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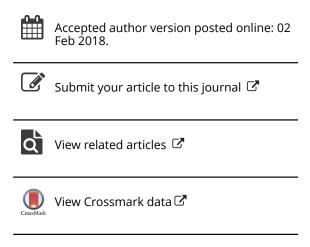
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Enhancing Food Processing by Pulsed and High Voltage Electric Fields:

Principles and Applications

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Abstract

Improvements in living standards result in a growing demand for food with high quality attributes including freshness, nutrition and safety. However, current industrial processing methods rely on

traditional thermal and chemical methods, such as sterilization and solvent extraction, which could induce negative effects on food quality and safety. The electric fields (EFs) involving pulsed electric fields (PEFs) and high voltage electric fields (HVEFs) have been studied and developed for assisting and enhancing various food processes. In this review, the principles and applications of pulsed and high voltage electric fields are described in details for a range of food processes, including microbial inactivation, component extraction, and winemaking, thawing and drying, freezing and enzymatic inactivation. Moreover, the advantages and limitations of electric field related technologies are discussed to foresee future developments in the food industry.

This review demonstrates that electric field technology has a great potential to enhance food processing by supplementing or replacing the conventional methods employed in different food manufacturing processes. Successful industrial applications of electric field treatments have been achieved in some areas such as microbial inactivation and extraction. However, investigations of HVEFs are still in an early stage and translating the technology into industrial applications need further research efforts.

Keywords: Electric fields, polarization, PEF, HVEF, mass transfer, food processing

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1. Introduction

There is an imperative need for the food industry to enhance process efficiency and productivity. maintain the nutritional and sensory properties of the food products and reduce processing cost, responding directly to the growing demands for foods of higher quality. It is widely known that traditional food processing strongly relies on thermal and chemical methods, such as the use of thermal sterilization, enzyme inactivation, direct-heat drying, solvent extraction, etc. These thermal treatments can have negative effects on heat sensitive components and flavor substances in food, causing losses of nutrients and sensory deterioration (Knoerzer, 2016). In addition, the overuse of organic solvents will cause environmental issues and the addition of antimicrobial agents like nitrates during meat processing will inevitably cause food safety concerns. Over the last decade, novel processing technologies have become a hot topic in the food industry. With the aim to delivering high quality food products, various alternative technologies have been investigated and developed, some of which have become available to the industry, such as processing using or assisted by high hydrostatic pressure, ultrasonic wave, electric field and magnetic field, which utilize no thermal or chemical treatments (Knoerzer et al., 2015). Therefore, in this paper, an overview on the principles of electric field technologies is given, and its latest applications in enhancing food processes are presented, leading to proposing future research directions.

The early utilization of electric field technology in food processing can be traced back to the

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early 1920s when a low-voltage alternating electric field was first applied to milk pasteurization (Prescott, 1927). Since then, studies have proved the positive effects of electric field on the inactivation of microorganisms due to electroporation and electric breakdown of the cell membranes (Tsong, 1991; Zimmermann, 1986). In recent years, investigations on electric field technologies have indicated their applications in the inactivation of microorganisms and enzymes, freezing and thawing, extraction of valuable compounds, acceleration of drying and winemaking. The application of the EFs can be performed in two modes: pursed electric fields and high voltage electrostatic fields (Sun, 2014). With the efforts of researchers, some industrial applications of PEFs have been achieved, for example, PEFs for microbial inactivation and extraction. However, development of HVEFs are at very early stage and their applications in the food industry still requires extensive research efforts.

2. Generation and Working Principles of Electric Fields

2.1 Generation of PEFs and HVEFs

Loeffler (2006) presented a comprehensive review on the generation and application of high intensity PEFs. A PEF processing system mainly consists of four parts: 1) a high voltage power supply, 2) a capacitor bank for energy storage and discharge handled by the on/off-switche, 3) a pulse generator providing pulses of given voltage, shape and duration by using a pulse forming network (PFN), 4) a control system for setting and monitoring the conditions, and 5) a treatment

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chamber installed with one or more electrodes inside. The electrode gaps are filled with liquid or aqueous substance to be treated. The high voltage power supply (1~100 kV) is converted from a public line (110 V or 220 V). The high-power switch is the connecting element between the storage capacitor and treatment chamber. After the completion of energy storage in the capacitor charged by the power source (with an opening switch), the switch should be closed to discharge the energy across the material to be treated. The construction of electrodes should follow the principle of planar, coaxial, and axial electrode geometries. The design and construction of pulsed power systems should focus on the field strength, treatment time, and pulse waveform that can affect the outcome of PEF treatment (Mohamed & Eissa, 2012). Typical types of pulses allowing the generation of high intensity PEF are monopolar or bipolar, and the exponentially decaying or square waves are among the commonly used waveforms. Monopolar pulses are a set of either positive or negative waves, whereas bipolar pulses are a set of pulse pairs that composed of one positive and one negative wave. Compared to monopolar pulses, bipolar pulses are more effective on the permeabilization of the cell membranes (Qin, et al., 1994), due to the structural fatigue of the membrane caused by the alternating stress. This alternating stress is produced by the high-frequency reverse of the pulse polarity.

The generation system of HVEFs is quite simple compared to that of PEFs. At present, the most commonly used equipment for generation of HVEFs mainly consists of three core devices, a direct current-power supply, a high voltage generator and a treatment chamber (Orlowska *et al.*, 2009). A

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low voltage (0 ~ 30 V) is provided by the power supply and then is converted to high voltage (500 ~ 50,000 V) through the high voltage generator. This high voltage is finally applied to the electrodes installed in the chamber, forming a HVEF with passage of negligible microcurrent through the area covered by the field. Generally, the electrodes are designed to be parallel-plate, pin-to-plate or wire-plate forms for different purposes.

