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Microbiological and physicochemical stability of raw, pasteurised or pulsed electric field-treated milk

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A B S T R A C T

Pulsed electric field (PEF) processing was investigated as an alternative dairy preservation technology that would not compromise quality yet maintain safety. PEF processing of raw whole milk (4% fat) was conducted at two processing conditions (30 kV/cm, 22 μs, at either 53 or 63 °C outlet temperature) and compared with two thermal treatments (15 s, at either 63 or 72 °C) and a raw milk control and replicated twice. Milk bottles (2 L) from each treatment were incubated for two weeks, at 4 and 8 °C, and assessed for total plate count, pH, colour, rennetability, plasmin activity and lipid oxidation. The microbial quality of the thermal (72 °C/15 s) and PEF (63 °C) were similar. A drop in pH occurred after a change in counts was observed. Rennetability was not different between the treatments. Short chain acids dominated the volatile profile of the milk samples. Concentration of volatiles derived from microbial activity, namely 2,3-butanedione, acetic acid and other milk lipid derived short chain free fatty acids (e.g. butanoic and hexanoic acids), followed the trend of microbial activity in milk samples.

Industrial relevance: Research on the application of PEF to control spoilage and pathogenic microorganisms and enzyme systems in milk spans a wide array of processing equipment and reaction conditions. While industrial scale PEF processing of liquid milk for preservation and improved quality seems generally possible, substantiation of lower thermal damage under safe and scalable PEF conditions is required to enable economic feasibility.

1. Introduction

In many countries, raw milk is required to undergo thermal processing so that the milk is safe to consume (Roberts et al., 2005). Recent interest in the consumption of raw milk and raw milk products has led to the consideration of alternative dairy processing technologies that will not compromise milk quality and safety. One technology being investigated is pulsed electric field (PEF) processing, which can be used alone or in combination with thermal processing. PEF may reduce the detrimental effect that conventional thermal treatment has on milk sensory and physio-chemical properties (Buckow, Chandry, Ng, McAuley, & Swanson, 2014). In the current study, PEF was investigated at two processing conditions and compared with two thermal treatments and a raw milk control for their effect on microbial counts, pH, colour, rennetability, plasmin activity and lipid oxidation.

Continuous PEF processing for approximately 20–50 μs, at approximately 25–30 kV/cm, in combination with mild temperature (50–60 °C), has been reported to deliver reductions in microbial load comparable to conventional thermal pasteurisation at 72 to 75 °C for a few seconds (Sharma, Bremer, Oey, & Everett, 2014; Walter, Knight, Ng, & Buckow, 2016). PEF application to milk under these PEF conditions can minimise thermal damage and affect protein functionality, colour and nutritional properties (Bermudez-Aguirre, Fernandez, Esquivel, Dunne, & Barbosa-Canovas, 2011). For example, PEF treatment of reconstituted skim milk (at its natural pH), at 35 kV/cm, 19.2 μs and up to 70 °C, did not affect the physico-chemical properties of milk caseins or whey proteins (Liu et al., 2015; Sui, Roginski, Williams, Versteeg, & Wan, 2011). Under PEF conditions (30 kV/cm for up to 240 μs at 50 °C) suitable for 5 $\log_{10}$ reduction of pathogenic microorganisms, milk can exhibit improved rennetability compared to heat pasteurised milk (Yu, Ngadi, & Raghavan, 2012).

Dairy enzymes are generally regarded as highly stable under mild PEF processing conditions (i.e. <60 °C) which can have advantages for flavour development in cheese and other dairy products, but can also lead to instability problems in long shelf-life of dairy products (Buckow et al., 2014). For example, bovine alkaline phosphatase or lactoperoxidase largely remain active after PEF treatment for 20–25 μs at up to 38 kV/cm and <60 °C (Jaeger, Meneses, Moritz, & Knorr, 2010). However, very little is known on the effects of PEF processing on plasmin, which is relatively heat-stable and can survive pasteurisation temperatures (e.g., 15 s at 72 °C). Recently, Sharma, Oey, Bremer, and Everett (2014) reported a decrease in plasmin activity in whole milk using PEF at up to 26 kV/cm for 20 μs and 55 °C pre-

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treatment temperature. It is likely, however, that the temperature development during PEF processing under these conditions will have significantly contributed to the partial inactivation of the enzyme.

Scale-up of PEF processes for the extension of milk shelf-life to pilot-scale (i.e., >100 L/h) or full commercial-scale (i.e., >1000 L/h) life continues to be an engineering challenge as most PEF studies are conducted using small sample volumes under either batch or laminar flow set-up. Inherently, treatment conditions at a larger scale are different in electric field uniformity, flow behaviour (laminar vs turbulent), uniformity, heat conduction and residence times. However, to make PEF a commercially viable, alternative preservation technology for milk it is important that milk PEF-treated under commercial (high-throughput) conditions maintains the flavour and nutritional value of fresh milk better than pasteurised milk.

Thus, the main objective of this study was to evaluate the microbiological, physicochemical (pH, colour, rennetability and gelation parameters), chemical (GC–MS) and selected enzymatic characteristics of milk treated by PEF or heat under scalable (i.e. pilot-scale) processing conditions in order to compare both treatments and analyse their effects during storage. To the best of our knowledge this is the first study that reports on PEF processing of raw milk at a constant flow of 2.4 L/min.

