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TRENDS IN FOOD SCIENCE & TECHNOLOGY

Review

Effect of pulsed electric field processing on the functional properties of bovine milk

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Thermal pasteurization ensures safety and enhances the shelf-life of milk. Exposure to heat can modify labile milk components and alter the functional properties of milk proteins. This has driven the development of non-thermal food preservation techniques such as pulsed electric field (PEF) processing, primarily for the inactivation of spoilage microorganisms. Milk components, in particular fat and protein, affect the functionality, yield and quality of dairy products, requiring a clear understanding of the structural and chemical changes occurring due to PEF processing. This review critically discusses current knowledge of the impact of PEF treatment on the functional properties of milk, namely, the physicochemical changes of milk components, changes in technological properties, shelf-life, and sensory and nutritional properties.

Introduction

Milk fat is encapsulated within a milk fat globule membrane (MFGM), consisting of proteins, glycoproteins, polar lipids, phospholipids, enzymes, and cholesterol. This membrane provides emulsion stability and inhibits the coalescence of milk fat globules (MFGs), thus preventing triglyceride hydrolysis through native or bacterial lipases (Keenan & Mather, 2006; Keenan & Patton, 1995). The size of the MFGs, the fat content and membrane composition provide a vital function to preserve the texture, flavour and taste of cheese (Métais, Cambert, Riaublanc, & Mariette, 2006). Dairy products made from unpasteurized milk, such as cheese, have a different flavour profile to that of pasteurized milk products; however, primarily for public health reasons, and as norms and regulations demand, milk must be thermally pasteurized to reduce the number of pathogenic and spoilage microorganisms.

Treatments given to raw milk in dairy processing, despite the obvious benefit of ensuring safety and improving the quality of milk for human consumption, can induce changes to milk components. It has been reported that heating milk above 70 °C for 20 min and homogenisation of milk (Darling & Butcher, 1978; Dufour & Riaublanc, 1997), and ultrafiltration of milk all alter the size and structure of the MFGM, as well as cause the denaturation of whey proteins (Lopez & Dufour, 2001). Heat impacts upon chemical and physical reactions in milk, such as a drop in pH through a decrease in ionic Ca^{2+} (100 °C and above for 30 min), flavour changes due to formation of sulphydryl groups (above 73 °C for 30 s), the transfer of copper from the plasma phase to the MFGM, thus affecting autoxidation (72 °C for 15 s), denaturation of serum proteins (above 70 °C), a reduction of creaming by 25% (73 °C for 20 s), and degradation of labile vitamins (73 °C for 30 s) (Walstra, Wouters, & Geurts, 2006). Whey proteins, such as β-lactoglobulin and α-lactalbumin, are denatured upon heating at temperatures above 85 °C and associate themselves with the MFGM proteins through disulphide bonds (Corredig & Dalgleish, 1996; Kim & Jimenez-Flores, 1995; Ye, Anema, & Singh, 2004). Hillbrick, McMahon, and McManus (1999) established that ultra-high-temperature (UHT) treatment (140 °C for 4 s) induces binding of κ-casein and β-lactoglobulin to the surface of the MFGM. When whole milk is heated at higher temperatures (≥85 °C for 2 min), usually prior to homogenization, denatured serum proteins bind to κ-casein on the surface of the casein micelle as well as to the MFGM surface (Dalgleish & Banks, 1991).

Wiking, Bjorck, and Nielsen (2003) reported mechanical damage to MFGs under the shear and temperature conditions found during pumping of milk in a commercial

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processing environment. A fat globule size reduction increases the total emulsion surface area, hence alters the MFGM composition due to the adsorption of casein micelles and whey proteins onto the MFG surface (Ye *et al*., 2004). Heating (85 °C for 15 min) also induces change in the viscosity of raw milk, which is credited to the binding of denatured whey proteins to casein micelles (Jeurnink & de Kruif, 1993). Although these treatments are important for improving milk quality, changes in the functional properties of raw (unpasteurised) milk will occur. Therefore, concerns about the changes induced by heating of raw milk, and growing consumer trends for fresh-like quality foods, have inspired research on emerging non-thermal food preservation techniques.

Pulsed electric field (PEF) processing is a promising non-thermal technique which applies high voltage electric fields for a short duration (order of microseconds) to reduce the numbers of both spoilage and pathogenic microorganisms, thus providing consumers with safe, nutritious, and potentially fresh-like quality foods (Mertens & Knorr, 1992; Sale & Hamilton, 1967). Similar to the heat treatments discussed above, PEF treatment can also induce changes in milk quality while inactivating bacteria and spoilage enzymes. There is little published information available on PEF-induced changes that affect the functional properties of milk. This review article discusses the structural and functional changes of milk after PEF processing and focuses on further investigations necessary to have a better understanding of functional changes induced by PEF processing of milk.

An overview of PEF technology

The main components of typical PEF equipment are a high voltage pulse generation system, a treatment chamber assembly, and a pump for subjecting liquid food, such as milk, to enable continuous PEF treatment. Heat exchangers can be installed for pre-heating and cooling of the food before and after PEF treatment. Exponentially decaying pulses and square wave pulses (monopolar, bipolar) are the two most common wave shapes generated during PEF processing (Fig. 1A), which are normally monitored during PEF treatment using an oscilloscope. Bipolar square wave pulses are reported to be more lethal as these lead to a charge reversal across the cell membrane, hence inducing more cellular damage (Ho, Mittal, Cross, & Griffiths, 1995; Zhang, Monsalve-González, Qin, Barbosa-Cánovas, & Swanson, 1994). Barbosa-Cánovas, Góngora-Nieto, Pothakamury, and Swanson (1999) reported several developments in the design of PEF treatment chambers; however, parallel plates, coaxial and co-linear configurations are the most commonly used (Fig. 1B).

The effectiveness of PEF treatment at inactivating microorganisms depends on various factors such as process parameters (electric field intensity, number of pulses, pulse shape, frequency, and duration of pulse), product parameters (composition, conductivity, pH, and water activity), and microbial characteristics (type of microorganism, growth conditions, growth phase, and recovery conditions) (Wouters, Alvarez, & Raso, 2001). The potential of PEF treatment to inactivate microorganisms and enzymes in milk for improving quality has been extensively studied in the last decade (Bendicho, Barbosa-Cánovas & Martín, 2002; Mittal & Griffiths, 2005; Sampedro, Rodrigo, Martínez, Rodrigo, & Barbosa-Cánovas, 2005). PEF-induced microbial inactivation is hypothesized to be due to dielectrical breakdown and electroporation of the cell membrane (Fig. 1C), based on microbial and other physicochemical studies using phospholipid vesicles and planar bilayers model systems (Ho & Mittal, 2000; Pagán & Mañas, 2006; Toepfl, Heinz, & Knorr, 2005). Despite this, the mechanism of PEF-induced inactivation of enzymes is not clearly understood although some authors have suggested that it is due to the structural and configurational changes induced under the influence of high electric fields (Ho, Mittal, & Cross, 1997; Mañas & Vercet, 2006).

Functional properties of milk

It is well understood that milk components such as proteins and the MFGM are susceptible to changes after heat and mechanical treatments; however, little is known about PEF-induced effects. PEF technology can provide new opportunities for maintaining the integrity of milk components during processing because the treatment works at relatively moderate temperatures compared to conventional heating under pasteurisation conditions. Although there have been some reports of the consequences of PEF processing on the physicochemical properties of milk, there are relatively few studies of the effect on sensory and nutritional properties. A summary of these properties is provided in Table 1, including the processing details. Conflicting results in the literature on the impact of physicochemical properties are likely due to variations in the type of sample, such as whole milk, skim milk, or simulated milk ultrafiltrate, the type of PEF processing conditions, or the design of the PEF equipment.

