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Factors affecting the inactivation of the natural microbiota of milk processed by pulsed electric fields and cross-flow microfiltration

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Prior to processing milk and cream were standardised and homogenised. Skim milk was cross-flow microfiltered (CFMF) prior to treatment with pulsed electric fields (PEF) or high temperature short time (HTST) pasteurization. The effect of temperature of the skim milk and product composition on the efficacy of PEF treatment was determined. The electrical conductivity of the product was related to fat and solids content and increased 5% for every g/kg increase of solids and decreased by nearly 0·7% for every g/kg increase of fat. From the three microbial groups analyzed (mesophilic, coliform, and psychrotroph) in milks differences ($P<0·05$) in the inactivation of mesophilic microorganisms were observed between the counts following PEF treatment, while HTST pasteurization resulted in higher reductions in all different counts than those obtained after PEF. Increasing the skim milk temperature prior to PEF treatment to about 34 °C showed equivalent reductions in microbial counts to skim milk treated at 6 °C in half the time. The reductions achieved by a combination of CFMF and PEF treatments were comparable to those achieved when CFMF was combined with HTST pasteurization. A higher reduction in coliform counts was observed in homogenised products subjected to PEF than in products that were only standardised for fat content.

Keywords: Milk natural microbiota, hurdle technology, pulsed electric field, cross-flow microfiltration, fluid milk, fat content, electrical conductivity.

The dairy industry still relies on thermal processes to ensure safety and quality of fluid milk. However, there is still a perception that these products have diminished nutritional quality. Thus, there has been a resurgence of interest in the adoption of non-thermal processing methods but it is unlikely that a single method can effectively replace thermal pasteurization.

Ross et al. (2003) and Raso & Barbosa-Cánovas (2003) have reviewed combinations of existing non-thermal technologies that can be used to achieve microbial inactivation in foods without damaging quality. Among these technologies, pulsed electric fields (PEF) have been used to reduce microbial loads in milk (Michalac et al. 2003; Fernández-Molina et al. 2006).

Using a hurdle processing approach, PEF has been combined with moderate heat and antimicrobials (Smith et al. 2002), thermosonication (Noci et al. 2009), and with heat in sequence (Walkling-Ribeiro et al. 2009). As PEF is more effective at moderate than low temperatures (Toepfl et al. 2006; Walkling-Ribeiro et al. 2010), a process called high electric field short time (HEST) was proposed by Sampedro et al. (2007).

Reviews by Sampedro et al. (2005), and Sobrino-López & Martín-Belloso (2010) suggest that different approaches are necessary to analyse the effect of milk nutrients on PEF effectiveness, with processing conditions, microbial analysis, and the food system as main factors.

Cross-flow microfiltration (CFMF) consists of the cross-flow of skim milk through a filter (1·4 µm pore size) under uniform trans-membrane pressure (Gesan-Guiziou, 2010). Following microfiltration, bacterial populations in the milk are reduced by up to 99·7% (Pedersen, 1992). Microfiltration has been incorporated into the Bactocatch system in combination with centrifugation, and an ultra-high-temperature (UHT) treatment (Kessler, 2002).

The objective of this study was to evaluate the effectiveness of PEF processing at different steps of milk manufacture to reduce numbers of the natural microbiota in milk with different fat levels. The impact of CFMF prior to PEF and the effects of cream homogenisation were investigated as well as the correlation between the fat and solids content and electrical conductivity.

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Materials and Methods

Conventional and alternative milk processing

Milk was obtained from the Elora-Ponsonby Dairy Research Station, University of Guelph. Prior to fat separation (Westfalia LWA 205, Centrico Inc., San Francisco, CA, USA), pre-heating (50 °C) was conducted in a dual stage heat exchanger (UHT/HTSTLab-25EDH, Micro-Thermics, Raleigh, NC, USA).

Following the conventional processing sequence shown in Fig. 1, milk was standardised to three fat contents (11, 20 and 31 g/kg). Homogenisation (NS2006H, GEA NiroSoavi S.p.A, Hudson, WI, USA) was conducted at 20 MPa (first stage) and 5 MPa (second stage), 15 Hz and a minimum supply pressure of 0·4 MPa. The heat exchanger was combined with the homogeniser to process milk at 50 °C, and subsequently cool it to 12 °C.

In the alternative processing sequence (Fig. 1), skim milk (5 g fat/kg) was passed through a CFMF pilot plant system (MFS-1, TetraPak, Aarhus, Denmark) with a membrane pore size of 1·4 µm and inlet and outlet pressures of 90, and 20 kPa, respectively. Creams were homogenised under the same conditions described above.

