Pulsed electric field processing of egg products: a review

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Revised: 7 September 2015 / Accepted: 7 October 2015 / Published online: 16 October 2015
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Abstract Thermal processing ensures safety and enhances the shelf-life of most of the food products. It alters the structural-chemical composition, modifies heat labile components, as well as affects the functional properties of food products. This has driven the development of non-thermal food processing techniques, primarily for extending the shelf-life of different food products. These techniques are currently also being evaluated for their effects on product processing, quality and other safety parameters. Pulsed electric field (PEF) is an example of non-thermal technique which can be applied for a variety of purpose in the food processing industry. PEF can be used for antimicrobial treatment of various food products to improve the storability or food safety, for extraction and recovery of some high-value compounds from a food matrix or for stabilization of various food products through inactivation of some enzymes or catalysts. Research on the application of PEF to control spoilage or pathogenic microorganisms in different egg products is being currently focused. It has been reported that PEF effectively reduces the activity of various microorganisms in a variety of egg products. However, the PEF treatment also alters the structural and functional properties to some extent and there is a high degree of variability between different studies. In addition to integrating findings, the present review also provides several explanations for the inconsistency in findings between different studies related to PEF processing of egg products. Several specific recommendations for future research directions on PEF processing are well discussed in this review.

Keywords PEF · Liquid whole egg · Pasteurization · Microbial inactivation · Egg powder · Antioxidant activity

Abbreviations

PEF	Pulsed electric field
TMP	Transmembrane potential
TMPc	Critical transmembrane potential
DC	Direct current
AC	Alternate current
SS	Stainless steel
HTST	High-temperature short-time
LWE	Liquid whole egg
SPC	Soluble protein content
TC	Triethyl citrate
EWP	Egg white powder
EDTA	Ethylenediaminetetraacetic acid
USDA	United States Department of Agriculture
SM	Skim milk
EO	Essential oil
MW	Molecular weight
DPPH	2, 2 diphenyl – 1 – picrylhydrazyl
FRAP	Ferric reducing antioxidant potential
RP	Reducing power
-SH	Sulphydryl

Introduction

Thermal processing is the most effective processing technique for killing spoilage or pathogenic microorganisms to improve the shelf-life of food as well as to ensure food safety. It requires a lot of energy to produce any form of heat and some forms of heat also act as an environmental hazard. Thermal treatment also alters the structural and chemical components

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of food while destroys heat labile components to a considerable amount (Lechevalier et al. 2005). It deteriorates the functionality of bioactive compounds present in certain food items and alters the nutritional and flavor characteristics as well (Hermawan et al. 2004; Plaza et al. 2006). It can also change the form of food from raw to cook stage if applies for longer duration; however, certain food stuffs are consumed or utilized as an ingredient for secondary processing, only in the raw stage. Thus, there is a need of novel alternate technology for safe and economical food processing to obtain quality raw food products. Non-thermal technologies are such possible alternate to overcome the negative implications of thermal processing up to a certain limit (Cappelletti et al. 2015; Espina et al. 2014; Omer et al. 2015; Wu et al. 2014). These non-thermal technologies are environmentally sound, economical and can produce nearly fresh-like food (Wang-Ke et al. 2013; Wang-Ke et al. 2012).

Pulsed electric field (PEF) processing is an example of non-thermal technology because it usually does not enhance the temperature of food during processing or the temperature elevation is much lesser than the thermal pasteurization (Lin et al. 2011). PEF was first used to increase the permeability of fruits to facilitate the subsequent extraction of juice, which is currently an important application of this technology (Evrendilek et al. 2016; Jaeger et al. 2012; Loginova et al. 2011). Recent research evidences show that PEF technology is very effective for inactivation of spoilage and pathogenic microorganisms, increasing the pressing efficiency, enhancing the activity of bioactive compounds, extraction of fruit juices, food stabilization, and intensification of food dehydration or drying (Boussetta et al. 2012; Boussetta et al. 2013; Evrendilek et al. 2016; López-Giral et al. 2015; Luengo et al. 2015; Milani et al. 2015; Puertolas et al. 2013; Sagarzazu et al. 2010; Saldana et al. 2009; Turk et al. 2012).

PEF processing involves the application of very short, high-voltage electrical pulses to a food item, which is placed between or passed through two electrodes. Usually, some pulses of 20–1000 μ s duration with several thousand electric field strength (kV/cm) between some specialized electrodes are required for an effective PEF treatment. Under the influence of this external electrical field biological membranes are punctured (Gaskova et al. 1996) through formation of hydrophilic pores and forced opening of protein channels as well (Tsong 1991). This phenomenon is called as ‘electroporation’ and due to this cell membrane loses its structural functionality. PEF processing is now being analyzed in combination (hurdle approach) with some other mild thermal or non-thermal processes for enhancing the efficiency of microbial inactivation, extraction of bioactive compounds and food stabilization (Amiali et al. 2007; Bazhal et al. 2006; Espina et al. 2014; Huang et al. 2006).