Milk fat globules

MFGs are surrounded by a phospholipid membrane which provides emulsion stability and inhibits coalescence. A structure of the MFGM proposed by Lopez *et al*. (2011) is shown in Fig. 2. The MFGM is considered to be similar in composition to a biological cellular membrane (Danthine, Blecker, Paquot, Innocente, & Deroanne, 2000). As viable microorganism numbers are reduced during PEF processing due to electroporation of the cell membrane (Fig. 1C), changes to the MFGM are also expected. The electro-kinetic potential at the surface of the MFGM, measured as the zeta (ζ)-potential, has been widely used to evaluate the extent of change to the MFG system. Garcia-Amezquita, Primo-Mora, Barbosa-Cánovas, and Sepúlveda (2009) found similar ζ-potential values for raw and PEF-treated milk which indicates that

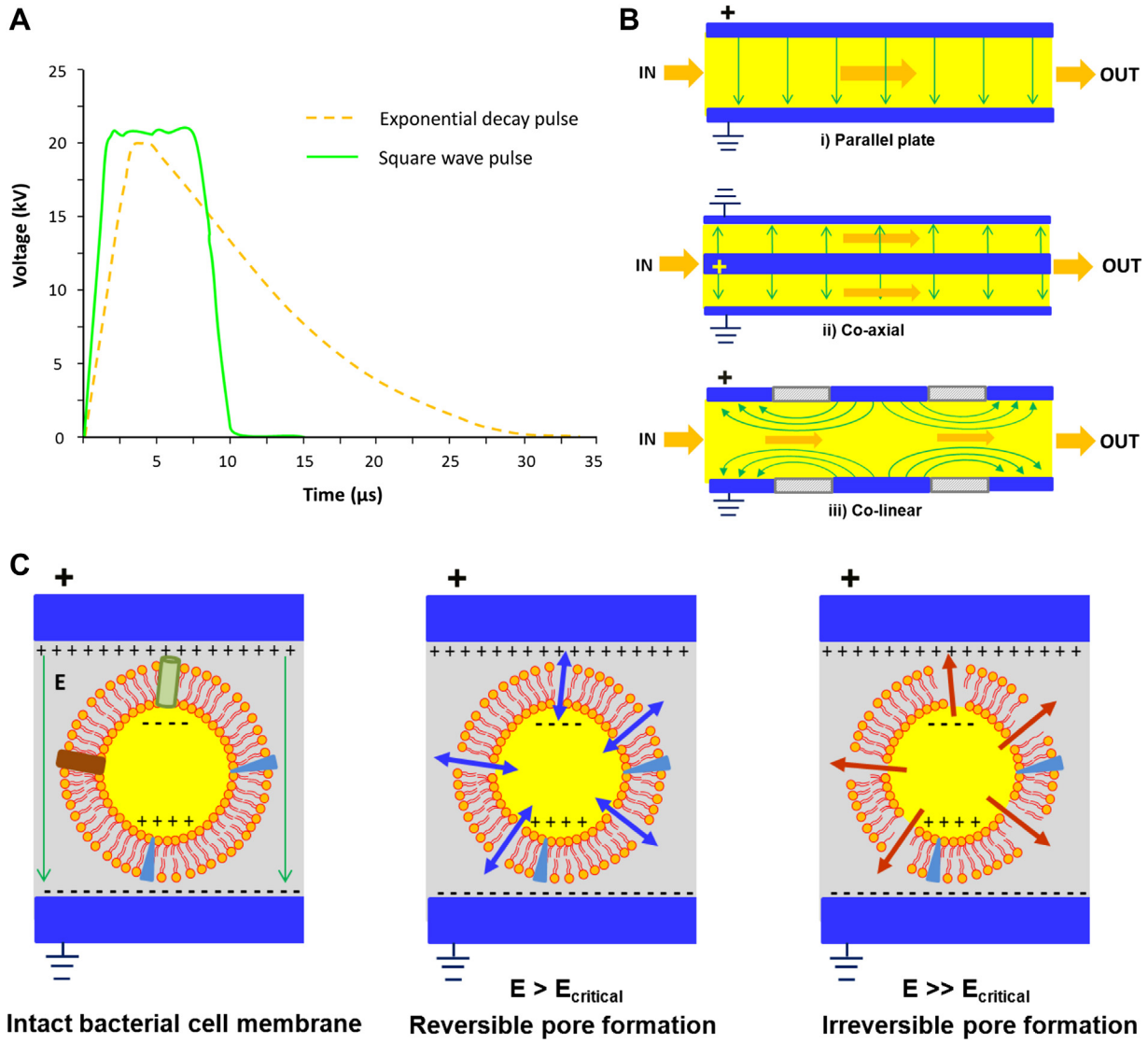

Fig. 1. Schematic depiction of, (A) the voltage patterns of exponential decay and square wave pulses, (B) configuration of treatment chambers for continuous PEF treatment, (C) mechanism of membrane permeabilisation by electro-compressive forces induced by an external electrical field. *E*: electric field intensity; Reversible: electrical breakdown when pores are smaller compared to membrane area; Irreversible: electrical breakdown when pores are large, causing mechanical destruction of the membrane leading to cell death. Adapted from (Toepfl *et al.*, 2005).

the average ratio of mass to surface electrical charge was unchanged, with no subsequent damage to the MFGM. Nevertheless, these authors also found that the MFG size distribution of PEF-treated (36 kV cm^{-1} and 42 kV cm^{-1}) milk was similar to heat-treated (63 °C for 30 min) milk, and an increase in electric field intensity or pulse number did not significantly ($P < 0.05$) affect the particle size. Similarly, the size measurements showed no significant ($P < 0.05$) difference between the average MFG size in PEF-treated skim milk compared to raw milk, regardless of the field intensities and temperatures applied (Shamsi, 2008).

In contrast, Xiang, Simpson, Ngadi, and Simpson (2011) reported an increase in the effective volume of the fat globule due to interaction with denatured skim milk serum proteins. Fat globule size in model solutions, such as β-lactoglobulin-stabilized oil-in-water emulsions (pH 7.1), and pasteurized skim and whole milk did not significantly change ($P > 0.01$) after PEF treatment at 29–36 kV cm^{-1} (Barsotti, Dumay, Mu, Diaz, & Cheftel, 2002). Under similar treatment conditions, these authors reported a slight decrease in the aggregates of PEF-treated liquid dairy cream; however, photon microscopic examination of PEF-treated cream did not reveal any changes in size. The emulsion stability index of PEF-treated cream also decreased slightly.

These studies on assessing changes to the MFGM in PEF-treated milk reveal apparently conflicting results which might be due to the use of different PEF equipment and measurement techniques. Most studies on PEF-induced changes to the MFG were carried out by examination of the

Table 1. Summary of functional properties of pulsed electric field (PEF)-treated products[a].

