RECENT TRENDS IN COLD PASTEURIZATION OF FRUIT JUICES USING PULSED ELECTRIC FIELDS: *A REVIEW*

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Abstract
Thermal processing of fruit juices is widely practiced today due to the exceptional ability to inactivate numerous spoilage and pathogenic microorganisms and inherent enzymes. However, unfavorable effects on sensory and nutritional food characteristics often accompany the application of thermal treatment. Increase in consumer interest for fresh-like and quality foods has given rise to the development of innovative non-thermal food processing technologies such as pulsed electric fields (PEF). PEF has been evidenced that it caused inactivation of pathogenic and spoilage microorganisms resulting in better retention of flavors and nutrients and fresher taste compared to heat pasteurized food products. Moreover, PEF exerted its preservation ability by inactivation of selected enzymes due to their major role in the shelf life of food products. In the PEF system, pasteurization of foods is achieved within microseconds and thus attracted much attention of researchers and manufacturers worldwide. In recent years, there has been a great deal of research and a growing commercial interest with this alternative pasteurization technique, also called cold pasteurization. Therefore, in the present review, advances in pulsed electric field processing for cold pasteurization and its impact on the physicochemical and sensory qualities of fruit juices is discussed and points of further studies are highlighted.

Keywords: Cold pasteurization; pulsed electric fields (PEF); fruit juices; physicochemical quality; sensory quality.

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1. INTRODUCTION

In the past, fresh fruits and fruit juices were considered as safe from pathogenic bacteria due to their low pH, which may usually prevent the growth of pathogenic bacteria (Zhao, 2005). However, changes in consumer diets have led new public health problems associated with the presence of pathogenic microorganisms (Balla and Farkas, 2006). These changes have shifted towards the consumption of fresh fruit juice, owing to their high nutritional, sensory qualities and their health benefits. However, fruit juices can be favorable growth media for various emerging pathogenic microorganisms including *Salmonella* spp. and *Escherichia coli* O157:H7. Although, thermal pasteurization is the most widely used technology for fruit juices to eliminate pathogenic microorganisms and spoilage bacteria, it may adversely affect the organoleptic, nutritional and physicochemical properties of foods (Espachs-Barroso *et al.*, 2003; Martin-Belloso and Elez-Martínez, 2005). This is why today's consumers demand high quality, fresh-like and microbiologically safe foods (Mittal and Griffiths, 2005). Therefore, a great interest in the development of novel technologies offering the advantages of using low processing temperatures, low energy usage, and preservation of nutritional and sensory attributes, while inactivating pathogenic microorganisms to levels that do not cause a public health risk, is being tested (Smith *et al.*, 2002).

The pulsed electric field (PEF) technology can be considered as a potential alternative to traditional thermal processing of foods, whereby a high-intensity electric field produced between two electrodes creates a large flux of electrical current that flows through a food without significant changes in nutrients and the sensory properties of the food (Dunn, 2001). In the PEF system,

pasteurization of foods is accomplished within microseconds, and thus, the technology has received much interest from researchers around the world resulting in various publications in different PEF application areas.

There is a rising demand in the application of PEF in food processing. PEF applications in food processing have typically been directed to two major categories: microbial inactivation and preservation of liquid foods and enhancement of mass transfer and texture in solids and liquids. A large part of PEF work focused on reducing microbial loads in liquid or semi-solid foods in order to extend their shelf life and ensure their safety. Orange juice, apple juice, tomato juice, milk and liquid egg commonly studied liquid products (Hermawan *et al.*, 2004; Evrendilek and Zhang, 2005; Amiali *et al.*, 2006). Compared to heat-pasteurized products, PEF has been shown to inactivate pathogenic and food spoilage microorganisms as well as selected enzymes, resulting in better flavor and nutrient retention and fresher taste (Espachs-Barroso *et al.*, 2003; Sepulveda *et al.*, 2005). The other areas where PEF has potential applications in the future are juice extraction and dehydration. In view of the above, this review provides a summary of the applications of pulsed electric field in cold pasteurization of fruit juices.

2. PULSED ELECTRIC FIELDS PROCESSING OF FOODS

2.1. Overview of PEF

Rising consumer interest for food with a high nutritional value and a "fresh-like" taste has led to the development of new mild food preservation processes and alternatives to improve or replace conventional techniques, such as heat treatment. Many non-thermal pasteurization methods, such as high hydrostatic pressure or pulsed electric fields (PEFs), have been developed to achieve sufficient microbial reduction while retaining food quality. A rapid damage of internal cell structures and membrane can be caused by exposure to an external electrical field within few microseconds. Several applications of PEF were investigated for the past few tens of years based on a phenomenon called electroporation. According to Neumann (1996), PEFs are used in the field of plant and microbial genetics to induce electroporation of cell membranes to allow external substance, like DNA, enter the cell. It is necessary to control the activity of reversible electroporation to maintain growth and development of the organisms throughout the PEF treatment. Cells rebuild their membranes because of the reversible permeabilization through resealing the electropores instantly after the PEF treatment. It is also possible to use this principle to induce stress reactions and biosynthesis of desirable secondary metabolites of foods. Inactivation of microorganisms can be achieved by using higher treatment intensities due to the irreversible breakdown of the cell membrane. This irreversible pore formation due to PEF treatment can be used in food technology as a mild preservation technique for liquid food as well as an alternative for conventional methods of cell disintegration, such as grinding or enzymatic treatment as a pretreatment step for mass transfer improvement before dehydration, extraction, or pressing.

