Use of Microfiltration to Improve Fluid Milk Quality^{1,2}

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ABSTRACT

The objectives of the research were to determine the growth characteristics of bacteria in commercially pasteurized skim milk as a function of storage temperature; to determine the efficacy of a microfiltration and pasteurization process in reducing the number of total bacteria, spores, and coliforms in skim milk; and to estimate the shelf life of pasteurized microfiltered skim milk as a function of storage temperature. For the first objective, commercially pasteurized skim milk was stored at 0.1, 2.0, 4.2, and 6.1°C. A total bacterial count >20,000 cfu/mL was considered the end of shelf life. Shelf life ranged from 16 d at 6.1°C to 66 d at 0.1°C. Decreasing storage temperature increased lag time and reduced logarithmic growth rate of a mixed microbial population. The increased lag time for the mixed microbial population at a lower storage temperature was the biggest contributor to longer shelf life. For the second objective, raw skim milk was microfiltered at 50°C using a Tetra Alcross M7 Pilot Plant equipped with a ceramic Membralox membrane (pore diameter of 1.4 µm). The 50°C permeate was pasteurized at 72°C for 15 s, and cooled to 6°C. Bacterial counts of raw skim milk were determined by standard plate count. Bacterial counts of microfiltered and pasteurized microfiltered skim milk were determined using a most probable number method. Across 3 trials, bacterial counts of the raw milk were reduced from 2,400, 3,600, and 1,475 cfu/mL to 0.240, 0.918, and 0.240 cfu/mL, respectively, by microfiltration. Bacterial counts in the pasteurized microfiltered skim milk for the 3 trials were 0.005, 0.008, and 0.005 cfu/mL, respectively, demonstrating an average 5.6 log reduction from the raw count due to the combination of microfiltration and pasteurization. For the third objective, pasteurized microfiltered skim milk was stored

at each of 4 temperatures (0.1, 2.0, 4.2, and 6.1°C) and the total bacterial count was determined weekly over a 92-d period. At 6 time points in the study, samples were also analyzed for noncasein nitrogen and the decrease in casein as a percentage of true protein was calculated. After 92 d, 50% of samples stored at 6.1°C and 12% of samples stored at 4.2°C exceeded a total bacterial count of 20,000 cfu/mL. No samples stored at 0.1 or 2.0°C reached a detectable bacterial level during the study. When the bacterial count was <1,000 cfu/mL, shelf life was limited because sufficient proteolysis had occurred at 32 d at 6.1°C, 46 d at 4.2°C, 78 d at 2.0°C, and >92 d at 0.1°C to produce a detectable off-flavor in skim milk produced from a raw milk with a 240,000 somatic cell count.

Key words: microfiltration, shelf life, bacterial removal

INTRODUCTION

There is a desire in the fluid milk processing industry for an HTST pasteurization process that will produce fluid milk with a refrigerated shelf life of 60 to 90 d. Currently, bacterial spoilage is the most limiting factor in extending the shelf life of conventionally pasteurized HTST-processed fluid milk products beyond 14 d (Boor, 2001). Most fluid milk processing plants use HTST temperatures and hold times that are well above (Douglas et al., 2000) the minimum requirements in the Pasteurized Milk Ordinance (PMO) to extend shelf life. Ultrapasteurization (UP) processes can extend refrigerated fluid milk shelf life to 45 d. Although this type of heat treatment can extend the shelf life of milk with respect to bacterial growth, there is a disadvantage to this practice. Ultrapasteurization imparts a distinct cooked flavor to milk, which may be undesirable to some people. For example, children aged 6 to 11 have been found to prefer the flavor of HTST milk over UP milk due to the cooked flavor in the UP milk (Chapman and Boor, 2001).

Microfiltration (MF) in combination with HTST is an alternative to UP for extending refrigerated milk shelf life. The most common commercial microfiltration process for removing bacteria from skim milk is the Bactocatch process developed by Tetra Pak (Pully, Switzerland). In this process, initially proposed by

