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Current and future prospects for the use of pulsed electric field in the meat industry

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ABSTRACT

Pulsed electric field (PEF) is a novel non-thermal technology that has recently attracted the attention of meat scientists and technologists due to its ability to modify membrane structure and enhance mass transfer. Several studies have confirmed the potential of pulsed electric field for improving meat tenderness in both pre-rigor and post-rigor muscles during aging. However, there is a high degree of variability between studies and the underlying mechanisms are not clearly understood. While some studies have suggested physical disruption as the main cause of PEF induced tenderness, enzymatic nature of the tenderization seems to be the most plausible mechanism. Several studies have suggested the potential of PEF to mediate the tenderization process due to its membrane altering properties causing early release of calcium ions and early activation of the calpain proteases. However, experimental research is yet to confirm this postulation. Recent studies have also reported increased post-mortem proteolysis in PEF treated muscles during aging. PEF has also been reported to accelerate curing, enhance drying and reduce the numbers of both pathogens and spoilage organisms in meat, although that demands intense processing conditions. While tenderization, meat safety and accelerated curing appears to be the areas where PEF could provide attractive options in meat processing, further research is required before the application of PEF becomes a commercial reality in the meat industry. It needs to deal with carcasses which vary biochemically and in composition (muscle, fat, and bones). This review critically evaluates the published reports on the topic with the aim of reaching a clear understanding of the possible applications of PEF in the meat sector in addition to providing some insight on critical issues that need to be addressed for the technology to be a practical option for the meat industry.

KEYWORDS
Pulsed electric field; meat; tenderization; meat safety; supercooling; accelerated curing; restructuring; sodium reduction; protein digestibility; mineral content

1. Introduction

Pulsed electric field (PEF) is considered as a very promising non-thermal technique of preserving foods and improving food quality. The technology involves the use of electric field pulses of short duration (several nanoseconds to several milliseconds) with electric field strength of 0.1–80 kV/cm applied to a food placed between or passed through two electrodes (Barba et al. 2018; Puértolas and Barba 2016; Koubaa et al. 2015; Buckow et al. 2014; Buckow et al. 2013). Electric field strength, treatment temperature and energy delivery are the three most important parameters identified for PEF processing (Toepfl et al. 2014b; Amiali et al. 2007; Lebovka et al. 2005; Heinz et al. 2003). As a non-thermal technology, pulsed electric field processing causes less degradation of nutritional and sensory characteristics of foods than traditional thermal processing technologies (Buckow et al. 2013; Walkling-Ribeiro et al. 2010; Rivas et al. 2006). It exhibits many advantages such as lower treatment temperature, shorter processing time and potential continuous flow in comparison to traditional processing technologies (Walkling-Ribeiro et al. 2011; Puertolas et al. 2010), making it a very appealing technology for food manufacturers. Although, it has been widely investigated for its industrial pasteurization and sterilization potential for liquid foods like milk, dairy products, liquid eggs, fruit juice, wine, beer and other alcoholic beverages (Milani et al. 2015; Delsart et al. 2014; Buckow et al. 2014; Timmermans et al. 2014; Monfort et al. 2010), the use of PEF as food processing technology in solid foods (Liu et al. 2017a; Aguiló-Aguayo et al. 2017; Ignat et al. 2015), particularly meat, has only recently emerged (Khan et al. 2017a; Ma et al. 2016; Bekhit et al. 2016; Suwandy et al. 2015a; 2015b; 2015c; 2015d).

2. Applications in food industry

Electric pulses of very short duration and high intensity can induce irreversible structural changes in cell membranes resulting in increased membrane permeability (Zimmermann et al. 1976), enhanced mass transfer (Janositz et al. 2011) and disruption or breakdown of cellular tissue (Toepfl et al. 2014a). Thus, PEF can be used to replace or improve existing processes for food preservation, food modification and tissue disintegration (Bekhit et al. 2017; Toepfl et al. 2014a). The first commercial application of PEF started with the installation of PEF system in USA in 2006 for fruit juice preservation (Clark 2006) which was followed with the installation of first commercial PEF juice line in 2009 in

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Europe (Toepfl et al. 2014b). The first commercial PEF preservation system for vegetable processing was installed in 2010. Systems with capacities up to 5000 litres/h are currently installed and available for fruit juice preservation in Europe (Toepfl et al. 2014b; Toepfl 2012). PEF technology has potential application in several food processes, like cold pasteurization and sterilization (Barba et al. 2015; Uchida et al. 2008), inactivation of enzymes (Zhao et al. 2010), promotion of extraction and recovery of bioactive compounds (Rodríguez-Roque et al. 2015; Abenoza et al. 2013), reduction of the allergenicity of certain food products (Johnson et al. 2010), potential reduction of food contaminants and pesticide residues (Zhang et al. 2012), food dehydration (Wiktor et al. 2014), and freezing processes (Wiktor et al. 2013). While the technology has been recognized as a non-thermal technology, as its effects do not require heat addition, ohmic heating can be generated under high treatment intensity. While this can be useful for synergistic effect on microbial inactivation and controlled by rapid cooling post-treatment, this can have negative effects on the quality and appearance of solid food materials such as cooking effect in fresh meat (Bekhit et al. 2014c).

3. Mechanism of action

In the absence of an external electric field, there is a naturally occurring perpendicular transmembrane potential of about 10 mV in a cell due to the accumulation of charges of opposite polarity on each side of a membrane (Toepfl et al. 2014b). When exposed to an external electric field, an additional potential is induced which depends on the strength of the applied field surrounding the cells. During PEF processing, the food is placed between two electrodes and an external electric field is applied which induces the movement of ions along the direction of lines of force of the applied electric field inside as well as outside the cells. This causes the accumulation of ions on the membranes causing the polarisation of the cell (Teissie et al. 1985) which results in a reduction in the thickness of the membranes due to the forces of attraction between oppositely charged ions on either side of the membrane (electro-compressive forces). When electric field strength exceeds the critical threshold value of transmembrane potential of approximately 1 volt (Weaver 2000; Zimmerman 1996; Hamilton and Sale 1967), these electro-compressive forces cause electrical breakdown or viscoelastic deformation of the cell membrane which can be observed as pores. This electrical breakdown or pore formation, also known as electroporation, increases the permeability of membranes (Zimmermann et al. 1976).

In addition to this electromechanical explanation of electroporation, another theory explains the electroporation based on formation of hydrophilic pores from hydrophobic pores in the membrane. The hydrophobic pores, which are naturally formed due to thermal fluctuations, exceed a critical size and become hydrophilic because these require less energy to maintain the structure and are more stable under the conditions. When PEF is applied, energy needed for the formation of pores gets reduced due to the increased transmembrane potential and Joule heating effect; this increases the number and size of pores and reduces the critical size. This expansion and accumulation of hydrophilic pores is believed to be responsible for electroporation (Joshi et al. 2002; Weaver and Chizmadzhev 1996; Barnett and Weaver 1991).

The intensity of electric field must exceed a critical strength for electroporation to occur (Barbosa-Canovas and Sepulveda 2005). The electric field strength above which the permeability increases is known as critical electric field and assuming a biological membrane of 5 nm thickness, this translates to a dielectric strength of 2 kV/cm (Glaser et al. 1998). The electric field strength to achieve the electroporation depends on several factors like food properties (whether a food is liquid, viscoelastic or solid, and its dielectric characteristics), process parameters (temperature, pulse duration, amplitude, and number of pulses), cell parameters (type, size, shape, and orientation) and membrane characteristics (ionic strength, thickness, and structure) (Toepfl, et al. 2014b; Saulis 2010). At low PEF intensity treatment, electroporation is often reversible; however, membrane breakdown will occur when the ratio of pore size to membrane surface area becomes too large (Buckow et al. 2014). For irreversible pore formation, a critical electric field strength of 1–2 kV/cm is required for plant cells and 10–14 kV/cm for microbial cells (1–10 μm) (Toepfl et al. 2005). Electroporation of microbial cell membranes (microbial inactivation) such as those of *Listeria innocua* or *Escherichia coli* can be achieved by using high electric field strengths of 10–40 kV/cm and energy input of more than 40 kJ/kg (Toepfl et al. 2014a). A lower field strength and energy input is required for the disintegration of plant and animal tissue due to their larger cell size. Pore formation in animal tissue, such as meat, can be achieved by using electric field strengths of 1–10 kV/cm and energy input of 0.5–10 kJ/kg (Toepfl et al. 2014a) whereas an electric field strength of 0.7–3 kV/cm and energy input of 1–20 kJ/kg are sufficient for the treatment of plant tissues (Corrales et al. 2008; Zimmermann et al. 1976). Studies on fresh meat indicated that at 10 kV/cm intensity, cooking effects start to appear on the meat edges (Bekhit et al. 2014c). This information collectively suggests that PEF could be used to create structural changes in fresh meat, i.e. tenderization, but is unlikely to be useful for microbial inactivation as that requires more intensive treatment.

