32
	30.0	21.06	3.97	1.33
	12.5	23.70	8.63	1.07
Potassium sorbate	50.0	21.10	2.18	1.73
	30.0	24.79	4.53	1.24
	12.5	28.55	6.67	1.03
Hydrogen peroxide	50.0	19.57	3.80	2.50
	30.0	22.21	4.25	1.20
	12.5	26.10	5.28	1.18

Table 4. Constants for the Weibull distribution for inactivation of *E.coli* (ATCC 11775) in apple cider after exposure to PEF and selected antimicrobials.

(E_f: electric field intensity, b: scale parameter, n: shape parameter, A_f: accuracy factor)

	Charging voltage (kV)	E_f (kV/cm)	b	n	A_f
PEF-only	35	40	3.98	0.96	1.09
	30	35	4.00	0.96	1.06
	25	30	4.01	0.79	1.04
	20	25	7.87	0.67	1.01
Cinnamic acid	35	40	0.052	0.43	1.05
	30	35	0.096	0.44	1.05
	25	30	0.27	0.50	1.10
	20	25	0.95	0.65	1.16
Sodium benzoate	35	40	3.00	0.97	1.05
	30	35	4.03	1.03	1.04
	25	30	4.99	0.98	1.04
	20	25	5.53	0.59	1.01
Potassium sorbate	35	40	8.53	1.58	1.10
	30	35	8.98	1.63	1.16
	25	30	10.47	1.42	1.10
	20	25	13.62	0.95	1.08
Hydrogen peroxide	35	40	1.96	0.87	1.04
	30	35	2.18	0.90	1.06
	25	30	4.30	0.74	1.03
	20	25	7.17	0.60	1.06

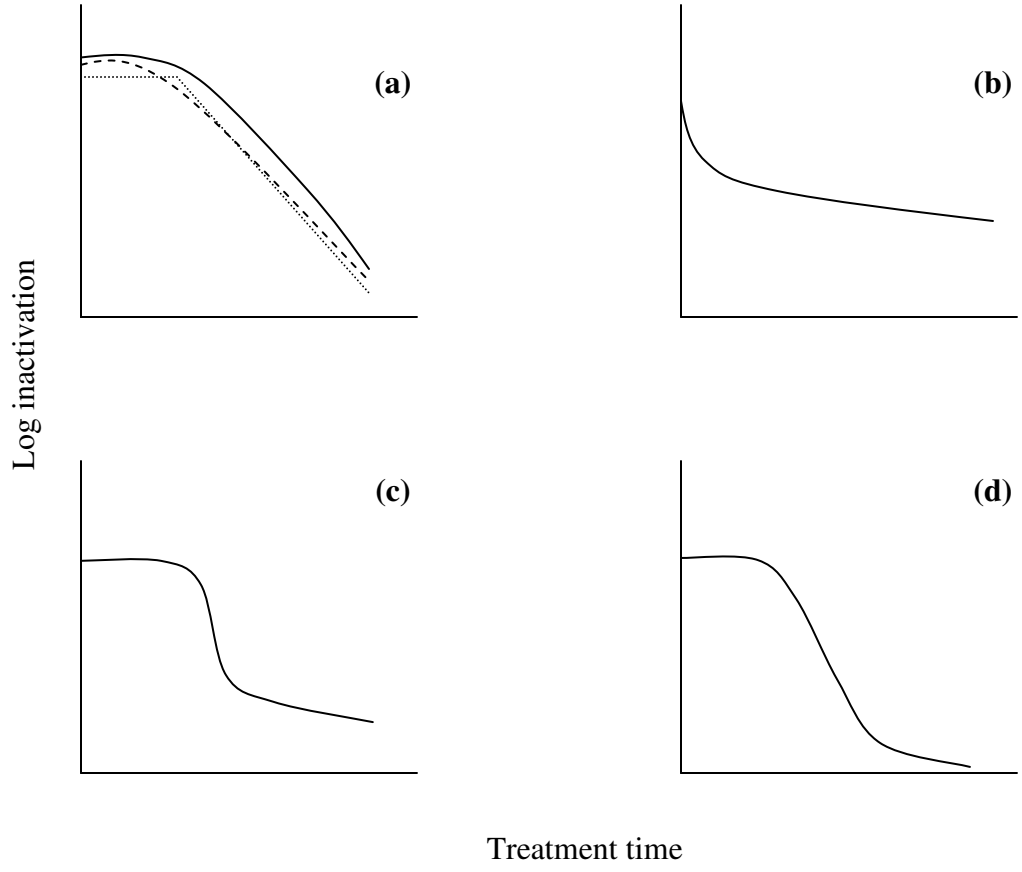


Figure 1. Examples of thermal death curves (McKellar and Lu, 2000): (a) lag or shoulder, with either linear (dotted line), power law where p>1 (broken line), or monophasic logistic (solid line) models; (b) concave with power law where p<1; (c) biphasic logistic; and (d) sigmoidal.

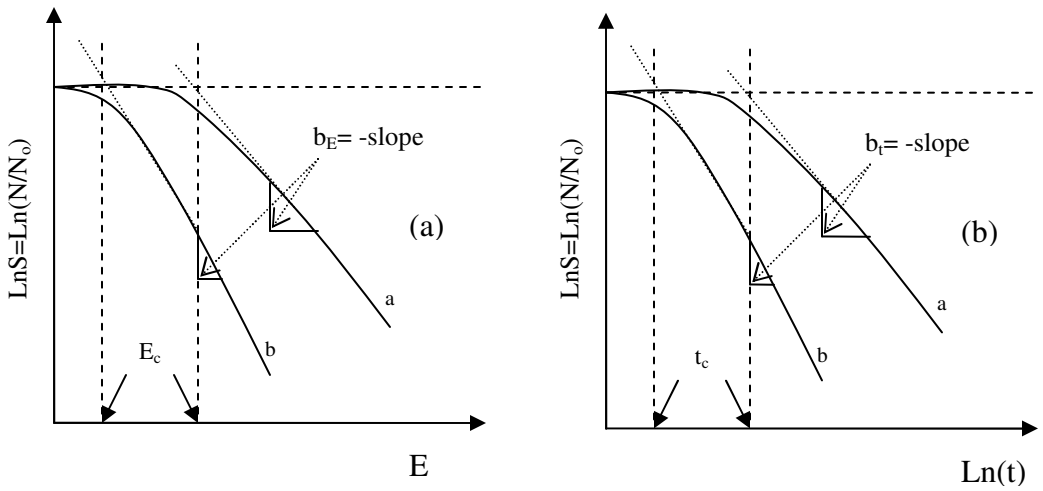


Figure 2. Dependence of microbial survival fraction on the: (a) electric field, and (b) treatment time. Curves, a, correspond to resistant microorganisms and curves, b, to sensitive microorganisms S, survival fraction; N, microbial count; E, electric field; b, kinetic constant; t, time. Subscripts: 0, initial; c, critical; t, time; E, electric field (Hülsheger et al., 1981).

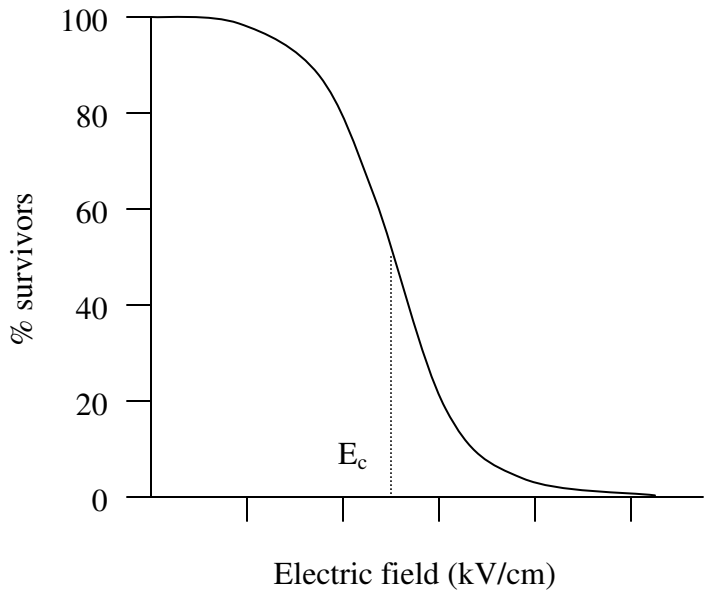


Figure 3. Peleg model based on Fermi's distribution (*E_c corresponds to 50 % inactivation*) (Alvarez et al., 2006).

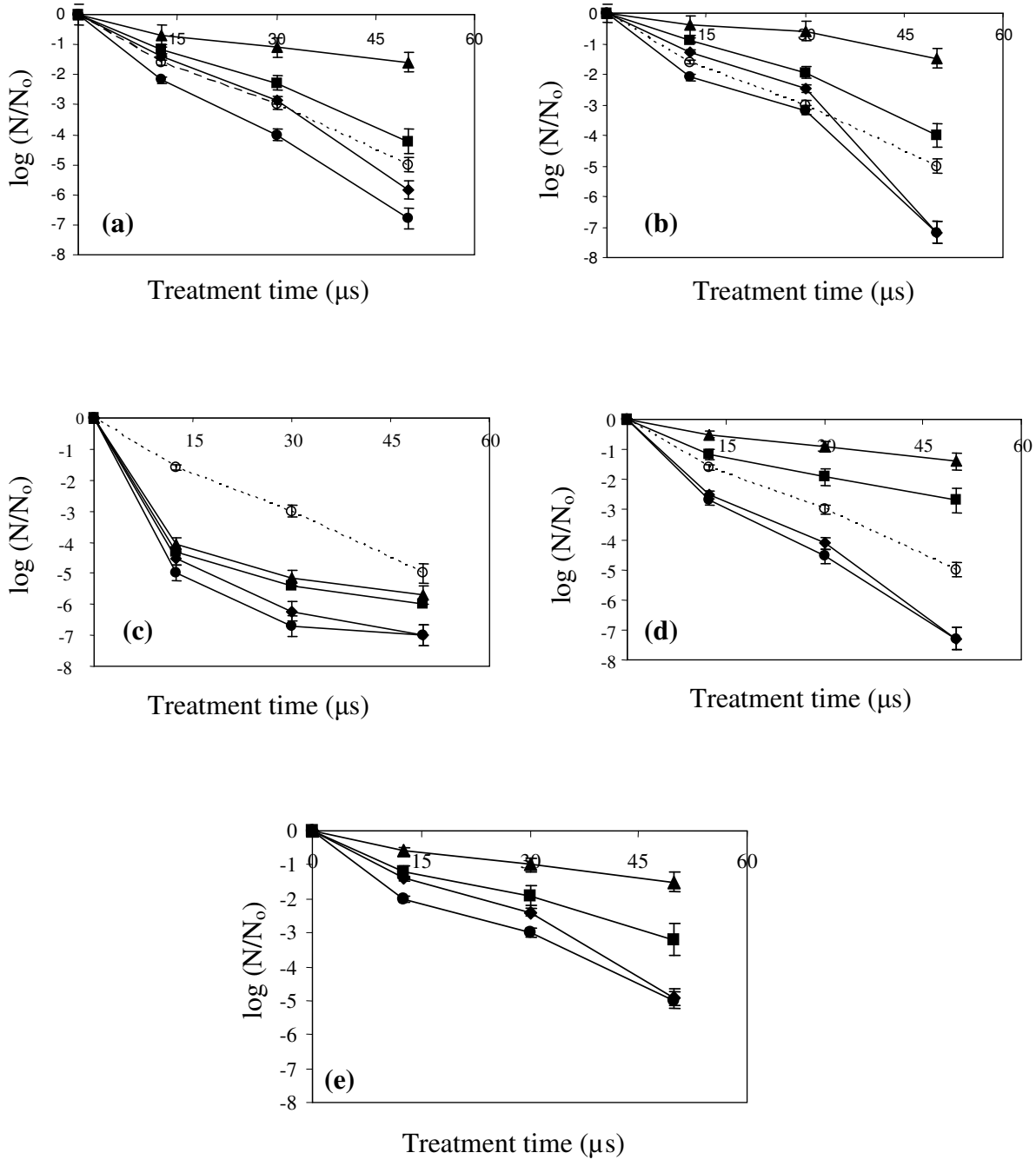


Figure 4. Survival of *E.coli* (ATCC 11775) in apple cider after PEF (40, 35, 30 and 25 kV/cm for 12.5, 30 and 50µs) and 1000 ppm of the selected antimicrobials. (●) 40 kV/cm; (♦) 35 kV/cm; (■) 30 kV/cm; (▲) 25 kV/cm and (a) sodium benzoate; (b) potassium sorbate; (c) cinnamic acid; (d) hydrogen peroxide; (e) PEF-only.

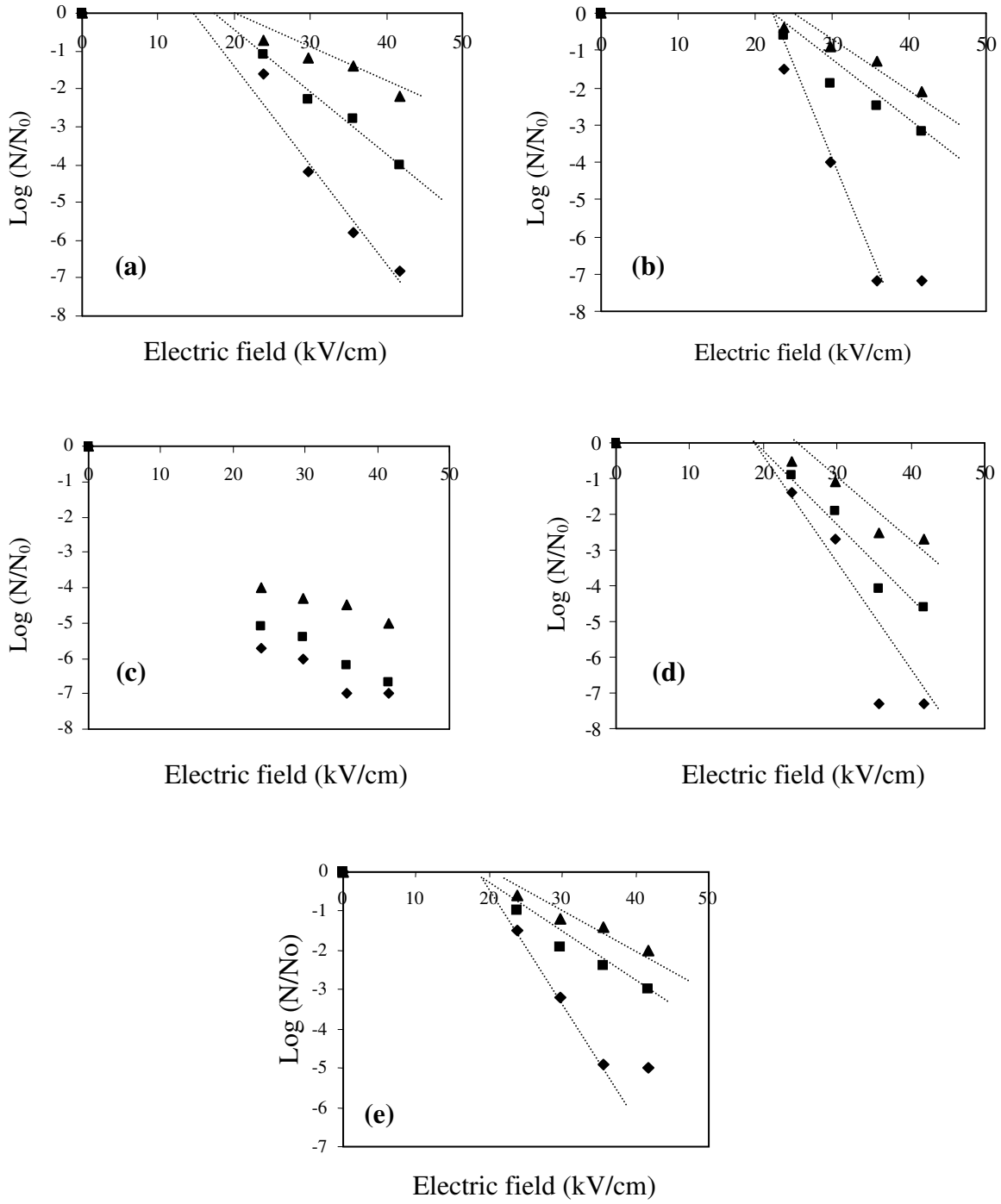
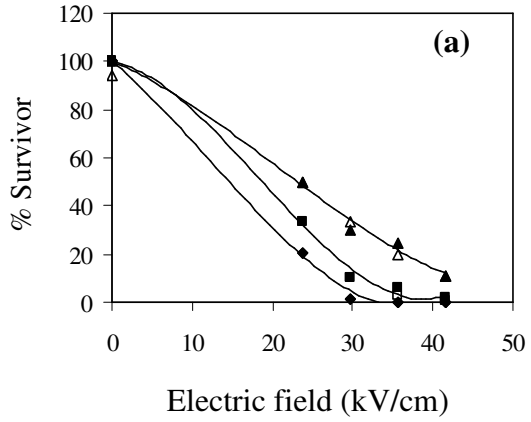
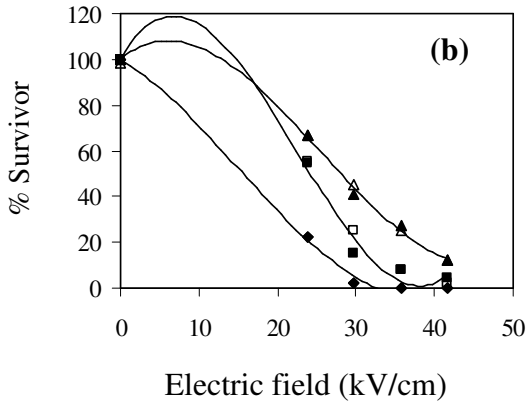
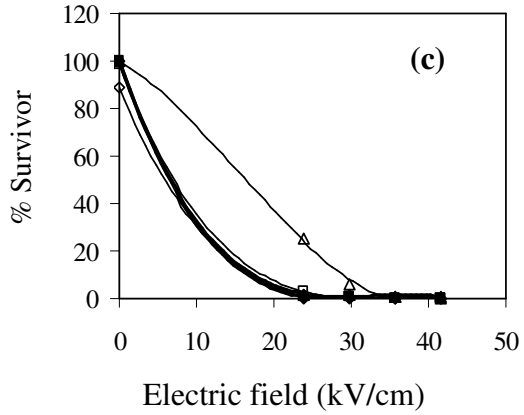
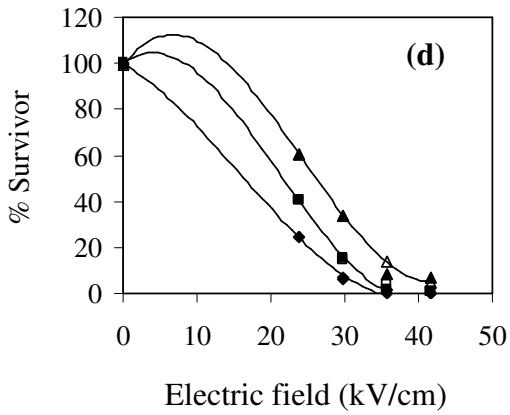


