Shelf life and quality of skim milk processed by cold microfiltration with 1.4- μ m pore size membrane, with or without heat treatment

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ABSTRACT

The objective of this study was to evaluate the effectiveness of cold microfiltration (MF), alone or in combination with heat treatment, in extending the shelf life of skim milk. Raw skim milk underwent MF at $6 \pm 1^\circ\text{C}$ with a ceramic membrane of 1.4- μm pore size, at a transmembrane pressure of 75.8 kPa and a crossflow velocity of 7 m/s. Samples of raw skim milk; MF skim milk; high-temperature, short-time (HTST)-pasteurized milk; and MF+HTST-pasteurized skim milk were stored at 6°C for 92 d. During the shelf-life study, the total bacterial count and degree of proteolysis were evaluated weekly. The study was replicated 3 times. Cold MF was very effective in reducing the microbial load in skim milk, and an average of 3.4 log reduction in vegetative bacteria was obtained. Although HTST pasteurization reduced the bacterial load by ~ 2 log, the MF+HTST process resulted in near complete elimination of vegetative microflora, with a total of ~ 4 log reduction. A 9-member sensory panel found no significant differences between skim milk samples processed with or without MF. The MF+HTST skim milk had only minor microbial growth after 92 d at 6°C , but its proteolytic shelf life was limited by plasmin activity. A reduction of plasmin activity and a slower rate of proteolysis were obtained by increasing the heat treatment temperature to 85°C . The results of this study can be used to make decisions regarding processing strategies that lead to increased skim milk shelf life.

Key words: skim milk, cold microfiltration, pasteurization, plasmin, proteolysis, shelf life

INTRODUCTION

The quality and shelf life of fluid milk depend greatly on the microbiological quality of the raw milk and that of the pasteurized product when it leaves the process-

ing plant. The Pasteurized Milk Ordinance (PMO) requires HTST-pasteurized fluid milk to have a total standard plate count lower than 20,000 cfu/mL (U.S. Food and Drug Administration, 2015). From a consumer's perspective, however, the end of fluid milk shelf life can be defined as the point at which consumers perceive the milk to be no longer acceptable based on its sensory attributes. For instance, 50% of sensory panelists detected off-flavors due to proteolysis when the decrease in casein as a percentage of true protein (CN%TP) exceeded 4.76% (Santos et al., 2003b). Some of the chemical changes in milk that lead to milk spoilage are caused by gram-negative psychrotrophic bacteria, which represent the predominant microflora in refrigerated raw milk. Although these bacteria do not survive heat treatment, they produce heat-resistant extracellular proteases and lipases that break down milk components (Law, 1979; Cousin, 1982). A high SCC can also lead to increased proteolytic and lipolytic activity that results in off-flavors and thus limit the quality and shelf life of milk (Azzara and Dimick, 1985; Ma et al., 2000; Santos et al., 2003a). Another cause for proteolytic degradation of milk is plasmin, a heat-stable blood enzyme that passes into milk and is optimally active at about pH 7.5 and 37°C (Driessen and Van der Waals, 1978; Bastian and Brown, 1996).

Microfiltration (MF) physically removes bacteria, spores, and somatic cells from skim milk, and it can thus be used to extend shelf life of fluid milk (Elwell and Barbano, 2006; Fritsch and Moraru, 2008). Elwell and Barbano (2006) reported that with respect to proteolysis, the shelf life of skim milk processed by MF (at 50°C) followed by HTST was 32 d at 6.1°C . Interestingly, Elwell and Barbano (2006) reported that although MF using 1.4- μm pore size led to undetectable levels of somatic cells in the permeate, about 75% of the somatic cells present in the raw milk were likely broken by the high shear during MF. However, the authors concluded that neither somatic cell removal nor breakage affect the shelf life of milk, and proteolysis in milk may be due to the activation of plasmin before processing.