2.2. Mechanisms of action

In general, the inactivation effects of PEF on microorganisms rely on electroporation of the cell membranes and organelles (Raso *et al.*, 2014). Cell membrane is a semi-permeable and a very essential element of biological cell for mass transfer and takes a significant part in the production of DNA and RNA, protein and cell wall constituents in addition to a lot of extra complex metabolic activities (Rogers *et al.*, 1980). On the other hand, instability of intracellular organelles and other structural alterations were also reported (Harrison *et al.*, 1997). Depiction of membrane permeabilization not easy in the real-time process since the area of pore formation is only within the range of 0.1 per cent of the total surface of membrane surface and pores are created in the scale of sub-microsecond. The permeabilization of a cell membrane involves two basic steps: firstly, the applied electric field applied must induce the formation of a

pore and, secondly, the pore must be sufficiently stable to let the intra- and extracellular media interact.

Sale and Hamilton (1968) developed a theory based on the formation of a transmembrane potential, $\Delta\psi$, across the cell membrane when exposed to an external electrical field. Crowley (1973) suggested an electromechanical instability theory to describe decontamination of microorganisms using PEF. "The cell membrane is considered as a capacitor filled with dielectric material of low electrical conductance and a dielectric constant in the range of 2" (Zimmermann *et al.*, 1974). Accumulation of charges on the two sides of the membrane with opposing polarity results in a normally happening, perpendicular transmembrane potential of around 10mV. An additional potential is generated by movement of charges along the electric field lines when exposed to an external electrical field which eventually results in a viscoelastic deformation of the cell membrane. The transmembrane potential generated on a cell membrane by an externally applied electric field depends on the applied intensity of the electric field strength and the cell size and shape. The external electric field strength required to reach the transmembrane voltage threshold is known as critical electric field strength (*Ec*) as shown in Figure 1, and when $E > Ec$ electroporation is induced (Álvarez *et al.*, 2006).

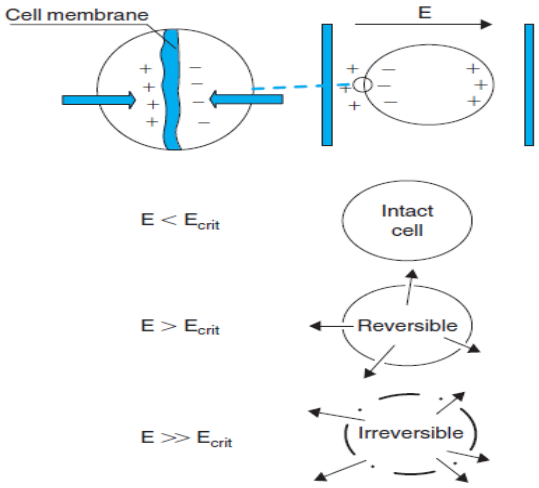

Figure 1. Schematic description of membrane permeabilization process with electrocompressive forces caused by an external electrical field (Toepfl *et al.*, 2014)

When the overall potential exceeds a critical value of around 1V, a local dielectric rupture of the membrane will occur due to the electrocompressive force and form a pore which acts as a conductive channel (Crowley, 1973; Zimmermann and Neil, 1996). Consequently, the compressibility, permittivity and initial thickness of the membrane depend on this phenomenon (Schoenbach *et al.*, 1997). Given a 5 nm membrane thickness, it turns into a 2000 V/cm dielectric strength. A massive increase in permeability restores the balance between the cell plasma and the extracellular medium of the electrochemical and electric potential differences to form a Donnan-equilibrium (Glaser *et al.*, 1988).

The breakdown of pores with electric filed treatment is reversible if the sizes of pores formed are small as compared to the area of the membrane. Increase in pulse width and/or number will increase the strength of electric field and treatment intensity which facilitate the formation of large pores. This enables the shift of reversible damage to irreversible breakdown along with mechanical damage of the cell membrane and leads to cell death. When the electric field intensity across the membrane exceeds threshold, permeabilization of microbial cells will be irreversible and subsequently results in a leakage of intercellular compounds and cell lysis (Jaeger *et al.*, 2014; Raso *et al.*, 2014).

2.3. PEF treatment system

A pulsed electric field processing system contains a power source, a charging current limiting resistor, an energy storage capacitor bank, a treatment chamber and a switch for discharging energy from the capacitor through the food. The pulse wave form is monitored by using an oscilloscope. The voltage DC generator, which is the main power source, converts voltage from a utility line to high voltage alternate current and transforms to a high voltage direct current. Energy is stored from the power source and discharged into the capacitor to produce an electric field via the treatment chamber in the food material. The voltage throughout the generator is

proportional to the capacitor's highest possible voltage. Amplified and rectified regular AC main source charges the bank of capacitors using a DC power source. Food is held in the treatment chamber and energy is discharged (instantly in millionth of a second) across the system from the capacitor storage bank using an electrical switch. Besides the main parts, other components such as pumps are required to move the food via the treatment chamber in case of continuous systems (Figure 2). During the treatment of food, a cooling chamber system could be required to minimize heating due to ohmic effect and control the temperature. High-current probes are used to determine the current and high-voltage probes are used to determine the voltage applied to the chamber (Amiali *et al.*, 2006).

2.4. Key factors affecting inactivation system by PEF

PEF inactivation are influenced by many factors, determined by the process, the food matrix in which the microorganism resides and the microorganism itself. Most literature focused on the process parameters, since they can be controlled in real food products. However, the microbial and product properties definitely need attention in order to evaluate whether PEF treatment is a suitable technology to obtain a safe application and provides the benefits that are aimed for.

