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Application of Electric Fields to Reduce Microorganism Contamination for Animal-Based Products

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ABSTRACT

Non-thermal preservation has become popular recently for the preservation of animal-based products. One of the non-thermal preservation methods is the electric field (EF) treatment of food, which can suppress enzyme and microorganism activity. The EF treatment itself consists of various types, including pulsed electric fields (PEF), high-voltage electric fields (HVEF), and alternating capacitive electric fields (ACEF and DCEF). This review discusses the EF treatment effects, especially PEF, on animal-based product preservation, including mechanisms, advantages and disadvantages, PEF application, and its preservation effect. PEF has the potential to be widely applied in food processing. The application of PEF (Pulsed Electric Field) can be applied to animal-based products and has an effect on population age and microbial production. This is because PEF can inhibit microbial activity and growth depending on process parameters, including pulse waveform, treatment time, pulse width, pulse frequency, EF strength, and food matrix type. PEF can be used for pretreatment of liquefaction processes.

Keywords: electric field; food processing; PEF; preservation

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INTRODUCTION

Non-thermal preservation is famous for food preservation because it preserves food quality better than thermal pasteurization and sterilization. Novel preservation techniques involving lower temperatures and/or brief holding times have attracted much attention. According to van Wyk et al. (2019), the electric field (EF) technique has significant potential for food sterilization and preservation due to its environmental friendliness, low energy consumption, economic viability, ability to preserve the food's original taste, aroma, and color, and lack of detrimental effects on the nutritional components. Changing the type of plate electrode also changes the characteristics of the EF (Mendes-Oliveira et al., 2020).

In their development, electric field applications can be categorized into pulsed electric fields (PEF), high-voltage electric fields (HVEF), alternating capacitive electric fields (ACEF), and direct current electric fields *(*DCEF). They have an antimicrobial effect on the food that is subjected to the treatment. The intensity and on-or-off characteristics of the HVAEF, which is a non-uniform electric field, continuously change over time. EF can suppress bacterial growth by stopping their reproduction (Mirzaii et al., 2015). HVEF processing involves almost no electric current flow and uses very little energy (Qi et al., 2021). HVEF had a microbial inactivation effect known as electroporation that was comparable to PEF. HVEF effects on the cells, which may include pH changes and the transference of extra ions into the cells brought on by free radical oxidant formation and electrolysis, were also thought to contribute to the inactivation effect (Mirzaii et al., 2015). The phenomenon of corona discharge is the main feature, which distinguishes each treatment EF by displacing the electrode plate with plate puncture electrodes. Local self-discharge from a gaseous medium in an irregular EF is referred to as a corona discharge (Wang et al., 2020). Based on Yawootti et al. (2015), corona discharge can produce small levels of ozone, which can have a substantial oxidative effect on cells. It can also produce charged particle movement, which can have a bioelectric effect on cell membranes. This phenomenon has the potential to suppress the activity of contaminating microorganisms so that it can provide a preservation effect. This review discusses the PEF effects on the preservation of animal-based products.

This review uses literature based on searches from various journals in the scientific database, such as Google Scholar, Scopus, and others. Mainly from references to the last five years. The lack of several scholarly articles or journals on this idea led to the combination of these references from the scientific community. Furthermore, because the papers' areas of concentration fell beyond the purview of this study, those that particularly addressed the PEF application in animal-based products were chosen over those that highlighted other items. It is anticipated that this review will be used to further investigate and create PEF, particularly for the preservation of animal-based products.

PEF TREATMENT

PEF can operate at low to moderate temperatures for pasteurization of heterogeneous food materials and preservation (Zderic & Zondervan, 2016; Picart & Cheftel, 2003). It also has some advantages over traditional thermal methods. PEF is a technique that makes use of high-amplitude electric waves and high voltage. The food is put between the electrodes by brief electrical impulses of high voltage (Deeth et al., 2007). These pulses cause plant, animal, and microbial cells to operate, disrupt the integrity of cell membranes temporarily or permanently, increase their permeability, and cause cell death and microbial inactivation (Nowosad et al., 2021). The properties of food and the desired effects, as well as the process conditions, such as exposure time, shape of the pulse wave, pulse width, pulse frequency, and EF strength, can be appropriately modified.