4. How PEF can affect meat quality

Studies on the utilization of PEF in solid foods like meat are generally lacking (O'Dowd et al. 2013; Gudmundsson and Hafsteinsson 2001), however, Topfl (2006) identified meat as one of the most promising applications to achieve a broad industrial exploitation of PEF in a study comparing the energy requirements to induce pore formation in different biological membrane systems. PEF treatment of meat was reported to enhance mass transfer during drying as well as brining of the products. Further, an improvement in water binding was noticed during cooking due to improved micro-diffusion of brine and water binding agents. Several papers have been published since then on the applications of PEF processing in meat technology highlighting several areas like meat safety, tenderization, supercooling and accelerated brining (Mok et al. 2017; Khan et al. 2017a; Ma et al. 2016; Bekhit et al. 2016; Suwandy et al. 2015a; 2015b; 2015c; 2015d; McDonnell et al. 2014; Faridnia et al. 2015; Haughton et al. 2012). Table 1 and 2 summarizes findings of different

Table 1. Findings of different studies elucidating the effect of pulsed electric field on the tenderness of different muscles.

Authors	Muscle Studied	Muscle status	PEF Treatment	Findings
Suwandy et al. (2015b)	Beef Longissimus lumborum	Pre-rigor	5 kV, 10 kV × 20, 50, 90 Hz	Toughness increased with treatment intensity
Suwandy et al. (2015b)	Beef Semimembranosus	Pre-rigor	5 kV, 10 kV × 20, 50, 90 Hz	Tenderness increased regardless of intensity, Shear force reduced by 21.6%
Bekhit et al. (2016)	Beef Longissimus lumborum	Pre-rigor	Repeated (1 ×, 2 ×, 3 ×) PEF treatment (10 kV, 90 Hz, 20 μs)	Tenderness reduced with 3 × PEF treatment, 1 × and 2 × treatment had no effect
Bekhit et al. (2016)	Beef Semimembranosus	Pre-rigor	Repeated (1 ×, 2 ×, 3 ×) PEF treatment (10 kV, 90 Hz, 20 μs)	Tenderness increased with lowest shear force in 3 × PEF treatment
Bekhit et al. (2014c)	Beef Longissimus lumborum	Post-rigor	5 kV, 10 kV × 20, 50, 90 Hz	Tenderness increased regardless of intensity, Shear force reduced by 19.5%
Bekhit et al. (2014c)	Beef Semimembranosus	Post-rigor	5 kV, 10 kV × 20, 50, 90 Hz	Tenderness increased with treatment intensity, Shear force reduced by 4.1, 10.4, 19.1% for 20, 50, 90 Hz
Suwandy et al. (2015d)	Beef Longissimus lumborum	Post-rigor	Repeated (1 ×, 2 ×, 3 ×) PEF treatment (10 kV, 90 Hz, 20 μs)	Tenderness increased with repeats, Shear force decreased by 2.5 N for each additional treatment
Suwandy et al. (2015d)	Beef Semimembranosus	Post-rigor	Repeated (1 ×, 2 ×, 3 ×) PEF treatment (10 kV, 90 Hz, 20 μs)	Tenderness was not affected by PEF, Shear force not affected by PEF
Suwandy et al. (2015c)	Beef Longissimus lumborum	Post-rigor	(10 kV, 90 Hz, 20 μs)	No effect on shear force due to fibre direction or initial muscle pH
Suwandy et al. (2015a)	Beef Longissimus lumborum	Post-rigor	5 kV, 10 kV × 20, 50, 90 Hz	Tenderness increased regardless of intensity, Shear force reduced by 19.0%
Suwandy et al. (2015a)	Beef Semimembranosus	Post-rigor	5 kV, 10 kV × 20, 50, 90 Hz	Tenderness increased with treatment intensity, Shear force reduced by 19.0%
O'Dowd et al. (2013)	Beef Semitendinosus	Post-rigor	1.9 kV/cm, 65 Hz, 20 μs	Tenderness was not affected by PEF, Shear force not affected by PEF
Arroyo et al. (2015b)	Beef Longissimus thoracis et lumborum	Post-rigor	1.4 kV/cm, 10 Hz, 20 μs, 300 and 600 pulses	Samples showed tendency towards reducing toughness, Shear force not affected by PEF
Faridnia et al. (2014)	Beef Longissimus thoracis	Post-rigor	0.2–0.6 kV/cm, 1–50 Hz, 20 μs	Tenderness was not affected by PEF, Shear force was not affected by PEF
Faridnia et al. (2015)	Beef Semitendinosus	Post-rigor	1.4 kV/cm, pulse width 20 μs, 50 Hz	Freezing-thawing + PEF improved tenderness, Tenderness was not improved by PEF alone
Faridnia et al. (2016)	Biceps femoris	Post-rigor	1.7kV/cm, 50 Hz, 20μs	Tenderness increased in treated samples, Shear force reduced in PEF samples
Khan et al. (2017)	Beef Longissimus et lumborum	Post-rigor	2.5 kV, 200 Hz, 20 μs-LPEF 10 kV, 200 Hz, 20 μs-HPEF	Tenderness was not affected by PEF, Shear force of HPEF samples was higher than LPEF and untreated samples
McDonnell et al. (2014)	Pork Longissimus thoracis et lumborum	Post-rigor	1.2 or 2.3 kV/cm x100 or 200 Hz x 150 or 300 pulses	No significant effect of PEF was observed on the texture profile analysis
Arroyo et al. (2015a)	Turkey Breast Meat	Post-rigor	7.5, 10, 12.5 kV (fresh meat), 14, 20, 25 kV (frozen meat) x 10, 55, 110 Hz	Tenderness was not affected by PEF in both fresh and frozen samples, Shear force not affected by PEF
Toepfl (2006)	Pork haunches and Shoulder, Beef meat	Post-rigor	0.5–5 kV/cm, 50–1000 pulses, 1–25 kJ/kg	Tenderness increased in treated samples

Table 2. Findings of different studies elucidating the effect of pulsed electric field on the quality characteristics of different muscles.