2.2 Working Principles

Foods are complex multiphase/multicomponent systems that allow for the formation of dipole moments or exhibit net charges. This electrochemical characteristics of the polar or charged particles can produce dipolar vibration, reorientation, translation and rotation when subjected to external electric fields. For instance, water molecules tend to be polarized, charged even reshuffled and realigned along the field, contributing to the reduction of free energy of food system. For proteins, especially enzymes, the bioactivity might be reduced due to the changes of molecular conformation (Pereira et al., 2011). With exposure to specific electric stimuli, some of the electrochemical and physicochemical properties change responsively (Bateni et al., 2004). For example, the hydrogen bonds along the electric field are strengthened while the others orthogonal to the field are weakened, causing a decrease of the dielectric constant and an increase of the surface tension of the water. The modification of electrically-sensitive components by electric field is capable of accelerating the mass and heat transfer, which becomes evident in the resulting

biological effects, mainly referred to 1) permeabilization of biomembranes induced by high intensity PEFs, 2) corona wind generated in non-uniform HVEFs, 3) molecular modification catalyzed by strong electric fields, and 4) polarization and realignment of molecules with dipole moments. All these biological effects are expected to play an effective role in food processing.

3. Permeabilization Effects of EFs

3.1 Mechanism

At present, PEF is receiving increased attention due to effective lethality on microorganisms and remarkable extraction efficiency for valuable components. However, the exact mechanism of the permeabilization effect of EFs is still in debate. The most widely accepted mechanisms responsible for the permeabilization effect are electric breakdown (Zimmermann, 1986) and electroporation (Tsong, 1991), leading to disruption of the cell membranes. The schematic of electric breakdown of cell membranes is shown in Fig. 1 (a). Under an externally applied electric field, the difference of transmembrane potential is formed on the cell membrane. When the electric field intensity across the membrane exceeds a threshold, permeabilization of microbial cells will be irreversible and subsequently results in a leakage of intercellular compounds and cell lysis (Jaeger et al., 2014; Farid, 2016). The hypothesis of electroporation is based on the polarization effect of high-voltage PEF on the frame structure of cell membrane. As shown in Fig. 1 (b), the phospholipid bilayer and proteins of the cell membrane temporarily get unstable under the applied

electric field, resulting in the formation of holes, which is followed by an permeability enlargement of cell membrane. Small molecules such as water can easily pass through the membrane into the cell driven by the colloid osmotic pressure of cytoplasm and cause the cell expansion, leading to the membrane rupture and death of the cells (Golberg *et al.* 2010).

3.2 Inactivation of Microorganisms

Inactivation of microorganisms is one of the most critical steps directly affecting the preservation of quality and safety attributes. Subjected to PEF treatments, pathogenic microorganisms including Escherichia coli, Salmonella Enteritidis, Listeria monocytogenes, Staphylococcus aureus, Pseudomonas fluorescens, lactobacillus plantarum Zygosaccharomyces bailii can be significantly inactivated (Table 1). For instance, Mosqueda-Melgar et al. (2008) combined high-intensity PEF with natural antimicrobials (citric acid and cinnamon bark oil) to inactivate E. coli O157:H7, S. Enteritidis and L. monocytogenes, showing that under the PEF treatment (35 kV/cm at 193 Hz and 4 ms pulse duration), reductions of more than 5.0 log₁₀ CFU/mL of the microbial populations in melon juice (for 1709 ms) and water melon juice (for 1682 ms) containing 2.0% and 1.5% of citric acid, respectively, or 0.2% of cinnamon bark oil were obtained. Recently, similar finding was expressed by Moody et al. (2014) working at a field strength of 30.76 kV/cm at 40 °C on apple juice inoculated with E. coli ATCC 11775, whereas at lower temperatures (32.5 °C) and higher intensity (40 kV), the same inactivation

effect (about 5 log reduction) was observed on milk subjected to PEF treatments. This finding reveals that the increase of electric field intensity has the capacity of reducing the requirement of high temperature. Moreover, other pathogenic bacteria such as *S. Aureus* and *P. fluorescens* have also been studied for the sensitivity to PEF. Cregenzán-Alberti *et al.* (2015) reported reductions of about 5 log cycles for *S. Aureus* and *P. fluorescens* under the PEF treatments (40 kV/cm for 89 μs and 42.5 kV/cm for 106 μs, respectively) at 32.5 °C. In general, the general field intensity of PEFs involved ranges from 20 kV/cm to 45 kV/cm, and the reduction of the microorganisms is close to 5 log₁₀ CFU/mL for fresh fruit and vegetable juices. Furthermore, these references reveal the fact that it is not possible to reach sterilization with PEF treatment at a temperature slightly higher than the room temperature.

Compared to single traditional thermal or PEF treatment, the combination of PEF with thermal treatment has been proved to be more effective on microbial inactivation, not only shortening the treatment time but refucing the temperature and population of microorganisms significantly. This is crucial to inactivation of spores. For example, Bermúdez-Aguirre *et al.* (2012) demonstrated that PFF treatment (40 kV/cm, 144 pulses) combined with thermal treatment (65 °C) could achieve 3.6 log reduction of *Bacillus cereus* spores in skim milk containing nisin (50 IU/mL). In addition, Siemer *et al.* (2014) reported 4.4 log reduction of *Bacillus subtilis* spores in Ringer solution with conductivity of 4 mS/cm and pH value of 7 treated by PEF (9 kV/cm, 20 µs) with thermal energy input of 195 kJ/kg (80 °C), which was significantly higher than the reductions of 1.15 log and 3.25

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log for thermal and PEF related inactivation, respectively. However, the inactivation mechanism of spores by PEFs is unclear. Some researchers assumed that PEFs might induce germination of the spores (Shin *et al.*, 2010). Furthermore, the type and growth stage of the microorganisms also influence the inactivation rate. PEF treatment is more effective on the inactivation of gram-negative (G') bacteria and vegetative cells than grampositive (G⁺) bacteria yeasts, and spores (Barbosa-Cánovas & Altunakar, 2006; Siemer *et al.*, 2014). All these findings validate the PEF treatment as a supplementary but not an alternative tool to overcome the disadvantages of conventional thermal sterilization. PEF combined with thermal treatment can reduce the population of microorganisms in food significantly and minimize the sensory deterioration caused by high temperatures.

3.3 Extraction of Valuable Compounds

Conventional extraction methods based on organic solvents or physical press are known to be energy intensive, environmental unfriendly, time consuming and high cost. Relevant literatures proved that PEFs could enhance the extraction process significantly by increasing the permeability of cell membranes that facilitates passage of intracellular compounds to the surrounding solution, subsequently accelerating the mass transfer and thus extraction rate (Yu et al., 2015).