Figure 5. Hülsheger model for (♦) 50 µs; (■) 30 µs; (▲) 12.5 µs (a) sodium benzoate; (b) potassium sorbate; (c) cinnamic acid; (d) hydrogen peroxide; (e) PEF-only.


(a)
% Survivor
120
100
80
60
40
20
0
0
10
20
30
40
50
Electric field (kV/cm)


(b)
% Survivor
120
100
80
60
40
20
0
0
10
20
30
40
50
Electric field (kV/cm)


(c)
% Survivor
120
100
80
60
40
20
0
0
10
20
30
40
50
Electric field (kV/cm)


(d)
% Survivor
120
100
80
60
40
20
0
0
10
20
30
40
50
Electric field (kV/cm)

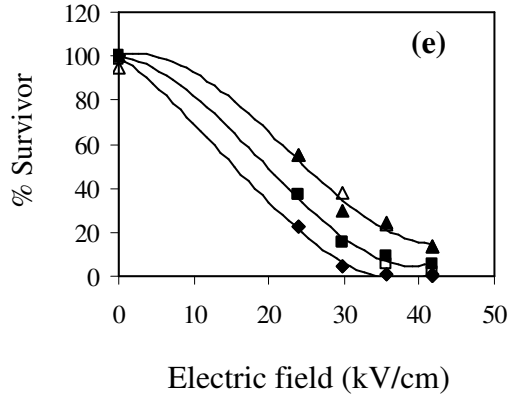

(e)
% Survivor
120
100
80
60
40
20
0
0
10
20
30
40
50
Electric field (kV/cm)

Figure 6. Survivor fraction versus electric field to fit Peleg's model based on Fermi's distribution for observed: (♦) 50 µs; (■) 30 µs; (▲) 12.5 µs; and predicted: (◊) 50 µs; (□) 30 µs; (Δ) 12.5 µs values. (a) Sodium benzoate (b) potassium sorbate; (c) cinnamic acid; (d) hydrogen peroxide; (e) PEF-only.

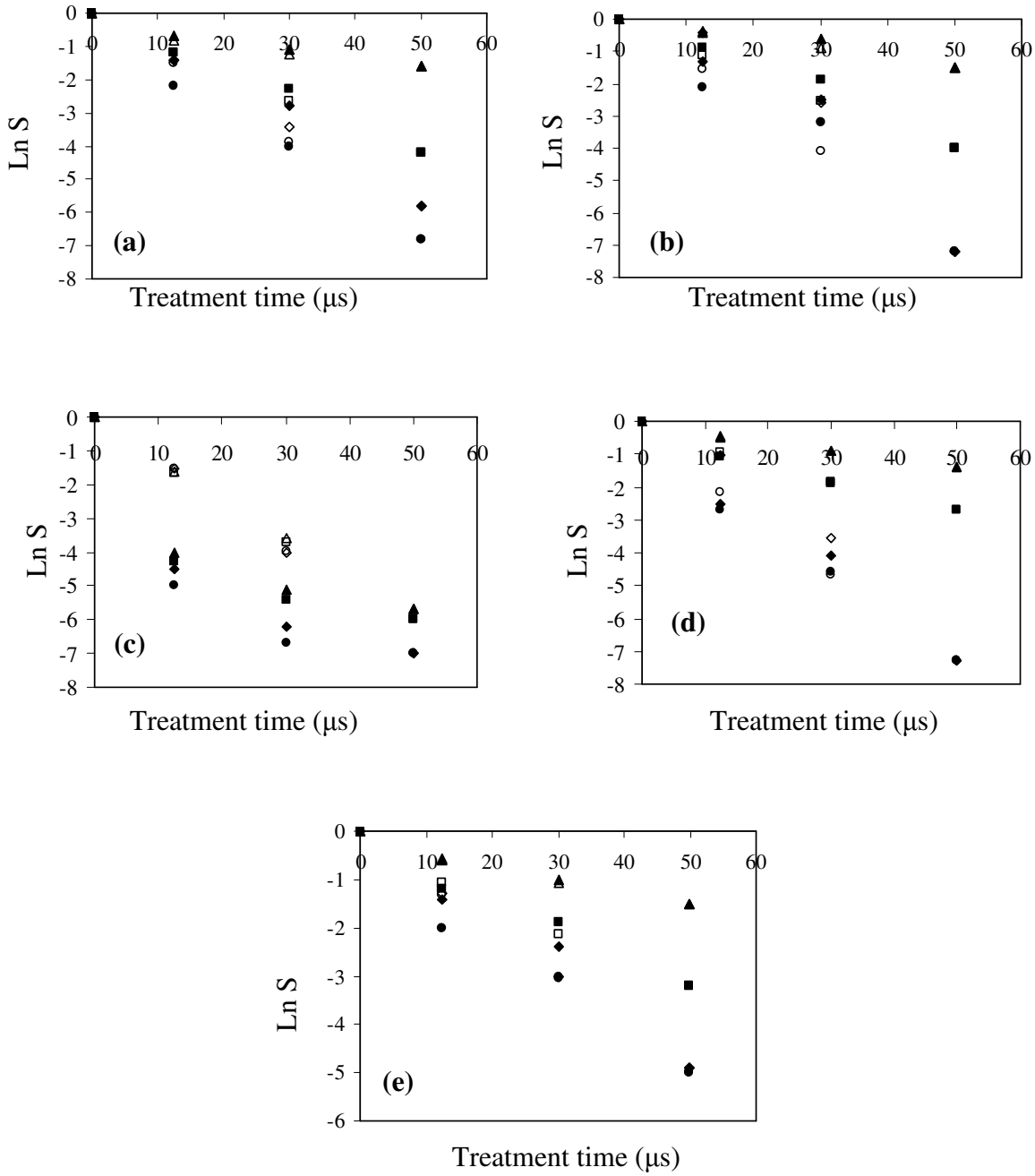


Figure 7. Weibull distribution for PEF and 1000 ppm of the selected antimicrobials :(a) sodium benzoate; (b)potassium sorbate; (c)cinnamic acid; (d)hydrogen peroxide;(e)PEF-only observed: (●) 40 kV/cm; (♦) 35 kV/cm; (■) 30 kV/cm; (▲) 25 kV/cm; predicted: (○) 40 kV/cm; (◊) 35 kV/cm; (□) 30 kV/cm; (Δ) 25 kV/cm.

CHAPTER FIVE

Engineering Aspects of Pulsed Electric Fields

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Abstract

Successful utilization of PEF technology requires good understanding of engineering aspects since one of the most important drawbacks of this emerging nonthermal technology is the lack of studies exploring electrical components, specific energy, temperature rise and flow characteristics of the treated food. This research explored the impact of electrical properties as a function of temperature and concentration, the effect of energy density and fluid flow rate on microbial inactivation and the deviations from ideality of the PEF system as a function of charging voltage and number of pulses (treatment time) by comparing the actual and theoretical energy densities in a PEF system. The effect of conductivity on PEF system performance showed that the temperature dependency of buffer systems was higher in concentrated solutions. As the flow rate of apple cider was reduced, the energy density increased, achieving 5 log cycle reductions with the use of 0.8 L/min flow. Increasing pulse frequency and voltage both resulted in temperate rise; however, the impact of pulse frequency was stronger than the voltage impact, especially at high voltages (30-35 kV). The ratio of voltage in the treatment chamber to the charging voltage (φ) decreased as the charging voltage was increased, leading to greater deviation of PEF system performance from ideality.

1. Introduction

Pulsed electric fields (PEF) is one of the most appealing nonthermal technologies for preservation of liquid foods due to reduced heating effects compared to traditional pasteurization methods (Barbosa-Cánovas et al., 1999). The common interest of academia and food industry on optimizing the PEF system to assure food safety and product quality have led the technology improve in recent years and successful results were obtained to be utilized for industrial implementation. From an engineering point of view, as in the case of most novel technologies, PEF technology is still in the early stages of commercialization and scaling up to cost-effective industrial operations is highly dependent on further research on the engineering principles behind this technology to fully understand the mechanisms at play (Góngora-Nieto et al., 2002).

A typical PEF system is based on a high voltage pulse generator with a treatment chamber with a suitable fluid handling system and necessary monitoring and controlling devices. Food product is pumped through the treatment chamber, either in a static or continuous design, where two electrodes are connected together with a nonconductive material to avoid electrical flow from one to the other. Generated high voltage electrical pulses are applied to the electrodes and high intensity electrical pulses are conducted to the product placed between the two electrodes. The food product experiences a force per unit charge, the electric field, while the dose of the application is adjusted by means of electric field intensity (peak voltage and the gap between electrodes) and the number of pulses (treatment time). Treated product is then removed or subjected to subsequent pulses with recirculation until the treatment dose is complete. The main process parameters that determine PEF treatments are

electric field strength, shape and width of the pulse, treatment time, frequency, specific energy density, and temperature. Among all, electric fields intensity and treatment time (number of pulses) are the basic control parameters affecting energy density applied during PEF process. The intensity of these parameters determines the final lethal effect on the microbial population while width and frequency of the pulses contribute to define the process time (Wouters et al., 2001).

There is substantial variety in PEF equipments operating in different laboratories around the world and the need for unification of the process parameters to assess equivalency among different system as well as reducing the difficulty in comparing experimental results is certain (Wouters et al., 2001). Currently, the majority of research reported on PEF defines process conditions in terms of two common variables: intensity of electric fields calculated from voltage and treatment time, which does not help to assess the feasibility or the efficiency of the system. Instead, if the energy density or energy per pulse together with the process conditions were provided, comparable and reproducible data could be obtained. Moreover, unless the voltage value measured with a probe in the treatment chamber, most of the studies assume perfectly efficient operating systems, that is, the electrical energy at the input to flow is equal to calorimetric heat out of the system. A proper energy balance in order to accurately calculate the treatment intensity requires evaluation of the system efficiency and behavior of the system components during PEF since several factors including the change of the electrical properties during the process, system configuration, treatment chamber or pulse shape may introduce inefficiency to the system. This inefficiency results in effective electric field intensity and treatment time being different from the reported values.

The purpose of this research is to verify the effect of electrical and flow properties of the system components on energy consumption during PEF process as well as the interaction of these components and their contribution to the overall energy balance. Therefore the objectives are threefold: (1) to understand the impact of electrical properties on system efficiency as a function of temperature and concentration, (2) to quantify microbial inactivation with energy density as a function of fluid flow rate, (3) to estimate the inefficacy of the system as a function charging voltage, number of pulses (treatment time) by comparing the actual and theoretical energy densities in a PEF system. In order to achieve the objectives of this study, we should begin with a review on the basic engineering aspects involved in this research as well as the mathematical relationships utilized to design an optimum PEF process.

1.1. Electrical Components

The high intensity pulsed electric field processing system is a simple electrical system consisting of a high voltage source, capacitor bank, switch, and treatment chamber. Generation of pulsed electric fields requires a fast discharge of electrical energy within a short period of time. This is accomplished by the pulse-forming network (PFN), an electrical circuit consisting of one or more power supplies with the ability to charge voltages (up to 60kV), switches (ignitron, thyratron, tetrode, spark gap, semiconductors), capacitors (0.1-10 μF), inductors (30 μH), resistors (2Ω -10MΩ), and treatment chambers (Góngora-Nieto et al., 2002). High voltage pulses are supplied to the system via a high voltage pulse generator at required intensity, shape, and duration. The simplest PFN is an RC (resistance-capacitance) circuit in which a power supply charges a capacitor that can deliver its stored energy to a

resistive load (treatment chamber) in a couple of microseconds, by activation of a switch (Góngora-Nieto et al., 2002).

1.1.1. Coaxial Treatment Chamber

Electrically pulsing devices generally have an electrode gap filled with a more or less conductive liquid acting as resistors. In the case of pulsed electric fields the treatment chamber represents the electrical load consisting of two or more electrodes filled with the liquid food product treated. The chamber has to be designed in such a way that electrical field acting on the liquid is homogeneous across the entire active region. This can be adjusted in principle with planar, co-axial and axial geometries. Coaxial treatment chamber design was composed of a continuous treatment chamber with coaxial conical electrodes and introduced by Bushnell et al. (1993), later followed by the continuous treatment chamber with parallel electrodes coaxial cylindrical electrodes (Qin et al., 1997). The coaxial arrangement has inner and outer cylindrical electrodes, shaped to minimize the electrical enhancements with uniform fluid flow (Figure 1).

1.1.2. Electric Field Intensity

Electric field intensity has been identified as the most relevant factor affecting microbial inactivation by PEF treatment. Electric field intensity in combination with total treatment time during PEF is mainly effective on the extent of membrane disruption in bacterial cells (Hamilton and Sale, 1967). Understanding the electrical principles behind PEF technology is essential for a comprehensive analysis of the PEF system. The electrical field concept, introduced by Faraday, explains the electrical field force acting between two charges. When

unit positive charge q located at a certain point within the electric field is generated in the treatment gap (E_r), it experiences force F identified by position vector r (Blatt, 1989). The electrical field per unit charge is then defined as:

$$E_r = \frac{F_{qr}}{q} = \frac{Newton}{Coulomb} \tag{1}$$

The electrical potential difference (V) between voltage across two points, separated by a non-conductive material, results in generation of an electric field between these points, with an electrical intensity (E) directly proportional to the magnitude of potential difference (V) and inversely proportional to the distance (d) between points as:

$$E = \frac{V}{d} = \frac{Volt}{Meter} \tag{2}$$

Transferring Eqn.2 into the form in Eqn.3:

$$V = E.d = \frac{Newton.Meter}{Coulomb} \tag{3}$$

The Laplace equation can be used as a general expression to describe the general electric field, depending on the voltage under different conditions within boundary conditions, where φ represents the electrical potential:

$$\nabla \varphi^2 = 0 \tag{4}$$

In the case of coaxial treatment chamber arrangement (Figure 1), the inner and outer cylindrical electrodes are shaped to minimize the electrical enhancements with uniform fluid flow. In a simple coaxial chamber design, the intensity of the electric field varies with respect to the radial distance from the inner electrode as shown in the following equation (Zhang et al., 1995b):

$$E = \frac{V}{r \ln\left(\frac{R_2}{R_1}\right)} \tag{5}$$

where r is the radius of electric field measurements and R_2 and R_1 are the radii of the outer and inner electrodes, respectively.

1.1.3. Treatment Time and Dose

PEF treatments are applied in the form of short pulses to avoid excessive heating or undesirable electrolytic reactions (Barbosa-Cánovas and Sepúlveda, 2005). Liquid foods are electrical conductors for carrying electrical charges. High voltage pulsed electric fields are created when a large flux of electrical current flows through the treatment chamber in a very short period of time (microseconds). Time elapsed between each pulse is much larger than the pulse width since charging of the capacitor is slower than instant discharging (Zhang et al., 1995).