PEF treatment

Exponential decay pulses of 1·5 µs width were formed with a generator unit (PPS 30, University of Waterloo, Waterloo, ON, Canada) and applied in a three electrode co-axial treatment chamber, which has been described by Walkling-Ribeiro et al. (2011a, b).

Skim milk was pumped (Masterflex pump drive 7524-40 and pump head 77201-60, Cole Parmer Instrument Co., Vernon Hills, IL, USA) through silicone tubing to the PEF chamber. The product was either pre-cooled in a stainless steel coil submerged in ice, or preheated in the same coil submerged in a water bath (Isotemp 10L, Fisher Scientific, Hampton, NH, USA) as in Table 1. The milk at the chamber outlet following PEF processing was cooled to 12 °C in a coil submerged in a refrigerated water bath (NESLAB RTE-7, Thermo Scientific, Newington, NH, USA).

To measure temperature in the tubing, thermocouples were connected to stainless steel flow-through chambers and monitored using a wireless temperature data logger (OM-SQ2020-2F8, Omega, Stamford, CT, USA). Pulses were recorded with a two-channel digital storage oscilloscope using a bandwidth of 100 MHz and a sample rate of 1 GS/s (TDS1012B, Tektronix Inc., Beaverton, OR, USA).

Based on the PEF treatment conditions (Table 1), specific energy density (w_{PEF}) was calculated according to Zhang et al. (1995) considering chamber resistance (Ω), and product electrical conductivity. For total energy calculation, the heating and cooling energy were calculated based on specific heat capacity (3·9 kJ/kg °C) and temperature change as described by Sepúlveda et al. (2009).

Heat treatment of milk

High temperature short time (HTST) treatment was conducted in the aforementioned dual stage heat exchanger using a pre-heating temperature of 50 °C in the first stage. The heating and cooling energy required by the HTST treatment was calculated as explained above.

Microbiological Analysis

The raw milk initial counts were below 2,500 CFU/ml for mesophiles, 300 CFU/ml for coliforms and the psychrotrophic count was below 300 CFU/ml. To achieve a reliable estimate of the inactivation rates accomplished by the treatments, a portion of the milk (100 ml/l) was incubated at 18 °C for 24 h. This inoculation rate produced milks with populations of about 7·0 log CFU/ml for mesophiles, and 6·0 log CFU/ml for both coliforms and psychrotrophs. The upstream processing conditions (white boxes in Fig. 1) decreased the initial microbial population by less than 1·0 log CFU/ml.

Milk (1·5 ml) was collected before and after each processing stage, and cooled on ice prior to serial dilution in Ringers solution (Oxoid, Thermo Fisher Scientific, Basingstoke, UK), and spread-plating on media. Mesophile counts were performed on plate count agar (BD Difco, Sparks MD, USA) after incubation for 48 h at 32 °C (Laird et al. 2004), coliforms were enumerated on MacConkey agar (Remel, Thermo Fisher Scientific, Lenexa, KS, USA) after incubation for 24 h at 35 °C (Henning et al. 2004), and psychrotroph counts were carried out on plate count agar (BD Difco, Sparks, MD, USA) after incubation for 10 days at 7 °C (Frank & Yousef, 2004). Recovered microorganisms were counted and log reductions were evaluated subsequently. Reductions in microbial population were calculated by subtracting the microbial counts of treated samples from the counts of non-treated samples. The detection limit for these counts was 2·4 log CFU/ml.

Physico-chemical properties

Electrical conductivity was measured at room temperature with a handheld conductivity meter (CON 11, Oakton Instruments, Vernon Hills, IL), and pH with a pH meter (AB 15 Accumet Basic, Fisher Scientific, Hampton, NH). Compositional analysis of milk was conducted at the Laboratory Services Division, University of Guelph using a MilkoScan (FT 120 type 71200, Foss Analytics, Copenhagen, Denmark).

Statistical analysis

Statistical analyses were conducted using R version 2.10·1 (R Foundation for Statistical Computing, Vienna, Austria) in conjunction with the R Commander (McMaster University, Hamilton, Ontario, Canada) package for each set of data with a minimum of two batches and two repetitions per

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Table 1. Applied intensities of high intensity pulsed electric fields to milk products, and resulting temperature profiles

