LWT - Food Science and Technology 42 (2009) 735–739

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Shelf life of whole milk processed by pulsed electric fields in combination with PEF-generated heat

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ARTICLE INFO

Article history:
Received 8 January 2008
Received in revised form
29 September 2008
Accepted 1 October 2008

Keywords:
Pulsed Electric Fields
PEF
Whole moilk
Thermization

ABSTRACT

Application of pulsed electric fields (PEF) in combination with mild thermal treatment was studied to extend the shelf life of whole milk. Five pulses with peak electric field strength of 35 kV/cm and pulse width of around 2.3 μs were applied to milk at 65 °C and sustained for less than 10 s. Shelf life of the milk was extended by a minimum of 24 days. A synergistic interaction between PEF and mild thermal treatment was found. Neither the severe PEF treatment applied at lower temperatures, nor the mild thermal treatment equivalent, including longer treatment times than used in this study, could significantly extend the shelf life of milk. However, the combination of both PEF and mild temperature extended milk's shelf life adequately. The use of a thermal regeneration system improved the energy efficiency of the studied preservation process making it highly competitive with pasteurization.

1. Introduction

Thermal pasteurization of milk is a preservation technique that has been employed commercially and enforced in the U.S. for more than fifty years, and has been used successfully to control milk-transmitted diseases (Steele, 2000). Besides eradicating pathogenic bacteria, thermal pasteurization also can extend the shelf life of refrigerated milk for up to three weeks. Nevertheless, after the storage period the high microbial content and/or other undesirable characteristics (sensory) make the milk unacceptable for human consumption (Richter, Ledford, & Murphy, 1992). The development of a suitable technology capable of substituting such a well-established preservation process involves identifying a process capable of producing microbiologically safe products with extended shelf life and superior quality attributes. This alternative preservation technique needs to be accomplished at a reasonable energy expenditure level. PEF treatment is a novel food preservation process valued for its ability to eliminate bacteria from foods without increasing their temperature (Barbosa-Cánovas, Góngora-Nieto, Pothakamury, & Swanson, 1999). Preservation of milk and fluid dairy products seems to be one of the main market niches for PEF technology since it is mainly intended for preservation of pumpable fluid or semi-fluid foods (Qin, Pothakamury, Barbosa-Cánovas, & Swanson, 1996). Commercial application of PEF technology, however, has not been implemented yet, mainly due to lack of regulatory approval, high initial investment, and elevated processing costs (Góngora-Nieto, Sepulveda, Pedrow, Barbosa-Cánovas, & Swanson, 2002).

Application of PEF in combination with mild thermal treatment has been identified as a sound strategy to efficiently inactivate bacteria in food products at reasonable energy-consumption levels (Sepúlveda, Góngora-Nieto, & Barbosa-Cánovas, 2003). Simultaneous application of PEF and thermal treatments could be accomplished economically by employing a system where raw milk is preheated using a thermal regeneration system. In this system, cold raw milk flows through one side of a heat exchanger while hot processed milk flows through the other side. Pre-heated raw milk is then subjected to PEF treatment, where the desired temperature inside the PEF chamber is reached via electrically induced heating. Following, the PEF treated milk is cooled down in the cooling section of the heat regeneration system. This strategy makes the PEF system the sole source of energy in the process and takes advantage of the nonthermal and thermal effects of the applied energy. The increased efficiency of PEF application in combination with mild temperature can be explained at two levels. On one hand, it has been demonstrated that the bactericidal effectiveness of PEF treatment increases as a function of treatment temperature (Dunn & Pearlman, 1987; Hülsheger, Pottel, & Niemann, 1981). This effect is supposedly due to the thermal modification of the cell

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0023-6438/$34.00 © 2008 Published by Elsevier Ltd on behalf of Swiss Society of Food Science and Technology.
doi:10.1016/j.lwt.2008.10.005

membrane's mechanical resistance to permeabilization that takes place under PEF treatment (Jayaram, Castle, & Margaritis, 1992). On the other hand, simultaneous application of mild temperature during PEF treatment increases the overall energy efficiency of the process by allowing thermal regeneration, as explained earlier. An added advantage of this approach is that the treated product requires less cooling after treatment than treated products processed in a typical low-temperature PEF process. Finally, it is also important to mention that energy conversion from electricity to heat is highly efficient (over 95%), and does not involve heat exchange surfaces (Wilcox, 1928) where fouling and reduced efficiency are often encountered in traditional thermal pasteurization systems (Sandu & Singh, 1991). As a result, additional benefits are accrued using the heating effect of PEF technology.