Treatment media	PEF processing conditions	Functional properties		References
		Unchanged	Changed	
Raw skim milk (2% fat)	E[b]: 40 kV cm^{-1}; 20 pulses; treatment time: 2 μs; T_i: <12 °C; T_p: <55 °C	Whey protein, taste and flavour did not change.	Shelf-life was 2 weeks	Qin *et al.* (1995)
Raw milk	E[b]: 20–80 kV cm^{-1}; 2 μs width pulses; treatment time: 1–10 μs; T_p: 55 °C	Fat & protein integrity, starter growth, rennet clotting, yield, calcium distribution & casein structure. No detrimental flavour degradation.		Dunn (1996)
Raw milk	E[c]: 21.5 kV cm^{-1}; 5 μF; 1–20 exponentially decaying pulses; T_p: 45–50 °C	No substantial change in whey protein and taste.	At high energy inputs (>200 kJ L^{-1}) ~90% destruction of vitamin C.	Grahl and Märkl (1996)
Raw milk	E[b]: 35 kV cm^{-1}; 30 exponentially decaying pulses of 3.3 Hz; flow rate: 600 mL min^{-1}; T_o: <30 °C	Adhesiveness & cohesiveness of cheese made from PEF-treated milk did not change.	Hardness & springiness increased. Sensory attribute of HTST-treated (72 °C, 15 s) milk was similar to raw milk.	Sepúlveda *et al.* (2000)
β-lactoglobulin-stabilised model emulsions; partial skim milk (1.5% fat); whole milk (3.5% fat) & cream (35% fat)	E[b]: 21–36 kV cm^{-1}; 200 exponentially decaying pulses of 0.8–1.6 μs at 1 Hz; T_o: <30 °C	MFG size in partial skim milk & whole milk did not change. No detrimental effect on functional properties.	In cream, larger MFG dissociated into smaller ones & stability index decreased.	Barsotti *et al.* (2002)
Skim milk and simulated milk ultrafiltrate	E[b]: 18.3–27.1 kV cm^{-1}; exponentially decaying pulses; treatment time: 400 μs; T_p: 20–25 °C & 50–55 °C	Fat-soluble & other water-soluble vitamins remained unaffected.	Ascorbic acid was the only vitamin destroyed however retention was greater in skim milk.	Bendicho, Espachs, *et al.* (2002)
Raw skim milk	E[b]: 34.7 kV cm^{-1}; 64 bipolar square wave pulses; treatment time: 188 μs; flow rate: 60 mL min^{-1}; T_i: 22 °C; T_o: 52 °C	Total solids, protein, pH, conductivity, colour, particle size, viscosity and density did not change significantly.		Michalac *et al.* (2003)
Bovine β-lactoglobulin solution (10%)	E[c]: 12.5 kV cm^{-1}, 40 μF; 1–10 exponentially decaying pulses after 15 s interval; T_p: <35 °C		Proteins transition temperatures decreased and gelation rate increased. Covalent aggregates were formed at higher intensities.	Perez and Pilosof (2004)
Raw skim milk (0.2% fat)	E[b]: 25 & 28 kV cm^{-1}; bipolar square waved pulses of 2 μs at 200 & 400 Hz; flow rate: 120 mL min^{-1}; T_p: <45 °C; T_o: <22 °C	Total solids, pH, & yield of cottage cheeses made from PEF-treated milk were comparable to raw milk Aroma of 25 kV cm^{-1} treated sample was similar to raw milk curd aroma	Gel strength decreased at 200 Hz with a change from 25 to 28 kV cm^{-1}.	Wüst *et al.* (2004)
HTST-pasteurized milk	E[b]: 35 kV cm^{-1}; five exponentially decaying pulses of 2.3 μs; flow rate: 1200 mL min^{-1}; T_i: 50 °C; T_o: 65 °C	Olfactory and visual parameters did not change.	Shelf-life increased up to 78 days at 4 °C.	Sepúlveda *et al.* (2005)
Raw skim milk (0.2% fat)	E[b]: 30–50 kV cm^{-1}; 10–30 exponentially decaying pulses of 2 μs at 3–4 Hz; flow rate: 500 mL min^{-1}		Shelf-life was >22 days (73 °C followed by 50 kV cm^{-1}), or 30 days (80 °C followed by 30 kV cm^{-1}) at 4 °C.	Fernández-Molina, Barbosa-Cánovas, *et al.* (2005)

Raw skim milk (0.2% fat)	E[b]: 28–36 kV cm^{-1}; 30 exponentially decaying pulses of 2.8 μs at 3 Hz; flow rate: 8.33 mL min^{-1}		PEF-(36 kV cm^{-1}) and heat (65 °C for 21 s)-treatment extended the shelf-life to>30 days at 4 °C.	Fernández-Molina, Fernández-Gutiérrez, *et al.* (2005)
Raw skim milk	E[b]: 45–55 kV cm^{-1}; square waved monopolar pulses of 500 & 250 ns, frequency, 40–120 Hz; flow rate: 83.3 mL min^{-1}; T_o: <50 °C	No change in pH No modification in the micellar mineral partition.	Casein micelle size, viscosity & clotting time decreased significantly.	Floury *et al.* (2006)
Raw whole milk (3.6% fat)	E[b]: 35.5 kV cm^{-1}; bipolar pulses of 7 μs at 111 Hz; treatment time: 300 or 1000 μs with flow rate: 60 mL min^{-1}; T_o: <40 °C	No proteolysis & lipolysis for one week.	pH decreased slightly during storage. FFA increased. Whey protein denaturation: α-lactalbumin (40%) > bovine serum albumin (24.5%) > β-lactoglobulin (20.1%).	Odriozola-Serrano *et al.* (2006)
Raw milk (3.8% fat)	E[b]: 15–35 kV cm^{-1}; treatment times: 12.5–75 μs; T_p: 30 °C	Vitamins did not change significantly.		Riener *et al.* (2008)
Raw whole milk	E[b]: 35 kV cm^{-1}; five exponentially decaying pulses of 2.3 μs; flow rate: 1200 mL min^{-1}; T_i: 50 °C; T_o: 65 °C	Olfactory and visual parameters did not change.	Shelf-life was extended up to 24 days at 4 °C.	Sepúlveda *et al.* (2009)
Raw, skim & whole milk	E[b]: 35 & 38 kV cm^{-1}; monopolar square waved pulses of 2 μs at 200 Hz; treatment time 19.2 μs; flow rate: 60 mL min^{-1}; T_i: 15 & 45 °C & corresponding T_o: 30 & 60 °C	Casein micelle & MFG size was unaffected.	Rheological and coagulation properties changed much less compared to heat treatments (LTLT, HTST, 97 °C for 10 min).	Shamsi (2008)
Whole milk	E[b]: 36 & 42 kV cm^{-1} with 24–64 & 8–24 exponentially decaying pulses, respectively; treatment time: 2.6 μs; flow rate: 383.3 mL min^{-1}; T_o: <25 °C	No significant effect of electric field intensity & pulse number on ζ-potential & MFGs particle size distribution.	Changes in MFG size were similar to LTLT-treatment.	Garcia-Amezquita *et al.* (2009)
Raw milk (3.8% fat & 3.1% protein)	E[b]: 20 & 30 kV cm^{-1}; 40–120 bipolar square waved pulses of 2 μs at 2 Hz frequency; T_p: up to 50 °C	Coagulation properties were better preserved at 30 kV cm^{-1} & 50 °C.	Curd firmness (CF) decreased & rennet coagulation time (RCT) increased with increase in the treatment intensity. CF & RCT were in between raw & thermally-treated (63 °C, 30 min) milk.	Yu *et al.* (2009)
Whey proteins (β-lactoglobulin, α-lactalbumin, IgG and lactoferrin)	E[c]: 37.6 kV cm^{-1}; 50, 100 & 200 exponentially decaying pulses of 2 μs at 1 Hz; T_o: <35 °C	No change in immunoreactive protein concentration after PEF treatments.	Lactoferrin reduced to 53–58% after thermal treatments (65 °C, 30 min & 75 °C, 15 s).	De Luis *et al.* (2009)
Lactoferrin (native: 98.3% protein; iron depleted: 95.7% protein; iron saturated: 89.5% protein) with different iron saturation levels 24.5, 6 & 78.7%, respectively	E[b]: 35 kV cm^{-1}; 200 mono & 100 bipolar square waved pulses of 2 μs; treatment time: 19.2 μs; flow rate: 60 mL min^{-1}; T_o: 30–70 °C	Physicochemical properties did not change after PEF treatment at ≤65 °C.	Lactoferrin decreased and proteins aggregated at higher temperatures due to thermal effects.	Sui *et al.* (2010)