Principle of microbial inactivation, cell damage and enzyme inhibition

PEF treatment alters the structure of cell membrane by electroporation and breakdown of the membrane (permeabilization). This affects the biological function of the membrane because it is responsible for transfer of biological molecules by acting as a semipermeable barrier. It also involves in the synthesis pathway of RNA, DNA, protein and other cell components. In some studies the intracellular components disruption have also been noticed in the PEF-treated cells (Katsuki et al. 2007; White et al. 2004). The process of electroporation involves consecutive three steps: a) increase in the transmembrane potential (TMP), b) pore formation, c) evolution in the number and size of pores. Finally, the integrity of the membrane recovers when the PEF treatment is stopped. This recovery depends on the effect of the electric field, if the electric field disrupts the membrane profoundly then the process is called irreversible and electroporation leads to death of the cell.

In terms of electrical properties a cell membrane functions as a combined resistor and capacitor. The membrane impedes the movement of charges across it (resistance) and the lipid bilayer is so thin that the accumulation of charged particles on one side gives rise to an electrical force that pulls oppositely charged particles toward the other side (capacitance). The capacitance of the membrane is also called as dielectric constant and has a more or less invariant value estimated at about 2 μ F/cm² (the total capacitance of a patch of membrane is proportional to its area). The accumulation of opposite charges on both sides of the membrane generates perpendicular TMP. The membrane damage occurs when the TMP is greater than some critical limit, which results in the destruction of normal biological functions of the membrane. The maximum value of this additional potential across a cell membrane can be calculated by the following equation (Saulis 2010):

$$\Delta \phi_g = f_s E_0 a \cos \theta - \Delta \phi_0$$

where $\Delta \phi_g$ and $\Delta \phi_0$ are the TMP produces by the application of external electric field and the resting potential, respectively; E_0 is the applied external electric field; a is the radius of spherical cell; θ is the angle between the membrane surface and the direction of field; and f_s is a factor that depends on the form of cell, conductivities of plasma membrane, intracellular and extracellular medium.

This transmembrane or induced potential across the sides of membrane is also calculated by various other workers considering other factors as well. Hülshager et al. (1983) proposed the equation for induced potential as $V_m = 1.5 a E_c$, where a is the cell radius (m) of spherical cell surrounded by non-conducting membrane and E_c is the applied external electric field (V). The equation for nonspherical cell was given as

$V_m = L (1 - 0.66a) a E_c$ (Zimmermann et al. 1974), where L is the cell length. The maximum TMP for spheroid cell (a and b axis) was given as $V_m = f(v, \theta_o) R E_o$ (Kinosita and Tsong 1977), where R is the largest semiaxis ($R = a$ for an oblate and $R = b$ for a prolate cell); f is a geometric factor which depends on the axial ratio $v = b/a$; and θ_o is the angle between the symmetry axis b and the direction of the field (E_o).

The critical transmembrane potential (TMPc) was given as $\Delta V_c \approx 1.1 \text{ aE}_{1/2}$ (Kinosita and Tsong 1977) and the formation of pores takes place when the TMP exceeds from 0.2 to 1.0. The increase in the TMP leads to viscoelastic deformation of the cell membrane due to the attraction of opposite charges (electromechanical model). If the applied electric field exceeds a critical value (critical electric field) the electrocompression forces are increased at both side of the membrane and the permeability increases. Electromechanical process produces compressive force by which the thickness of membrane is reduced. The membrane is composed of bilayer lipid volume which is incompressible resulting in the increase in area per unit of lipid which causes destabilization of lipid bilayer. The potential difference for this breakdown is given by $V_c = [0.3679 G h^2 / (\epsilon \epsilon_o)]^{0.5}$ (Coster and Zimmermann 1975), where G is the membrane shear elastic modulus; h is the unstrained membrane thickness; ϵ is the relative electric permittivity; and ϵ_o is the free space electric permittivity. The surface tension also provides some sort of protective effect on destabilization of the membrane hence the equation $V_c = [24 \gamma G h^3 / (\epsilon^2 \epsilon_o^2)]^{0.25}$ was proposed (Dimitrov 1984) which takes into account the effect of surface tension (γ) of the membrane. The increase in the field intensity and size of cell increases the TMP across the membrane which also depends upon the direction of applied field (θ) as proposed by the equation $\Delta \phi = 1.5 a E_f \cos \theta$, where E_f is the field intensity (V/m) (Newmann 1989). Thus, higher field strength is required for microbial inactivation while plant and animal cells are inactivated at very low field strength due to their larger size than microbes. Usually, a field strength of $>10 \text{ kV/cm}$ is required for microbial inactivation while it requires only $\sim 2 \text{ kV/cm}$ for plant or animal cell destruction. This type of hydrophilic pore formation and the number of pores also depend upon the pulse shape, pulse width, temperature, and membrane fluidity.

