Microfiltration of skim milk and modified skim milk using a 0.1- μ m ceramic uniform transmembrane pressure system at temperatures of 50, 55, 60, and 65°C¹

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ABSTRACT

Increasing the temperature of microfiltration (MF) to >50°C may allow for operation at higher fluxes and reduce the bacterial growth during MF. However, there is a concern that operating at higher temperatures could cause calcium phosphate precipitation that would lead to membrane fouling. Our objective was to determine the effect of operating a 0.1- μ m ceramic uniform transmembrane pressure MF unit at temperatures of 50, 55, 60, and 65°C on membrane fouling and serum protein (SP) removal from skim milk with and without removal of low-molecular-weight soluble milk components by ultrafiltration (UF) before MF at a flux of 54 kg/m² per hour. For each replicate, 1,000 kg of pasteurized skim milk was split into 2 batches. One batch was ultrafiltered (with diafiltration) to remove an average of 89 $\pm$ 2% of the lactose and a percentage of the soluble calcium and phosphorus. The retentate from UF was diluted back to the protein concentration of skim milk, creating the diluted UF retentate (DUR). On subsequent days, both the DUR and skim milk were run on the MF unit with the flux maintained at 54 kg/m² per hour and a concentration factor of 3 $\times$ and the system run in recycle mode. The temperature of MF was increased in 5°C steps from 50 to 65°C, with a 1-h stabilization period after each increase. During the run, transmembrane pressure was monitored and permeate and retentate samples were taken and analyzed to determine if any changes in SP, calcium, or phosphorus passage through the membrane occurred. Increasing temperature of MF from 50 to 65°C at a flux of 54 kg/m² per hour did not produce a large increase in membrane fouling when using either skim milk or a DUR as the MF feed type as measured by changes

in transmembrane pressure. Increasing the temperature to 65°C only caused a slight reduction in calcium concentration in the permeate (11 $\pm$ 3%) that was similar between the 2 MF feed types. Increasing processing temperature reduced the percentage of SP removal by the process, but the increased temperature also caused a decrease in casein contamination in the permeate with no evidence of membrane fouling.

Key words: ceramic microfiltration, serum protein removal, processing temperature

INTRODUCTION

Much of the research using microfiltration (MF) to separate serum protein (SP) and micellar casein in skim milk has been conducted at temperatures of 50 to 55°C. Whereas some researchers have looked at MF at lower temperatures (15°C; Samuelsson et al., 1997; Lawrence et al., 2008), temperatures above 55°C have not been explored. Several possible advantages for operating at higher temperatures exist. It may allow the process to operate at a higher flux, as research has found that increasing the temperature of filtration increased the flux (Samuelsson et al., 1997). Operation at a higher flux would reduce the membrane area required at the same production rate, reducing the system cost. Energy costs may also be reduced, as the pumping energy required to maintain a constant recirculation rate will decrease as retentate viscosity decreases (Cheryan, 1998). Another advantage is a possible reduction in microbiological growth. During the process any bacteria in the feed will be concentrated in the retentate along with the micellar casein. Operation at higher temperatures may reduce the bacterial growth in the retentate, improving product quality.

Despite the possible advantages of operating a MF at higher temperatures, possible disadvantages may arise when operating at temperatures above 50°C; including decreased membrane stability, mineral precipitation, and protein denaturation. Different membrane materials have different stability in regard to temperature. Spiral-wound polymeric membranes typically have a

Received August 23, 2014.

Accepted November 1, 2014.

¹Use of names, names of ingredients, and identification of specific models of equipment is for scientific clarity and does not constitute any endorsement of product by authors, Cornell University, or the Northeast Dairy Foods Research Center.

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temperature limit of around 75°C (Cheryan, 1998), though manufacturer recommendations on specific membranes are often less (63°C for Parker-Hannifin polyvinylidene fluoride MF membranes; Parker-Hannifin, 2014). In contrast, ceramic membranes can operate at much higher temperatures (120°C; Cheryan, 1998).

Calcium phosphate solubility decreases as temperature increases and operating the MF process at higher temperatures may cause precipitation. Precipitation of calcium phosphate could cause severe membrane fouling. In simulated skim milk ultrafiltrate (containing the mineral concentration of the permeate phase of skim milk, but no protein) at 55°C, precipitation of calcium phosphate was seen and the amount precipitated increased as temperature increased (Spanos et al., 2007).

In skim milk it is not as clear if increasing the temperature in the range of 55 to 65°C would cause calcium phosphate precipitation. Heating of milk at 60°C for 1 h caused a roughly a 30% decrease in the concentration of calcium and phosphorus in the permeate portion of milk isolated by UF that was attributed to calcium phosphate precipitation (Pouliot et al., 1989). However, milk proteins have been found to have a protective effect against calcium phosphate precipitation, when researchers separated the permeate portion of skim milk before heat treatment little precipitation was seen when the protein concentration was 0.8% (Brule et al., 1978). Additionally, in the fouling of heat exchangers at temperatures from 75 to 110°C, 50 to 70% of the foulant was protein (30 to 40% mineral), and it was not until temperatures above 110°C that 70 to 80% of the foulant was mineral (Bansal and Chen, 2006).

Increasing the temperature of MF from 50 to 65°C might also cause denaturation of SP (β -LG and α -LA) and possible association with CN micelles. Long et al. (1963) found that only 3.4% of β -LG associated with κ -CN after 20 min at 65°C. Any β -LG covalently associated with the CN micelles would not be removed by MF. If β -LG was covalently associated with κ -CN at higher temperatures, then the yield of SP in the permeate would be reduced. β -Lactoglobulin and α -LA have also been found to form aggregates with each other when heated at 75°C (Dalglish et al., 1997), and these aggregates might not be able to pass through the MF membrane. In contrast to SP, CN is relatively stable to heat treatment, but increasing the temperature could reduce the amount of nonmicellar CN in the serum phase. Rose (1968) found that increasing the temperature from 4 to 35°C reduced the amount of CN in the serum phase by more than 60%. A reduction in soluble CN at temperatures above 50°C may reduce the CN contamination in the SP concentrates produced from MF permeates.