The objective of this study was to evaluate the effectiveness of PEF treatment in combination with thermal treatment (thermal regeneration and PEF-induced heating) in extending the shelf life of whole milk.

2. Materials and methods

2.1. Milk

Three batches of raw whole milk were obtained on three separate days from the Washington State University dairy farm for processing by selected methods. Each batch was divided under sterile conditions in three sub-samples of approximately 20 l each. Treatments were assigned in random order. Four treatment scenarios were carried out for the milk: a) raw whole milk (stored at 4 °C), b) thermal treatment (65 °C for 10 s), c) treatment by high-temperature short-time (HTST) pasteurization, and d) treatment by PEF processing in combination with self-generated electric heat (65 °C for less than 10 s).

2.2. Thermal treatment

Two milliliter of raw whole milk were placed into test tubes. Tubes were immersed into a water bath (set at 97 °C) for 35 s or until milk reached 65 °C. Samples were removed from the water, left stand for 10 s and then immersed in an ice-water bath. The treated milk samples were cooled down and stored at 4 °C for microbial analysis for the duration of shelf life.

2.3. HTST pasteurization

High-temperature short-time thermal pasteurization of the raw whole milk was carried out in a plate heat exchanger operated at the Washington State University Creamery. A temperature of 72 °C for 15 s was applied in compliance with the Grade "A" Pasteurized Milk Ordinance (HHS/PHS/FDA, 2001). The treated milk was cooled down, bottled in 2-l glass containers, and stored at 4 °C for microbial analysis during shelf life.

2.4. Pulsed electric fields treatment

A pilot plant PEF system manufactured by Physics International (San Leandro, CA) and a cylindrical concentric-electrodes treatment chamber (Qin, Zhang, Barbosa-Cánovas, Swanson, & Pedrow, 1995) were used for PEF treatments. Based on preliminary trials it was decided that 5 exponentially decaying electric field pulses, with an approximate pulse width of 2.3 μs at FWHM (Full Width at Half Maximum), would be applied at 35 kV/cm. The raw whole milk was pumped at a flow rate of 72 l/h to avoid fouling of the electrodes (also based on preliminary findings). Heat produced in the treatment chamber was recovered from the outgoing milk stream and incorporated into the incoming stream by means of a tube-in-tube concentric heat exchanger in counter-current flow. The PEF treated milk reached a maximum temperature of 65 °C (<10 s); the average time required for the milk to flow from the treatment chamber to the end of the regeneration heat exchanger was 10 s. Treated samples left the regeneration system after reaching a temperature of 25 °C. After treatment, the milk was immediately cooled down, bottled in sterile two-liter glass containers and stored under controlled temperature conditions (4 °C) for microbial analysis during shelf life.

Relevant parameters such as inlet and outlet temperatures, thermal regeneration effectiveness, pulse shape, voltage, current, and energy expenditure were monitored throughout the process. Electrical parameters were measured with a digital oscilloscope (Hewlett-Packard 54530A, Colorado Springs, CO) connected to the treatment chamber using high voltage probes. Temperatures were monitored with digital thermometers (Cole-Palmer, Vernon Hills, IL).

2.5. Shelf life and quality assessment

Samples of milk treated at different conditions were taken at selected times to evaluate certain bacteriological (i.e. enterobacteria, mesophile, and psychrotroph counts), chemical, and visual and olfactory characteristics during storage at 4 °C. Mesophilic counts (quantified every other day), enteric bacteria counts (quantified every other day) and psychrotrophic counts (quantified once a week) as well as determination of titratable acidity were conducted during storage.

a) *Mesophilic and psychrotrophic bacteria.* Serial dilutions of milk were prepared with peptone water (0.1%) and pour-plated in Standard Plate Count Agar (Difco: Becton, Dickinson and Co., Sparks, MD). Mesophilic bacteria were incubated at 32 °C while psychrotrophic bacteria were incubated at 4 °C. Counting of colonies was performed after 2 and 7 days, respectively (Marshall, 1992).
b) *Enteric bacteria*. Serial dilutions of milk were pour-plated in Violet Red Bile agar (Difco: Becton, Dickinson and Co., Sparks, MD) with an over-layer and incubated at 32 °C. Counting of colonies was performed after 24 h (Marshall, 1992).
c) *Titratable acidity*. Acidity was quantified as indicated in the Standard Methods for the Examination of Dairy Products, and expressed as percentage of lactic acid (Marshall, 1992).
d) *Sensory observations*. Visual and olfactory inspections were carried out by authors daily to detect any abnormality (coagulation, thickening, curdling, change in color, sedimentation, off-odor, and/or bubbling.