2.4.1. Process parameters

The key mechanism of PEF inactivation of microorganisms is generally accepted as electroporation of the cell membrane (Zimmermann *et al.*, 1976; Chang and Reese, 1990; Ho and Mittal, 1996; Weaver, 2003), although intracellular organelle damage may also cause inactivation, particularly for pulses with a short pulse width (e.g. <100 ns) (Joshi *et al.*, 2001; Schoenbach *et al.*, 2001). Since the food matrix and the internal structures of a microorganism have a high conductivity compared to the cell membrane, the potential drop of an applied electric field will be concentrated on the cell membrane: the transmembrane potential. Above a critical transmembrane potential of approximately 150 mV (Chang, 1991), pores will be formed, which become irreversible above transmembrane potentials of approximately 1 V (Ho and Mittal, 1996). This irreversible pore formation process causes the microbial inactivation. The transmembrane potential (TMP) is described (Neumann, 1992) by Eq. 1:

$$TMP = k \cdot E \cdot r \cos(\varphi) \qquad \text{Eq. 1}$$

where k is a form factor (3/2 for spherical cells), E is electric field strength, r is cell radius and φ is the angle between the electric field and the point of interest on the membrane (1 at the poles).

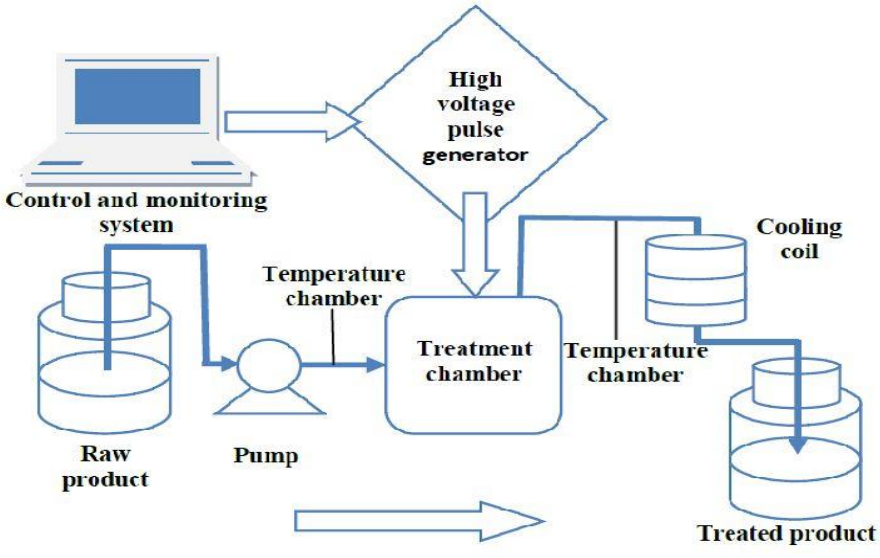

Figure 2. Components of a pulsed electric field system for food processing (Zimmermann and Benz, 1980)

The above equation implies that a higher electric field strength results in a higher transmembrane potential, thereby increasing the chance of irreversible pore formation and subsequently cell death. Higher inactivation rates at higher electric field strengths have been observed by many authors (Zimmermann *et al.*, 1976; Jayaram *et al.*, 1992; Qin *et al.*, 1998; Sampedro *et al.*, 2006). However, the applied electric field strength should be determined carefully. Many different treatment chamber designs have been applied to inactivate microorganisms. These designs usually have an inhomogeneous electric field distribution, which can cause an inhomogeneous treatment of the food sample (Donsì *et al.*, 2007). For a proper evaluation of the treatment, the electric field strength distribution in the treatment chamber should therefore be modelled carefully (Gongora-Nieto *et al.*, 2004), e.g. by application of multiphysics finite element models (Fiala *et al.*, 2001; Lindgren *et al.*, 2002) and it should be reported which electric field strength (at what place) is used for the description of the kinetics and whether it was homogeneous. The pore formation is a random process (Neumann, 1992), where pores are created which grow and finally become irreversible, leading to cell death (Weaver and Chizmadzhev, 1996). Therefore, a longer treatment time is a second factor that will lead to a higher chance of irreversible pore formation and cell death. The treatment time can be increased in two ways, either by increasing the pulse length or by increasing the number of pulses that are applied. Both approaches lead to higher inactivation levels (Hülsheger *et al.*, 1981).

A third important factor is the pulse shape. Pulses can be applied with several pulse forms and in a monopolar or bipolar fashion. In general, exponential decay or square wave pulses are used in PEF processing, with square wave pulses being more effective than exponential decay pulses (Zhang *et al.*, 1994; Ho and Mittal, 1996). The pulses can be applied in a monopolar or bipolar fashion, where a positive pulse is succeeded by a negative pulse. Bipolar pulses seem to be more effective than monopolar pulses, since the switch in polarity will cause a movement of charged molecules and therefore an increase of stress on the membrane (Qin *et al.*, 1994). However, contradictory results (Beveridge *et al.*, 2002), saying that bipolar pulses do not show any added inactivation, are also reported.

The last important process factor is the treatment temperature. Inactivation levels are higher with increasing temperatures (Pothakamury *et al.*, 1996), since the cell membrane turns into a liquid crystalline state at higher temperatures (Gášková *et al.*, 1996; Ho and Mittal, 1996). The treatment temperature is often hard to determine in experimental setups, since ohmic heating will cause an almost instant rise in temperature. Therefore, the starting or end temperature is often reported in the literature instead of the true treatment temperature (Wouters *et al.*, 1999). In addition, temperature differences can exist within the treatment chamber due to inhomogeneities in the electric field strength distribution. The inhomogeneities will cause a temperature distribution within the treatment chamber which can even be enhanced by the increase of the local conductivity at a higher temperature.