Plant cells permeabilize reversibly at 0.1 to 1 kV/cm of EF strength, irreversibly at 0.5 to 3 kV/cm in plant and animal tissue, and irreversibly at 15 to 40 kV/cm in microbial cells (Tsong, 1996). PEF has the potential to retain the physical and chemical properties of food, prolong shelf life, and alter the nutritional and sensory qualities of food (Jin et al., 2017). This is because heat is limited during PEF treatments. Additionally, affordable, ecologically secure, and scaleable for sustainable industrial applications is the PEF approach (Kumar et al., 2021). PEFs produce high-energy discharges over very brief timescales (s to ms). The food is placed in a chamber, which can have a variety of designs, and passed between two electrodes, leading to electroporation, producing pores on the membrane, derangement, and leakage of intracellular content. The high-intensity pulses enter the food primarily to inactivate enzymes and microorganisms. According to theory, the meal should maintain its fresh product's physicochemical, nutritional, and sensory qualities during delivery

(Guerrero-Beltrán & Welti-Chanes, 2015; Alam et al., 2018).

PEF MECHANISM

The efficacy of electroporation of cellular tissue is significantly influenced by EF strength. The PEF modes were categorized by Bazhal et al. (2003) as high (E>1500 V cm^{-1}), moderate (E=300–1500 V cm^{-1}), and low (E100–200 Vcm^{-1}). The treatment period for cellular membrane electroporation should be longer when the EF strength is weak. Experimental research has shown that EF strength has an inverse relationship with the time required to electroporate the cellular membranes of various biological tissues (Bouzrara & Vorobiev, 2003; Zderic & Zondervan, 2016). According to evidence provided by Tieleman (2004) and Nowosad et al. (2021), the electroporation procedure involves two steps. First, plant cell membranes undergo lysis, and intracellular material is gotten out of the tissue, allowing water molecules arranged in a single wire to pass through the bilayer hydrophobic core. The majority of this substance is water, but it also contains beneficial plant chemicals (vitamins, flavonoids, lipids, etc.) in the tissue rather than being locked up within the cell. This phenomenon can accelerate drying or improve material extraction. Second, the water wires lengthen and widen, forming pores that are filled with water. These pores are then stabilized by rearranging the lipid molecules in the cell membrane, which serves to maintain the cell walls. However, following electroporation, this pressure is reduced due to the release of intracellular material.

Biological cells, as electrolytes, are encased in a shell that is electrically damaged and exposed to an external EF that causes an induced transmembrane voltage, where the cytoplasm is enclosed by the plasma membrane (Kotnik et al., 2010). Due to the actions of potassium leak channels and sodium-potassium pumps, the membrane has an ionic gradient when it is functioning normally. Its value varies depending on the kind of cell and normally falls between 80 and 40 mV (Kotnik & Miklavčič, 2006). The cell membrane begins to permeabilize due to the electric field reaching 250 mV for the transmembrane potential difference. The cells' uptake of the indicator is proof that the membrane has become permeable. The membrane flaws close up when the electric field ceases operating, allowing the cells to keep the molecules or ions that were previously delivered. Both the length of exposure and the strength of EF are factors. Depending on the temperature, resealing can take anything from a few seconds to several hours. For instance, the membrane defects close quickly at 37 °C, quickly at 4 °C, and slowly on ice over the course of several hours. According to Golzio et al. (2002) and Nowosad et al. (2021), irreversible cell damage occurs when the field strength greatly exceeds the critical value.

The majority of a typical PEF machine is made up of monitoring equipment, a treatment chamber, and a high-voltage pulse generator (Figure 1). The product is put between electrodes in the treatment chamber, to which the generated pulses are transmitted (Mohamed & Eissa, 2012; Nowosad et al., 2021). PEF is used to form pores; the size and expansion of it affect the length of the electrical exposure and material properties (Demir et al., 2018). Cell membrane polarization produces cellular tissue that becomes permeable and is disrupted (Toepfl et al., 2014). Applications of PEF initially centered on its capacity for non-thermal pasteurization the ability to kill bacteria without heat (Kempkes & Munderville, 2018).