Treatment	Product	g/kg fat	Conductivity (S/m)	Flow Rate (L/h)	Frequency (Hz)	Treatment time (μs)	E. Field (kV/mm)	PEF Energy (kJ/L)	Total Energy (kJ/L)	Temperature (°C)	
										Inlet	Outlet
I. Standardised milks	Fluid milks	10–31	0·45–0·52	1·2	25	1913	3·2	1537	1670	14·3	46·0
				2·4	12	459	4·8	830	998	13·3	55·1
				1·2	12	918	4·8	1662	1830	17·0	55·1
II. Skim milk	Low temperature	5	0·50	2·4	20	765	4·0	924	1045	5·9	43·0
				1·2	20	1530	4·0	1848	1985	5·0	47·0
				2·4	12	459	5·6	1086	1258	6·3	56·1
	Mild temperature	5	0·50	4·8	20	383	4·0	462	722	34·3	50·3
				3·6	20	510	4·0	616	887	34·0	53·5
				4·8	12	230	5·6	543	826	34·3	56·3
III. Skim milk	CFMF + PEF	5	0·47	1·2	25	1913	3·2	1581	1736	19·1	42·3
				2·4	12	459	4·8	854	1063	18·9	56·2
				1·2	12	918	4·8	1709	1938	20·1	61·2
IV. Creams	Standardised	122	0·34	2·4	20	765	4·0	1359	1477	5·3	42·3
				1·2	20	1530	4·0	2718	2835	5·5	42·1
				2·4	12	459	5·6	1598	1748	5·7	50·4
	Homogenised	123	0·35	2·4	20	765	4·0	1322	1393	4·8	30·2
				1·2	20	1530	4·0	2644	2767	5·0	43·5
				2·4	12	459	5·6	1554	1704	5·0	50·5

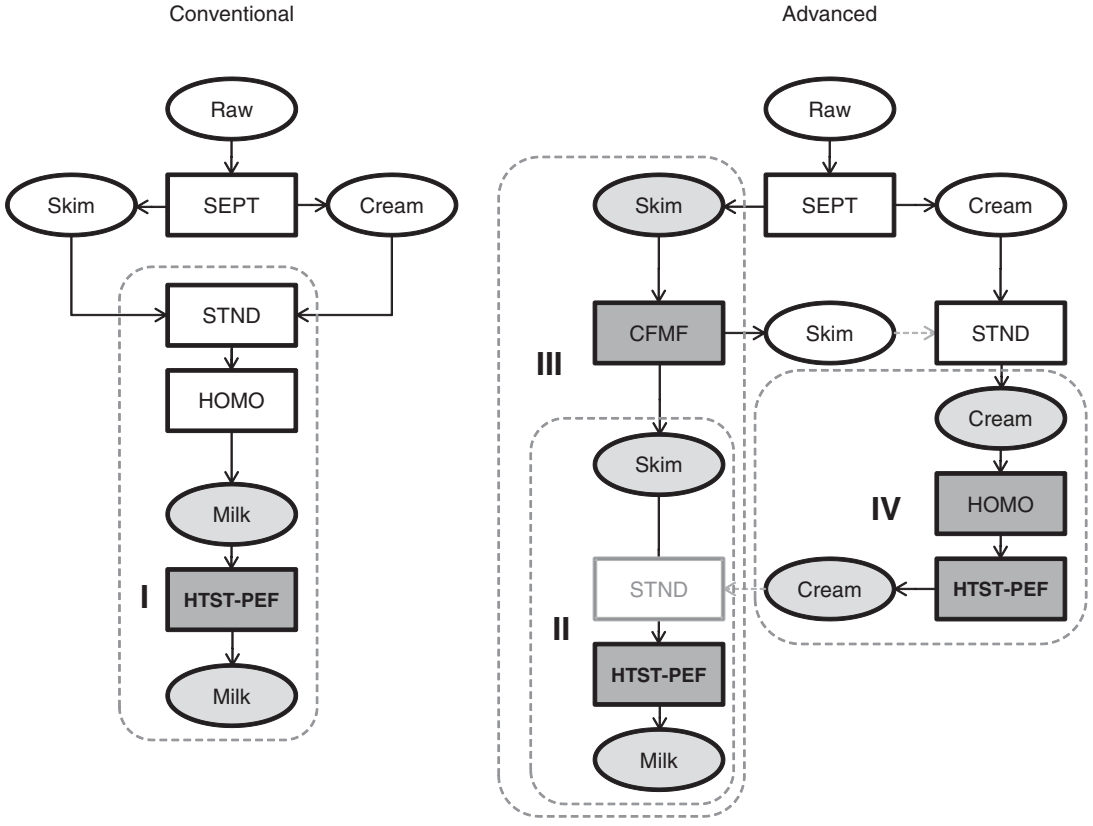

Fig. 1. Milk process sequence in conventional and advanced fluid milk processing. Roman numerals indicate the sequence of the studies. I. Standardised fluid milks; II. Skim milk; III. Combination of processes for skim milk treatment; IV. Cream. Products (ovals) and processes (rectangles) investigated in this study are highlighted in gray. SEPT = separation, STND = standardisation, HOMO: homogenisation, HTST = high temperature short time, PEF = pulsed electric fields, CFMF = cross-flow microfiltration. For description of treatment conditions see Table 1. Adapted from Pedersen (1992), and Walstra et al. (2006).

