Shelf life of pasteurized microfiltered milk containing 2% fat¹

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ABSTRACT

The goal of this research was to produce homogenized milk containing 2% fat with a refrigerated shelf life of 60 to 90 d using minimum high temperature, short time (HTST) pasteurization in combination with other nonthermal processes. Raw skim milk was microfiltered (MF) using a Tetra Alcross MFS-7 pilot plant (Tetra Pak International SA, Pully, Switzerland) equipped with Membralox ceramic membranes (1.4 μm and surface area of 2.31 m^2 ; Pall Corp., East Hills, NY). The unpasteurized MF skim permeate and each of 3 different cream sources were blended together to achieve three 2% fat milks. Each milk was homogenized (first stage: 17 MPa, second stage: 3 MPa) and HTST pasteurized (73.8°C for 15 s). The pasteurized MF skim permeate and the 3 pasteurized homogenized 2% fat milks (made from different fat sources) were stored at 1.7 and 5.7°C and the standard plate count for each milk was determined weekly over 90 d. When the standard plate count was $>20,000$ cfu/mL, it was considered the end of shelf life for the purpose of this study. Across 4 replicates, a 4.13 log reduction in bacteria was achieved by MF, and a further 0.53 log reduction was achieved by the combination of MF with HTST pasteurization (73.8°C for 15 s), resulting in a 4.66 log reduction in bacteria for the combined process. No containers of MF skim milk that was pasteurized after MF exceeded 20,000 cfu/mL bacteria count during 90 d of storage at 5.7°C. The 3 different approaches used to reduce the initial bacteria and spore count of each cream source used to make the 2% fat milks did not produce any shelf-life advantage over using cold separated raw cream when starting with excellent quality raw whole milk (i.e., low bacteria count). The combination of MF with HTST pasteurization (73.8°C for 15 s), combined with filling and packaging that was protected from microbial con-

tamination, achieved a refrigerated shelf life of 60 to 90 d at both 1.7 and 5.7°C for 2% fat milks.

Key words: microfiltration, shelf life, bacterial removal

INTRODUCTION

The fluid milk-processing industry would like the refrigerated shelf life of conventionally pasteurized HTST processed fluid milk to be longer than 14 to 21 d. Benefits of extended-shelf-life fluid milk include expanded distribution distances, fewer and more efficient processing plants, and increased product availability to consumers through convenience stores with slower product turnover rates. Extended shelf life could also deliver longer use time before spoilage once the consumer has purchased the product. Improved consumer perception of the quality of fluid milk products could lead to increased fluid milk consumption if consumers are less concerned with milk spoilage and more confident that fluid milk will retain its fresh taste longer.

United States regulatory standards require pasteurized fluid milk to have an SPC $<20,000$ cfu/mL (FDA, 2011). In practice, this criterion is applied on d 1 after pasteurization. From a consumer's perspective, the end of shelf life of fluid milk is the point at which they perceive the milk is no longer palatable based on sensory attributes such as flavor and aroma. Currently, bacterial spoilage is the limiting factor in extending the shelf life of conventionally pasteurized HTST processed fluid milk beyond 17 to 21 d (Boor, 2001; Martin et al., 2011). A published report found that in 1999, of 447 samples of commercially pasteurized milks, the percentages of samples with bacteria counts $<20,000$ cfu/mL during storage at 6°C were 61, 45, and 28 at 7, 10, and 14 d, respectively (Boor, 2001).

The Pasteurized Milk Ordinance defines legal minimal HTST pasteurization as heating milk to 72°C and holding at that temperature for at least 15 s (FDA, 2011). However, most milk processing plants use temperatures and holding times that exceed the minimum requirements (Douglas et al., 2000) to extend shelf life. One study found that pasteurization conditions of 79°C for 18 s, 79.4°C for 22 s, and 79.4°C for 28 s were being used at 3 New York state fluid milk processing plants (Fromm and Boor, 2004).

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Current shelf-life extension strategies include ultra-pasteurization (**UP**), UHT pasteurization, and micro-filtration (**MF**). Ultra-pasteurized milk is heated to 138°C for at least 2 s, and generally has a shelf life of several weeks under refrigerated conditions (Boor and Nakimbugwe, 1998) when it is packaged nonaseptically. Although UP milk has a longer shelf life than HTST milk, a sensory study reported that the mean degree of liking of UP milk was rated slightly below “good” by children ages 6 to 11 (Chapman and Boor, 2001). The study also reported that children preferred HTST milk to UHT milk, and preferred UHT milk to UP milk, although the authors had no explanation of why the flavor quality of the UHT milk was rated better than the UP milk. The thermal process for UHT milk is the same thermal process as for UP milk, but the milk is aseptically packaged to avoid recontamination (Boor, 2001). The UHT milk is shelf stable and has an approximate shelf life of 6 mo (Boor, 2001). The advantage of UHT processing and aseptic packaging is that it allows the milk to be transported over longer distances and could provide milk to parts of the world without access to refrigeration. The UHT process is known to impart a cooked flavor to the milk (Horner et al., 1980), but over the years the UHT process has improved by using direct steam injection.

Other processes (e.g., bactofugation, MF, and gravity separation) offer alternatives to using high heat to reduce the bacteria count of raw milk. Bactofugation is a centrifugal process for removing spores from milk and has been applied mostly before cheese making to control late gas formation in cheeses (Fox et al., 2000). Microfiltration physically removes a portion of bacteria, spores, and somatic cells from skim milk. The Bactocatch commercial MF process for removing bacteria from skim milk, developed by Tetra Pak International SA (Pully, Switzerland), begins with the centrifugal removal of cream from raw whole milk using commercial continuous cream separator. The remaining skim milk is MF using a 1.4- μ m ceramic membrane, and the bacteria are concentrated in the MF retentate (approximately 5% of the skim milk). A more recent version of this process further concentrates the MF retentate on a second MF apparatus, leading to permeation rates of proteins and TS >99.5 and 99.8%, and an MF retentate that is approximately 0.5% of the original volume of skim processed (Maubois and Schuck, 2005). The Bactocatch process uses cross-flow uniform transmembrane pressure to reduce membrane fouling (Sandblom, 1978). The MF retentate (concentrate of bacteria and spores) and cream undergo UP treatment and then can be combined with the low-bacteria MF skim permeate. The combination of MF permeate, cream, and MF retentate then receive a minimum HTST treatment.