ADVANTAGES AND DISADVANTAGES OF PEF

The PEF process can improve product quality while reducing costs and time. The drying of plant or animal tissue is one example of a sluggish mass transport process that can be accelerated to reduce processing times, such as for extraction,

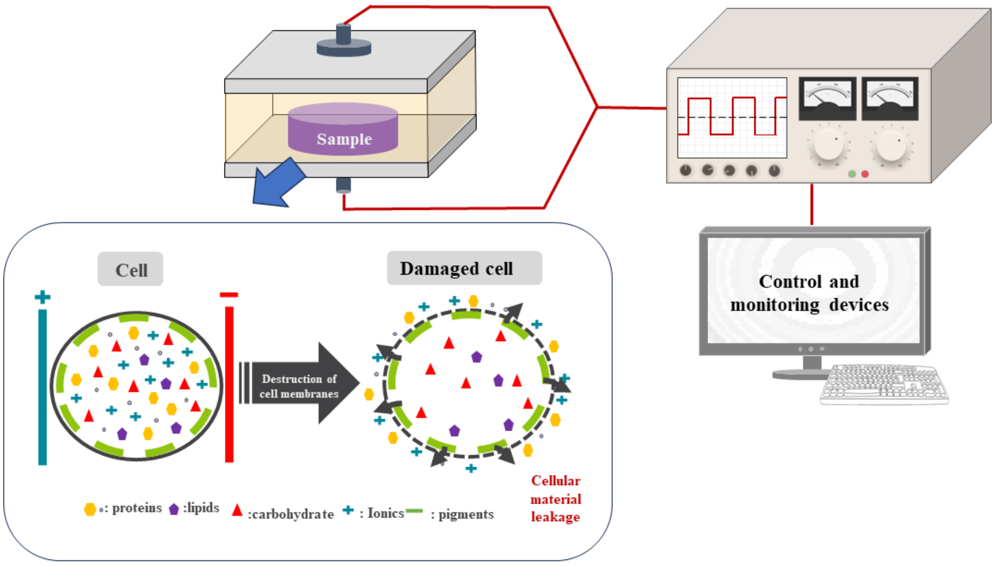

Figure 1. The schematic diagram of PEF treatment system

which can enhance yields. Later, better brining techniques will shorten the tumbling times in meat processing. The manufacture of dry sausages is improved by PEF technology, which reduces the required drying time (Golberg et al., 2016). Across a variety of food kinds, PEF can produce and precisely measure noticeable increases in yield, freshness, and flavor preservation. PEF transforms manufacturing in a constructive and significant way. Enhancing productivity, lowering labor costs, optimizing logistics for the supply chain, and decreasing retail waste (Lasekan et al., 2017). Even though there are numerous commercial PEF systems in use around the world and that this technology has undergone extensive research, the majority of the information gathered refers to laboratory-scale operations. The PEF method is widely regarded as safe for people because it has no harmful chemical reactions. However, corrosion causes elements of electrode materials to be discharged into liquid food. Carbon electrodes could be used to solve this issue. The electrodes corrosion is affected by pulse frequency and the makeup of the treated product. Undoubtedly, ideal PEF treatment settings are needed in order to prevent or at least reduce unfavorable electrode reactions (Nowosad et al., 2021).

PEF APPLICATIONS IN FOOD PROCESSING

Products processed during PEF treatment still require refrigeration to maintain low temperatures, and the electrical pulse energy generates heat. However, this phenomenon can be used in a delicate preservation procedure. The inactivation efficiency is enhanced when high temperatures and PEF membrane electroporation are combined (Nowosad et al., 2021). This method has been examined for its ability to inactivate germs, improve mass transfer, and alter the characteristics of bioactive substances like enzymes, among other things. PEF has also been studied for certain novel food science applications, including the increase of peeling efficiency, changes in starch characteristics, and the tenderization of muscles (Kumar et al., 2021).

A previous study shows that meat treated with PEF has a higher electrical conductivity than controls, respectively 13.01 ± 0.22 mS/cm and 10.63 ± 1.5 mS/cm. This indicates that PEF exerts a permeabilizing effect on cell membranes, and this is also associated with moisture redistribution and osmotic flow (Faridnia et al., 2015). This process can speed up mass transfer by weakening the structures of membranes. The degradation of the thermo-labile chemicals is minimized using PEF extraction, a nonthermal technique (Singh et al., 2021). For each unique application, the PEF parameters must be optimized (Shamsi & Sherka, 2009; Raso et al., 2016). A combination of the EF, duration of treatment, flow rate, type of food, and refrigeration systems should be taken into consideration in order to supply foods that preserve their nutritional and sensory properties similar to fresh foods (Guerrero-Beltrán & Welti-Chanes, 2015). However, due to the possibility of a sudden and rapid temperature increase during PEF processing, caution should be exercised.