Typically, MF of skim milk is conducted under warm conditions, at around 50°C . Conducting MF at low

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temperatures could offer some benefits, such as reducing the risk of thermophilic bacteria growth and biofilm formation on the membrane or in the recirculation loop of the MF system.

The main objective of this research was to evaluate the effect of cold MF with a pore size of 1.4 μm , as well as a combination of cold MF and pasteurization, on the microbial and proteolytic shelf life of skim milk and its sensory attributes. The results of this study can inform processing decisions regarding the best strategies for increasing fluid milk shelf life.

MATERIALS AND METHODS

Microfiltration Experiments

The pilot-scale MF unit used in this study consisted of a tubular ceramic membrane enclosed in a stainless steel housing, a tubular heat exchanger, and a centrifugal pump. The system was previously described by Fritsch and Moraru (2008). The tubular ceramic membrane of Tami design (GEA Filtration, Hudson, WI) had a pore size of 1.4 μm , 23 channels that were each 25 mm in diameter and 1,200 mm in length, and a total surface area of 0.35 m^2 . The MF unit was equipped with a counter-current tubular heat exchanger, which helped maintain the temperature of skim milk at $6 \pm 1^\circ\text{C}$ during the MF process, using chilled water as the cooling medium.

Before each MF run, the storage solution (HNO_3 solution, 0.5% vol/vol) was flushed from the membrane, which was then rinsed and simultaneously disinfected by circulating reverse osmosis water of 80°C for 20 min through the MF system.

Raw skim milk purchased from the Cornell Dairy Plant (Ithaca, NY) was stored at 6°C before use. All MF experiments were conducted with a cross-flow feed velocity of 7.0 m/s and a transmembrane pressure (TMP) of 75.8 kPa. These conditions were identified by Fritsch and Moraru (2008) as being optimal for cold MF of skim milk. The duration of each MF run was about 1 h.

Membrane Cleaning

After each MF run, the membrane was first flushed with reverse osmosis water, followed by alkaline cleaning with Ultrasil-25 (20 g/L; Ecolab, St. Paul, MN) for 30 min at 85°C . The membrane was then rinsed with 50°C reverse osmosis water, followed by cleaning with nitric acid solution of 5 mL/L concentration (HNO_3 solution, 0.5% vol/vol) for 20 min at 50°C . The cleaning efficiency was monitored by determining the water

flux of the membrane at 20°C , which was performed both after cleaning and before each MF run. The water fluxes recorded for the 3 processing runs for the shelf life study were 1,020 (new membrane), 804, and 795 L/m^2 per hour, respectively (values recorded immediately before MF processing).

Sample Collection, Pasteurization, and Preparation for the Shelf-Life Study

Using an inline sampling port, we aseptically collected 10 to 15 kg of MF permeate into 2 sterile 50-L polypropylene carboys (Cole Parmer Instrument Company, Vernon Hills, IL). Each carboy had a stainless steel sanitary ball valve attached to an outlet at the bottom, closed with a stainless steel end cap. The screw-cap lid for each carboy had 3 ports: 1 port for the inlet tube and 2 for high-efficiency particulate air (HEPA) filters. Tubing was extended from the inlet of the cap to the bottom of the carboy to reduce foaming during collection of the permeate (MF skim milk) and to facilitate pumping of the permeate out of the carboy. The end of the tube inside the carboy was weighed down with a stainless steel barbed insert. All tubing was firmly attached to the carboy cap using stainless steel hose clamps. The carboy setup, including all tubing, filters, and fittings, was autoclaved before use. After sample collection was completed, the carboy was disconnected from the MF unit and kept on ice until further use.