(*continued on next page*)

Table 1 (*continued*)

Treatment media	PEF processing conditions	Functional properties		References
		Unchanged	Changed	
Raw skim milk & milk concentrates (18% solids)	E[b]: 45 kV cm^{-1}; bipolar pulses of 2 μs at 1370 pulses s^{-1}; treatment time: 20 μs; flow rate: 240 mL min^{-1}; T_i: 25 °C; T_o: 30 °C		A reversible shear-induced effect appeared for casein micelles by circulating samples through the continuous PEF unit without applying treatment.	Hemar *et al.* (2011)
Raw skim milk	E[b]: 16–42 kV cm^{-1}; 0.31 μF; monopolar exponentially decaying pulses of 1.5 μs; treatment time: 612–2105 μs; flow rate: 4.2 mL min^{-1}; T_i: 16 °C; T_o: 40–49 °C		Shelf stability of PEF-microfiltration-treated milk was seven days at 4 °C.	Walkling-Ribeiro *et al.* (2011)
Skim milk	E[c]: 15–20 kV cm^{-1}; 0.33 μF; 20–60 exponentially decaying pulses at 0.50 Hz; T_o: <35 °C		Shear-thinning behaviour was noticed. Consistency index increased.	Xiang, Simpson, *et al.* (2011)
Whey protein isolate solution (3% & 5% w/v)	E[c]: 12–20 kV cm^{-1}; 0.33 μF; 10–30 exponentially decaying pulses at 0.50 Hz; T_o: <35 °C		Surface hydrophobicity increased, thus structural modifications.	Xiang, Ngadi, *et al.* (2011)
Raw milk (3.4% fat)	E[b]: 15–30 kV cm^{-1}; bipolar square waved pulses of 2 μs at 200 Hz; treatment time: 800 μs; T_o: <40 °C	No major differences in acids, lactones & alcohols in the volatile profile of PEF-treated milk.	Aldehydes increased & methyl ketones were lower than pasteurized milk (75 °C, 15 s). 2(5H)-furanone was only detected in PEF-treated milk.	Zhang *et al.* (2011)
Raw milk (3.8% fat & 3.1% protein)	E[b]: 30 kV cm^{-1}; 80 & 120 bipolar square waved pulses of 2 μs at 2 Hz; flow rate: 6 mL min^{-1}; T_o: 50 °C	FFA content was very close to raw milk during storage.	Intermediate proteolysis profile between raw and thermally-treated (63 °C, 30 min) milk.	Yu *et al.* (2012)
Raw milk	E[b]: 5–40 kV cm^{-1}; pulse duration, 5–35 μs; square waved pulses, frequency, 50–1000 Hz; flow rate: 500 mL min^{-1}; T_i: 20–45 °C; T_o: 39–72 °C	No change in the colour of PEF-treated milk.	Conductivity decreased. Maximum 70% reduction in the native form of milk proteins. Lactoferrin decreased with increasing specific energy. Shelf-life of 14 days at 4 °C.	Mathys *et al.*, (2013)

[a] E: electric field intensity; T_i: inlet temperature; T_o: outlet temperature; T_p: processing temperature; LTLT: low temperature long time; HTST: high temperature short time, MFG: milk fat globule; FFA: free fatty acids.
[b] Continuous mode of operation.
[c] Batch mode of operation.

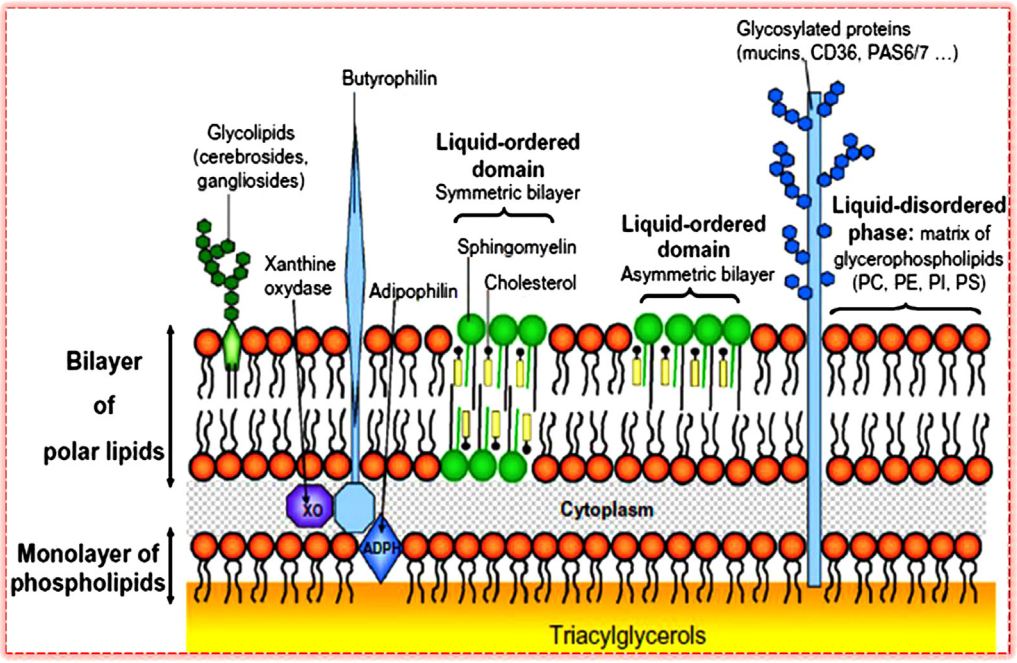

Fig. 2. Milk fat globule membrane structure. The drawing is highly schematic and relative sizes are not to scale. Adapted from (Lopez *et al.*, 2011).

fat globule size and interfacial ζ-potential of the MFGM. There are no reports on compositional changes in the MFG system due to PEF processing of milk. The changes in the MFG system after homogenization (Michalski, Michel, Sainmont, & Briard, 2002) and heating (Ye, Singh, Taylor, & Anema, 2005) are well-studied, where casein micelles and whey proteins were shown to be adsorbed to the MFGM (Fig. 3A, i–iii), but there are no reports revealing PEF-induced changes to the MFGM. PEF treatment of milk can also induce changes to the MFG system where milk proteins, such as caseins, adsorb and repair damage done to the MFGM. In a recent study in our laboratory, confocal microscopic examination of MFGs of PEF-treated (20 kV cm^{-1}) whole milk revealed the presence of structural changes to the MFGM (Fig. 3A, iv; B); however, these changes were found to be less damaging compared to thermal pasteurization methods (63 °C for 30 min and 72 °C for 15 s). Hence, standard microscopic techniques may provide a clear visualisation of PEF-induced damage to the MFGM. Moreover, a detailed examination and characterization of MFGM proteins and phospholipids in PEF-treated milk can help to understand PEF-induced effects to the MFG system.

Caseins

Studies on the structure of milk components reveal that PEF treatment induces protein structural changes. Higher flow rates employed during continuous PEF treatment appears to alter the casein micelle size; however, this change can be reversible. This indicates that casein micelles are perhaps very sensitive to the shear forces introduced in the PEF system while pumping. A decrease in casein micelle size was observed by Floury *et al.* (2006) after PEF treatment (45–55 kV cm^{-1} for 2.1–3.5 μs at a flow rate of 83.3 mL min^{-1}) compared to the raw skim milk. This was attributed to the apparent charge modification of micelles after exposure to an intense electric field, resulting in a reduction in the hydrodynamic volume of casein micelles. The authors concluded that PEF treatment of milk may alter the structure of proteins with concomitant modifications to the functional properties, such as during cheese manufacture. This could be due to the unfolding or orientation of proteins in the direction of the applied electric field. The molecular dynamic (MD) simulated modelling approach of soybean hydrophobic protein suggests that under the influence of an external electric field, proteins unfold or align themselves in the direction of the applied electric field, although it is dependent on the intensity of electric field (Singh, Orsat, & Raghavan, 2013). The transition between helical structures from α-helix to π-helix also plays an important role in defining its function (Budi, Legge, Treutlein, & Yarovsky, 2005). It is possible that milk proteins can also undergo similar conformational changes when exposed to external electric field.