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Table 2. Variation in microbial populations after pulsed electric fields (PEF) and high-temperature short-time (HTST) processing of fluid milks

Fat (g/kg)	Initial Population (log CFU/mL)	PEF Intensity			HTST Intensity	
		3·2 kV/mm × 1913 µs	4·8 kV/mm × 459 µs	4·8 kV/mm × 918 µs	72 °C × 15 s	85 °C × 20 s
		Reduction in mesophilics (log CFU/mL)				
11	$7·3 \pm 0·47^{A}$	$2·2 \pm 0·08^{a}$	$2·3 \pm 0·06^{a}$	$3·8 \pm 0·09^{c}$	$4·7 \pm 0·09^{d}$	$4·9 \pm 0·07^{d}$
20	$6·4 \pm 0·43^{B}$	$2·0 \pm 0·09^{a}$	$2·3 \pm 0·21^{a}$	$3·1 \pm 0·20^{b}$	$> 3·8 \pm 0·00$	$> 3·8 \pm 0·00$
31	$6·9 \pm 0·81^{A}$	$1·8 \pm 0·15^{a}$	$2·1 \pm 0·25^{a}$	$3·2 \pm 0·18^{bc}$	$4·7 \pm 0·07^{d}$	$4·6 \pm 0·03^{d}$
		Reduction in coliforms (log CFU/mL)				
11	$5·8 \pm 0·24^{B}$	$2·6 \pm 0·15^{abc}$	$3·0 \pm 0·11^{abc}$	$> 3·3 \pm 0·00$	$3·5 \pm 0·05^{c}$	$3·5 \pm 0·03^{c}$
20	$6·1 \pm 0·23^{B}$	$2·4 \pm 0·41^{ab}$	$2·6 \pm 0·09^{abc}$	$2·3 \pm 0·10^{a}$	$> 3·8 \pm 0·00$	$> 3·8 \pm 0·00$
31	$6·3 \pm 0·71^{A}$	$3·4 \pm 0·09^{bc}$	$3·4 \pm 0·17^{bc}$	$3·0 \pm 0·35^{abc}$	$3·3 \pm 0·00^{abc}$	$3·4 \pm 0·03^{bc}$
		Reduction in psychrotrophs (log CFU/mL)				
11	$6·2 \pm 0·10^{A}$	$> 3·9 \pm 0·00$	$> 3·9 \pm 0·00$	$> 3·9 \pm 0·00$	$> 3·8 \pm 0·00$	$> 3·8 \pm 0·00$
20	$5·9 \pm 0·16^{C}$	$> 3·5 \pm 0·03$	$> 3·3 \pm 0·23$	$> 3·5 \pm 0·00$	$> 3·4 \pm 0·00$	$> 3·4 \pm 0·00$
31	$6·1 \pm 0·16^{B}$	$> 3·5 \pm 0·00$	$> 3·1 \pm 0·40$	$> 2·8 \pm 0·67$	$> 3·8 \pm 0·00$	$> 3·8 \pm 0·00$

^{A,B,C,a,b,c,d}Superscripted letters indicate that means are statistically different within their microbial group, with uppercase indicating the initial population, and lowercase their reduction. SEM = Standard error of the mean. The energy inputs from heat treatment correspond to 530 kJ/L (72 °C × 15 s), and 632 kJ/L (85 °C × 20 s). For detailed PEF treatment conditions see Table 1

treatment. The mean values of reductions in microbial populations for each group were compared separately using a multi-way analysis of variance and multiple comparisons of treatments by Tukey's test (HSD) with an alpha value of 0·05. The relationship between electrical conductivity and fat or solids content was determined by a linear regression analysis. Differences between the upstream and downstream products were evaluated by an Analysis of Covariance. Means of pH values were compared for each product and processing step as for the microbial populations.

Results

Microbial reduction in standardised fluid milks

The reductions in microbial counts in fluid milks following PEF are shown in Table 2. Higher reductions of total mesophiles ($P < 0·05$) were observed at lower fat concentrations when a higher electric field and a longer treatment time were applied. A high energy level from a high electric field strength and low pulse frequency proved to be more effective for inactivation of mesophiles than lower electric field strength and higher frequency (Tables 1 & 2). Differences ($P \geqslant 0·05$) were also found between the minimum and the maximum reductions of coliforms, while numbers of psychrotrophs fell below the detection limit for all treatments and fat concentrations. Higher levels of inactivation were observed for the mesophiles after HTST treatment of all milks, while it resulted in similar coliform count reductions compared with that obtained using the highest electric field strength. The survival of psychrotrophs in milk subjected to HTST was similar to PEF.