Therefore, PEF technology has been applied in the extraction of bioactive substances such as proteins, carbohydrates, polysaccharides, lipids, etc. (Ganeva et al., 2003; Yu et al., 2015; Yin et

al., 2006; Guderjan et al., 2007) (Table 1). Ganeva et al. (2003) performed an experiment on electroextraction of intracellular enzymes from yeast treated by PEF and obtained a high yield of up to 80% ~ 90%. Yin et al. (2006) optimized a PEF extraction method for polysaccharide from Rana temporaria chensinensis David, in which the samples were immersed in 0.5% KOH solution rather than organic solvent and subjected to PEF treatment (20 kV/cm, 6 μs duration). The extracted polysaccharide showed a higher extraction rate (55.59%) and purity compared with conventional extraction methods using alkali, enzyme and complex enzymes. At a pilot-plant scale, Puértolas et al. (2010) utilized continuous PEF treatment (2 ~ 7 kV/cm, 10 μs duration) to extract anthocyanins and phenols during fermentation of red grapes. Compared with the control group, increments of the extraction rate of 34% and 40% for anthocyanins and phenols were obtained, respectively. Most recently, Bouras et al. (2016) reported the PEF treatment permitted to enhance extraction of total phenolic compounds and antioxidants.

It is worth stressing that the enhancement of extraction by PEFs depends on the rupture of cells. Thus, the stage at which the EF treatment is applied should be well controlled and it is usually used for pretreatment. For instance, Guderjan *et al.* (2007) reported that desired extraction effect was achieved with the assistance of PEF pretreatment (5 ~ 7 kV/cm) followed by hexane extraction. Both the yield and quality of the rapeseed oil and other functional food ingredients, such as tocopherols, polyphenols, and phytosterols, were improved notably. It is expected to achieve a higher extraction efficiency and lower consumption of organic solvents with the aid of PEF

pretreatment, as Yu et al. (2015) showed the positive effects of PEF pretreatment on extraction yields of polyphenols and proteins from rapeseed stems and leaves. Moreover, selective extraction of polyphenols can be induced by PEF-treatment at 5 kV/cm while proteins remain in the residues. which is of great significance for subsequent separation and purification. In addition, the synergistic effects of PEF (400 ~ 600 V/cm) and moderate thermal treatment (30 ~ 70 °C) showing a noticeable enhancement on extraction were also confirmed (Leboyka et al., 2004; Loginova et al., 2011; Medina-Meza & Barbosa-Cánovas, 2015). From the above results, it can be concluded that the cellular electroporation effect of PEF makes it an alternative technique to the solvent- and energy-consuming extraction. However, the single PEF treatment that is commonly conducted in water is limited and its application scope is quite narrow, mainly for extraction of water soluble components or mixture without subsequent separation and purification. Because it mainly relies on the rupture of cells induced by electroporation, which requires relatively strong PEF treatment. In comparison, PEF-assisted solvent extraction can effectively obtain the target components according to the similarity-intermiscibility theory. The permeabilization effect of PEF combined with the attraction from solvent makes it more effective in selective extraction. Despite the remarkable reduction of PEF intensity and amount of solvent, there still exist some drawbacks in particular solvent residue and pollution. Furthermore, the handling capacity of PEF extraction equipment is generally small, thus more optimization designs on both the equipment and the process are needed. Integration with other emerging techniques is also a potential solution for

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scale-up and industrial translation.

3.4 Acceleration of Winemaking

It is widely accepted that fresh wine tastes bitter and pungent, and only after aging for an extended period will it tastes mellow. However, the natural maturation period usually takes years. In order to shorten the aging time, electric fields have been utilized and proved to be efficient in the extraction of phenolic compounds from pomace, contributing to further stability of the wine (El Darra et al., 2016; Zeng et al., 2008). In EF pretreatment, the permeabilization effect is permitted to shorten the maceration time and enhance the extraction rate of phenolic compounds, mainly including flavan-3-ols, flavonols and hydroxycinnamic acids (López, et al., 2009). In the maturation period, the higher concentration of phenolic compounds allows the promotion of condensation reactions in freshly fermented wines, resulting in the formation of oligomeric and polymeric pigments. Among these phenolic compounds, anthocyanins, oligomeric and polymeric pigments mainly contribute to the color, while flavan-3-ols and flavonols are responsible for the bitterness, astringency and structure of wines (Boulton, 2001). A series studies were conducted on using EFs on wine for aging, microbial inactivation, extraction of components from wine and must, and improvement on sensory characteristics (López et al., 2009; Puértolas et al., 2011; Puértolas et al., 2010; Puértolas et al., 2009).

As reported by Puértolas *et al.* (2010), the total anthocyanic content, color intensity, total

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polyphenol index of Cabernet Sauvignon red wines treated by PEF were higher than the control at the end of both the maceration period and alcoholic fermentation for 4 months due to the primary permeabilization effect. The PEF treated wines also received a higher mouthfeel recognition for taste and astringency. This result is in agreement with Chen et al. (2010), who reported that PEF treatment could accelerate the aging process of young red wine and increase the concentration of most phenolic compounds. Most recently, a contrast experiment on PEF, enzymes and thermovinification pretreatments was carried out to investigate the changes in polyphenol profiles and color constitution of Cabernet Sauvignon wine. Through comparison and analysis, both the contents of phenolic compounds and flavonols in musts as well as the chromaticity in PEF-treated wine were significantly higher than the control, while these increases in enzymes treated wine were indistinctive. The results suggest that the PEF pretreatment is a promising non-thermal technique for vinification freshly fermented wine due to the negligible energy consumption (W = 48 kJ/kg) and temperature variation ($\Delta T = 7$ °C) compared to thermovinification (W = 418.5 kJ/kg, $\Delta T = 50$ °C) (El Darra et al., 2016). Besides the red wine, electric field can also be used to accelerate the maturation of distilled spirit. For example, Zhang et al. (2013) applied alternating current electric field treatment (1 kV/cm, 50 Hz) directly on the fermented brandy aged in eak barrels for 14 months, and found that the total phenolic content increased due to the enhanced extraction of tannins, volatile phenols and other phenols from the wood. Moreover, PEF treatment was proved to be efficient in the inactivation of spoilage flora and microorganisms.