Hydrophobic pores are also formed in the lipid membrane due to the thermal and molecule free site fluctuations. The energy requirement to maintain hydrophilic structure is less being more stable than the hydrophobic pores. Thus, hydrophobic pores become hydrophilic if the radius of the pore (R_p) exceeds the critical size (R_c). The increase in the TMP and temperature during application of external electric field reduces the R_c . Thus, the formation of pores increases with increase in the number and size of the pore. In short, the formation, accumulation and expansion of hydrophilic pores are responsible for the electroporation to occur. The pore

formation is of two types, reversible and irreversible, and this depends on the thickness of membrane, shape and size of cell and electric field quality and strength. In reversible pore formation pore resealing takes place and the pore size is reduced. This causes the PEF-treated cells to survive after the treatment if the favorable conditions exist (Fig. 1). However, if the electric field strength is more than $\sim 10 \text{ kV/cm}$ then irreversible electroporation occurs in which resealing of pores cannot be completed which leads to the loss of membrane functionality.

PEF is sometime applied for specific application like for microbial inactivation. The above described mechanism i.e. permeabilization or formation of pores under external electric field is also the basic mechanism of microbial cell inactivation. In irreversible type of pore formation the inside of cell may interact with the surrounding media which ultimately causes escape of cellular contents, organelle damage and ultimate death of the PEF-treated cell. The mechanism by which PEF inactivates enzymes (protein) is not fully understood yet. The irreversible unfolding of structure (denaturation) and permeabilization of the membrane are the possible mechanisms by which protein is mostly affected. The protein channels present in the membrane are having very low gating potential and dielectric strength as compared to the lipid bilayer. Thus, these channels are more sensitive to external electric field thus are denatured and opened before the pores of lipid bilayer. The alternation in the pH control mechanism and protein pumps also occurs due to the formation of these protein pores.

PEF equipment

The PEF assembly for food processing consists of two components: a PEF generator unit and a treatment unit. The generator unit includes a power supply, an energy storage capacitor, and pulse-forming and shaping circuit. The treatment unit includes treatment chamber with electrodes, food circulation pump, and a cooling mechanism. Some other components are also required to monitor the pulse wave, electric field, temperature etc. The power supply consists of a high voltage direct current (DC) generator which converts simple electric voltage (110 or 220 V) alternate current (AC) to high voltage DC. This energy is stored in the capacitors and is discharged when the circuit allows, to the treatment chamber. If a food matrix is present in the treatment chamber then this energy is transferred to the food cells through some specialized electrodes. The capacitors can store the energy according to a simple equation $W = CV^2/2$, where W is energy (J), C is capacitance (F) and V is the charging voltage. Some specialized type of treatment chambers are required for effective PEF treatment for solid and liquid food (Table 1). They are also designed according to the specific application of PEF. Like batch type chambers are suitable for extraction and dehydration and continuous type are used in food preservation purpose. A simple

Fig. 1 Reversible or irreversible effects induced by applied PEF (in this figure *Salmonella* cell inactivation is shown), increase in the transmembrane potential, pore formation and permeabilization of cell membrane and pore resealing or inactivation of cell occur

treatment chamber includes two parallel electrodes encased in an insulating medium. An insulated spacer is used to change the volume of chamber. Recent research on PEF food processing has been done with coaxial, parallel and collinear arrangement of electrodes. These electrodes are made up of stainless steel (SS), titanium-coated SS, solid titanium, or silver + germanium alloy etc. Different types of pulse forming network produce different waveforms like exponential decay, bipolar, square, or oscillatory decay etc. These waveforms also affect the efficiency of PEF treatment, and it has been found that the square waveforms are more lethal than the exponential decay and the bipolar are more lethal than the monopolar waveforms. It has been noticed that exponential decay waveforms could not provide a sufficient electrical potential gradient for the required length of time to cause the ion migration to accumulate at the cell membrane (Huang et al. 2006) hence the efficiency of square shape waveforms is more than the exponential decay waveforms (Monfort et al. 2010).

PEF processing of egg products

Egg products contain a high microbial population if are not produced aseptically. Moreover, they contain a high quality nutrients which are needed for growth of some microorganisms like *Salmonella*. Thus, being a favorable growth medium for several microorganisms the pasteurization of liquid egg products is mandatory to ensure the storability and food safety. The conventional high temperature short time (HTST) pasteurization damages the structural and functional properties of egg products while the PEF treatment is much safer regarding the retention of original quality of egg products (Table 2). Marco-Molés et al. (2011) observed less opaque and less

change in the lipoprotein matrix when PEF was applied on liquid whole egg (LWE) as compared to the HTST pasteurization. It was also noticed that the water soluble protein content and mechanical properties of egg gel was better in PEF-treated samples in contrast to the HTST-treated samples. The reduction in the soluble protein content (SPC) of liquid whole egg (LWE) was 6 and 25 % when heat pasteurization was done at 60 °C for 3.5 min and 64 °C for 2.5 min, respectively while the reduction was only 1 % when PEF was applied in combination with heat (55 °C) and other additives (triethyl citrate, TC) and nearly more than 8 log₁₀ cycle reduction in the *Salmonella enteritidis* count was observed in the LWE samples at 25 kV/cm, 200 kJ/kg energy.