The simplest configuration of a pulse forming network (PFN) is the direct discharge of the capacitor to a treatment chamber with purely resistive load and no other associated loads, which produces exponentially decaying pulses. In the case of a charged capacitor (C_0) discharging through a resistor (R), the voltage across the food in the treatment chamber decreases exponentially with a pulse width (Góngora-Nieto et al., 2002):

$$\tau = RC_0 \tag{6}$$

In RC circuits, the total pulse duration equals approximately (5τ) five time constants (Cogdell, 1999). Considering the exponential decaying behavior of the delivered energy, τ can be adopted as the effective pulse width, calculated as the time required for the input voltage to decay to 1/e (37%) of its maximum value (Zhang et al., 1995b; Grahl and Mark, 1996; Barsotti et al., 1999; Góngora-Nieto et al., 2002). Treatment time (t) for a PEF application is defined as a function of pulse width (τ) and number of pulses (n):

$$t = n\tau \tag{7}$$

Pulse repetition rate, maximum voltage and peak current are the key parameters in specifying a PEF generator. In order to establish the desired treatment dose in terms of number of pulses per treatment volume of the treated of fluid (n), treatment chamber volume (v), volumetric flow rate (F) and pulse repetition rate (f) can be adjusted as (Zhang et al., 1995):

$$f = \frac{nF}{v} \tag{8}$$

The electrical components of the PEF system and related mathematical expressions explaining their relationship were reviewed. Understanding the electrical properties (conductivity, resistivity) of the liquid food product affecting electrical components of the system is also essentially required.

1.1.4. Conductivity and Intrinsic Electrical Resistance

Among physical properties of biological materials, electric properties are the least known and described; however one can find the examples of their application in the food industry. The most important characteristics that alter the biological changes produced during PEF are conductivity, ionic strength, pH, the presence of particles, presence of gas bubbles, dielectric properties, sugar content (for juices), and temperature (Barbosa-Cánovas, 1999).

Electrical conductivity is the reciprocal of resistance through a unit cross-sectional area *A* over a unit distance *L*, or the reciprocal of resistivity. Intrinsic electrical resistance is one of the most important design criteria for PEF treatment chamber for defining the pulse width, peak electric field and power per pulse delivered to the treated product. The resistance of a treatment chamber can be analytically determined, provided the effective electrode area (*A*), the distance between the electrodes (*d*), and the electrical conductivity of the treated product (σ)are known, by:

$$R_{Ch} = \frac{d}{\sigma A} \tag{9}$$

When we simplify the PEF system into a basic electrical discharge circuit (Figure 2) we can see that the total resistance of the circuit (R_T) is a sum of individual resistances including treatment chamber resistance (R_{Ch}), transmission line resistance (R_t), switch resistance (R_s), and any other resistance present in the series circuit (Barbosa-Cánovas and Sepúlveda, 1995).

$$R_T = R_s + R_t + R_{Ch} \tag{10}$$

The circuit is basically a voltage divider therefore, the larger the chamber resistance in comparison with the total resistance, the higher the peak voltage reached at the chamber electrodes. If all resistance in the system except the treatment chamber resistance (R_{ch}) kept constant than the performance of the system is governed by the resistance of the treatment chamber according to the Ohm's law (Voltage is equal to current times resistance) (Blatt, 1989).

1.2. Specific Energy and Temperature

From and engineering point of view, in order to estimate energy requirements and temperature rise during the PEF process, complex energy balance calculations are required. Therefore, estimation of energy density or energy per pulse is infrequently reported; in a few cases it is only qualitatively correlated (Evrendilek et al., 2000; Giner at al., 2000). For a continuous single pass process without recirculation the energy Q (J) dissipated during the discharge of the capacitor C (μF) at a charging voltage V (kV) is given by:

$$Q = \frac{1}{2} C V^2 \tag{11}$$

Only under ideal conditions where the ratio of the chamber resistance to the total resistance is 1, all the energy discharged from the capacitor can be delivered to the treatment chamber. Generally, a fraction of the total energy is delivered to the food product which defines the peak voltage received by the treatment chamber (Barbosa-Cánovas and Sepúlveda, 1995):

$$V_{\text{chamber}} = V_{\text{charging}} \frac{R_{chamber}}{R_{\text{total}}} \tag{12}$$

Therefore the energy per pulse delivered to the treatment chamber can be describes as:

$$Q_{pulse} = \frac{1}{2} \frac{R_{Ch}}{R_T} CV^2 \tag{13}$$

where C is the capacitance of the charging capacitor (μF) and V (kV) is the charging voltage. Upon delivery of the energy to the food product, during PEF operation, the bulk temperature of the fluid increases due to ohmic heating (Mastwijk, 2006). The temperature increment is related to the input power and is the result of conversion of electrical energy into heat. Heating of the product is defined as:

$$\Delta T = \frac{Q}{mC_p} \tag{14}$$

where ΔT is the temperature rise, Q is the total energy delivered to the treated product, m is the mass of the product being treated, and C_p is the specific heat capacity of the product. For a continuous PEF process, considering the energy balance completed above:

$$fQ_{pulse} = \rho F C_p \Delta T \tag{15}$$

where mass (m) is replaced by density (ρ) times volumetric flow rate (F) of the product and pulsing frequency (f) is taken into account. The ratio between pulsing frequency f (1/s) and flow rate F (mL/s) defines the number of applied pulses (n) , when multiplied by the treatment volume v (mL) can be set by the processor to reach the desired degree of inactivation, as given in Eqn. 8 (Zhang et al., 1995).

1.3. Fluid Flow in Coaxial Treatment Chamber Design

The continuous PEF chamber with a coaxial geometry is shown in Figure 1. The flow regime within the chamber can be determined by the ratio of inertial forces to viscous forces, defined as the Reynolds' number:

$$\mathrm{Re} = \frac{\rho v L}{\mu} \tag{16}$$

where, ρ is the density of the fluid, v is the average fluid velocity, L is the characteristic treatment length and μ is the dynamic (absolute) fluid viscosity. The velocity profile in the coaxial treatment chamber can be determined by applying momentum balance equations in cylindrical coordinates for incompressible fluid flow through an annulus (Bird et al., 1960).

Figure 3 shows an incompressible fluid flowing in steady state in the annular region between two coaxial circular cylinders of radius r and R.

The steady state laminar flow (Re is less than 2100) of an incompressible (density ρ is constant) Newtonian [$\tau_{rz}=-\mu(dv_z/dr)$] fluid inside the treatment chamber while neglecting end effects and slip conditions at the wall (pure liquid) can be modeled by setting up a momentum balance over a thin cylindrical shell and arriving the differential equation (Bird et al., 1960):

$$\frac{d}{dr}(r\tau_{rz}) = \frac{(P_0 - P_L)r}{L} \tag{17}$$

where (P_0- P_L) is the ΔP which is the pressure drop through the effective length. Then $\tau_{rz}=-\mu(dv_z/dr)$ can be substituted and the equation becomes:

$$\frac{d}{dr}[r\mu(\frac{dv_z}{dr})] = \frac{-(P_0 - P_L)r}{L} \tag{18}$$

Taking out the constant viscosity and integrating the above equation:

$$-r\frac{dv_z}{dr} = \frac{-\Delta \mathrm{Pr}^2}{2\mu L} + C_1 \tag{19}$$

The constant C_1 cannot be determined immediately, due to the lack of information on the momentum flux at either of the fixed surfaces r=kR or r=R. But there will be a maximum in

the velocity curve at some plane r=λR at which the momentum flux will be zero. Solving the equation for C_1:

$$\frac{dv_z}{dr} = \frac{-\Delta PR}{2\mu L}[(\frac{r}{R}) - \lambda^2(\frac{r}{R})] \tag{20}$$

Integration with respect to r:

$$v_z = \frac{-\Delta PR^2}{4\mu L}[(\frac{r}{R})^2 - 2\lambda^2 \ln(\frac{r}{R}) + C_2] \tag{21}$$

Solving the equation for C_2 by using the boundary conditions:

Boundary condition 1: at r= kR v_z =0

Boundary condition 2: at r=R v_z =0

Substituting the boundary conditions and solving for C_2 and λ, respectively, the velocity distribution for steady incompressible flow in an annulus is:

$$v_z = \frac{-\Delta PR^2}{4\mu L}[1 - (\frac{r}{R})^2 + [(1-k^2)/\ln(\frac{1}{k})]\ln(\frac{r}{R})] \tag{22}$$

Therefore, by using the above equation for velocity distribution we can calculate:

The maximum velocity (the center line of the annulus r=λR):

$$v_{z,\max} = -\frac{\Delta P R^2}{4\pi L}\left([1-\frac{(1-k^2)}{2\ln(\frac{1}{k})}][1-\ln\frac{(1-k^2)}{2\ln(\frac{1}{k})}] \right) \tag{23}$$

The average velocity:

$$\overline{v_z} = \frac{-\Delta P R^2}{8\mu L}[\frac{(1-k^4)}{(1-k^2)} - \frac{(1-k^2)}{\ln\frac{1}{k}}] \tag{24}$$

The volumetric flow rate:

$$Q = \pi R^2 (1-k^2)\overline{v_z} \tag{25}$$

For designing a PEF process with optimum conditions, it is important to determine the residence time of the treated fluid within the effective electrode length inside the co-axial cylindrical treatment chamber where the electric fields are delivered to the flowing product. Based on the derived velocity profile, the velocity of the fastest moving particle (Eqn. 23) should be taken into account due to its minimum residence time inside the treatment chamber.

Up to this point, the basic engineering aspects involved in this research as well as the mathematical relationships utilized to design an optimum PEF process were reviewed to serve as a comprehensive tool to discuss the results obtained. Based on the objectives the utilized materials and methods will be mentioned next.

2. Materials and Methods

In the first part of the study, the effects of temperature and concentration on the conductivity of McIlvaine buffer solutions were evaluated to understand the impact of electrical properties on system efficiency. Electrical conductivity of the buffer solutions were adjusted by diluting the buffer solutions with deionized water. In the second part, microbial inactivation kinetics of *E.coli* (ATCC 11775) with respect to energy density as a function of fluid flow rate was explored. Lastly, the inefficacy of the system as a function charging voltage, number of pulses (treatment time) was estimated by comparing the actual and theoretical energy densities.

2.1. Conductivity of Buffer Solution

McIlvaine (citric-phosphate) buffer solution at pH 7.0 was prepared mixing 178 mL of 0.1 M citric acid solution (21.01 g$C_6H_8O_7 \cdot H_2O$ L^{-1}) and 822 mL of 0.2 M Na_2HPO_4 (28.40 g Na_2HPO_4 L^{-1}) (Perrin and Dempsey, 1974). Electrical conductivity of the buffer solution was adjusted by diluting 1 part buffer with 15 parts deionized water. A final conductivity of 0.23 S/m at 20^{o}C to resembles the electrical conductivity of apple cider. Conductivity of McIlvaine buffer solution diluted with deionized water at different ratios was measured with respect to increasing temperature by Orion-Thermo (Beverly, MA).

2.2. PEF Treatment

A pilot plant PEF system (Physics International, San Leandro, CA), with variable charging voltage and pulse frequency, was employed to discharge a 0.5 μF capacitor into a cylindrical concentric-electrodes treatment chamber (Qin et al., 1995), in order to apply PEF treatments

to the prepared samples. In order to prevent contamination of the lines downstream of the treatment chamber with the untreated product, during start-up, the system was pre-equilibrated with a sterile buffer matching the electrical properties of apple cider. The inlet line to the system is then switched from the sterile buffer to the test product (apple cider) without an interruption in pulsing. Processing parameters and the PEF system settings were defined during the study to ensure that a quantifiable bacterial inactivation was attained under the employed processing parameters. Relevant parameters such as initial temperature, treatment temperature, pulse shape, pulse width, voltage and current traces were monitored throughout the conducted experiments. Electrical parameters were measured with a digital oscilloscope (Hewlett-Packard 54530A, Colorado Springs, CO) connected to the treatment chamber through high voltage probes. Temperatures were monitored using digital thermometers (Cole-Palmer, Vernon Hills, IL). In order to evaluate the effect of flow rate on microbial inactivation, inoculated apple cider was pumped through the system in four different flow rates (2, 1.6, 1.2 and 0.8 L/min). Each flow rate was tested in a separate PEF run.

2.3. Microbiology

Two milliliters of *E.coli* (ATCC 11775) was added to 100 ml nutrient broth (Difco, Kansas MO) for growth on a rotary platform shaker (MSB-3322A-I, GS Blue Electric, Blue Island, IL) at 30°C and 225 rpm until reaching the early stationary phase or 27 hours maximum time. Once the desired bacterial concentration was reached (~10^9 CFU/ml), 100 ml of the inoculum were transferred into 10 liters of apple cider to obtain an initial bacterial concentration of approximately 10^7 CFU/ml. Bacterial concentration in treated samples was determined by the

pour plate method. Serial decimal dilutions in sterile 0.1% peptone (Difco, Kansas MO) water were plated on Eosin Methylene Blue (EMB) Agar. All samples were plated in duplicate and incubated at 35±0.5°C for 48 h in a controlled temperature chamber. After incubation, colonies were quantified following standard procedures (AOAC, 1995). Each study was conducted in triplicate using independent prepared batches of buffer and fresh bacteria for every replicate.

3. Results and Discussion

The effect of temperature on conductivity for buffer solutions for different concentrations is given in Figure 4. The conductivity of buffer solution increases in a range of 1-2.5 S/m when the temperature rise is from 10˚C to 40˚C. The increasing trend of electrical conductivity for ionic solutions as a function of temperature is of major concern affecting the electrical properties of the PEF system. The higher the conductivity of the food product treated in the treatment chamber, the lower the electrical resistance to the flowing current. This in turn, reduces the proportion of applied charging voltage in the form of electric field strength between the electrodes of the treatment chamber. The conductivity of the media, which is related to the material's capacity to conduct electric current, will influence the maximum electric field achievable for a given input power and the maximum temperature rise during processing. Lowering the conductivity (high resistivity) reduces the temperature rise and applied power, thus increasing the electric field intensity and overall microbial effectiveness as suggested by Vega-Mercado et al. (1996).

The rate of increase for conductivity as a function of temperature was higher for concentrated buffers where strong temperature dependency of ionic solutions is generally explained by their kinetic state where an increase in temperature increases the mobility of ions throughout the solution, causing an increase in the conductivity (Heinz et al., 2002).

For the second part of the study, inactivation kinetics with respect to energy input at 35 kV/cm is given in Figure 5. Energy density (J/ml) is calculated by adjusting the energy per milliliters for different flow rates of the apple cider inoculated with *E.coli* (ATCC 11775) as listed in Table 1. As the flow rate of the inoculated apple cider is decreased, residence time of the treatment volume inside the treatment chamber increased, therefore, energy density (per milliliter) increased. Table 1 indicates that, a decrease in flow rate from 2.0 L/min to 0.8 L/min resulted in temperature rise from 32.3˚C to 57.9˚C in outlet of the treatment chamber. It is possible to achieve 5 log cycle reductions with the use of 0.8 L/min flow rate however in order to design a PEF process with the optimum process conditions the energy density and temperature rise should also be considered.

Inactivation results at 35 kV/cm and 12 pulses (30 µs) indicate that at energy inputs lower than approximately 150 J/mL the microbial response is almost linear. However, at higher energy inputs an abrupt increase in the slope of the survival curve is observed. The observed shape of the survival curve is a typical PEF inactivation curve possessed exponential decay pulses due to negligible microbial inactivation at electric fields lower than the critical electric field. Representing inactivation studies with the use of specific energy consumed during the

process would certainly allow better quantification of the process feasibility as well as efficiency.

In the last part of the study, the calculated input energy and output energy in terms of measured heat output are represented in Table 2. In order to combine all factors influencing the efficiency of the system including conductivity of the fluid and temperature development as a function of flow rate, an energy balance comparing the electrical energy at the input to the heat dissipated out of the system was established. Based on the results, for a single pass continuous PEF operation where the apple cider with density (ρ) of 1013.7 kg/m^3 and specific heat capacity (C_p) of 3.8 kJ/kg˚C flowing through the system with a flow rate of 1.2 L/min, the theoretical energy balance equation is given in Equation 15. This equation holds when the input energy (Eqn. 11) is completely utilized by the treatment chamber to convert electrical energy to heat as output (Eqn. 14). However in the actual case, only one part of this energy will heat the fluid food that passes through the PEF chamber. This ratio (φ) is less than 1 and depends strongly on electrical conductivity of the food (Eqn. 12).

Based on Equation 10, it was mentioned that the PEF system resembles a basic electrical circuit and serves as a voltage divider and according to Ohm's law, the performance of the system is governed by the resistance of the treatment chamber. Therefore, if the resistance of the treatment chamber decreases as a result of increase in the conductivity of the food being treated, the ratio of chamber resistance to the constant total resistance is lower resulting in more discrepancy between the input charging voltage and the actual voltage in the treatment chamber.