2. Material and methods

Raw milk (2 × 1000 L) was obtained from a Victorian dairy factory on two occasions, one week apart. The milk was processed upon arrival, approximately 48 h after milking. Raw milk was homogenised with a Rannie high-pressure homogeniser (type 30.60, SPX Flow Technology, Soeborg, Denmark) at 21 MPa (with 17.5 MPa in the 1st stage and 3.5 MPa in the 2nd stage) and kept agitated until thermal or PEF treatment. There were four treatments, which included two thermal and two PEF treatments as well as a non-processed control (raw milk). The process conditions are specified in Table 1. Milk treatments were dispensed into 2 L sanitised milk bottles, in quadruplicate, and duplicate bottles were stored at both 4 and 8 °C. Analysis of the milk was conducted on day 0 and then daily for up to two weeks, excluding weekends. The entire experiment was replicated twice, designated Trial I and Trial II.

2.1. Thermal and PEF processing

Thermal thermisation and pasteurisation by heating to 63 or 72 °C for 15 s was achieved using an in-house manufactured 1500 L/h plate heat exchanger/pasteuriser (CSIRO, Highett, Victoria, Australia).

The PEF system (Diversified Technologies Power Mod™ 25 kW Pulsed Electric Field System (Diversified Technologies Inc., Bedford, MA, USA)) used in this study consists of two co-linear electrodes described elsewhere (Buckow, Schroeder, Berres, Baumann, & Knoerzer, 2010). Each set of electrodes had an electrode gap of 6 mm and an inner diameter of the insulator of 5 mm resulting in a volumetric capacity of 0.118 mL and an approximately residence time per chamber of 3.19 ms at a constant flow of 2.4 L/min. The applied peak voltage was approximately 19,500 V and the duration of each rectangular pulse was about 1.5 μs at a frequency of 1176 Hz. In total, approximately 15 pulses were applied to the milk over the two treatment chambers resulting in a total treatment time of approximately 22 μs. In our recent literature review on the effects of PEF processing on microbial and sensory quality of dairy products we identified some of these PEF conditions as being effective on inactivation of spoilage and pathogenic bacteria while retaining most of the functional, nutritional and sensory properties of whole milk (Buckow et al., 2014). Based on this literature data, PEF processing at approximately 30 kV/cm for 22 μs and 63 °C (T_{OUT}) will result in a > 4 $\log_{10}$ CFU/mL reduction of relevant dairy microorganisms whilst largely retaining nutritional and functional properties of raw milk. Dropping the PEF processing temperature to 53 °C (T_{OUT}) will not impact raw milk sensory and functional properties but will limit microbial reduction to 2–4 $\log_{10}$ CFU/mL. However, such a treatment might still allow industry to slightly extend the shelf-life of raw milk.

Three tubular heat-exchangers of 3.6 m length were connected to the PEF system for temperature control of the milk. The first heat-exchanger increased the milk temperature from 8.6 °C to 42 or 51.8 °C within 44 s. Due to heat loss in the pipe (~10.18 W/m^2) an estimated temperature drop of 0.8 °C occurred resulting in a real inlet temperature to the PEF system of 41.2 or 51.0 °C, respectively. Due to Ohmic heating during PEF treatment, the temperature of the milk increased by approximately 12 °C (Table 1). The PEF outlet temperature were approximately 63 (PEF63) and 53 °C (PEF53), respectively. The milk was at this temperature for approximately 12 s before it was cooled back to the respective inlet temperature in the second heat-exchanger within 22 s. The milk passed through the second co-linear set of electrodes where the temperature increased again to 63 (PEF63) and 53 °C (PEF53) where it was held for approximately 12 s before cooled to approximately 15 °C in the third heat exchanger. Milk samples were collected in a stainless steel tank from which they were aseptically sampled into 2 L plastic bottles.

2.2. Composition of milk

The milk composition was determined on day 0 by Mid-Infrared (IR) analysis technology (LactoScope, Advanced Instruments, Inc., Norwood, MA, USA). A single milk sample was analysed from each treatment for both trials.

2.3. pH

The pH measurements were performed in duplicate by direct immersion of the electrode in the sample, using a PHM210 pH Meter (Radiometer, Copenhagen, Denmark). Samples were collected daily during the week during the storage trial (14 days) until the pH fell below pH 6.

2.4. Colour

The L*, a*, b* colour parameters of samples were measured in triplicate with the CIELAB* scale using a Minolta CR-400 colorimeter (Konica Minolta, Shanghai, China), taking L* as the main value due to the bright nature of the product (milk). Colour was measured on day 0 for both trials as well as on day 13 for Trial II.

Table 1
Processing parameters used in this study.

Code	Treatment	T_{out}[a] [°C]	E[b] [kV/cm]	Treatment time	PEF ΔT[c] [°C]	Pulse energy [J]	PEF specific energy [kJ/L]
Raw	Untreated raw milk	–	–	–	–	–	–
Thermal72	"Pasteurised"	72	–	15 s			
Thermal63	"Thermised"	63	–	15 s	–	–	–
PEF63	PEF condition 1	63	30	22 μs	12.4	3.43	94.15
PEF53	PEF condition 2	53	30	22 μs	11.8	3.27	89.89

[a] Outlet temperature.
[b] Electric field strength.
[c] ΔT represents the temperature increase in each PEF treatment chamber.