Wu et al. (2014) concluded that PEF at 25 kV/cm for up to 800 μs had no effect on the oxidation of egg white protein (EWP) as protein carbonyl content was non-significant between PEF-treated and control samples. Zhao and Yang (2008) also showed that the structure and function of lysozyme was not affected by the PEF treatment at 35 kV/cm for 300 μs in sodium phosphate buffer. However, the unfolding of tertiary structure was induced by PEF treatment at 35 kV/cm for 1200 μs. The unfolding of lysozyme structure was accompanied by the cleavage of disulfide bonds (Zhao et al. 2007), and more tyrosine residues were buried inside the protein after PEF treatment, accompanied by the exposure of more tryptophan residues (Zhao and Yang 2008). These authors also suggested that the inactivation of lysozyme at higher electric field strength and duration was closely related to the loss of α-helix of secondary structure. Some physicochemical properties of LWE treated by PEF (25 kV/cm, 75 kJ/kg) followed by heat treatments (52 °C/3.5 min, 55 °C/2 min, or 60 °C/1 min) in the presence of 2 % TC, or by heat (60 °C/3.5 min, and 64 °C/2.5 min) was investigated by Monfort-S et al. (2012), and

Table 1 PEF equipment for processing of egg products

Product	Process	Electrode	Gap between electrode/area	PEF generator	Waveform shape	References ^a
LWE, yolk, egg white	Continuous	Parallel SS	1 cm	30 kV generator with internal impedance of 100 Ω	Bi-polar, instant reversal square	a
LWE	Batch	Parallel SS	1 cm	High voltage pulse generator Samtech Ltd., Glasgow, UK	Rectangular instant reversal bipolar pulse	b
EWP	Continuous	Six co-field flow tubular SS with 2.3 mm inner Diameter	2.92 mm	OSU-4 L Ohio State University, Columbus, OH, USA; 100 Hz, 2 μ s	Square	c
LWE-SM ^c beverage	Continuous	Eight co-field treatment chambers with a diameter of 0.23 cm	0.293 cm	OSU-4D Ohio State University (Columbus, OH, USA), 2.5 μ s	–	d
LWE	Batch	Parallel SS	0.4 cm 0.79 cm ² area	ScandiNova (Modulator PG, ScandiNova, Uppsala, Sweden), 3 μ s, max-30 kV, 300 Hz and 200 A	Square	e, f, g, h, i
Egg proteins	Continuous	–	–	OSU-4 L, Ohio State University, Columbus, OH, USA	Square	j
Antioxidant peptide from EWP ^d	Continuous	Coaxial	–	Custom, 2 μ s	Exponentially decaying bipolar triangle	k, l, m, n, o, p

LWE liquid whole egg, SS stainless steel, LWE-SM liquid whole egg-skim milk, EWP egg white powder

^a (a). (Amiali et al. 2006), (b). (Bazhal et al. 2006), (c). (Wu et al. 2014), (d). (Pina-Pérez et al. 2009), (e). (Monfort-S et al. 2010), (f). (Monfort et al. 2010), (g). (Monfort-S et al. 2011), (h). (Monfort et al. 2012), (i). (Espina et al. 2014), (j). (Zhao et al. 2009), (k). (Lin et al. 2011), (l). (Lin et al. 2012), (m). (Lin et al. 2013), (n). (Wang et al. 2012), (o). (Wang-Ke et al. 2012), (p). (Wang-Ke et al. 2013)

