Pulsed Electric Fields: Processing System, Microbial and Enzyme Inhibition, and Shelf Life Extension of Foods

Seacheol Min, Gulsun Akdemir Evrendilek, and Howard Q. Zhang

***Abstract*—Pulsed electric field (PEF) nonthermal food processing has been of growing interest owing to because of its excellent potential in providing consumers with microbiologically safe and fresh quality foods. Application of high-voltage electric fields at a certain level for a very short time by PEF not only inhibits pathogenic and spoilage microorganisms but also results in the retention of flavor, aroma, nutrients, and color of foods. This paper provides the most current information about PEF food processing. It reviews the systems for PEF processing and its effects on the inhibitions of microorganisms and enzymes and sensory and nutritional properties of foods. Regulatory issues of PEF processing are discussed as well.**

***Index Terms*—Enzyme inactivation, microbial inhibition, nonthermal process, pulsed electric field (PEF), shelf life.**

I. Introduction

PULSED electric field (PEF) is a food preservation method used to inhibit microorganisms in foods without causing significant loss of flavor, color, taste, and nutrients [1]. PEF processing has been successful in producing a variety of food products such as orange, apple, and cranberry juices, yogurt-based products, carbonated beverages, pea soup, skim milk, and liquid whole eggs [2]–[5]. PEF treatment uses a high-intensity electric field generated between two electrodes. A large flux of electrical current flows through food when a high-intensity electric field is generated. Nonthermal treatment is attained by use of a very short pulsewidth of treatment time (e.g., microseconds).

Commercialization of PEF technology has drawn the attention of people in the food industry and of food regulatory agents who wish to satisfy the consumer demands for fresh food products. PEF technology has developed rapidly, resulting in many publications regarding PEF treatment of foods. The objectives of this paper are to: 1) provide fundamental concepts of PEF processing; 2) review current reports on PEF research; and 3) suggest directions for future PEF research.

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II. PEF Systems

PEF treatment systems are composed of PEF treatment chambers, a pulse generator, a fluid-handling system, and monitoring systems.

A. Treatment Chambers

A PEF treatment occurs inside of a PEF treatment chamber, which houses electrodes and delivers a high voltage to a food material. Various types of PEF treatment chambers have been used for PEF treatments [6]. A uniform distribution of electric-field strength in PEF treatment chambers is desirable to ensure that each microbial cell within a population receives the same PEF treatment. The uniform distribution of electric-field strength also simplifies mathematical models, which predict microbial inhibition by PEF [7].

Treatment chamber design has improved from static treatment chambers to continuous treatment chambers.

1) Static Treatment Chambers: Static treatment chambers are mostly preferred in laboratory-scale experiments. Dunn and Pearlman [8] designed a circular parallel-plate chamber for both static and continuous PEF treatments with stainless steel electrodes and a nylon spacer. Zhang *et al.* [6] introduced a disk-shaped static PEF treatment chamber with two round-edged stainless steel electrodes with polysulfone or plexiglas spacer. This chamber design could support electric-field strengths up to 70 kV/cm.

2) Continuous Treatment Chambers: A continuous treatment chamber was initially invented by Dunn and Pearlman [8]. The chamber consisted of two parallel-plate electrodes and a dielectric spacer insulator. The electrodes were separated from food by conductive membranes made of sulfonated polystyrene and acrylic acid copolymers.

Coaxial and the cofield PEF treatment chambers are currently widely used due to their simplicity in structure [6]. Electrical current flows perpendicularly to food flow in coaxial PEF treatment chambers and in parallel to food flow in cofield flow PEF treatment chambers [1]. A cofield flow tubular PEF treatment chamber was invented by Yin *et al.* [9] and used in commercial-scale PEF systems [10], [11]. The chamber, introduced in the study in [10] and [11], consisted of two boron carbide tubular electrodes and a tubular ceramic insulator body. The chamber had an inner diameter of 0.808 cm in the cylindrical treatment zone and a distance of 1.270 cm between the electrodes. This PEF treatment chamber could be connected up to eight in

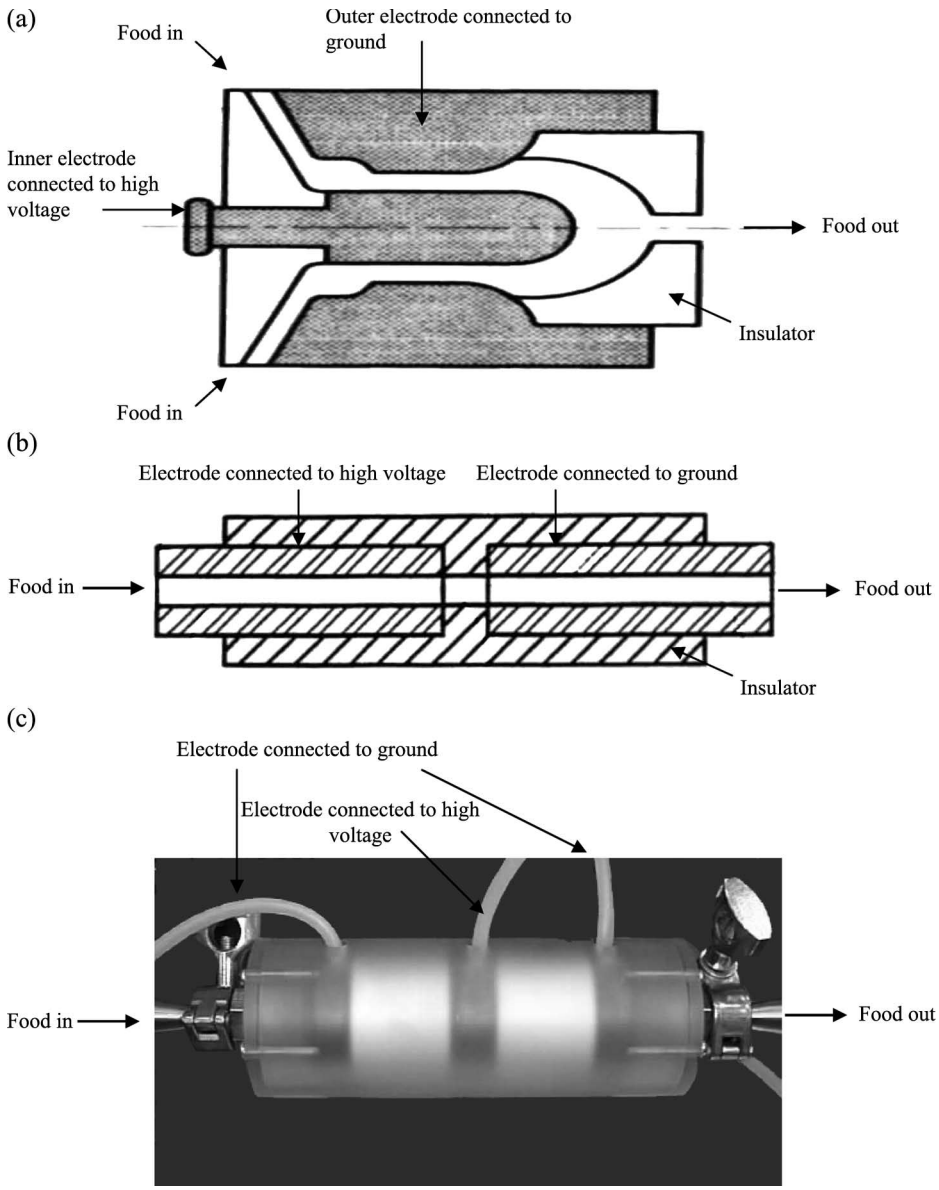

Fig. 1. Diagrams of (a) coaxial flow (Washington State University) and (b) cofield flow PEF treatment chambers. (c) Picture of a cofield flow PEF treatment chamber for a commercial-scale PEF system (The Ohio State University).

parallel for electrical flow and in series for fluid flow for the commercial-scale PEF system.