The MF permeate and raw skim milk were pasteurized using a laboratory-scale benchtop shell and tube pasteurization system, as described by Elwell and Barbano (2006). The stainless steel tubular heat exchanger (i.d. = 0.5 cm) consisted of 19 straight pieces of tube, each 105 cm long, connected by 18 U-turns, each 13 cm long. Flow entered at the lowest point in the system, with successive loops of tubes pitched upward to the system exit. The heat exchanger contained a heating section, a holding section, and a cooling section. The product was pumped through the heat exchanger at a flow rate of 640 mL/min. The skim milk was pasteurized either under HTST conditions ($72 \pm 1^\circ\text{C}$ for 25 s) or at a higher temperature (85°C for 25 s), as described later, then cooled to $4 \pm 1^\circ\text{C}$ before exiting the system.

Approximately 5 kg of pasteurized product was allowed to exit the system before sample collection began. The pasteurized product exiting the system was collected directly into sterile carboys, which were kept on ice until sampling for the shelf life study. The system was flushed with boiled reverse osmosis water at 80°C between treatments, with the cooling section turned off, to minimize any carryover or contamination between treatments.

Carboys containing unpasteurized MF skim milk, HTST-pasteurized MF skim milk, and HTST-pasteurized skim milk, respectively, were brought into a room with recirculated HEPA-filtered air, and connected to a peristaltic pump (Cole Parmer Instrument Company) in a HEPA-filtered air transfer hood sanitized with UV light (Labconco Corp., Kansas City, MO). Approximately 80 mL of each type of skim milk were pumped from each carboy into 42 sterile 100-mL amber serum bottles (Fisher Scientific, Chicago, IL). Each bottle was aseptically closed with sterilized Wheaton aluminum seals (Fisher Scientific), using an EZ crimper (Fisher Scientific).

Shelf-Life Study

Samples of raw skim milk, MF skim milk, HTST skim milk, and MF+HTST skim milk were kept under refrigeration at 6°C until the time of analysis. This temperature was selected because it was considered to realistically represent the temperature in retail dairy distribution (Boor, 2001) and has been used in shelf-life studies before (Caplan and Barbano, 2013). Three bottles from each treatment were used for initial microbial and chemical analysis. Subsequently, 3 bottles from each treatment were taken out for analysis every week. The refrigerated shelf-life study was conducted for a period of 13 wk.

Microbiological Analysis

The vegetative bacteria load in the skim milk samples was determined by standard plate counting. Samples were aseptically collected from each amber serum bottle using sterile disposable syringes (Fisher Scientific) and plated in duplicate on standard methods agar medium (Fisher Scientific). Plates were incubated at 32°C for 48 h, and the SPC method was used to quantify the colony-forming units (cfu/mL). Colony counts of less than 25 cfu/plate for the lowest dilution were deemed too low to count.

Somatic Cell Analysis

Somatic cell counts were determined using a somatic cell counter (Fossomatic 360 A/S N, Foss Electric, Hillerød, Denmark) at the DairyOne Laboratory (Ithaca, NY). The counter had a limit of detection of 3,000 cells/mL. Two replicate measurements were performed for each sample. For each replicate, 10 measurements were conducted for the raw skim milk and 15 measurements for the MF skim milk. A larger number of measurements for permeate was deemed necessary to

minimize the carryover of somatic cells by the equipment for samples with a very low SCC. Results were reported as thousands of somatic cells per milliliter.

Protein Analyses

The following analyses were performed: total nitrogen (TN; method number 991.20; AOAC International, 2010), NPN (method number 991.21, AOAC International, 2010), and noncasein nitrogen content (NCN), using the Kjeldahl method (method number 998.05, AOAC International, 2010). Nitrogen results were expressed as a protein equivalent, using 6.38 as the conversion factor. Total protein and casein (CN) were calculated as $(TN - NPN) \times 6.38$ and $(TN - NCN) \times 6.38$, respectively. Casein as a percentage of true protein was calculated as $(CN/TP) \times 100\%$. The change in CN%TP was used as an index of proteolysis. All protein measurements within a processing replicate were performed in duplicate.