The influence of the intensity of the charging voltage and number of pulses (treatment time) on the efficiency of the system can be better observed in Figure 6a and 6b. The theoretical values of temperature rise for an ideal system ($\varphi=1$) calculated by Eqn. 11 are shown in Figure 6a. The exponential curves for increasing pulse frequencies were expected due to the direct proportionality of temperature difference (ΔT) with the square power of charging voltage (V^2_{Ch}). On the other hand, for the actual case, in Figure 6b, where the observed temperature rise was plotted with respect to the same input charging voltage a different pattern was followed. Keeping in mind that φ is the ratio of voltage in the treatment chamber to the charging voltage (Eqn. 12), the concavity of the curves in Figure 6b indicates that, as the charging voltage increases, the value of φ gets smaller, possibly due to the increase in conductivity, leading to more deviation from ideality.

Figure 7 represents the effect of treatment time on temperature rise to be compared with the effect of charging voltage (Figure 6). Obviously, the effect of pulse frequency (treatment time) on temperature rise is stronger especially for higher voltages (30-35 kV). This approves previously explained change in voltage proportions with respect to fluid conductivity and treatment chamber resistance as result of temperature development.

Based on the data given in Table 2, the plot of energy per pulse versus charging voltage (Figure 8) for both theoretical and observed values provides evidence for the increasing inefficiency of the system for higher voltages. The ratio φ changes as a function of capacitor charging voltages and approaches to 1 only for smaller charging voltages at all three pulsing frequencies where the effect of temperature is negligible on the effectiveness of the PEF

system. As the number of pulses applied increases and reaches to its maximum value (20 pulses) the difference between input and the output increases possibly due to changing electrical properties of the system with increased temperature.

By using data in Table 2, the energy consumption by PEF can be estimated, compared to thermal pasteurization and the difference between the actual and the theoretical values can be determined. With the use of Eqn 14, the energy requirement for bringing 50 mL of water from room temperature (20˚C) to pasteurization temperature (71.7˚C) is calculated as 10.7 kJ. For the same amount of water, the actual and theoretical energy requirement for a PEF system with 35 kV and 20 pulses (50 μs) assuring 5 log cycles reduction of *E.coli* are 9.5 kJ and 12.65 kJ, respectively.

There is very limited data published regarding to the energy consumption of PEF systems and lethality with respect to energy received by the food (Heinz et al., 1999). The lack of studies is possibly due to the fact that even if the energy delivered to the treatment chamber is accurately measured, the energy received by the food may not be entirely used to inactivate the microbial flora. However before going into the efficiency on cellular basis, unknowns related to the optimum PEF conditions should be explored. In this study the obtained results enabled us to take a step forward by suggesting the possible factors that influence the system performance in terms of the discrepancy between input and output values. However, further research is required to evaluate the main source of inefficiency. The electrical resistance, current and voltage proportion of each component of the system should be further analyzed using electrical engineering principles.

4. Conclusions

The focus of this research was to investigate the effects of the possible factors contributing to the variation in PEF system efficiency. The impact of electrical properties as a function of temperature and concentration, the effect of energy density and fluid flow rate on microbial inactivation and the efficiency of the system as a function of charging voltage, number of pulses were explored by comparing the actual and theoretical energy densities in a PEF system. From the first part of study, the impact of temperature on conductivity was higher for concentrated buffer solutions contributing to the deviation of voltage in the treatment chamber from the charging voltage. Similarly, the flow rate of the apple cider contributed to the temperature rise inside the treatment chamber by increasing the energy density. *E.coli* (ATCC 11775) was inactivated by 5 log cycles with the use of 0.8 L/min flow for 35 kV/cm and 50 μs of PEF treatment consuming 167 J/ml of energy. Based on energy balance with the input electrical energy and energy output as the heat dissipation, the PEF system performance deviated from ideality for intense PEF conditions. As the charging voltage was increased, the ratio of voltage in the treatment chamber to the charging voltage (φ) became smaller, leading greater deviation of PEF system performance from ideality. Increasing pulse frequency and voltage both resulted in temperature rise; however, the impact of pulse frequency was stronger than the voltage impact, especially at high voltages (30-35 kV). In this study, the obtained results enabled us to take a step forward by suggesting the potential factors that influence the system performance in terms of the discrepancy between input and output energy values. However, further research is required to evaluate the main source of inefficiency by analyzing the electrical resistance, current and voltage proportion of each component of the system by using electrical engineering principles.

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Table 1. Treatment temperatures, inactivation and input energy density of PEF treated apple cider inoculated with *E.coli* (ATCC 11775) and pumped with selected flow rates at 35 kV/cm electric field strength and 12 pulses (30 microseconds).

(T_{in}: Inlet temperature of the product, T_{out}: outlet temperature of the product)

Flow rate (L/min)	T_{in}	T_{out}	Inactivation log(N/No)	Input energy (J/ml)
2.0	13.3	32.3	-1.4	67
1.6	13.5	36.4	-1.6	84
1.2	13.3	43.5	-2.3	111
0.8	13.6	57.9	-5.0	167

Table 2. Maximum input energy and output energy in terms of measured heat dissipation. (V_{Ch}:charging voltage, Q_{pulse}: Energy per pulse, E_{in}: Input energy as the charging voltage, E_{out}: measured heat dissipation from the PEF system, ΔT: temperature difference (T_{in}-T_{out}).

V_{Ch} (kV)	Q_{pulse} (J)	Number of pulses	E_{in} Q_{in}(J/s)	(J/ml)	ΔT	E_{out} Q_{out}(J/s)	(J/mL)
20	100		410	21	4	264	13
25	156	5	852	43	12	641	32
30	225		1022	51	14.4	923	46
35	306		1256	63	15	1064	53
20	100		990	50	8.9	632	32
25	156	12	1675	84	23.6	1547	72
30	225		2228	111	30.2	2143	107
35	306		3032	152	35.4	2512	126
20	100		1650	83	13.6	965	48
25	156	20	2725	136	38.4	2578	129
30	225		3713	186	49.5	3512	176
35	306		5053	253	53.6	3803	190

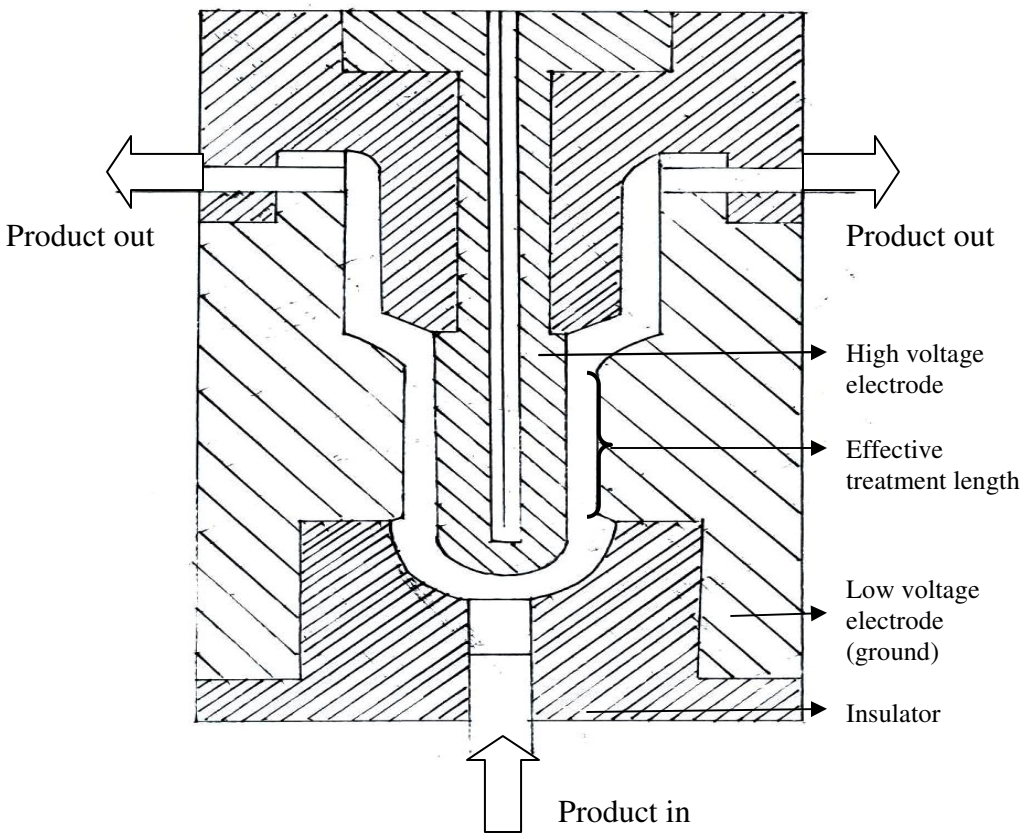
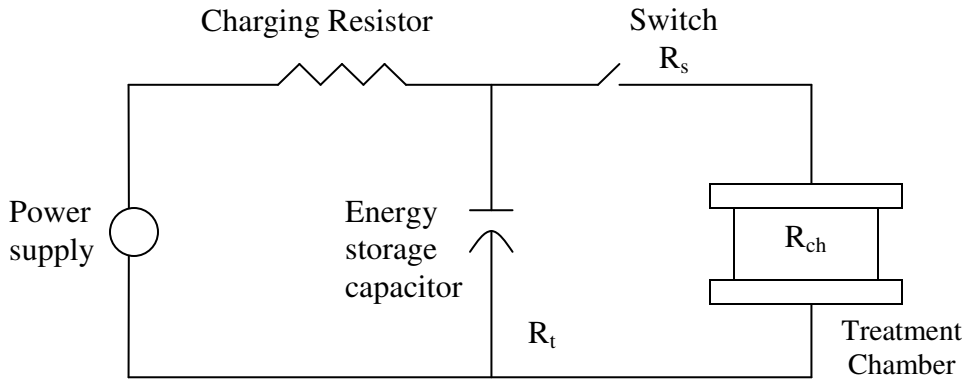
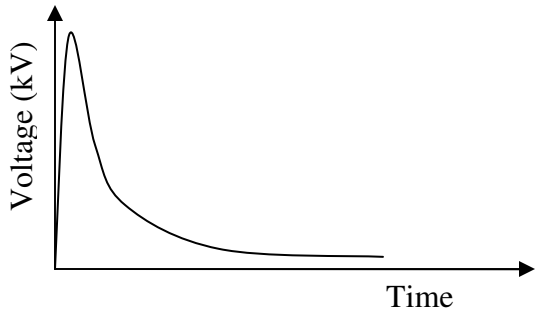


Figure 1. Longitudinal cross-sectional view of the modified coaxial treatment chamber designed, constructed, and tested for microbial inactivation at WSU (Martín-Belloso et al., 1997).



(a)



(b)

Figure 2. (a) Simplified electrical discharge unit of the PEF system,

(R_s: switch resistance, R_t: transmission line resistance, R_{ch}: treatment chamber resistance)

and (b) a typical exponentially decay pulse.

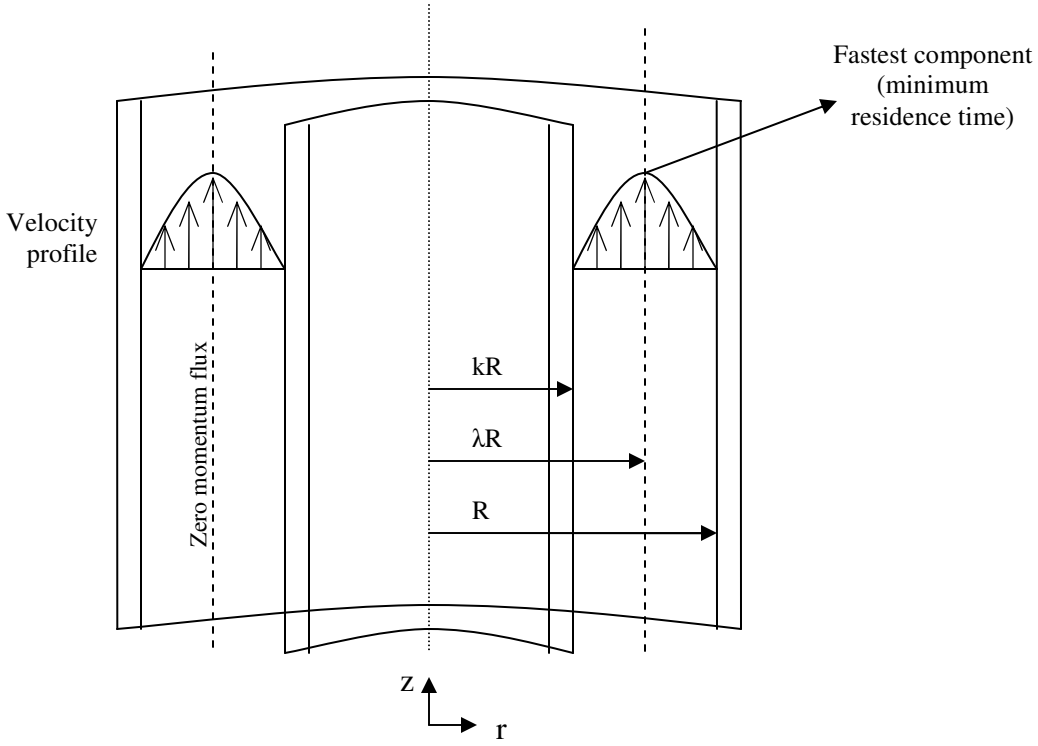


Figure 3. Cross-sectional view of the upward flow through coaxial cylinder treatment chamber with cylindrical annulus (Bird et al., 1960).

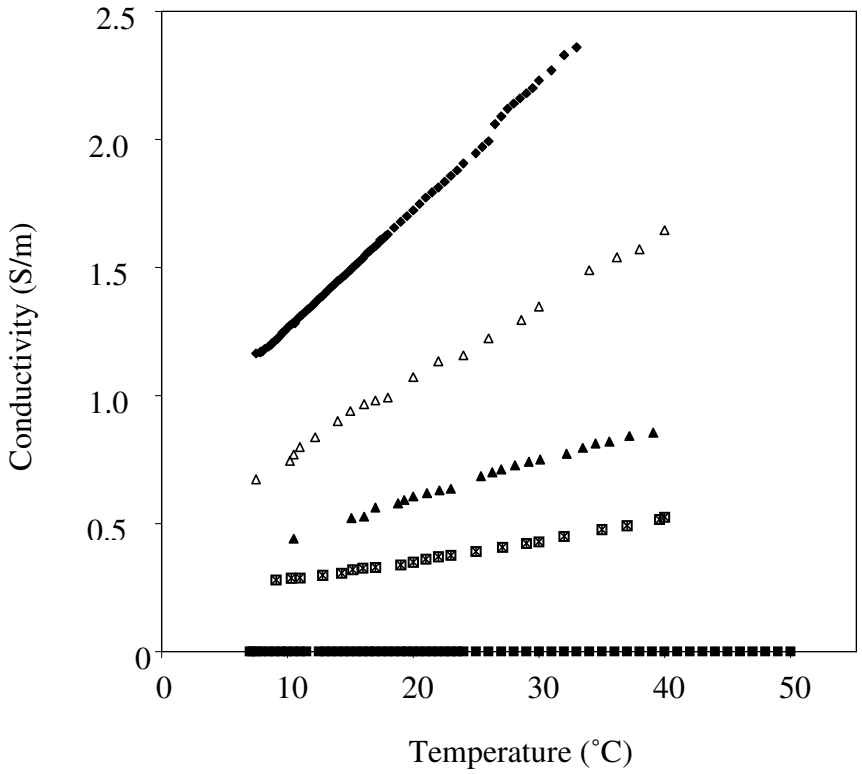


Figure 4. The effect of temperature on electrical conductivity of McIllvaine buffer for different ratio of dilutions with distilled water. Buffer dilution ratio (buffer:water): (♦) 1:0 (buffer only), (∆) 1:1, (▲) 1:3, (□) 1:15 (apple cider), (■) 0:1 (water).

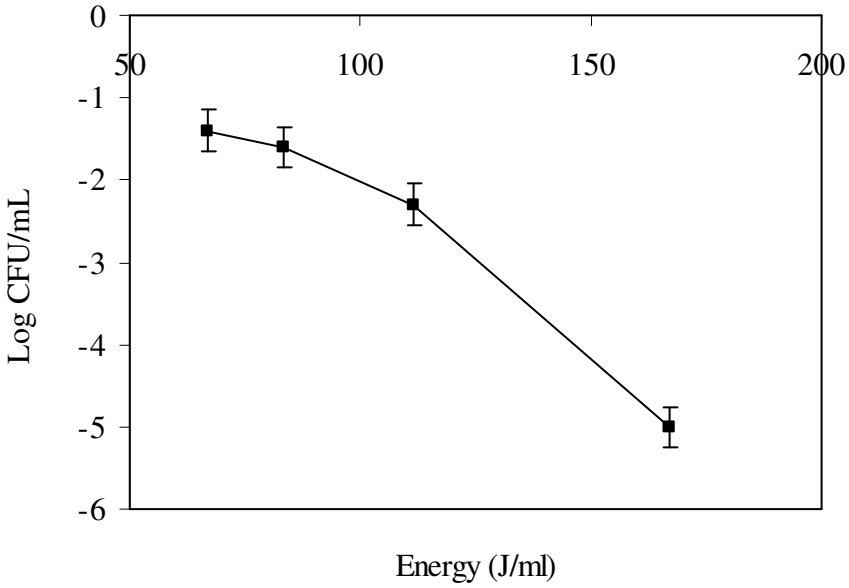


Figure 5. Inactivation of *E.coli* (ATCC 11775) in apple cider as a function of input energy at 35 kV/cm (■) for 50 microseconds of PEF with a coaxial treatment chamber.