Authors	Muscle studied	Muscle status	PEF Treatment	Findings
Suwandy et al. (2015b)	Beef Longissimus lumborum	Pre-rigor	5 kV, 10 kV × 20, 50, 90 Hz	Temperature rise of 0.6 – 4.4 °C, Purge loss (%) was not affected by PEF, Cooking loss (%) tended to increase
Suwandy et al. (2015b)	Beef Semimembranosus	Pre-rigor	5 kV, 10 kV × 20, 50, 90 Hz	Temperature rise of 0.7 – 8.1 °C, Purge loss (%) increased regardless of intensity, Cooking loss (%) was not affected by PEF
Bekhit et al. (2016)	Beef Longissimus lumborum	Pre-rigor	Repeated (1 ×, 2 ×, 3 ×) PEF treatment (10 kV, 90 Hz, 20 μs)	Temperature rise of 6.5 – 13.4 °C, Purge loss % increased by 1.38% for every extra treatment, Cooking loss (%) was not affected by PEF
Bekhit et al. (2016)	Beef Semimembranosus	Pre-rigor	Repeated (1 ×, 2 ×, 3 ×) PEF treatment (10 kV, 90 Hz, 20 μs)	Temperature rise of 1.8 – 6.7 °C, Purge loss (%) affected linearly by number of PEF repeats, Cooking loss (%) was not affected by PEF
Toepfl (2006)	Pork shoulder, haunches, sausage products	Post-rigor	0.5–5 kV/cm, 50–1000 pulses, 1–25 kJ/kg	Mass transfer enhancement in meat, Enhanced drying rates of raw ham and sausage products, Accelerated the curing process, Improved water binding capacity
Bekhit et al. (2014c)	Beef Longissimus lumborum	Post-rigor	5 kV, 10 kV × 20, 50, 90 Hz	Temperature rise of 0.4 and 7.7 °C, Purge loss (%) increased linearly with voltage and frequency, Cooking loss (%) lowered in PEF treated samples
Bekhit et al. (2014c)	Beef Semimembranosus	Post-rigor	5 kV, 10 kV × 20, 50, 90 Hz	Temperature rise of 0.4 and 8.0 °C, Purge loss (%) increased linearly with voltage, Cooking loss (%) was not affected by PEF
Suwandy et al. (2015d)	Beef Longissimus lumborum	Post-rigor	Repeated (1 ×, 2 ×, 3 ×) PEF treatment (10 kV, 90 Hz, 20 μs)	Temperature rise of 7.7 – 16.2 °C, pH and purge loss (%) was not affected by PEF, Cooking loss (%) increased regardless of PEF repeats Redness (a values) decreased with PEF repeats
Suwandy et al. (2015d)	Beef Semimembranosus	Post-rigor	Repeated (1 ×, 2 ×, 3 ×) PEF treatment (10 kV, 90 Hz, 20 μs)	Temperature rise of 7.7 – 15.6 °C, pH and cooking loss (%) was not affected by PEF, Purge loss (%) increased regardless of PEF repeats, Redness (a values) decreased with PEF repeats
Suwandy et al. (2015c)	Beef Longissimus lumborum	Post-rigor	10 kV, 90 Hz, 20 μs	No significant effect of PEF was found on total water loss, meat colour and lipid stability
O'Dowd et al. (2013)	Beef Semitendinosus	Post-rigor	1.9 kV/cm, 65 Hz, 20 μs	Temperature rise of 5–22 °C, Weight loss (%) increased linearly with temperature of PEF, No adverse effect on drip loss (%), moisture (%), water activity, total expressible moisture and solid (%), Muscle fibre bundles appeared smaller in diameter and Hunter L values were significantly lower for PEF samples
Arroyo et al. (2015a)	Turkey Breast Meat	Post-rigor	7.5, 10, 12.5 kV (fresh meat), 14, 20, 25 kV (frozen meat) x 10, 55, 110 Hz	No significant effect of PEF was observed on weight loss (%), cook loss (%), lipid oxidation, texture and colour of fresh and frozen meat samples
Arroyo et al. (2015b)	Beef Longissimus thoracis et lumborum	Post-rigor	1.4 kV/cm, 10 Hz, 20 μs, 300 and 600 pulses	Temperature rise of 7.7 °C (300 pulses), 14.5 °C (600 pulses), No significant effect of PEF on weight loss (%), cook loss (%) or storage loss (%), Colour L, a, and b values were not affected by PEF
Faridnia et al. (2014)	Beef Longissimus thoracis	Post-rigor	0.2–0.6 kV/cm, 1–50 Hz, 20 μs	No effect of PEF on pH, cooking loss and colour stability, Moisture (%) decreased significantly by 0.7–3.6%
Faridnia et al. (2015)	Beef Semitendinosus	Post-rigor	1.4 kV/cm, pulse width 20 μs, 50 Hz	Increased purge loss (%), No effect of PEF on free fatty acid profiles, omega6/omega 3, polyunsaturated/saturated fatty acids ratios, A two log-unit increase in aerobic microbial counts during log phase of frozen-thawed PEF-treated samples
Faridnia et al. (2016)	Biceps femoris	Post-rigor	1.7 kV/cm, 50 Hz, 20μs	Significant increase in conductivity, purge loss (%) and temperature (Mean temperature of PEF sample – 26.53°C), Significant decrease in pH, Cooking loss was not affected, Dramatic increase in the number of ruptured myofibrils along the z-lines making muscle more porous structure
McDonnell et al. (2014)	Pork Longissimus thoracis et lumborum	Post-rigor	1.2 or 2.3 kV/cm, 100 or 200 Hz, 150 or 300 pulses	No significant effect of PEF on cook loss (%), water holding capacity, total viable count and lipid oxidation (TBARS), Potential may exist for reduced curing time through PEF
Qianli et al. (2016)	Lamb shoulder (Infraspinatus), rib (Longissmus), loin (Longissmus, Psoas major)	Post-rigor	1–1.4 kV/cm, 90 Hz, 20μs	PEF induced significant changes in volatile compounds, PEF affected temporal flavour of meaty and oxidized flavour attributes, Significant effect on lipid oxidation in chilled meat but led no off flavour
Khan et al. (2017)	Beef Longissimus et lumborum	Post-rigor	2.5 kV, 200 Hz, 20 μs – LPEF 10 kV, 200 Hz, 20 μs – HPEF	Significantly higher L* values and lower a* values in HPEF samples, Significantly lower P, K and Fe concentrations in HPEF samples, Higher lipid oxidation in HPEF than LPEF

studies on effect of PEF on tenderness and other quality parameters of different muscles.

Because of its potential for cell membrane permeabilization, PEF is able to modify several quality traits of meat like texture, colour, and water-holding capacity and enhance mass transfer processes like curing and brining (McDonnell et al. 2014; Gudmundsson and Hafsteinsson 2001). Further, the moderate temperature rise (5–30°C) observed during PEF processing due to mild ohmic heating (Lindgren et al. 2002) could affect the meat quality (O'Dowd et al. 2013) and the combination of the two could have a thermo-electric effect on the muscle cell membranes (Ortega-Rivas 2011) and consequently on meat quality attributes. While studying the effect of PEF processing on volatile profile and sensory attributes of cooked lamb meats, Ma et al. (2016) reported that PEF treatment affected the temporal flavour profiles of meaty and oxidized flavour attributes. All PEF treated samples were associated with browned, juicy, livery, and meaty flavour attributes. Meaty, roast beef, juicy, browned, fatty, and salty are some of the terms associated with "positive" attributes (Ma et al. 2016). The following sections of the review will discuss the possibilities and opportunities that PEF can provide to the meat industry.

4.1. Meat tenderness

Given that the tenderness of meat largely depends on the overall integrity of muscle cells (Hughes et al. 2014), PEF processing, which has the potential to enhance cell disruption, presents an environment friendly and energy efficient tenderization technique that could be applied to muscles for cost effective alterations to the muscle cell structure (Toepfl et al. 2006). Unlike some other methods of tenderization (Bekhit et al. 2014a; Bekhit et al. 2014b), PEF processing does not cause side effects like severe structural and oxidative changes, and off-flavour development. Further, it does not generate environmental hazards and there is no evidence of toxicity (Pal 2017; Kumar et al. 2015). It is a well-established low-cost operational method for irreversible permeabilization of cell membranes without a significant rise in temperature within certain processing conditions (Toepfl et al. 2006). Unlike other technologies like electrical stimulation or Tenderstretch™, which do not affect all muscles equally, PEF is a stand-alone technology that can be applied to different muscles either pre-rigor or post-rigor (Suwandy et al. 2015a). This technology could be used to upgrade the less tender meat cuts by optimizing the technological inputs to different meat cuts and thereby optimizing the product quality (Bekhit et al. 2016).

4.1.1. Mechanism of action for PEF on meat texture

Given the fact that electroporation in animal tissues, such as meat, could be achieved by using electric field strengths of 1–10 kV/cm and energy input of 0.5–10 kJ/kg (Toepfl et al. 2014a), PEF treatment of muscles before aging could help in the tenderization process through early activation of calpains by release of calcium ions from cell organelles due to enhanced membrane permeability. This probable mechanism is further supported by evidence that a post-mortem aging period is required for gaining the tenderization benefit of PEF through increased proteolysis (Warner et al. 2017; Bekhit et al. 2016; Suwandy et al. 2015a, b, c; Bekhit et al. 2014c). However, other factors like release of cathepsins from lysosomes, accelerated glycolysis due to calcium release (pre-rigor muscles) and physical disruption of muscles may contribute.

