



# Interfacial properties and transmission electron microscopy revealing damage to the milk fat globule system after pulsed electric field treatment



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## ABSTRACT

Changes to the bovine milk fat globule membrane (MFGM) were assessed after a combination of pre-heating at 55 °C for 24 s followed by pulsed electric field (PEF) treatment at 20 kV cm<sup>-1</sup> or 26 kV cm<sup>-1</sup> for 34 μs, and compared to changes after thermal treatments at 63 °C or 73 °C. A decrease in milk fat globule size, increase in ζ-potential and specific surface area, and adsorption of plasma proteins onto the surface of the MFGM occurred after pre-heating and PEF and thermal treatments. The increase in ζ-potential was correlated with the surface coverage of MFGM with the plasma proteins which was further confirmed by transmission electron microscopy. PEF treatment of whole milk had less impact on the structure of milk fat compared to high temperature thermal treatments alone. This study suggests that a combination of PEF and low heat treatment at 55 °C may have less detrimental effect on the fat globule surface in whole milk.

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## 1. Introduction

Thermal pasteurisation is used in the dairy industry to ensure food safety and to increase the shelf-life of milk. The impact of heat treatment on milk has been thoroughly studied (Corredig & Dalgleish, 1999; Dalgleish & Banks, 1991; Houlihan, Goddard, Nottingham, Kitchen, & Masters, 1992; Kim & Jimenez-Flores, 1995; Lee & Sherbon, 2002; Ye, Singh, Taylor, & Anema, 2004). The consequences of heat treatment on milk components have raised concerns about its use and prompted alternative non-thermal approaches to pasteurising milk, such as pulsed electric field (PEF) processing. The use of PEF in combination with heat (Guerrero-Beltran, Sepulveda, Gongora-Nieto, Swanson, & Barbosa-Cánovas, 2010; Shamsi, Versteeg, Sherkat, & Wan, 2008; Sharma, Bremer, Oey, & Everett, 2014; Sharma, Oey, Bremer, & Everett, 2014) or antimicrobial agents (Bermúdez-Aguirre, Dunne, & Barbosa-Cánovas, 2012; Smith, Mittal, & Griffiths, 2002) offers advantages to ensure milk safety with lower heat treatment effects.

Milk fat globules (MFGs) are suspended in milk and protected from enzymatic attack by a phospholipid tri-layer membrane known as the milk fat globule membrane (MFGM) (Lopez et al., 2011; Mather, 2011). This is an important constituent of milk due to its technological, nutritional and health properties; however, the structure is not yet fully understood (Lopez et al., 2011; Mather, 2011; Singh, 2006). The native structure of the MFGM can be damaged by heat or mechanical treatments. Heat treatment can lead to the adsorption of casein micelles and whey proteins to the MFGM surface (Corredig & Dalgleish, 1999; Dalgleish & Banks, 1991; Kim & Jimenez-Flores, 1995; Michalski, Michel, Sainmont, & Briard, 2002; Ye et al., 2004). Damage to the MFG system can affect the stability and functional properties of milk (Huppertz & Kelly, 2006). Therefore, it is essential to investigate the effect of any new alternate approaches, such as PEF processing, on MFG structure.

There are few studies of PEF-induced changes to milk components; most of these have focused on studying the integrity of milk proteins (De Luis et al., 2009; Mathys et al., 2013; Perez & Pilosof, 2004; Qin et al., 1995; Xiang, Ngadi, Ochoa-Martinez, & Simpson, 2011) and cheese-making quality of PEF-treated milk (Dunn, 1996; Floury et al., 2006; Sepúlveda, Ortega-Rivas, & Barbosa-Cánovas, 2000; Yu, Ngadi, & Raghavan, 2009). PEF-induced changes to the size of fat globules and casein micelles have been examined

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(Barsotti, Dumay, Mu, Diaz, & Cheftel, 2002; Flourey et al., 2006; Garcia-Amezquita, Primo-Mora, Barbosa-Cánovas, & Sepúlveda, 2009), but we are unaware of any studies on the impact of PEF on the MFGM structure.

The purpose of this research was to investigate the effect of thermal and PEF treatments on milk components. PEF-induced changes to the MFGM and interactions between the MFG system and skim milk proteins were compared with the effects due to the use of high temperature thermal treatment of milk. Structural damage to the MFG system after PEF treatment was also visualised by transmission electron microscopy and compared to thermal treatments. This study is the first to report adsorption of caseins and whey proteins onto the MFGM as a consequence of PEF treatment of whole milk.

## 2. Materials and methods

### 2.1. Sample collection

Bovine whole milk samples were collected in the morning of each day from a Belted Galloway–Jersey cross cow at a local dairy farm (Port Chalmers, New Zealand). The cow was pastured and milked using a vacuum milking machine. Milk was put into glass bottles and transported to the laboratory at ambient temperature with minimal mechanical disruption. Milk fat content was  $4.4 \pm 0.1\%$  as measured using the Babcock method (AOAC, 2005). The conductivity of raw milk before experiments was  $4.81 \pm 0.22 \text{ mS cm}^{-1}$  at  $20.3 \text{ }^\circ\text{C}$ , measured using a conductivity/temperature meter (Eutech Instruments Pte. Ltd., Singapore).