Plasmin Activity

Plasmin activity in skim milk was assayed using the spectrophotometric method of Korycka-Dahl et al. (1983), with a slight modification. Plasmin activity, defined as the change in absorbance per minute, was determined by mixing 100 μ L of skim milk with 1,400 μ L of 50 mM Tris buffer in pH 7.4 at 37°C. The Tris buffer (Fisher Scientific) contained 110 mM NaCl (Fisher Scientific), 0.6 mM *H*-D-valyl-L-leucyl-L-lysine-*p*-nitroanilide dihydrochloride (Sigma Chemical Co., St. Louis, MO), and 3.3 mM ϵ -amino caproic acid (Sigma Chemical Co.). The reaction mixture was incubated for 60 min at 37°C. The absorbance at 405 nm was measured at 30-min intervals using a ThermoSpectronic spectrophotometer (Fisher Scientific). The rate of *p*-nitroanilide formation was computed from the linear part of the absorbance versus time curves. After a lag of about 30 min, the increase in absorbance was linear at least up to 3 h. A similar mixture without skim milk was used as a control.

Sensory Analyses

Sensory tests were performed at the sensory testing facility in the Department of Food Science at Cornell University. Milk for sensory testing was processed separately from the milk for the shelf-life study, using enhanced cleaning and sanitary handling conditions throughout the process. Samples of raw skim milk and MF skim milk to be used in the sensory testing were pasteurized (on d 1) using the previously described

shell and tube mini-pasteurizer to ensure that they were safe for consumption. The skim milk entered the mini-pasteurizer at $\sim 5^{\circ}\text{C}$ at a flow rate of 640 mL/min. The skim milk was heated from 5°C to 75°C in about 13 s, held at 75°C for 18 s, and then cooled to $\sim 3.5^{\circ}\text{C}$ before exiting the system. Approximately 1,000 mL of pasteurized skim milk was allowed to exit the mini-pasteurizer before collection into commercial 1-gallon (3.8-L) plastic jugs. The plastic jugs containing the pasteurized samples were then stored below 6°C until sensory testing.

Before sensory analyses, the skim milk samples were evaluated for standard plate counts, alkaline phosphatase tests, and coliforms at the DairyOne Laboratory and Eastern Laboratory Services (Waverly, PA), to verify that they were safe for human consumption. In parallel, in-house testing of the microbial load of all the samples was also performed.

Triangle tests were used to determine if the skim milk processed by MF significantly differed from skim milk that was not microfiltered after 5 and 24 d of refrigerated storage. The test consisted of each of the 90 panelists evaluating 3 samples, 2 of which were the same and 1 different. Samples were evaluated in a specified order, and sample order was randomized for the panelists. Panelists were asked to identify the sample that differed from the others, and if the correct answer was given the panelist was prompted to describe the perceived difference. The minimum number of correct identifications required for statistically valid test results for the desired significance level and number of panelists in the test was determined according to Bodyfelt et al. (1988).

For the samples stored under refrigeration for 24 d, a trained sensory panel performed a descriptive test immediately following the triangle test. The descriptive evaluation was conducted by 9 trained panelists associated with the Milk Quality Improvement Program at Cornell University who were trained to identify flavor defects in milk according to the Committee on Flavor Nomenclature and Reference Standard of the American Dairy Science Association (Shipe et al., 1978). Trained panelists rated the 2 skim milk samples on the following attributes: astringent, bitter, cooked, cowy, feedy, flat, foreign/chemical, fruity/fermented, high acid, lacks freshness, malty, milk carton, light oxidized, rancid, unclear, coagulated, other, and an overall flavor score.

Replication and Statistical Analyses of Data

All processing runs and sample analyses were replicated 3 times, with the exception of the sensory evaluation and the shelf-life study for the milk samples pasteurized at 85°C , which were performed only once.

Data were analyzed statistically with the ANOVA test, using Excel software (Microsoft Corp., Redmond, WA).