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replacing the role of SO₂ used in wines production and storage (Garde-Cerdán *et al.*, 2008; Puértolas *et al.*, 2009). In summary, the utilization of electric fields under optimum conditions can effectively accelerate the aging process and enhance the quality of the wine.

4. Corona Wind Generated in Non-uniform HVEFs

4.1 Mechanism

In recent years, corona discharge has shown great potential in food processing especially for acceleration of drying and thawing processes. The driving force is defined as "corona wind" (He et al., 2013; Bai et al., 2013). Corona wind is the product of corona discharge occurred in a strong and non-uniform HVEF. The system most commonly used for the generation of corona wind is electrohydrodynamic (EHD) system, typically shown as Fig. 2 (He et al., 2013; Bai et al., 2013). The EHD system is simple with two electrodes connected to a high voltage power supply. As shown by Janda et al. (2016), the corona electrode with small curvature radius could form a non-uniform HVEF and realize a corona discharge in the gas-filled gap. Thus the configuration of the electrodes is commonly adopted in a form of pin-to-plate or wire-plate. Driven by high voltage source, the gas occupying the surface of the sharp electrode tends to be ionized primarily, resulting in the generation of energetic electrons and ions. Collisions of these high-velocity electrons with the other gas molecules constantly happen and create a large amount of secondary excited species, carrying a current flow from the corona electrode towards the other one (Lieberman & Lichtenberg,

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2005). The stream of charged particles is called corona wind containing electrons, active atoms and molecules, free radicals, photons, and ions. The energy carried by these excited gas particles acting on the food materials has been proved to speed up the dehydration and thawing processes. However, relevant studies mainly focus on the exploration of effects and technical parameters but the exact mechanism is still not clear, because currently there is no strong evidence to explain how the corona wind works.

It should be stressed that the ozone and free radicals generated by discharge make the corona wind a weakly ionized and oxidized medium, which can initiate oxidation of lipids and other substances such as anthocyanin. Therefore this negative effect should be considered when conducting food drying and thawing assisted by HVEFs.

4.2 Electrohydrodynamic Drying

In terms of the electric field-assisted drying, the most commonly used method at present is convective EHD drying based on the synergistic effect of voltage and corona wind velocity. The EHD drying characterized by drying rate, power consumption, sensory and nutritional quality, has been investigated intensively. Cao *et al.* (2004) studied the drying kinetics of wheat using HVEF (5, 7.5 and 10 kV/cm) produced by a pin-to-plate corona discharge electrode, and revealed a significant increase of drying rate by 1.7 ~ 2.1 times with the increase of field intensity, as compared with the air-dried control. Bai *et al.* (2013) made a comparison between EHD drying of

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sea cucumber at 18 °C, air drying at 18 °C and oven drying at 80 °C in aspects of energy consumption, drying rate, sensory and nutritional quality. The result showed that EHD drying generated the best quality with an average drying rate of 194.4 mg/min, which was around 3.6 times of that of ambient air drying (54.2 mg/min), and consumed only 21.31% of the electric energy required for oven drying. Taghian Dinani and Havet (2015a, 2015b) designed a convective-EHD system with a wire-plate electrode to study the synergistic effect of HVEF and airflow velocity on the dehydration of mushroom slices. It was found that 30 kV - 0.4 m/s represented the most promising condition for its industrial applications, which could enhance the drying rate and simultaneously reduce the energy consumption of the combined convective-EHD system. This result conformed to the findings of Martynenko and Zheng (2016), who performed a similar study on EHD (15 kV) combined with convective airflow (0-1 m/s) drying of apple slices and observed appreciable enhancement of drying rate. However, at a high air velocity of 2.2 m/s, the drying rate of the mushroom slices treated by 20 kV, 25 kV, and 30 kV EHD remained fairly constant compared to the 0 kV treatment. Therefore no improvement on the drying rate was observed from the corona wind. This result is consistent with the findings of Ramachandran and Lai (2010), who stated that cross-flows with high air velocity would suppress the effect of corona wind produced in EHD process. It should be noted that the smaller the curvature radius of the high-voltage electrode, the easier the formation of corona wind, leading to higher drying rates. Therefore, the pin-to-plate electrode is expected to be more effective than the wire-plate electrode.

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4.3 Electrothawing

Conventional thawing methods, such as thawing in air or water, are normally time-consuming and have a tendency to deteriorate the post-thawing quality, and thawing with the aids of electric fields (Mousakhani-Ganjeh et al., 2016a, 2016b) have been conducted to accelerate the thawing rate with high retention of product quality attributes. Relevant study (Shevkunov & Vegiri, 2002) showed that very high field intensity up to 5×10⁹ V/m was required to reorient water molecules in ice and modify the crystal morphology, so that freezing was inhibited, leading to the acceleration of the thawing process. However, such a high electric field intensity is not so easy to reach in practice. Therefore, other researchers investigated alternative way to accelerate the thawing process by using corona wind (Table 2). He et al. (2013) reported that a multiple points-to-plate electrode operating at voltages above 6 kV could shorten the thawing time effectively at -5 ~ 0 °C, with an microbial reduction of 0.5 ~ 1 log₁₀ CFU/g. Mousakhani-Ganjeh et al. (2015) utilized a pin-to-plate electrode operating at voltages of (4.5, 7.5, and 10.5 kV), (6, 10.5, and 13.5 kV) and (7.5, 10.5, and 14 kV) for electrode gaps of 3, 4.5, and 6 cm, respectively, in thawing of frozen tuna fish at 20 °C. The highest thawing rate was improved by 1.8 times at 10.5 kV-3 cm compared to the control whereas the values of thiobarbituric acid-reactive substances (TBA) and total color difference (ΔE) increased significantly (p<0.05), indicating that the lipid oxidation and color changes induced by HVEF might restrict its application to maintain the freshness of frozen fish

rich in lipids. Most recently, Mousakhani-Ganjeh et al. (2016b) combined HVEF with still air, and obtained higher efficiency in thawing. These researches suggest a great potential of HVEFs in accelerating the thawing process of frozen products, avoiding undesired problems of conventional thawing methods such as slow rate, high drip loss, microbiological spoilage, overheating and high cost. Despite all these improvements, there are also some limitations posed by lipid oxidation on thawing of frozen meat. Moreover, as the corona wind can accelerate the drying process significantly, it may cause off-flavor and apparent deterioration to some extent.