Concluding, it can be said that treatment at a higher electric field strength with a longer treatment time will give the best inactivation results. However, the product temperature will increase due to ohmic heating (also giving an increasing risk of bubble formation and arcing), which limits the electric field strength, the pulse width and the number of pulses that can be applied. In this respect, bipolar pulses (due to the higher efficiency) and higher treatment temperatures are beneficial, although the temperature of the incoming product will be limited by the temperature rise due to ohmic heating.

2.4.2. Microbial parameters

The PEF process is also greatly influenced by the microorganism to be inactivated. As has been stated above, the transmembrane potential is linearly correlated to the cell radius. This implies that larger cells have higher transmembrane potentials at a given electric

field strength, which makes them more vulnerable.
Therefore, in general, plant cells are more susceptible to PEF treatment than yeast cells, which, in turn, are more susceptible than bacterial cells (Sale and Hamilton, 1967). In the case of bacteria, gram positive ones are in general more resistant than gram negative ones (Hülsheger *et al.*, 1983; García *et al.*, 2007). It should be noted, however, that the inactivation kinetics of equally sized microorganisms can differ widely, as has been demonstrated for example by (Álvarez *et al.*, 2003).
Finally, the growth stage of the spoilage organism, i.e. whether it is in a logarithmic or stationary phase, is of importance during PEF treatment. During the cell division stage, microorganisms are more susceptible to electroporation (Wouters *et al.*, 2001) (Wouters *et al.*, 2001b). Therefore, cells in the logarithmic growth phase are more sensitive than stationary phase cells (Gášková *et al.*, 1996).

2.4.3. Product parameters

In addition to the process and microbial parameters, the inactivation kinetics are also influenced by product parameters. One of the most important of these is the conductivity of the product. Products with a high conductivity will cause a decrease of the treatment chamber resistance. Because of the lower chamber resistance, the pulse width will be reduced, which in turn causes lower inactivation rates (Vega-Mercado *et al.*, 1996; Wouters *et al.*, 1999).
This can be compensated, but at the expense of a higher energy input. Therefore, products with high ionic concentrations, such as vegetable juices (Barbosa-Cánovas and Zhang, 2001), seem to be less suitable for PEF treatment than lower conductivity products, such as fruit juices.
Other product parameters, for example pH (Vega-Mercado *et al.*, 1996; García *et al.*, 2005) or certain ions such as Mg^{2+} (Hülsheger *et al.*, 1981) may also affect the inactivation, but the effect varies with the microorganism.
Other molecules in the product can also affect the microbial inactivation kinetics. Nisin, for example, is a natural food preservative that works synergistically with PEF (Pol *et al.*, 2000).
Application of 50×2 µs pulses of 1.67 kV mm^{-1} to *Bacillus cereus* results in a log 1.2 log reduction, while nisin treatment with 0.06 µg ml^{-1} results in a log 0.8 reduction. A combined treatment results in a log 3.8 reduction, which clearly shows the impact of the nisin on the PEF treatment. The combined treatment of PEF with nisin and/or lysozyme has been reported by several authors (Dutreux *et al.*, 2000; Terebiznik *et al.*, 2002; Pol *et al.*, 2001; Iu *et al.*, 2001; Smith *et al.*, 2002; Liang *et al.*, 2002; Ulmer *et al.*, 2002; Zhang and Mittal, 2005; Wu *et al.*, 2005; Gallo *et al.*, 2007).

3. COLD PASTEURIZATION OF FRUIT JUICE USING PEF

3.1. Inactivation of microorganisms and enzymes in fruit juices using PEF

During PEF processing of fruit juices, inactivation of contaminating microorganisms can be achieved when the treatment is implemented properly (Figure 3).
Several researchers studied the influence of PEF on microbial decontamination in fruit juices. For example, Ertugay *et al.* (2013) evaluated the influence of PEF on an unclarified apple juices pre-treated at 40 °C and at electric field strengths of 30 and 40 kV/cm with pulses (2 µs each) of 50, 100, 150, and 200. The authors stated that complete inactivation of total yeast/mold and mesophilic bacteria would be possible with the studied PEF treatment conditions.
These studies were conducted over a storage time of three month at 25 °C. In addition, the authors also noted that PEF treated juices didn't show microbial activity throughout the storage time when compared with the untreated sample which was observed to have higher than 6 log CFU/ml at the end of the three month time.