2.6. Statistical analysis

The Excel program was used to compute averages and standard deviations (Microsoft Corporation USA, 2000).

3. Results and discussion

3.1. Raw milk

Table 1 presents the enteric, mesophilic and psychrotrophic bacteria counts in raw whole milk during storage under refrigeration. Although the raw milk used in this study was within the

Table 1
Logarithmic units for some microbial types in milk stored at 4 °C.

	Log (CFU/mL)			
	Raw whole milk		Treated at 65 °C/10 s	
0 day	6/7 days	0 day	6/8 days	
Enteric bacteria	2.55 ± 0.47	2.50 ± 0.39	0.83 ± 0.71	2.61 ± 0.31
Mesophiles	3.72 ± 0.39	5.50 ± 0.31[a]	2.80 ± 0.10	6.00 ± 0.10[a]
Psychrotrophic	1.44 ± 0.47	3.25 ± 0.27	0.00 ± 0.00	1.55 ± 0.39

[a] Six days.

bacterial limits required for raw milk pasteurization (HHS/PHS/FDA, 2001), the mesophiles count reached 2×10^4 CFU/ml (4.3 log units) after four days (Fig. 1). There was a significant load of enteric bacteria in the milk during storage as well; however, its population remained practically unchanged after eight days. The growth was probably delayed by the low storage temperature and presence of a larger number of lactic acid bacteria. It was also observed that psychrotrophic bacteria started to grow much earlier than the enteric bacteria, however, its growth was not significant during the storage period.

3.2. Thermal treatment

Enteric and psychrotrophic bacteria growth in the stored thermally treated whole milk (65 °C/10 s) is listed in Table 1. Mesophiles grew rapidly from 2.8 log units to 6 log units in seven days, however, fewer log units were observed for enteric (6 days) and psychrotrophic (8 days) bacteria at similar storage times (Table 1). The heat produced in this treatment obviously exceeded the thermal treatment produced during the PEF processing; however, due to practical considerations, it was decided to use this treatment as control.

3.3. Mesophilic bacteria

Total mesophilic counts registered for the milk during storage are depicted in Fig. 1, for the selected treatments. The shelf life examination was conducted for 54 days until the longest-lasting milk exceeded the bacterial limits established by the Grade "A" Pasteurized Milk Ordinance (PMO) which states that this type of bacteria in milk cannot exceed 2×10^4 CFU/ml (4.3 log units). It can be observed that the maximum log units permitted by the PMO were reached after 3, 4, 24, and 44 days of storage, respectively, for thermally treated, raw, PEF treated, and HTST treated milks, respectively. Therefore, the HSTS and PEF treated milk lasted more than the "regular" storage time (7–9 days) at low temperature (4 °C).

3.4. HTST pasteurization

Fig. 1 illustrates the mesophilic bacteria counts for the HTST pasteurized whole milk during storage. No enteric (<10 CFU/mL) and psychrotrophic (<10 CFU/mL) bacteria counts were found after the HTST treatment during the entire storage time. Based on its microbial content and sensory characteristics, the shelf life of the refrigerated commercially HTST pasteurized whole milk is usually estimated to be from 14 to 21 days (Richter et al., 1992). However, in this study the HTST pasteurized milk lasted up to 44 days before reaching a total bacterial count of 2×10^4 CFU/ml. This shelf life extension was probably due to the aseptic conditions used for packaging in the lab, which usually cannot be achieved in commercial processes. The increase in bacterial load after 44 days of storage was exclusively due to the increase in mesophilic bacteria.

Spoilage of refrigerated milk is normally caused by psychrotrophic bacteria polluting the pasteurized product after thermal treatment. Craven and Macauley (1992) stated that these types of microorganisms do not survive pasteurization temperatures. It has also been reported that in the absence of psychrotrophic bacteria, that thermoduric bacteria, which are capable of surviving pasteurization temperatures, are the main bacteria responsible for spoilage of pasteurized milk (Bodyfelt, 1980).