Effect of PEF processing temperature on microbial reduction in skim milk

Increasing milk temperature prior to PEF and lowering exposure time affected the survival of the three populations (Table 3). Increasing the temperature of milk from about 6 °C to 34 °C had almost the same effect on the inactivation of mesophilic bacteria at the two higher treatment intensities but the effect was achieved in half the time at the higher temperature, confirming that microbial inactivation is more effective at mild temperatures, and that colder products require additional energy (Tables 1 & 3). A reduction of 2·0 log CFU/ml for coliforms was achieved at the lowest PEF treatment conditions, which is a different result from the mild temperature conditions of a 2·7 log reduction ($P < 0·05$) (Table 3). The reduction of the psychrotrophs was higher at the low intensity treatment conditions. The data suggested that the increase in inactivation is related to temperature rise, but the difference between outlet temperatures was not significant ($P \geqslant 0·05$).

Milk processing with a combination of CFMF and PEF

Using CFMF and PEF in a hurdle approach resulted in higher reductions of the skim milk bacterial load yielding reductions of $\geqslant 4·0$ log CFU/mL for all three microbial groups when the highest electric field (4·8 kV/mm) was applied (Table 4). Doubling the treatment time at 4·8 kV/mm and 12 Hz led to the same results compared with 459 µs, but the latter resulted in lower total energy use (Tables 1 & 4).

Effect of cream homogenisation on the inactivation of native bacteria

The reduction in mesophiles was similar between the non-homogenized and homogenised creams, with significant increments ($P < 0·05$) in reduction with treatment time and electric field (Table 5). The results indicate that there was a greater inactivation ($P \geqslant 0·05$) of the coliforms and psychrotrophs in homogenised cream when treatment time was doubled at 4·0 kV/mm; whereas similar high levels of

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Table 3. Effect of the pulsed electric fields inlet processing temperature on the reduction of different bacteria groups with pulsed electric fields in skim milk

Temperature		PEF Intensity		Mesophilics	Coliforms	Psychrotrophs
				Initial population (log CFU/mL)		
Inlet (°C)	Outlet (°C)	(kV/mm)	(μs)	6·2 ± 0·13^{A}	6·3 ± 0·18^{A}	6·2 ± 0·08^{A}
				Reduction in microbial population (log CFU/mL)		
5·9	43·0^{M}	4·0	765	1·5 ± 0·09^{a}	2·0 ± 0·41^{a}	3·1 ± 0·18^{a}
5·0	47·0^{M}	4·0	1530	3·7 ± 0·10^{c}	> 3·9 ± 0·00	> 3·8 ± 0·00
6·3	56·1^{M}	5·6	459	3·4 ± 0·05^{bc}	> 3·9 ± 0·00	> 3·8 ± 0·00
34·3	50·3^{M}	4·0	383	2·8 ± 0·21^{b}	2·7 ± 0·10^{b}	2·9 ± 0·29^{a}
34·0	53·5^{M}	4·0	510	3·0 ± 0·23^{b}	> 3·9 ± 0·00	> 3·8 ± 0·00
34·3	56·3^{M}	5·6	230	3·3 ± 0·07^{bc}	> 3·9 ± 0·00	> 3·8 ± 0·00

^{A,M,a,b,c} Superscripted letters indicate that means within the microbial groups are statistically different with uppercase indicating the initial population, and lowercase their reductions. SEM = Standard error of the mean. For detailed PEF treatment conditions see Table 1

Table 4. Reduction of microbial populations after processing of skim milk with cross-flow microfiltration (CFMF) and pulsed electric fields (PEF) or high-temperature short-time (HTST) pasteurisation in a combined approach

Treatment	Intensity		Mesophilics	Coliforms	Psychrotrophs
			Initial Population (log CFU/mL)		
	(kV/mm)	(μs)	6·9 ± 0·47^{A}	7·0 ± 0·36^{A}	6·3 ± 0·39^{B}
			Reduction in microbial population (log CFU/mL)		
CFMF	†		2·1 ± 0·11^{a}	2·9 ± 0·07^{b}	3·2 ± 0·09^{b}
CFMF/PEF	3·2	1913	4·2 ± 0·10^{b}	> 4·7 ± 0·00	> 3·7 ± 0·00
CFMF/PEF	4·8	459	> 4·7 ± 0·00	> 4·7 ± 0·00	> 3·7 ± 0·00
CFMF/PEF	4·8	918	> 4·7 ± 0·00	> 4·7 ± 0·00	> 3·7 ± 0·00
	(°C)	(s)			
CFMF	†		2·0 ± 0·18^{a}	2·3 ± 0·08^{a}	2·0 ± 0·03^{a}
CFMF/HTST	72	15	> 4·4 ± 0·00	> 4·3 ± 0·00	> 4·1 ± 0·00
CFMF/HTST	85	20	> 4·4 ± 0·00	> 4·3 ± 0·00	> 4·1 ± 0·00