Diagrams of most current coaxial flow and cofield flow PEF treatment chambers and a picture of a cofield flow PEF treatment chamber used for a commercial-scale PEF system (OSU-6, The Ohio State University, Columbus) are shown in Fig. 1.

B. Pulse Generator

Pulse generators convert low voltage into high voltage and provide the high voltage to PEF chambers [12]. Square, exponential decay, or oscillatory pulses are generally used for PEF treatment [6]. A low-level voltage is collected and stored in a capacitor. Stored voltage at a high level is discharged instantaneously. Generation of a high-voltage PEF within a food in a treatment chamber requires a large flux of electrical current through the food for microseconds [6]. Due to a very short period of discharge time (i.e., microseconds), heating of the foods is minimized [6].

Fig. 2 illustrates the circuit diagrams for a bench top-scale, a pilot plant-scale, and a commercial-scale pulse generators (The Ohio State University), respectively. The laboratory-scale PEF system and a later model of pilot plant-scale PEF systems were based on a patented design [13] using semiconductor H-bridge switches coupled with an output pulse transformer. The rated voltage for the laboratory systems is 15 kV and for the pilot systems is 35 kV, both with bipolar square wave pulse shape. The commercial-scale pulse generator provided bipolar square waveform pulses with a maximum peak voltage of $\pm$60 kV and a maximum peak current of 600 A into multiple PEF chambers during PEF processing [10], [11]. The 60-kV power supplies

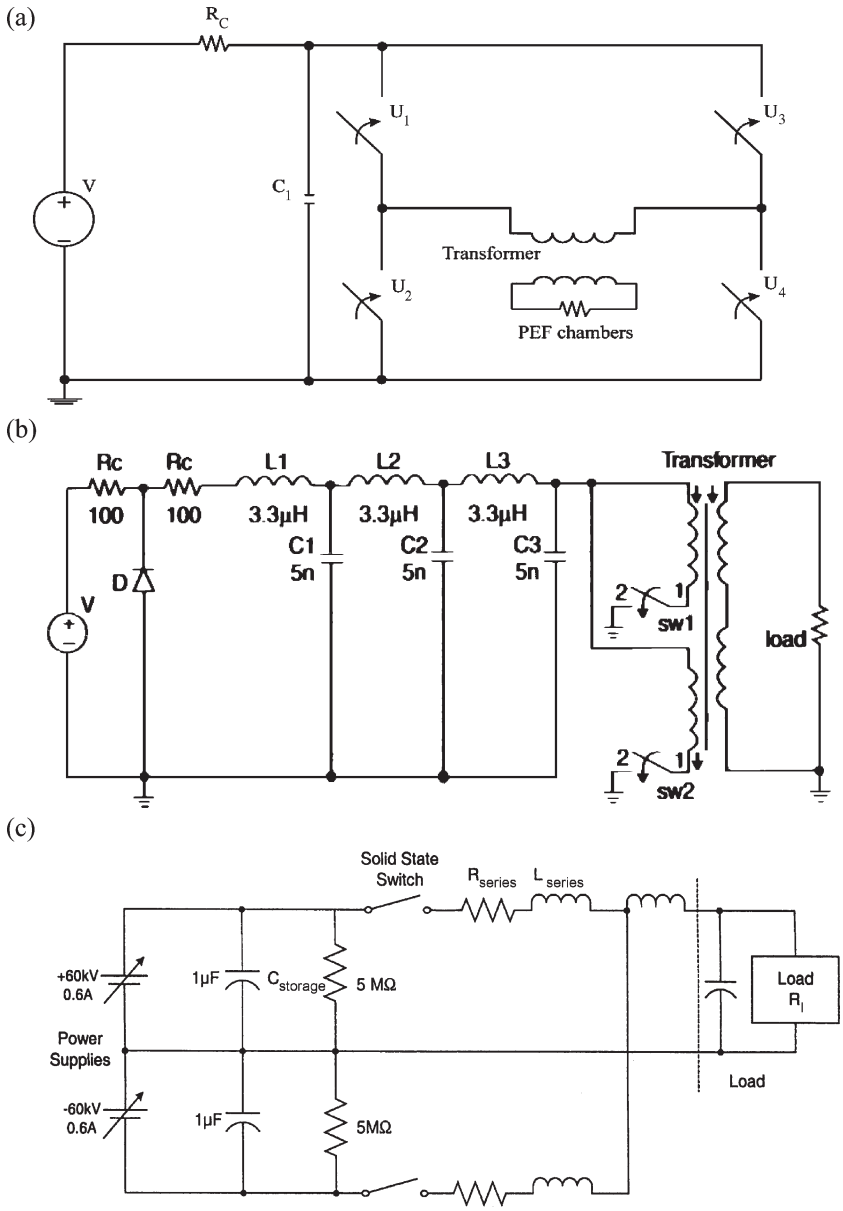

Fig. 2. Circuit diagrams for (a) a bench top-scale, (b) a pilot plant-scale, (c) and a commercial-scale pulse generators (The Ohio State University). R, C, U, and L are resistor, capacitor, switch, and inductance, respectively.

charged storage capacitors that were partially discharged by a series of solid-state switches to form square wave bipolar pulses. The maximum repetition rate of the pulse generator was 2000 pps [10], [11].

C. Fluid-Handling System

The basis of fluid-handling systems is to transfer products for processing and packaging. Fluid-handling systems also monitor and control flow rate, temperature, and pressure during processing. For bench top-scale PEF units, the fluid-handling system is generally formed by stainless steel tubing and a pump, providing continuous flow of a product to be treated. For pilot plant- or commercial-scale PEF systems that are integrated with aseptic packaging system, the fluid-handling system must have mobility and flexibility of processing sequence and allow system cleaning and sterilization (e.g., cleaning-in-place and sterilization-in-place).

D. Temperature- and Pulse-Monitoring Systems

Bench top-scale PEF systems usually have thermocouples (e.g., K type) connected to stainless steel tubes, in which food product flows, to monitor temperature changes during PEF treatments. Temperatures of products before and after the treatment are controlled by placing the tubes in water baths set at target temperatures. Pilot plant- or commercial-scale PEF systems employ a set of tubular heat exchangers for temperature control before and after PEF treatments. A series of resistance-temperature-detector probes with dual-sensing elements may be placed in the inlet and outlet of the PEF treatment chambers and the outlets of heat exchangers to monitor the system

temperatures. Pulses were monitored with high-voltage probes, current monitors, and oscilloscopes [10].

III. Inhibition of Microorganisms by PEF

A. Mechanism

Structural damages of cell membrane, which lead to ion leakage, metabolite losses, protein releases, and increased uptake of drugs, molecular probes, and DNA, have been used to explain the microbial inhibition by PEF [14], [15]. Primary effects of PEF on microbial cells include structural fatigue due to induced membrane potential and mechanical stress. Secondary effects include material flow after the loss of the integrity of cellular membrane by the electric field, local heating, and membrane stress. Tertiary effects include cell swelling or shrinking and disruption due to the unbalanced osmotic pressure between the cytosol and external medium [16].

The electric potential causes an electrostatic charge separation in the microbial cell membrane due to the dipole nature of the membrane molecules [17]. The cell membrane is regarded as an insulator shell to the cytoplasm due to its electrical conductivity, which is six to eight times weaker than that of the cytoplasm. Electrical charges are accumulated in cell membranes when microbial cells are exposed to electric fields. The accumulation of negative and positive charges in cell membranes forms transmembrane potential. The charges attract each other and generate compression pressure, which causes the membrane to decrease in thickness. A further increase in the electric-field strength beyond a critical transmembrane potential leads to pore formation (electroporation). Cell lysis with loss of membrane integrity occurred when transmembrane potential was approximately 1 V [18]. This critical electrical potential varies depending on pulse duration time, number of pulses, and PEF treatment temperature [6].