3.5. PEF and mild temperature processing

Fig. 2 shows the enteric, mesophilic, and psychrotrophic bacteria counts in whole milk processed by PEF in combination with mild temperature and stored under refrigeration conditions. The PEF processed milk lasted up to 24 days before exceeding the established total bacterial limits (4.3 log units).

The increase in microbial load was also due to an increase in psychrotrophic bacteria. This fact suggests that although PEF treatment was effective in the eradication and control of enteric bacteria, it cannot totally eradicate psychrotrophic or mesophilic bacteria, unlike the more effective HTST thermal treatment. Although these results could indicate the resistance of psychrotrophic bacteria to PEF treatment, it seems more probable that the limited growth of mesophilic bacteria and the outgrowth by psychrotrophic bacteria were promoted by storage temperature. Both types of microorganisms were significantly reduced by PEF treatment at day zero. A synergistic interaction between PEF and mild thermal treatment (65 °C × 10 s) produced in this study a remarkable shelf life extension of whole milk in comparison with other similar PEF treatments. Previous studies of PEF processing of raw whole milk by Qin et al. (1995), applying twenty exponentially decaying pulses of 2 μs with a peak electric field of 40 kV/cm at a maximum temperature of 54 °C were able to extend the shelf life of whole milk stored at 4 °C for only 14 days.

3.6. Titratable acidity and sensory observations

Titratable acidity (lactic acid) (Fig. 3) throughout the entire storage period for both HTST and PEF treated milks remained below the minimum sensory detection level (0.20%) (Bassette, Fung, & Mantha, 1986). Control samples surpassed the acid limit after eight days of storage.

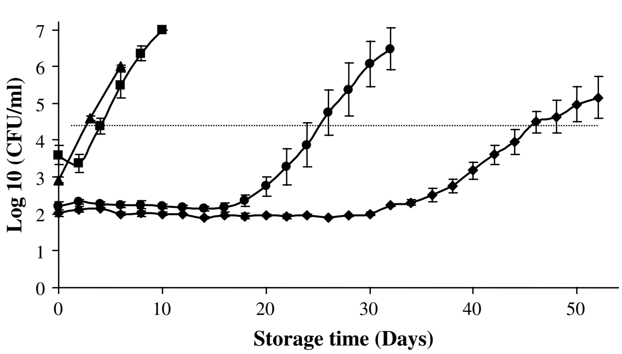

Fig. 1. Standard plate counts (mesophiles) for raw (■), thermally treated (▲), PEF treated (●), and HTST pasteurized whole milks stored at 4 °C (◆). Doted line indicates the maximum total count (4.3 log) limit.

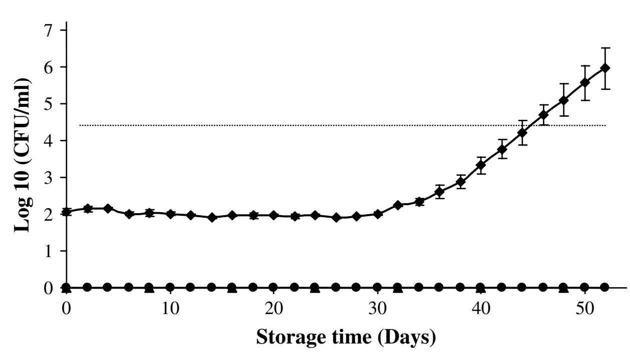

Fig. 2. Enterobacteria (●), mesophiles (◆), and psychrotrophs (▲) in PEF treated whole milk stored at 4 °C. Doted line indicates the maximum total count (4.3 log) limit.

Although no formal sensory analysis was carried out for this study, visual and olfactory inspection of both HTST and PEF treated whole milk was evaluated by authors every other day. No abnormalities were detected up to the end of the storage period in any of the treated samples. Mild thermal treatments, like those applied in combination with PEF in this study, did not cause significant changes in the natural characteristics of raw whole milk (Morales, Romero, & Jimenez-Perez, 2000). It is generally accepted that bacterial populations of around 1×10^7 CFU/ml are required before changes in the sensory perception of milk can be detected (Griffiths & Phillips, 1986; Schroder, Cousins, & McKinnon, 1982). Sweet curdling observed in HTST milk at the end of its shelf life may be a confirmation of the development of spore-forming bacteria.