Various studies have shown the effect of MF on reducing bacteria counts of skim milk. Kelly and Tuohy (1997) found that the total bacteria count of MF skim milk was, on average, 1 log cycle lower than the pasteurized skim control. Hoffmann et al. (1996), using an MF process with 1.4- μ m membranes, reduced the total bacteria count of raw skim milk by 99.85%, with an average logarithmic reduction of 2.8. Elwell and Barbano (2006) found a 3.79 log reduction in total bacteria of raw skim milk was achieved by MF with 1.4- μ m membranes, and a further 1.84-log reduction was achieved by following MF with HTST pasteurization (72°C for 15 s). These logarithmic reductions in bacteria counts may have the ability to lengthen the refrigerated shelf life of pasteurized milk. Schaffner et al. (2003), using Monte Carlo simulations, reported that reducing the average initial microbial contamination level of pasteurized milk by 0.5 log can significantly reduce the fraction of milk samples that spoil after 14 d of refrigerated shelf life when either mesophilic or psychotrophic microbes are present.

It is well known that if raw milk is left unmixed it will gravity separate or cream. This approach has been used commercially to prepare milk for the production of Grana Padano and Parmigiano-Reggiano cheeses (McSweeney et al., 2004). According to Rossi (1964), the number of total bacteria, coliform, thermophilic bacteria, and spores in partially skim milk is decreased to 5 to 10% of that in the starting raw whole milk when milk is gravity separated for 6 h at 15°C. Dellagio et al. (1969) showed that gravity separation of raw whole milk, to which a variety of pure bacterial cultures were added, caused *Clostridium tyrobutyricum* BZ15, *Streptococcus cremoris* 760 and 803, *Acinetobacter* 12-2 and R66, *Escherichia coli* NCDO 1246, *Pseudomonas fluorescens* P442, and *Flavobacterium* 8-9 to rise to the top. Caplan et al. (2013) found that in raw and HTST pasteurized (72.6°C for 25 s) whole milk, the milk fat, bacteria, and somatic cells rose to the top of columns during gravity separation. About 50 to 80% of the milk fat and bacteria, and 90 to 96% of somatic cells were present in the top 8% of the milk weight in the column after gravity separation for 22 h of both milks. Therefore, gravity separation can also be used to physically remove bacteria from raw whole milk.

Elwell and Barbano (2006) reported that following MF with HTST pasteurization (72°C for 15 s) produced skim milk with extended shelf life (i.e., <20,000 cfu/mL) of up to 90 d at 2°C. The next step in research is to extend the shelf life of 2% fat milk to 60 to 90 d of refrigerated shelf life without using high heat. A combination of gravity separation to produce cream with low bacteria count, MF of skim milk, and HTST pasteurization might make this possible.

The goal of our research was to produce homogenized milk containing 2% fat with a refrigerated shelf life of 60 to 90 d using HTST pasteurization in combination with other nonthermal processes. The objective of our study was to determine the efficacy of an MF process, in combination with gravity separation, followed by minimum time and temperature HTST pasteurization to reduce the total bacteria and spore count and extend the refrigerated shelf life of 2% fat milks.

MATERIALS AND METHODS

Experimental Design

Microfiltration and minimum HTST pasteurization were used to reduce total bacteria, spores, coliforms, and somatic cells in 2% fat milks made from MF skim milk permeate combined with 3 fat sources: raw centrifugal cream (raw cream 1), raw centrifugal cream produced from the lower 90% of gravity-separated milk (raw cream 2), or pasteurized butter oil prepared from raw cream 2. The MF skim permeate and each fat source was blended to achieve 2% fat, and each mixture was homogenized and HTST pasteurized. The pasteurized MF skim permeate and the 3 pasteurized homogenized 2% fat milks were stored at 1.7 and 5.7°C and the SPC for each milk was determined weekly over 90 d. When the bacteria count was $>20,000$ cfu/mL, it was considered to be the end of shelf life for the purpose of this study. This experiment was replicated 4 times.

Processing

Preparation of Butter Oil Ingredient. Prior to the experiment, one batch of butter oil was produced for use in all 4 replicates. Raw whole milk (470 kg) was pasteurized (72°C for 16 s), cooled to 4°C, and gravity separated in cone-bottom tanks in a cold room (4°C) overnight (about 22 h). The lower 90% portion (approximately 425 kg) was drained through the bottom valve of each tank, heated to 50°C with a plate heat exchanger, and separated into skim at 50°C and cream using a centrifugal cream separator (model 619; De Laval, Poughkeepsie, NY) operating at about $5,000 \times g$. The lower layer of the gravity separated whole milk contained about 2 to 2.5% fat with reduced bacteria and SCC. The cream produced from this lower gravity layer produced a centrifugally separated cream with reduced bacteria and SCC. Approximately 60 L of centrifugal cream was warmed to approximately 10°C and churned (model LR 61850; Berry Hill Ltd., St. Thomas, ON, Canada) into butter (Ip et al., 1999). The butter was collected, melted, and allowed to separate into a lipid and an aqueous layer. The lipid layer was collected

and further clarified at $27,485 \times g$ for 8 min at 25°C using a GSA centrifuge rotor and centrifuge (Sorvall Evolution RC, Newtown, CT). The clarified butter oil was commingled, split into four 500-g batches (1 for each replicate), and held frozen at -40°C.