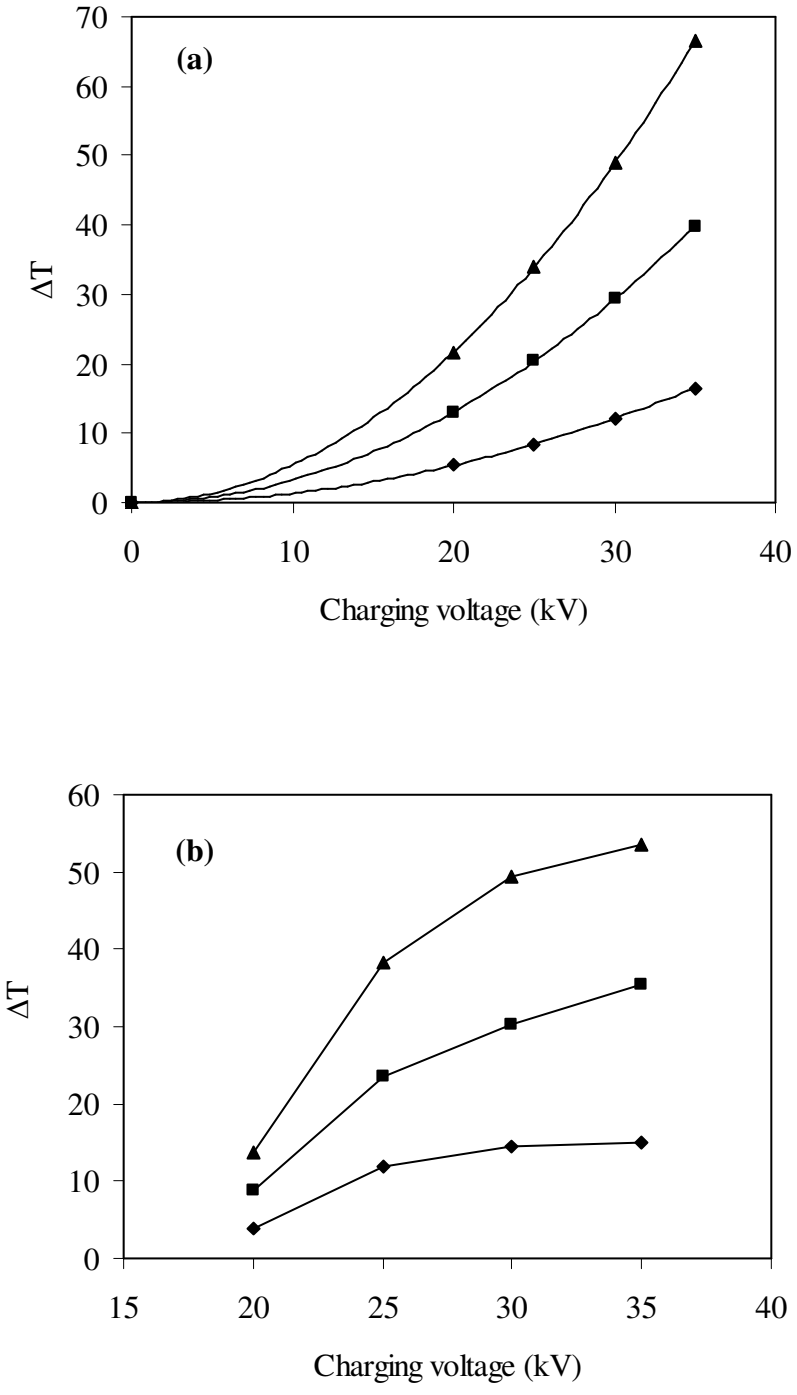


Figure 6. Temperature increase as a function of charging voltage for (▲) 20, (■) 12, (♦) 5 pulses. (a) Theoretical temperature rise when φ=1, (b) Observed temperature rise.

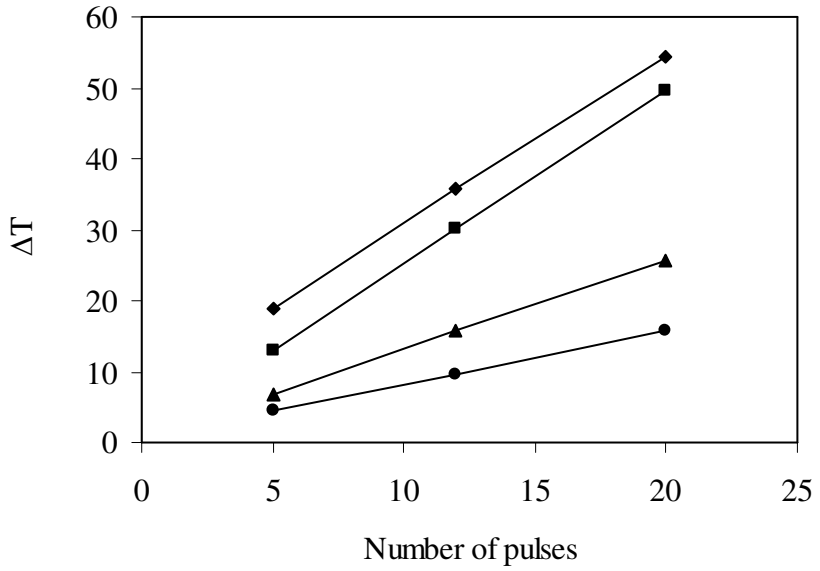


Figure 7. Temperature increase (observed) as a function of applied pulses for (♦) 35 kV, (■) 30 kV, (▲) 25 kV, (●) 20 kV.

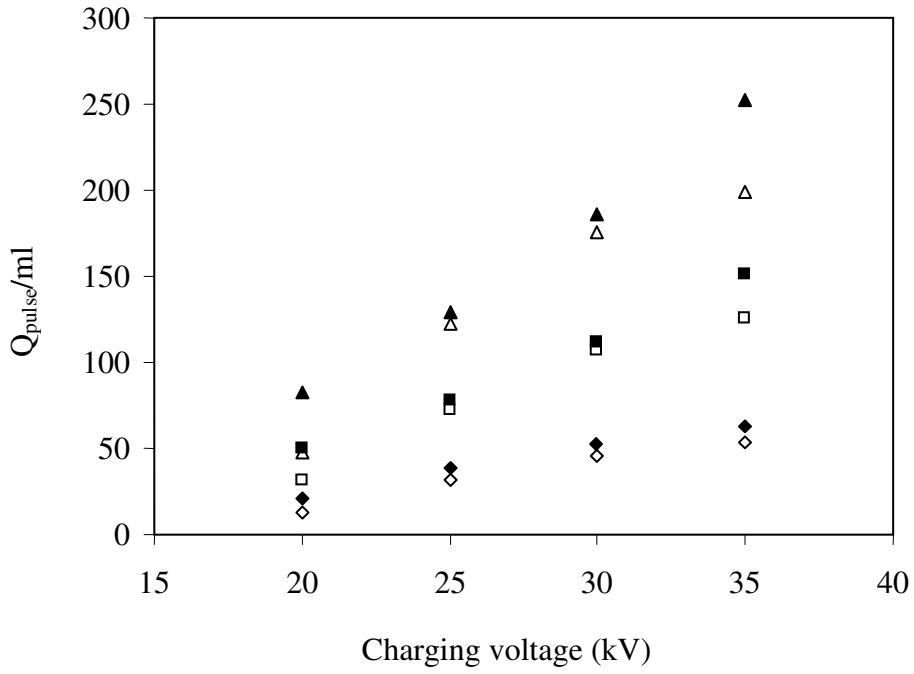


Figure 8. Energy densities (energy per pulse) as a function of charging voltage for input at (▲) 20 pulses, (■) 12 pulses, (♦) 5 pulses; and output at (Δ) 20 pulses, (□) 12 pulses, (◊) 5 pulses.

CHAPTER SIX

Combined Effect of Pulsed Electric Fields and Selected Antimicrobials On Shelf-life of Apple Cider

Bilge Altunakar, Subba Rao Gurram, Gustavo V. Barbosa-Cánovas

Abstract

The combined effect of PEF and selected antimicrobials on shelf-life of apple cider in terms of microbiological and chemical changes during refrigerated and room temperature storage were investigated. Apple cider was initially inoculated with *S. cerevisiae* (ATCC 16664) in view of the common appearance of this yeast as the most common spoilage agent in refrigerated fruit juices. 1000 ppm (parts per million) of selected antimicrobial was added to the apple cider prior to PEF treatment at 40 kV/cm electric field strength and 50 μs (20 pulses) treatment time. PEF treatment in the presence of selected antimicrobials achieved 6 log cycles reduction in yeast populations where the PEF treatment alone reduced yeast populations by 5.5 log cycles. The initial quality of apple cider after PEF treatment together with selected antimicrobials retained initial quality more than six weeks in refrigeration (4˚C) storage. Based on observed changes in microbial and physicochemical quality of apple cider during storage (color, pH, titratable acidity and PPO activity), the use of cinnamic acid, sodium benzoate and potassium sorbate were favored in combination with optimum PEF conditions. The potential use of selected antimicrobials in combination with PEF in juice pasteurization providing evidence for retained microbial safety and physiochemical quality.

1. Introduction

Consumer demand for fresh, minimally processed, and nutritious food products with long shelf life is challenging traditional heat pasteurization treatments as well as supporting the interest for the development of new food preservation technologies such as nonthermal or minimal processing. Among the novel methods of nonthermal processing, pulsed electric fields (PEF) has been extensively studied as a practical and promising preservation method to inactivate both spoilage and pathogenic organisms while minimizing the loss in volatile compounds and color during processing of liquid food products without the need for high temperatures (Barbosa-Cánovas et al., 1999).

Traditional thermal processing such as pasteurization and retorting can result in adverse changes to the flavor, taste and nutrient content of foods (Jarayam et al., 1992). Recently, with the rapid increase in the market segment of fruit juices, manufacturers began seeking ways to overcome the thermal effects of pasteurization that increase their shelf life as well (Hodgins et al., 2002; Sadler et al., 1992). Currently, juices are thermally processed to be commercialized either at refrigeration temperatures or at room temperature. The changes in fruit juice quality are generally due to microbiological growth, enzymatic activities and chemical reactions, therefore, the stability of the product is assured by heat treatment, the acidity of the product or by refrigeration temperatures. However, compounds responsible for flavor in fruit juices are heat-sensitive and heat-treated juices are perceived significantly different from raw juices (Manas and Vercet, 2006). Among fruit juices, apple cider has been produced and consumed in most of the apple producing regions of the United States for many years. Demand for apple cider is due to its fresh apple flavor and aroma as well as its full-

bodied texture where both qualities are susceptible to degradation by heat, microorganisms, enzymes, oxygen, and light during processing and storage. Cider producers have traditionally relied upon the acidity of cider and refrigerated storage for preservation of the product. Commercial pasteurization of apple juice is by heating to 77-78˚C for 25-30 seconds where degradation of nutritional and sensory qualities are normally associated with this process (Tressler and Joslyn, 1954).

Fruit juices are acidic foods with pH below 4.6, which enables the growth of yeasts, molds and a few groups of aciduric bacteria (Raso et al., 1998). PEF application is especially promising due to concerns over spoilage microorganisms leading to the production of off-flavor compounds such as lactic acid bacteria (Hendrix and Redd, 1995). PEF processing (25-75 kV/cm) for only a few microseconds has been successful in a variety of fruit juices having low viscosity and electrical conductivity such as orange, apple, and cranberry juice (Sizer & Balasubramaniam, 1999). Studies carried out so far have shown adequate inactivation levels after very short treatment periods at low temperatures for a number of spoilage and pathogenic microorganisms, molds, and yeasts, such as *S. cerevisiae* (Grahl et al., 1992; Grahl and Mark, 1996), *E. coli* (McDonald et al., 2000; Rodrigo et al., 2003), and *L. plantarum* (Rodrigo et al., 2000). Minimal quality and nutritional changes in fruit juices after PEF treatment were reported in literature (Zhang et al., 1996; Yeom et al., 2000; Min et al., 2002a). Simpson et al. (1996) evaluated the physical and chemical attributes of PEF-treated apple juice stored at 4˚C and found no physical or chemical changes in ascorbic acid or sugars (glucose, fructose, sucrose). The shelf life of treated apple juice lasted four weeks and the sensory panel found no significant difference between the PEF treated juice and

freshly prepared juice. Additionally, the color change in PEF-treated orange juice stored at 4°C for 112 days was less than that of thermally pasteurized juice, where the color change mainly attributed to browning due to the conversion of ascorbic acid to furfural (Yeom et al., 2000).

Counted as an important source of quality deterioration in fruit juices, enzymes have to be controlled in order to maintain quality and extended shelf life (Manas and Vercet, 2005). Among important enzymes contributing to the quality deterioration of fruit juices, polyphenoloxidases (PPO) play important role in quality due to enzymatic reactions leading to discoloration in fruit products. PPO can react with monophenols and diphenols undergoing oxidation and generate orthoquinones, which polymerize to form brown pigments (melanins) (McEvily et al., 1992). The literature over PEF inactivation of PPO is conflicting since there are studies reporting successful inactivation of this enzyme as well as the studies mentioning no effect at all. Ho et al. (1997) reported an inactivation of mushroom PPO up to 60% of the original activity after 60 μs at 50 kV/cm in a buffer system. Giner-Segui et al., (1997) studied the effect of PEF on PPO from pear, peach and apple, concluding that apple PPO activity was reduced to 3% of the original activity after 6000 μs at 24 kV/cm while the temperature of the process did not exceed 24˚C. An opponent study reported 10% inactivation of PPO, peroxidase and lipoxygenase after PEF treatment with 10-30 kV/cm and 5-40 μs (van Loey et al., 2002). This latter study observed inactivation of PPO with extended treatment times regardless of the electric field intensity, where the inactivation of this metal containing enzyme, PPO, would be due to electrochemical reactions with the electrodes surface rather than the electric field itself (Manas and Vercet, 2006).

PEF application has extensively been explored for improved effects on juice safety and quality as well as shelf life extension and economic feasibility. Moreover, the use of antimicrobial agents such as nisin and organic acids in combination with PEF has been more effective in microbial inactivation and extending shelf life of refrigerated products than PEF treatment alone due to increased sensitivity of cells to antimicrobials by electric fields (Kalchayanand et al., 1994). The simultaneous combination of antimicrobials and PEF may provide an additive effect on microbial inactivation, and a synergistic effect can be observed when the hurdles take up different targets in the microbial cell (Leistner, 2000). However not sufficient studies reported for the comparative effect of antimicrobials tested in this study in combination with optimum PEF conditions on microbiological and physicochemical shelf life of juices.

The objective of this research was to determine the combined effect of PEF and selected antimicrobials on shelf-life of apple cider in terms of microbiological and chemical changes during storage at refrigerated and room temperatures. Apple cider was initially inoculated with *S. cerevisiae* in view of this yeasts' common appearance as the most common spoilage agent in refrigerated fruit juices (Molinari et al., 2004). The concentration of antimicrobials was selected according to their maximum regulatory permitted levels and average use in commercial food products (Cords and Dychdala, 1993; Conner, 1993; Sofos, 1989) and PEF process parameters were adjusted based on effective inactivation studies of *S.cerevisiae* obtained from literature.

2. Materials and Methods

The combined effect of PEF and four selected antimicrobials was studied during refrigerated (4˚C) and room temperature (22˚C) storage of apple cider. 1000 ppm of the selected antimicrobial was added to the 10^5 CFU/ml *S. cerevisiae* (ATCC 16664) inoculated sample prior to PEF treatment at 40 kV/cm electric field intensity and 50 µs (20 pulses) treatment time. Antimicrobial combined PEF treated apple cider was filled in 100 ml sterile containers and stored at both refrigeration (4˚C) and room (22˚C) temperatures until tested. PEF is generally viewed as an alternative to heat treatment for pasteurized products and PEF treated products generally recommended to be kept in refrigeration due to insufficient data reported on efficiency of PEF against mold ascospores (Raso et al., 1998). Shelf life conditions for this study were selected as refrigeration storage (4˚C) and room temperature storage (22˚C) which will provide information on prolonged storage of juices under extreme temperature. Microbiological (*S. cerevisiae*, total bacteria), physicochemical (pH, titratable acidity, color) and chemical (polyphenoloxidase activity) changes were measured every five days during 6 weeks. PEF-only treatment without the selected antimicrobials and fresh apple cider were used as control groups.