RESULTS AND DISCUSSION

Effect of MF on the Composition and Quality of Skim Milk

Removal of Microorganisms and Somatic Cells by Cold MF. For all cold MF runs, permeate fluxes of around 30 L/m² per hour were recorded at the beginning of the run, with the flux dropping to about 70% of this value after the first hour. It should be noted that, because of the low temperature used for MF in this study, the permeate was significantly lower than for MF under warmer conditions. For example, Elwell and Barbano (2006) reported a permeate flux of 200 L/m² per hour during MF of skim milk at 50°C .

The average vegetative bacteria count for the raw skim milk was 4.11 ± 0.48 log cfu/mL, and SCC were $\sim 115,000$ cells/mL (Table 1); both were well below the maximum regulatory limits prescribed by the PMO. The MF process very effectively reduced the microbial load of the raw skim milk, and an average reduction in vegetative bacteria of 3.38 ± 1.40 log was achieved. High-temperature, short-time pasteurization resulted in a reduction of 2.24 ± 0.17 log of vegetative bacteria in the raw skim milk. No vegetative cells were detected in the HTST-pasteurized MF skim milk, which indicates a > 4 -log reduction of the vegetative bacteria in skim milk after the combined process. Somatic cells were also efficiently removed from skim milk by MF. Although the initial raw skim milk contained over 100,000 SCC/mL, a very low SCC was found in the MF permeate—only 200 cells/mL, which is below the detection limit of the instrument (Table 1). As a note, no somatic cells were expected to pass through the 1.4- μm MF membrane. These values are consistent with data reported by Fritsch and Moraru (2008).

Effect of MF on Skim Milk Composition. Protein content was used to evaluate the effect of MF on skim milk composition because all other components in skim milk (lactose, minerals, other minor components) are water soluble or in concentrations too small to be significantly affected by the large pore size MF process. As seen in Table 1, MF resulted in approximately 15% reduction of the total protein content in the skim milk. This rather significant reduction occurs due to membrane fouling (Tang et al., 2014), but measures to counteract fouling can be applied to minimize protein losses. For example, Fritsch and Moraru (2008) demonstrated how backpulsing the membrane with CO₂ gas can physically disrupt the fouling layer and thus stabilize the flux during cold MF of skim milk.

Table 1. Vegetative bacteria, SCC, and protein content of skim milk subjected to different processing methods¹

Sample	Vegetative bacteria (cfu/mL)	SCC (cells/mL)	Protein content (%)
Raw skim	4.11 ^a ± 0.48	114,800 ± 6,657	3.33 ^a ± 0.14
Microfiltration skim milk (permeate)	1.10 ^b ± 1.19	200 ± 67 ²	2.84 ^b ± 0.59
HTST pasteurized skim	1.87 ^b ± 0.64	Same as raw skim	3.31 ^a ± 0.15
HTST pasteurized permeate	ND ³	Same as permeate	2.72 ^b ± 0.47
85°C pasteurized skim	1.48 ^d	Same as raw skim	3.19 ^d
85°C pasteurized permeate	ND ⁴	Same as permeate	2.20 ^d

^{a,b}Values with different letters within the same column are significantly different from each other ($P < 0.05$).

¹Values represent averages ± SD ($n = 3$); values for samples heat treated at 85°C were not included in the statistical analysis.

²Values are below the limit of detection for the equipment used for somatic cells analyses (3,000 cells/mL).

³ND = none detected (no growth observed on plates at the lowest dilution plated).

⁴Only one processing run available. Protein analyses were conducted in duplicate and microbiological analyses in triplicate.