Transmission electron microscopy (TEM) micrographs of PEF-treated *Saccharomyces cerevisiae* in apple juice exhibited disruption of organelles and lack of ribosomes, which were proposed as other ways in explaining microbial inhibitions by PEF [19].

B. Critical Factors

The factors determining the efficiency of the microbial inhibition by PEF can be classified with treatment parameters, product parameters, and microbial characteristics.

1) Treatment Parameters: The main treatment parameters that affect microbial inhibition by PEF are electric-field strength, PEF treatment time, pulsewidth, pulse shape, and treatment temperature [20], [21]. Generally, as the intensity of each of these parameters increases, the microbial inhibition by PEF also increases. A log-linear relationship between electric-field strength and inhibition of *Escherichia coli* was reported [21]. The rate of microbial inhibition by PEF at a constant electric-field strength increased as the PEF treatment time increased [22].

High efficiency of PEF treatments on the inhibition of microorganisms can be obtained with a long pulsewidth (in the range less than 10 μs). The PEF treatment time is calculated by multiplying the number of pulses applied by the pulsewidth. As the pulsewidth increases, the PEF treatment time also increases, which results in an increased microbial inhibition. However, if the pulsewidth is too long, food temperature rises to an undesirable level for PEF treatment. Thus, the pulsewidth must be determined within the range that does not ramp up the temperature [6].

Electric-field pulses are generally applied in the form of square wave, exponentially decay, or oscillatory pulses. The square wave pulse minimizes energy absorption in foods [20] and is more effective for inhibiting microorganisms than other types of pulses [23]. During PEF processing, a shielding layer can be formed on electrodes in the PEF treatment chamber when charged molecules (e.g., proteins) migrate to the surface of electrodes. The shielding layer reduces the efficiency of the PEF treatment. Bipolar pulses are used to prevent the formation of the shielding layer [6]. Bipolar pulses are likely more lethal than monopolar pulses because a reversal in the orientation or polarity of the electric field changes the direction of charged molecules in the cell membrane, which causes a stress in the cell membrane of microorganisms.

Synergistic effects between PEF treatments and thermal treatments at moderate temperature (20 °C–50 °C) on the inhibition of microorganisms, including *E. coli* and *Salmonella dublin*, have been reported [13], [24]. The increased lethal effects might be due to the temperature-related phase transition of cell membranes from a gel to a liquid-crystalline and the associated reduction in the bilayer thickness of cell membranes [25]. The phase transition reduces the transmembrane potential needed for the breakage of cell membrane [26]. Thus, the combination of PEF treatment with a moderate thermal treatment was recommended for the efficient inhibition of microorganisms in foods [27]. In the combined treatment (heat + PEF), the food temperature is generally raised before a PEF treatment takes place. The food product gets less thermal load in this way compared to the product undergoing a PEF treatment without preheating [28]. The combined treatment also showed advantages over a conventional method in juice extraction from food plants. The combined treatment of mild heat (45 °C–65 °C) with PEF (500–1100 V/cm) resulted in an improved softening effect for carrots, potatoes, and apples [29].

2) Product Parameters: Critical product parameters include electric conductivity, density, viscosity, pH, and water activity. Information on the physical properties of foods over a wide range of temperature is needed to find optimum PEF treatment conditions and design PEF processing units [30].

PEF treatment is most effective for the microbial inhibition of foods with low electrical conductivity. Increase in electrical conductivity of a treatment medium causes a decrease in inhibition of microorganisms at constant energy input [31]. Low electrical conductivity increases the difference in electrical conductivity between a medium and microbial cytoplasm. This increased difference in electrical conductivity weakens the membrane structure of microorganisms due to an increased flow of ionic substances across the membrane during PEF treatments [26]. Dependency of electric-field strength on the electrical conductivity of fruit juices was also reported [32].

The lowest electrical conductivity caused the highest electric-field strength and, thus, resulted in the highest efficiency in microbial inhibition.

The temperature inside the PEF treatment chambers increases during PEF treatments. A low electrical conductivity of food results in a small temperature change during PEF treatments. The temperature change is also low with high-density foods. The temperature increase during PEF treatments can decrease the solubility of air in foods [6]. Air generated by the temperature increase can cause dielectric breakdown under electric field (arching). Thus, the temperature increase should be minimized for efficient PEF treatments.

Food viscosity determines the flow characteristics in PEF systems. A uniform flow of a food product in the PEF treatment chamber results in a uniform PEF treatment [30].

Enhanced efficiency in the inhibition of microorganisms in an acid environment was reported. *E. coli* was more inhibited by PEF at pH 5.7 than at pH 6.8 [31]. At low pH, the lethal effect of PEF on mold spores in fruit juices was increased [32]. Effect of sublethal injury of *E. coli* by PEF on the pH of treatment media was studied [33]. The 99.95% of survivors were injured when cells were PEF-treated at pH 4 (19 kV for 400 μs), while only slight-sublethal injury was detected at pH 7. The injured cells were progressively inhibited by subsequent holding at pH 4. The effect of low pH was more significant than microbial characteristics such as cell size, shape, and type of the cell envelopes in inhibiting *Bacillus subtilis*, *Listeria monocytogenes*, *Lactobacillus plantarum*, *Staphylococcus aureus*, *E. coli*, *E. coli* O157:H7, and *Yersinia enterocolitica* by PEF [34]. However, no significant effects of pH on the inhibition of microorganisms by PEF were also reported in early publications [35], [36]. The effect of pH on the inhibition of microorganisms by PEF might depend on the characteristics of the microorganisms to be investigated.

Microbial resistance to PEF treatments was high in a low water activity (a_{w}) environment. The level of inhibition of *Enterobacter cloacae* inoculated in chocolate liquor or in model system increased as the a_{w} of the liquor or the model system increased. *E. cloacae* that survived at low a_{w} environment had high resistance to PEF [37]. The resistance of microorganisms in a low a_{w} environment may need to be considered when the inhibition of microorganisms by PEF is evaluated.

3) Microbial Characteristics: Bacteria are generally more resistant to PEF than yeasts. Among bacteria, gram-positive bacteria are more resistant to PEF than gram-negative bacteria [38]. The higher resistance to PEF of gram-positive bacteria may be related to the rigidity of the teichoic acids in the peptidoglycan layer of gram-positive cell walls [39]. Bacterial spores and mold ascospores are more resistant to PEF treatment than vegetative cells [32], [40].

The growth stage of microorganisms is also related to the effectiveness of microbial inhibition by PEF. Bacteria and yeasts at their logarithmic stage are more sensitive to PEF than those at the stationary or lag growth stage [26]. The growth stage of microorganisms needs to be considered when developing mathematical kinetic models that describe the inhibition kinetics of microorganisms by PEF.

Microbial cell size or shape may influence the efficiency of PEF inhibition. *Lactobacillus* species in different sizes or shapes had different membrane permeabilization by PEF. Larger cells were more easily permeabilized than smaller cells [41].

C. Microbial Stability of PEF-Treated Foods

The inhibition of microorganisms in foods by PEF treatments is summarized in Table I. The PEF treatment is advantageous for the pasteurization of juice products due to their high acidity and low protein concentration. The high acidity provides a hurdle for recovery of some cells that were not sufficiently inhibited by PEF. The low protein content of juice products does not cause the formation of a protein-deposited shielding layer on electrodes, which reduces efficiency of PEF treatments in inhibiting microorganisms [32].

1) Apple Juice: A PEF treatment at 40 kV/cm reduced the number of *S. cerevisiae* inoculated in apple juice from 8×10^7 to 4×10^4 CFU/mL [19]. TEM micrographs of PEF-treated *S. cerevisiae* in apple juice revealed that PEF treatment disrupted yeast cells and resulted in the almost total absence of ribosome bodies [19].