4.1.2. Effect of PEF on post-rigor (cold-boned) muscles

In general, it appears that PEF has an influence on the tenderization process of meat during aging as several workers have reported a positive effect of the technology on the tenderization process (Ma et al. 2016; Suwandy et al. 2015a; 2015c; 2015d; Faridnia et al. 2015, Bekhit et al. 2014c), however, there is no agreement in the literature on whether it significantly enhances tenderness. The potential of PEF to improve meat tenderization in cold-boned muscles seems to depend on several factors including electric field strength, muscles under study, and the use of an aging period. Although, it is independent of initial muscle pH and fibre direction (orientation in the field) (Suwandy et al. 2015c), there appears to be a minimum electric field intensity above which PEF induces an improvement in the tenderization process in each muscle during a proper aging period.

No significant effect of PEF treatment was observed by O'Dowd et al. (2013) on beef tenderization process, however, Bekhit et al. (2014b) attributed these results to the absence of an aging period. Likewise, Arroyo et al. (2015b) also observed no significant gain of the PEF processing on the tenderness following a short aging period of 2 days. Although, both O'Dowd et al. (2013) and Arroyo et al. (2015b) used PEF treatments of high electric field intensity [(1.9 kV/cm, 65 Hz, 20 μs); (7.5, 10, 12.5 kV for fresh meat; 14, 20, 25 kV for frozen meat; 10, 55, 110 Hz), respectively], absence of a proper aging period following the PEF treatment could be the possible reason for the non-significant response, since it is generally agreed that meat tenderness is a biochemical process (Koohmaraie and Geesink 2006). Physical disruption may also be a contributing factor since mechanical tenderization is a well-documented technology to improve meat tenderness (Bekhit et al. 2014a), however, the amount of energy required is enormous. PEF is mostly believed to mediate tenderness through proteolysis (Suwandy et al. 2015a, b) which is not an instantaneous process and requires time (aging) to achieve improvement in tenderness. This may be supported by the finding of O'Dowd et al. (2013) who observed some additional myofibril breakdown in the PEF treated samples that resulted in smaller particle sizes as determined by laser diffraction particle size analysis. Although, no significant improvement was observed in tenderness by O'Dowd et al. (2013) and Arroyo et al. (2015b), it is worth mentioning that both these studies had observed a tendency towards reducing toughness for PEF treated samples. Where no significant effects were observed on the tenderization in the above studies, a significant improvement was observed in tenderness of cold-boned beef *Biceps femoris* treated with PEF of strength of 1.7–2.0 kV/cm, 50 Hz, 185 kJ/kg followed by an aging period of 21 days at 4°C (Faridnia et al. 2016).

The importance of a post-treatment aging period for PEF-induced tenderness was, however, rejected by a study which included an aging period of 26 days and no significant improvement was observed in the tenderness of cold-boned beef *Longissimus thoracis et lumborum* subjected to PEF treatment (Arroyo et al. 2015a). Similarly, Faridnia et al. (2014) reported no effect of PEF on the tenderness of beef *Longissimus thoracis* followed by vacuum aging. Unlike other studies, which have reported a significant effect of PEF on tenderness, one thing common among these two studies was the use of low intensity PEF. Faridnia et al. (2014) used PEF treatment of 0.2 to 0.6 kV/cm, 1–50 Hz, 20 μs for the cold-boned beef *Longissimus thoracis* and Arroyo et al. (2015a) used PEF treatment of 1.4 kV/cm, 10 Hz, 20 μs for the *Longissimus thoracis et lumborum* muscle. Although, no significant improvement was observed in tenderness by Arroyo et al. (2015a) and Faridnia et al. (2014), both these studies reported a tendency towards reducing toughness for PEF treated samples. Further, these studies have suggested that the PEF treatment used may not have been strong enough to induce physical disruption of muscle fibres, suggesting physical disruption may play an important role in the tenderization process. There is also, however, a possibility that low intensity PEF used in these studies may not have induced an irreversible membrane permeabilization required to cause the release of calcium ions and enzymes from the cell organelles and result in the significant tenderization observed in other studies. Thus, treatment intensity as well as aging period appears to play an important role in determining the effect of PEF on tenderization.

The conflicting results of different studies regarding the tenderization effect of PEF were further explained by Bekhit et al. (2017) on the grounds of sample size, origin, statistical validity and inaccurate design and reporting of studies. Variations and contradictions in the results obtained in some of the studies were attributed to various reasons like use of insufficient number of experimental units or small size of meat samples and use of meat of unknown background. Due to inevitable variation in tenderness observed within groups of animals raised together (Devine et al. 2006), use of statistically valid and sufficient number of experimental units (animals or carcasses) is required to generate meaningful results. Further, variation in tenderness among and within the muscles makes it necessary to include blocking and randomization in the experiment design to avoid the effects of carcass sides and location within muscles. Small size samples, used in some studies, makes it impossible to involve blocking and randomization in the experiment design. Studies involving use of samples of unknown origin makes it difficult to understand and replicate the work and ignores the biochemical background of the materials used (Bekhit et al. 2017).

While PEF of low electric field intensity has mostly failed to produce significant effects on tenderization, PEF with similar electric field intensity has generated some significant and interesting results in other studies. Several studies have been conducted on evaluating the effect of PEF on cold-boned beef *Semimembranosus* and *Longissimus lumborum* muscles using electric field intensity of 5, 10 kV (equivalent to 0.625 and 1.25 kV/cm) at 20, 50, or 90 Hz. Both Suwandy et al. (2015a) and Bekhit et al. (2014c) reported a decrease of 19% in the shear force of *Semimembranosus*. This decrease in the shear force of *Semimembranosus* was observed to be dependent on the PEF frequency (20, 50, or 90 Hz) and showed a decreasing trend with increasing level of frequency (4.1, 10.4 and 19.1% reduction at 20, 50 and 90 Hz, respectively). Yet another study (Suwandy et al. 2015d) showed no significant effect of repeated (1 ×, 2 ×, 3 ×) PEF treatment (10 kV, 90 Hz, 20 μs) on the shear force of beef *Semimembranosus*. The *Longissimus lumborum* muscle appeared to behave differently with PEF treatment but like *Semimembranosus* showed a decrease in shear force with PEF treatment by up to 19.0% (Suwandy et al. 2015a). Bekhit et al. (2014c) also reported a similar decrease in shear force in *Longissimus* by up to 19.5%, however, unlike *Semimembranosus* the decline in shear force was observed to be independent of frequency (20, 50, or 90 Hz) of the PEF treatment (Suwandy et al. 2015a; Bekhit et al. 2014c). A significant effect of repeated (1 ×, 2 ×, 3 ×) PEF treatment (10 kV, 90 Hz, 20 μs) was also observed on the shear force of beef *Longissimus lumborum* which showed a decrease of 2.5 N with every extra application of PEF treatment (Suwandy et al. 2015d). The differences in the behaviour of two muscles with PEF treatment may be attributed to the anatomical and physiological differences between the two muscles which influence the factors like heat generation, conductivity, fibre-type composition and membrane properties.

No significant effect of PEF was observed by Khan et al. (2017a) in cold-boned beef loins (*M. Longissimus et lumborum*) treated with high intensity of 2.5 kV, 200 Hz (LPEF) and 10 kV, 200 Hz (HPEF) at 1 and 14 days of aging who rather observed a toughening effect of the PEF as the shear-force of the muscles increased with PEF treatment compared to untreated control. They suggested that this toughening, which was generally observed more in HPEF treated samples than LPEF samples, was due to the denaturation of the proteins and enzymes involved in the tenderization process by the heat produced due to the Joule effect (Ohmic heating) which supports the enzymatic nature of the PEF induced tenderization. A moderate rise in temperature (5–30°C) has already been reported during PEF processing due to mild ohmic heating (Lindgren et al. 2002). Further, high intensity PEF treatments have also been reported to produce heat and increase the temperature of beef muscles (Khan et al. 2017a; Bekhit et al. 2014c; Suwandy et al. 2015c; 2015d).