5. Molecular Polarization and Realignment Induced by EFs

5.1 Initiation Mechanism of ice nucleation

During freezing, with the removal of sensible heat, the liquid water turns into a supercooled state and then ice nuclei occur suddenly when initial freezing takes place. Thereafter, the ice crystals grow (Sun et al., 2008).

It should be highlighted that the electric field mainly acts on the supercooling degree and initial freezing point. Clearly, the evolution of the Gibbs free energy (ΔG_0) during this supercooled period is the key to clarify the mechanism of electric field-assisted freezing. In the absence of EF, ΔG_0 is given by (Mullin, 2001):

$$\Delta G_0 = \Delta G_S + \Delta G_V = 4\pi r^2 \gamma - \frac{4}{3}\pi r^3 \Delta G_V \tag{1}$$

where ΔG_0 is the sum of the surface free energy (ΔG_S) and volume free energy (ΔG_V), r is the

radius of the sphere, γ is the surface free energy of the crystal fluid interface. However, under the influence of electric field, the Gibbs free energy ΔG_E of the supercooled water can be written as (Marand *et al.*, 1988):

$$\Delta G_0 = 4\pi r^2 \gamma - \frac{4}{3}\pi r^3 \left(\Delta G_V + PE\right) \tag{2}$$

where P is the permanent polarization of the system and E is the external electric field intensity. Therefore, by comparing the above two equations, it can be noted that the application of EF can modify the free energy and subsequently affect the nucleation.

5.2 HVEF-assisted Freezing

Ice crystals formed during freezing play an important role in the quality of frozen food products. The formation of large ice crystals may bring about irreversible damages to tissues such as structural ruptures and cell deformation, leading to significant drip loss upon thawing. Therefore, more uniformly distributed small ice crystals should be preferred for maintaining better microstructure in frozen products. To address these issues, a variety of researches were carried out on innovative electric field-induced ice nucleation (Hsieh & Ko, 2008; Orlowska *et al.*, 2009).

Generally, the crystallization could be more controllable under the effects of electric fields, since the polarization and realignment of water molecules make the formed ice crystals more uniform and smaller. Moreover, the energy needed for electric field-assisted freezing is very low compared to conventional freezing technologies (Xanthakis *et al.*, 2013). Table 3 lists the

applications of electric fields in freezing processes, indicating the potential of HVEF-assisted freezing on enhancing the initiation of ice nucleation

In last decade, electric field treatments were widely introduced for freezing of biological tissues to extend their shelf life. However, most studies focused on the use of electric field technology as pretreatments prior to freezing while the exact mechanisms and conditions for controlling the degree of supercooling, phase transition time, size and shape of ice crystals are not clear (Ade-Omowaye et al., 2003; Amami et al., 2006; Ammar et al., 2010; Jalté et al., 2009). To examine the effects of HVEF on ice nucleation, Sun et al. (2008) employed electrostatic field of different intensities in the range of 10 ~ 1000 V/cm to assist the freezing process of distilled water. It was found that HVEF could induce ice nucleation and when the strength reached 1000 V/cm, the supercooling temperature increased by 1.6 °C. However, similar research demonstrated that externally applied uniform HVEF up to (1.6±0.4) ×10⁵ V/m produced no effect on the homogeneous nucleation of ice. Furthermore, thermodynamic simulation indicated that electric fields ranging from 10^7 V/m to 10^8 V/m might cause an observable increase in the rate of nucleation (Stan et al., 2011). Recently, the effect of HVEFs on the freezing of pork meat was evaluated through the freezing curve and microstructure in regard to the supercooling degree and size of ice crystals (Xanthakis et al., 2013). In the absence of HVEF, the supercooling degree and the average equivalent circular diameter of the ice crystals obtained were about 3.93 °C and 32.79 μm, respectively, while in the presence of 12 kV SEF were about 1.92 °C and 14.55 μm. Therefore,

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the supercooling degree and ice crystal size tend to decrease with the increase of HVEF strength, which was in agreement with previous study on electrofreezing of pure water (Orlowska *et al.*, 2009). Recently, Dalvi-Isfahan *et al.* (2016) found that the utilization of HVEF (5.8 × 10⁴ V/m) during freezing could decrease the size of ice crystals and maintain the quality of the lamb meat. Instead of using external voltage and gap to represent the field strength, the application of simulation tools such as COMSOL Multiphysics, Ansoft Maxwell, ANSYS, etc. can be more accurate to estimate the electric field strength.

5.3 Inactivation of Enzymes

As mentioned previously, biomolecules are characterized by dipole moments and net charges, which are sensitive to external electric fields, resulting in different levels of molecular modification. It is widely known that enzymes are bioactive polymers and most of them are proteins in essence, which are highly efficient in catalysis. Thus the inactivation of enzymes is similar to denaturation of proteins: unfolding the native proteins first followed by irreversible aggregation of unfolded protein molecules. Under physiological conditions, most enzymes can be assumed as a globular polymer with an active center buried in the hydrophobic interior of the proteins. Compared to non-enzymatic proteins, the most notably of enzymes is the active center with specific metal ions as cofactor and then the tertiary and quaternary structures, which are strongly stabilized by hydrogen bonds, non-covalent interactions and the balance of sulfhydryl

and disulfide bonds (Zuniga *et al.*, 2010). In particular, enzymes are amphoteric, whose net charges and dipole moments must undergo translation and rotation responding to an externally applied electric field (Samaranayake & Sastry, 2016). Furthermore, conformational changes might occur in the enzymes subjected to electric fields (Pereira *et al.*, 2011).