A bench top-scale PEF treatment at 34 kV/cm for 166 μs (total treatment time) reduced the number of *E. coli* O157:H7 in apple juice by 4.5-log cycles [42]. A pilot plant-scale PEF treatment at 35 kV/cm for 94 μs significantly increased the microbial shelf life of apple juice and apple cider. Shelf life of an apple cider treated by a combination of PEF at 35 kV/cm for 94 μs and a thermal treatment at 60 °C for 30 s was more than 67 d at both 4 °C and 22 °C [42].

2) Cranberry Juice: The effect of a pilot plant-scale PEF treatment at 35 kV/cm for 195 μs on the inhibition of microorganisms in reconstituted cranberry juice was investigated [43]. The PEF treatment decreased the number of total aerobic plate count and yeast and mold count of cranberry juice by more than 4-log cycles. The PEF-treated cranberry juice had the shelf life of 8 mo, 37 d, and 30 d at 4 °C, 22 °C, and 37 °C, respectively.

A PEF treatment at 40 kV/cm for 150 μs reduced the total aerobic plate count and the yeast and mold count in cranberry juice by about 5-log cycles. The PEF treatment prevented the growth of yeasts and molds in the cranberry juice during 14 d of storage at 4 °C [44].

Both PEF treatment (32-kV/cm electric-field strength, 500-pps frequency, 1.4-μs pulse duration, and 47-μs total treatment time) and heat treatment (60 °C for 32 s) combined with the PEF provided a 97-d shelf life of cranberry juice stored at 4 °C or 22 °C. Both treatments did not alter the color of cranberry juice [45].

3) Orange Juice: A pilot plant-scale PEF treatment at 29.5 kV/cm for 60 μs inhibited aerobic microorganisms in reconstituted orange juice by 4.2-log cycles. The PEF-treated orange juice had a 7-mo shelf life at 4 °C in aseptic packages [46].

A pilot plant-scale PEF treatment at 32 kV/cm for 92 μs reduced the yeast and mold counts of whey protein-fortified orange juice from 1.4×10^5 CFU/mL to less than 40 CFU/mL.

TABLE I
OVERVIEW OF MICROORGANISM INHIBITION IN FOODS BY PEF TREATMENTS

Food	Microorganism	PEF system	PEF treatment condition	Log reduction	Source
Apple juice	*S. cerevisiae*, *E. coli*	Batch parallel plate	12 kV/cm, 20 pulses, exponential decay, < 30°C	*S. cerevisiae*: 3-4, *E. coli*: 3	[72]
Apple juice	*S. cerevisiae*	Batch parallel plate	25 kV/cm, 558 J, exponential decay, < 25°C	3-4	[86]
Apple juice	*S. cerevisiae*	Continuous flow coaxial	50 kV/cm, square wave, 29.6°C	6.3	[87]
Apple juice	*S. cerevisiae*	Continuous recirculating parallel plate	40 kV/cm, 64 pulses, exponential decay, 15°C	3.3	[19]
Apple juice	*E. coli* O157:H7	Co-field flow tubular	29 kV/cm, square wave	5	[88]
Apple juice	Bench top-scale: *E. coli* O157:H7, Pilot plant-scale: aerobic microorganisms, yeasts & molds	Co-field flow tubular	Bench top-scale: 34 kV/cm, 166 μs of treatment time, 1.5 mL/s, 800 pps[a] Pilot plant-scale: 35 kV/cm, 94 μs of treatment time, 85 L/h, 952 Hz	Lab scale: 4.5 Pilot plant scale: aerobic microorganisms – 2.1, yeasts & molds – 1.5	[42]
Apple juice	*S. cerevisiae*	Co-field flow tubular	20 kV/cm, 10.4 pulses, square wave	4	[89]
Cranberry juice	Total aerobic microorganisms, yeasts & molds	Pilot plant-scale	35 kV/cm, 195 μs	> 4	[43]
Cranberry juice	*Byssochlamys fulva* canidiospores, *Neosartorya fischeri*	Coaxial	• *Byssochlamys fulva* canidiospores: 36.5 kV/cm, 22°C • *Neosartorya fischeri*: 51.0 kV/cm, 34°C	• *Byssochlamys fulva* canidiospores: 5.9, • *Neosartorya fischeri*: Not inhibited	[32]
Cranberry juice	Aerobic microorganisms, yeasts & molds	Co-field flow tubular	40 kV/cm, 150 μs of treatment time, square wave	Aerobic microorganisms: 4.8, Yeasts & molds: 4.9	[44]
Cranberry juice	*E. coli*	Continuous parallel plate	0-40 kV/cm, 69-80 μs treatment time	6.4	[90]
Orange juice	Aerobic microorganisms	Co-field flow tubular	29.5 kV/cm, 60 μs of treatment time, square wave	4.2	[46]
Whey protein fortified orange juice	Aerobic microorganisms, yeasts & molds	Pilot plant-scale system, co-field flow tubular	32 kV/cm, 92 μs of treatment time, 3.3 μs of pulse width, 800 Hz, 79 L/h	Aerobic microorganisms: 0.5 Yeasts & molds: 3.5	[47]
Orange juice	Aerobic microorganisms, yeasts & molds	Pilot plant-scale system, co-field flow tubular	30 kV/cm, 240 μs of treatment time, 2 μs of pulse width, 1000 Hz, 2 mL/s	Aerobic microorganisms: 2.5 Yeasts & molds: 2.5	[48]
Orange juice	*L. mesenteroides*, *E. coli*, *L. innocua*, *S. cerevisiae* ascospore	CPS1 system, cathode (PurePulse Tech)	30 kV/cm or 50 kV/cm (*S. cerevisiae* ascospores), 100 L/h	*L. mesenteroides*, *E. coli*, *L. innocua*: 5, *S. cerevisiae* ascospore: 2	[49]
Orange juice	Aerobic microorganisms, yeasts & molds	Pilot plant-scale system, co-field flow tubular	35 kV/cm, 59 μs of treatment time, 1.4 μs of pulse width, 600 pps, 98 L/h	Aerobic microorganisms: 7 Yeasts & molds: 7	[74]
Orange juice	Aerobic microorganisms, yeasts & molds	Commercial-scale system, co-field flow tubular	40 kV/cm, 97 μs of treatment time, 2.6 μs of pulse width, 1000 pps, 500 L/h	Aerobic microorganisms: 6 Yeasts & molds: 6	[10]
Orange juice	*S. cerevisiae*		8-11kV/cm, 3 pulses, 50μF capacitance or 12.5kV/cm, 40 pulses, 1 μF capacitance, exponential decay pulses	2-5.8	[91]
Orange juice	*L. brevis*	Co-field flow	8 chambers, 1-10μs pulse width, 1000Hz, 1-12kV electric field strength, mono-bipolar pulse	5.8	[92]
Grape juice	Naturally occurring microorganisms		20 pulses of 65 kV/cm	• PEF only: 5.9 • PEF + nisin (400 U/mL) treatment: 6.2	[93]
Tomato juice	Aerobic microorganisms, yeasts & molds	Commercial-scale system, co-field flow tubular	40 kV/cm, 57 μs of treatment time, 2 μs of pulse width, 1000 pps, 500 L/h	Aerobic microorganisms: 6 Yeasts & molds: 6	[11]
Orange-carrot juice	*L. plantarum*, *E. coli*	Continuous co-field flow	28, 25,22x10^5 Vm^{-1}, 11.5 , 7and 9x10^{-5} s total treatment time	1.3 (*L. plantarum*), 2.6 (*E. coli*)	[94]
Apple juice (AJ), orange juice (OJ), grape juice (GJ), pineapple juice (PJ), cranberry juice (CJ)	*Zygosaccharomyces balii* ascospores Vegetative cells (V), ascospores (A)	Coaxial	• AJ: 32.3 kV/cm, 19°C • OJ: 34.3 kV/cm, 20°C • GJ: 35.0 kV/cm, 20°C • PJ: 33.0 kV/cm, 20°C • CJ: 36.5 kV/cm, 22°C	• AJ (V): 4.8 AJ (A): 3.6 • OJ (V): 4.7 OJ (A): 3.8 • GJ (V): 5.0 GJ (A): 3.5 • PJ (V): 4.3 PJ (A): 3.4 • CJ (V): 4.6 CJ (A): 4.2	[95]
Milk	*E. coli*	Circular parallel stainless steel electrode	33 kV/cm, 35 pulses, 43°C	3	[8]
Milk	*S. dublin*	Circular parallel stainless steel electrode	36.7 kV/cm, 36 μs of treatment time, 40 pulses, 63°C	4	[8]
Simulated milk ultrafiltrate (SMUF)	*E. coli*	Batch parallel plate	25 kV/cm, 20 pulses, exponential decay, < 25°C	3	[86]