4.1.3. Effect of PEF on pre-rigor (hot-boned) muscles

PEF could be of importance in improving the quality of hot-boned muscles that are separated from the hot carcasses in pre-rigor state and are generally less tender than cold-boned muscles (Troy and Kerry 2010; White et al. 2006). Hot-boning offers several benefits for the meat industry, such as less space required, less handling of carcasses and faster production. Hot-boned muscles could be treated separately with different process parameters to obtain optimal results (Bekhit et al. 2016). Two recent studies (Bekhit et al. 2016; Suwandy et al. 2015b) were reported on the effect of PEF treatment on the tenderization of pre-rigor beef muscles during aging. Both used beef topsides (*M. Semimembranosus*) and beef loins (*M. Longissimus lumborum*) as the study model and evaluated the effect of different electric field intensities (5, 10 kV at 20, 50, or 90 Hz) and repeated (1 ×, 2 ×, 3 ×) PEF treatment (10 kV, 90 Hz, 20 μs) on the tenderization during aging. The PEF treatments (5, 10 kV at 20, 50, or 90 Hz) affected the tenderness of hot-boned beef topsides with up to 21.6% reduction observed in the average shear force. The reduction in shear force was independent of the treatment intensity, though the shear force tended to decrease with increasing frequency (20, 50, or 90 Hz) of the PEF treatment. The same pattern was observed with the repeated PEF treatment as the shear force kept on decreasing with repeated PEF treatment (10 kV, 90 Hz, 20 μs) and the lowest shear force was observed with 3 × PEF treatment at 3 days post-treatment time, however, this beneficial effect was reported to disappear with longer aging periods. A negative effect of the PEF treatments (5, 10 kV at 20, 50, or 90 Hz) was reported on the tenderness of hot-boned loins as average shear force tended to increase, indicating increased toughness of muscle with an increase in the PEF frequency (20, 50, or 90 Hz). Further, repeated 3 × PEF treatment was also observed to reduce the tenderness, although no effect was seen on the tenderization with 1 × and 2 × PEF treatments. This result was different from what was reported for cold boned loins where an improvement in tenderness was observed regardless of the treatment intensity (Bekhit et al. 2014c). This suggests that the effect of PEF treatment will also depend on the post-mortem status of the muscle. The differences in the tenderization behaviour of these two muscles with PEF treatment may be attributed to the differential effect of PEF on the water holding capacity of these muscles (Bekhit et al. 2016; Suwandy et al. 2015b).

These results demonstrated that the tenderizing effects of PEF will be dependent on the biochemistry of the treated meat as evident by the muscle type and rigor time. Thus, complete profiling for PEF effects on various muscles will be required. Furthermore, these results suggest that meat cuts composed of various muscles will be affected differently; therefore, application of the technology to small animals such as lamb may not be practical.

4.1.4. Proteolysis of structural proteins

Troponin-T and desmin are important structural proteins of the muscle and their post-mortem degradation has been associated with the loss of myofibrillar integrity (Sun et al. 2014; Kitamura et al. 2005; Geesink et al. 2000; Ho et al. 1996). Their proteolysis (Han et al. 2009; Kitamura et al. 2005; Claeys et al. 2004; Geesink et al. 2000; Ho et al. 1996; Ho et al. 1994) has been significantly correlated to shear force values (Marino et al. 2013). Therefore, both troponin-T and desmin have been used as markers of myofibrillar protein degradation and meat tenderization (Sun et al. 2014; Wheeler and Koohmaraie 1999; O'Halloran et al. 1997; Koohmaraie and Shackelford 1991). Several studies have reported changes in the proteolysis patterns of troponin-T and desmin in pre-rigor and post-rigor beef muscles treated with PEF during aging (Bekhit et al. 2016; Suwandy et al. 2015a; b; c; d). Table 3 shows findings of different studies on effect of PEF on proteolysis of different muscles.

Suwandy et al. (2015a) studied the effect of PEF treatments (5, 10 kV at 20, 50, or 90 Hz) on the shear force and proteolysis of cold-boned beef *Longissimus lumborum* muscle for an aging period of 21 days. Where a significant reduction was observed in the shear force of the muscle (up to 19%) unaffected by the treatment intensity, a significant proteolysis of troponin-T was observed in 5 kV–90 Hz and 10 kV–20 Hz treated samples at day 3 and day 7 post-treatment in addition to 10 kV–50 Hz in subsequent post-treatment times (day 14 and 21). The desmin degradation also followed a similar pattern with higher proteolysis observed in 5 kV–50 Hz and 10 kV–20 Hz treated samples at day 3 and day 7 post-treatment. Increased degradation of desmin was observed in all PEF treated samples, except 10 kV–90 Hz treatment, at 14 and 21 days. Collectively, these degradation patterns of troponin-T and desmin provided evidence for improved proteolysis in PEF treated meat, both early post-mortem and during subsequent post-mortem storage. These proteolysis results agreed with the shear force results of the muscle and support the enzymatic basis of PEF induced tenderization. However, the tenderization effect observed in PEF 10 kV–90 Hz treated samples indicates some other mechanism, like physical disruption, also operates.

In a similar study, Suwandy et al. (2015b) studied the effect of PEF treatments (5, 10 kV at 20, 50, or 90 Hz) on the proteolysis patterns of hot-boned beef *Longissimus lumborum* muscle. While shear force tended to increase with treatment frequency, a significant proteolysis of troponin-T was observed in 5 kV–20 Hz and 10 kV–20 Hz treated samples at 3, 7, and 14 days post-treatment with prominent bands on day 21. Degradation of troponin-T was observed to be higher in all PEF treated samples compared to control on all day's post-treatment except in 5 kV–50 Hz treated sample on day 7 and 14. The desmin degradation showed a similar pattern with most prominent proteolysis in 5 kV–20 Hz and 10 kV–20 Hz treated samples at 3, 14 and 21 days post-treatment. Desmin proteolysis was also prominent in 10 kV–90 Hz treated sample at 3 days post-treatment. Increased degradation of desmin was observed in all PEF treated samples compared to controls at 7 and 21 days post-treatment except 5 kV–90 Hz sample at day 7. The difference in the degradation pattern of cold-boned and hot-boned beef *Longissimus lumborum* muscle was suggested to be due to the higher final temperature of the hot-boned muscles post-treatment (Suwandy et al. 2015b).

The effect of repeated (1 ×, 2 × or 3 ×) PEF treatment (10 kV, 90 Hz, 20 μs) on the proteolysis of cold-boned beef *Longissimus lumborum* muscle has also been evaluated (Suwandy et al. 2015d). While shear force of the muscle was found to decrease by 2.5 N with every extra application, increased proteolysis of desmin and troponin T was reported only in muscles subjected to 1 × PEF treatment. Less degradation of desmin and troponin T observed with increasing number of PEF treatments suggest another operational mechanism, like physical disruption, for PEF induced tenderisation in beef. In a similar study evaluating the effect of repeated (1 ×, 2 × or 3 ×) PEF treatment (10 kV, 90 Hz, 20 μs) on the proteolysis of hot-boned beef *Longissimus lumborum* (Bekhit et al. 2016), increased proteolysis was observed in muscles subjected to 1 × PEF treatment in terms of troponin T degradation and the pattern observed was 1 × PEF samples > 2 × PEF samples > untreated samples > 3 × PEF samples. Increased proteolysis was observed in 1 × PEF treated samples over the entire 21 days of aging period compared to control samples whereas 2 × and 3 × PEF treated samples showed less degradation than the control. Since 3 × PEF treatment was reported to produce toughest hot-boned meat and 1 × and 2 × PEF treatments didn't affect the tenderization in *Longissimus lumborum*, therefore this proteolysis pattern supported the suggestion that high intensity PEF treatment produced unfavourable conditions for

Table 3. Findings of different studies elucidating the effect of pulsed electric field on proteolysis of different muscles.