In contrast, Shamsi (2008) reported that PEF treatment (38 kV cm^{-1} and 60 °C at a flow rate of 60 mL min^{-1}) did not show any significant ($P > 0.05$) change to casein micelle size. There were no substantial differences in the particle size after PEF treatment (34.7 kV cm^{-1} at a flow rate of 60 mL min^{-1}) compared to thermal pasteurization at 73 °C and 30 s (Michalac, Alvarez, Ji, & Zhang, 2003). These studies suggest that the applied electric field strength and the shape of pulses were not considerable

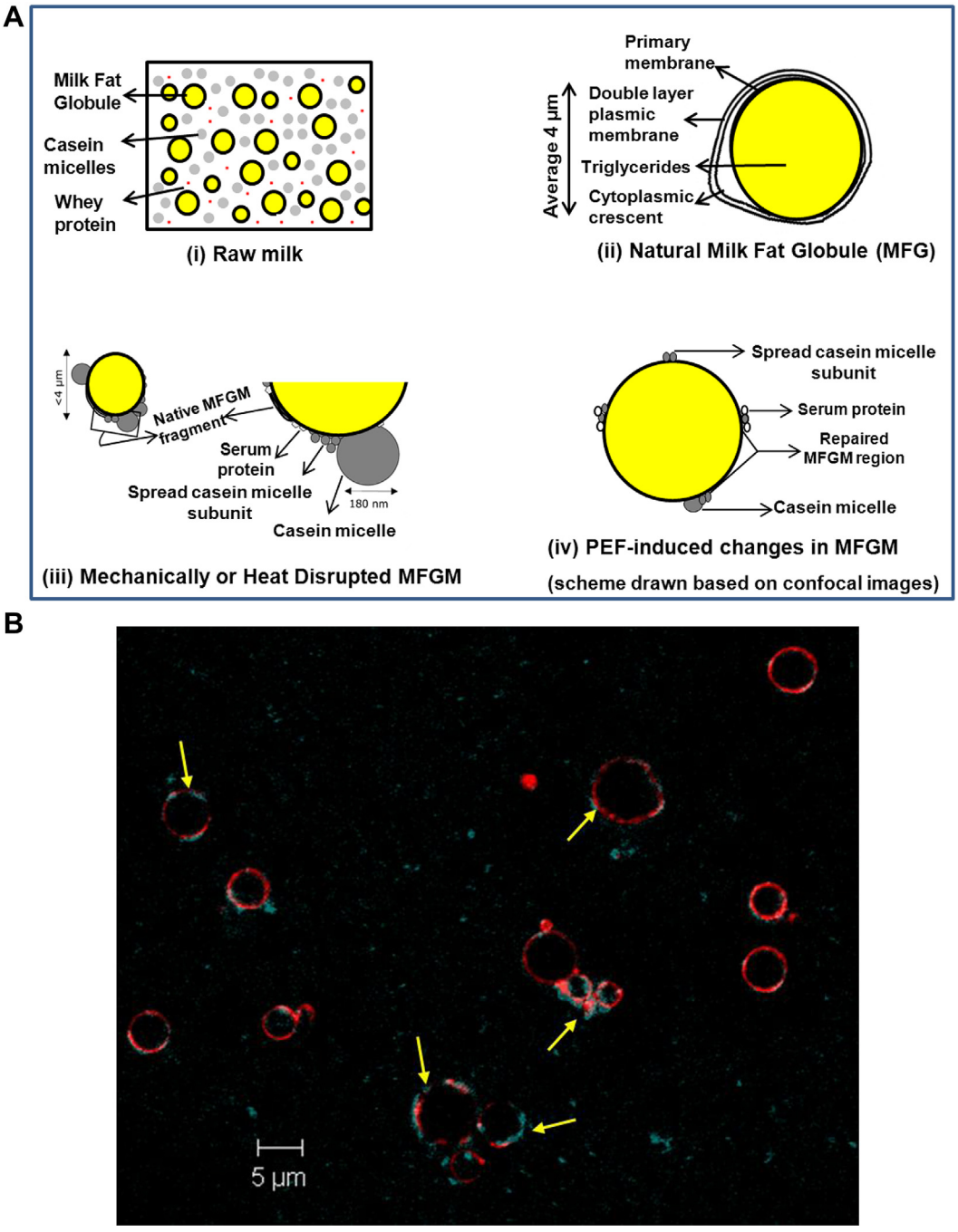

Fig. 3. Milk fat globules (MFGs): (A i) raw milk, (A ii) native MFG, (A iii) disruption by mechanical or heat treatment; adapted from (Michalski *et al.*, 2002), (A iv) disruption due to pulsed electric fields. (B) confocal micrograph of PEF-treated milk (electric field intensity, 20 kV cm^{-1}; pulse width, 20 µs; frequency, 20 Hz; flow rate, 4.2 mL s^{-1}), arrows indicate PEF-induced changes to the membrane of MFGs, red colour emission fluorescence for phospholipid binding analogue Rd-Dope (1, 2-dioleoyl-sn-glycero-3-phosphoethanolamine-N-(lissamine rhodamine B sulfonyl)) and green colour for protein binding Fast Green FCF. MFGM: milk fat globule membrane; PEF: pulsed electric field.

enough to induce changes in the conformation of caseins. In contrast, Hemar *et al.* (2011) reported that PEF treatment (45 kV cm^{-1} at a flow rate of 240 mL min^{-1}) reduced the casein micelle size in various milk systems; however, upon overnight storage at 4 °C, the particle size reverted to that of the untreated sample. Similar changes to the average diameter were observed without applying PEF treatment, indicating that changes were due to the high shear encountered by casein micelles from the feed pump rather than the PEF treatment. Casein micelles are known to partially dissociate during homogenization of milk (Sandra & Dalgleish, 2005). The effect of shear from the feed pump in a continuous PEF device, without applying any PEF treatment, must be considered.

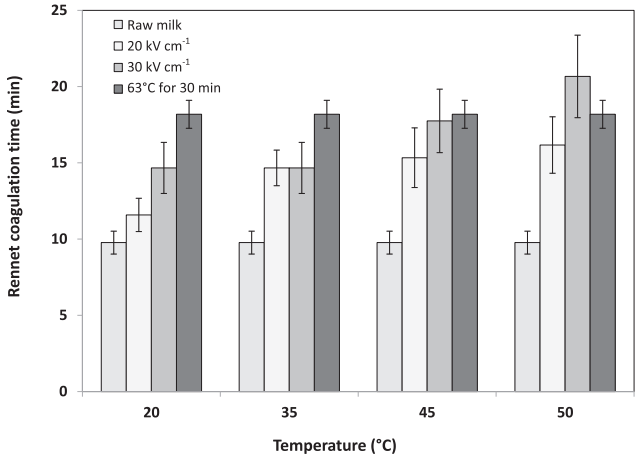

Fig. 4. Effect of electric field intensity and temperature on rennet coagulation time. Adapted from (Yu *et al.*, 2009).