On the first day of processing, one 500-mL batch of butter oil was melted and poured into a 2-L Nalgene container (C-06040-70; Cole Parmer Instrument Co., Vernon Hills, IL) with a screw cap lid and fittings that had been autoclaved. The lid of the container had 3 openings: one port with tubing (Masterflex 0.6-cm diameter Norprene food pump tubing, catalog no. SA-06402-15; Cole Parmer Instrument Co.) attached, one port with a high-efficiency particulate air (HEPA) filter (Whatman HEPA-Vent, 0.6- to 1-cm tapered barb, catalog no. 09-744-79; Fisher Scientific, Hampton, NH) attached, and one port with a removable plastic cap. The container was placed in a steam kettle (model DN 5 SP; Groen, Chicago, IL) and submerged in 65 to 70°C water to the bottom of the screw cap lid. A temperature probe (HH64 thermometer; Omega Engineering Inc., Stamford, CT) was inserted through the uncapped port in the screw cap lid to monitor the temperature of the butter oil. Once the temperature reached 63°C, the probe was removed and the port was capped. After 30 min of batch pasteurization, the container of pasteurized butter oil was removed from the steam kettle, cooled in ice, and refrigerated (3°C) for use the next day.

Equipment Preparation. The MF unit, homogenizer, and benchtop HTST pasteurizer were setup and approximately 800 L of reverse osmosis (RO) water was boiled on the first day and held at room temperature for use in cleaning and flushing of the equipment on the second day of processing. All 0.6-cm needle valves (catalog no. SS-1VS4; Swagelok Co., Solon, OH) on the MF unit and HTST pasteurizer were checked for leaks around the stems by pressurizing the system with compressed air and using a soap solution to check for leaks. Valves with leaks were repaired or replaced before the processing run.

Microfiltration of Raw Skim Milk. Raw skim milk (approximately 440 L) was produced from raw whole milk by cold (4°C) centrifugal separation (about $6,000 \times g$; model 590; Equipment Engineering, Indianapolis, IN) at the Cornell University Dairy Plant (Ithaca, NY). The raw skim milk was heated to 51°C with circulating 65°C water in a covered, jacketed stainless steel hinged tank (model RE6801; Pfaudler Inc., Rochester, NY) that was connected directly to the MF unit. Milk samples were taken from the tank before heating for microbial and SCC. Skim milk was MF using a Tetra Alcross MFS-7 pilot plant (Tetra Pak International SA) in uniform transmembrane pressure

mode equipped with Membralox ceramic membranes (1.4 μm and surface area = 2.31 m^2 ; Pall Corp., East Hills, NY). All RO water used in the study was boiled and cooled to room temperature before use. The membranes were flushed of their storage solution (0.3% vol/vol HNO_3) with 25°C RO water for 15 min, heated to 80°C by adding 90°C RO water to the balance tank, cleaned with a caustic solution (1.5% vol/vol Ultrasil 25; Ecolab, St. Paul, MN) by recirculating for 25 min at 80°C, and cooled to 40°C by circulating cool water through the heat exchanger built into the retentate circulation loop. The membranes were then flushed for 15 min with 50°C RO water, cleaned with a nitric acid solution (0.3% v/v HNO_3 ; Fisher Scientific, Fairlawn, NJ) by recirculating for 25 min at 50°C, cooled to 40°C with the heat exchanger, and flushed with 25°C RO water for 15 min. During each step of the cleaning process, all valves were worked through their range of open and closed positions to clean all internal surfaces. Next, RO water at 50°C was pumped into the MF unit and recirculated to warm the membranes and piping before the start of milk processing. Just before starting milk processing, the MF feed inlet valve, MF permeate outlet valve, MF retentate outlet valve, and MF inline sample port were swabbed for microbial analysis. Once the skim milk reached 51°C, it was immediately processed. During processing, the MF skim permeate and MF skim retentate collection rates were approximately 462 and 25 L/h (i.e., approximately 5% of the feed rate), respectively. These rates provided a calculated flux of approximately 200 L/m^2 per hour. Inlet and outlet pressures were maintained at approximately 400 kPa and 200 kPa, respectively. The difference in transmembrane pressure (TMP) between the inlet and outlet of the membrane (ΔP) was maintained between 22 and 35 kPa, with an inlet TMP of 37 to 46 kPa and an outlet TMP of 10 to 18 kPa. To allow for the transition from water to milk at the beginning of processing, approximately 35 L of MF skim permeate was allowed to exit the permeate outlet valve of the MF unit before beginning collection of MF skim permeate and MF skim retentate. The MF skim retentate was collected in a sanitized stainless steel milk can.

Approximately 20 to 25 kg of MF skim permeate was collected aseptically from an inline sample port in the permeate recirculation loop of the MF unit into each of 4 separate sterile, HEPA-filtered polypropylene carboys (one 20 L and three 50 L) for further processing. The first 20-L carboy (catalog no. C-06063-22; Cole Parmer Instrument Co.) was immediately disconnected from the MF unit and moved to a 51°C water bath. Each of three 50-L carboys (catalog no. C-06064-70; Cole Parmer Instrument Co.) had a stainless steel sanitary ball valve (cat no. SS-67TSC24; Swagelok Co.) attached to

an outlet at the bottom of the carboy. The end of each sanitary ball valve was closed with a stainless steel end cap. The screw cap lid for each carboy had 3 openings: 1 port for the inlet tube and 2 ports for HEPA filters. Tubing was extended from the inlet of the cap to the bottom of the carboy with a piece of tubing to reduce foaming as the MF skim permeate was collected and to allow for later pumping of MF skim permeate back out of the carboy. The end of this tube inside the carboy was weighted with a stainless steel barbed insert (catalog no. SS-4-HC-A-401; Swagelok Co.). All tubing was firmly attached to the carboy cap by stainless steel hose clamps. The entire carboy apparatus, including all tubing, filters, and fittings, was autoclaved together before use. Each 50-L carboy, containing 20 to 25 kg of MF skim permeate at 51°C, was disconnected from the MF unit and immediately moved to a 51°C water bath, where it remained before and after the fat addition step. A 20-mL sample of MF skim permeate was taken directly from the permeate outlet valve of the MF unit for fat content determination.