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Holm et al. (1986), cream is centrifugally removed from whole milk. Skim milk is microfiltered using a 1.4- μ m ceramic membrane. The bacteria are concentrated in the MF retentate, which represents approximately 5% of the skim milk. The Bactocatch system utilizes cross-flow uniform transmembrane pressure (Sandblom, 1978) to minimize membrane fouling. The MF retentate and cream undergo UP treatment (130°C for 4 s) and then are combined with the nearly bacteria-free MF skim permeate. The milk then receives a minimum HTST treatment. In a similar process (Krabsen et al., 1997) developed by APV (Aarhus, Denmark) called Invensys (te Giffel and van der Horst, 2004), the high-bacteria retentate, instead of being high-heat-treated, is fed back into the raw whole milk entering the centrifugal separator. The accumulation of bacteria in the MF retentate is removed in the separator sludge. Microfiltration has been used commercially in Canada by Natrel (a division of Agropur cooperative, Longueuil, Quebec) and in the United Kingdom by Cravendale (a subsidiary of Arla Foods, Leeds, UK). These companies tout the fresh taste, nutritional quality, and long shelf life of their milk. Natrel (formerly Ault Foods) achieves a refrigerated shelf life of 32 d (Eino, 1997). Microfiltration has been shown to be effective in reducing the number of bacteria in skim milk (Kelly and Tuohy, 1997). Reduction in total bacteria of 2.8 logs (Bindith et al., 1996; Hoffmann et al., 1996) has been reported for the Bactocatch MF process. Microfiltration utilizing a 1.4- μ m membrane provides complete removal of somatic cells from skim milk (Saboya and Maubois, 2000; te Giffel and van der Horst, 2004).

Our goal through this research and ongoing studies is to produce milk with a refrigerated shelf life of 60 to 90 d using minimum pasteurization heat treatment while retaining the flavor quality of fresh milk. Therefore, the objectives of our study were to determine the rate of bacterial growth in commercially pasteurized skim milk as a function of storage temperature, to determine the efficacy of a process of microfiltration followed by pasteurization in reducing the number of total bacteria, spores, and coliforms in skim milk, and to determine the effect of the process on extending skim milk shelf life.

MATERIALS AND METHODS

Spoilage Rate of Commercial Skim Milk

Experimental Design. Pasteurized skim milk (approximately 4 L) was obtained from the Cornell Dairy Store in 946-mL paperboard containers. Before packaging, this milk was processed by HTST with a pasteurization temperature of 79°C and a hold time of 16 s. The skim milk was transferred from the retail

containers and commingled into a single sterile polypropylene 10-L carboy. From this carboy, 1 L of skim milk was transferred into each of 4 sterile 1-L wide-mouth, screw-cap Nalgene polypropylene bottles (cat. no. 03-311-2E, Fisher Scientific, Hampton, NH), each containing a 3.18-cm magnetic stir bar. All transfers were carried out in a HEPA-filtered, UV-sterilized Labconco Purifier Class II Safety Cabinet (Labconco Corporation, Kansas City, MO). The sample bottles were stored at each of 4 temperatures (0.1, 2.0, 4.2, and 6.1°C) for 92 d.

Shelf-Life Storage Conditions. Milk samples in 1-L polypropylene bottles were immersed to 2.5 cm below the cap in water baths. To minimize temperature fluctuations, and as an added safeguard in case of a malfunction, all 4 water baths were operated in a walk-in cooler maintained at 6°C. The temperature of each water bath was monitored continuously using an 8-channel, thermocouple-based, temperature data logger (OM-CP-OCTTEMP, Omega Engineering Inc., Stamford, CT) that was NIST-certified to within 0.1°C and equipped with Type E thermocouples. The 4 target storage temperatures for shelf-life tests were 0, 2, 4, and 6°C.

Sampling. Samples were taken for total bacterial measurement semiweekly over the course of the 92-d storage period. Sample vials were immediately placed in ice until time of analysis (within 2 h). The total bacterial count (cfu/mL) of the samples was determined by flow cytometry using a Foss BactoScan FC (A/S N, Foss Electric, Hillerød, Denmark). Four replications of this experiment were carried out starting with milk from different days of processing in the Cornell Dairy.

Removal of Bacteria with Microfiltration

Equipment Cleaning and Validation. To validate the efficacy of the standard operating procedure for equipment cleaning, the microfiltration unit was cleaned and evaluated in the following manner. The storage acid solution (HNO₃, 0.3% vol/vol) was flushed from the system using reverse osmosis (RO) water. The system was then heated to 80°C by adding heated RO water to the balance tank. Ultrasil 25 caustic solution (Ecolab, St. Paul, MN) was added (1.5% vol/vol) and the system was recirculated at 80°C for 25 min. The system was slowly cooled to 40°C by circulating cool water through the heat exchanger built into the retentate circulation loop, and then flushed clear for 15 min with RO water at 25°C that had previously been boiled. The length of the rinse was previously determined to be sufficient time for the caustic soap to be completely flushed from the system. The system