TABLE I *(Continued.)*
OVERVIEW OF MICROORGANISM INHIBITION IN FOODS BY PEF TREATMENTS

SMUF	*E. coli, B. subtilis*	Batch parallel plate	• *E. coli*: 40 kV/cm, oscillatory decay, < 30°C • *B. subtilis*: 16 kV/cm, 180 μs of treatment time, bipolar	3	[72]
SMUF	*L. delbrueckii* ATCC 11842	Batch parallel plate	16 kV/cm, 300 μs of treatment time, 40 pulses, exponential decay, < 30°C	4-5	[96]
SMUF	*L. subtilits* ATCC 9372	Batch parallel plate	16 kV/cm, 300 μs of treatment time, 50 pulses, exponential decay, < 30°C	4-5	[96]
SMUF	*L. delbrueckii* ATCC 11842, *L. subtilits* ATCC 9372	Batch parallel plate	16 kV/cm, 300 μs of treatment time, 40 pulses or 50 pulses (*L. subtilits* ATCC 9372), exponential decay, < 30°C	4-5	[96]
Skim milk	*E. coli*	Batch parallel plate	50 kV/cm, 62 pulses, square wave, < 30°C	2.5	[97]
SMUF	*E. coli*	Continuous parallel plate	50 kV/cm, 48 pulses, square wave, < 30°C	3.6	[97]
UHT milk	*E. coli*	Plain parallel carbon electrode	22.4 kV/cm, 300 μs of treatment time	4.8	[40]
Skim milk	*E. coli*	Batch parallel plate	40 kV/cm, exponential decay, 15°C	6	[57]
Skim milk	*S. dublin*	Co-field flow	30°C,50°C, 25kV/cm, 100pulses	1, 2	[24]
Pasteurized whole, 2%, and skim milk	*L. monocytogenes* Scott A	Co-field flow tubular	30 kV/cm, 600 μs of treatment time, square wave, 50°C	4	[2]
Skim milk	*L. innocua*	Continuous concentric cylindrical	50 kV/cm, 64 μs of treatment time, 36°C	2.5	[3]
Fat free milk Phosphate buffer	*E. coli, L. innocua*	Co-field flow	2.5 μs pulse width, 3Hz frequency, 0-60 pulses 41 kV/cm electric field strength,	2.3-6.5 (*E. coli*), 0.7-2.8 (*L. innocua*)	[98]
Skim milk	*L. innocua, P. fluorescens*		50 kV/cm for 200 μs	2.6 (*L. innocua*), 2.7 (P. fluorescens)	[99]
Skim milk	*S. aureus*	Bench top-scale, co-field flow tubular	3.7μs pulse duration time, 250Hz frequency, 35kV/cm electric field strength, 450 μs of treatment time (stepwise and circulation mode fluid handling system)	3.3 and 3.5	[4]
Liquid whole egg	*L. innocua*	Concentric cylindrical	2 ms pulse duration, 3.5 Hz, 10.6, 21.3 an 32 pulses, 30, 35, and 40 kV, exponentially decay pulse	3.5	[100]
Liquid whole egg	*S.* Enteritidis	Bench top-scale,	200pps frequency,2.12 μs pulse duration, 25kV/cm electric field strength, 250 μs total treatment time, PEF+55C for 3.5 min	1 and 4.3	[52]
Liquid egg	*E. coli*	Coaxial	26 kV/cm, 37°C	6	[101]
Yogurt	*S. cerevisiae*	Circular parallel stainless steel electrode	18 kV/cm, 55°C	3	[8]
Spices (dry)	Yeasts	Batch cylindrical	65 kV/cm, 750 μs of treatment time	4.2	[102]
Phosphate buffer Whole milk Skim milk Dairy cream	*L. innocua*		17-46kV/cm electric field strength, 545 pulses, 1.1 or 100Hz frequency, exponentially decay pulses	~5	[103]

[a]pps: Pulses Per Second

The PEF-treated protein-fortified orange juice was microbiologically stable for 5 mo at 4 °C [47].

Total aerobic plate counts and yeast and mold counts in freshly squeezed orange juice were reduced by a PEF treatment at 30 kV/cm for 240 μs. The PEF treatment was as effective as a thermal treatment at 90 °C for 1 min in reducing the total aerobic plate count and the yeast and mold count in single strength orange juice. The total aerobic plate count and the yeast and mold count of the PEF-treated orange juice were under a detection limit (< 1 CFU/mL estimated) at 4 °C for 6 wk [48].

Leuconostoc mesenteroides, *E. coli*, and *L. innocua* were inoculated into orange juice, and the inoculated juice was treated by PEF at 30 kV/cm [49]. More than 5-log reductions of *L. mesenteroides*, *E. coli*, and *L. innocua* were obtained. A PEF treatment at 50 kV/cm resulted in 2.5-log reductions of *S. cerevisiae* ascospores in the juice [49].

The effects of PEF treatment by a pilot plant-scale PEF system at 35 kV/cm electric-field strength for 59 μs on the microbial stability of orange juice was compared to those of thermal treatment at 94.6 °C for 30 s. The PEF treatment reduced and maintained the number of endogenous microorganisms in orange juice at about 1-log cycle at 4 °C, 22 °C, and 37 °C for 112 d, which was as effective as the thermal treatment [50].

The effects of a commercial-scale PEF treatment at 40 kV/cm for 97 μs on the inhibition of endogenous microorganisms in orange juice were studied [10]. Commercial-scale PEF treatment reduced the total aerobic plate count and the yeast and mold count of orange juice by 6-log cycles. Fresh orange juice treated by a commercial-scale PEF system showed microbial shelf life (< 4-log cycles of both total aerobic plate count and yeast and mold count) of 196 d at 4 °C [10].

4) Tomato Juice: A PEF treatment at 40 kV/cm for 57 μs by a commercial-scale PEF system inhibited 6-log cycles of endogenous microorganisms in tomato juice. The PEF-treated juices showed microbial shelf life of 112 d at 4 °C (< 4-log cycles of both total aerobic plate count and yeast and mold count) [11]. A higher rate of microbial growth in the PEF-treated tomato juice than the tomato juice thermally treated at 92 °C for 90 s during storage at 4 °C for 112 d was observed.

This might be due to a relatively less inhibition of the spores by PEF than the thermal treatment and the germination of the survived spores during the storage [11].

5) Milk: Ultra-high-temperature processed (UHT) milk was inoculated with *E. coli*, *L. brevis*, *Pseudomonas fluorescens*, or *S. cerevisiae* and then treated by PEF at 10–30 kV/cm with 1–22-Hz frequency. The threshold electric-field strength was 10 kV/cm, below which no inhibition of microorganisms occurs, for all the tested pathogenic microorganisms. High fat content of milk reduced the lethal effect of PEF [40]. UHT milk containing different types of microbial spores was treated with PEF at 22 kV/cm. The PEF treatment did not significantly inhibit the endospores of *Clostridium tyrobutyricum* and *B. cereus* and the ascospores of *Beauveria nivea* [40].