^{A,B,a,b} Superscripted letters indicate means within the microbial groups that are statistically different with uppercase indicating the initial population, and lowercase their reductions. †The standard CFMF conditions were inlet and outlet pressures of 90 (at 120 L/h), and 20 kPa (at 12 L/h) at 35 °C with a membrane pore size of 1·4 μm, with an estimated energy consumption of 37 kJ/L. SEM = Standard error of the mean. The energy inputs from heat treatment correspond to 530 kJ/L (72 °C × 15 s), and 632 kJ/L (85 °C × 20 s). For detailed PEF treatment conditions see Table 1

Table 5. Effect of pulsed electric fields processing on different groups of native bacteria in cream

Pre-treatment	PEF Intensity		Mesophilics	Coliforms	Psychrotrophs
	Initial population		Initial Population (log CFU/mL)		
NON-HOMO			6·5 ± 0·10^{A}	6·3 ± 0·05^{A}	6·2 ± 0·13^{A}
HOMO			5·6 ± 0·10^{B}	5·5 ± 0·05^{B}	5·5 ± 0·05^{B}
	E. field (kV/mm)	T. time (μs)	Reduction in microbial population (log CFU/mL)		
NON-HOMO	4·0	765	0·7 ± 0·00^{ab}	0·4 ± 0·05^{a}	0·5 ± 0·06^{a}
	4·0	1530	1·7 ± 0·05^{b}	1·6 ± 0·07^{b}	0·5 ± 0·00^{a}
	5·6	459	3·4 ± 0·06^{c}	3·6 ± 0·15^{c}	> 3·8 ± 0·00
HOMO	4·0	765	0·1 ± 0·05^{a}	0·2 ± 0·09^{a}	0·2 ± 0·03^{a}
	4·0	1530	1·2 ± 0·49^{b}	2·8 ± 0·19^{c}	2·0 ± 0·40^{b}
	5·6	459	2·9 ± 0·15^{c}	> 3·1 ± 0·03	> 3·1 ± 0·00

^{A,B,a,b,c} Superscripted letters indicate means within the microbial group that are statistically different with uppercase indicating the initial population, and lowercase their reductions. SEM = Standard error of the mean, NON-HOMO = non-homogenised, HOMO = homogenised. Homogenisation was conducted at two pressure stages of 20 MPa and 5 MPa respectively. For detailed PEF treatment conditions see Table 1

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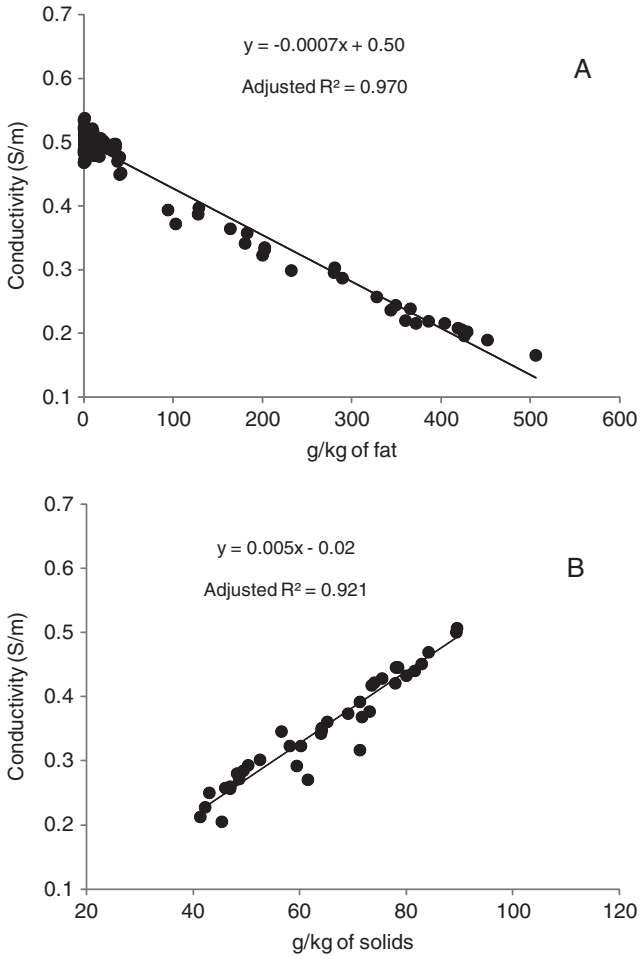

Fig. 2. Correlation between electrical conductivity and fat or solids content (A = non-homogenised products; B = non-fat milk products). Lines represent the linear regression established.

inactivation for all microbial populations in both homogenised and non-homogenised cream were achieved at 5·6 kV/mm.