PEF can help shorten the process and lower the price of hydrophobic extraction. PEF can hasten the penetration of the solvents into the cells by cracking up the cell membranes. Second, a wet extraction procedure can use less volatile solvents and fully skip the drying step because the solvent no longer needs to pass through the cell membranes.

PRESERVATION EFFECT OF EF TREATMENT

According to published studies, PEF treatment increased the shelf life by inhibiting pathogens and deteriorating several liquid foods (Aadil et al., 2018). PEF also altered the structural properties of several solid meals to obtain desirable texture changes or enhanced extraction methods (Zhang et al., 2019). Different specific energy and EFintensity effects affect the results, including enzyme and microorganism inactivation, freezing, structure modification, recovery of bioactives, etc (Arshad et al., 2021). Applications for PEF in solid food have included preservation, recovery of bioactive substances, physical quality alterations, and structural enhancements. Dehydration of food and alteration of water activity have long been effective methods of food preservation. PEF causes structural changes such as better meat tenderization and microbe inactivation (Arshad et al., 2021). In drying methods, PEF removes that restriction by opening the cell membrane and lowering the necessary processing temperature for a shorter drying duration (Ostermeier et al., 2018). HVEF prolongs the shelf life of food by producing negative air ions and ozone, preventing pathogens from growing and causing apple decay (Atungulu et al., 2005).By far the most researched PEF, which has a potent antibacterial impact (Mendes-Oliveira et al., 2020). PEF at 25–40 kV/cm can suppress 3-6 logs of pathogenic and spoilage microorganisms (Zhao et al., 2012). According to Tanino et al. (2020), this had a significant impact on *Escherichia coli* inactivation. Boosting the EF intensity and reducing the flow rate were successful ways to boost the inactivation efficiency.

The intensity and on-or-off properties of the high voltage alternating electric field (HVAEF), which is a non-uniform EF, continuously change over time. Low-voltage alternating electric fields can suppress bacterial activity by stopping their reproduction via an effect on the bacterial division during cytokinesis, even though they have no electroporation effects and do not form free radical oxidants (Mirzaii et al., 2015). HVEF processing involves almost no electric current flow and uses very little energy. It is uniform and considerably more stable than PEF and HVAEF. It was discovered that the field and duration have a significant impact on *B. Subtilis* survival ratio. *Bacillus* viability was also seen to decrease with increasing duration. Furthermore, when the applied field reaches a sufficiently high value of more than 15 Kv/cm, the comparative *B. subtilis* survival ratio drops below 35% in a short period of time. These results revealed that the uniform field inhibited the *B. Subtilis* viability. Based on the inactivation impact of the applied field on *B. subtilis*, the efficacy of charged polypropylene films on *B. subtilis* inactivation was assessed (Bu et al., 2005).

Table 1. EF application on animal-based product

Animal-based food	Objective	Operating parameters	Equipment	Findings/Effects	Reference
Beef	Frozen-Thawed	1.4 kV/cm, 20 µs pulse width, 50 Hz, and 250 kJ/kg	Square-wave bipolar pulses (Elcrack-HVP 5, DIL Quakenbruck, Germany)	PEF increased purge loss and significantly altered the microstructure of the beef tissue, but did not affect cooking loss.	(Faridnia et al., 2015)
Turkey breast	Preservation	4.4-12 kV/cm, 26 -194 kJ/kg, 20 µs, 5 Hz, and 300 pulses	Modified batch PEF (ELCRACK-HPV5, DIL, Germany)	None of the conditions considered caused variations in the instrumentally observed weight loss, cook loss, lipid oxidation, texture, or color in either fresh or frozen samples.	(Arroyo et al., 2015)
Purple sea urchin	Preservation (Freezing)	10 kV, 50 Hz, intensity 0.1 - 1 kV/m.	Modified ACEF (P6015A, Tektronix).	The freshness of this perishable fish can be effectively preserved by freezing it using AC power.	(Ito et al., 2014)
Chicken meat	Preservation (Thawing)	Voltage 20 kV, temperature -3 to 4°C, field strength 100 kV/m)	HVEF refrigerating system (Model Chargemaster CM-60, Taiwan Hon-lycon, Inc., Taiwan)	The HVEF accelerates and slows down the thawing process by moving the water molecules in food.	(Hsieh et al., 2010)
Carp	Preservation (Thawing)	Voltage 12 kV.	Generator (DW-N303-1AC, Tianjin, China)	Thawing under HVEF reduced microorganisms, inhibited inosine monophosphate degradation, and decreased the water loss of fish.	(Li et al., 2017)
Shrimp	Drying	45 kV for 8 h at 15 ±1℃, 65±3% RH.	---	Compared to oven-dried shrimp, HVEF-dried shrimp showed a softer body, lesser distortion, better color, better rehydration, and less shrinkage.	(Bai & Sun, 2011)
Tuna	Frozen-Thawed	10.5 kV	Maximum output current of 5 mA (LS50KV-5 mA, China)	HVEF treatment considerably accelerated the thawing of frozen tuna fish cubes.	(Mousakhani-Ganjeh et al., 2015)