Preparation of Fat Sources for Standardization. The first fat source (raw cream 1) was cream that had been separated directly from raw whole milk using a cold bowl centrifugal cream separator (at about 6,000 $\times g$ and 4°C; model 590; Equipment Engineering, Indianapolis, IN) in the Cornell University Dairy Plant. The second fat source (raw cream 2), starting from the same raw whole milk in each replicate, was cream isolated from the lower layer (i.e., reduced fat milk with about 2.35% fat) of the gravity separated milk. Raw whole milk (approximately 240 L) was obtained from the Cornell University Dairy Plant and weighed into a 400-kg stainless steel cone-bottom tank (model P2-CB; Walker Stainless Equipment, New Lisbon, WI) jacketed with circulating chilled water. Milk samples were taken from the tank for microbial and SCC before gravity separation. The raw whole milk was gravity separated for 22 h at 3°C. After 22 h of gravity separation, the lower 90% of the gravity separated milk was removed by weight, commingled in a 380-L stainless steel vat, heated to 51°C (with water at 65°C) using a plate heat exchanger, and separated with a centrifugal cream separator (model No. 3-G; International Harvester Co., Chicago, IL). Samples of raw cream 1 (conventional cream), raw cream 2 (cream with bacteria count reduced by gravity separation), and the butter oil were taken for fat content determination before milk standardization. The butter oil was prepared as described above.

Standardization, Homogenization, and Pasteurization of 2% Fat Milks. Before standardization, there were 7 carboys in 51°C water baths: one 20-L carboy filled with approximately 20 kg of MF skim

permeate, three 50-L carboys filled with approximately 25 kg of MF skim permeate, and three 2-L carboys filled with 0.5 to 2 L of 1 of the 3 fat sources. The 2 creams were pumped directly into 2 of the 50-L carboys containing MF skim permeate. The 2-L containers were placed on a balance, so that by subtraction, the correct amount of cream was added to the MF skim permeate to produce milks containing approximately 2% fat. The 50-L carboys now containing 2% fat milks from raw creams 1 and 2 were held at 51°C until homogenization.

To produce the third 2% fat milk, the pasteurized butter oil was dispersed in a portion of the MF skim permeate with a high-shear mixer before homogenization. Approximately 3 kg of MF skim permeate from the third 50-L carboy was pumped into a chlorine sanitized (200 mg/kg) blender (model CB-5; Waring Products Co., Winsted, CT). The weight of butter oil needed to produce a 2% fat milk was pumped from the 2-L container (at 51°C) into the sanitized blender with the MF skim permeate. The blender lid was sealed tightly on top of a sanitized sheet of plastic wrap (Reynolds 905 film; Reynolds Food Packaging LLC, Richmond, VA), and the mixture was blended as follows: 60 s on low, 30 s off, 60 s on low, 30 s off, and 30 s on low. The blended mixture of MF skim permeate and butter oil was pumped out of the blender directly into the 50-L carboy from which the original 3 kg of MF skim permeate was removed. The carboy was held at 51°C until homogenization.

To achieve the desired fat globule size distribution, and to prevent creaming throughout the shelf-life study, all three 2% fat milks were homogenized (first stage at 17 MPa and second stage at 3 MPa) at 51°C (Gaulin homogenizer type laboratory 60/120-5TBS; PMS, Philadelphia, PA). The homogenizer was heated with 80°C RO water until the system exit temperature reached 80°C before recycling the RO water back to the feed funnel. Swab samples were taken at the connections to the homogenizer system for microbial analysis before milk processing began. Between treatments, the homogenizer system was flushed with 80°C RO water to prevent cross-contamination among treatments within the same processing session. Just before attachment to the homogenizer, each carboy was manually swirled 20 times, while supporting the valve and outlet nozzle to prevent leaks. The three 2% fat milks were homogenized in the following order: butter oil, raw cream 2, and raw cream 1. The carboys containing the homogenized 2% milks were held at 51°C before HTST processing.

The MF skim permeate and all three 2% fat milks were pasteurized using a benchtop shell and tube HTST pasteurizer system, as described previously (Ma and Barbano, 2003). Milk entered the HTST system at approximately 51°C and a flow rate of 770 mL/min. Swab

samples were taken at the connections to the pasteurizer system for microbial analysis before milk processing began. The milk was heated to $73.8 \pm 0.3^\circ\text{C}$, held for 15 s, and then cooled to 4 to 5°C before exiting the HTST system. Approximately 5 kg of each pasteurized milk was allowed to exit the system before collection began. The pasteurized milk exiting the system was collected directly into a sterile 20-L carboy. The system was flushed with boiled RO water at 80°C between each treatment, with the cooling section turned off, to minimize any carryover or contamination between treatments. The MF skim permeate was pasteurized before the 2% milks, and the 2% milks were pasteurized in the order that they were homogenized. Approximately 5 kg of the MF skim permeate was not pasteurized, and was held in a 3°C cooler until sampling for chemical and microbial analysis.

Sampling

Initial Microbial and Chemical Analysis. The carboys containing the unpasteurized MF skim permeate, pasteurized MF skim permeate, and all 3 pasteurized 2% fat milks were moved to a 3°C cooler. Each carboy was brought into a 25°C room with recirculated HEPA-filtered air, and connected to a peristaltic pump (model 7554-90; Cole Parmer Instrument Co.) in a UV light sanitized HEPA-filtered air transfer hood (Labconco Purifier class II safety cabinet; Labconco Corp., Kansas City, MO). The product was pumped from each carboy into sterile 60-mL plastic snap-top vials (Capital Vial Inc., Fultonville, NY) for initial bacteria counts, fat determination, verification of pasteurization, and milk fat globule size distribution. Milk samples were held refrigerated until time of analysis, except the samples for microbial analysis, which were stored in ice.