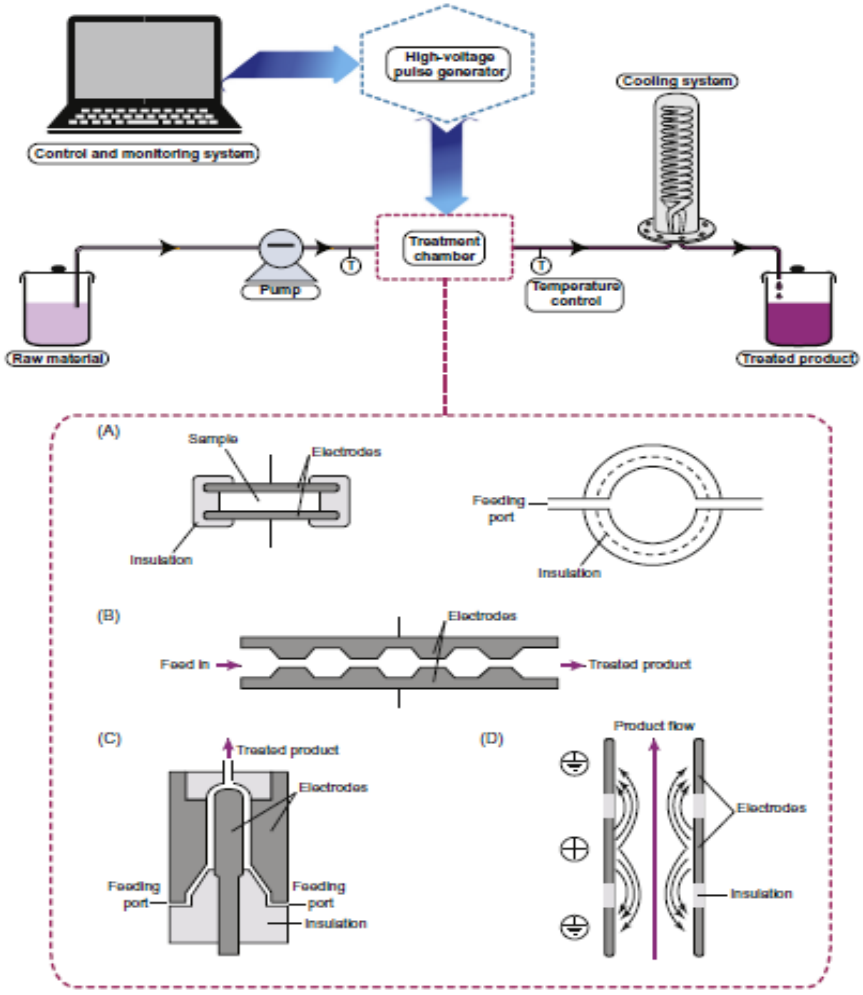

Figure 3. Typical pulsed electric field system used for fruit juice treatment. (A) Static chambers; (B) Side-view of a basic continuous design; (C) Coaxial chamber; and (D) Colinear chamber (Koubaa *et al.*, 2018)

In another work on banana juice quality, Gao *et al.* (2015) determined the impact of Maillard reaction products (MRPs) in the fructose-lysine model system in combination with PEF. The authors investigated the inhibitory potential of MRPs polyphenol oxidase activity in banana and the changes in color. Besides, they evaluated the minimum inhibitory concentration (MIC) and minimum bactericidal concentration of MRPs for growth inhibition of *Staphylococcus aureus* and *E. coli* with/without combination with PEF. Based on the findings of the study, PEF treatment of banana juice inactivated the two microbes and significant improvements were obtained on the inhibitory effects of MRPs. The MICs of MRPs against *S. aureus* and *E. coli* decreased to 1.56 and 3.125 mg/mL, respectively, with PEF treatment at 30 kV/cm for 1200 µs.

In line with inactivation of microorganisms by PEF, the impact of *Stevia rebaudiana Bertoni* extract on inoculated/non inoculated mango and papaya juices with *Listeria monocytogenes* were evaluated (Belda-Galbis *et al.*, 2016). According to these authors, PEF treated juices were found to have lower microbial load and its reduction was proportional to the electric field intensity and treatment duration. In a different study, Huang *et al.* (2014) investigated the resistance of *S. aureus*, *E. coli* DH5α, and *S. cerevisiae* to PEF treatments in grape juice with variable biological factors. The authors employed electric field strengths of 12-24 kV/cm and treatment times of 30-180 µs, where the temperature of the grape juices was initially 30 °C. Based on this study, *S. aureus* was the most resistant microorganism followed by *E. coli* DH5α whereas *S.*

cerevisiae was observed to demonstrate the most sensitive nature. Similar studies on the influence of PEF on fruit juices at various conditions are summarized in Table 1.

Timmermans *et al.* (2014) investigated the impact of continuous-flow PEF system on pH, temperature, and inactivation of pathogenic and spoilage microorganisms, in apple, orange, and watermelon juices. Treatment conditions of 20 kV/cm with varying frequencies were used to inactivate *Salmonella panama*, *Saccharomyces cerevisiae*, *L. monocytogenes*, and *E. coli* in the fruit juices. Based on the findings, kinetic data revealed that *E. coli* was the least sensitive followed by *S. panama* and *S. cerevisiae* was reported to be highly sensitive to the treatments at similar conditions. Moreover, among the studied microbes, *L. monocytogenes* was reported to show highest resistance under similar conditions of treatments. Studies with combined treatments of electric field strength and temperature revealed a synergistic effect when the temperature was higher than 35 °C. Thus, at higher temperatures, a lower energy was needed to inactivate microorganisms.

The very definition of the enzymes as proteinaceous catalysts that change the rate of a biochemical reaction without undergoing any qualitative and quantitative change provides an answer for the effect of PEF on enzymes. It is well established that proteins carry an electric charge, and the application of an electric field induces association or dissociation of functional groups, movements of charged chains, and changes in alignment of helices, and this change in conformation results in enzyme inactivation.

This mechanism is supported by a number of studies, wherein papain was inactivated by the loss of helical structure; alkaline phosphatase was inactivated by polarization followed by aggregation and loss of native structure (Castro *et al.*, 2001). Similar to microbial inactivation, enzyme inactivation also depends on parameters such as strength of the electric field, exposure time, pulse width, treatment temperature, and structure of the enzyme and product characteristics (Yeom and Zhang, 2001).