Table 2 Recent studies on quality alteration of egg products by PEF treatment

Product	PEF conditions	Properties studied	Effects	References ^a
EWP	25 kV/cm for 200–800 μ s	Colloidal properties and protein oxidation	SPC $\downarrow$, Z-average size $\uparrow$ and protein aggregation $\uparrow$ at 25 kV/cm and >400 μ s	c
EWP	25, 30, and 35 kV/cm for 400 μ s, 200 Hz and 2 μ s		Insoluble protein aggregation $\uparrow$ at 35 kV/cm for 800 μ s; insoluble lysozyme aggregates $\uparrow$ at PEF (35 kV/cm, 1200 μ s) and heat (100 °C, 3.5 min); Protein denaturation $\uparrow$ by 16.5 % at 35 kV/cm, 400 μ s	j
LWE	25 kV/cm 75 kJ/kg 52, 55, 60 °C 3.5, 2, 1 min	Physicochemical, functional properties	Conductivity $\uparrow$ by 8.8 % of raw LWE, <i>a</i> value $\downarrow$ by 4.7 %, <i>b</i> value $\downarrow$ by 8.9 %, viscosity $\uparrow$ by 7.4 %, SPC $\downarrow$ by 1.8 %, foaming capacity $\uparrow$ by 15.2 %, foaming stability $\uparrow$ by 133.8 %, foaming stability $\uparrow$ by 133.8 %, emulsifying capacity $\uparrow$ by 8.9 %, emulsifying stability $\uparrow$ by 8.3 %, Reducing power $\uparrow$ (1.18 fold)	q ^b
Antioxidant peptide from EWP	10 kV/cm 3000 Hz 300 pulses	Reducing power	DPPH inhibition activity $\uparrow$	k, l, m
Antioxidant peptide (m. wt. = 1–10 kDa) from EWP	10 kV/cm 2400 Hz	DPPH inhibition activity		n
Antioxidant peptide (m. wt. = 10–30 kDa) from EWP	10–30 kV/cm 2000, 2350, 2700 Hz 300 pulses	DPPH inhibition activity	DPPH inhibition activity $\uparrow$ by 28.44 % at 10 kV/cm, 2000 Hz	p
Antioxidant peptide (m. wt. = 10–30 kDa) from EWP 300 pulses	10, 20, 30 kV/cm 2000 Hz	FRAP assay	FRAP activity $\uparrow$ by 44.23 % at 10 kV/cm, 2000 Hz	o
LWE	25 kV/cm 100 and 200 kJ/kg 52, 55, 58 and 60 °C; 10 mM disodium EDTA, 2 % Triethyl citrate	Microbial inactivation	SPC $\downarrow$ by 1.8–5 % at 25 kV/cm; 75–100 kJ/kg; 2 % TC	h

EWP egg white powder, SPC soluble protein content, LWE liquid whole egg, DPPH 2, 2-diphenyl-1-picrylhydrazyl, FRAP ferric reducing antioxidant potential

^aThe letters in this column correspond to the literature mentioned in Table 1

^b(Monfort-S et al. 2012)

found no change in the pH of LWE, suggesting some other reasons for the modification of functional properties of LWE. The loss of protein solubility after PEF treatment (25 kV/cm for 200–800 μ s) and upto ~10 % reduction in the SPC in the liquid egg white (LEW) was observed (Wu et al. 2014). This might be due to the aggregation of protein at higher electric field strength as revealed by Zhao et al. (2009), who concluded that the formation of EWP insoluble aggregates was more at 35 kV/cm for 400 μ s. The formation of protein aggregates was correlated with the increase in the Z-average particle size as well. Wu et al. (2015) concluded that when lysozyme (0.05 %) was present in ovalbumin (0.2 %) solution, insoluble protein aggregation occurred due to electrostatic interactions and disulphide bonds; and lysozyme (34.4 %), ovalbumin (36.2 %) and ovotransferrin (24.1 %) existed in the aggregates after PEF treatment at 25 kV/cm for 800 μ s. These authors also showed the protective effect of citric acid on protein aggregation as addition of citric acid (0.20 %) could effectively inhibit lysozyme from interacting with other proteins during PEF processing.

If the soluble protein loss is more than 5 % then the product is not acceptable as a functional product. Thus, the PEF conditions that produce more than 5 % soluble proteins are not suitable for producing functional egg products. PEF treatment caused decrease in the foaming capacity and gelling properties of liquid egg products (Marco-Molés et al. 2011). The moderate PEF conditions alone or extreme PEF conditions with some modifiers may be used to overcome this negative effect. In another study the oxidation of lecithin was observed (Zhao et al. 2012) in the PEF-treated liquid egg samples. These authors were of opinion that the use of an antioxidant with PEF treatment may reduce the oxidation of lecithin. The efficiency of PEF also depends on the conductivity of medium as higher conductivity results in low-peak electric field across the treatment chamber due to the generation of higher current during PEF treatment. The LWE possesses higher conductivity which reduces the pasteurization efficiency of PEF in this product (Amiali et al. 2006). This fact is supported by the studies of Saldaña et al. (2012) who reported that the application of PEF at 50 °C inactivated 5 log₁₀ cycles (more than the observed reduction in the microbial count at higher conductivity) of the population of *Salmonella typhimurium* and *Escherichia coli* 0157:H7 in buffer media, at the range of pH of most foods (pH 3.5–7), adjusted to an electrical conductivity of 0.10 $\pm$ 0.01 mS/cm. It is also evident from the study of Monfort-S et al. (2012) that some modifiers (triethyl citrate, TC) reduced the conductivity of medium (LWE) significantly. This may enhance the efficiency of PEF which was confirmed in the later studies conducted by Monfort et al. (2012). The reduction in the conductivity of TC supplemented LWE samples might be due to the fact that TC might bind the divalent metal ion (Ca²⁺/Mg²⁺) of LWE which are mainly responsible for the ion-induced conductivity.