The effect of increasing the temperature on membrane fouling can be monitored by measuring the transmembrane pressure (**TMP**). According to Darcy's law, flux is equal to TMP divided by permeate viscosity and resistance (Belfort et al., 1994). At a constant flux and viscosity, an increase in fouling would increase the resistance and the TMP required to maintain a constant flux. Increasing the temperature is expected to decrease the viscosity of the permeate (Morison and Mackay, 2001), and if no change in membrane resistance occurs the TMP required to maintain a constant flux is expected to decrease as temperature is increased. Membrane fouling may also change the rejection characteristics of the membrane. Gesan-Guizieu et al. (1999) found that as membrane resistance (fouling) increased, transmission of SP decreased during the MF of skim milk.

If operation at elevated temperatures is not feasible with skim milk as the MF feed because of mineral precipitation and fouling of MF membranes, then it may be possible to use UF to remove some of the soluble minerals from skim milk before MF to minimize the effect of heat on fouling of MF membranes at temperatures higher than 50°C. Our objective was to determine the effect of operating a 0.1- μ m ceramic uniform transmembrane pressure (**UTP**) MF unit at temperatures of 50, 55, 60, and 65°C on membrane fouling and SP removal from skim milk with and without removal of low-molecular-weight soluble milk components by UF before MF at a flux of 54 kg/m² per hour.

MATERIALS AND METHODS

Experimental Design and Statistical Analysis

Each replicate consisted of 4 d of processing. On the first day, approximately 1,000 kg of raw skim milk was pasteurized at 72°C for 16 s, cooled to 4°C, and stored overnight. The skim milk was split into 2 portions. The first portion of milk was UF on the second day to reduce the low-molecular-weight solutes in skim milk by approximately 90%; the UF retentate was then diluted with reverse osmosis (**RO**) water to achieve the protein concentration of original skim milk. The second portion of skim milk was MF directly with no prior UF. In this way the effect of lactose and soluble minerals on MF could be determined independent of protein concentration.

On the third and fourth processing days the diluted UF retentate (**DUR**) and skim milk were microfiltered. The MF was operated at a concentration factor (**CF**) of 3 $\times$ in total recirculation mode (i.e., the permeate and retentate were returned to the MF feed tank). The temperature was increased sequentially from 50 to 55

to 60 to 65°C. The MF system was operated for 1 h at each temperature. After 1 h at each temperature, samples of the MF retentate and permeate were collected for chemical analysis. The experiment was replicated 3 times.

All data were analyzed by ANOVA using the Proc GLM (general linear model) procedure of SAS (SAS version 8.02, SAS Institute Inc., Cary, NC). A split plot model was used. Replicate and type of MF feed (i.e., DUR or skim milk) were categorical whole plot variables. The feed by replicate type III mean sum of squares was used as the error term to test for the significance of the whole plot terms (feed and replicate). Temperature was transformed to a mean-centered continuous variable. Temperature and the interactions with feed and replicate were the split plot terms. To determine the effect of type of MF feed and temperature on permeate and retentate composition, along with interactions, the following model was used: dependent variable = feed + replicate + feed $\times$ replicate + temperature + temperature $\times$ feed + temperature $\times$ replicate + temperature $\times$ feed $\times$ replicate + temperature $\times$ temperature + temperature $\times$ temperature $\times$ feed + temperature $\times$ temperature $\times$ replicate + temperature $\times$ temperature $\times$ feed $\times$ replicate.

For the analysis of retentate and permeate composition measured by SDS-PAGE, only the 50 and 65°C samples were analyzed and temperature was a categorical variable, but feed and replicate remained whole plot terms. The model used was dependent variable = feed + replicate + feed $\times$ replicate + temperature + temperature $\times$ feed + temperature $\times$ replicate. The type III mean sum of squares for feed $\times$ replicate was used as the error term to test for the significance of the feed and replicate. For both models, the full model was run, then higher order nonsignificant terms in the model were discarded one by one (if they did not appear in significant terms), and the model was rerun until all split plot terms were either significant or appeared in higher order significant terms.

Microfiltration Feed Preparation

Skim Milk. Raw whole bovine milk (about 1,000 kg) was separated in the Cornell University dairy plant at 4°C using a model 372 Air Tight Centrifuge (DeLaval Co., Chicago, IL); if the skim milk contained more than 0.15% fat as measured using an infrared spectrophotometer (IR; Lactoscope FTIR, Delta Instruments, Drachten, the Netherlands) the milk was re-separated before pasteurization. Raw skim milk was pasteurized with a plate heat exchanger with 3 sections: regeneration, heating, and cooling (model 080-S, AGC Engineering, Manassas, VA) at 72°C with a holding time

of 16 s. The pasteurized skim milk was cooled to 4°C and stored at $\leq 4^\circ\text{C}$ until processing. The pasteurized skim milk was split into 2 portions; one portion was UF before MF (as described herein), the other portion was MF as skim milk.