Table 1. Summary of the studies conducted on fruit juices using PEF

Juice	PEF conditions	Microorganisms studied	Reference
Amla	26 kV/cm, 1 Hz, 1 µs, 500 µs, <41°C	*Zygosaccharomyces bailii* (MTCC 257)	Bansal *et al.* (2015)
Apple	29 kV/cm, square wave, 34 kV/cm, 166 µs of treatment time, 1.5 mL/s, 800 pps	*E. coli* O157:H7	Evrendilek *et al.* (2000)
Apple	20 kV/cm, 10.4 pulses, square wave	*S. cerevisiae*	Cserhalmi *et al.* (2002)
Blueberry	30 kV/cm, 54 μs and 20 to 35 kV/cm, 27 to82 μs	*E. coli*	Chen *et al.* (2014)
Cranberry	40 kV/cm, 150 µs treatment time, square wave	Aerobic microorganisms, yeasts and molds	Jin and Zhang (1999)
Grape	65 kV/cm, 20 pulses, 50 °C	Aerobic microorganisms, yeasts and molds	Wu *et al.* (2005)
Orange	40 kV/cm for 150 µs, 55 °C	Aerobic microorganisms, yeasts and molds	Walkling-Ribeiro *et al.* (2009)
Pineapple	33.0 kV/cm	*Z. bali* ascospores, Vegetative cells (V), Ascospores (A)	Raso *et al.* (1998)
Prickly pear	27 to 36 kV/cm, 25 and 50 Hz, 11 to 15 μs, 25 °C	*S. cerevisiae*	García-García *et al.* (2015)
Tomato	80 kV/cm, 20 pulses, 50 °C	Aerobic microorganisms, yeasts and molds	Nguyen and Mittal (2007)

In general, enzymes are more resistant to PEF treatment. The three-dimensional molecular structure of globular protein is stabilized by hydrophobic interactions, hydrogen bonding, van der Waal interactions, ion pairing, electrostatic forces, and steric constraints. The conformational nature of protein can be influenced via different forms of chemical reactions either by charge, dipole, or induced dipole when subjected to high PEF treatment. The charged groups and structure are highly susceptible to various types of electric field perturbations. Association and dissociation of ionizable groups, movement of charged side chains, changes in structure, and alignment of helices and, thus, the overall shape of the protein may all be influenced by external electric fields. Because high PEF can be used to inactivate enzymes, which are responsible for development of oxidative off-flavor and color in the foods, the quality of the foods treated with PEF can be preserved. The reaction kinetics of the enzyme activation follows the first-order reaction kinetics which can be expressed by Eq. 2.

$$EA = EA_o e^{-(k_1 t)} \quad \text{Eq. 2}$$

where EA = % residual enzyme activity, and $t = n\,\tau$. For instance, in the case of peach PPO, the first order constant of inactivation k_1 varied from 9 (at E = 3 kV cm^{-1}) to 234 μs^{-1} (at 24 kV cm^{-1}) (Kotnik and Miklavcic, 2000). The value of k_1 is expressed as function of the electric field strength E and can be expressed by Eq. 3.

$$k_1 = k_{o1} e^{-(\omega E)} \quad \text{Eq. 3}$$

where k_{o1} and ω are constants. The value of EA can be expressed as a function of the total energy density ω_t supplied by Eq. 4 as follows:

$$EA = EA_o e^{-(K\omega_t)} \quad \text{Eq. 4}$$

where K is a constant whose value varies depending upon the shape of the pulse, i.e., bipolar exponential decay pulses, mono- or bipolar pulses. In general, bipolar pulses are more effective than monopolar pulses.

3.2. Impact of PEF on fruit juice qualities

Various research groups have studied the influence of electric pulses on food matrix, particularly the physicochemical and nutritional parameters in liquid foods, such as fruit and vegetable juices, milk and other beverages (Barba *et al.*, 2012; Zulueta *et al.*, 2013). It was shown that PEF impact depends on the food matrix; hence it is necessary to evaluate each product individually before it can be marketed. The evaluation of the sensorial and nutritional quality of these kinds of beverages is very important for the consumers, since it determines the product's acceptance (Riener *et al.*, 2009; Soliva-Fortuny *et al.*, 2009). The adverse effects of PEF on the physicochemical and sensorial properties of the concentrated fruit juices are mainly referred to the content and to the evaluation of sugar content, pH, acidity, and browning degree (Evrendilek *et al.*, 2000; Yeom *et al.*, 2000). With regard to bioactive compounds and antioxidant capacity, vitamin C is the main vitamin studied in relation to PEF and fruit juices (Barba *et al.*, 2017). It is one of the most abundant thermolabile vitamins in foods; hence it could be among the best-preserved compounds in processing by non-thermal treatments. Various studies have shown that juices treated by PEF retain a greater quantity of vitamin C than the thermally processed ones (Elez-Martinez and Martin-Belloso, 2007; Min *et al.*, 2003). However, there are only few studies on the influence of PEF on other biologically active compounds, such as phenolics (Agcam *et al.*, 2014; Ertugay *et al.*, 2013).

3.2.1. Impact of PEF on the physicochemical properties of fruit juices

Physical properties are important measures of food quality in the food and bioprocess industries, as they influence the consumer's choice and preferences (Putnik *et al.*, 2017b). The °Brix value is used to indicate the percentage of soluble solids content (SSC), and it is one of the main factors for juice quality assessment (Nagy and Attaway, 1980). Several studies have been carried out to evaluate the effects of PEF on the physicochemical characteristics in different fruit and vegetable juices. For instance, (Morales-de La Peña *et al.*,

2010) did not observe significant changes in fruit juice-soy milk beverage after PEF treatment (35 kV/cm, 800 or 1400 μs) on SSC, pH, and acidity values. However, regardless of the applied treatment, beverage viscosity increased over time. (Elez-Martínez *et al.*, 2006) studied the effects of PEF processing (35 kV/cm, 1000 μs) on various physicochemical properties in orange juice, observing no significant changes in pH, acidity, or °Brix. Similar results were observed when processing apple juices with PEF (Ertugay *et al.*, 2013). The authors reported merely slight variations in the levels of pH, conductivity, color, °Brix and physical appearance. On the other hand, a slight decrease was observed by (Cserhalmi *et al.*, 2006) in pH, °Brix, and enzymatic browning for orange juice treated with PEF (28 kV/cm, 100 μs).