As shown in Table 4, numerous investigations have proved that electric field is effective in inactivating enzymes. Giner-Seguí et al. (2009) and Aguiló-Aguayo et al. (2008) reported that the inactivation effect on enzymatic activity increased with the treatment time and field intensity. Moreover, bipolar pulses were more effective than monopolar ones. Except the electrical stimuli, another possible reason is that the sudden reverse of the filed direction of bipolar pulses can change the orientation of the charged groups in particular the metal ions in the active center of the enzymes. The high-frequency reverse of the bipolar pulse polarity inevitably results in an alternating stress, which causes structural fatigue and inactivation of the enzymes. It is undeniable that enzymes are sensitive and vulnerable to extreme physicochemical environment (high temperature, strong acid or base, high salinity, etc.). To this point, despite sensory deterioration and off-flavor, the literatures on inactivation of enzymes using thermal treatment and chemical agents are still instructive. Consequently, the combination of electric field with moderate thermal treatment is a promising way to inactivate enzymes while avoiding excessive denaturation. Espachs-Barroso et al. (2006) performed an exploration on the the roles of PEF strength and temperature in the inactivation of pectin methylesterase (PME) from different sources, and their

results indicated that the longer the treatment time, the higher the intensity and pulse frequency, the higher the inactivation rate of PME. Under the most intense conditions (19.1 kV/cm, 5 Hz at 81 % for 120 min), the maximum inactivation rate was up to 87% for orange and tomato PME 83% for carrot PME and 45% for banana PME. Furthermore, the synergistic effect of moderate electric field (MEF) and thermal treatment on inactivation kinetics of tomato PME was also investigated by (Samaranayake & Sastry, 2016), who showed increasing activity at lew temperatures (<70 °C) and inactivation effect at high temperatures (>70 °C), and with MEF (10.5 V/cm, 60 Hz, sine wave) at 75 (±1) °C, a marked reduction (60%) of the PME activity compared to the control (40%) was achieved. In the experiment on inactivation of enzymatic activity in apple juice performed by Riener et al. (2008), a maximum reduction of 71% and 68% were obtained for peroxidase (POD) and polyphenoloxidase (PPO), respectively, which were higher (p< 0.05) than those (46% and 48%, respectively) treated by mild pasteurization. In general, the combination of electric field and thermal treatment has proved to be more effective in enzymatic inactivation compared to individual thermal treatment. Although most researchers considered that such inactivation of enzymes is induced directly by the electric field, Meneses et al. (2011) considered that it was induced by pH changes during PEF treatments.

6. Conclusions and Future Trends

This paper gives an overview on recent investigations and applications of pulsed and high

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voltage electric field technologies to assist or enhance food processes. Firstly, from the technical characteristics, electric fields as effective and non-thermal treatments can maintain the heat-sensitive components and provide products of high quality. Secondly, several effects of electric fields have been widely used in different scenarios of food processing including permeabilization effect of PEFs for microbial inactivation and component extraction, corona wind generated in non-uniform HVEFs for thawing and dehydration, molecular polarization and realignment for freezing, and the combination of these effects for enzymatic inactivation and winemaking. With the aids of electric field treatments, all these processes perform better in maintaining the freshness, extending the shelf life and improving the flavor of products. Finally, electric field technologies are proved to be energy saving and environmental friendly with low operating power for HVEFs and a very short response time for PEFs due to intermittent operation. The amount of chemical substances such as preservatives and organic solvents can be greatly reduced with the assistance of electric field treatments.

Although great progress has been made on utilization of electric field technologies for the food industry, in particular, in the area of PEF permeabilization effect on biomembrane and the development of PEF processing equipment, the mechanisms HVEFs are still not clear. More efforts are needed to reveal the thermodynamic properties of various food materials under electric fields, to clarify the mechanisms of HVEFs-assisted freezing/thawing, and to realize the industrial translation of laboratory results. With the development of theoretical understanding and

experimental applications, it is hoped that significant progress could be made in the future for the food industry to fully utilize the electric field technologies for enhancing food processes.

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Table 1. Applications of permeabilization effects of electric fields on cell membranes

Applicatios	Materials	Targets to be treated	Treatments	Effects	References
	Apple juice	E. coli, G	PEF: 30.76 kV/cm, 0.2 k Hz, 40 °C	About 5 log reduction	Moody et al. (2014)
	Melon juice, watermelon juice	E. coli O157:H7, G S. Enteritidis, G L. monocytogenes, G +	PEF: 35 kV/cm, 193 Hz, 2.0% citric acid or cinnamon bark oil	About 5.0 log ₁₀ CFU/InL reduction	Mosqueda-Melgar et al. (2008)
Microbial inactivation	Milk	E. coli, G ⁻ , S. aureus, G ⁺ , P. fluorescens, G ⁻	PEF: 40 kV/cm, 89 µs for E. coli and S. Aureus, and 42.5 kV/cm, 106 µs for P. fluorescens, 32.5 °C	About 5.0 log ₁₀ CFU/mL reduction	Cregenzán-Alberti et al. (2015)
	Cell suspensions	Lactobacillus plantarum 564, G ⁺	PEF: 31.6 kV/cm, 5 μs ×100 pulses	2.65 Log ₁₀ units reduction	Seratlić <i>et al.</i> (2013)
	Emblica officinalis juice	Z. bailii	PEF: 26 kV/cm, 1 µs ×500 pulses	5.1 log ₁₀ cycles reduction	Bansal <i>et al.</i> (2015)
	Potato peels	Steroidal alkaloids	PEF: 0.75 kV/cm, 3 μs ×200 pulses	The maximum yield (1856.2 μg) was 99.9% higher than the control.	Hossain <i>et al.</i> (2015)
Extraction	Rapeseed stems and leaves	Polyphenols, proteins	PEF: 5 kV/cm, 10 μs ×200 pulses	The purity increased from 57.0% to 83.6%	Yu et al. (2015)