DUR. On the second day of processing, about 540 kg of pasteurized skim milk was UF to remove approximately 90% of the lactose and soluble minerals. The UF system was run in batch recirculation mode using a polyethersulfone (PES) spiral-wound UF membrane (model 3838, Gea Niro Inc., Hudson, WI; nominal molecular weight cutoff: 10,000 Da, surface area: 13.6 m²). Before processing, the UF membrane was cleaned and sanitized, following the procedure of Evans et al. (2009). After the cleaning step the membrane was then flushed with 50°C RO water to neutral pH and the clean water flux was determined by operating only the inlet pump with an inlet pressure of 172 kPa. The initial clean water flux (typically about 41.6 L/m² per hour) was measured by weight by collecting permeate for 30 s.

The skim milk was heated to 51°C before being transferred to the UF feed tank. The UF feed pump was started and approximately 20 L of retentate and permeate were collected to remove most of the water from the system, then the retentate was returned to the UF feed tank while the permeate was collected. Next, the retentate recirculation pump was turned on and the inlet pressure adjusted to 276 kPa, the outlet retentate pressure was approximately 124 kPa for the entire run, with no back pressure on the permeate. Permeate was collected, weighed, and discarded. Samples of permeate and retentate were taken every 15 min for analysis using an IR (Lactoscope FTIR) to monitor retentate and permeate composition. The UF feed tank could only hold approximately 315 kg of skim milk; thus, as permeate was collected, the remaining skim milk was added to the UF feed tank. When a CF of 2 $\times$ had been achieved, the second stage (first diafiltration step) began. The RO water was heated to 50°C and added to the UF feed tank, the weight of water added was equal to the weight of permeate removed in the previous stage. For the first diafiltration, the target CF was 2 $\times$; when a 2 $\times$ CF was reached, the second diafiltration began with RO water addition equal to the weight of permeate removed in the second stage. For the second diafiltration, concentration continued until the UF feed tank composition achieved a ratio of lactose to protein by IR (Lactoscope FTIR) >6.78. A lactose-to-protein ratio of 6.78 corresponds to an approximate 90% reduction in lactose and other low-molecular-weight compounds. At this point, the UF was stopped and the UF retentate was collected and weighed. The collected UF retentate was then adjusted to a lactose-to-protein ratio of 6.78 by the addition of UF permeate saved from

the first stage. The retentate was diluted by the addition of RO water such that the DUR had the protein concentration of skim milk as measured by IR. This DUR then had the approximate protein concentration of skim milk, but with a greatly reduced lactose and soluble mineral concentration. The DUR was chilled to $\leq 4^{\circ}\text{C}$ and stored at $\leq 4^{\circ}\text{C}$ overnight. Immediately after processing, the UF system was cleaned as described in Evans et al. (2009).

MF Operation

A pilot-scale UTP MF system (Tetra Alcross M7, TetraPak Filtration Systems, Aarhus, Denmark) equipped with ceramic Membralox (EP1940GL 0.1 μA , Alumina, Pall Corp., Cortland, NY) membranes (pore diameter: 0.1 μm ; surface area: 1.7 m^2) and variable area flow meters (models 57/-/23 and 55/-/23 for the permeate and retentate, respectively, GEMÜ, Atlanta, GA) were used. The membranes in a tubular stainless module consisted of 7 ceramic tubes, 19 channels each with 4 mm of channel diameter. The permeate section of the stainless steel module was filled with polymeric beads (3.72 to 3.78 mm in diameter) to reduce dead volume, act as buffer for pressure changes, and produce a larger pressure decrease from inlet to outlet on the permeate side of the membrane to match the pressure decrease from inlet to outlet on the retentate side of the membrane. The UTP MF system consisted of a feed pump (type LKH 10/110 SSS, 1.75 kW), a retentate recirculation pump (type LKH 20/125 SSS, 6.3 kW) with a variable-frequency drive (MC Series, Model M12100C, Lenze AC Tech, Uxbridge, MA), a magnetic flow transmitter (I/A Series, IMT25, Foxboro, Foxboro, MA) on the recirculation loop so that the cross-flow velocity could be monitored, and a permeate recirculation pump (type LKH 10/130 SSS 2.5 kW) with all pumps from Alfa Laval (Kansas City, MO). The membranes were 1.02 m in length and mounted vertically in the MF system with permeate and retentate flow co-current from the top to the bottom of the module. Because the membrane was mounted vertically, the inlet and outlet gauge pressures had to be corrected for the difference due to the weight of the vertical column of liquid. The correction was measured with 50°C RO water in the system and only the feed pump turned on and the retentate and permeate outlet valves closed. Retentate inlet pressure (Rp_i), permeate inlet pressure (Pp_i), retentate outlet pressure (Rp_o), and permeate outlet pressure (Pp_o) were measured under these conditions. A correction factor for calculating TMP was calculated for each gauge pressure as follows: the Rp_i gauge pressure correction was Pp_o minus Rp_i , the Rp_o gauge pressure correction was Pp_o minus Rp_o ,

the Pp_i gauge pressure correction was Pp_o minus Pp_i , and the Pp_o gauge pressure correction was zero. This correction factor was determined at the beginning of each day's processing. Next, retentate and permeate recirculation pumps were turned on and the retentate bleed flow was set to 45 L/h and the permeate bleed flow was set to 90 L/h. The elevation-corrected inlet and outlet pressures were measured and the TMP from the retentate to the permeate side of the membrane at the retentate inlet (TMP_i) and outlet (TMP_o) ends of the membrane were calculated. The goal was to have a ΔTMP ($\Delta\text{TMP} = \text{TMP}_i - \text{TMP}_o$) of 25 ± 3 kPa for a membrane length of 1.02 m. A diaphragm valve in the permeate recirculation loop was used to adjust the recirculation flow rate on the permeate side of the membrane. The permeate recirculation flow rate was adjusted with the diaphragm valve until the ΔTMP was 25 ± 3 kPa.