was heated to 50°C by adding RO water at 90°C. Nitric acid (0.3% vol/vol) was added and the system was recirculated at 50°C for 10 min. The system was slowly cooled to 40°C with the heat exchanger and flushed clear with RO water at 25°C that had previously been boiled. During each step of the cleaning process, all valves were worked through their range of positions to allow the cleaning of every surface in the system, including valve seats. Valve cycling was not part of the standard operating procedure for cleaning at the time of this evaluation but was determined to be necessary based on prior experience and was added. Swabs (Puritan sterile cotton-tipped applicators, no. 25-806-1WC, Hardwood Products Company, Guilford, ME) were taken at each outlet of the three 3-way valves (valves 01, 03, and 08) and at the permeate outlet of the membrane. A 1-cm² surface area at each of these points was swabbed (Evancho et al., 2001). Swabs were stored in 10 mL of 0.02 M PBS, pH 7.4, and refrigerated until analysis (<2 h). Valve 01 directed flow of the feed, either from the internal or external balance tank. Valve 03 directed flow of the permeate bleed-off to either the balance tank or external collection vessel. Valve 08 directed flow of the retentate bleed-off to either the balance tank or external collection vessel. These points were chosen because they were the areas of the equipment that appeared most likely to harbor bacteria in the case of insufficient cleaning, due to their configuration or flow pattern. After a milk-processing run was completed, the system was cleaned again. Following the second cleaning, swabs were taken from the same areas. The bacterial analysis consisted of plating 1 mL of buffer on Standard Methods agar using the pour-plate method (Houghtby et al., 1992). The cleaning procedure was performed before each processing run, but validation of the efficacy of the cleaning procedure was conducted only once.

Experimental Design. Raw skim milk (approximately 265 L) was produced from whole raw milk by cold separation using a centrifugal separator (model 590, Equipment Engineering, Indianapolis, IN). The microfiltration unit used in this work was a Tetra Alcross MFS-7 pilot plant (TetraPak, Pully, Switzerland). The unit was fitted with Membralox ceramic membranes with a pore size of 1.4 μ m and surface area of 2.31 m². Prior to the study, the MF system was disassembled, inspected, and all gaskets were replaced. Following equipment cleaning, the MF system was heated to 50°C with RO water and recirculated until the start of processing. The raw skim milk was placed into a covered, jacketed stainless steel feed tank attached to the feed inlet of the MF plant. The milk was agitated and heated in this tank to 50°C by circulating 70°C water through the tank jacket. Once the milk

reached 50°C, it was processed immediately. The permeate collection rate was maintained at approximately 462 L/h and the retentate collection rate was maintained at approximately 25 L/h (i.e., approximately 5% of the feed rate). These rates gave a calculated flux of approximately 200 L/m²·h. This relatively slow flux was chosen to allow adequate time for sampling, monitoring, and data logging. The pressures at the inlet and the outlet of the membrane were approximately 400 and 200 kPa, respectively. The difference in transmembrane pressure between the inlet and the outlet of the membrane (ΔP) was maintained at 25 to 30 kPa, with an inlet transmembrane pressure of 49 to 53 kPa and an outlet transmembrane pressure of 23 to 27 kPa. To allow for the transition from water to milk at startup of the MF system, approximately 38 L of permeate was allowed to exit the system before permeate and retentate were collected. All of the retentate (approximately 12.5 L) was collected into a stainless steel milk can. Of the total permeate exiting the system, approximately 50 L was collected aseptically from an inline sample port on the permeate side of the MF into a sterile 50-L polypropylene carboy (cat. no. 02-960-20B, Fisher) for further processing. The screw-cap lid of the carboy had 3 openings, 1 for the inlet tube (Nalgene 1/4 i.d. $\times$ 3/8 o.d. 180 PVC tubing, cat no. 14-176-22, Fisher), and 2 ports with HEPA filters (Whatman HEPA-Vent, 1/4- to 3/8-inch tapered hose barb, cat no. 09-744-79, Fisher) attached. From the inlet port, tubing was extended to the bottom of the carboy to reduce foaming while pumping into the carboy and to allow for later pumping of milk out of the carboy. The entire carboy apparatus, including all tubing and fittings, was autoclaved together before use. The carboy, containing 50 L of MF permeate at 50°C, was disconnected from the MF plant and immediately attached to a peristaltic pump at the inlet of a laboratory-scale countercurrent stainless steel tubular heat exchanger (i.d. = 0.5 cm). The system consisted of 19 straight pieces of tube that were each 105 cm long, interrupted by eighteen 13-cm U-turns with flow entering at the lowest point in the system and having an upward pitch on successive loops of tubes until reaching the system exit. The heat exchanger was made up of 3 sections: a heating section, a holding section, and a cooling section. The MF permeate entered the heat exchanger at 50°C and was pumped through at a rate of 770 mL/min. The milk was heated to 72°C, held for 15 s, and then cooled to 6°C before exiting the system. The comeup time from 50 to 72°C was approximately 9 s and the comedown time from 72 to 6°C was approximately 22 s. The milk exiting the pasteurizer was collected into a second sterile 50-L, HEPA-filtered polypropylene carboy packed in ice.