These results imply that the shear force generated while pumping is a critical factor leading to changes in the casein micelle structure. The time of measurement after PEF treatment should also be taken into account, as Hemar *et al.* (2011) noticed a reversibility in casein micelle size after overnight storage. The changes in the conformation of casein micelles observed by other researchers (Floury *et al.*, 2006) could be due to the high electric field strength (45–55 kV cm^{-1}) applied. Additionally, it may also be possible due to the measurements made immediately after PEF treatment. Thus, it becomes a prerequisite to report the waiting time between PEF treatment and measurement of the change in casein micelles, as the change observed immediately after the treatment can be transient.

Whey proteins

Whey proteins are a class of high-quality nutritive proteins present in milk that are gaining importance because of putative health benefits. These proteins are also susceptible to denaturation after heat-processing (Fox & McSweeney, 1998). PEF treatment of raw milk was reported to have little effect on whey protein tertiary structure (Dunn, 1996; Grahl & Märkl, 1996; Michalac *et al.*, 2003; Qin *et al.*, 1995) whereas Odriozola-Serrano, Bendicho-Porta, and Martín-Belloso (2006) reported the occurrence of whey protein denaturation in the order of α-lactalbumin > bovine serum albumin > β-lactoglobulin. Odriozola-Serrano *et al.* (2006) found that the occurrence of thermal denaturation (75 °C for 15 s) of whey proteins was higher compared to PEF in the order of α-lactalbumin > β-lactoglobulin > bovine serum albumin. In contrast, the order of heat stability of whey proteins was reported as α-lactalbumin > β-lactoglobulin > bovine serum albumin (Celestino, Iyer, & Roginski, 1997; Donovan & Mulvihill, 1987). The observed difference in the stability of whey proteins is likely due to the different pH values during the measurements. Donovan and Mulvihill (1987) reported that at a higher pH value of 6.7, β-lactoglobulin has a greater degree of denaturation, thermal denaturation of α-lactalbumin is independent of pH, and bovine serum albumin is more stable. Xiang, Ngadi, Ochoa-Martinez, and Simpson (2011) suggested that electric field intensity, number of pulses, and concentration of proteins altered the structure of whey proteins, and as such, PEF treatments can be used to create new products with particular functional properties. The exposure of proteins to an external electric field strength might induce changes in the polarity of the microenvironment of protein amino acid residues owing to the unfolding of the native molecule structure.

The folded protein structure of heat-sensitive lactoferrin is maintained following PEF treatment, regardless of the mode of operation, indicating the potential of PEF to maintain the stability of this protein during treatment of milk. De Luis *et al.* (2009) reported that heat treatments (65 °C for 30 min and 75 °C for 15 s) produced a severe loss of immune reactivity of IgG and lactoferrin, but no considerable differences were observed for untreated and PEF-treated (37.6 kV cm^{-1}) samples in a batch mode of operation using exponential decay pulses. Similarly, Sui, Roginski, Williams, Versteeg, and Wan (2010) found that the physicochemical properties of bovine lactoferrin were not significantly ($P > 0.05$) affected by PEF treatments (35 kV cm^{-1}) in a continuous mode using square wave pulses. However, at higher temperatures (60–70 °C) during PEF processing, the native folded lactoferrin concentration decreased, the protein aggregated, and surface hydrophobicity increased, largely due to the associated thermal effects and perhaps partially due to the stress induced by PEF treatment alone. The effective loss of native lactoferrin due to partial denaturation through PEF treatment was less than that caused by thermal treatments (63 °C for 30 min and 72 °C for 15 s). Likewise, Mathys *et al.* (2013) reported about a 70% reduction in the native form of milk proteins (IgG, IgA, and lactoferrin) after PEF treatment (40 kV cm^{-1} and 1000 Hz) using a continuous mode of operation and square wave pulses, which may be attributed to the high processing temperatures (up to 72 °C) coupled with the intense PEF treatment conditions. The native lactoferrin concentration decreased with increasing specific energy; however, at 244 kJ kg^{-1} (the energy required to inactivate inoculated *Escherichia coli* and *Listeria innocua*) the extent of lactoferrin denaturation was only about 15% (Mathys *et al.*, 2013). The findings from these authors suggest that intense PEF processing conditions (≥35 kV cm^{-1}) combined with a higher heat (>60 °C) treatment in a continuous mode of operation with square wave pulses could lead to lactoferrin denaturation, probably due to the unfolding of protein molecules or their alignment in the direction of applied electric field. PEF treatment alone has a less pronounced effect compared to thermal pasteurization treatment of milk.

Milk protein-based model systems

In model systems, PEF treatment of β-lactoglobulin proteins (10%, w/w) at 12.5 kV cm^{-1} in a batch mode of operation can induce changes such as polarization, dissociation of non-covalently linked sub-units, change in the protein conformation, attraction between protein structures by electrostatic forces, and, if the electric field pulses are induced for a sufficiently long period, hydrophobic interactions or covalent bonds may result in the formation of aggregates (Perez & Pilosof, 2004). In contrast, Barsotti *et al.* (2002) found that PEF treatment of β-lactoglobulin-stabilized oil-in-water emulsions and β-lactoglobulin solution at 21–36 kV cm^{-1} in a continuous mode of operation did not result in any noticeable unfolding or aggregation of this protein. However, higher concentrations of β-lactoglobulin solution (17% w/v) were more affected after PEF treatment (~32 kV cm^{-1}) compared to lower concentrations (2% w/v). These studies suggest that the differences in native protein structure are likely due to the different modes of operation (continuous or batch) of the PEF devices indicating that protein molecules are more likely to be unfolded in a batch system with more intense electric field environment in the treatment chamber. This suggests that a shorter duration at higher field strength is less destructive to the β-lactoglobulin structure than longer duration at lower electric field strength.

PEF as a non-thermal technique induces changes to milk components; however, these changes are reported to be less compared to thermal treatments. PEF-induced changes to milk components may be due to the PEF or the thermal effect depending on the treatment conditions. A longer exposure of milk to elevated temperatures during PEF processing at high electric field intensities or treatment temperatures may lead to the unfolding of protein molecules, their alignment in the direction of electric field and transition in the native structure. PEF-induced conformational changes to proteins may be due to the denaturation and formation of inter-molecular complexes such as β-lactoglobulin/κ-casein after cross-linking between casein and serum proteins, as found in heat-treated milk (Mohammad & Fox, 1987). To avoid longer exposure of milk to elevated temperatures, effective intermediate cooling systems may be employed between multiple treatment zones, as well as at the end of the PEF treatment assembly. In future, a detailed understanding of conformational changes to dairy proteins under the influence of an external electric field using MD simulated modelling approach would also be required.

Rennet-coagulated milk gels

Viscoelastic and coagulating properties of milk gels are considerably affected by the electric field intensity and treatment temperature during PEF processing of milk. The storage (G', elastic response) and loss (G'', viscous response) moduli of gels made from PEF-treated milk using square wave pulses decreases with an increase in the electric field intensity and temperature, and PEF (35 and 38 kV cm^{-1}) combined with heat treatment (60 °C) has a cumulative effect that further reduces G' and G'' (Shamsi, 2008). These authors concluded that PEF and heat treatments cause similar changes to milk protein structure due to the similarity in the gel properties. Furthermore, they observed that the rennet coagulation time (RCT) of milk increased with an increase in the field intensity (35–50 kV cm^{-1}), which was in contrast to the findings of Dunn (1996) who did not find any change. On the other hand, Floury *et al.* (2006) reported a decrease in coagulation time after PEF treatment (45 and 55 kV cm^{-1}) with a treatment temperature of <50 °C, concomitant with a decrease in the size of casein micelles, with increased specific area and increased possibility of micelle inter-particle collisions. The RCT of PEF-treated milk using square wave pulses at 60 °C was significantly ($P < 0.05$) longer (38.8 min) than untreated (32.3 min) and PEF-treated milk at 30 °C (35.4 min), indicating the additional effect of heat on RCT (Shamsi, 2008).