Most Probable Number Bacteria Counts. Because the initial bacteria counts in the unpasteurized and pasteurized MF skim permeate, and pasteurized 2% fat milks were expected to be <1 cfu/mL, a most probable number (MPN) technique (Garthright and Blodgett, 2003) was used to estimate these counts. Samples of both MF skim permeates and all three 2% fat milks were pumped into 5 sterile containers at each of 4 volumes: 500, 100, 50, and 1 mL. The 500-mL volumes were pumped into 1-L wide-mouth Nalgene polypropylene bottles (catalog no. 03-311-2E; Fisher Scientific, Fairlawn, NJ). The 100-mL volumes were pumped into sterile 120-mL plastic snap-top vials (Capital Vial Inc.). A separate 120-mL vial was also filled with each milk, and from this vial, the 10- and 1-mL samples were pipetted using sterile, disposable pipettes into sterile 60-mL vials. A BactoScan FC analyzer (Foss Electric A/S, Hillerød, Denmark) requires a minimum sample

volume of 20 mL; therefore, the 10- and 1-mL samples were diluted to 20 mL with autoclaved UF permeate from skim milk.

Shelf-Life Microbial Counts. Portions of pasteurized milk from each of the 4 treatments were also prepared for a shelf-life study. Approximately 0.5 L of the pasteurized MF skim permeate and each pasteurized 2% fat milk was pumped into each of 6 sterile 1-L wide-mouth Nalgene polypropylene bottles (catalog no. 03-311-2E; Fisher Scientific, Fairlawn, NJ) per treatment. Each bottle contained a 3.2-cm Teflon-coated magnetic stir bar (catalog no. C-08551-00; Cole Parmer Instrument Co.), and the bottles, with stir bars, were autoclaved before use.

Microbial and Chemical Analysis

Initial Bacteria Counts. Samples of the raw whole milk, raw skim milk, raw cream 1, raw cream 2, pasteurized butter oil, both MF skim permeates, and all 3 pasteurized 2% milks were spread plated for total bacteria count (**TBC**). About 200 μ L of milk from each sample was spread on each of 5 brain heart infusion (**BHI**) agar (EMD Biosciences, Darmstadt, Germany) plates for a total of 1 mL of sample across the plates. When counted, the plate count from the 5 plates was summed to give the number of colony-forming units per milliliter of sample. Both raw cream 1 and 2 were diluted in a bottle of 99 mL sterile phosphate buffer (catalog no. 3127-27; Weber Scientific, Hamilton, NJ) before plating as described above. The pasteurized butter oil was warmed in a 40 to 42°C water bath to melt the sample, and then diluted and plated in a similar fashion as the creams. All plates were incubated at 32°C for 24 h before being counted.

Coliform counts were done by plating 1 mL of sample on *Escherichia coli*/coliform count plate (catalog no. 6404; 3M Petrifilm, St. Paul, MN). For both creams and the pasteurized butter oil, 1 mL of diluted sample was plated. All films were incubated at 32°C for 24 h before being counted (Wehr and Frank, 2004; method number 7.070).

Swab Samples. Each microbial swab sample (catalog no. 3664-30, Copan swab rinse kit; Weber Scientific) was aseptically transferred into a test tube containing 5 mL of BHI broth (Bacto brain heart infusion; Becton, Dickinson, and Co., Sparks, MD). The BHI broth (37 g of powder in 1 L of purified water) was used as an enrichment phase to recover greater numbers of bacteria for plating, as bacteria numbers were expected to be very low (i.e., below detection for direct plating procedures). The tubes were incubated at 32°C for 24 h and then plated and counted for TBC as above.

Spore Counts. Spore counts (Huck et al., 2007) were done by activating each sample in an 80°C, agitating water bath for 12 min. Samples were cooled to 4°C, with the exception of the pasteurized butter oil, and refrigerated overnight before plating. The pasteurized butter oil was tempered to 45°C, diluted, and plated immediately. Both creams were diluted and plated on BHI agar as described above for initial total bacteria counts. All plates were incubated at 32°C for 24 h before being counted.

MPN Counts. Bacteria counts (cfu/mL) were determined using a BactoScan FC analyzer (Foss Electric A/S; Suhren et al., 2001; Walte et al., 2005). All samples (500, 100, 10, and 1 mL) for MPN analysis were incubated at 32°C for 5 d to ensure that any bacteria present would grow to sufficient numbers to be detected by the BactoScan FC analyzer. After the incubation period, samples containing bacteria should have grown to high counts, whereas samples containing no bacteria should have no growth. Samples analyzed by the BactoScan FC analyzer had either a very high (>1,000,000 cfu/mL) or a very low bacteria count (<1,000 cfu/mL, the detection limit). When samples differing greatly in bacteria count are analyzed in succession on the BactoScan FC analyzer, carryover between samples can elevate the count of the sample with lower bacteria count (Bolzoni et al., 2001). A 60-mL vial of autoclaved UF permeate from skim milk was run on the BactoScan FC analyzer as a blank between successive milk samples to avoid carryover of counts from a high to a low count sample analyzed in succession. The number of high count and low count samples at each dilution was used to calculate the MPN estimate of the SPC of the unpasteurized and pasteurized MF skim permeates, and pasteurized 2% fat milks. This calculation was performed using an Excel spreadsheet provided by the Food and Drug Administration Center for Food Safety and Applied Nutrition (Garthright and Blodgett, 2003).