An alkaline phosphatase assay (Charm PasLite, Charm Science, Inc., Malden, MA) was used to determine the efficacy of pasteurization and the consistency of the pasteurization treatment across all replications. Three replications of this experiment were carried out on different days with different batches of raw milk.

Sampling. All samples were collected for analysis in sterile 60-mL plastic snap-top vials (Capital Vial, Inc., Fultonville, NY). Samples of RO water from both the permeate and the retentate side of the membrane were taken for microbial analysis before the start of milk processing. Samples of the raw skim milk, retentate, permeate, and the pasteurized permeate were taken for bacterial and chemical analysis. The raw skim milk was sampled from the feed tank just before processing and the retentate was sampled from the stainless steel collection can following processing. The 50-L carboys containing the MF permeate and the pasteurized MF permeate were each connected to a peristaltic pump under the sterile transfer hood. The product was pumped from the carboys into the sterile plastic vials. All samples for bacterial analysis were stored on ice until time of analysis (within 2 h). For the purpose of comparison of the pasteurization process, a 237-mL container of pasteurized skim milk was purchased from the Cornell Dairy store and analyzed for microbial content. This skim milk was processed from the same raw skim milk used in the microfiltration experiment on the same day as the experiment. Microbial analysis of the commercial sample was conducted simultaneously with analysis of experiment samples.

Chemical Analysis. Somatic cells were enumerated (AOAC, 2000; method number 978.26; 17.13.01) using a Fossomatic 360 (A/S N, Foss Electric). Fat was determined by the Mojonnier method (AOAC, 2000; method number 989.05; 33.2.26). Kjeldahl analysis was used to measure total N (AOAC, 2000; method number 991.20; 33.2.11), NPN (AOAC, 2000; method number 991.21; 33.2.12), and noncasein nitrogen (AOAC, 2000; method number 998.05; 33.2.64). True protein and CN were calculated by difference. The decrease in casein as a percentage of true protein (CN%TP) was used as an index of proteolysis during storage.

Microbial Analysis. Samples of raw skim milk, retentate, permeate, and pasteurized permeate were analyzed for total bacteria, coliforms, and spores. The RO water was analyzed for total bacteria and coliform. Samples for total bacterial count were plated with Standard Methods agar using the pour-plate method, and incubated at $32 \pm 1^\circ\text{C}$ (Houghtby et al., 1992). Samples for coliform count were plated with violet red bile agar using the pour-plate method (Christen et al., 1992), and incubated at $32 \pm 1^\circ\text{C}$. Follow up coliform

confirmation tests were not performed and all coliform results were reported as presumptive. Spore analysis was performed according to Frank et al. (1992). All samples were plated in duplicate. Dilutions were carried out according to the standard method (Houghtby et al., 1992) using Butterfield's phosphate dilution buffer, pH 7.2 (AOAC, 1998).