Sensory Attributes of Skim Milk Processed by MF and HTST. To assess whether MF significantly affected the sensory properties of skim milk, triangle tests were performed on skim milk samples processed by MF versus non-MF skim milk at d 5 and 24 of refrigerated storage at 6°C, with 88 and 90 panelists, respectively. Both types of skim milk subjected to sensory analyses were pasteurized to ensure that they were safe for consumption. For samples stored under refrigeration for 5 d, the panelists did not notice any significant differences induced by the MF process ($P > 0.05$; Table 2). However, the triangle test performed after 24 d of refrigerated storage indicated that panelists perceived a difference ($P < 0.05$) between the skim milk that underwent MF compared with the non-MF skim milk. Some panelists characterized the MF skim milk as having a “smoother milky taste” and a “fresher taste” compared with the non-MF skim milk, but the majority of comments seemed to pertain to compositional differences. The MF samples were characterized using terms such as “less flavor, less sweet, thinner, lighter taste, less creamy, different aftertaste, more watered down, does not taste as strong” compared with the non-

MF samples. These latter remarks seem to be related to compositional differences between milk samples due to the slight reduction in milk solids by the MF process.

To further understand the differences between these samples, we submitted them to testing by 9 trained panelists. Table 3 shows the overall flavor scores according to each panelist. Overall, the trained panel evaluation did not indicate a significant difference between the MF and non-MF skim milk and provided no directional information. Interestingly, 7 out of 9 trained panelists chose the odd sample correctly during the triangle test session, but when tasting samples side by side they were not able to detect differences based on the sensory defect attributes they had been trained on. The main conclusion of the sensory testing is that MF did not seem to have a significant effect on the sensory properties of skim milk within 3 wk of processing.

Shelf Life of MF Skim Milk, with and without Heat Treatment

During refrigerated storage, the microbial load of raw skim milk exceeded the PMO limit for raw skim

Table 2. Triangle test results for refrigerated pasteurized skim milk, with and without microfiltration¹

Item	Time in refrigerated storage	
	Day 5	Day 24
Total panelists	88	90
Incorrect answers	64	49
Correct answers	24	41
Confidence level	0.16	0.99
<i>P</i> -value	>0.05 ($P = 0.84$)	<0.05 ($P = 0.01$)

¹Samples for the sensory testing were processed separately from the samples for the shelf-life study. The number of correct answers necessary to establish a significant difference in sensory properties between the 2 types of skim milk by 90 panelists is 38 for a significance level of 5%.

Table 3. Overall flavor score breakdown for pasteurized skim milk samples after 24 d of refrigerated storage, determined by a 9-member trained panel¹

Panelist	No microfiltration	Microfiltration
1	8.90	8.90
2	7.40	9.00
3	8.50	8.50
4	9.20	9.20
5	7.00	6.60
6	8.80	9.00
7	9.00	5.00
8	9.20	9.10
9	8.60	6.10
Mean ± SD	8.51 ± 0.79	7.93 ± 1.59

¹Samples were rated on a scale ranging from 0 (lowest) to 10 (highest).

milk (300,000 cfu/mL) first, followed by that of the MF permeate (Figure 1). The HTST-pasteurized skim milk had a slower microbial growth rate than the raw and MF skim milk and reached the PMO limit for HTST-pasteurized milk (20,000 cfu/mL) at 59 d. No quantifiable bacterial growth took place in the MF+HTST-pasteurized skim milk during the 92 d of storage at 6°C. Despite the insignificant microbial growth in the MF+HTST skim milk, proteolysis still occurred in these samples, and the decrease in CN%TP reached the 4.76% threshold after about 3 wk (Figure 2). As previously mentioned, this value coincided with the level of proteolysis at which 50% of panelists could detect sensory changes in skim milk samples (Santos et al., 2003b). As anticipated, the rate of proteolysis was the fastest for the raw samples (non-MF skim milk and MF skim milk). Although MF reduced the microbial load considerably, proteolysis was not affected, as indicated by the proteolysis rates for the HTST-pasteurized versus MF+HTST-pasteurized samples. A statistical analyses of the proteolysis data revealed an average rate of proteolysis across the 3 processing replicates of $0.23 \pm 0.03\%$ decrease in CN%TP/day for HTST-pasteurized milk and $0.22 \pm 0.01\%$ decrease in CN%TP/day for the MF+HTST-pasteurized skim milk, respectively, which were not statistically different from each other ($P = 0.58$). These findings are consistent with the data reported by Elwell and Barbano (2006) for milk microfiltered at warm temperatures. The practical implication is that, although the MF process can ex-

tend significantly the microbial shelf life of skim milk, MF-HTST skim milk underwent proteolysis, which was expected to lead to noticeable sensory changes.