3.7. Energy saving by PEF processing

Use of regenerative heat to increase the initial temperature of milk, and the enhanced killing effectiveness of PEF applied in combination with mild thermal treatments makes this preservation process highly competitive in comparison with conventional pasteurization. Heating milk in the range of 5–73 °C, assuming a specific heat (*Cp*) of 3.9 kJ/kg °C, theoretically takes 265.2 kJ/kg, as defined by:

$$Q = mCp\Delta T$$

where *Q* is heat (kJ), *m* is mass (kg), and *T* is temperature (°C). It is estimated that the total steam consumption used by an industrial pasteurization plant is about 70 kJ/kg (Sandu & Singh, 1991), implying a thermal regeneration efficiency of around 74%. However, if the efficiency of steam production is included into the calculations, and fuel consumption is considered instead of that steam consumption, the energy requirements in commercial pasteurization would increase up to 300 J/ml (Miller, 1986). On the other hand, the energy consumption of PEF treatment applied in this study to whole milk was 82 J/ml at a flow rate of 72 l/h. This lower energy usage involved the discharge of a 0.5 μF capacitor (*C*) charged at 40 kV (*V*) at a frequency of 4.1 Hz, resulting in delivery of 1640 W as defined by:

$$Q = \frac{CV^2}{2}$$

The inherit electrical resistance of the PEF system caused that only 74 J/ml reached the treated product, nevertheless, the use of electric energy instead of fossil fuel resulted in large energy savings. Modification of the PEF process, in regard to thermal regeneration, could accomplish that the precise thermal treatment required by law (heating the product to at least 72 °C for 15 s) (HHS/PHS/FDA, 2001) is achieved, eliminating or reducing the legal hurdles involved in the commercial adoption of this preservation process. Moreover, such increase in treatment temperature in the PEF process could easily be attained at practically no extra cost, without added energy expenditure, simply by a slight increase in the efficiency of the thermal regeneration system. The thermal regeneration system used in this study was able to heat the product to 65 °C, with a thermal regeneration efficiency of 71%, however, a regeneration efficiency of around 73.3% would have been enough to reach the required treatment temperature of 72 °C. Since thermal regeneration efficiencies up to 90% are technically possible and economically suitable (Reay, 1979), such modification of the system would not represent a major effort. Further studies employing this processing strategy would most likely demonstrate the feasibility and convenience of PEF/thermal processing of milk and its adoption by the fluid milk industry.

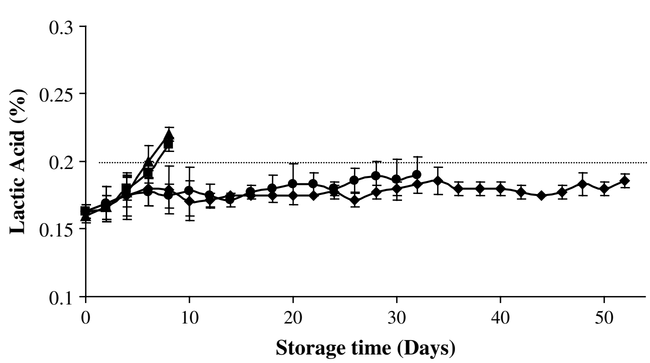

Fig. 3. Titratable acidity of raw whole milk (■), thermally treated (▲), PEF treated (●), and HTST pasteurized whole milks stored at 4 °C (◆). Doted line indicates the maximum acidity (0.2%) limit.

4. Conclusions

The combination of PEF and mild temperature effectively extended the shelf life of whole milk up to 24 days, which compares well to the shelf life of commercially pasteurized milk. PEF/thermal treatment was able to effectively eradicate enteric bacteria and reduce the population of mesophilic bacteria. Limited control of psychrotrophic bacteria in PEF/thermal treated milk was credited for milk's reduced shelf life when compared to HTST thermally pasteurized milk. PEF/thermal treatments also effectively controlled lactic acid bacteria, as titratable acidity remained low. Use of PEF in combination with thermal treatment is a promising energy-efficient method for the preservation of milk. Further studies using higher temperatures and holding times in combination with PEF could possibly improve this preservation process and would be a sound strategy to extend the shelf life of refrigerated milk.

Acknowledgements

Financial support for this study was provided by Avista Utilities and the Washington Technology Center.

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