Similarly, Yu, Ngadi, and Raghavan (2009) observed that an increase in electric field intensity, treatment temperature and number of square wave pulses increased the RCT (Fig. 4), thus considerably affecting rennet coagulation properties of PEF-treated raw milk. These authors found that a PEF treatment at 30 kV cm^{-1} and 50 °C may result in similar coagulating properties as after thermal pasteurization, such as gel firmness, and is a potential alternative pasteurization method for cheese milk. Shamsi (2008) reported that the pores in the protein matrix of gels from PEF-treated milk became larger as the field intensity and temperature increased. This was attributed to weaker interactions between the casein micelles of the gel network which resulted in lower gel firmness. However, compared to PEF effects, the impact of heat on gel structure was much more pronounced, showing larger pores and weaker gels.

These results suggest that an intense PEF treatment (≥30 kV cm^{-1}) using square wave pulses combined with heat (≥50 °C) leads to an increase in RCT, and this increases further with an increase in treatment intensity. The observed changes are possibly due to the thermal effects from higher treatment temperatures, or the temperature rise during PEF treatment, which may have caused proteins to aggregate. Therefore, appropriate cooling systems can be installed around the treatment zone of a treatment chamber or intermediately between two treatment chambers to minimize these thermal effects.

Textural properties of cheese

The texture of cheese is dictated by the composition of milk and the processing methods employed to make the cheese. Cheeses prepared from PEF-treated milk are harder than those prepared from raw milk, mainly due to the thermal effects during the treatment; however, low intensity

PEF treatment of milk can still produce cheese with comparable textural attributes to raw milk cheese.

Sepúlveda, Ortega-Rivas, and Barbosa-Cánovas (2000) observed that hardness and springiness of cheddar cheese prepared from PEF-treated milk increased whereas adhesiveness and cohesiveness were not statistically different compared to raw milk cheese. In contrast, cheese made from thermally-treated milk (63 °C for 30 min) was reported to have the highest degree of adhesiveness, which might be due to increased water retention within an open protein structure (Bryant, Ustunol, & Steffe, 1995). These authors suggested that denaturation of proteins was due to a rise in temperature during PEF processing, and heating could be one reason for increased hardness and springiness as these textural attributes are known to be largely due to the protein phase (Chen, Larkin, Clark, & Irwin, 1979).

Wüst, Pearce, Ortega-Rivas, and Sherkat (2004) found that the textural characteristics of cottage cheese prepared from thermally pasteurized milk and PEF-treated milk at 28 kV cm^{-1} and 400 Hz were similar, whereas at 200 Hz, cheese from treated milk was similar to raw milk cheese. These authors reported that increasing the electric field intensity decreased the hardness, and increasing the frequency raised the cheese solids content, which could be due to a faster rate of acidification and increased rate of whey expulsion. Similarly, Shamsi (2008) reported an inverse relationship between hardness with increasing field intensity and treatment temperature (30 °C and 60 °C). Long exposure of milk to heat has an adverse effect on gel structure owing to whey protein denaturation, and the subsequent binding to the surface of casein micelles hinders the rennet coagulation process, resulting in a soft gel (Dalgleish, 1990). In contrast, Sepúlveda *et al.* (2000) reported that rennet-induced gels of PEF-treated skim milk were firmer than those of the non-PEF controls. Yu *et al.* (2009) found that cheese hardness increased following a rise in the electric field intensity, treatment temperature, and pulse number.

Viscosity of milk

Viscosity of milk and model solutions following PEF treatment is also impacted by the PEF mode of operation (batch or continuous) and electric field strength; however, there are apparent conflicting results. The viscosity of milk after PEF treatment in batch mode increases, whereas, in a continuous mode of operation some authors did not see any change whereas others noticed a decrease with the treatment intensity. The mechanism for this decrease in viscosity after continuous operation is confounded by the impact of pumping, which may cause coalescence of MFGs that will increase viscosity (Walstra *et al.*, 2006). An increased viscosity after batch mode operation of PEF could be due to an increase in the voluminosity of casein micelles, and MFGs through aggregation.

PEF-treated skim milk (batch mode at 15–20 kV cm^{-1}) shows a shear-thinning flow behaviour (Xiang, Simpson, *et al.*, 2011). These authors reported an increase in apparent viscosity with increasing electric field intensity and number of pulses which was attributed to inter-molecular interactions amongst contiguous denatured milk serum proteins and MFGs, subsequently causing an increase in the effective volume of the MFGs. In contrast, the viscosity of skim milk (Michalac *et al.*, 2003) and β-lactoglobulin solutions (Barsotti *et al.*, 2002) was unaffected by PEF treatment in continuous mode at 34.7 kV cm^{-1} and 32 kV cm^{-1} (200 pulses), respectively. A significant ($P < 0.05$) decrease in viscosity has been reported, thought to be due to the diminution of the hydrodynamic volume of casein micelles when PEF treatment was conducted in continuous mode at higher electric field intensities in the range of 45–55 kV cm^{-1} (Floury *et al.*, 2006). Similarly, Hemar *et al.* (2011) observed that PEF treatment at 45 kV cm^{-1} decreased the viscosity of raw skim milk, reconstituted skim milk (10% total solids), concentrated skim milk, and milk protein concentrate (18% total solids). A comparable decline in the viscosity was observed for the samples passed through the feed pump of the unit without applying PEF treatment, indicating that the viscosity decrease was not due to the PEF treatment, but more likely by the high shear encountered by the casein micelles during pumping (240 mL min^{-1}). Consequently, the viscosity of PEF-treated milk considerably decreases in continuous operational mode when higher flow rates and electric field strengths ($\geq$45 kV cm^{-1}) are used.

The PEF-induced effects on bovine milk suggest that intense PEF treatments can affect the functional properties of milk. Considering the potential improvement in food safety and increased shelf-life due to PEF treatment, these changes may be minimized by controlling the temperature rise in the treatment zone and using low treatment temperatures. Alternatively, PEF treatment may also be combined with other hurdle approaches using cold operation such as antimicrobial agents or microfiltration.

Shelf-life properties

Reported studies suggest that PEF treatment, with or without heat treatment, have the potential to preserve milk comparable to that of thermal pasteurization techniques. Different conclusions by different authors on the stability of PEF-treated milk in continuous mode is likely due to differences in the design of equipment; however, PEF-treated milk from all reported studies has been found to be stable for at least one week, post-treatment.

Michalac *et al.* (2003) established that pH, total solids, electrical conductivity, and density of skim milk do not change with either PEF or thermal treatment. Likewise, Floury *et al.* (2006) found that pH does not change, regardless of the energy applied after PEF treatment of skim milk. Odriozola-Serrano *et al.* (2006) reported that acidity, pH, and free fatty acid content do not change following PEF treatment of whole milk, and no proteolysis and lipolysis was observed within one week after treatment at

35.5 kV cm^{-1} and 1000 μs. Yu, Ngadi, and Raghavan (2012) reported a gradual increase in the total water-soluble peptide content of cheese slurries during incubation at 30 °C for five days, indicating that protease enzymes retain activity after PEF treatment of milk (Van-Loey, Verachtert, & Hendrickx, 2002). Yu *et al*., (2012) found that the free fatty acid content of cheeses prepared from raw milk and PEF-treated milk was similar throughout the ripening period.