Physicochemical properties of milk and conductivity

The non-homogenised products showed the highest correlation between conductivity and fat content (Adj-R^2 of 0·970) (Fig. 2A). When all the products were included, the correlation decreased slightly (Adj-R^2 of 0·824). This decrease in correlation may have been due to physicochemical variations caused by homogenisation and heating. A similar trend was observed for the relation between solids content and electrical conductivity for CFMF products, which resulted in a high Adjusted R^2 (0·706 to 0·966). When combined with skim milk, all the products gave a higher correlation (0·921) as shown in Fig. 2B.

From the slopes it can be estimated that for each 100 g/kg increase in fat the conductivity of the non-homogenised products decreased by 0·07 S/m in the range of 0·5 and 500 g fat/kg (Fig. 2A), while the conductivity increased 0·05 S/m for each 10 g/kg increase of solids content (Fig. 2B). These electrical conductivity measurements can be also related to temperature rise (Table 1). For example, applying 2·4 kV/mm at 12 Hz in skim milk (6·3 °C) resulted in a temperature rise to 56·1 °C compared with standardised cream (5·7 °C), which reached a maximum temperature of 50·4 °C, and homogenised cream (5·0 °C), the temperature of which was raised to 50·5 °C.

The pH of all milk products investigated ranged from 6·71 to 6·98 with no differences ($P \geqslant 0{\cdot}05$) between products.

Discussion

Microbial reduction in milks

The low impact of the fat content on the PEF effectiveness in fluid milks was confirmed (Table 2). Various reports describing the effect of milk fat content on microbial inactivation by PEF reach different conclusions (Sobrino-López & Martín-Belloso, 2010). Grahl & Märkl (1996) treated milks containing *Escherichia coli* (15 and 35 g fat/kg) and estimated regression coefficients between survivors and electrical field (B_E) or treatment time (B_t). Differences were found for the effect of these parameters on the two milks; suggesting that milk fat protected *Esch. coli* from inactivation during PEF treatment. Sobrino-López et al. (2006) inactivated *Staphylococcus aureus* in milks (0 and 30 g fat/kg) and applied a response surface quadratic model considering fat content, electric field, and pulse number, width, and type. They concluded that fat did not affect microbial inactivation. Reina et al. (1998) treated *Listeria monocytogenes* in milks (2 to 35 g fat/kg) and reported no differences in survival. Walkling-Ribeiro et al. (2009) suggested that for the protective effect of fat to be observed a threshold level must be attained. Therefore, the application of mathematical models like surface response analysis or tertiary models considering composition could aid the optimization of milk processing.

The sensitivity of the microbial groups to PEF inactivation agrees with previous reports in that mesophilic microorganisms are more resistant than coliforms and psychrotrophs. Sepúlveda et al. (2009) PEF treated whole milk and subsequently obtained high populations of mesophiles, followed by coliforms and psychrotrophs. The same trend has also been reported by Sepúlveda et al. (2005).

In the case of HTST the microbial reductions obtained are within the range quoted in previous studies. While Odriozola-Serrano et al. (2006) obtained a 2 log reduction in counts in whole raw milk at 75 °C for 15 s, Walkling-Ribeiro et al. (2009) obtained a 6·7 log CFU/ml reduction at 72 °C for 26 s. Higher reductions in count could have been expected at higher temperatures (85 °C × 20 s), but the presence of thermoduric microorganisms shown to be present in the milk might have caused a high post-pasteurization count.

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Effect of the PEF inlet temperature

The results of this study support the conclusion that PEF is more effective at temperatures above 30 °C by demonstrating a decrease in almost half the PEF energy applied to reduce microorganisms to the same level achieved in milks treated at lower temperatures (Tables 1 & 3). Reina et al. (1998) observed that increasing the initial temperature from 43 to 50 °C increased the inactivation of *List. monocytogenes*. Sampedro et al. (2007) reduced the energy required to inactivate *Lactobacillus plantarum* in an orange juice/milk-based beverage by increasing the product processing temperature from 35 to 55 °C in a PEF system with six co-field chambers. Advanced systems for pre-heating milk include the one used by Sepúlveda et al. (2005) in which the heat in the outgoing stream of the PEF chamber was recovered and incorporated into the incoming by using a tube-in-tube heat exchanger. Hoogland & de Haan (2007) estimated that PEF could be feasibly applied using pre-heating from 5 to 35 °C, and post-cooling from 50 to 5 °C, thereby, representing a more eco-friendly alternative for the inclusion in a PEF-based Bactocatch system.