Because the bacterial counts in the skim MF permeate samples were so low (<1 cfu/mL), a most probable number (MPN) technique (Garthright and Blodgett, 2003) was used to estimate the bacterial count. Permeate from MF was placed into 5 containers at each of 4 volumes: 500, 100, 10, and 1 mL. The 500-mL volumes were placed into the same type of sterile 1-L plastic bottles described earlier for the shelf-life study. Under the sterile transfer hood, the peristaltic pump was used to transfer MF permeate from the carboy into the 500- and 100-mL sample containers. A separate 120-mL vial was also filled with MF skim permeate, and from this the 10- and 1-mL samples were pipetted using sterile, disposable pipette tips into the 60-mL vials. Because the Bactoscan FC instrument requires a minimum sample volume of 20 mL, the 10-mL and 1-mL samples had to be diluted with a sterile medium. The Bactoscan FC instrument is designed for milk samples and this made it necessary to use a sterile dilution medium that had similar mineral content as milk. For this reason, autoclaved permeate from UF of skim milk was used as the diluent. All samples for MPN testing were incubated at 32°C for 6 d to ensure that any bacteria present would grow to sufficient number to produce a positive result. Positive or negative growth in the samples was determined using the Bactoscan FC. At the end of the incubation period, any samples containing bacteria should have grown to very high count, whereas samples containing no bacteria should have shown no growth. Therefore, samples analyzed by Bactoscan had either a very high count (i.e., $>10^6$ cfu/mL) or a very low count (i.e., $<10^3$ cfu/mL, the limit of detection). The number of positive and negative tubes at each dilution was used to calculate the most probable number estimate of the bacterial count in the original sample. This calculation was performed using an Excel spreadsheet provided by the FDA/Center for Food Safety and Applied Nutrition (Garthright and Blodgett, 2003).

Estimation of Shelf Life of MF Pasteurized Skim Milk

Experimental Design (Bacterial Growth). Following the collection of pasteurized permeate in the 50-L carboy and immediately following the aseptic collection of analysis samples for MPN analysis, 1 L of

the pasteurized permeate was transferred using the peristaltic pump into each of 8 sterile 1-L wide-mouth Nalgene polypropylene bottles (cat. no. 03-311-2E, Fisher), each containing a 3.18-cm magnetic stir bar. Two sample bottles were stored at each of 4 temperatures (0.1, 2.0, 4.2, and 6.1°C). The storage and sampling protocol were the same as previously described for evaluating the spoilage rate of commercial skim milk. The bacterial analysis by flow cytometry was also the same. For the third replication of the experiment, 4 bottles of sample, instead of 2, were stored at each temperature.

Experimental Design (Proteolysis). On the day of the MF with pasteurization experiment (d 0), the pasteurized MF skim permeate was analyzed by Kjeldahl for total N, NPN, and noncasein N. True protein and CN were calculated by difference. During the storage period, samples were taken from the stored pasteurized permeate at d 37, 51, 65, 79, and 93. The samples were frozen (at -80°C and transferred to -40°C) until the end of the study and analyzed by Kjeldahl for noncasein N. At the time of analysis, the samples were thawed using a microwave oven in 15-s increments on medium power. At the completion of thawing, the samples did not exceed 10°C and were placed on ice until analysis (within 1 h). The decrease in CN%TP was calculated for each time point in the study.

Statistical Analyses. Data for proteolysis were analyzed using the GLM procedure of SAS (version 8.02, 2001, SAS Institute, Inc., Cary, NC) using the split-plot model with temperature and replicate in the whole plot as category variables and days of storage in the subplot as a continuous variable. The day term was transformed as follows: day = day - (total days of storage/2). This transformation made the data set orthogonal with respect to time. This transformation directs SAS to determine the effect of storage temperature in the whole plot at the midpoint of the storage time (i.e., d 46.5) instead of d 0.

RESULTS

Spoilage Rate of Commercial Skim Milk

Decreasing storage temperature from 6.1 to 0.1°C increased the length of the apparent lag phase and decreased the logarithmic growth rate of the mixed populations of bacteria (Figure 1) resulting in longer microbial shelf life. The apparent lag phase and logarithmic growth rate of the mixed microflora in commercial pasteurized skim milk varied among the 4 replicates (Figures 1a to 1d) presumably due to random difference in the populations of microflora surviving in the milk. The end of shelf life was defined as total

bacterial count >20,000 cfu/mL, based on the Pasteurized Milk Ordinance requirement for pasteurized milk. Figure 2 is an example of the Arrhenius plot that was used to calculate the effect of storage temperature on the rate of bacterial growth in the skim milk for replicate 2. The natural log of k was plotted against $1/T \times 10^3$, where k is the logarithmic growth rate (log cfu/mL per d) from Figure 1b and T is the absolute storage temperature in Kelvin. The equation of the line from this plot describes the effect of storage temperature on bacterial growth rate. The calculated equations for each of the 4 replicates were used to predict the microbial shelf life of commercial pasteurized skim milk given the starting bacteria concentration and storage temperature across the range of temperatures examined in this study. The predicted shelf-life values for each trial are presented in Figure 3, including lines for the separate contributions of lag time and log growth rate of the mixed microbial population to the overall shelf life. The calculation for predicting the effect of storage temperature on apparent lag time was similar to that for determining the effect of storage temperature on growth rate. The lag time of the mixed microbial population for each storage temperature in each trial was determined by calculating the intercept of the growth curves in Figure 1 with the baseline value (bacteria concentration on d 0). The lag time was transformed by taking the natural log twice and plotting this against storage temperature (°C). The equation of the resulting line was used to predict lag time of the surviving mixed microflora in the pasteurized skim milk across the range of temperatures examined in this study.