PEF treatment directly after heating (72 °C for 15 s) increases the shelf-life of milk up to 60 days whereas applying PEF after eight days extended the shelf-life to 78 days (Sepúlveda, Góngora-Nieto, Guerrero, & Barbosa-Cánovas, 2005). These authors reported that the acidity of PEF-treated milk remained below the sensory detection level throughout the storage period (Sepúlveda *et al*., 2005; Sepúlveda, Góngora-Nieto, Guerrero, & Barbosa-Cánovas, 2009). Sepúlveda *et al*. (2009) concluded that a combination of PEF and mild heat treatment (thermal regeneration and PEF-induced heating) successfully extended the shelf-life of milk up to 24 days, and was comparable to that of commercially pasteurized milk. PEF-treated skim milk acidity was least affected after a combination of PEF and heat treatment, and this extended the shelf-life to 30 days at 4 °C (Fernández-Molina, Barbosa-Cánovas, & Swanson, 2005; Fernández-Molina, Fernández-Gutiérrez, *et al*., 2005). PEF was also used in combination with other non-thermal technologies such as microfiltration for milk processing which resulted in a shelf stability of seven days at 4 °C, similar to that of thermal treatment at 75 °C for 24 s (Walkling-Ribeiro, Rodríguez-González, Jayaram, & Griffiths, 2011). Mathys *et al*. (2013) reported a milk shelf-life of 14 days at 4 °C when PEF treatment was carried out with mild inlet temperatures of 20–45 °C.

Sensory properties

Most studies report that milk retains its sensory characteristics after PEF treatment. No differences were found in the taste and flavour of PEF-treated milk compared to untreated milk (Dunn, 1996; Grahl & Märkl, 1996; Qin *et al*., 1995). Dunn (1996) reported that it may be possible to manufacture dairy products such as cheese, butter and ice-cream with less flavour degradation comparable to heat-treated products. Likewise, Sepúlveda *et al*. (2005) found no apparent changes in the olfactory or visual characteristics after PEF treatment of heat-pasteurized milk. Mathys *et al*. (2013) also did not find any change in the colour of PEF-treated milk.

Changes have been reported in the types of volatile organic compounds found in PEF-treated milk compared to the raw milk. The level of aldehydes in PEF-treated milk was observed to be increased whereas thermal pasteurization increased both aldehydes and methyl ketones (Zhang *et al*., 2011). These authors suggested that membranes of larger MFG lead to aldehyde formation, probably due to autoxidation of unsaturated fatty acids and decomposition of hydroperoxides through the action of heat (Grosch, 1982). Volatile compound formation under PEF treatment is also believed to occur from electrochemical reactions at the electrode surface in the presence of milk (Morren, Roodenburg, & de Haan, 2003), which would result in free radical formation to initiate the peroxidation of unsaturated fatty acids with subsequent aldehyde formation.

In the case of cheeses prepared from PEF-treated milk, Sepúlveda *et al*. (2000) found significant ($P < 0.05$) differences in the flavour of cheddar cheese prepared from PEF-treated milk compared to the raw milk. The observed differences in flavour could be due to the thermal effects from the temperature rise during PEF treatment or high flow rate (600 mL min^{-1}) for pumping of milk since shear alters the size of casein micelles, as discussed previously. The aroma of cottage cheese prepared from milk treated at 28 kV cm^{-1} and 400 Hz, was similar to that prepared from pasteurized milk, whereas the 200 Hz treated cheese showed similar aroma to raw milk cheese (Wüst *et al*., 2004). The changes in sensory parameters after PEF treatment of milk are not clearly understood in terms of employed flow rates and electric field intensities and therefore, may be attributed to different PEF equipment used.

Nutrition

The type of treatment media, such as milk, skim milk, or various model systems, may result in different destruction levels of nutritional components. Amongst all of the vitamins, ascorbic acid is the most sensitive to PEF treatment. Grahl and Märkl (1996) found ~90% destruction of ascorbic acid at high energy levels (>200 kJ L^{-1}) and 20 pulses, whereas vitamin A did not change markedly. Bendicho, Espachs, Arántegui, and Martín (2002) reported that PEF treatment of skim milk or simulated milk ultrafiltrate at room and at moderate temperatures did not affect thiamine, riboflavin, cholecalciferol or tocopherol content, but destroyed ascorbic acid. These authors found that PEF treatment at 22.6 kV cm^{-1} and 400 μs retained ascorbic acid (~93%) to a greater extent compared to thermal processing (63 °C for 30 min and 75 °C for 15 s). The retention was greater in PEF-treated skim milk than in simulated milk ultrafiltrate, which could be due to a protective effect from milk components, such as caseins. Riener, Noci, Cronin, Morgan, and Lyng (2008) reported that PEF treatment did not induce significant ($P < 0.05$) changes in thiamine, riboflavin, retinol, and α-tocopherol levels compared to raw milk.

PEF application in the dairy industry

The ability to provide a safe, shelf-stable, cost effective and better quality product to the consumer are the main criteria to evaluate any new technology to replace thermal processing methods. As discussed earlier, PEF has the potential to inactivate vegetative bacterial cells and spoilage

enzymes in milk. García *et al.* (2005) demonstrated the presence of sub-lethally injured cells after PEF treatment and suggested that bacterial membrane damage is an important part of the inactivation mechanism. These authors suggested the synergistic use of PEF with other techniques to enhance the inactivation of enzymes and reduction of bacterial numbers. Attempts have been made to combine PEF treatment with hurdles such as antimicrobial agents (Smith, Mittal, & Griffiths, 2002) and heat (Shamsi, Versteeg, Sherkat, & Wan, 2008; Sharma, Bremer, Oey, & Everett, 2014) to decrease bacterial numbers. Inactivation of bacterial spores in milk using PEF processing is a topic of current investigation. A recent study (Bermúdez-Aguirre, Dunne, & Barbosa-Cánovas, 2012) reported inactivation of *Bacillus* spores (3.6 log) using PEF processing conditions (40 kV cm^{-1} and 144 pulses) combined with other hurdles such as heat (65 °C) and an antimicrobial agent (nisin, 50 IU mL^{-1}). Such an intense treatment may induce changes in the functional properties and impair the final product quality. These approaches have shown the potential of PEF treatment combined with hurdles to replace thermal treatment in the dairy industry. The capital cost and probable changes in the functional properties should be considered along with consumer safety. The major concerns of PEF operation include variability in the equipment design, occurrence of flashovers (electric arching) and a rise in temperature during intense treatment conditions.

Conclusions and research outlook

PEF treatment in combination with other hurdles (such as heat) has the potential to increase the preservation of milk while retaining sensory attributes. Intense PEF treatment conditions (electric field intensity, treatment temperature and square wave pulses), higher flow rates, and the mode of operation (batch or continuous) can induce changes to proteins that may affect the functional properties of milk; however, these changes after PEF treatment are often less compared to thermal treatments. Variability in equipment design and differences in treatment conditions and medium used will impact upon functional properties.

Further studies on PEF-treated whole milk should be considered to provide more understanding of the changes that take place in more complex dairy products containing fat. The mechanism of volatile compound formation and partitioning between the fat and aqueous phases in PEF-treated milk is not well-studied. This will have an impact on the flavour of dairy products.

The direct equivalence of PEF processing to thermal pasteurization of milk with defined quality assurance indices is necessary before up-scaling and commercialization. Preservation of the integrity of labile milk components and associated functional properties can be addressed by stepwise intermediate cooling between PEF treatment zones. Uniform pumping systems with minimised generation of shear forces should be implemented to reduce the impact of shear-induced structural changes. Mathematical models, similar to those developed for microorganism count reductions and enzyme inactivation, need to be developed as tools to predict the effect of PEF treatment on the structural changes of the MFGM. These structural changes will have a profound impact upon the development of texture and flavour in dairy products.

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