In apple juice, Ortega-Rivas *et al.* (1998) observed no changes concerning SSC, pH, and acidity. However, a slight browning was observed after 50, 58, and 66 kV/cm of PEF treatment using 2-16 pulses and with 40 kV/cm over 100 μs (Walkling-Ribeiro *et al.*, 2008).

Cortés *et al.* (2005) studied the behavior of a refreshing drink from tiger nuts, "horchata" after PEF treatment (20, 25, and 35 kV/cm, 100-475 μs) with no changes in pH and total fat. Similar results were found by Rivas *et al.* (2006) in the assessment of PEF processing effects (25-40 kV/cm, 30-340 μs). No significant changes in pH, °Brix, or hydroxymethylfurfural were reported in an orange-carrot beverage. Garde-Cerdán *et al.* (2007) observed neither significant changes in pH, nor reducing sugar content or total acidity in grape juice treated by the PEF and high field strengths for longer treatment times (35 kV/cm, 1 ms). In a tomato juice, (Min and Zhang, 2003) found no significant changes in pH, °Brix, or viscosity when PEF was applied (40 kV/cm, 57 μs). Nevertheless, an opposite result was found by Aguilo-Aguayo *et al.* (2008), a viscosity increase being observed in the PEF-treated tomato juice (35 kV/cm, 1700 μs) as compared to the thermally processed and untreated samples. Similar findings were observed in strawberry juices in comparison with heat treatment (Aguiló-Aguayo *et al.*, 2009).

In a recent study performed by Aadil *et al.* (2015), the effect of various levels of electric field strength was investigated on the qualities of grape juice. Experimental conditions employed in the study include: electric field strengths of 0, 5, 10, 15, 20, and 25 kV/cm, flow rate of 80 mL/min, pulse frequency of 1 kHz, and pulse duration of 600 μs at 40 °C. The authors studied antioxidant activity (DPPH), total phenols, total anthocyanins, total antioxidant capacity (TAC), total carotenoids, and sugars, and the physico-chemical characteristics of the juice. The obtained results showed that with increasing electric field strength, changes in °Brix, titratable acidity, pH, total anthocyanins, sugars or color attributes were not significant as compared to control. However, a decrease in viscosity and non-enzymatic browning was significant along with an increase in TAC, total phenols, DPPH and total carotenoids cloudiness value under similar treatments compared to control. According to the authors' conclusion, the qualities of grape juice could be improved by applying PEF treatment at 25 kV/cm and may successfully be employed for the processing of grape juice at industrial scale. The effects of PEF processing on the physicochemical properties of different kinds of juices are summarized in Table 2 below.

3.2.2. Impact of PEF on color, aroma and flavor of fruit juices

The visual color is an important quality attribute of foods as it is usually the first property evaluated by the consumer (Putnik *et al.*, 2017b; Putnik *et al.*, 2017a). Detrimental changes in color during processing, primarily caused by nonenzymatic browning, reduce the consumer's acceptance of the juices. Researchers have also used color as an indicator of the organoleptic and nutritional qualities of food during storage and processing. It has been reported that many reactions could take place during thermal processing that affect the color.

Table 2. PEF processing effects on the physicochemical properties of different juices

Juice	PEF conditions	Key findings	Reference
Apple	16 Hz, 0 to 35 kV/cm, 0.2 and 2 μs, 75 μs, <42 °C	The content of ascorbic acid decreased significantly during PEF treatment, the highest loss was 36.6% at 30 kV/cm and 2 μs-pulse rise time.	Bi *et al.* (2013)
Carrot	200 Hz, 20 to 35 kV/cm, 6 μs, 300 to 2000 μs, <40 °C	POD reduced by 93% at 35 kV/cm for 1500 μs.	Quintão-Teixeira *et al.* (2013)
Date	100 Hz, 35 kV/cm, 4 μs, 1,000 μs, <35 °C	Lower HMF concentration than pasteurized samples. Beneficial effect on turbidity, soluble solids and pH. The content of total phenols increased after treatment compared to untreated juice.	Mtaoua *et al.* (2017)
Longan	10 Hz, 32 kV/cm, 3 μs, 90 s, <40 °C	High retention of ascorbic acid and flavor compounds.	Zhang *et al.* (2010)
Mango	200 Hz, 35 kV/cm, 4 μs, 1500 μs, <40 °C	PEF treatment did not negatively influence the antioxidant activity.	Odriozola-Serrano *et al.* (2016)
Mango, papaya blend sweetened with *Stevia rebaudiana*	32 and 256 kJ/kg, <35 °C	High retention of the ascorbic acid content (80% to 83%). Total carotenoids were significantly higher than untreated sample; reduction of carotenoids at 256 kJ/kg, total carotenoid diminished significantly. Phenolic concentration was significantly higher at 256 kJ/kg.	Carbonell-Capella *et al.* (2016)
Peach	55 Hz, 36 kV/cm, 21 μs, 25 °C	Ascorbic acid retention increased more than 10%.	Meneses *et al.* (2011)
Tomato	100 Hz, 35 kV/cm, 4 μs, 1500 μs, <40 °C	An enhancement of 63% to 65% in 15-*cis*-lycopene content.	Vallverdú-Queralt *et al.* (2013)
Twistspine pricklypear	35 kV/cm	Good preservation of antioxidant activity.	Moussa-Ayoub *et al.* (2011)