Once pores of 0.5 nm are formed after applied EF, disrupting the flow of the cell (Sale & Hamilton, 1967). The breakdown of membrane integrity leads cell materials to diffuse into their environment, resulting in the cell death, a process known as irreversible electroporation (Gavahian et al., 2018). One of the most critical parameters influencing the sterilizing impact of an electric field is electrical conductivity. It determined the chamber's resistance, which might affect the temperature and EF intensity rise of the product (Álvarez et al., 2000). The release of DNA, proteins, and other biological components from cells was associated with bactericidal activity (Huang et al., 2017). Their findings revealed that the protein content in the treated bacteria's supernatant enhanced linearly with increasing voltage, whereas the protein concentration in the cells steadily declined. HVPEF process may potentially improve intracellular membrane permeability, develop membrane pore size, and result in intracellular DNA and proteins release necessary for survival. High-voltage electrostatic field application affects the composition of *Pseudomonas*, LAB, and halophilic bacteria in fish samples. Freshwater fish spoilage is typically caused by *Moraxella*, *Micrococcus*, *Shewanella*, *Flavobacterium*, *Aeromonas*, *Pseudomonas*. LAB, *Pseudomonas*, and halophilic bacteria had initial concentrations of 2.84, 2.86, and 3.00 log CFU/g in CK samples, respectively, while H_2S-producing bacteria and *Aeromonas* were not found once the samples had thawed. The sample that was thawed at -12 kV had fewer microbes than the control. This outcome might be the consequence of the HVEF producing a lot of ozone, which could prevent the growth of these spoilage germs throughout the thawing process. Second, the -12 kV HVEF decreased the thawing period, which prevented the growth of internal microorganisms and the invasion of external microbes. According to this finding, the populations of LAB, HB, H_2S-producing bacteria, *Pseudomonas*, and *Aeromonas* in carp were reduced after thawing with -12 kV HVEF (Li et al., 2017).

Flow cytometry was utilized to study the relationship between cell membrane integrity and membrane permeability. HVPEF dramatically reduced esterase activity and cell membrane integrity. HVPEF disrupted membrane integrity and permeability and triggered intracellular material release as well as the esterase activity reduction, ultimately leading to *S. aureus* cell death. The cell membrane became rough after 6 minutes of HVPEF treatment, with filaments and protrusions on the cell surface and a small percentage of cells damaged. After 15 minutes, more filaments and protrusions emerged on the cell surface, and a substantial number of cells were fractured, with many irregularly shaped holes forming on the collapsed membranes. The cell debris gathered around and produced reticulations. The images revealed that HVPEF could cause cells to collapse, discharge intracellular material, and inflict serious injury to the cells' basic activities, which is similar to prior findings (Qi et al., 2021).

The compound 2′,7′-dichlorofluorescin - diacetate (DCFH-DA) can permeate past the cellular membrane and be deacetylated to dichlorofluorescin (DCFH) by an intracellular esterase. DCFH can be oxidized by ROS (ROO. and HO.) and transformed into DCF, which is easily visible due to its intense fluorescence (Gomes et al., 2005). The overall amount of intracellular ROS in *S. aureus* grew as the HVPEF treatment period increased, reaching relative stability after 12 minutes. According to studies, the ROS accumulation produced many types of cell death and cell damage. Antibiotics could kill bacteria by increasing ROS production (Grant et al., 2012).