Currently, the heat pasteurization of LWE is being done at 60 °C for 3.5 min (USA) and 64 °C for 2.5 min (UK) which provides a reduction of 5–9 log₁₀ cycles in *Salmonella* serovar count (Monfort-S et al. 2012; Monfort et al. 2012). Ultrapasteurization is a process in which LWE is treated at 70 °C for 1.5 min for providing higher level of microbial safety. However, due to the precipitation of the soluble proteins of LWE at >57 °C and complete coagulation at 73 °C (Hamid-Samimi et al. 1984; Herald and Smith 1989), and due to the alteration in the functional properties of egg products (heat sensitive) at high temperature, the application of PEF is gaining its importance for inactivation of microbial population and pasteurization.

Most of the microbial inactivation studies have been focused on *Salmonella* serovar (Gram –ve) (Table 3) in the LWE, yolk or egg white. The inactivation of *Salmonella enteritidis* and *Salmonella senftenberg* 775 W was studied by Monfort-S et al. (2010) and approximately 3 log₁₀ cycles reduction of these microorganisms was observed at 25 kV/cm for 105 μ s (441 kJ/kg) and 35 kV/cm for 45 μ s (369 kJ/kg), respectively. The lethal effectiveness of PEF to eliminate *Salmonella typhimurium* and *Salmonella aureus* in LWE has been investigated by Monfort et al. (2010), and the maximum inactivation levels of 4 and 3 log₁₀ cycles of the population of *S. typhimurium* and *S. aureus* were achieved with treatments of 45 kV/cm for 30 μ s and 419 kJ/kg, and 40 kV/cm for 15 μ s and 166 kJ/kg, respectively. Monfort-S et al. (2011) observed maximum reduction of 4 log₁₀ cycles in *Salmonella* count when the PEF was applied at 45 kV for 30 μ s. *Salmonella enteritidis* count was reduced by 8 log₁₀ cycles in the LWE samples when PEF (25 kV for 48 μ s) was used in combination with temperature (55 °C for 2 min) (Monfort-S et al. 2011). The efficiency of microbial inactivation was increased if some modifiers were used; for instance, 10 mM ethylenediaminetetraacetic acid (EDTA) or 2 % TC was used in combination with PEF (25 kV/cm, 100 kJ/kg) and mild heat (60 °C, 1 min), and 9 log₁₀ cycles reduction in *S. enteritidis* count and 5 log₁₀ cycles reduction in *S. dublin*, *S. typhimurium*, *S. typhi*, *S. senftenberg*, and *S. virchow* was observed (Monfort-S et al. 2011; Monfort et al. 2012).

Similarly, the lethal effectiveness of PEF in static and continuous conditions followed by the heat treatment in LWE with additives (EDTA or TC) on 7 different *Salmonella* serovars was evaluated by Monfort et al. (2012). The PEF treatment (25 kV/cm and 75–100 kJ/kg) followed by heat (52 °C/3.5 min, 55 °C/20 s, or 60 °C/10 s) in LWE with 2 % TC permitted the reduction of heat treatment time from 92 fold at 52 °C to 3.4 fold at 60 °C, and 4.8 fold at 52 °C in LWE with EDTA for a 9 log₁₀ cycles reduction of the population of *Salmonella enteritidis* as compared to the heat treatment alone. This combined treatment inactivated more than 5 log₁₀ cycles of *Salmonella* serovars *dublin*, *enteritidis*, *typhimurium*, *typhi*, *senftenberg*, and *virchow*, both in static

