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Thermal properties of milk fat, xanthine oxidase, caseins and whey proteins in pulsed electric field-treated bovine whole milk

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ABSTRACT

Thermodynamics of milk components (milk fat, xanthine oxidase, caseins and whey proteins) in pulsed electric field (PEF)-treated milk were compared with thermally treated milk (63 °C for 30 min and 73 °C for 15 s). PEF treatments were applied at 20 or 26 kV cm^{-1} for 34 μs with or without pre-heating of milk (55 °C for 24 s), using bipolar square wave pulses in a continuous mode of operation. PEF treatments did not affect the final temperatures of fat melting ($T_{melting}$) or xanthine oxidase denaturation ($T_{denaturation}$), whereas thermal treatments increased both the $T_{melting}$ of milk fat and the $T_{denaturation}$ for xanthine oxidase by 2–3 °C. Xanthine oxidase denaturation was ~13% less after PEF treatments compared with the thermal treatments. The enthalpy change (ΔH of denaturation) of whey proteins decreased in the treated-milk, and denaturation increased with the treatment intensity. New endothermic peaks in the calorimetric thermograms of treated milk revealed the formation of complexes due to interactions between MFGM (milk fat globule membrane) proteins and skim milk proteins. Evidence for the adsorption of complexes onto the MFGM surface was obtained from the increase in surface hydrophobicity of proteins, revealing the presence of unfolded hydrophobic regions.

1. Introduction

Pulsed electric field (PEF) technology has been extensively explored since the 1990s as a non-thermal method of food preservation. The use of PEF for pasteurising milk has efficacy in combination with a lower heat treatment, compared to higher temperature pasteurisation alone (Sharma, Oey, Bremer, & Everett, 2014). Thermal treatments applied to milk have been extensively studied, and heat-induced denaturation of milk proteins is well-understood (Corredig & Dalgleish, 1996; Ye, Singh, Taylor, & Anema, 2004). Heat treatment of milk causes unfolding of globular whey proteins and exposes the hydrophobic sites previously buried inside the native structure, as well as previously buried thiol groups in cysteine-containing proteins (Paulsson & Dejmek, 1990). A disulphide complex can be formed in this way between β-lactoglobulin and κ-casein after heating of milk (Dalgleish, 1990). Heat or mechanical treatments given to milk can disrupt the native structure of the milk fat globule (MFG) and lead to adsorption of casein micelles and whey proteins onto the milk fat globule membrane (MFGM) surface (Ye et al., 2004).

The changes that occur through heating, such as denaturation of proteins, interaction of denatured whey proteins with casein micelles, and casein micelle dissociation, can affect the functionality of milk (Anema, 2008). There is scarce literature on the effects of PEF treatment on milk components and the subsequent impact upon functionality (Sharma, Oey, & Everett, 2014, 2015). There is only one study (Xiang, Ngadi, Gachovska, & Simpson, 2007) reporting on the denaturation of proteins in whole milk after PEF-treatment. Milk protein functionality plays a major role in the rheological, structural and sensorial attributes of dairy products (Lucey & Singh, 1997), therefore, knowledge of PEF-induced effects on milk proteins is required.

Fat (triacylglycerols) is a major component of milk, with a melting point range of −40 to 40 °C due to the different fatty acids, resulting in a mixture of fat crystals and oil at room temperature (Lopez et al., 2002). Milk fat crystals show polymorphism in three different forms, α, β′, β, and the ratios depend upon the thermal history of the sample (Garti & Sato, 1988). Alongside milk proteins, fat globules play a key role in determining the textural, sensorial and technological properties of dairy products. The refrigeration

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temperature during supply chain movements and storage can crystallise milk fat and impact upon the final product attributes. In whipping cream, the presence of fat crystals can pierce the interfacial membrane leading to partial coalescence of fat globules and may eventually cause aggregation and phase inversion (Everett, 2007). In contrast, fat crystals inducing emulsion destabilisation may be desirable, as in the churning of butter (Narine & Marangoni, 1999). Considering the technical importance of milk fat crystallisation, it is important to understand the thermal properties of fat after PEF-treatment of milk.

Differential scanning calorimetry (DSC) has widely been used for investigating the thermal properties of milk proteins (Paulsson & Dejmek, 1990; Rüegg, Moor, & Blanc, 1977) and milk fat (Lopez, Briard-Bion, Camier, & Gassi, 2006; Lopez et al., 2002). Rüegg et al. (1977) reported that disruption of hydrogen bonds and hydrophobic interactions can lead to endothermic and exothermic reactions, respectively. These enthalpy changes can be measured by DSC. Heat-induced denaturation (unfolding) of globular proteins is an endothermic process whereas aggregation is an exothermic process. (Fitzsimons, Mulvihill, & Morris, 2007).

Knowledge of the impact of PEF treatment on milk fat and proteins will help to design dairy products where milk component functionality plays an important role in product quality attributes. In the present study, the crystallisation behaviour and polymorphism of milk fat from PEF-treated milk were investigated using DSC. The thermal transition characteristics and denaturation of proteins in milk after PEF treatment were also evaluated using DSC and compared with the effects due to thermal treatments. The extent of skim milk protein adsorption to the MFGM surface was examined by measuring surface hydrophobicity.