Furthermore, the results revealed that HVPEF treatment, as an efficient antibacterial approach, may enhance the overall amount of ROS, hence inducing injury and death of S. aureus cells (Qi et al., 2021). Another potential electroporation mechanism involves an externally applied EF of sufficient power to produce an increase in cell membrane permeability. This rise is linked to the production of pores, or aqueous channels, in the membrane's lipid bilayer (Mahnič-Kalamiza et al., 2014).

The EF and process duration, as well as the microorganism itself, medium, and temperature parameters, will all influence PEF lethality (Barba et al., 2015; Morales-de la Peña et al., 2019). In general, increasing the number of pulses enhance microbial inactivation but can also cause significant product heating (Oziembłowski & Kopeć, 2005). It is worth noting that inactivation of larger microbial cells requires less intense field strength than inactivation of smaller cells. In fact, the field modulates the transmembrane potential in a cell-size-dependent manner. Furthermore, cells in the exponential growth phase are more sensitive to PEF treatments than cells in the lag or stationary phases (Álvarez et al., 2000). As a result, the minimal EF intensity needed for correct inactivation is determined by cell size and geometry (Heinz et al., 2001).

Applications of EF treatment in animal-based products have been carried out, including beef, turkey, sea urchin, chicken, carp, shrimp, and tuna. A study showed that during 16 days of storage at 4°C, the quantity of bacteria on beef muscle was counted at 2.69–2.77 log CFU/g, represented the initial total viable count (TVC). From day 8, there was an increase in growth, which reached 6.89 log CFU/g and 7.2 log CFU/g for untreated fresh beef samples and PEF-treated samples, respectively. Furthermore, since samples of genuine meat are varied and contain areas with various electrical resistivities. The preparation of turkey breasts also involves PEF, which results in variations in lipid oxidation and sensory. Both the fresh and frozen turkey samples' lipid oxidation was not increased or decreased by PEF compared to the control. Fresh samples (5.0 mg MDA/kg) of the untreated samples exhibit higher levels of lipid oxidation ($P < 0.05$) than frozen samples (2.4 mg MDA/kg). The softness of PEF-treated samples is "neither tough nor tender" (scoring 5.3), but the control samples are "tender" (score 6.19) (Arroyo et al., 2015).

The PEF application can be used as a method to speed up the thawing process, and this will affect the microbial count (Faridnia et al., 2015). Thawing chicken meat with PEF showed differences in microbial properties compared to controls. The initial TVC was 3.3×10^3 and 4.6×10^3 CFU/g for the E-group (thawed at 4 ^{o}C) and R-group (thawed in the common refrigerator), respectively. The TVC values for the R-group were already above the value of 107 CFU/g, which was regarded as the upper microbiological limit for good-quality fresh poultry meat (6 days), and the R-group began to leak mucus and release offensive odors. On 8 days of storage, the E- and R-group microbial counts were 4.5×10^7 and 4.9×10^8 CFU/g, respectively (Hsieh et al., 2010).

Apart from influencing microbial growth in animal-based products, EF also influences drip loss during processing, which in this case will affect the texture of the product. When the AC electric field was used during freezing, a large amount of protein was eluted into the drip. While defrosting, however, introducing an AC electric field had no such impact. Because freezing food typically takes a long time, proteins break down throughout the freezing process. Perishable foods' freshness and quality can suffer as a result of decomposition. When meals are frozen, adding an AC electric field can stop proteins from breaking down due to the digestive response associated with putrefaction (Ito et al., 2014). TVC were initially 3.17 and 3.27 log CFU/g in thawing air (CK) and -6 kV samples, respectively. Additionally, the high-voltage electrostatic field causes oxygen to leave and nitrogen to coat the flesh's surface, increasing the concentration of ozone and limiting the growth of bacteria (Li et al., 2017).

CONCLUSION

PEF applications can be applied to animal-based products. In general, PEF has a direct impact on the microbial population in this product, which will affect its shelf life. Moreover, the level of PEF treatment will affect other properties such as lipid oxidation, texture, and sensory. Different animal-based products may have different processing parameters for preservation purposes. PEF technology can also be used for pretreatment of other processes, such as the thawing process. In general, PEF is able to inhibit microbial activity and growth depending on process parameters, including the shape of the pulse wave, treatment time, pulse width, pulse frequency, EF strength, and food matrix type. The PEF instrument can be modified and adjusted during application.

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