Initial Composition Analysis. Fat content was determined using mid-infrared analysis (AOAC International 2000; method number 33.2.31, 972.26) using a MilkoScan 605 (Foss Electric A/S). Approximately 3-g samples of the 2 raw creams were taken and diluted with approximately 45 g of UF permeate from skim milk before being run on the mid-infrared analyzer. This dilution ensured that the fat content values of the samples fell within the range of the instrument. These values were then adjusted to account for the dilution. Fat content of the butter oil was determined using an ether extraction method (AOAC International, 2000; method number 33.2.26, 989.05). Somatic cell counts were done using an optical fluorescence method (AOAC International, 2000; method number 17.13.01, 978.26)

with a Fossomatic 360 (Foss Electric A/S). Milk fat globule size distribution was measured (Smith et al., 1995) using laser light scattering (Mastersizer/E; Malvern, Worcestershire, UK). A reverse Fourier optical lens with a 45-mm focal length was used and only forward light scattering was measured. When using only forward light scattering minimal influence of light scattering by casein micelles occurs when measuring the particle size distribution of milk fat globules. The parameters used to describe milk fat globule size distribution were the volume mean diameter [**d(4,3)**] and the mean fat globule diameter below which 90% of fat volume is contained [**d(0.9)**]. Efficacy of pasteurization and consistency of the pasteurization treatment across all replications were determined using a phosphatase test (Wehr and Frank, 2004; method 14.060).

Shelf-Life Testing. Three 1-L bottles of milk for each of the 4 treatments (pasteurized MF skim permeate, and the 3 pasteurized 2% fat milks made by addition of raw cream 1, raw cream 2, or pasteurized butter oil to MF skim permeate) were incubated for shelf-life testing at 1.7 and 5.7°C for 90 d or until the SPC on the BactoScan FC analyzer was >20,000 cfu/mL. Each 1-L shelf-life bottle was immersed in a water bath with the water level at least 2.54 cm above the milk level. Sample bottles were placed on a raised platform within each bath, beneath which a small submersible pump (model NK-1; Little Giant Pump Co., Oklahoma City, OK) circulated the water to maintain uniform temperature throughout the bath. To minimize temperature fluctuations, and as an added safeguard in case of a malfunction, all water baths were operated in a walk-in cooler maintained at 6°C. The temperature of each water bath was monitored continuously using a National Institute of Standards and Technology-certified (to nearest 0.1°C) 8-channel thermocouple-based temperature data logger (OM-CP-OCTTEMP; Omega Engineering Inc.) equipped with Type E thermocouples. The mean temperatures and standard deviations of the water baths were $1.7 \pm 0.1^\circ\text{C}$ and $5.7 \pm 0.1^\circ\text{C}$.

Milk from each 1-L bottle for each treatment was sampled for SPC weekly over 90 d. Each bottle was removed from its water bath and mixed on a stir plate (Thermix stirring hot plate, model 310T; Fisher Scientific, Fairlawn, NJ) for 60 s inside the UV light sanitized HEPA-filtered air transfer hood. A 20-mL sample was aseptically pipetted from each shelf-life bottle into a 60-mL sterile snap-top vial that was immediately placed on ice until time of analysis (within 24 h). Each container of milk for each treatment was sampled up to 14 times over the 90-d study, so care was taken to minimize the risk of contamination. This was a worst-case scenario versus having a separate 1-L container for each of the 13 sampling times for each treatment. The

SPC (cfu/mL) of each sample was determined by flow cytometry using the BactoScan FC analyzer (Suhren et al., 2001; Walte et al., 2005).

RESULTS AND DISCUSSION

Microbial and Chemical Analysis

The mean TBC of the raw whole milk was 1,100 cfu/mL, which is below the regulatory limit (FDA, 2011) of 300,000 cfu/mL for commingled raw milk (Table 1). The mean spore and coliform counts of the raw whole milk were 25 and 68 cfu/mL, respectively (Table 1). The mean SCC of the raw whole milk was determined to about 200,5000 cells/mL, which is below the regulatory limit (FDA, 2011) of 750,000 cells/mL for grade A raw milk (Table 1). The raw skim milk had a higher ($P < 0.05$) TBC and lower SCC than the original raw milk (Table 1).

The mean TBC and spore count of raw cream 1 was determined to be 25 and 60 cfu/mL, respectively (Table 1). These values were higher than the mean TBC and spore count of raw cream 2 and the pasteurized butter oil, whose counts were all <10 cfu/mL. It was expected that raw cream 1 would have higher mean TBC and spore counts than the other 2 fat sources. Raw cream 2 was produced from the centrifugal separation of the lower 90% of gravity separated raw whole milk, which had a reduced microbial count due to the concentration of bacteria and spores in the upper cream layer during gravity separation. The butter oil, produced from raw cream 2, also had a reduced microbial count due to batch pasteurization. All 3 fat sources had coliform counts <1 cfu/mL.

The mean fat content of the raw skim milk and pasteurized MF skim permeate was determined to be 0.12 and 0.06%, similar to the values of 0.10 and 0.03% reported by Elwell and Barbano (2006), using the same MF process (Table 2). The mean fat contents of raw cream 1, raw cream 2, and the pasteurized butter oil were 40.55, 33.86, and 99.00%, respectively. The higher fat content of raw cream 1 compared with raw cream 2 was due to the reduced efficiency of the centrifugal cream separator when processing gravity “skim” (with approximately 2.35% fat) from the lower 90% of the gravity-separated whole milk compared with whole milk with 3.3% fat.