Among them, the most common are pigment degradation (especially carotenoids, anthocyanins, and chlorophylls), and browning reactions such as Maillard reactions, enzymatic browning, and oxidation of ascorbic acid. The CIE Lab parameters: (1) lightness (L^*); (2) $-a^*$ (green), $+a^*$ (red); and (3) $-b^*$ (blue), $+b$ (yellow) (Sharma, 2003) have been widely used to describe the color changes during processing in fruit and vegetable products (Bouaziz *et al.*, 2016; Herceg *et al.*, 2016). Non-significant changes in the CIE Lab parameters were found by Walkling-Ribeiro *et al.* (2008), and by Evrendilek *et al.* (2001) in PEF-treated apple juice and chocolate-milk beverage. However, the PEF treatment (35 kV/cm, 1700 µs) of strawberry juices resulted in greater L^* values than the heat treatment (Aguiló-Aguayo *et al.*, 2010). Xiang *et al.* (2011) also reported significant alteration in the CIE Lab parameters of a soy beverage, with increased a* value after PEF processing (18-22 kV/cm). Similarly, Bi *et al.* (2013) demonstrated that PEF-treated (0-35 kV/cm) apple juice had significantly higher lightness and yellowness than the control sample.

Aguiló-Aguayo *et al.* (2010) reported that during storage, PEF-treated watermelon juice (35 kV/cm, 1727 μs, 188 Hz in bipolar mode) maintained a brighter red color than the thermally processed juices.

The flavor of fruit and vegetable juices is easily influenced during processing and storage. Irreversible changes are produced in the flavor of juices as a result of chemical reactions that are initiated or occur during thermal processing. The changes in flavor are also associated with a number of deteriorative reactions that take place during storage, giving rise to the development of off-flavors (Putnik *et al.*, 2017a). Several authors have studied the effects of PEF processing on the flavor of orange juice, where no differences in sensory acceptability from the original fresh juice were observed (Min *et al.*, 2003; Yeom *et al.*, 2000). For instance, it was reported that PEF-treated juice showed better ratings in a sensory analysis in comparison with untreated tomato juice (Qin, 1995). Similarly, Mosqueda-Melgar *et al.* (2012) did not find significant changes in the aroma after PEF processing in apple, pear, tomato, strawberry, and orange juices. However, significant changes in flavor could occur during PEF treatment of juices as previously reported by Min and Zhang (2003), when processing tomato juice with PEF (40 kV/cm, 57 μs). A similar result was observed by Jia *et al.* (1999), who found that PEF-treated orange juice with an electric field of 30 kV/cm for 240 and 480 μs resulted in a loss of the aromatic compounds between 3% and 9%, respectively. This was also observed by Sampedro *et al.* (2009) when using PEF in continuous treatment. The authors reported a reduction in volatiles (8.3%-13.7% at 25 °C; 5.8%-21% at 45-C; and 22.9%-42.3% at 50 °C) after application of PEF (15-30 kV/cm, 50 μs, 25-50 °C) in an orange juice mixed with milk. In a different study by Evrendilek (2016), PEF treatment affected aroma-active compounds in apricot and peach nectars, and sour cherry juice. Findings revealed that PEF treatment preserved 70% of the physical and 94% of the sensory characteristics. Moreover, from a total of 73 aroma-active chemical components identified from both juices, significant amount (57%) was affected. The authors demonstrated that PEF could be applied with various treatment times for pasteurization of apricot and peach nectars, and sour cherry juice, along with minimum loss of aroma-active compounds, sensory and physical properties.

4. CONCLUSION AND FUTURE OUTLOOK

The use of PEF technology in food processing has demonstrated immense potential for preserving premium quality products, such as fruit juices at lower temperatures and short residence periods while maintaining the product's fresh-like property and nutritional characteristics. The effects of the PEF process, however, depend largely on the treatment conditions and chemical composition of juices such as macronutrient contents. In addition, the effect PEF on physicochemical properties can differ during processing, but the viscosity of juices tends to increase. During treatment with PEF, smell, taste and color changes are within the appropriate industrial range. Such treatment can therefore be an alternative to thermal treatment (e.g. pasteurization), with the benefit of maintaining no or limited physicochemical modifications and degradation of bioactive compounds. As a result, application of PEF processing at industrial scale can be an option to retain the physicochemical and nutritional qualities of foods.

The effectiveness of PEF processing to inactivate microorganisms and enzymes in fruit juices has been reported. Yet, there are several fundamental questions about this technology that have not been addressed or clarified to date. These are the detailed mechanism that PEF exerts at the molecular level, which is associated with the existence of sublethal cell injury, and engineering challenges that are linked to PEF treatment chamber design or optimization of multivariable processing conditions to provide more uniform food treatment. Future works may be essential to investigate interaction of food matrices with electric field strength on molecular level, screening of spoilage and pathogenic

microorganisms, standardization and quantification of PEF treatment variables and their role to the inactivation mechanism, and the influence of PEF treatment on enzymes and inactivation mechanism. Because of equipment variations (continuous vs static), applied pulse wave forms (exponential, logarithmic, monopolar vs bipolar), and scaling variations (bench scale vs pilot scale), sometimes it is difficult to compare the experimental results. Besides, because of the uniqueness of some liquid food systems, a substantial amount of optimization will have to be undertaken to render the process efficient and to produce the desired quality. Industry and consumer acceptance is important for success of PEF technology, which will be driven by marketing and awareness campaigns. Overall, it may be pointed out that PEF process has immense potential for use as a preservation treatment for various fruit juices and liquid foods.

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