A pilot plant-scale PEF system was used to treat high-temperature short-time (HTST) milk with PEF, having a peak electric field of 35 kV/cm and 2.3 μs of pulsewidth, at 65 °C for less than 10 s [51]. Application of the PEF immediately after an HTST pasteurization extended the shelf life of milk to 60 d. Extension of shelf life was more noticeable (78 d) when the PEF was applied after 8 d from the HTST pasteurization.

6) Liquid Whole Egg: A 4.3 log CFU/mL reduction in *S. Enteritidis*, inoculated in liquid whole egg, was obtained by a PEF treatment combined with a mild heat treatment at 55 °C for 3.5 min [52]. The combined treatment did not cause significant changes in viscosity, electrical conductivity, color, pH, and Brix, relative to controls without any treatments. The liquid whole egg samples treated by the combination exhibited significantly longer shelf life at 4 °C compared with the controls and only heat-treated samples [52].

D. Kinetic Models

The study of mathematical kinetic models that describe the inhibition of spoilage and pathogenic microorganisms by PEF is required to find PEF treatment conditions for desired levels of microbial inhibition. Models and parameters that are determined in studies can be used for quantitative risk assessments, which could be useful for food hazard analysis and critical control point (HACCP) systems [53]. To develop inhibition kinetic models, microbial inhibition data must be obtained from reliable experiments where artifacts of the experimental procedures are avoided [54].

Microbial inhibition by PEF has been described by first-order kinetic models [(1) and (2)]. The natural logarithm of a survivor fraction (S), microbial count after PEF treatment/microbial count before PEF treatment, is expressed as the function of PEF treatment time (1) or electric-field strength (2).

$$\ln(S) = -k_{\mathrm{E}} t \tag{1}$$

where t is the PEF treatment time (in microsecond) and k_{E} is the first-order kinetic constant for a given electrical field strength.

$$\ln(S) = -k_{\mathrm{N}} E \tag{2}$$

where E is the electric-field strength (in kilovolt per centimeter) and k_{N} is the first-order kinetic constant for a given number of pulse treatment.

The inhibition of *E. coli*, *S. aureus*, and *S. cerevisae* by 5- to 6-log cycles yielded log-linear kinetic data, and the data were fit to the first-order kinetic models of inhibition with respect to PEF treatment time and electric-field strength [55]. A study on the inhibition kinetics of *S. dublin* in skim milk revealed that *S. dublin* followed first-order kinetics with respect to electric-field strength ($R^2 = 0.97-0.98$) over 4-log cycles of survivor fractions [24].

The Hulsheger's kinetic model [35] shown in (3) describes the kinetics of survival curves, assuming a linear relationship between the natural logarithm of the survivor fraction and the electric-field strength as well as a linear relationship between the natural logarithm of the survivor fraction and the natural logarithm of the PEF treatment time.

$$S = \left(\frac{t}{t_c}\right)^{-(E-E_c)/k} \tag{3}$$

where t is the PEF treatment time (in microsecond); t_{c} is the critical treatment time, below which no inhibition of microorganism occurs (in microsecond); E is the electric-field strength (in kilovolt per centimeter); E_{c} is the critical electric-field strength, below which no inhibition of microorganisms occurs (in kilovolt per centimeter); and k is the specific rate constant.

The kinetic model parameters (E_{c}, K, and t_{c}) were dependent on microbial species. The Hulsheger's kinetic model fits lots of experimental data [56]. The Hulsheger's kinetic model accurately predicted the PEF inhibition of *E. coli*, *L. brevis*, and *P. fluorescens* in sodium-alginate and UHT milk of up to 4-log cycles ($R^2 = 0.97 - 1.00$) [40]. The inhibition of *E. coli* in skim milk was also successfully described by the Hulsheger's kinetic model [57].

Peleg [22] proposed a kinetic model for the microbial inhibition by PEF based on Fermi's equation. The Fermi's kinetic model shown in (4) represents the survivor fraction as a function of electric-field strength and the number of pulses. The Fermi's kinetic model provides a sigmoid-shaped curve.

$$S = \frac{1}{1 + e^{(E-E_h)/a}} \tag{4}$$

where E is the electric-field strength (in kilovolt per centimeter), E_{h} is the electric-field strength (in kilovolt per centimeter), and a is the parameter indicating the slope of the curve around E_{h}.

The Fermi's kinetic model can explain low inhibition rates of microorganisms after very short PEF treatment times and the tailing effect at long PEF treatment times due to its sigmoid-shaped curve [22]. This model is also useful with microbial inhibition data that spans several log cycles of inhibition. The Fermi's kinetic model with published microbial inhibition data showed very good fits ($R^2 = 0.973 - 0.999$) [22], [24].

A log-logistic kinetic model and a Weibull distribution-based model have also been used [53], [58]. Survival curves of *S. senftenberg* that covered 6–7-log cycles were modeled by the log-logistic kinetic model [58]. The experimentally measured inhibition and the estimated inhibition from the log-logistic kinetic model showed a very good agreement ($R^2 = 0.99$). A Weibull distribution-based model provided better estimation

on the influence of electric-field strength on the inhibition of *E. coli* than a sigmoidal equation [53].

IV. Inactivation of Enzymes by PEF

A. Mechanism

The effects of electric fields on proteins include the association or dissociation of functional groups, movements of charged chains, and changes in alignment of helices [59].

Alkaline phosphatase molecules treated with PEF at 22.3 kV/cm with pulsewidth of 0.78 ms tended to associate and aggregate. The aggregates might be formed by the polarization created by electrical charges of dipoles on the enzyme. The polarization, leading to the aggregation of the enzyme, was proposed as the mechanism of the inactivation of alkaline phosphatase by PEF [60].

B. Critical Factors

The factors that mainly influence PEF enzymatic inactivation are: 1) electric parameters (e.g., electric-field strength, total treatment time, and pulsewidth); 2) enzymatic structures (e.g., secondary and tertiary structures); 3) PEF treatment temperatures; and 4) treatment media.

The effect of electric-field strength on the inactivation of tomato juice lipoxygenase by PEF treatment with 0.3 kJ/mL of energy input was studied [61]. Three treatment conditions with different levels of electric-field strength were selected to provide an identical level of energy input (0.3 kJ/mL). The temperature change was controlled at 25 °C for the all three PEF treatments. The percentages of lipoxygenase inactivation were 4.7%, 46.3%, and 60.0%, with the electric-field strength of 9.0, 17.8, and 30.1 kV/cm, respectively. The inactivation of tomato juice lipoxygenase by PEF increased as the electric-field strength increased at the same level of energy input. Electric-field strength was a primary variable for the inactivation of tomato juice lipoxygenase by PEF [61].

Differences in the secondary or tertiary structure among enzymes resulted in the diverse sensitivity of enzymes to PEF [62]. Conformational changes in α-helical structures were thought to cause inactivation of papain by high electric fields [63].

Synergistic effects in inactivating enzymes were observed when PEF treatments were combined with a mild heating (50 °C) for inactivating pectin methyl esterase (PME) and lipoxygenase [61], [64].

The level of enzyme inactivation by PEF was also affected by the treatment media. For example, protease is protected from PEF-induced unfolding by the presence of casein in the treatment medium [65].

C. Enzymes Inactivated by PEF

An overview of the inactivation of enzymes by PEF treatment is shown in Table II. A 90% of plasmin (milk alkaline protease) activity in a simulated milk ultrafiltrate was reduced by a PEF treatment at 45 kV/cm with 50 pulses. Synergistic effects were shown among electric-field strength, number of pulses, and PEF treatment temperature on the inactivation of plasmin. The activity of PEF-treated plasmin was not restored after 24 h of storage at 4 °C [66].