Reducing storage temperature had a much larger effect on lengthening the lag time than on decreasing the log growth rate. The increase in lag time of the mixed microbial population was the major contributor to the increase in total shelf life of pasteurized skim milk in this study (Figure 3). The mean time to reach a microbial count of 20,000 cfu/mL ranged from 16 d at 6.1°C to 66 d at 0.1°C across the 4 replicates. Results among the 4 replicates were variable (Figures 3a to 3d), most likely due to variations in the surviving microflora present in the pasteurized skim milk.

Removal of Bacteria with Microfiltration

Across 3 replicates, total bacterial counts of the raw skim milk were reduced from 2,400, 3,600, and 1,475 cfu/mL to 0.240, 0.918, and 0.240 cfu/mL, respectively, by microfiltration (Table 1). Bacterial counts in the pasteurized microfiltered skim milk for the 3 trials were 0.005, 0.008, and 0.005 cfu/mL, respectively. Microfiltration achieved an average 3.79 log reduction

Figure 1. Logarithmic bacterial growth in commercially pasteurized skim milk. Treatments are storage temperatures of 0.1 (○), 2.0 (●), 4.2 (□), and 6.1°C (■). Panels a through d represent replications 1 through 4 of the experiment, respectively.

Figure 2. Example of Arrhenius plot for calculating the effect of storage temperature on bacterial growth rate, where k is the logarithmic growth rate at each of the 4 storage temperatures and T is the absolute temperature in Kelvin.

and pasteurization of the MF skim milk achieved an additional average 1.84 log reduction, producing an average 5.6 log reduction from the raw count due to the combination of MF and pasteurization (72°C for 16 s) across the 3 replicates (Table 2). The log reduction in bacterial count due to MF was comparable to that reported by Maubois (1997). The microbial inactivation achieved by pasteurization following MF (i.e., 1.84 log) was slightly greater than the inactivation achieved by pasteurization without MF (i.e., 1.67 log) done by the Cornell Dairy plant using the same source milk, even though Cornell Dairy uses a pasteurization treatment of 79°C for 16 s. This indicates that the bacteria remaining after MF were as susceptible to heat treatment as the typical bacteria population in Cornell raw skim milk. Both coliforms and spores were reduced by MF to an undetectable level (Table 1). Some of the fat in the skim milk was removed by MF. Before MF, the fat content of the skim was about 0.1% and after MF it was about 0.03%.

Figure 3. Shelf life of commercially pasteurized skim milk as a function of storage temperature. Contribution of lag phase (□) and log phase (○) are shown with total shelf life (◆). Panels a through d represent replications 1 through 4 of the experiment, respectively.

Estimation of Shelf Life of MF Pasteurized Skim Milk

Bacterial Growth. Microbial shelf life of the pasteurized MF skim milk was characterized in terms of the percentage of the total samples stored at each temperature that exceeded a total bacterial count of 20,000 cfu/mL. Following 92 d of storage, 50% of samples stored at 6.1°C and 88% of samples stored at 4.2°C had counts <20,000 cfu/mL (Figure 4a). At 0.1 and 2.0°C, none of the stored samples reached a detectable bacterial count (i.e., >1,000 cfu/mL) by 92 d. As a means of comparison, the microbial data from the commercial pasteurized skim milk described in objective 1 was analyzed in the same way (Figure 4b). Commercial skim milk reached a total bacterial count >20,000 cfu/mL much earlier than the MF pasteurized skim milk. At 6.1, 4.2, 2.0, and 0.1°C, 100% of the stored samples had reached a total bacterial count >20,000 cfu/mL by d 22, 29, 57, and 92, respectively.