Causes for Proteolysis: The Roles of Plasmin and Heat Treatment

For refrigerated skim milk with a low microbial load, proteolysis is most likely due to native milk proteases, which cause the development of off-flavors (Santos, et al., 2003b). Another reason for proteolysis in skim milk is the presence of somatic cells. In this work, the MF skim milk was virtually free of somatic cells (Table 1), which was expected based on the pore size of the membrane. Additionally, Elwell and Barbano (2006) demonstrated that some of the somatic cells in the initial milk can be physically disrupted by the high shear exerted on them during MF, which would release their contents into the MF milk. However, a previous report showed that somatic cells are not significant contributors to proteolysis unless present at concentrations of about 2×10^6 somatic cells/mL (Saeman et al., 1988).

The comparable rate of proteolysis for HTST skim milk and MF+HTST skim milk suggested that proteolysis may have been caused by the activation of plasmin (Saeman et al., 1988; Elwell and Barbano, 2006). Plasmin activity in skim milk samples subjected to MF and heat treated under different conditions was tested. The temperatures for HTST pasteurization were shown to increase plasmin levels in milk, which was attributed

Figure 1. Bacteria count in skim milk samples subjected to different processing conditions (raw, MF, HTST pasteurization, and MF + HTST pasteurization) over 13 wk of storage at 6°C. MF = microfiltration; Past = pasteurization; PMO = Pasteurized Milk Ordinance. Error bars represent SD (± 1 SD).

Figure 2. Proteolysis in skim milk samples subjected to different processing conditions (raw, MF, HTST pasteurization, and MF + HTST pasteurization) over 13 wk of storage at 6°C, measured by the decrease in casein as a percentage of true protein (CN%TP). Values represent averages $\pm$ 1 SD ($n = 3$). MF = microfiltration.

to inactivation of plasmin inhibitors and plasmin activator inhibitors (Ismail and Nielsen, 2010). However, higher heat treatments have been shown to reduce plasmin levels. Therefore, besides the 72°C heat treatment discussed in the previous section (Figures 1, 2), a heat treatment at 85°C was also evaluated for both plasmin activity and proteolysis during refrigerated shelf life.

Figure 3 shows the changes in plasmin activity of the processed samples compared with raw skim milk samples from the same batch. Changes in plasmin activity rather than absolute values were used due to some differences in plasmin activity between batches of raw skim milk (i.e., plasmin activity in the raw skim milk varied between 3.8 and 5.1 U/mL).

Plasmin activity in the MF skim milk was slightly higher than in raw skim milk, but the differences between the 2 types of skim milk were not statistically significant. Samples that were heat treated at 72°C, with or without MF, had significantly higher plasmin activity than the raw skim milk samples, but plasmin activity decreased by almost half the activity in raw milk ($P < 0.05$) after heat treatment at 85°C (Figure 3). These data are consistent with previous reports about the changes in plasmin activity in milk as a result of heat treatment. According to Humbert and Alais (1979), heat-labile plasmin inhibitors are inactivated by pasteurization, which could lead to an increase in plasmin activity after pasteurization. Lu et al. (2009) reported that heating milk to 75°C enhanced plasminogen activation and led to a considerable increase in

plasmin activity, while heating at 85°C and 90°C caused a significant decrease in plasmin and plasminogen-derived plasmin activities. Prado et al. (2007) also found that half the tissue plasminogen activator activity was lost in milk heated at 85°C for 15 or 30 s, and 30% of urokinase-type plasminogen activator activity was lost in milk heated at 85°C for 30 s. However, Lu et al. (2009) reported that during storage of processed milk, plasmin levels can be restored significantly because of the activation of remaining unfolded plasminogen. This activations would have implications for the proteolytic shelf life of milk.