Authors	Muscle Studied	Muscle Status	PEF Treatment	Findings
Suwandy et al. (2015b)	Beef Longissimus lumborum	Pre-rigor	5 kV, 10 kV × 20, 50, 90 Hz	Significant proteolysis of Troponin-T and Desmin, Prominent proteolysis was observed in 5 kV-20 Hz and 10 kV-20 Hz samples
Bekhit et al. (2016)	Beef Longissimus lumborum	Pre-rigor	Repeated (1 ×, 2 ×, 3 ×) PEF treatment (10 kV, 90 Hz, 20 μs)	Increased proteolysis of Troponin T in 1 × PEF treatment, Decreased proteolysis of Troponin T in 2 × and 3 × PEF samples
Suwandy et al. (2015a)	Beef Longissimus lumborum	Post-rigor	5 kV, 10 kV × 20, 50, 90 Hz	Increased proteolysis of Troponin-T and Desmin both early and subsequent post-mortem, Prominent proteolysis was observed in 5 kV-90 Hz, 10 kV-20 Hz and 10 kV-50 Hz samples
Suwandy et al. (2015d)	Beef Longissimus lumborum	Post-rigor	Repeated (1 ×, 2 ×, 3 ×) PEF treatment (10 kV, 90 Hz, 20 μs)	Increased proteolysis of Troponin T and Desmin in 1 × PEF treatment, Decreased proteolysis of Troponin T and Desmin in 2 × and 3 × PEF samples
Suwandy et al. (2015c)	Beef Longissimus lumborum	Post-rigor	10 kV, 90 Hz, 20 μs	Increased proteolysis of Troponin-T and Desmin in PEF treated samples, More increase in proteolysis in low-pH (5.5–5.8) than high-pH (>6.1) samples

post-mortem proteolysis, likely by inactivation of proteases as a result of heat generated at high treatment intensity.

The effect of initial pH and meat structural arrangement (fibre orientation) on the proteolysis pattern of PEF treated (10 kV, 90 Hz, 20 μs) cold-boned beef *Longissimus lumborum* muscle has also been studied (Suwandy et al. 2015c). Although, no significant change was observed in the shear force of PEF treated samples in comparison to untreated samples, increased proteolysis of troponin-T and desmin was observed in the PEF treated samples. Increased degradation of troponin-T and desmin was also observed in the low-pH muscle samples (5.5–5.8) in comparison to the high-pH (>6.1) samples which agreed with the results of the tenderness measurements as the low-pH muscles tended to have lower shear force. No effect of fibre orientation was reported on the proteolysis of PEF treated muscle samples.

Based on the available literature about the various aspects of meat tenderness, it is evident that PEF has a potential to influence the tenderization process of muscle and could be utilized as a post-mortem intervention to maximize the tenderness gain. However, a great deal of research is required before it becomes a commercial reality in the meat industry.

4.2. Meat safety

At high electric field strengths (>20 kV/cm), PEF has been shown to be lethal to many spoilage and pathogenic bacteria at or near atmospheric temperature (Zhao et al. 2013; Haughton et al. 2012; Moritz et al. 2012; Rodríguez-González et al. 2011) and can be used as an alternative to conventional thermal pasteurization processes to inactivate food microbes and quality related enzymes while retaining the nutritional, sensory and health-promoting characteristics of the products (Sánchez-Vega et al. 2014). Sufficient microbial inactivation depends on the properties of food matrices and intensity of the pulses in terms of energy, field strength and number of pulses applied on the bacterial strain under study (Toepfl et al. 2007a). It is one of the most validated non-thermal food preservation techniques (Sanz-Puig et al. 2016) and is mostly used as an alternative to thermal pasteurization for liquid and semi-solid stuffs like milk, egg liquid, juices and potato dextrose agar (Zhao et al. 2013, Haughton et al. 2012; Zhang et al. 1994). Treatment at 25–40 kV/cm has been reported to reduce 3–6 logs of pathogenic and spoilage bacteria and extend the shelf-life to 3–4 weeks for milk and 5–8 weeks for milk beverages under refrigerated storage (Zhao et al. 2012).

PEF causes inactivation of enzymes as well as destruction of spoilage and pathogenic microorganisms through formation of hydrophilic pores as well as the forced opening of protein channels in the membrane (Buckow et al. 2014; Sharma et al. 2014). The preservative potential of the technology has been studied under commercial conditions on the microorganisms of public health importance, such as *Salmonella typhimurium, Staphylococcus aureus, Escherichia coli* and *Listeria monocytogenes*, achieving log reductions of 2.0–4.2, 0.5–4.0, 1.5–3.3, and 0.6–1.5, respectively, depending on processing conditions and food type (Saldaña et al. 2014). Due to their low conductivity and high protein-fat content, PEF seems to have limited applicability on solid foods like meat and meat products. PEF was reported to be ineffective at controlling *E. coli* O157:H7 in beef burgers or on beef trimmings (Bolton et al. 2002), however, application of PEF resulted in 2-log reduction of *E. coli* K12 suspended in a meat injection solution (Rojas et al. 2007) although, high electric field strength of ≥7 kV/cm was required which resulted in arcing. A reduction of 8-log in *E. coli* O157:H7 was observed on goat meat immersed in a brine solution using a pulsed DC square wave electric signal for a treatment time of 32 minutes (Saif et al. 2006). Test meat pieces of 25 × 25 × 30 mm sizes weighing approximately 20.0 ± 2.0 g were cut from thawed goat meat (frozen at −20°C for up to 4 weeks) inoculated with *Escherichia coli* O157:H7 on the surface and covered with a thin film of 0.15 M sodium chloride solution. Stachelska et al. (2012) investigated the efficacy of PEF for inactivation of *Y. enterocolitica* (6.7 $\log_{10}$ CFU/g of meat) in minced beef meat using pulse frequency of 28 to 2800 MHz and electric field strength of 300 V/m. PEF treatment with the pulse frequency of 28 MHz was reported to be ineffective for inactivating *Y. Enterocolitica* in beef samples; however, the pulse frequency of 280 MHz was effective for inactivating *Y. Enterocolitica* in beef samples stored at −20°C for 30 days. PEF treatment with 2800 MHz pulse frequency was highly effective in controlling the bacteria in meat stored both at +4°C and at −20°C. The authors concluded that PEF is a safe and effective method of meat decontamination and can be successfully carried out on frozen meat for enhancing the meat safety. Recently, a two log-unit increase in aerobic microbial counts was reported by Faridnia et al. (2015) during log phase of frozen-thawed PEF-treated beef samples in comparison to untreated control. Significantly higher microbial counts for the treated samples were explained by the increased purge loss observed in these samples. Haughton et al. (2012) studied the efficacy of PEF to inactivate a range of microorganisms (*Campylobacter isolates, Escherichia coli and Salmonella enteritidis*) on raw chicken meat and in liquid media. No significant reductions were observed in total viable counts of *Enterobacteriaceae, C. jejuni, E. coli* or *S. Enteritidis* in inoculated samples of raw chicken treated with PEF (3.75 and 15 kV/cm, 5 Hz, 10 ms). Isolates of *Campylobacter* in liquid were susceptible to PEF treatment (65 kV/cm, 500 Hz, 5 ms) with reductions of between 4.33 and 7.22 $\log_{10}$ CFU/mL. The authors concluded that PEF technology may have potential to reduce contamination of process water; however, it is not suitable as an intervention measure for food safety for the control of microbial contaminants on broilers during processing. To achieve better results in terms of food safety and quality, novel combinations of PEF with other hurdle technologies were recently proposed which included the addition of antimicrobial agents (Bermúdez-Aguirre et al. 2012; Smith et al. 2002) and other emerging physical hurdles such as high intensity light pulses and manothermosonication (Palgan et al. 2012). Clearly, PEF treatment of intact meat cuts is not causing significant impact on microorganism's load and better effects can be found in meat immersed or suspended in solutions, therefore, the technology is unlikely to be useful for the meat industry to be practical for improving the safety of fresh meat cuts.