Microbiologically, the synergistic effect of PEF and high temperature is attributed to increased membrane fluidity caused by phase transition of the phospholipids from gel to a liquid-crystalline state (Heinz et al. 2003; Jaeger et al. 2009). In milk, the liquefaction of colloidal particles that affect resistivity may add to the synergism. From a physico-chemical perspective a decrease in sample viscosity, and increase in conductivity may increase treatment efficiency and can be a consequence of the upstream operations (Walstra et al. 2006; Kessler, 2002).

Combining CFMF and PEF

Walkling-Ribeiro et al. (2011a) also studied the variation in natural microflora subjected to a combination of CFMF and PEF. A microbial reduction of > 4·0 log CFU/ml was obtained using this combination (Table 4). Studies on milk CFMF by Olesen & Jensen (1989) reported a reduction in the total count of almost 4 log CFU/ml. Saboya & Maubois (2000) reviewed milk CFMF and mention an average microbial reduction of 3·5 log CFU/ml. Madec et al. (1992) observed similar results for mesophiles, and also noted that the microbial reduction could be constant and independent of the initial population level. The reduction in coliforms and psychrotrophs were closer to those observed by Saboya & Maubois (2000). Elwell & Barbano (2006) reported a reduction in total counts of 3·8 log CFU/ml with CFMF, and a total reduction in count of 5·6 log CFU/ml was achieved when CFMF was combined with HTST pasteurization. This process has the potential of increasing efficacy by increasing the processing temperature.

Microbial inactivation by PEF in cream

The microbial reductions achieved in cream by PEF processing in our study were higher than those obtained by Mañas et al. (2001), and Picart et al. (2002). Mañas et al. (2001) conducted their study on cream with 330 g fat/kg (< 30 °C), and suggested that a higher fat content did not protect *Esch. coli* against PEF inactivation. Similarly, Picart et al. (2002) obtained a maximum reduction of 2·0 log CFU/ml when processing cream (200 g fat/kg) inoculated with a cocktail of three *List. innocua* strains (< 45 °C). Toepfl et al. (2007) suggested that agglomeration of microbial cells or between cells and insulating particles might impair the lethal effect of PEF, resulting in a reduction of the PEF effect by 45%. To compare the effect of fat content on the effectiveness of PEF, Otunola et al. (2008) treated *Esch. coli* and indigenous microorganisms in milk (20 g fat/kg) and cream (180 g fat/kg) and found no differences in rates of inactivation between the two products.

The PEF treatment of cream at temperatures around 5 °C (Table 1) resulted in lower microbial reductions than skim milk (Tables 3 & 5), and also in lower final temperatures under comparable PEF conditions (Table 1), which was most likely due to the lower electrical conductivity of creams. In conclusion, PEF treatment of cream may require an increase in the PEF intensity as in the commercial practice of heat pasteurisation, where cream needs to be treated at temperatures 5 to 7 °C higher than whole milk (Kessler, 2002).

Effect of composition in electrical conductivity

This study improves our understanding of the effect of milk fat and solids content on electrical conductivity and its implication for microbial inactivation, and product temperature rise during PEF processing. Mabrook & Petty (2003) and Sobrino-López et al. (2006) observed slightly higher conductivity values and a higher decrease in conductivity with increasing fat content compared with this study. In contrast, Ruhlman et al. (2001) observed a lower conductivity in skim compared with whole milk. These observations may be a result of the variation in the analytical methods. Michalac et al. (2003) observed no differences in electrical conductivity or pH of raw, UHT or skim milk after PEF, or heat pasteurisation, and Saboya & Maubois (2000) obtained similar pH values for CFMF products. The studies on PEF treatment of cream have not reported variation in pH.

The effect of conductivity on temperature rise during PEF treatment has been reported previously (Zhang et al. 1995; Heinz et al. 2001; El-Hag et al. 2006), but these earlier studies did not relate electrical conductivity with temperature rise and microbial inactivation.

Conclusions

In the present study the most effective PEF treatment of milk inoculated with a natural microbiota led to lower microbial reductions than those obtained with HTST. Treatment efficacy of PEF against the microorganisms was enhanced

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when higher electric field strengths were applied. Moreover, a lower milk fat content enabled higher bacterial inactivation by both PEF and HTST. Combining CFMF and PEF in a hurdle strategy increased the antimicrobial effect to a similar level as HTST treatment and consequently, further research on CFMF/PEF processing is recommended. PEF proved to be slightly more effective for homogenised than for standardised cream. The conductivity of the PEF-treated milk was primarily affected by the solids content and secondarily by the fat content, while no variation in the pH of the milk products was observed during processing. These results complemented with the observations in temperature rise, and microbial reduction will aid to design effective PEF treatments for processing milk and milk products.

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