We evaluated the kinetics of proteolysis during refrigerated storage of skim milk processed with and without MF or heat treatment at the temperatures. As seen in Figure 4, proteolysis of the skim milk samples followed linear kinetics for all processing conditions. The rate of proteolysis, expressed as the change in CN%TP per day (the slope of the linear regression lines in Figure 4), was virtually identical for the samples heated at HTST temperature (72°C, open symbols and solid trend lines in Figure 4): a drop of 0.22 CN%/TP per day for skim milk that was not MF, compared with 0.23 CN%/TP per day for skim milk that was MF. For samples heated at 85°C (closed symbols and dotted trend lines in Figure 4), the rate of proteolysis was lower: 0.14 CN%/TP per day for skim milk that was not MF, compared with 0.19 CN%/TP per day for skim milk that was MF. It is important to note that only 1 processing replicate was available for the 85°C treatment, which also explains

Figure 3. Plasmin activity in processed skim milk compared with raw skim milk. Values represent averages $\pm$ 1 SD ($n = 3$). MF = micro-filtration; temp. = temperature.

the variability of the data and the low coefficient of determination values for these 2 treatments. For this reason, no statistical evaluation of differences between the 2 heating temperatures can be made. Nonetheless, some discussion about the observed differences is still possible.

Most importantly, it appears that heat treatment at temperatures higher than those used for HTST pasteurization can potentially lead to gains in shelf life, even if proteolysis is not slowed as drastically as the differences in plasmin activity observed in Figure 3, possibly due to the partial restoration of plasmin levels. Yet

Figure 4. Rate of proteolysis (decrease in casein as a percentage of true protein, CN%TP) for processed skim milk samples stored at 6°C. Error bars represent standard deviations for the 72°C heat treatments ($n = 3$). Only one replicate was available for the 85°C heat treatments. MF = microfiltration.

an increase in pasteurization temperature needs to be properly evaluated because, while reduction of plasmin activity only occurs at temperatures above 85°C (Lu et al., 2009), an increase in pasteurization temperature can have unwanted consequences on the microbiological quality of milk (Martin et al., 2012). For example, Martin et al. (2012) found significantly lower bacterial counts after processing and at 21 d post processing in milk pasteurized at 76.1°C versus milk pasteurized at 79.4°C, while Ranieri et al. (2009) found lower bacterial numbers in milk heat processed at 72.9°C compared with milk processed at 85.2°C. Regarding the effect of MF, although it did lead to significant reduction of bacterial counts, it did not affect the rate of proteolysis. This finding suggests that MF may have additional benefits for treating cheese milk, which would combine the advantage of removing bacterial spores from milk while maintaining a high level of plasmin activity, which is beneficial in the cheese-ripening process (Ana et al., 2006).

CONCLUSIONS

The data presented here confirm that cold MF can effectively remove microorganisms and somatic cells from skim milk, without significantly affecting the sensory properties of the product. The addition of a minimal pasteurization treatment step led to a total microbial reduction of about 4 log and a microbial shelf life of skim milk that exceeded 92 d at 6°C. The limiting factor for the shelf life of the processed skim milk was proteolysis, which was primarily attributed to plasmin. Reduction of plasmin activity to increase the proteolytic shelf life of refrigerated fluid milk could benefit from an increase in pasteurization temperature, but the exact range of beneficial temperatures has to be evaluated because elevated pasteurization temperatures may have detrimental effects on the sensory quality and even the microbiological quality of the product.

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