Cleaning Prior to Processing. The MF system was cleaned before each week's processing. Storage solution (0.55% vol/vol aqueous solution of 70% nitric acid, Fisher Scientific, Waltham, MA) was flushed out of the system with room-temperature RO water until the pH was neutral. The MF flow system was heated with RO water to 80°C and then Ultrasil 25 (Ecolab Inc., Food and Beverage Division, St Paul, MN) liquid alkaline membrane cleaner (1.95% vol/vol) was added to the water to reach pH 11. The alkaline solution was recirculated for 25 min at a permeate removal rate of approximately 1,000 L/h and a retentate removal rate of approximately 160 to 180 L/h, with all pumps running. After cleaning, the membrane system was slowly ($<10^{\circ}\text{C}$ per min) cooled to 50°C with a tubular heat exchanger in the recirculation loop. The MF system was then flushed with RO water (about 300 kg at 30°C) until neutral pH was reached.

On the first day of MF processing, the membrane was flushed with 50°C RO water until the system temperature was 50°C (about 60 kg) and the initial clean water flux was determined. During flux measurement, the retentate removal outlet valve was closed and permeate outlet valve was fully open and only the feed pump was running.

Processing: Diluted UF Retentate. The DUR (about 320 kg) was processed at a $3\times$ CF (a $3\times$ CF being 2 kg of permeate removed for every 1 kg of retentate) at 50, 55, 60, and 65°C using the UTP MF system described previously. The temperature was controlled by changing the flow of cooling water to the tubular heat exchanger in the MF retentate recirculation loop. The system was started on 50°C RO water and a transition from water to DUR occurred with all the pumps running; the retentate recirculation rate was approximately 648 L/min with a linear velocity of ap-

proximately 6.5 m/s. To flush the 50°C water out of the system with DUR at the beginning of the process, about 122 kg of 50°C DUR was processed with the retentate and permeate discarded. After this start up, about 320 kg of 50°C DUR was added to the MF feed tank with the retentate and permeate being returned to the feed tank. Target retentate and permeate removal rates were 45 and 90 L/h, respectively, and were selected to achieve a 3× CF. If the Δ TMP was not 25 ± 3 kPa after switching from water to DUR, then the permeate recirculation diaphragm valve was adjusted while processing to achieve and maintain a Δ TMP of 25 ± 3 kPa.

After flushing with DUR, the retentate and permeate were returned to the feed tank to run the system in total recirculation mode at 50°C for 1 h. The flux (kg/m² per hour) and CF by weight were measured every 15 min. If the CF was not 3.0 ± 0.05 , then the permeate or retentate removal rates were adjusted depending upon whether the target flux of 54 kg/m² per hour was met. For example, if the permeate removal rate was such that the flux was 54 kg/m² per hour but the CF was off target, then the retentate removal rate would be adjusted to achieve a 3× CF. Samples of permeate and retentate were taken every 15 min for analysis using an IR (Lactoscope FTIR) to monitor retentate and permeate composition. After 1 h at 50°C, permeate and retentate were collected for 15 min to produce samples for chemical analysis. After sampling (about 500 mL of retentate and permeate), the remaining 50°C retentate and permeate that were collected during the 15 min were returned to the MF feed tank along with the flow of retentate and permeate. Next, the temperature was increased to 55°C by decreasing the amount of cooling water flowing in the tubular heat exchanger in the MF retentate recirculation loop. The MF was operated in recirculation mode for 1 h at 55°C under the same operating conditions (flux and CF) and sampling regimen used at 50°C. After 1 h at 55°C, a 15-min collection of retentate and permeate was done and composite samples were taken, as described previously. This procedure was repeated for 60 and 65°C. During the entire run, as temperatures increased, the diaphragm valve was adjusted as necessary to maintain a Δ TMP of 25 ± 3 kPa. The frequency on the retentate recirculation pump was also adjusted to maintain a recirculation rate of approximately 648 L/min. After processing, the membrane system was cleaned immediately.

Processing: Skim Milk. The MF system was flushed with room temperature RO water to remove the 0.55% (vol/vol) nitric acid storage solution from the previous day's cleaning. The system was then flushed with 50°C RO water until the system was at 50°C. The starting flux and pressure correction factors were then

determined as described previously. Skim milk (about 335 kg) was processed at a 3× CF at 50, 55, 60, and 65°C using the UTP MF system described herein, using the same operating conditions and parameters as for the DUR. To flush the 50°C water out of the system with skim milk at the beginning of the process, about 116 kg of 50°C skim milk was processed with the retentate and permeate discarded.

Cleaning After Processing. Immediately after processing, 50°C RO water (about 150 to 200 L) was flushed through the MF system with all pumps on. The retentate and permeate removal rates were set at approximately 160 and 120 L/h, respectively. The MF system was flushed until no skim milk or DUR was visible in the flush water on the retentate side. When the water flush was complete, the fouled membrane water flux was determined (retentate outlet valve closed, permeate outlet valve completely open, with only the feed pump running with temperature maintained at 50°C). Typically, fouled membrane flux was about 90% of the clean membrane water flux (740 vs. 830 L/m² per hour). Next, the MF flow system was heated with RO water to 80°C. Ultrasil 25, liquid alkaline membrane cleaner (Ecolab Inc.) was added (1.95% vol/vol) to the water to reach pH 11. This solution was recirculated for 25 min with the permeate and retentate bleed at approximately 1,000 and 160 to 180 L/h, respectively, with all pumps on. After cleaning, the membrane system was slowly (<10°C per min) cooled to 50°C using the heat exchanger in retentate recirculation loop. The membrane was then flushed with approximately 30°C RO water until neutral pH was reached. The MF flow system was heated to 50°C by flushing with 50°C RO water and the postrun clean water flux was determined. During the flux determination the retentate outlet valve was closed and permeate outlet valve was fully open with only the feed pump running and the temperature maintained at 50°C. The postrun clean water fluxes were close to prerun clean water flux (about 860 to 830 L/m² per hour). After determination of clean water flux, a 0.55% (vol/vol) aqueous solution of 70% nitric acid was recirculated through the membrane at 50°C for 10 min. Permeate and retentate outlet flows were approximately 1,000 and 160 to 180 L/h, respectively. After 10 min of the nitric acid solution recirculation, the permeate and retentate outlet valves were closed and the pumps turned off. The membrane was stored in 0.55% (vol/vol) dilution of the 70% nitric acid solution.