The effect of PEF on the inactivation of lipase, lactoperoxidase, and alkaline phosphatase in raw milk was studied [40]. The inactivation of lipase, peroxidase, and alkaline phosphatase were 65%, 25%, and $< 5\%$, respectively, after a PEF treatment at 21.5 kV/cm with 400 kJ/L. A higher fat content of the milk provided a higher protection effect against PEF to alkaline phosphatase.

Lipase, glucose oxidase, α-amylase, peroxidase, polyphenol oxidase, and phosphatase were treated with PEF (21.5 kV/cm, 400 kJ/L) [62]. Lipase, glucose oxidase, and heat stable α-amylase exhibited a large reduction in activity (75%–85%). Peroxidase and polyphenol oxidase were inactivated by 30%–40%. Alkaline phosphatase was reduced by 5%.

Papain in a 1-mM EDTA solution was inactivated by a PEF treatment at 50 kV/cm at 10 °C [63]. A linear relationship between residual activity and electric-field strength was observed in this paper.

A 94% reduction of PME activity was obtained by a PEF treatment at 24 kV/cm for 8 ms [67]. Inactivation of the enzyme was described by a classical exponential decay model as well as Hulsheger's and Fermi's empirical models [67]. Reduction of pectinesterase activity by a PEF treatment, using exponential decay pulses up to 463 μs at electric-field intensities ranging from 19 to 38 kV/cm, fitted on Fermi, Hulsheger, and Weibull equations to describe PEF inactivation kinetics [68].

A PEF treatment at 30 kV/cm for 60 μs at 50 °C inactivated 88.1% of lipoxygenase obtained from tomato juice [61]. The first-order kinetic models and the Hulsheger's and Fermi's models adequately described the lipoxygenase inactivation by PEF. Calculated D-values for the lipoxygenase were 161.0, 112.9, 101.0, and 74.8 μs at 15, 20, 30, and 35 kV/cm at 30 °C, respectively. A commercial-scale PEF treatment at 40 kV/cm for 57 μs reduced 53% activity of lipoxygenase in a cold break tomato juice [61].

The activity of a horseradish peroxidase in a buffer solution was reduced by 16.7% and 34.7% after the treatment at 25 kV/cm for 207 pulses and 22 kV/cm for 1214 pulses at < 40 °C, respectively [69].

The issue of the inactivation of enzymes by PEF is controversial [70]. The diversity of employed PEF systems, such as PEF treatment chamber design, limits the comparability among inactivation data.

V. PEF-Treated Food Products

Thermal treatment has been conventionally used to inhibit spoilage and pathogenic microorganisms and enzymes to extend shelf life of food products. However, thermal treatment can lower the sensory and nutritional qualities of juices [71]. PEF has been intensively studied as a nonthermal agent in providing microbiological safety, while reducing the loss of flavor, color, and nutrients of juices from heat [1], [27].

TABLE II
OVERVIEW OF THE INACTIVATION OF ENZYMES BY PEF TREATMENTS

Enzyme	Medium	PEF system	PEF treatment condition	Reduction (%)	Source
NADH dehydrogenase, succinic dehydrogenase, hexokinase, acetylcholinesterase, lipase, α-amylase	NADH dehydrogenase, succinic dehydrogenase, hexokinase: extract of pulse treated *E. coli* 8196 Acetylcholinesterase: bovine erythrocytes	Carbon electrode, polyethylene spacer with air contact	Pulse length: 20 μs, 1 Hz NADH dehydrogenase, succinic dehydrogenase, hexokinase: 20 – 25 kV/cm lipase, α-amylase: up to 30 kV/cm	No inactivation	[104]
Plasmin	SMUF	A continuous flow chamber, two parallel stainless steel electrodes	30 or 45 kV/cm, 2 μs of pulse width, 50 pulses, 0.1 Hz	90%	[66]
Lipase, peroxidase, alkaline phosphatase, lactoperoxidase	Raw milk	High voltage pulse generator with 5-15 kV d.c., Two plain parallel carbon electrodes, electrode gap: 0.5 cm	21.5 kV/cm, 1 – 22 Hz, Energy input (Q) = 400 (kJ/L)	Lipase: 65% Peroxidase: 25% Alkaline phosphatase: < 5% Lactoperoxidase: 0%	[40]
Peroxidase, Alkaline phosphatase, α-amylase, lipase, lysozyme, glucose oxidase, polyphenol oxidase, pepsin	Buffer solution or deionized water	High voltage pulse generator with ≤ 30 kV d.c., batch circular shape treatment chamber, Two circular and parallel stainless steel electrodes, electrode gap: 0.3 cm	2 μs of pulse width, 30 of number of pulses, 0.5 Hz Peroxidase: 73.3 kV/cm Alkaline phosphatase: 83.3 kV/cm α-amylase: 80 kV/cm Lipase: 88 kV/cm Lysozyme: 13.3, 50 kV/cm Glucose oxidase: 50 kV/cm Polyphenol oxidase: 50 kV/cm Pepsin: 40 kV/cm	Peroxidase: 30% Alkaline phosphatase: 5% α-amylase: 85% Lipase: 85% Lysozyme: 13.3kV/cm: 60%, 50 kV/cm: 10% Glucose oxidase: 75% Polyphenol oxidase: 40% Pepsin: 150% increase	[62]
Papain	1 mM EDTA solution	Co-field flow tubular PEF treatment chamber, stainless steel electrode, electrode gap: 0.2 cm	50 kV/cm, 4 μs of pulse width, 1500 Hz, 10°C	With activators (L-Cys and DTT): 50% Without activators: 90%	[63]
Lactate dehydrogenase	100 mM potassium phosphate buffer, pH 7	Batch, stainless steel electrodes, electrode gap: 0.5 cm, volume 5.7 mL	31.6 kV/cm, 0.96 μs of pulse width, 1.1 Hz, 200 of number of pulse, 30°C, exponential decay	No inactivation	[105]
Pectin methyl esterase (PME)	Tomato	Gene electroporator (Bio-Rad Laboratories)	24 kV/cm, 800 μs of treatment time, exponential decay	93.8%	[67]
PME	Orange juice	Pilot plant-scale system, co-field flow tubular PEF treatment chamber, stainless steel tubular electrode, electrode gap: 1.0 cm	35 kV/cm, 59 μs of treatment time, 1.4 μs of pulse width, 600 pps[a], 98 L/h	88%	[74]
Milk alkaline phosphatase	Modified SMUF, raw milk, pasteurized and homogenized 2% milk		18.8-22.3 kV/cm	65% in modified SMUF, 59% in raw milk, pasteurized and homogenized 2% milk	[60]
PME	Orange juice	Co-field flow tubular PEF treatment chamber, stainless steel electrode, electrode gap: 0.2 cm	20-35 kV/cm, 2.0 or 2.2 of pulse width, 700 pps, 0.42, 0.31 mL/s	90%	[64]
Lipoxygenase	Tomato juice	Commercial-scale system, co-field flow tubular PEF treatment chamber, boron carbite tubular electrodes, electrode gap: 1.27 cm	40 kV/cm, 57 μs of treatment time, 2 μs of pulse width, 1000 pps, 500 L/h	54%	[77]
Lipoxygenase	Tomato juice	Co-field flow tubular PEF treatment chamber, electrode gap: 0.292 cm	30 kV/cm, 60 μs of treatment time, 3 μs of pulse width, 1 mL/s, 50°C	88.1%	[61]

[a] pps: Pulses Per Second

A. Juice Products

1) Apple Juice: A PEF-treated apple juice had sensory characteristics similar to those of freshly squeezed apple juice. Panels could not find significant differences in sensory properties between the PEF-treated and freshly squeezed apple juice [72].

An apple juice from concentrate was treated by PEF at 50 kV/cm with ten pulses, 2-μs pulse duration, and maximum processing temperature of 45 °C. The PEF-treated juice had a shelf life of 28 d, while freshly squeezed apple juice had a shelf life of 21 d [73]. A sensory panel did not find any significant differences in sensory properties between the PEF-treated and freshly squeezed apple juices [73].