Removal of Bacteria with Microfiltration and Pasteurization

Most swab samples of valves and other connections indicated no detectable microbial contamination after enrichment. When contamination was found, that loca-

Table 1. Total bacteria, spore, coliform, and somatic cell counts of milk ingredients (n = 4)

Item	Raw whole milk	Raw skim milk	Unpasteurized MF skim permeate ¹	Raw cream 1 ²	Raw cream 2 ³	Pasteurized butter oil
Bacteria count (cfu/mL)						
Mean	1,128 ^b	1,691 ^a	0.13 ⁴	25	<10	<10
SD	44	209	0.04 ⁴	13	—	—
Spore count (cfu/mL)						
Mean	25 ^a	13 ^a	<10	60	<10	<1
SD	11	11	—	87	—	—
Coliform count (cfu/mL)						
Mean	68 ^a	80 ^a	<1	<1	<1	<1
SD	47	47	—	—	—	—
SCC (cells/mL)						
Mean	199,500 ^a	106,000 ^b				
SD	25,000	19,000				

^{a,b}Means for raw whole milk and raw skim milk within a row not sharing a common superscript are significantly different ($P < 0.05$).

¹MF = microfiltered.

²Raw cream 1 obtained from cold centrifugal separation of raw whole milk.

³Raw cream 2 obtained from hot centrifugal separation of the lower 90% of gravity-separated raw whole milk.

⁴Determined using the most probable number method.

tion was disassembled and carefully inspected before use for the next replicate.

The mean fat content of the pasteurized 2% fat milks made with unpasteurized MF skim permeate plus raw cream 1, raw cream 2, and pasteurized butter oil was determined to be 2.06, 2.24, and 1.88%, respectively (Table 2), and these values were not different ($P > 0.05$). The volume mean diameter [i.e., d (4,3)] values of the homogenized pasteurized 2% fat milks made with unpasteurized MF skim permeate plus raw cream 1, raw cream 2, and pasteurized butter oil, were 0.58, 0.60, 0.62 μm , respectively, and the d (0.9) values were 1.02,

1.00, and 1.05 μm , respectively (Table 2). Using the same particle size analysis method used in the current study, a survey of homogenized whole milks from 16 commercial fluid milk processing plants in New York State was done. The average d(4,3) and d(0.9) were 0.88 and 1.40 μm , respectively, with a range of 0.72 to 1.18 μm for d(4,3) and 1.19 to 1.81 μm for d(0.9). Therefore, the particle size distribution of milk fat in the samples for the current study reflect a more efficient homogenization than typically seen in many commercial homogenized whole milks. All 3 treatments with very different fat sources were sufficiently homogenized, and

Table 2. Fat content, fat globule particle size distribution, and Charm test (Charm Sciences Inc., Lawrence, MA) result of pasteurized microfiltered (MF) skim permeate and pasteurized 2% fat milks made from MF skim permeate plus a fat source (n = 4)

Item	Pasteurized MF skim permeate	Pasteurized 2% milk (raw cream 1 ¹)	Pasteurized 2% milk (raw cream 2 ²)	Pasteurized 2% milk (butter oil)
Fat (%)				
Mean	0.06	2.06	2.24	1.88
SD	0.01	0.18	0.37	0.04
d(4,3) ³ (μm)				
Mean	NA ⁴	0.58	0.60	0.62
SD		0.02	0.07	0.06
d(0.9) ⁵ (μm)				
Mean	NA	1.02	1.00	1.05
SD		0.07	0.09	0.06
Charm ⁶ (mU/L)				
Mean	34	20	19	19
SD	11	5	5	8

¹Raw cream 1 obtained from centrifugal separation of raw whole milk.

²Raw cream 2 obtained from centrifugal separation of the lower 90% of gravity-separated raw whole milk.

³Volume mean diameter.

⁴NA = not applicable.

⁵Mean fat globule diameter below which 90% of fat volume is contained.

⁶Efficacy of pasteurization was determined using an alkaline phosphatase test. A Charm value of <350 mU/L indicates proper pasteurization.

Table 3. Initial bacteria counts (cfu/mL) in pasteurized microfiltered (MF) skim permeate and pasteurized 2% fat milks made from MF skim permeate plus a fat source (n = 4)

Item	Pasteurized MF skim permeate	Pasteurized 2% milk (raw cream 1 ¹)	Pasteurized 2% milk (raw cream 2 ²)	Pasteurized 2% milk (butter oil)
Bacteria count				
Mean	0.08 ³	18 ⁴	59 ⁴	92 ⁴
SD	0.11	19	12	78
Spore count				
Mean	<1	<10	11	29
SD	—	—	7	29
Coliform count				
Mean	<1	<1	<1	<1

¹Raw cream 1 obtained from centrifugal separation of raw whole milk.

²Raw cream 2 obtained from centrifugal separation of the lower 90% of gravity-separated raw whole milk.

³Determined using the most probable number method, expressed as SPC.

⁴Determined using the total bacteria count method.

no difference ($P > 0.05$) in fat globule size distributions [d(4,3) and d(0.9)] was detected among treatments. All pasteurized milks were phosphatase negative, with alkaline phosphatase values by the Charm test (Charm Sciences Inc., Lawrence, MA) below 350 mU/L (Table 2).

Across 4 replicates, the average TBC of the raw skim milk was reduced from 1.6×10^3 cfu/mL before MF to 0.13 cfu/mL after MF, resulting in an average 4.13 log reduction due to MF (Table 1). The TBC of the unpasteurized MF skim permeate and the logarithmic reduction in TBC from the raw skim milk due to MF were similar to the mean values of 0.47 and 3.80 cfu/mL reported by Elwell and Barbano (2006). The mean SPC of the pasteurized MF skim permeate (Table 3), as determined by MPN, was 0.08 cfu/mL and this was similar to the 0.06 cfu/mL reported by Elwell and Barbano (2006). Spore and coliform counts of the pasteurized MF skim permeate were <1 cfu/mL immediately after processing (Table 3). Pasteurization of the unpasteurized MF skim permeate achieved a mean 0.53 log reduction in bacteria count. This value is less than the logarithmic reduction from pasteurization of 1.84 reported by Elwell and Barbano (2006) and may be due to a different mixture of microflora in the starting milks. The mean TBC for the pasteurized 2% fat milks made with unpasteurized MF skim permeate plus raw cream 1, raw cream 2, and pasteurized butter oil were 18, 59, and 92 cfu/mL, respectively, immediately after processing (Table 3), and these counts were not significantly different, but were higher than the pasteurized MF skim milk permeate. The mean spore counts for the 2% fat milks made with unpasteurized MF skim permeate plus raw cream 1, raw cream 2, and pasteurized butter oil were <10, 11, and 29 cfu/mL, respectively, and the mean coliform count for each 2% fat milk was <1 cfu/mL immediately after processing (Table 3). Neither the spore nor the coliform counts differed ($P > 0.05$) among treatments.