Fresh apple cider was obtained from Tree Top Inc. (Selah, WA) and stored at -20°C for the entire period of the experiments. Prior to experimentation, required amount of juice was thawed and diluted with water to obtain Brix° value of commercial apple juices (12.6°Brix). 1000 ppm of each antimicrobial, sodium benzoate (NaB), cinnamic acid (CA) (Sigma-Aldrich Inc., St. Louis, MO, 63103), potassium sorbate (KS) and hydrogen peroxide (HP) (Mallinckrodt Baker Inc., Phillipsburg, NJ) were added to apple cider 30 minutes prior to

PEF process. In order to determine optimum PEF treatment conditions, first, the inactivation of *S. cerevisiae* (ATCC 16664) in apple cider (inoculated with 10^6 CFU/ml) before and after PEF treatment (35 and 40 kV/cm for 50 and 30 μs) was analyzed in the presence of (1000 ppm) each antimicrobial where PEF only sample was used as the control.

2.1. Pulsed Electric Fields Treatment

A pilot plant PEF system (Physics International, San Leandro, CA), consisting of a continuous concentric cylindrical treatment chamber with 0.6 cm gap and 25 mL effective treatment volume (Qin et al., 1995) was used for treatment. 12, and 20 exponentially decaying pulses, corresponding to 30 and 50 microseconds, at electric field intensities of 35, 40 kV/cm and 2.5±0.1 μs pulse width were delivered to the treatment chamber by discharging a 0.5 μF capacitor. The pulse width approximated by the electrical circuit's time constant, time taken for the voltage to decay 37% (i.e. 1/e) of its maximum value, and the treatment time was calculated by multiplying pulse width (τ) with the number of pulses (n) applied (Góngora-Nieto et al., 2004). Chilled apple cider (4ºC) was pumped at a flow rate of 72 L/h. Inlet and outlet temperatures, pulse shape, voltage, current, and energy flow were monitored throughout the process. Electrical parameters were monitored with a digital oscilloscope (Hewlett-Packard 54530A, Colorado Springs, CO) connected to the treatment chamber through high voltage probes.

2.2. Microbiology

Two milliliters of frozen *S. cerevisiae* (ATCC 16664) were obtained from stock stored at -21°C. Thawed *S. cerevisiae* (ATCC 16664) was then added to 100 ml Sabouraud Dextrose Broth (Difco, Becton Dickinson, Sparks, MD) for growth on a rotary platform shaker (MSB-3322A-I, GS Blue Electric, Blue Island, IL) at 30°C and 225 rpm until reaching the early stationary phase.

Bacterial concentration was determined by the streak plating on standard methods agar (BBL, Becton Dickinson, Sparks, Md.) and dichloran-rose bengal-chloranphenicol agar (Difco, Benton Dickinson), for total microbial count and *S. cerevisiae*, respectively. Total microbial count samples were incubated at 35±0.5°C for 48 hours, and *S.cerevisiae* samples were incubated at 22±0.5°C (room temperature) for 5 days. Inactivation effectiveness was quantified by determining the logarithmic difference between the numbers of bacteria before and after treatment, and expressed as logarithmic cycle's reduction. Each study was conducted triplicate using independent prepared batches and fresh bacteria for every replicate.

2.3. Color

CIE Lab color parameters, light to dark (L^*) (100 to 0), red (+) to green (-) (a^*), and yellow (+) to blue (-) (b^*) were determined by using a Minolta CM-2002 spectrophotometer (Minolta, Camera Co., Osaka, Japan). 25 milliliters of apple cider were measured into a glass container (diameter 40 mm; height 10 mm) and a HunterLab standard white plate, (L=96.93, a^*=-0.12, b^*=-0.17) was used as background for measuring color parameters. The deviation

of color indices (L, a and b) of the apple cider sample from the indices of reference white plate was used to calculate the total color difference. The total color difference (ΔE^*) was calculated as (Marcus, 1998):

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \qquad (1)$$

2.4. Titratable Acidity and pH

Titratable acidity was determined according to the AOAC 942.15B (AOAC, 1990) method described by Sadler (1994). 5 milliliters of apple cider sample was diluted with 5 milliliters of deionized water and titrated with 0.1 N NaOH to a pH beyond 8.1 in the presence of phenolphthalein as the indicator. The predominant acid in apple, malic acid is expressed as the acid percentage:

$$\%acid = \frac{\text{base normality (mEq/mL) x mL base x Eq. Wt. of acid (mg/Eq)}}{\text{sample weight (g) x 10}} 100 \qquad (2)$$

Before and after each test, pH values of apple cider were recorded with a digital bench top pH meter (Orion-Thermo, Beverly, MA) standardized by using buffer solutions of pH 4.0 and 7.0.

2.5. PPO Assay

Polyphenoloxidase (PPO) activity was evaluated at 25˚C using an 8452A diode array spectrophotometer (Hewlett-Packard, Palo Alto, CA) at 420 nm. The reaction mixture

consisted of 1 ml buffer, 0.5 ml catechol (0.175 M) and 0.25 ml apple cider. The linear portion obtained by plotting the reaction time versus absorbance was used to compute the enzyme activity units (EAU). One unit of PPO activity was defined as 0.001 ΔA_{420} $min^{-1}ml^{-1}$ (Pizzocaro et al., 1993). All samples were analyzed in triplicate.

2.6. Statistical Analysis

Each PEF treatment was performed in triplicate and the data obtained was analyzed by linear regression using Microsoft Excel program (Microsoft Inc., Redmond, WA.). The analysis of variance (ANOVA) and least significant difference multiple comparison test (P=0.05) were conducted by SAS (SAS Institute, 1999) to determine the significant difference between shelf life samples of different treatments and within each sample during the shelf life storage.

3. Results and Discussion

PEF treatment in the presence of selected antimicrobials enhanced yeast inactivation in apple cider (Table 1). Yeast reduction (CFU/ml) after PEF treatment at 40 kV/cm and 30 µs (12 pulses) without antimicrobials was 4.5 log cycles. After PEF treatment (40 kV/cm and 30 µs) in the presence of sodium benzoate, potassium sorbate, cinnamic acid and hydrogen peroxide additional reductions of 0.5, 0.6, 1.2 and 0.6 log cycles were obtained, respectively. The effectiveness of selected antimicrobials differentiated from each other only when the treatment time was 30 microseconds for both electric field intensities tested (35-40 kV/cm). Even for the lower level of electric field and treatment time, the lethality of cinnamic acid in combination with PEF was significantly higher than the other antimicrobials tested in this study. Cinnamic acid is a naturally occurring phenolic antimicrobial with an aromatic ring

and hydroxyl groups in its structure, showed the most effective inactivation regardless of the electric field strength applied. The mechanism of phenolic compounds for microbial inactivation is generally attributed to their effects on cellular membranes (Davidson, 1993) where their interference with cell membrane permeability causes leakage of cellular components and inhibition of active transport of nutrients (Eklund, 1985). The suggested mode of action for cinnamic acid on cellular membranes was enhanced with PEF due to the influence of electric fields on cell membrane.

S. cerevisiae is one of the two yeasts in particular relevance for juice spoilage (Barbosa-Cánovas et al., 1999). In general *S. cerevisiae* cells have been found less resistant to PEF treatment than other vegetative cells which is mostly attributed to their large size (Harrison et al., 1996). It is possible to increase effectiveness of PEF on yeast inactivation with the use of most convenient antimicrobial in terms of microbial safety and juice quality. If the proper antimicrobial that retains quality over prolonged storage could be selected it is possible to assure legal microbial reduction requirements without over processing the product.

The aim of this part of the study was to select the optimum PEF conditions (electric field intensity and treatment time) to be used in the shelf life study. *S. cerevisiae* population reduced by 6 log cycles after treatment with 40 kV/cm and 50 µs (20 pulses) regardless of the antimicrobials used; therefore, PEF treatment with these conditions in the presence of selected antimicrobials would provide basis to compare the changes (microbiological, chemical and physicochemical) in apple cider samples containing different antimicrobials during shelf life storage.

3.1. Microbial Shelf-life

Shelf life study was conducted for six weeks. *S. cerevisiae* (ATCC 16664) and total bacterial count during refrigerated and room temperature storage of apple cider in the presence of selected antimicrobials with control (fresh apple cider) are shown in Figure 1. Neither yeast nor microbial growth was detected in PEF treated samples during six weeks of storage. Untreated fresh apple cider was considered as spoiled when the initial microbial load of 10^2 has reached 10^4. The shelf life of untreated apple cider was less than 10 days at refrigerated storage (4˚C) and in 5 days at room temperature (22˚C). Compared to untreated apple cider sample, all PEF treated samples regardless of the selected antimicrobials used, achieved 6 weeks of shelf life stability in all storage conditions tested. Further observation of apple cider samples after 90 days of storage in refrigeration showed that the PEF treated samples in the presence of hydrogen peroxide spoiled first among all samples with different antimicrobials.

The origin and mechanism of action of hydrogen peroxide is different from other antimicrobials tested in this study. Among several different theories proposed to explain the mechanism involved in the cell inactivation by hydrogen peroxide, according to Brock et al. (1984) the decomposition products of hydrogen peroxide such as singlet or superoxide oxygen are responsible for the bacterial inactivation. Superoxide oxygen for being a higher energy form of oxygen is extremely reactive against microbial cells, yet it is also generated via specific enzyme systems that naturally occur in milk, honey, and living cells such as lactoperoxidase and myeloperoxidase enzyme systems (Fridovic, 1975). In another theory proposed by Gould and Dring (1962), the antimicrobial activity of hydrogen peroxide is due to the oxidation of sulfhydrly groups and double bonds in proteins, lipids, and surface

membranes. The proposed theories for the antimicrobial activity of hydrogen peroxide suggest that this antimicrobial is effective as a lethal agent when the initial microbial load is high; however, for prolonged storage of juices the preservative effect is not as efficient as the other antimicrobials tested in this study probably due to the unstable nature of the compound. In order to assess the efficiency of selected antimicrobials to preserve storage quality of apple cider, other attributes including color, titratable acidity, pH and PPO activity were measured.

3.2. Color

The initial color parameters L* (light), a* (green) and b*(yellow) of apple cider after PEF treatment (40 kV/cm, 50 μs) without antimicrobials were measured as 36.11, -0.44 and 16.46, respectively. The total color deviation (ΔE*) of this sample from the white reference point was 65.98 (Figure 2). Color parameters of PEF-only sample indicated that, in the absence of selected antimicrobials, PEF treated apple cider was the lightest and the least yellow sample among all other samples ($P<0.01$). A comparison of the overall color change (ΔE*) of samples at the beginning of the shelf life storage showed that PEF treatment without selected antimicrobials resulted in loss of color for being the closest to the white reference point. This result could be explained by the color fading effect of PEF due to the inactivation of browning enzymes by electric fields and generally accepted as insignificant and unnoticeable (Zárate-Rodríguez et al., 2000). Color fading was not significant for the samples treated by PEF in the presence of antimicrobials, suggesting that tested antimicrobials might have prevented color loss by enhancing the remaining enzyme activity.

PEF treatment in the presence of selected antimicrobials did not significantly changed the overall color (ΔE*) of apple cider during six weeks of refrigeration storage at 4˚C. Additionally, no significant difference among selected antimicrobials were observed in terms of their contribution to the color (ΔE*) development in apple cider after six weeks of storage (4˚C) (Figure 2a). In the case of room temperature storage (22˚C), ΔE* value of all samples significantly increased representing gradual increase in color development with respect to the reference point (white). Among selected antimicrobials, PEF treated sample in the presence of potassium sorbate was significantly different ($P<0.05$) than other antimicrobials with the highest ΔE* value representing the color change of this sample was significant after six weeks of room temperature storage (Figure 2b).

Results obtained from this part of the study were in accordance with the literature where the color of PEF treated apple juice remained unchanged in refrigerated storage (Evrendilek et al., 2000). Regarding to color change in room temperature storage, we know that the major contributor of ΔE* value is the L* (lightness) parameter (Guerrero-Beltrán and Barbosa-Cánovas, 2005). The observed darkening during extreme temperature storage might be due to the conversion of ascorbic acid to furfural under prolonged storage as suggested by (Zhang et al., 1997).

Exploring the individual color parameters contributing to the overall color development, we observed that L*, a* and b* values of the PEF treated apple cider in the presence of antimicrobials did not significantly changed during six weeks of refrigeration storage (Figures 3a, 4a, and 5a). A comparison between the tested antimicrobials at (4˚C) showed

that sample with potassium sorbate was significantly darker than the PEF-only treatment sample. In the case of room temperature (22˚C), after six weeks of storage, apple cider with hydrogen peroxide and cinnamic acid observed to be the darkest and the lightest, respectively (Figure 3b).

For being the most efficient antimicrobial for inactivating yeast in combination with PEF, cinnamic acid, is the least water soluble compound among tested antimicrobials. Addition of this antimicrobial to the apple cider and mechanical mixing prior to the PEF process resulted in a suspension with stability for less than 24 hours. Therefore, after 24 hours of storage, sedimentation was observed in samples containing cinnamic acid. The consistency of color measurements might have partially affected due to the presence of sediments in the sample, as observed in the color parameter trends with respect to storage time.

Samples containing hydrogen peroxide and potassium sorbate were significantly different from others in terms of a* and b* parameters in both storage conditions (Figures 4 and 5). Hydrogen peroxide is a disinfecting agent mostly effective reducing the initial microbial load of the samples while preservation of quality during storage might not be that successful due to the adverse affects of hydrogen peroxide on enzymes after prolonged exposure (Gerald et al., 1998). The observed discoloration with lower a* (green) and b* (yellow) values in hydrogen peroxide containing samples could be due to the highly reactive decomposition products of hydrogen peroxide such as singlet or superoxide oxygen and possible reaction of these compounds with color pigments (Nassau, 1998) in apple cider might have resulted color change during room temperature storage.

Consumer preference for the clarity and color of apple cider can be variable based on regional differences where golden yellow and cloudy apple cider is generally preferred. Therefore noticeable darkening achieved by the addition of antimicrobials would be preferable if legally required juice safety is ensured. In general, the overall contribution of selected antimicrobials to color development in apple cider was insignificant in refrigerated storage for six weeks. However, for room temperature conditions (22˚C), which can be accepted as an estimation for accelerated shelf life testing, all antimicrobials tested in this study significantly contributed darkening of the apple cider.

3.3. pH

ANOVA for pH change data after six weeks of storage in 4˚C and 22˚C showed that pH did not significantly changed for the selected antimicrobials except cinnamic acid (Figures 6). The pH of the cinnamic acid containing sample increased from 3.63 to 3.78 which could be explained by the sedimentation of the antimicrobial at the beginning of the shelf life storage. After six weeks of storage, potassium sorbate and hydrogen peroxide containing samples became the less (3.91) and the most acidic (3.67) samples, respectively, while the initial pH of the fresh apple cider was 3.69. By increasing the confidence level of statistical analysis, such differences may be considered negligible for practical purposes, since the measured pH values vary between 3.7 and 3.9. The results obtained from this part of the study were in accordance with the microbiological results since pH change is generally correlated with microbial spoilage during shelf life studies of PEF treatment (Rodrigo et al., 2005).

The influence of treatment medium pH on microbial resistance to PEF treatment has been studied by Hülsheger et al. (1980) first and no correlation was reported. However, later studies proved that inactivation rate of several microorganisms were significantly higher under acidic conditions where low pH of the medium affects the cytoplasm disturbing the homeostasis of the cells, increasing the sensitivity of the microbial membranes to permeabilization during PEF (Vega-Mercado et al., 1996). Additionally, recovery of sublethal injury after PEF treatment is prevented by acidic conditions (Wouters el al., 2001). The lower pH of the apple cider maintained by cinnamic acid during the PEF process might have contributed to the microbial effectiveness of this compound.

3.4. Titratable Acidity

Acidity in apple juice is an important sensory attribute associated with its characteristic flavor and astringency. Malic acid is the predominant acid in apple cider and titratable acidity is expressed as the malic acid percentage. Malic acid composition of apples is reported in the range of 0.27-1.02 % with typical Brix of 9.12-13.5. When acids compose more than 0.2 percent of a food, they have a strong impact on flavor perception (Sadler, 1994).

The contribution of selected antimicrobials tested in this study to the titratable acidity of apple cider was not significantly different and no significant statistical difference was observed during shelf life storage as shown in Figure 7 ($P<0.05$). The acidity increase was limited to 0.1% and when the confidence limit is reduced to determine individual impact of each antimicrobial on the titratable acidity of apple cider, only cinnamic acid containing sample observed to be significantly different than the PEF-only treatment. The acidity of

apple cider samples containing antimicrobials varied in the range of 0.35-0.45% indicating that they all have impact on flavor perception. Therefore, the results obtained from this part of the study highlights the need for a sensory evaluation especially for the cinnamic acid containing sample (0.45%).