2. Materials and methods

2.1. Milk collection

Milk was collected from a single pasture-fed Belted Galloway-Jersey cross cow at a local dairy farm (Port Charmers, New Zealand) on the morning of each experimental day between 9 and 10 a.m. directly after milking. The cow was milked using a vacuum milking machine and the milk was transported to the laboratory in 500 mL glass bottles at ambient temperature with minimal mechanical disruption. The raw whole milk was stored at 20.3 ± 1.8 °C before treatments.

2.2. Milk processing

Thermal treatments of milk were carried out at 63 °C for 30 min and 73 °C for 15 s, and PEF treatments using a process design described in previous studies (Sharma, Bremer, Oey, & Everett, 2014; Sharma, Oey, Bremer et al., 2014). Milk was pumped at 4.2 mL s^{-1}, pre-heated to 55 °C for 24 s, and then PEF-treated at an electric field intensity of 20 or 26 kV cm^{-1} for 34 μs using bipolar square wave pulses at a constant pulse width of 20 μs and frequency of 20 Hz. Milk samples that were collected after circulating through the PEF system at a constant flow rate (4.2 mL s^{-1}) and after pre-heating (55 °C for 24 s) were referred to as 'pumped milk' and 'pre-heated milk', respectively. PEF process parameters, such as the total average residence time (ms), process energy per unit volume (J m^{-3}), and treatment time (μs) were calculated as reported before (Sharma, Bremer et al., 2014). PEF-treated samples were cooled to ~23.1 ± 1.6 °C using two cooling systems containing chilled water (12.8 ± 2.5 °C) in the process line. Milk samples were collected after 3 min of PEF treatment in duplicate for each independent treatment. The conductivity of fresh whole milk was 4.70 ± 0.17 mS cm^{-1} at 19.5 ± 2.2 °C, measured using a conductivity/temperature meter (Eutech Instruments Pte. Ltd., Singapore). The fat content of raw whole milk, as measured by the Babcock method (AOAC, 2005), was 4.5 ± 0.3%.

2.3. Isolation of milk fat globules

Raw whole milk or treated milk was centrifuged at 2200*g* for 20 min at 20 °C to obtain a cream fraction. The cream phase obtained from about 30 mL of milk was then re-suspended in an equivalent volume of simulated milk ultra-filtrate (SMUF) (Jenness & Koops, 1962), to remove loosely bound skim milk proteins from the surface of the MFGM. This suspension of MFGs in SMUF was centrifuged again under the same conditions, and the top cream layer was collected. The re-suspension and removal of the skim milk proteins by washing in SMUF was carried out immediately after the thermal and PEF treatments. The collected cream fraction after two SMUF washings was used for analysing the denaturation of whey proteins and adsorption to the surface of MFGs by DSC and surface hydrophobicity measurements. An aliquot was stored at −20 °C for DSC experiments and analysed within one week.

2.4. Differential scanning calorimetry

The thermal profile of the cream samples was determined by DSC (TA Instruments, New Castle, DE, USA). Individual milk proteins were used as references for identifying the thermal transitions in the cream components. The following bovine milk proteins were purchased from Sigma–Aldrich (St. Louis, MO, USA): α-lactalbumin (α-LA; ⩾85% purity), β-LG (⩾90% purity), and β-casein (⩾98% purity). Xanthine oxidase (0.4–1 units mg^{-1}) from buttermilk was obtained from Sigma–Aldrich. Each individual protein or cream sample was accurately weighed (8–10 mg) in an aluminium pan and hermetically sealed. An empty hermetic aluminium pan was used as a reference. The cream samples were equilibrated at 20 °C and then heated to 120 °C at a programmed heating rate of 2 °C min^{-1} followed by cooling back to 20 °C at the same rate.

DSC parameters, such as the onset temperature (T_{onset}, °C) and enthalpy change (ΔH, J g^{-1}) of fat melting or protein denaturation, and the final temperatures of fat melting ($T_{melting}$, °C) and protein denaturation ($T_{denaturation}$, °C) were calculated from each endotherm using the DSC software (TA Instruments). The amount of native protein in the PEF- or thermally-treated samples was defined in terms of enthalpy values (Xiang et al., 2007) using Eq. (1):

$$\text{Np } (\%) = \frac{\Delta \text{Ht}}{\Delta \text{Ho}} \times 100 \quad (1)$$

where N_p is the native protein in the cream fraction of treated milk on a weight basis, ΔH_t is the enthalpy change of the treated sample (J g^{-1}), and ΔH_o is the enthalpy change of the untreated milk (J g^{-1}), calculated from the area under the endothermic peaks. In the case of peaks seen only in the treated milk, the ΔH of individual proteins closest to the transition midpoint temperature was used.

Accordingly, the amount of denatured protein in the cream fraction of the treated milk samples was calculated using Eq. (2), as given by Manji and Kakuda (1987):

$$\text{Dp}(\%) = \frac{\Delta H_o - \Delta H_t}{\Delta H_o} \quad (2)$$

where D_p is the denatured protein in the cream fraction of treated milk on a weight basis.