Shelf Life of Microfiltered Pasteurized 2% Milks

The shelf life of the pasteurized MF skim permeate and pasteurized 2% fat milks is reported as the percentage of containers stored at each temperature that had SPC >20,000 cfu/mL (Figure 1) to make it easier to see differences among treatments. For the pasteurized MF skim permeate, following 90 d of storage, 8.3% of containers at 1.7°C and 0% of containers at 5.7°C had counts >20,000 cfu/mL (Figures 1a and b, respectively). For the pasteurized 2% fat milk made with unpasteurized MF skim permeate plus raw cream 1, following 90 d of storage, 0% of containers at 1.7°C and 8.3% of containers at 5.7°C had counts >20,000 cfu/mL (Figures 1a and b, respectively). For the pasteurized 2% fat milk made with unpasteurized MF skim permeate plus raw cream 2, following 90 d of storage, 25% of containers at both 1.7 and 5.7°C had counts >20,000 cfu/mL (Figures 1a and b, respectively). The 25% of containers across the 4 replicates (i.e., each replicate was a separate processing run) that had growth >20,000 cfu/mL were all from the same replicate. For the pasteurized 2% fat milk made with unpasteurized MF skim permeate plus pasteurized butter oil, following 90 d of storage, 25% of containers at 1.7°C and 0% of containers at 5.7°C had counts >20,000 cfu/mL (Figures 1a and b, respectively). The 25% of containers for this treatment across the 4 replicates stored at 1.7°C that had growth >20,000 cfu/mL were also from a single replicate. We did not observe any clear advantage in shelf life of 2% fat pasteurized homogenized milk when using cream that had been reduced in initial bacteria and spore count by gravity separation or by using pasteurized butter oil as the fat source. However, if the starting bacteria and spore count of the original raw milk was higher, some benefit of these treatments may have existed to reduce the bacteria and spore count of the cream source used to make the 2% fat milks.

A chance of contamination existed during the repeated weekly sampling of each container. Each container was opened and sampled 14 times during the 90-d period. It is likely that some of the growth observed in the containers was due to contamination during sampling, particularly when some containers from a treatment had microbial growth at 1.7°C and not 5.7°C. The results of this study represent an improvement in shelf life when compared with data from Elwell and Barbano (2006), who found that 0% of commercially pasteurized skim milk samples remained at <20,000 cfu/mL by d 57 and 22, after storage at 2.0 and 6.1°C,

respectively. The shelf-life results for the pasteurized MF skim permeate samples also were better than those reported by Elwell and Barbano (2006), who found that for pasteurized MF skim permeate samples after 92 d of storage, 100% of containers stored at 2.0°C and 50% of containers stored at 6.1°C remained at <20,000 cfu/mL. In the current study, all the skim milk samples were at <20,000 cfu/mL for 90 d at 5.7°C (Figure 1b). A study on the shelf life of commercially pasteurized 2% fat milks conducted by Fromm and Boor (2004), found that after 7, 14, and 17 d of storage at 6°C, 92, 42, and 8% of the samples had bacteria counts <20,000

Figure 1. Percentage of all samples with SPC >20,000 cfu/mL for pasteurized microfiltered (MF) skim milk and pasteurized homogenized 2% fat milks made from microfiltered skim milk plus a fat source (made from raw cream 1, raw cream 2, and pasteurized butter oil) stored at 1.7°C (a) and 5.7°C (b).

cfu/mL, respectively. Our study found that, for the pasteurized 2% fat milk made with unpasteurized MF skim permeate plus raw cream 1, 92% of containers stored at 5.7°C remained at <20,000 cfu/mL for 90 d. An opportunity clearly exists for the fluid milk industry to extend the shelf life of refrigerated HTST fluid milk by use of MF of skim milk to double and even triple the shelf life currently achieved. Further work is needed to calculate the increased cost of processing versus the benefits of increased fluid milk shelf life.

CONCLUSIONS

By reducing the total bacteria, spore, coliform, and somatic cell counts in raw skim milk to low levels, the MF and HTST pasteurization (78.3°C at 15 s) process produced skim milk and 2% fat milks with extended refrigerated shelf life (i.e., bacteria count <20,000 cfu/mL at 90 d). A 4.13 log reduction in total bacteria was achieved by MF, and a further 0.53 log reduction was achieved by HTST pasteurization of MF skim permeate, resulting in a total 4.66 log reduction in bacteria count for the combined process. No containers of MF skim milk that was HTST pasteurized after MF exceeded 20,000 cfu/mL SPC during the 90 d of storage at 5.7°C. The approaches used in the current study to reduce the initial bacteria and spore count of the cream source used to make the 2% fat milks in combination with MF skim permeate did not produce any shelf-life advantage over cold separated raw cream when starting with excellent quality raw milk (i.e., low bacteria count). The combined process of MF plus HTST pasteurization (78.3°C for 15 s), followed by filling and packaging that is protected from microbial contamination, can achieve a refrigerated shelf life of 60 to 90 d of storage at both 1.7 and 5.7°C for milk containing 2% fat. Further work is needed to evaluate the sensory quality of long-shelf-life fluid milk produced with this process.

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