Chemical Analysis

Samples of skim milk, DUR, permeate, and retentate collected during processing were analyzed using an IR (Lactoscope FTIR) for fat, lactose, and true protein

(**TP**) content (Kaylegian et al., 2006). The MF feeds (DUR and skim milk) were analyzed for TS, fat, and anhydrous lactose using forced-air oven-drying (AOAC International, 2000; method 990.20; 33.2.44), ether extraction (AOAC International, 2000; method 989.05; 33.2.26), and enzymatic lactose (AOAC International, 2000; method 984.15; 33.2.67), respectively. The DUR, skim milk, retentates, and permeates were analyzed for total N (**TN**; AOAC International, 2000; method 991.20; 33.2.11) and NPN (AOAC International, 2000; method 991.21; 33.2.12) content by Kjeldahl. Noncasein nitrogen (**NCN**) content of DUR, skim milk, and retentates was determined using a Kjeldahl method (AOAC International, 2000; method 998.05; 33.2.64) modified so that 5.5 mL of acetic acid (10% vol/vol) was added instead of 1 and 5.5 mL of sodium acetate (1 *N*), to ensure that all of the CN was precipitated at the higher protein concentrations found in the retentates. Total protein was calculated by subtracting NPN from TN and multiplying by 6.38, CN was calculated by subtracting the NCN from TN and multiplying by 6.38, and SP content was calculated by subtracting NPN from NCN and multiplying by 6.38. The calcium and phosphorus content of the DUR, skim milk, MF retentates and MF permeates were measured at Dairy One Forage Analysis Laboratory (Ithaca, NY) using a Thermo IRIS Advantage HX Inductively Coupled Plasma Radial Spectrometer (Waltham, MA). The samples were prepared by predigesting a 5-g sample with 8 mL of nitric acid and 2 mL of hydrochloric acid for 15 min at 20°C. The samples were then heated to 190°C and held for 15 min using microwave digestion [CEM Microwave Accelerated Reaction System (Mathews, NC) with MarsXpress Temperature Control using 50 mL of calibrated Xpress Teflon PFA vessels with Kevlar/fiberglass insulating sleeves], the samples were then diluted to 50 mL using a buffer consisting of 1.5 *N* HNO₃ and 0.5 *N* HCl and aliquots used for analysis.

SDS-PAGE

A 10 to 20% polyacrylamide gradient was used to determine the relative proportion of protein types in retentates and permeates from the MF of both skim milk and DUR at 50 and 65°C; the MF feeds (skim milk and DUR) were also analyzed. The MF feed and permeate samples (0.1 mL) were diluted with sample buffer (0.9 mL); retentate samples (0.1 mL) were diluted with 2.9 mL of the sample buffer. The sample buffer consisted of 10 *mM* Tris-HCl pH 6.8, 1.0% SDS, 20% glycerol, and 0.02% bromophenol blue tracking dye and 50 *mM* dithiothreitol. The prepared samples were stored frozen (−17°C) in glass vials (Target DP Vials

C4000–1W, National Scientific Company, Rockwood, TN) sealed with DP Blue Cap (C4000–51B, National Scientific Company). Diluted samples were thawed, heated to 100°C with steam, and held at 100°C for 3 min and then cooled to about 25°C. The loading was 8.5 µL for retentates and MF feeds and 35 µL for permeates. The samples were loaded onto an SDS-PAGE gel (Verdi et al., 1987) and the procedure of Verdi et al. (1987) was used for running, staining, and destaining the gels. Gels were scanned with USB GS 800 Densitometer using Quantity 1 1-D Analysis software (Bio-Rad Laboratories Inc., Hercules, CA) to obtain a relative protein composition of samples. Loading of the samples was chosen to achieve an optical density of the predominant protein in the sample in the range of 1.0 to 1.4. A milk sample was run on each gel as a reference for proper resolution of milk proteins and a check for consistency of quantitative analysis from gel to gel. The background was adjusted separately for each lane using the rolling disk method of subtraction to obtain a flat base on the pop-up trace. The line that defined each lane was adjusted using the lane tool function (add, adjust anchors) in the software so that the lane line crossed each band at the center. The adjust band function of the densitometer software was used with brackets to set the leading and trailing edge for each band as visually observed on the image of the gel, not based on the beginning and end of the peak in the pop-up trace.

SP Removal Calculation

Percentage SP removal was calculated at each temperature using the formula $100 \times \frac{2}{3} \times (\text{SP concentration in the permeate}) / (\text{SP concentration in the MF feed})$. The value $\frac{2}{3}$ is related to the CF, for each 1 kg of MF feed $\frac{2}{3}$ kg of permeate is removed. For this calculation it was assumed that the CF remained constant at $3\times$.

RESULTS

Composition of MF Feeds

The composition of the skim milk and DUR prepared by UF of skim milk used as MF feeds is shown in Table 1. The UF process was expected to remove the low-molecular-weight components in the serum phase of skim milk, such as lactose and NPN, but not higher-molecular weight components such as protein or fat. The TS, lactose, and NPN concentrations were lower ($P < 0.05$) in the DUR than skim milk (Table 1). The concentration of lactose was reduced by $89.2 \pm 0.2\%$.