				The oil yield increased	
	Domosoad	D1-:1	PEF: 7.0 kV/cm, 30 μs ×120	from 23% to 32% for	
					Guderjan et al.
	Rapeseed	Rapeseed oil		hulled rapeseed and from	(2007)
			pulses	43% to 45% for non-hulled	
			DEE OF LV	rapeseed.	
			PEF: 25 kV,	The yield of anthocyanins	
	Plum and		26 mm gap,	and flavonoids increased	Medina-Meza and
	grape peels	Phytochemicals	6 μs pulse	several folds, while that of	Barbosa-Cánovas
			width, 10	ascorbic acid decreased.	(2015)
			Hz		_))
			PEF: 25		
	Chlorella	Lutein	kV/cm, for	The yield increased around	Luengo et al.
	vulgaris		100 μ s, 25 \sim	3.5 ~ 4.2 folds.	(2015)
			30 °C		
				Aging for 14 months,	
	Brandy	_	ACEF: 1 kV/cm, 50 Hz	higher tannin	
				concentration (+54.4% and	
				+43.9%) and chromatic	Zhang et al.
				aberration (+19.2% and	(2013)
			NIZ	+24.9%) in 5-L and 2-L	
				barrels were obtained than	
				the control, respectively.	
				The maceration time	
		^\\//>		reduced for PEF wine was	
Acceleration			PEF: 5	48 h. Higher total	
of			kV/cm, 122	anthocyanic content	
winemaking	Cabernet	~	Hz, 3 μs	(+11%), color intensity	Puértolas et al.
	Sauvignon		pulse	(+38%), and total	(2010)
			duration,	polyphenol index (+22%)	
			below 2 °C	were obtained than the	
) `			control after aging for 4	
				months.	
			PEF:	Higher polyphenols	
	A =1:=. *		1.5 ,3.0	(+100%), anthocyanins	
	Aglianico	_	kV/cm,	(+30%), color intensity	Donsì <i>et al.</i> (2011)
	grapes		1 kHz, 10	(+20%) and the	
			MHz,	antioxidant activity	

	25±3 °C	(+40%) were obtained than the control.	
Cabernet Sauvignon	PEF: 5 kV/cm, 1 Hz, 50 monopolar exponential decay pulses	The maceration time reduced for PEF wine was 196 h. The anthocyanic profiles of PEF wine were similar to the control indicating that the permeabilization of the cell membranes had no selective effect on any anthocyanin.	López et al. (2009)

Note: G⁺: Gram-positive bacterium; G⁻: Gram-negative bacterium; PEF: Pulsed electric field;

ACEF: Alternating-current electric field.

Table 2. Applications of corona wind generated in non-uniform electric fields

Applications	Materials	Treatments	Effects	References
	Wheat	HVEF: Pin-to-plate electrode, 10, 7.5, and 5 kV/cm, 30, 40, 50, and 60 mm gap	The average drying rate was increased by 2.1, 2.0, and 1.7 times, respectively for 10, 7.5, and 5 kV/cm compared with the air-dried control.	Cao et al. (2004)
EHD drying	Sea cucumber	electrode.	The average drying rate was 194.4 mg/min, around 3.6 times of the ambient air drying (54.2 mg/min); EHD drying generated the best quality and consumed only 21.31% of the electric energy required for oven drying.	Bai <i>et al.</i> (2013)
	Mushroom slices	HVEF: Wire-to-plate electrode,	The highest drying rate was obtained for 30 kV - 0.4 m/s, 1.78 times as high as that for 0 kV - 0.4 m/s.	Taghian Dinani and Havet (2015a)

	Apple slices	20, 25, 30 kV, 6 cm gap, 45 °C, 0.4 m/s and 2.2 m/s air velocity HVEF: Pin-to-plate electrode, 0, 5, 10 and 15 kV, 25 mm gap, 1~5 m/s air velocity	Voltage of 10-15 kV increased the drying rate by 1.5-4 times at high (5 m/s) and low (1 m/s) air velocity compared to the air control. At higher air velocities, effect of EHD was retarded by convective crossflow.	Martynenko and Zheng (2016)
	Frozen distilled water	HVEF: Point-to-plate electrode, ±8 ~ ±13 kV, 2.7 cm gap, 20 °C HVEF: Pin-to-plate	The shortest thawing time at -13 kV/cm was 13.5 min, much shorter than the control air thawing of 37 min. Under 4, 6, 8, and 10 kV, thawing times were 70, 52, 46, and 40 min,	He et al. (2016)
	pork tenderloin meat	electrode, 4, 6, 8, 10 kV, 0.05 m gap	respectively, versus 64 min for the control, indicating voltages above 6 kV significantly shortened the thawing time.	He et al. (2013)
Thawing	Frozen tuna fish	Pin-to-plate electrode, 4.5, 7.5, 10.5 kV, 3 cm gap; 6, 10.5, 13.5 kV, 4.5 cm gap; 7.5, 10.5, 14 kV, 6 cm gap, 20°C	The highest thawing rate was improved by 1.8 times for 10.5 kV - 3 cm compared to the control (0 kV - 3 cm) whereas the TBA and ΔE values were increased significantly ($p < 0.05$);	Mousakhani-Ganjeh et al. (2016a)
	Apple tissue	PEF: Pair-wise combination of intensity (1.85, 3,5 kV/cm) and (10, 50, 100	The shortest total thawing time (1508.40 s) was reduced by 26.8% compared with the control. Maximal (8.9%) and minimal mass loss (1.6%) were noticed for samples treated by 50 pulses at 5	Wiktor <i>et al.</i> (2015)

	pulses)	kV/cm and 10 pulses at 3 kV/cm,	
		compared to the control (2.3%).	

Note: HVEF: High voltage electrostatic field; PEF: Pulsed electric field.