A PEF treatment at 35 kV/cm for 94 μs did not change the concentration of vitamin C in apple juice. Sensory test results showed that acceptability of fresh apple juice was not affected by the PEF treatment [42].

2) Cranberry Juice: Cranberry juice, collected immediately after a PEF treatment (40 kV/cm for 150 μs), showed similar flavor profiles as the untreated cranberry juice [44]. No significant differences were observed in the content of anthocyanin and lightness (Hunter L) values between PEF-treated cranberry juice and untreated cranberry juice. The shelf life of cranberry juice at 4 °C and 22 °C was extended by the PEF treatment [45].

3) Orange Juice: Orange juice treated with PEF at 29.5 kV/cm for 60 μs retained more volatile flavors and vitamin C than orange juice treated thermally at 90 °C for 15 s. The PEF treatment reduced 5%–9% of volatile flavor compounds, while the thermal treatment reduced 25% of volatile compounds over freshly squeezed orange juice [46]. The vitamin C content of the PEF-treated orange juice was found 68% while that of the thermally treated orange juice was 46% after 90 d of storage at 4 °C when the vitamin C content of the freshly squeezed juice prior to storage on day zero was 100% [46].

A whey protein-fortified orange juice was treated by a PEF treatment (32 kV/cm for 92 μs). The PEF-treated juice retained its color longer than the juice thermally treated at 71 °C for 25 s [47]. The PEF treatment was considered to cause less protein denaturation in the orange juice than the thermal treatment. The PEF treatment denatured 6%–7% of whey protein in the protein-fortified orange juice, while the thermal treatment denatured 55% of the protein [47].

A PEF treatment at 30 kV/cm for 240 μs was as effective as a thermal treatment at 90 °C for 1 min in reducing the total aerobic plate count and the yeast and mold count [48]. Reductions in total flavor compounds after the PEF and after the thermal treatment were 3% and 22%, respectively. The concentration of ethyl butyrate in orange juice was decreased by 9.7% and 22.4% after the PEF and the thermal treatment, respectively. Decanal was not reduced by the PEF treatment, while 41% of decanal was reduced by the thermal treatment [48].

Microbial stability, sensory properties, and vitamin C contents of orange juice treated by a pilot plant-scale PEF system at 35 kV/cm for 59 μs were compared to those of thermally treated orange juice at 94.6 °C for 30 s [74]. Both PEF and thermal treatments provided microbial stability at 4 °C, 22 °C, and 37 °C for 112 d. The PEF-treated orange juice contained significantly higher concentrations of vitamin C and flavor compounds than the thermally treated orange juice during storage at 4 °C. The PEF-treated orange juice had lower browning index, higher lightness (L), and higher hue angle values than the thermally treated orange juice during storage at 4 °C.

The effects of a commercial-scale PEF processing on the quality of freshly squeezed orange juice were studied [10]. A PEF treatment (40 kV/cm for 97 μs) inhibited 6-log cycles of aerobic microorganisms and yeasts and molds. The concentration of vitamin C was not significantly changed by the PEF treatment while a thermal treatment (90 °C for 90 s) reduced 19% of the vitamin C. Orange juice processed by the commercial-scale PEF system retained more vitamin C than thermally treated orange juices at 4 °C for 84 d. Orange juice should contain at least 25 mg of vitamin C per 100 mL at the time of expiration date to provide 100% of the U.S. Recommended Daily Allowances requirement [75]. The concentration of vitamin C in the PEF-treated orange juice decreased to 25 mg/100 mL at 4 °C after 56 d, which is longer than the 42 d of thermally treated orange juice. The PEF-treated orange juice retained more flavor compounds of α-pinene, octanal, d-limonene, and decanal than the thermally treated orange juice. The PEF treatment decreased 12% myrcene, while the thermal treatment reduced 37% myrcene over freshly squeezed orange juice [10].

A PEF treatment of 28 kV/cm for 97 μs was applied to orange, tangerine, lemon, and grapefruit juices. The organic acid content, tested volatile content, visual color, pH, Brix, electric conductivity, viscosity, and nonenzymatic browning index of juices were not practically affected by the treatment [76].

4) Tomato Juice: Effects of a commercial-scale PEF processing on the quality of tomato juice were studied [77]. Tomato juice was prepared by hot break at 88 °C for 2 min and then treated by PEF at 40 kV/cm for 57 μs or thermally treated at 92 °C for 90 s. The PEF-treated tomato juice retained more vitamin C than thermally treated juice for 42 d at 4 °C. The flavor compounds of trans-2-hexenal, 2-isobutylthiazole, and cis-3-hexanol were retained more in the PEF-treated juice than in the thermally treated or untreated juice during storage at 4 °C for 112 d. The browning index and the concentration of 5-hydroxymethyl-2-furfural of the PEF-treated juice were significantly lower than those of the thermally treated or untreated juice during storage at 4 °C for 112 d. No significant differences were observed in the Brix, pH, viscosity, and concentration of lycopene between the PEF-treated and the thermally treated tomato juices during storage (4 °C for 112 d). Sensory evaluations indicated that the PEF-treated juice had higher flavor intensity and overall acceptability than the thermally treated juice [77].

B. Juice Extraction

PEF treatments on solid foods have been applied to increase yield of juice from food extractions. PEF induces permeabilization of food membranes within very short time (microsecond to millisecond range), leaving the product matrix largely unchanged while positively affecting mass transfer in juice extraction [78]. A PEF-aided extraction is considered less detrimental than a solely heat-dependent extraction for plant tissue ingredients such as pigments, vitamins, and flavoring agents. This advantage of the PEF-aided extraction can be achieved at a minimum power consumption [29].

The combination use of mild thermal and PEF treatments damaged apple tissue more effectively than application of PEF alone, resulting in enhanced juice extraction [79].

Efficiency of the PEF amplification for enhancing juice expression from soft vegetable tissues might be controlled by the consolidation characteristics of pressed materials [80]. To visualize the effect of a PEF treatment on plant tissue, a new method for *in situ* visualization of changes related to electropermeabilization of plant tissue was developed [81].

VI. Regulatory Issues

PEF food processing is currently subject to the Novel Food Regulation for its application within European countries. Safety is assumed if no additives are introduced in a food product and no changes in the composition of the product are made during processing. However, during PEF treatments, charged electrodes are in contact with a food product, which might inevitably lead to release of the electrode material into the product stream, depending on current magnitude, pulse duration, pulse shape, and product constitution [82]. Thus, investigation has been made into designing electrodes that have improved electrode durability and developing systems that generate pulse waveforms minimizing electrode corrosion [83]. Titanium was recently introduced as an alternative electrode material for durability [84].

A potential change in food compositions, induced by electrochemical actions of PEF, may be another factor which needs to be further studied for safety.

Commercially available PEF-processed juices and blends were introduced in the U.S. [85]. Commercial juice products include apple, strawberry, orange, and other flavors. They are packaged in glass bottles with full labels and sold from refrigerated cases. In order for PEF treatments to be applied to more foods, more studies demonstrating microbiological and chemical safety of various PEF-treated foods and the maintenance of fresh quality during extended storage need to be reported.

VII. Conclusion

PEF processing significantly increases microbiological safety and stability of foods, while reducing unfavorable changes in nutritional and sensory properties of the foods. PEF processing systems are available for a commercial production of PEF-treated foods. PEF-treated high-acid juices and blends are currently marketed in the USA. PEF has also been studied as a method in increasing efficiency in the extraction of cellular contents from plant materials. Development in PEF processing systems, verification of microbiological and chemical safety of PEF processed foods, and demonstration of freshlike quality of PEF processed foods have to be continuously made to gain popularity and broaden the category of commercial products of PEF-processed foods.

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