Table 1. Microfiltration feed composition: skim milk (SM) and diluted ultrafiltration retentate (DUR) microfiltration feed composition for each of the 3 replicates¹

Item	TS	Fat	Lactose	Ca	P	TN	NPN	NCN	TP	CN	SP	CN%TP
SM												
Replicate 1	9.23	0.17	4.77	1,063	1,002	3.30	0.20	0.78	3.10	2.51	0.59	81.07
Replicate 2	9.10	0.10	4.77	1,070	1,016	3.30	0.19	0.77	3.11	2.53	0.58	81.31
Replicate 3	9.13	0.07	4.80	1,015	978	3.34	0.18	0.78	3.16	2.56	0.60	81.06
Mean	9.15 ^a	0.11 ^a	4.78 ^a	1,049 ^a	999 ^a	3.31 ^a	0.19 ^a	0.78 ^a	3.12 ^b	2.53 ^a	0.59 ^a	81.15 ^a
SD	0.07	0.05	0.01	29.7	19.2	0.02	0.007	0.008	0.03	0.02	0.01	0.14
DUR												
Replicate 1	4.22	0.12	0.50	881	618	3.24	0.05	0.71	3.19	2.53	0.66	79.33
Replicate 2	4.26	0.10	0.52	889	615	3.26	0.04	0.74	3.22	2.52	0.70	78.34
Replicate 3	4.32	0.07	0.53	906	630	3.30	0.05	0.70	3.26	2.60	0.66	79.79
Mean	4.27 ^b	0.09 ^a	0.52 ^b	892 ^a	621 ^b	3.27 ^b	0.04 ^b	0.72 ^a	3.22 ^a	2.55 ^a	0.67 ^a	79.15 ^a
SD	0.05	0.023	0.02	12.8	7.9	0.03	0.003	0.019	0.03	0.04	0.02	0.74

^{a,b}Means in the same column not sharing a superscript are different ($P < 0.05$).

¹All values are percent by weight except calcium and phosphorus, which are given as milligrams per kilogram. TN = total nitrogen $\times 6.38$, NCN = noncasein nitrogen $\times 6.38$, NPN = nonprotein nitrogen $\times 6.38$, TP = true protein (TN minus NPN), CN = casein (TN minus NCN), SP = serum protein (NCN minus NPN), CN%TP = casein as a percentage of true protein, $100 \times \text{CN}/\text{TP}$.

As expected, the concentration of fat in the MF feeds were similar ($P > 0.05$).

No difference in calcium concentration ($P = 0.07$) among feeds was detected, but in each replicate the calcium concentration was lower in the DUR than in the skim milk and the phosphorus concentration was lower ($P < 0.05$) in the DUR than in the skim milk. The trend ($P = 0.07$) for reduction in calcium averaged $15 \pm 2\%$ and the reduction in phosphorus averaged $38 \pm 1\%$ for the DUR. The total reduction in calcium and phosphorus was less than the reduction in lactose, because only about one-third of the calcium and phosphorus in milk is in the serum phase of skim milk. A 90% reduction in the serum phase calcium or phosphorus concentration corresponds to about a 30% overall calcium or phosphorus reduction. Previous researchers have found that UF membranes reject some nonmicellar calcium (Ramachandra Rao et al., 1994), which may explain the lower than expected calcium removal.

The TN and TP concentrations were higher ($P < 0.05$) in the DUR than in the skim milk, as shown in Table 1, which was caused by under-diluting the UF retentate with RO water in the preparation of the DUR. No difference ($P > 0.05$) due to feed was detected for NCN, CN, SP, or CN as a percentage of TP (CN%TP; Table 1).

MF Process Control Parameters

Parameters Controlled During MF. For consistent operation, 4 processing parameters were controlled (Table 2). Flux was maintained at 54 kg/m^2 per hour by controlling the permeate removal rate. The CF was set at $3\times$ and controlled by changing the retentate removal rate. The retentate recirculation rate was kept constant at 648 L/min by decreasing the retentate recirculation pump frequency using an inverter as temperature increased. Finally, the ΔTMP was controlled to 25 ± 3

Table 2. Microfiltration processing control: mean ($n = 3$) flux, concentration factor (CF), recirculation rate, and recirculation pump frequency for the microfiltration of skim milk (SM) and diluted ultrafiltration retentate (DUR) at 50, 55, 60, and 65°C

Feed	Temperature (°C)	Flux (kg/m ² per hour)	CF	Recirculation rate (L/min)	Recirculation pump frequency (Hz)
SM	50	53.5	3.04	647.2	59.0
	55	53.8	3.02	648.8	58.7
	60	54.2	3.00	648.2	58.0
	65	54.7	2.98	646.4	57.7
	Mean	54.0 ^a	3.01 ^a	647.6 ^a	58.35 ^a
DUR	50	53.0	3.03	647.5	58.6
	55	53.2	3.03	648.3	58.2
	60	54.0	3.04	647.4	57.7
	65	54.5	3.05	647.9	57.3
	Mean	53.7 ^a	3.04 ^a	647.8 ^a	57.95 ^a

^aMeans in the same column for each feed did not differ ($P > 0.05$).