4.3. Supercooling

Defined as the process of lowering the temperature below the usual freezing point of a product without the formation of ice crystals, supercooling is a metastable state of water (Stonehouse and Evans 2015) that could be utilized to prevent the quality loss in meat due to the formation of ice crystals during freezing. Supercooled meat products could be processed or consumed at freezing storage conditions without quality deterioration by ice crystallization. Recently, a combination of electric field and magnetic field treatments was reported to affect the mobility of water molecules (Mok et al. 2015; Wowk 2012) and the combination of both these technologies was explored to achieve an extension of a supercooled state in chicken breast (Mok et al. 2017). PEF, a promising technology to improve the freezing and drying processes (Carbonell-Capellaet et al. 2016; Puertolas and Barba 2016; Koubaa et al. 2015), was applied in combination with oscillating magnetic field to vibrate water molecules and inhibit sudden ice nucleation in chicken breast (Mok et al. 2017). Temperature of chicken breast samples decreased to $-6.5°C$ in a supercooling state during the whole testing period of 12 hours at the freezer temperature of $-7°C$ (± 0.5). The samples were also analysed for various quality parameters like microstructure, drip loss, colour, texture, pH and lipid oxidation to evaluate the impact of supercooling on the quality of meat. PEF-assisted supercooling was reported to be highly effective in maintaining the original meat quality without significant physical damage or chemical changes.

4.4. Processed meats

4.4.1. Accelerated diffusion processes

Cell membranes play an important role in the curing and brining process of the cured meats as they resist the free diffusion and equalisation of salt (NaCl) in the muscle tissue (Janositz et al. 2011). PEF is reported to enhance mass transfer processes because of its membrane permeabilization potential (Toepfl et al. 2014a; Siemer et al. 2012) and this property finds it an application in the meat industry in the curing process. Several techniques have been used to accelerate the curing process and reduce processing time but have not considered the resistance offered by the membranes. Toepfl and Heinz (2007a) were first to show the potential of PEF in the brine acceleration process when they observed that prior treatment with PEF (3 kV/cm) could improve the diffusion of salt and nitrite in pork. Recently, McDonnell et al. (2014) confirmed the potential of PEF in accelerating the brining process in pork. PEF treatments of varying energy densities (22.6–181.1 kJ/kg) were used as pre-treatments and evaluated for rate of saline diffusion. Two treatments viz. 1.2 kV/cm 100 Hz for 300 pulses and 2.3 kV/cm 100 Hz for 300 pulses were reported to increase the salt content (NaCl) significantly in comparison to control. These findings confirm the potential of PEF in accelerating the salting process and reducing the curing time. Although, the exact mechanism of how PEF improved the salt diffusion process was not deciphered, it was suggested that PEF could fragment the myofibrils (O'Dowd et al. 2013) and create gaps within the muscle structure (Gudmundsson and Hafsteinsson 2001) which could aid in the diffusion process (McDonnell et al. 2014). A similar mechanism was suggested by Gudmundsson and Hafsteinsson (2001) while studying the effects of PEF on the texture of salmon, demonstrating that gaps in the microstructure of salmon caused collagen leak into extracellular space. PEF has been reported to cause a porous structure in ham that holds brine through capillary forces (Klonowski et al. 2006). Faridnia et al. (2016) reported a dramatic increase in the number of ruptured myofibrils along the Z-lines in PEF treated beef samples which resulted in a muscle with a more porous structure that accounted for the observed increase in electrical conductivity and purge loss. PEF has also been reported to accelerate the fermentation process of raw sausages (salami type) by improving the availability of intracellular liquid for bacterial cultures in minced beef (Raso and Heinz 2006).

Due to its potential to accelerate diffusion processes, one of the positive effects of PEF treatment is the reduction of the processing time during dry ageing of meat through increased water movement. It has been reported to facilitate a faster rate of moisture loss and accelerate the dry ageing process in venison without any detrimental effect on the total weight loss (Mungure et al. 2017).

4.4.2. Restructured meat products

PEF has been reported to enhance the recovery of compounds, food additives and nutraceuticals from different matrices by electrically piercing the cell membrane which loses its semi-permeability temporarily or permanently under its influence (Rodríguez-Roque et al. 2015; Deng et al. 2014; Barba et al. 2015; Abenoza et al. 2013). The semi-permeable membrane which separates the cell cytoplasm from its surroundings is considered as the main barrier for mass transport processes aiming for diffusion of soluble products out of the food tissues. The techniques, like PEF, which cause the physical disintegration of the membranes facilitate diffusion and improve mass transport processes (Toepfl et al. 2014a). The permeabilization of cellular tissue has been reported to improve all mass transfer processes, such as distillation or extraction (Siemer et al. 2012). PEF has been investigated as a cold process (non-thermal) for extraction of intracellular compounds (Barba et al. 2012; Gachovska et al. 2010) and has recently been reported to be very effective for improving the extraction from borage leaves (Segovia et al. 2015), sugar beet (Lebovka et al. 2007; Vorobiev and Lebovka 2008) and betalains (red purple pigments) from red beets (Loginova 2011). It has been proposed to be a promising future industrial application for extraction of soluble matter from chicory roots (Loginova et al. 2010). Given the fact that restructuring process involves extraction of proteins from the membrane bound muscle fibres, PEF could find an application in restructured meat products as pre-treatment of meat is expected to improve the release of myofibrillar and sarcoplasmic proteins and potential re-establishment of protein network.

4.4.3. Sodium reduction

Low sodium meat products are suitable for persons with high blood pressure disorders and help to improve the health of the population (Kunnath et al. 2015; Cardoso et al. 2014). Strategies to reduce salt in processed meat products have been especially focused around restructured meat products and many low sodium ingredient options have been successfully produced in restructured meat products like sausages (Inguglia et al. 2017). The structural functions of salt-soluble proteins in these products have been replaced by the addition of other ingredients like milk or soy proteins, starches and gums (Fellendorf et al. 2016). In addition, the use of the microbial enzyme transglutaminase, which improves the quality parameters like textural and emulsifying properties, heat stability, and gelation (Gaspar and De Góes-Favoni 2015; Moreno et al. 2010), also allows to reduce the salt content of restructured meat products (Cardoso et al. 2014).

One of the current approaches to reduce the sodium content of processed foods and meat products is by improving the salt diffusion by employing novel processing technologies like high pressure processing or ultrasound (Inguglia et al. 2017). A natural increase in the saltiness was reported in dry-cured pork loin treated with high pressure in a study due to a change in interaction between sodium ions and protein structures which may have caused a higher release of sodium to the taste receptors on the tongue (Clariana et al. 2011). Salt reduction using ultrasound is based on the understanding that it can modify cell membranes and affect the mass transfer processes like curing and brining. Use of ultrasound leads to a better distribution of salt during brining which delivers a higher salt perception even with lower overall salt content (Alarcon-Rojo et al. 2015). Thus, a rapid curing technology along with the increased salt gain rate could allow benefits like salt reduction (Tao and Sun 2015). No such attempt has been made using pulsed electric field which also has the potential to enhance mass transfer and has been recently reported to accelerate the curing process by improving the salt diffusion in the pork (McDonnell et al. 2014). The PEF processing of meat may result in a better salt distribution which could deliver a higher salt perception. Thus, PEF is expected to aid in protein extraction during restructuring process and may also help to reduce sodium content of the restructured meat products. Future research needs to focus on these unexplored areas.

4.5. Concerns

4.5.1. Digestibility of meat proteins

Meat proteins are susceptible to aggregation during high-temperature thermal processing which causes unfolding of the tightly coiled polypeptide chains and form large aggregates due to the formation of intermolecular cross-links (Santé-Lhoutellier et al. 2008; Morzel et al. 2006; Stadtman 1993). Accordingly, the use of non-thermal processing technologies, like pulsed electric field, for processing of meat is of interest. Unlike liquid foods (milk, wine, juices), severe PEF conditions are required to achieve sufficient microbial reduction in protein-fat based matrices, like meat, due to protective effects of food components like proteins and lipids (Monfort et al. 2011; Martín-Belloso et al. 1997). Use of high intensity electric fields, however, could disrupt the electric interactions of protein peptide chains (Park and Boxer 2002) which may initiate subsequent macroscopic changes such as interactions with other proteins through exposure of susceptible regions. PEF has been reported to induce partial unfolding of the proteins. This depends on the total energy input and on electric field strength and plays a key role in the subsequent intermolecular interactions that are responsible for formation of intermolecular cross-links and protein aggregates (Liu et al. 2017b).