Proteolysis. In the absence of high bacterial numbers in milk, it has been demonstrated that proteolysis increases during storage and that these increases in proteolysis are faster in high SCC milk (Santos et al., 2003b). The primary milk proteins being broken down are caseins and the enzyme causing this is plasmin (Saeman et al., 1988). With respect to proteolysis, the end of shelf life was defined as a decrease in CN%TP greater than 4.76%. This level of proteolysis has previously been established (Santos et al., 2003a) as the level at which an off-flavor due to proteolysis was detected by 50% of sensory panelists. There was a significant ($P < 0.01$) influence of storage temperature, days of refrigerated storage, and the interaction of storage temperature and days of storage (Table 3). At 6.1, 4.2, 2.0, and 0.1°C the mean time for the MF pasteurized skim milk to reach a decrease in CN%TP >4.76% was 32, 46, 78, and >92 d, respectively, across the 3 replicates (Figure 5). The rate of proteolysis in-

Table 1. Bacteria and somatic cell counts in skim milk before and after microfiltration and pasteurization

Item and replicate	Sample			
	Raw skim milk	Retentate	Permeate	Pasteurized permeate
Total bacteria (cfu/mL)				
Replicate 1	2,400	18,250	0.240 ¹	0.005 ¹
Replicate 2	3,600	23,000	0.918 ¹	0.008 ¹
Replicate 3	1,475	9,250	0.240 ¹	0.005 ¹
Spore count (cfu/mL) ²				
Replicate 1	87	465	<25	<25
Replicate 2	74	380	<25	<25
Replicate 3	13	100	<25	<25
Coliform count (cfu/mL) ³				
Replicate 1	455	2,775	<10	<10
Replicate 2	194	715	<10	<10
Replicate 3	60	335	<10	<10
SCC (cells/mL)				
Replicate 1	138,000	669,500	<3,000 ⁴	—
Replicate 2	121,000	631,500	<3,000 ⁴	—
Replicate 3	127,000	595,500	<3,000 ⁴	—

¹Determined using most probable number method.

²The spore count given for the permeate and pasteurized permeate samples is the lowest reportable level; however, it should be noted that in the analysis there were no spores detected.

³The coliform count given for the permeate and pasteurized permeate samples is the lowest reportable level; however, it should be noted that in the analysis there were no coliforms detected.

⁴The reported level of somatic cells in the permeate is the lower limit of detection for the equipment used. Based on the size of somatic cells relative to the membrane pore size, it was assumed that there were no somatic cells present in the permeate or the pasteurized permeate.

creased with increasing storage temperature. Plotting the proteolysis rate as a function of storage temperature gave a quadratic equation with the rate of proteolysis increasing at an increasing rate with increasing temperature (Figure 6). Based on this equation we predict that an off-flavor due to proteolysis for MF pasteurized skim milk would occur at about 49 d when the original SCC of the milk is 240,000 cells/mL.

The MF process reduced the SCC in the permeate to an undetectable level (Table 1), indicating complete retention by the membrane. In spite of this, there was not a 20× concentration of somatic cells in the retentate as expected. Instead, the SCC in the retentate was approximately 5× the original level, demonstrating a loss of approximately 75% of the somatic cells originally present in the skim milk. Because the somatic cells remain on the retentate side of the membrane, they are exposed to high shear during their residence time in the recirculation loop. It is possible that many

somatic cells are physically destroyed by shear forces. The bacterial counts in the MF retentate also were not concentrated 20×. It would be expected that there would be some death of the more heat-sensitive mixed microflora during the MF process at 50°C, and therefore, a lower than 20× concentration of bacteria in the retentate would be observed (Table 1).

In spite of what appears to be complete removal of somatic cells from the skim milk, it was observed that the rate of proteolysis during shelf-life storage was higher than expected given that no somatic cells were present in the MF pasteurized skim milk during the storage period. It has been demonstrated previously that growth of a mixed microbial population in pasteurized milk can be blocked by the use of preservatives (Santos et al., 2003b) or CO₂ (Ma et al., 2003). When microbial growth is kept very low in fluid milk, proteolysis during shelf life is due to native milk proteases, which causes the development of off-flavors (San-

Table 2. Log reduction in total bacteria achieved by the stages of the process

Process	Reduction in total bacteria (log cfu/mL)		
	Replicate 1	Replicate 2	Replicate 3
Microfiltration	4.00	3.59	3.79
Pasteurization	1.72	2.07	1.72
Microfiltration + pasteurization	5.72	5.66	5.51

Figure 4. Microbial shelf life of pasteurized microfiltered skim milk (a) and commercial pasteurized skim milk (b).

tos et al., 2003a). The data collected in the present study were compared with data collected by Santos et al., 2003a (Figure 7) for 2% milk made from a whole

Table 3. Type III sum of squares (SS) and probability values for ANOVA of the impact of storage temperature on the rate of proteolysis in pasteurized microfiltered skim milk

Independent variables ¹	df	Analyzed as	Type III SS	P-value
Whole plot				
T	3	Category	272.6	<0.0001
R	2	Category	8.6	<0.0001
T × R	6	Interaction	0.9	0.07
Subplot				
D	1	Continuous	546.1	<0.0001
D × T	3	Interaction	113.6	<0.0001
D × R	2	Interaction	2.6	<0.0001
Total SS	71		1068.9	
Error	54		4.2	
R ²			0.996	

¹T = Storage temperature; R = experiment replication; and D = days of storage.