Table 3. Microfiltration processing control: ANOVA df and type III sum of squares to determine the effect of feed type (feed), replicate (rep), and temperature (temp) on flux, concentration factor (CF), recirculation rate, and recirculation pump frequency¹

Model term	df	Flux	CF	Recirculation rate	Recirculation pump frequency
Whole model	—	7.359*	0.054*	NS	6.865*
Whole plot					
Rep	2	0.356	0.0002	NS	0.207
Feed	1	0.641	0.0016	NS	0.961
Rep × feed ²	2	0.272	0.0076*	NS	0.185*
Subplot					
Temp	1	6.055*	0.0007	NS	5.627*
Feed × temp	1	NS	0.0078*	NS	NS
Rep × temp	2	NS	0.0093*	NS	NS
Temp × temp	1	NS	0.00004	NS	NS
Temp × feed × rep	2	NS	0.0020*	NS	NS
Temp × temp × rep	2	NS	0.0054*	NS	NS
Temp × temp × feed	1	NS	NS	NS	NS
Temp × temp × rep × feed	2	NS	NS	NS	NS
Reduced model df		6	17	—	6
Reduced error df		17	6	—	17
R ²		0.72	0.98	—	0.96

¹Temperature was transformed to a mean centered continuous variable.

²Used as whole-plot error term for rep and feed.

* $P < 0.05$.

kPa by using a diaphragm valve in the permeate recirculation loop to change the permeate recirculation rate.

Effect of MF Feed Type. The mean values for process control parameters for DUR and skim milk at each temperature are shown in Table 2. No effect of the MF feed type on flux, CF, retentate recirculation rate, or recirculation pump frequency was detected, as shown in Table 3.

Effect of MF Temperature. During each run, the temperature was increased in 5°C steps. The actual temperatures averaged $50 \pm <0.1$, 54.9 ± 0.2 , 60.1 ± 0.5 , and $64.8 \pm 0.6^\circ\text{C}$ and did not vary with replicate or feed ($P > 0.05$). As temperature increased, a slight increase in flux was observed (Tables 2 and 3). The increase in flux was similar for both feeds as confirmed by the fact that the feed by temperature interaction in Table 3 was not significant. If the permeate removal rate had not been controlled, the increase in flux as temperature increased would have been greater.

Recirculation rate was independent ($P > 0.05$) of feed type and temperature (Tables 2 and 3). To maintain a constant recirculation rate as temperature increased, the retentate recirculation pump frequency had to be decreased ($P < 0.05$), as shown in Tables 2 and 3. The decrease in pumping energy needed to maintain constant recirculation rate was likely due to the decrease in viscosity and density of the retentate as temperature increased. Based on principles of fluid mechanics, it is expected that the pressure drop required to maintain a constant flow rate decreases as viscosity and density decrease (Denn, 1980).

Retentate Composition

Effect of MF Feed Type. No effect of feed type ($P > 0.05$) on calcium, TN, NCN, TP, and CN concentration (Table 4) was detected, as shown in Table 5. The reason a difference in calcium concentration between skim milk and DUR was not detected was probably because much of the calcium in skim milk and DUR was associated with the CN micelles and CN concentration was similar between feeds ($P > 0.05$). Feed type had an effect ($P < 0.05$), with phosphorous and NPN concentrations being lower (consistent with the MF feed composition in Table 1) and SP being higher in the DUR retentate (Table 4). A feed type by temperature interaction ($P < 0.05$) with NCN (and SP) concentration in the MF retentates was noted, with the NCN (and SP) content of the skim milk retentate increasing with temperature and the reverse happening for the DUR retentate.

Effect of MF Temperature. Temperature had a nonlinear effect on calcium, phosphorus, TN, NCN, TP, and CN concentration (Table 4), as shown by the significant temperature by temperature interactions (Table 5). From Table 4, it appears that as temperature increased the concentrations of calcium, phosphorus, TN, NCN, TP, and CN initially decreased and then increased again as temperature continued to increase. The NPN increased slightly with temperature ($P < 0.05$), as shown in Tables 4 and 5. A feed type by linear and feed type by quadratic temperature interaction on SP concentration was seen, with SP concentration in

Table 4. Retentate composition: mean ($n = 3$) composition of retentates from the microfiltration of skim milk (SM) and diluted ultrafiltration retentate (DUR) at 50, 55, 60, and 65°C¹

Feed	Temperature (°C)	Ca (mg/kg)	P (mg/kg)	TN (%)	NPN (%)	NCN (%)	TP (%)	CN (%)	SP (%)
SM	50	2,919	2,191	8.79	0.17	0.95	8.62	7.84	0.78
	55	2,843	2,137	8.52	0.18	0.97	8.34	7.56	0.78
	60	2,832	2,159	8.44	0.19	0.98	8.26	7.46	0.80
	65	2,949	2,187	8.74	0.19	1.02	8.55	7.72	0.84
	Mean	2,886 ^a	2,166 ^b	8.62 ^a	0.18 ^a	0.98 ^a	8.44 ^a	7.64 ^a	0.80 ^b
DUR	50	2,792	1,773	8.72	0.06	1.03	8.66	7.69	0.97
	55	2,648	1,708	8.49	0.07	1.06	8.43	7.44	0.99
	60	2,711	1,727	8.64	0.06	1.04	8.58	7.60	0.98
	65	2,753	1,750	8.82	0.07	0.99	8.75	7.83	0.92
	Mean	2,726 ^a	1,739 ^a	8.67 ^a	0.06 ^b	1.03 ^a	8.60 ^a	7.64 ^a	0.96 ^a

^{a,b}Means in the same column for each feed not sharing a common superscript are different ($P < 0.05$).

¹TN = total nitrogen $\times 6.38$, NCN = noncasein nitrogen $\times 6.38$, NPN = nonprotein nitrogen $\times 6.38$, TP = true protein (TN minus NPN), CN = casein (TN minus NCN), SP = serum protein (NCN minus NPN).

the skim milk retentate increasing with temperature and SP concentration in the DUR retentate decreasing with increasing temperature.