3.5. PPO Activity

Figure 8 represents the remaining PPO activity of apple cider after combination and PEF-only treatments during refrigerated storage. The initial PPO activity of 1,200 EAU in apple cider was reduced to 30% after all PEF treatments regardless of the antimicrobial added and the remaining enzyme did not change significantly during the entire storage period of six weeks at 4°C. Considering the insignificant difference between PEF-only and antimicrobial added PEF treatments, the results show that the important enzyme activity inhibition mechanism is the PEF itself rather than the tested antimicrobials. Electric field intensity of 40 kV/cm for 50 microseconds applied in this study was enough to reduce the PPO activity by 70%. Similarly, Giner et al. (2002) reported 95 % reduction of PPO activity in apple juice after 24.6 kV/cm electric field for 6 ms with treatment temperature at 15˚C. In another study reported by Van Loey (2002) the inactivation of PPO was related to the thermal effects of PEF process with higher pulse frequency.

This study focused on the possible adverse effects of selected antimicrobials used in combination with optimum PEF conditions on the storage quality of apple cider. Comparative evaluation of difference between selected antimicrobials would enable selection of the most convenient antimicrobial to be used in combination with PEF. Any antimicrobial

could provide synergistic inactivation with PEF but the effects on quality attributes under different storage conditions should be considered. Based on findings in this study, we can conclude the requirement for refrigeration storage for PEF treated juices in the presence of selected antimicrobials as the quality attributes monitored remained unchanged through out six weeks of shelf life. Among all antimicrobials tested, cinnamic acid, sodium benzoate and potassium sorbate were favored in combination with optimum PEF conditions for pasteurization of apple cider. Hydrogen peroxide, for being a disinfectant rather than a preservative with the ability of rapid killing of microorganism did not provide long-lasting action. Considering no adverse effects were observed during shelf life storage, and successful microbial and yeast inactivation, further research eliminating the solubility problem of cinnamic acid was recommended as well as a sensory panel for flavor perception of the PEF treated apple cider in the presence of selected antimicrobials.

4. Conclusions

Antimicrobials tested in this study achieved significant synergistic response to yeast inactivation when combined with optimum PEF conditions while refrigeration storage was essentially required for PEF processed apple cider in the presence of selected antimicrobials. Microbial and physicochemical quality attributes of apple cider remained unchanged more than 6 weeks in refrigeration storage. Based on observed changes in microbial and physicochemical quality during storage (color, pH, titratable acidity and PPO activity), the use of cinnamic acid, sodium benzoate and potassium sorbate were favored in combination with optimum PEF conditions for pasteurization of apple cider. Hydrogen peroxide, for being a disinfectant rather than a preservative with the ability for rapid inactivation of

microorganism, did not provide long-lasting preservative action during prolonged storage. This study provides evidence for the successful use of PEF in combination with selected antimicrobials as a potential alternative to thermal pasteurization. Further research to eliminate the insolubility problem of cinnamic acid is recommended as well as a sensory panel for flavor perception of the PEF treated apple cider in the presence of selected antimicrobials.

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Table 1. Mean inactivated *S.cerevisiae* (ATCC 16664*)* population (log CFU/ml) in fresh apple cider treated by PEF in combination with selected antimicrobials (1000 ppm). (PEF-only treatment was used as the control)

Electric Field (kV/cm)	Treatment Time (μs)	PEF-only	Sodium benzoate	Potassium sorbate	Cinnamic acid	Hydrogen peroxide
40	50.0	-5.5±0.1	-6.0±0.0	-6.0±0.0	-6.0±0.0	-6.0±0.0
	30.0	-4.5±0.1	-5.0±0.1	-5.1±0.1	-5.7±0.1	-5.1±0.1
35	50.0	-5.3±0.1	-6.0±0.1	-6.0±0.1	-6.0±0.1	-6.0±0.1
	30.0	-3.3±0.1	-3.6±0.1	-3.7±0.2	-5.5±0.2	-4.0±0.2

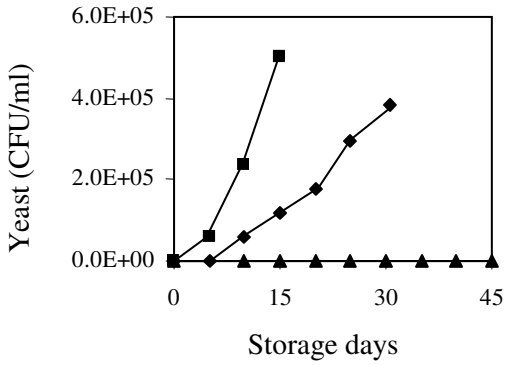
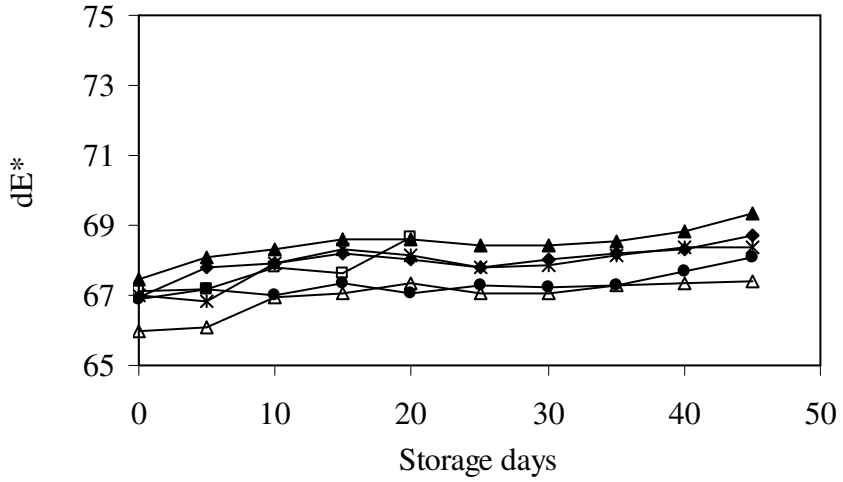


Figure 1. Yeast growth of apple cider during storage for:

(■) fresh (untreated) apple cider at 22˚C, (♦) fresh (untreated) apple cider at 4˚C, and

(▲) PEF treated apple cider (with and without antimicrobials) at 4˚C and 22˚C.



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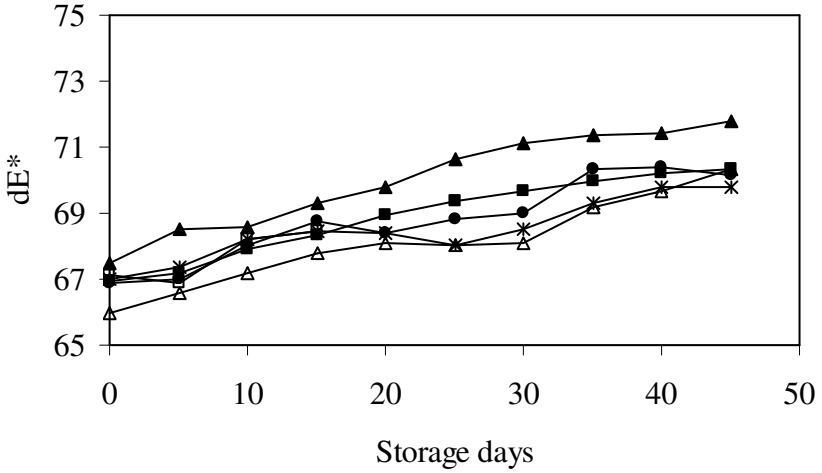
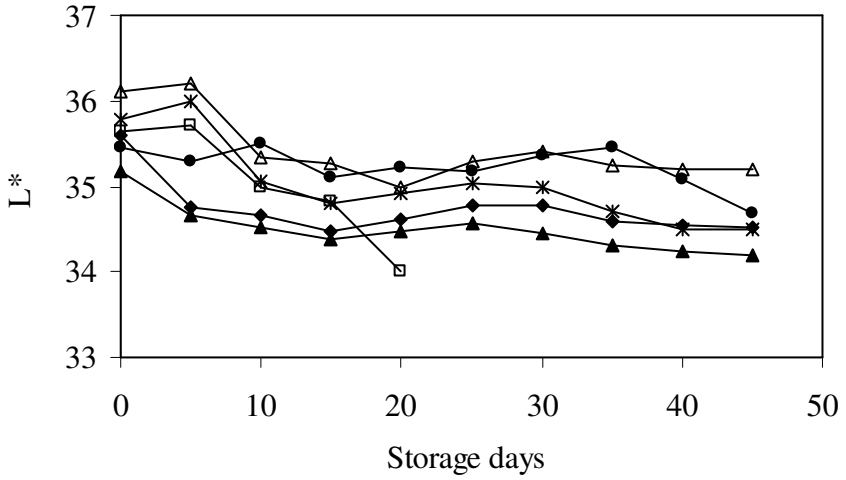


(b)

Figure 2. ΔE* color change of apple cider treated with PEF (40kV/cm, 50μs) in the presence of: (♦) sodium benzoate; (▲) potassium sorbate; (*) hydrogen peroxide; (●) cinnamic acid, during storage at: (a) 4˚C and (b) 22˚C.

(Δ) PEF-only treatment (40kV/cm, 50μs), (□) non-treated (fresh) apple cider.



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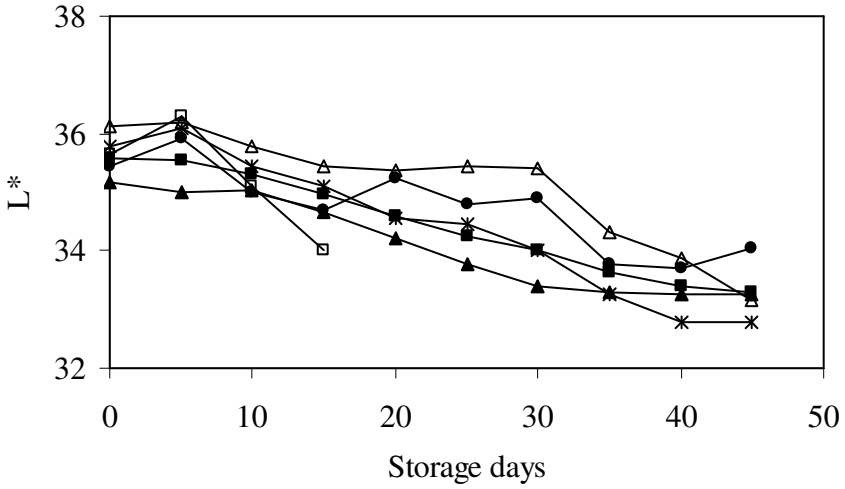
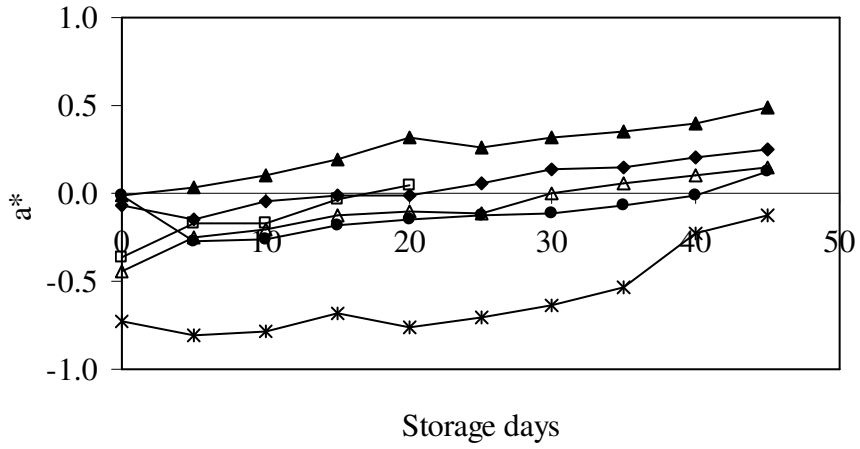


(b)

Figure 3. L* color change of apple cider treated with PEF (40kV/cm, 50µs) in the presence of: (♦) sodium benzoate; (▲) potassium sorbate; (*) hydrogen peroxide; (●) cinnamic acid, during storage at: (a) 4˚C and (b) 22˚C.

(Δ) PEF-only treatment (40kV/cm, 50µs), (□) non-treated (fresh) apple cider.



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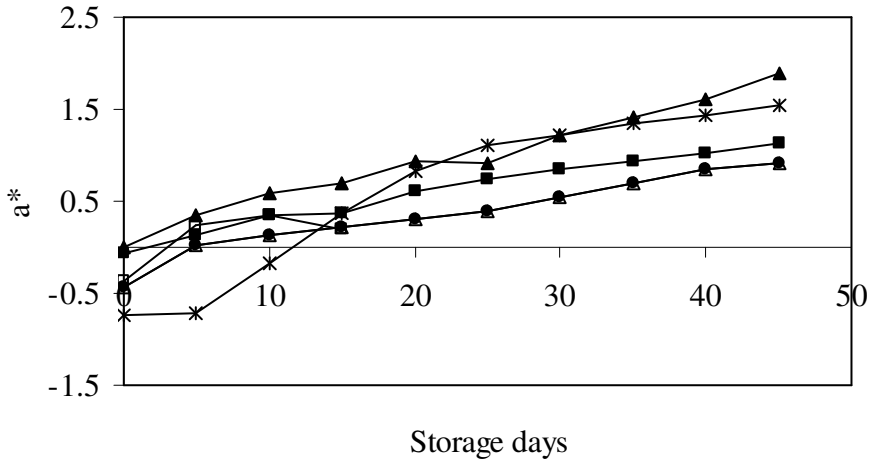
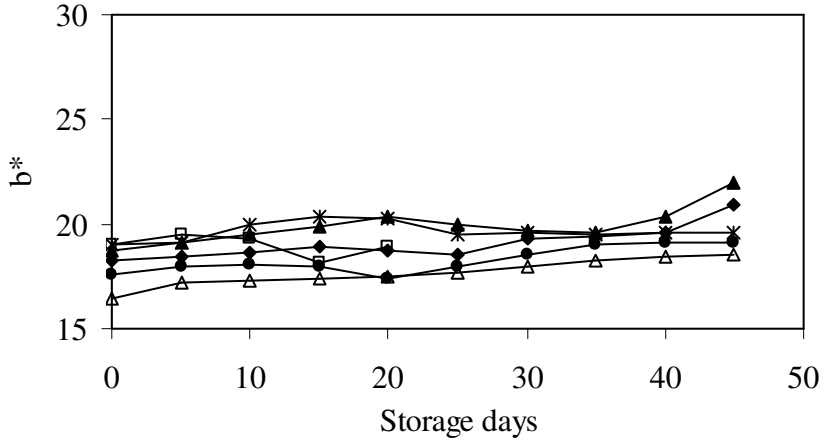


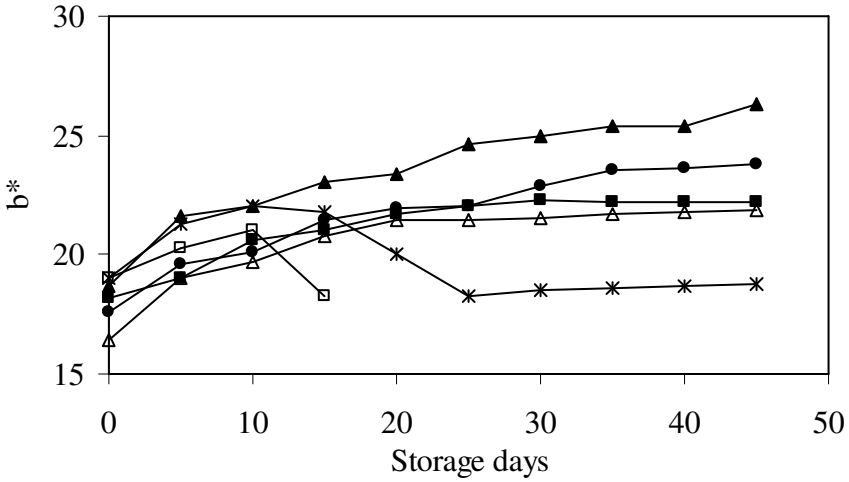
(b)

Figure 4. a* color change of apple cider treated with PEF (40kV/cm, 50µs) in the presence of: (♦) sodium benzoate; (▲) potassium sorbate; (*) hydrogen peroxide; (●) cinnamic acid, during storage at: (a) 4˚C and (b) 22˚C.

(Δ) PEF-only treatment (40kV/cm, 50µs), (□) non-treated (fresh) apple cider.