Generally, meat proteins should be digested into small peptides by enzymes such as pepsin, trypsin and α-chymotrypsin which are further broken down into amino acids by enzymes like carboxypeptidases and absorbed in the intestine. However, the digestibility of PEF treated meat by digestive tract enzymes has not been elucidated. Further, PEF treatment has already been reported to reduce the availability of some protein-bound minerals in meat (Khan et al. 2017a) and increase the temperature of the muscles during processing (Bekhit et al. 2014c; O'Dowd et al. 2013), indicating potential denaturation at high treatment intensities. Studies have confirmed that PEF treatment can cause denaturation and aggregation of egg proteins and both pulse intensity and energy input are decisive factors in determining PEF-induced denaturation and aggregation (Liu et al. 2017b; Wua et al. 2014; Zhao et al. 2009). Wua et al. (2014) observed partial protein unfolding in PEF treated egg proteins and reported insoluble aggregates resulting from covalent and non-covalent binding between heterogeneous proteins. PEF is known to cause the inactivation of endogenous food enzymes (Li et al. 2008; Riener et al. 2008) and protein denaturation and aggregation in soybean protein isolates (Li et al. 2007). Sharma et al. (2016) also reported an increase in surface hydrophobicity of milk proteins with increase in intensity of PEF treatment. Thus, PEF may induce intermolecular cross-links and aggregation of meat proteins. This intermolecular cross-linking and aggregation of meat proteins can reduce their degradation by digestive enzymes (Gatellier and

Santé-Lhoutellier 2009; Santé-Lhoutellier et al. 2008; Santé-Lhoutellier et al. 2007) as digestive process largely depends on the structure and physicochemical state of proteins (Kaur et al. 2014; Kong and Singh 2008). No information is available in the literature about the effect of PEF processing on the digestibility of meat proteins and needs immediate scientific attention.

4.5.2. Mineral content

Given the fact that PEF causes electroporation in meat and has potential to enhance cellular permeability and mass transfer processes, it could affect the mineral content of meat during processing and storage. Meat is generally regarded as an excellent source of minerals like iron, zinc and phosphorus and any change in the mineral content would be of commercial interest. Khan et al. (2017a) studied the effect of low (2.5 kV, 200 Hz and 20 μs) and high PEF (10 kV, 200 Hz and 20 μs) on the concentration of four nutritionally important minerals (Fe, Zn, K, P) of raw and cooked cold-boned beef M. *Longissimus et lumborum* at 1 and 14 days post-treatment. The concentration of K and P was significantly decreased by PEF treatment and cooking as well as by post-treatment aging period. The concentration of Fe was significantly decreased by PEF treatment only with more loss observed in high PEF than low PEF treated samples. The concentration of Zn was not affected by PEF treatment. These results suggest that low and high PEF treatments can lead to changes in beef muscles with different outcomes on quality. In addition to lowered levels of mineral (P, K and Fe) concentrations, high PEF has also been reported to negatively affect the shear force and colour stability of beef *Longissimus et lumborum* (Khan et al. 2017a). Thus, there is an optimal PEF treatment for beef cuts within a range of processing parameters. Khan et al. (2017b) studied the effect of low (2.5 kV, 200 Hz and 20 μs) and high PEF (10 kV, 200 Hz and 20 μs) on the mineral content of raw and cooked chicken breast muscles. No significant effect of PEF was reported on the mineral content of the muscles; however, a significant effect of cooking was observed as the concentration of minerals viz. P, K and Zn decreased upon cooking. The concentration of Fe was not affected by PEF treatment, storage or cooking. These results suggest that PEF technology has no effect on concentrations of P, K, Fe and Zn in chicken breast muscles and will not affect the nutritional value of these minerals.

Khan et al. (2018) studied the effect of low (2.5 kV, 200 Hz and 20 μs) and high PEF (10 kV, 200 Hz and 20 μs) on the levels of 40 macro- and micro-minerals in raw and cooked cold-boned beef loins at 1 and 14 days of post-treatment and in chicken breasts at 1 and 4 days. PEF treatment was reported to reduce the concentration of Ca (calcium), Na (sodium) and Mg (magnesium) and increase the concentration of Cr (chromium) in beef compared to control. Chicken breast treated with high PEF showed significantly higher Ni (nickel) concentration than control and samples treated with low PEF. This increase in Cr and Ni concentration of the meat is within the safety limits and there are no chances of toxicity (Meditext 2005; EPA 2016). Both high and low PEF treated samples had higher Cu (copper) concentrations than control samples. These results suggest a differential effect of PEF on mineral content according to the type of meat and the possible release of elements from the PEF electrodes to meat samples. Pataro et al. (2014) also studied the release of Fe, Cr, Ni and Mg from stainless steel electrodes in buffers and reported an increased metal release with an increase in total specific energy input and in the presence of halides in the treated material. The above studies document the migration of minerals from the electrodes of standard PEF systems to the treated food. The electrodes used in these systems were of high quality. However, higher levels of sample contamination may occur if PEF systems are made in-house or engineered by independent labs where the quality of the electrodes is unknown.

5. Limitations of PEF

Continuous PEF systems are considered as efficient, high-speed and low maintenance, however, high initial capital investments and cost involved in changing the plant design are viewed as highly prohibitive (Jeyamkondan et al. 1999). The reliability and cost effectiveness of the equipment are being constantly improved and the technology is heading for wider industrial application (Toepfl and Heinz 2007b). However, nonthermal processing technologies, like PEF and HPP, have higher environmental impact (in terms of CO_2 production) than traditional thermal pasteurization (Sampedro et al. 2014). While PEF is a novel non-thermal technology for production of microbiologically safe liquid foods under high-speed continuous process without affecting their sensorial and nutritional quality, it appears to be an ineffective technology for microbial decontamination of fresh meat. The high intensity PEF treatments required to inactivate the microbial load in meat have an adverse impact on sensorial and nutritional quality of meat. Further, heat produced during such intense treatments in batch mode could denature the endogenous proteolytic enzymes and affect the tenderization process during aging. This technology also fails to inactivate bacterial spores that prevents its use for food and water sterilization (Pillet et al. 2016) and the primary applications of this technology in food preservation are thus focused on pasteurization (Saldaña et al. 2014). A potential solution to this limitation may be the combined use of other hurdle technologies, such as natural preservatives (lactic acid or essential oils) or antimicrobial agents, to improve the safety of PEF treated fresh meat. Another limitation of PEF is its uneven treatment distribution in non-uniform and complex food matrices in continuous PEF systems. This will be particularly challenging under the factory settings where bone-in meat cuts with variations in fat content will provide a matrix of non-uniform tissue density resulting into unevenly treated product with variations in effective electropermeabilization. The non-homogenous distribution of the electric field was demonstrated by Golberg et al. (2015) who observed less than 40% of the PEF strength in the cells near vascular structures in a rat liver due to the formation of “electric fields sinks”. The implementation of PEF technology in meat industry requires guaranteeing that all parts of the meat cuts receive the established treatment during the process to achieve a permeabilization level that assures tenderization. Currently, all published reports available on application of PEF used almost homogeneous meat samples devoid of fat and bones. The available information can only be applicable to the lean muscles stated in the reported studies and therefore, a great deal of research is still needed before a commercial system becomes a reality. Moreover, different muscles and cuts in different species would need different PEF treatments for optimum results. Research is required to standardize the optimum treatment parameters for different cuts and muscles for different species.

6. Conclusions

PEF technology has provided more environmentally friendly and energy efficient options in diverse variety of foods with some possible options in the meat sector too. PEF appears to have some potential tenderization effect which may find a commercial application in future provided further research comes up with optimized technological inputs for different muscles, cuts and species. Loss or gain of minerals during PEF processing have no commercial significance and there is no scientific evidence regarding the toxic or hazardous effects of the technology. Meat safety, accelerated curing and brining, supercooling, drying and restructuring are some of the promising areas which can deliver PEF assisted options in future, however, these are still in their infancy and need a significant body of research before finding an application in the meat industry.

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