Figure 5. Proteolysis (decrease in casein as % of true protein, CN%TP) in pasteurized microfiltered skim milk containing <1,000 cfu/mL total bacteria. Treatments are storage temperatures of 0.1 (○), 2.0 (●), 4.2 (□), and 6.1°C (■).

raw milk containing 340,000 somatic cells/mL. The pasteurized MF skim milk in the present study contained 240,000 somatic cells/mL before MF. Both milks were stored at approximately 6°C. Despite the apparent removal of somatic cells from the skim milk by MF, the rate of proteolysis for these 2 milks was similar, indicating that the effect that the SCC of the original milk had on the rate of proteolysis in milk in the present study may have been due to activation of plasmin before the milk is removed from the cow as shown by Saeman et al. (1988), and its subsequent activity during shelf life. Plasmin survives pasteurization. Somatic cells themselves do not have a significant direct effect on proteolysis rate unless present at $>2 \times 10^6$

Figure 6. The effect of storage temperature on the rate of proteolysis (decrease in casein as % of true protein, CN%TP) in pasteurized microfiltered skim milk containing <1,000 cfu/mL total bacteria.

Figure 7. Comparison of proteolysis (decrease in casein as % of true protein, CN%TP) between 2% milk made from raw milk with 340,000 somatic cells/mL (■) and MF pasteurized skim milk made from raw milk originally containing 240,000 somatic cells/mL (□). Both milks were stored at 6°C.

somatic cells/mL (Saeman et al., 1988). Therefore, to maximize the length of acceptable flavor of MF pasteurized skim milk, the initial raw milk SCC should be kept as low as possible (e.g., <100,000 somatic cells/mL); this will keep the active plasmin concentration in milk low.

CONCLUSIONS

Lower storage temperature resulted in longer shelf life (i.e., time to reach 20,000 cfu/mL total bacterial count) in commercial pasteurized skim milk. The days of shelf-life extension were progressively larger (i.e., not linear) with each incremental decrease in storage temperature. Most of the benefit of decreasing storage temperature on control of microbial growth came from the extension of microbial lag time of the mixed microbial population, but there was also some benefit from reduced logarithmic growth rate. A 3.79 log reduction in total bacteria was achieved by MF and a further 1.84 log reduction was achieved by following MF with minimum pasteurization, resulting in a 5.63 log reduction for the combined process. By reducing the total bacteria in skim milk to very low levels, the MF and pasteurization process produced skim milk with extended microbial shelf life.

Shelf life with respect to proteolysis was defined as the time for samples to demonstrate a decrease in CN%TP >4.76%, a level that corresponds to sensory detection of off-flavors due to proteolysis. Pasteurized MF skim milk stored at 0.1 or 2.0°C exceeded 92 d of microbial shelf life, but at 2.0°C, the milk was limited to a 78-d shelf life due to proteolysis. Some MF pasteur-

ized skim milk stored at 4.2 and 6.1°C achieved a 92-d microbial shelf life, but was limited to 46 and 32 d, respectively, due to proteolysis.

Although there was apparently complete retention of somatic cells by the MF, there was a substantial loss of somatic cells in the mass balance for the process, possibly indicating destruction of somatic cells by shear forces within the MF plant. Despite what appears to be complete removal of somatic cells from the skim milk, the rate of proteolysis during refrigerated storage was not reduced by MF, suggesting that most of the effect that somatic cells have on the rate of proteolysis in milk may be due to activation of plasmin before processing. Had the raw milk used in this study contained fewer somatic cells before processing, the shelf life with respect to proteolysis would have been further extended. If a storage temperature lower than 6°C is used for fluid milk, then a shelf life of 60 d may be achievable with a higher SCC. The requirement of higher quality raw milk to achieve shelf-life extension for fluid milk underscores the need for processors to provide milk producers with financial incentives to produce lower SCC milk for fluid milk use.

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