Table 3. Applications of molecular polarization and realignment induced by electric fields

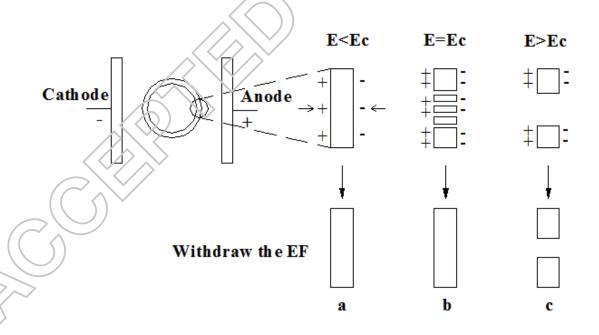
Applications	Materials	Targets to be treated	Treatments	Effects	References
	0.9% NaCl solution	_	PEF (1.78 V/cm, duty ratio of 0.5, 20 kHz) combined with repulsive SMF	Uniformly round and small ice crystais were formed in the phase transition time of 1004 s, 30.4% for the control.	Mok et al. (2015)
	Distilled water	-	DCEF: 0-6 0 ×10° V/m	With the increase of applied voltage, mean nucleation temperature increased from -12.28 to -5.90 °C.	Orlowska <i>et al.</i> (2009)
Freezing	Distilled water		UEF: 0-(1.6±0.4)×10 ⁵ V/m	Amplitudes up to 1.6 $\times 10^5$ V/m had no effect on the homogeneous nucleation of ice. Estimations suggest that 10^7 - 10^8 V/m might enhance the nucleation rate significantly.	Stan <i>et al.</i> (2011)
	Pork tenderloin muscle	_	SEF: 12 kV, 8 mm gap	Both the supercooling degree and average equivalent circular diameter of the ice crystals reduced from 3.93 °C and 32.79 µm for the control to 1.92 °C and 14.55 µm	Xanthakis et al. (2013)

				for the SEF-treated	
				sample.	
	Tomatoe	Pectin methylesterase (PME)	MEF: 8 V/cm, 60 Hz, sine wave, 70 (± 1) °C	Activation effect (≤70 °C) and inactivation effect (>70 °C) increased with field strength.	Samaranayake and Sastry (2016)
	Carrot	Ascorbic acid oxidase (AAO)	PEF: 1-520 kJ/kg	1-400 kJ/kg had no effect whereas more than 500 kJ/kg could lower the activity of AAO. Treated by PEF (0.2-0.5 kV/cm), the AAO became ther molabile.	Leong and Oey (2014)
Enzymatic inactivation	Brown rice	Lipase	PEF: 8.96 kV, 716.9 Hz, residence time: 6.12 s. pulse width: 12.94 µs	The remaining lipase activity was only 2.01 mg/g (38.29% of the starting value).	Qian et al. (2014)
macuvation	Gazpacho	Pectinesterase (PE)	PEF: 5~35 kV/cm, 200 Hz, 4 μs monopolar or bipolar square-wave pulses, < 40 °C	Inactivation effect on PE activity increased with treatment time and field intensity. Bipolar pulses were more effective than monopolar ones.	Giner-Seguí et al. (2009)
	Apple juice	Peroxidase (POD), polyphenoloxidase (PPO)	PEF: 40 kV/cm, 15 Hz, 1 μs pulse duration, 50 °C	Reductions of 71% and 68% were obtained for PPO and POD activity, respectively, higher (<i>p</i> < 0.05) than the control processed by mild pasteurization (46% and 48%).	Riener <i>et al.</i> (2008)
	Carrot,	Pectin	PEF: 19.1	Maximum enzyme	Espachs-Barroso
	tomato,	methylesterase	kV/cm, 5 Hz,	inactivation was: 87%	et al. (2006)

banana, orange	(PME)	for 120 min, 81 °C	for orange and tomato PME, 83% for carrot	
			PME and 45% for banana PME.	
Tomato juice	Peroxidase (POD)	PEF: 35 kV/cm, >200 Hz, monopolar or bipolar pulse of 1 ~ 7 μs duration, <35 °C	Bipolar pulses were more effective than monopolar ones and the longer the treatment time, the greater the reduction.	Aguiló-Aguayo et al. (2008)
Bovine whole milk	Plasmin, xanthine oxidase, lipolysable fat	PEF: pre-heated to 55 °C for 24 s, and PEF-treated at 26.1 kV/ cm for 34 μs	Reductions of 12%, 32% and 82% for plasmin, xanthine oxidase and lipolysable fat were obtained, respectively.	Sharma <i>et al</i> . (2014)

Note: PEF: Pulsed electric field; DCEF: Direct-current electric field; UEF: Uniform electric field;

SEF: Static electric field; MEF: Moderate electric field.



⁴⁵ ACCEPTED MANUSCRIPT

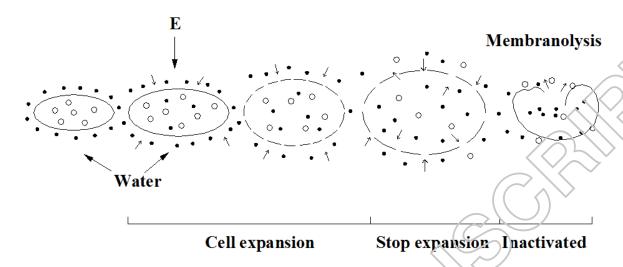


Fig. 1. (a) Schematic diagram of electric breakdown of cell membrane, where E represents the intensity of applied electric field (Zimmermann, 1986), a: An external electric field (E>0) increases the transmembrane potential, causing membrane compression but no pore formation; b: Further increase of E leads to a critical transmembrane potential E_C and subsequent pore formation, causing reversible break down; c: When E becomes too high, larger pores are formed, causing irreversible break down. (b) Schematic diagram of electroporation of cell membrane (Tsong, 1991). Under an applied electric field (E), the phospholipid bilayer and proteins of the cell membrane temporarily became unstable, causing electroporation of the cell membrane. Then the colloid osmotic pressure of cytoplasm drove the influx of water and small solutes (small dark circles) via the holes. The cell swells and eventually the cell membrane ruptures leading to cell lysis.

⁴⁶ ACCEPTED MANUSCRIPT

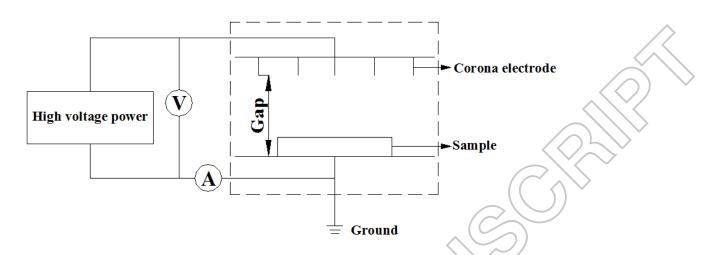


Fig. 2. Schematic diagram of the electrohydrodynamic (EHD) system (Bai et al., 2013; He et al.,