Table 3 Recent studies on PEF inactivation of microorganisms in egg products

Product	PEF conditions	Combined hurdle	Microorganisms	MI (log10 cycle)	MI conditions	+ve effects	-ve effects	References ^a
LWE	9–15 kV/cm 2 μ s, 1 Hz upto 138 pulses	50–60 °C for 20 s	<i>Escherichia coli</i> O157H7	4.0	11 kV/cm 40 pulses 60 °C	na	na	b
LWE-SM beverage	15, 25, and 35 KV/cm 60 to 3895 μ s	CocoaOX 12 %	<i>Bacillus cereus</i>	3.3	40 kV/cm 360 μ s 20 °C	na	na	d
LWE	25 kV/cm 100 and 200 kJ/kg	55 °C, 10 mM EDTA, 2 % triethyl citrate	<i>Salmonella enteritidis</i>	> 8.0	25 kV/cm 200 kJ/kg 55 °C for 2 min 2 % TC	na	na	g
LWE	25 kV/cm 100 and 200 kJ/kg 0.5 Hz	60 °C for 3.5 min, 200 μ L/L carvacrol, citrate, (+) limonene, rosemary EO, Lemon EO, mandarin EO	<i>Salmonella senftenberg</i> 775 W <i>Listeria monocytogenes</i>	4.0 4.0	25 kV/cm 100 kJ/kg 60 °C/3.5 min 200 μ L/L lemon EO	HA $\uparrow$ (products made from treated LWE)	na	i
LWE	20–45 kV/cm	–	<i>Salmonella enteritidis</i> <i>S senftenberg</i> 775 W	~ 3	25 kV/cm 105 μ s 441 kJ/kg	na	na	e
LWE	25 kV/cm 100 and 200 kJ/kg	52, 55, 58 and 60 °C, 10 mM NA2 EDTA, 2 % Triethyl citrate	<i>Salmonella serovar Enteritidis</i> , <i>S typhimurium</i> , <i>S senftenberg</i> 775 W, <i>S typhi</i> , <i>S dublin</i> , <i>S virchow</i> , <i>S enteritidis</i>	~ 3 ~ 9, > 5, > 5, > 5, > 5, > 5, > 5	35 kV/cm 45 μ s 369 kJ/kg 25 kV/cm 75–100 kJ/kg 2 % TC	na	na SPC $\downarrow$ (1.8–5 %)	e h
LWE	30–45 kV/cm	–	<i>S typhimurium</i>	4	45 kV/cm 30 μ s 419 kJ/kg	na	na	f
			<i>Staphylococcus aureus</i>	3	40 kV/cm 15 μ s 166 kJ/kg	na	na	f

LWE liquid whole egg, na not analyzed, LWE-SM liquid whole egg-skim milk, EO essential oil, HA hedonic acceptability, SPC soluble protein content, MI maximum inactivation

^a The letters in this column correspond to the literature mentioned in Table 1

and continuous conditions. Conversely, the heat pasteurization treatments of 60 °C/3.5 min and 64 °C/2.5 min reduced 5 log₁₀ cycles of various *Salmonella* serovars but only 2 and 3–4 log₁₀ cycles of *Salmonella senftenberg* and *Salmonella enteritidis*, respectively with 9.4 % reduction of SPC after heat pasteurization at 64 °C. A 8 log₁₀ cycles reduction was observed at 25 kV/cm with 200 kJ/kg energy when heat (55 °C for 2 min) was applied after PEF treatment in the presence of 2 % TC (Monfort-S et al. 2011). This reduction in the *Salmonella* count is nearly similar to the efficiency of heat pasteurization at 60 °C for 3.5 min. It is evident from these studies that the lethal effectiveness of PEF treatment at temperatures lower than 35 °C is far from the 5 log₁₀ cycles inactivation (recommended by the USDA) of *Salmonella* (Monfort et al. 2012) and could be insufficient to guarantee the absence of *Salmonella* in 25 g or ml of LWE, as indicated by the European Regulation (Directive 89/107/CE).

PEF treatment is also used for inactivation studies of microorganisms other than *Salmonella* species in different egg products. Bazhal et al. (2006) reported reduction in *Escherichia coli* (gram –ve) count by ~2.5 log₁₀ cycles at 30 pulses of 15 kV/cm, 68 pulses of 11 kV/cm and 138 pulses of 9 kV/cm when mild heat (55 °C) was also used. If the treatment temperature was increased by 5 °C (60 °C) then at the same electric field strength and at 10, 40 and 72 corresponding pulses the inactivation of *E. coli* was increased by around 40–60 % (~1–1.5 log₁₀ cycles). The increase in the inactivation of microbial count at higher temperature was explained by increase in the conductivity ($\sigma = 0.277 + 0.012 T$), as in the liquid medium the conductivity is a function of temperature.

The effect of PEF in combination with some antimicrobials (CocoanOX 12 %) against *Bacillus cereus* (gram –ve) inactivation kinetics in LWE – skim milk (LWE – SM) beverage was studied by Pina-Pérez et al. (2009). The maximum inactivation of 3.03 log₁₀ cycles was observed at 35 kV/cm for 200 µs (20 °C). This inactivation was directly proportional to the applied external electric field strength and duration. A synergistic antimicrobial effect was observed when CocoanOX was supplemented in the beverage hence maximum inactivation was observed. However, the synergism was observed more at lower electric field strength due to the possible reason that the reversible pore formation was maximum at lower field strength, and during the rearrangement of these pore structures the antimicrobial acted to inactivate the cells more efficiently. This scenario was again confirmed by the studies of Monfort-S et al. (2011), when the use of some antimicrobials (dimethyl dicarbonate, nisin, TC and EDTA) increased the inactivation efficiency more at 100 kJ/kg rather than at 200 kJ/kg. Alternative pasteurization strategy using PEF, heat and essential oil (EO) is recently studied by Espina et al. (2014). The application of PEF at 25 kV/cm, 100 kJ/kg followed by heat treatment at 60 °C for 3.5 min on LWE supplemented with 200 µL/L of lemon EO

inactivated 4 log₁₀ cycles of *S. senftenberg* 775 W and *Listeria monocytogenes* without affecting the sensory properties of LWE. This provides similar microbial safety for these microorganisms in the LWE samples to that of ultrapasteurization treatment.