### 2.2. Pulsed electric field and thermal treatments

Thermal treatments of  $63 \text{ }^\circ\text{C}$  for 30 min (T1) and  $73 \text{ }^\circ\text{C}$  for 15 s (T2), with lag times for heating and cooling, and PEF treatments were applied to whole milk as described by (Sharma, Bremer, et al., 2014; Sharma, Oey, Bremer, et al., 2014). The times given refer to the time at each temperature and do not include times for heating up and cooling down. These are not standard commercial time and temperature conditions for pasteurisation. Milk kept at  $20.3 \pm 1.8 \text{ }^\circ\text{C}$  was first pre-heated to  $55 \text{ }^\circ\text{C}$  for 24 s by circulating milk through a tubular coil ( $200 \text{ cm} \times 0.8 \text{ cm}$ , internal diameter) designed as a heat exchanger followed by PEF treatments at two different electric field intensities of  $20 \text{ kV cm}^{-1}$  or  $26 \text{ kV cm}^{-1}$  for a treatment time of 34  $\mu\text{s}$  using bipolar square wave pulses at a constant pulse width of 20  $\mu\text{s}$  and frequency of 20 Hz.

PEF process calculations for total energy per unit volume ( $\text{J m}^{-3}$ ) and treatment time ( $\mu\text{s}$ ) were carried out as previously reported (Sharma, Bremer, et al. 2014; Sharma, Oey, Bremer, et al., 2014). Milk after PEF treatment was cooled to  $\sim 17\text{--}22 \text{ }^\circ\text{C}$  (measured by an inbuilt k-type thermocouple) using two cooling systems containing chilled water ( $11.2 \pm 2.2 \text{ }^\circ\text{C}$ ) in the process line. PEF-treated samples were collected after 3 min of continuous flow from the PEF assembly after each treatment to ensure that the collected sample had undergone the specified level of treatment. Milk samples after circulating in the PEF system at an average flow rate of  $4.2 \text{ mL s}^{-1}$  without PEF treatment were designated as 'pumped milk', and after pre-heating to  $55 \text{ }^\circ\text{C}$  for 24 s were designated as 'pre-heated milk'.

### 2.3. Fat globule size

The MFG size distribution was determined using Mie scattering theory with a laser scattering particle size distribution analyser (Partica LA-950V2, Horiba Ltd., Kyoto, Japan) equipped with a red wavelength diode laser (5 mW) at 650 nm, a blue light emitting

diode (3 mW) at 405 nm, and a silicon photo diode detector. The refractive index of MFGs was taken to be 1.460 for the diode laser and 1.470 for the light emitting diode (Michalski, Briard, & Michel, 2001) at  $20 \text{ }^\circ\text{C}$ . The particle size analyser was set at 70–90% transmittance with the circulation speed at level 3. Deionised water was used as the continuous medium. Milk samples were diluted (1:1) in ethylenediaminetetraacetic acid (EDTA, 35 mM, pH 7) to dissociate casein micelles. The sample mixture containing MFGs was then diluted (1:0.5) with 0.1% (w/v) sodium dodecyl sulphate (SDS) buffer solution to disperse aggregated MFGs. From the size distribution, volume mean diameter ( $d_{43}$ ), surface mean diameter ( $d_{32}$ ), modal diameter ( $d_m$ ) representing the peak of frequency distribution, and specific surface area (S) which is defined as the total surface area of MFGs per unit mass of fat ( $\text{m}^2 \text{ g}^{-1} \text{ fat}$ ), were calculated (Huppertz & Kelly, 2006) using the Horiba diffraction software.

The surface fraction ( $\Phi$ ) of MFGs covered by additional proteins after treatment was calculated using Eq. (1):

$$\Phi = \frac{(S_d - S_o)}{S_o} \quad (1)$$

where  $S_o$  is the original specific surface area of MFGs before treatment, and  $S_d$  is the specific surface area of the MFGs after treatment (Michalski, Michel, et al., 2002). The total surface area of MFGs before treatment ( $A_o$ ) and the damaged surface area after treatment ( $A_d$ ) in units of  $\text{m}^2$  were calculated using Eqs. (2) and (3):

$$A_o = FS_o \quad (2)$$

$$A_d = A_o\Phi \quad (3)$$

where F is the milk fat content (Michalski, Cariou, Michel, & Garnier, 2002).

### 2.4. Zeta ( $\zeta$ ) potential of fat globules

The  $\zeta$ -potential, as an indicator of the degree of change to the milk fat globule surface, was determined from the method of Michalski, Michel, et al. (2002), a method where unadsorbed casein micelles has no significant effect on measuring the value of  $\zeta$ -potential for fat globules. The measurements were carried out by laser doppler micro-electrophoresis using a Zetasizer Nano ZS90 (Malvern Instruments Ltd., Worcestershire, UK) equipped with standard He–Ne laser (4 mW) at 632.8 nm. The Smoluchowski approximation ( $f(\kappa a) = 1.5$ ) was used to calculate the  $\zeta$ -potential from the electrophoretic mobility of MFGs as the thickness of the electric double layer, known as the Debye length ( $\kappa^{-1}$ ), in raw milk is smaller (1.1 nm) than the radius ( $a$ ) of the MFGs (Walstra, Wouters, & Geurts, 2006). Milk samples were diluted to  $8 \times 10^{-3}$  in freshly prepared simulated milk ultrafiltrate (Jenness & Koops, 1962) and the measurements were immediately carried out at  $25 \text{ }^\circ\text{C}$  and pH 6.67 as five replicates.

The fraction of MFG surface ( $\Phi$ ) covered with the plasma proteins was compared to the  $\zeta$ -potential using a linear regression analysis. This analysis was based on the assumption that  $\Phi = 0$  for MFGs in raw milk (Michalski, Michel, et al., 2002). The measured  $\Phi$  values from the laser diffraction technique were also compared to the estimated values from the model by Michalski, Michel, et al. (2002). This model was used to assess the surface coverage of MFGs with plasma proteins based on the relative increase in  $\zeta$ -potential of MFGs:

$$\Phi = 1 - e^{-1.082 \times 10^{-3} \zeta_R^2} \quad (4)$$

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