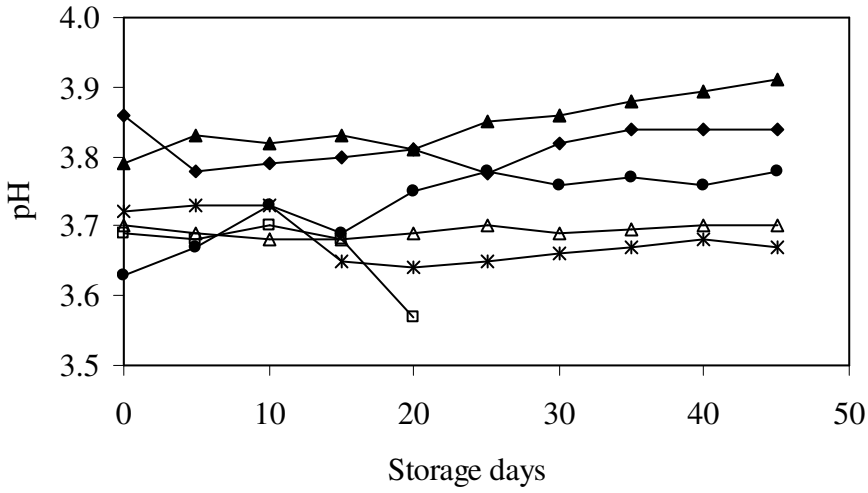
(a)



(b)

Figure 5. b* color change of apple cider treated with PEF (40kV/cm, 50µs) in the presence of: (♦) sodium benzoate; (▲) potassium sorbate; (*) hydrogen peroxide; (●) cinnamic acid, during storage at: (a) 4˚C and (b) 22˚C.

(Δ) PEF-only treatment (40kV/cm, 50µs), (□) non-treated (fresh) apple cider.



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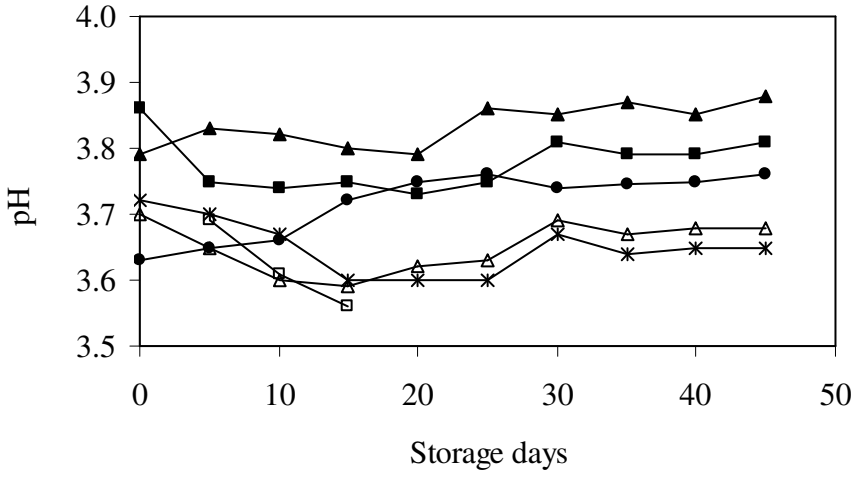
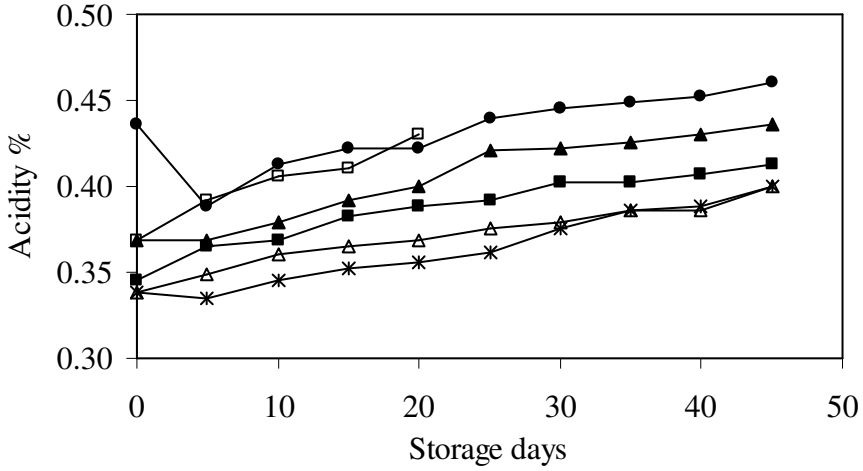


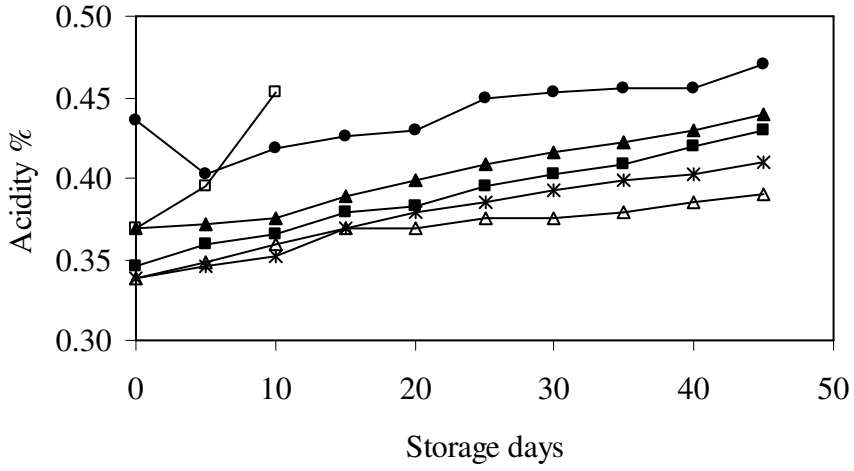
(b)

Figure 6. pH change of apple cider treated with PEF (40kV/cm, 50µs) in the presence of: (♦) sodium benzoate; (▲) potassium sorbate; (*) hydrogen peroxide; (●) cinnamic acid, during storage at: (a) 4˚C and (b) 22˚C.

(Δ) PEF-only treatment (40kV/cm, 50µs), (□) non-treated (fresh) apple cider.



(a)



(b)

Figure 7. Titratable acidity change of apple cider treated with PEF (40kV/cm, 50µs) in the presence of: (♦) sodium benzoate; (▲) potassium sorbate; (*) hydrogen peroxide; (●) cinnamic acid, during storage at: (a) 4˚C and (b) 22˚C.

(Δ) PEF-only treatment (40kV/cm, 50µs), (□) non-treated (fresh) apple cider.

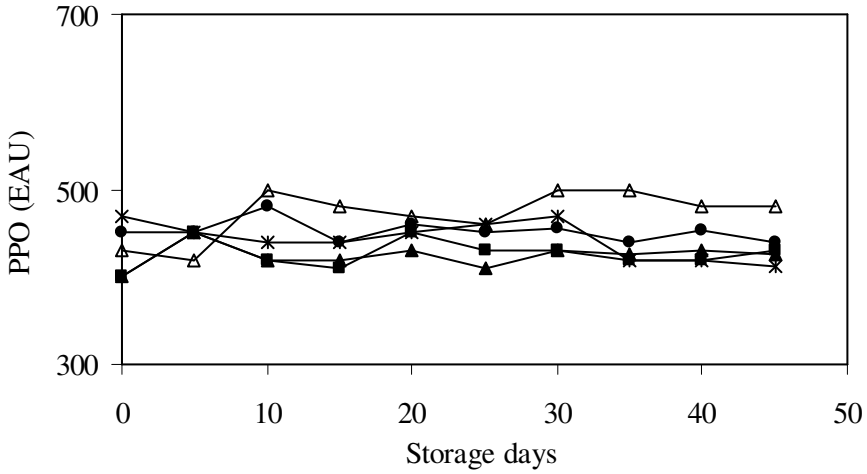


Figure 8. Polyphenoloxidase activity change of apple cider treated with PEF (40kV/cm, 50μs) in the presence of: (♦) sodium benzoate; (▲) potassium sorbate; (*) hydrogen peroxide; (●) cinnamic acid, during storage at 4˚C.

(Δ) PEF-only treatment (40kV/cm, 50μs)

CHAPTER SEVEN

CONCLUSIONS AND RECOMMENDATIONS

Pulsed electric fields (PEF) technology is under research for nearly 45 years as a method to preserve food products and its effectiveness in reducing microbial populations is already established. This study demonstrates the possibility of increasing the efficiency of PEF by using this technology in combination with selected antimicrobials to preserve apple cider while retaining the quality attributes during refrigerated storage. Combination processes were developed by testing selected levels of PEF treatment conditions in the presence of selected antimicrobials. The impact of each antimicrobial together with PEF on microbial inactivation was quantified and compared by using selected mathematical models. This study also points out the need for understanding engineering principles behind PEF technology by exploring the effect of electrical components, energy density and fluid flow rate on microbial inactivation as well as the potential source of variation in PEF system efficiency. The findings identified in this study can be grouped into four main areas.

Microbial Inactivation

In the first part of this dissertation, following an extensive literature review on the fundamental aspects of PEF technology and applications for food preservation, it was hypothesized that if proper antimicrobials are used in combination with optimum PEF conditions, it is possible to achieve significant bacterial inactivation without over processing the food. Antimicrobials tested in this study, sodium benzoate, potassium sorbate, cinnamic acid and hydrogen peroxide, achieved 7 log cycles reduction of *E.coli* (ATCC 11775) when used in combination with 35-40 kV/cm electric field intensity and 50 microseconds treatment

time. Inactivation of *E.coli* (ATCC 11775) significantly increased when higher electric field intensities and longer treatment times were used regardless of the antimicrobial type and concentration. Among the four selected antimicrobials, cinnamic acid was the most effective antimicrobial in terms of bacterial inactivation, followed by hydrogen peroxide, potassium sorbate and sodium benzoate. The extent of antimicrobial activity varied among selected antimicrobials and the difference between inactivation efficiencies was suggested to be due to their mechanism of action on bacterial cells. Moreover, the electrical properties of antimicrobial solutions may contribute to antimicrobial lethality. Considering solubility, regulatory issues and ease of processing, potassium sorbate and sodium benzoate were recommended for use in combination with PEF treatment for enhanced inactivation of *E.coli* (ATCC 11775) in apple cider. This research presents combinations of PEF and selected antimicrobials successfully met FDA requirements for juice pasteurization by ensuring a 5 log-cycle reduction of *E. coli* (ATCC 11775) cells in fresh apple cider for electric fields greater than 35 kV/cm, at 20 pulses. The results obtained in this study can be extrapolated to juices with similar properties if proper antimicrobials and optimum treatment conditions are satisfied.

Modeling

In the second part of this research, the individual impact of each selected antimicrobial in combination with selected PEF conditions on inactivation of *E.coli* (ATCC 11775) was explored. Four mathematical models (Bigelow, Hülsheger, Peleg and Weibull) commonly used to predict microbial response against PEF treatment were used and the accuracy factors of models for describing the combined inactivation effect of selected antimicrobials and PEF

were compared. The Weibull survival function provided the best model to describe the inactivation of *E.coli* (ATCC 11775) by PEF treatment in the presence of selected antimicrobials as verified by accuracy factors in a range of 1.06-1.21. Inactivation of *E.coli* by PEF in the presence of cinnamic acid resulted in a completely concave upward survival curve where the survival curves for the other three antimicrobials tested were generally concave downward at higher electric fields (35-40 kV/cm) and almost linear at lower electric fields (25-30 kV/cm). The observed difference between the concavities of the survival curves suggested to be due to the different mechanism of inactivation, i.e. the effects on cellular membranes and intracellular components, dominated by selected antimicrobials during PEF. Being the simplest model used, the Bigelow model was insufficient to describe antimicrobial synergism to PEF. The Hülsheger model was also unable to predict the microbial response; however, provided critical electrical field and treatment time values to be used as insightful and quick comparison tools for the process with less accuracy (2.23-4.76). Among two-parameter models, Peleg's model based on Fermi's distribution successfully predicted the microbial response against combined stress factors with accuracy factors (1.18-2.5); however, this was limited to certain antimicrobials (hydrogen peroxide, sodium benzoate, potassium sorbate). The ability of Peleg's model to describe concavities of the survival curves suggested that the system's resistance to stress factors is changing throughout the process and a more flexible model with more fit parameters is required. The use of Weibull distribution model with three parameters enabled the observation of varying microbial response against different antimicrobials under intense PEF conditions (35-40 kV/cm and 50 μs). Weibull distribution model is recommended for being a flexible and accurate model with the ability to fit inactivation curves attained from both thermal and nonthermal processes.

Shelf-life

The third part of this research explored the effects of selected antimicrobials on quality attributes of apple cider during refrigeration and room temperature storage. For its common occurrence in spoiled juices, *S. cerevisiae* was selected as the target microorganism. Antimicrobials tested in this study achieved significant synergistic response to yeast inactivation when combined with optimum PEF conditions. Refrigeration storage was recommended for PEF processed apple cider in the presence of selected antimicrobials. Microbial and physicochemical quality attributes of apple cider remained unchanged more than 6 weeks under refrigeration storage. Based on observed changes in quality during storage (color, pH, titratable acidity and PPO activity), the use of cinnamic acid, sodium benzoate and potassium sorbate were favored in combination with optimum PEF conditions for pasteurization of apple cider. Hydrogen peroxide, for being a disinfectant rather than a preservative with the ability of rapid inactivation of microorganism did not provide long-lasting preservative action during prolonged storage. This study provided evidence for the successful use of selected antimicrobials in combination with PEF as a potential alternative to thermal pasteurization.

Engineering Aspects

Finally, based on the experiments conducted in the first three parts of this research, the engineering aspects contributing to the efficiency of the PEF system were evaluated. The electrical properties of the product, energy density (energy per pulse) with respect to temperature rise and product flow rate were assessed to be some of the factors affecting microbial inactivation and influencing the efficiency of the PEF system.

The electrical properties as a function of temperature and concentration were investigated first. The electrical conductivity of buffer solution varied 1-2.5 S/m for a temperature rise of 30˚C where the rate of increase was higher for concentrated solutions. Based on the fact that conductivity is inversely proportional to resistivity, energy delivered to the treated product is governed by the resistance of the treatment chamber. Therefore, the temperature dependence of electrical conductivity was highly effective on the PEF efficiency.

The flow rate of the apple cider contributed to the observed temperature rise inside the treatment chamber by increasing the energy density. 5 log cycles of *E.coli* (ATCC 11775) were inactivated by using a flow rate of 0.8 L/min with PEF (35 kV/cm and 50 μs). With the selected PEF conditions and flow rate, the energy consumption during the PEF process was calculated as 167 J/ml.

Based on an energy balance over the PEF system by comparing the input electrical energy and energy output as the heat dissipation, the PEF system performance deviated from ideality for intense PEF conditions. As the charging voltage increased, the value of φ (ratio of voltage in the treatment chamber to the charging voltage) became smaller, possibly due to the increase in conductivity. Increasing pulse frequency and voltage both resulted in temperature rise; however, the impact of pulse frequency was greater than the impact of the voltage rise, especially at high voltages (30-35 kV).

In this study, the obtained results enabled to take a step forward by pointing out the potential factors and their relation that influence the PEF system performance. However, further

research investigating the electrical circuit of the PEF system is required. Measuring the distribution and proportion of the charging voltage around each component of the electrical circuit by using the principles of electrical engineering would help to optimize PEF parameters.

Overall, this study proves that the use of PEF in combination with the most suitable food antimicrobial providing synergistic effect on microbial inactivation, stability during storage, ease of processing, regulatory approval and feasible cost, has great potential for industrial level. Such combination process will yield microbiologically safe products with improved quality attributes and extended shelf-life.

Continued research on the use of pulsed electric fields for food preservation with more effective and affordable processing systems will help to overcome hurdles and make commercial processing available in the near future. The specific areas of interest identified during the conduction of this dissertation that require further research are summarized:

- Application of PEF in combination with other natural antimicrobials providing synergism on microbial inactivation. Mild heat treatment may also be included to the process as a complementary step to increase the efficiency of both PEF and antimicrobials for food safety and quality.
- Evaluation of sensory properties of apple cider treated with PEF in the presence of natural antimicrobials and determination of consumer acceptance.

- Formulation of cinnamic acid derivatives in the form of soluble salts to solve insolubility problem of cinnamic acid. Salts of organic acids, potassium sorbate or sodium benzoate can be used in relatively low concentrations together with cinnamic acid to provide stability and ease of processing during PEF.
- Conduct of in depth studies for the effect of PEF on specific food components such as functional enzymes, vitamins, minerals and proteins.
- Standardization of protocols that clearly define PEF processing conditions. Electric field intensity and number of pulses are insufficient to describe the efficiency of a PEF process alone. Unique parameters such as energy density (energy per milliliter) should be used to facilitate communication among research groups.
- Verification of flow dynamics of the fluid inside the treatment chamber. Depending on the flow rate of the treated product, the characteristic of flow might change (switch from laminar to turbulent) and this would affect the distribution of electric fields inside the treatment chamber.
- Inline determination of metal release from electrodes to the treated product. High energy electrical pulses may cause metal release from the electrodes to the treated food product. Loading a metal detector to the current PEF system would be useful to detect metal release without interrupting the process.