Some other applications of PEF have recently been investigated (Table 2). Wang et al. (2012) studied the effect of PEF treatment on the antioxidant activity of 1–10 kDa molecular weight (MW) peptides derived from EWP. These authors concluded that 2, 2-diphenyl-1-picrylhydrazyl (DPPH) inhibition activity of these peptides were maximum at 10 kV/cm of 2400 Hz electric field with 8 mg/ml concentration. Under somewhat similar conditions Wang-Ke et al. (2012) reported 28.4 % increase in the DPPH inhibition activity and 44.2 % increase in the ferric reducing antioxidant potential (FRAP) of 10–30 kDa MW peptides produced from EWP. These authors have also demonstrated the negative effect of electric field strength on the FRAP activity of peptides. Thus, increase in the external electric field does not always result in better effect. This phenomenon may be considered to design economical PEF treatment modules for such applications. Some workers (Lin et al. 2012; Lin et al. 2013) studied the effect of PEF on the reducing power (RP) of alcalase hydrolysates with MW < 1 kDa produced from EWP. After PEF treatment, the hydrolysates showed a significant improvement in the RP (1.18-fold) within 4–6 h. PEF might cause the polarization of the peptide molecule, destroy the noncovalent bonds, dissociate more subunits leading to a disorganization of protein structure. As a result, more and more free reducing amino acid and small peptides were released. Thus, the exposure of His, Pro, Cys, Tyr, Try, Phe and Met residues might be involved in the enhanced antioxidant activity of peptides. Moreover, the smaller antioxidant peptides might have exerted better biological effects (Hernández-Ledesma et al. 2005). Barsotti et al. (2001) reported that a series of 20 exponential decay pulses of 31.5 kV/cm at 1 Hz applied to ovalbumin in a buffer solution induced at least a partial unfolding of the protein structure or enhanced the ionization of sulphhydryl (–SH) groups into a more reactive (–S) form. However, the specific mechanism for increase in the antioxidant potential needs further study.

Limitations and challenges

PEF is a technology which is establishing its applications in egg processing industry. Thus, following points need to be answered or worked upon before concluding PEF a safe alternative of conventional egg processing technology:

- The sustainability of egg or any food processing industry mainly depends on the economics of various operations. Hence, the economics of PEF processing operations should be calculated and compared to that of

conventional processing technologies so that the viability of new PEF method should be confirmed in the egg processing industry. PEF processing of relatively high conductivity products such as LWE leads to ohmic heating of the product, thus, some cooling energy is required. The economics of such secondary operation should also be confirmed.

- The design and development of low-cost PEF equipment with higher efficiency is required. The determination of electrical conductivity of different liquid egg products over a wide range of temperature is critical for design and optimization of effective PEF process. The systems (including treatment chambers and power supply equipment) need to be scaled up to the commercial level.
- The high electrical conductivity and thermal sensitivity of LWE limit the application of PEF at elevated temperatures due to the occurrence of arcing. Appropriate treatment chamber that would match the characteristic impedance of a PEF unit (in the case of a square waveform) should be designed from product electrical conductivity. Matching impedances of the load and PEF unit is required in order to allow higher energy transfer and proper treatment of the food.
- If PEF is used for pasteurization of egg products then a). establishing and comparing the efficiency of new PEF method to the efficiency of heat pasteurization, b). assessing the level of inactivation, c). validating the efficiency of the pasteurization process, and d). defining the specific equipment and operating parameters for the proposed PEF pasteurization process is needed.
- PEF efficiency depends upon various factors, hence the effect of PEF should be confirmed at various processing parameters like electric field strength, duration, pulse wave form, type of microorganism (type, genus, species, strain etc.), pH of medium, temperature and conductivity level.
- The presence of bubbles in the treatment medium leads to non-uniform treatment as well as operational and safety problems. If the applied electric field exceeds the dielectric strength of the gas bubbles, partial discharges take place inside the bubbles that can volatilize the sample and therefore increase the volume of the bubbles. It may become big enough to bridge the gap between the two electrodes and may produce a spark. Therefore, air bubbles in the samples must be removed by vacuum degassing or pressurizing the treatment media during processing.
- It is also important to note here that a threshold level of 250 kJ/kg provides a corresponding temperature $\sim 65^{\circ}\text{C}$ (Heinz et al. 2001) which affects the functional properties of egg products. Hence, a low level of energy is suitable for PEF processing without affecting other functional properties of egg products. However, it has also been

concluded that at low energy level complete microbial inactivation may not be achieved (Monfort-S et al. 2010). Hence, PEF technology alone would not be suitable for complete pasteurization of liquid egg products. Thus, a possible hurdle approach should be identified in order to increase the efficiency of PEF at low electric field level.

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