The retentate composition depended on both the CF and rejection characteristics of the membrane. The change in TP, TN, NCN, SP, and CN with temperature could be a result of changes in CF, rejection characteristics of the membrane, and the effect of heat on SP and their classification in the Kjeldahl analysis.

Permeate Composition

Effect of MF Feed Type. The calcium and phosphorus concentrations in the permeate provide an estimate of the soluble calcium and phosphorus in the MF

feed. Both the calcium and phosphorus concentrations were lower ($P < 0.05$) in permeate from DUR than skim milk (Tables 6 and 7). Because DUR had some calcium and phosphorus removed by UF, a lower concentration of calcium and phosphorus in permeate from DUR than permeate from skim milk was expected. Both TN and NPN were lower ($P < 0.05$) in DUR permeate than skim milk permeate (Tables 6 and 7), but the TP was higher ($P < 0.05$) in the DUR permeate than the skim milk permeate. The DUR permeate was expected to have a lower concentration of NPN because DUR feed had a lower concentration of NPN (Table 1). The higher concentration of TP in the DUR permeate is consistent with the trend (though not significant) for a higher SP concentration in the DUR MF feed (Table 1).

Table 5. Retentate composition: ANOVA df and type III sum of squares to determine the effect of feed type (feed), replicate (rep) and temperature (temp) on the composition of microfiltration retentates¹

Model term	df	Ca	P	TN	NPN	NCN	TP	CN	SP
Whole model	—	513,187*	1,239,429*	2.37*	0.083*	0.069*	2.53*	2.08*	0.22*
Whole plot									
Rep	2	47,019	19,058	0.588	<0.0001	0.032	0.590	0.363	0.03
Feed	1	161,873	1,100,242*	0.010	0.08*	0.016	0.149	0.0003	0.10*
Rep $\times$ feed ²	2	67,386*	36,357*	0.802*	0.0002	0.01*	0.800*	0.645*	0.01*
Subplot									
Temp	1	121	83.74	0.002	0.0005*	0.001*	0.0003	0.0002	<0.0001
Feed $\times$ temp	1	NS	NS	NS	NS	0.01*	NS	NS	0.009*
Rep $\times$ temp	2	NS	6.830*	0.211*	NS	NS	0.210*	0.217*	NS
Temp $\times$ temp	1	50,215*	11,403*	0.312*	NS	0.0007*	0.322*	0.351*	0.0003
Temp $\times$ feed $\times$ rep	2	38,581*	11,460*	0.324*	NS	NS	0.329*	0.372*	NS
Temp $\times$ temp $\times$ rep	2	26,103*	11,484*	NS	NS	NS	NS	NS	NS
Temp $\times$ temp $\times$ feed	1	NS	NS	NS	NS	0.005*	NS	NS	0.006*
Temp $\times$ temp $\times$ rep $\times$ feed	2	NS	NS	NS	NS	NS	NS	NS	NS
Reduced model df		14	14	12	6	9	12	12	9
Reduced error df		9	9	11	17	14	11	11	14
R ²		0.97	>0.99	0.90	0.99	0.98	0.90	0.90	>0.99

¹Temperature was transformed to a mean-centered continuous variable. TN = total nitrogen $\times 6.38$, NCN = noncasein nitrogen $\times 6.38$, NPN = nonprotein nitrogen $\times 6.38$, TP = true protein (TN minus NPN), CN = casein (TN minus NCN), SP = serum protein (NCN minus NPN).

²Used as whole-plot error term for rep and feed.

* $P < 0.05$.

Table 6. Permeate composition: mean ($n = 12$) composition of permeate from microfiltration of skim milk (SM) and diluted ultrafiltration retentate (DUR)¹

Feed	Temperature (°C)	Ca (mg/kg)	P (mg/kg)	TN (%)	NPN (%)	TP (%)
SM	50	262	389	0.79	0.20	0.59
	55	257	402	0.78	0.20	0.58
	60	256	410	0.76	0.20	0.56
	65	243	405	0.71	0.20	0.51
	Mean	254 ^a	401 ^a	0.76 ^a	0.20 ^b	0.56 ^a
DUR	50	115	112	0.72	0.04	0.68
	55	114	113	0.72	0.05	0.67
	60	107	109	0.70	0.04	0.66
	65	99	103	0.61	0.06	0.55
	Mean	109 ^b	109 ^b	0.69 ^b	0.05 ^a	0.64 ^b

^{a,b}Means in the same column for each feed not sharing a common superscript are different ($P < 0.05$).

¹TN = total nitrogen $\times$ 6.38, NPN = nonprotein nitrogen $\times$ 6.38, TP = true protein (TN minus NPN).

Effect of MF Temperature. Calcium concentration in the permeate decreased ($P < 0.05$) as temperature increased (Tables 6 and 7). The feed by temperature interaction term was not significant (Table 7), indicating that the decrease in calcium in the permeate with increase in temperature did not depend on the feed type (Table 6). If increasing the temperature of MF to 65°C when skim milk was the feed caused calcium phosphate precipitation, it was expected that the DUR feed with its lower concentration of calcium and phosphorus would not experience calcium phosphate precipitation (resulting in a larger decrease in calcium in the permeate from skim milk with increasing temperature than from DUR). If this had happened, then the temperature by feed interaction in Table 7 for calcium would have been significant. Calcium phosphate precipitation did

not appear to have occurred as temperature increased for either DUR or skim milk MF feeds based on the permeate composition data.

The phosphorus concentration in the permeate decreased as temperature increased when DUR was the MF feed, but not when skim milk was the feed (Table 6), as shown by the significant feed by temperature interaction (Table 7). But the magnitude of the decrease in phosphorus (in permeate from DUR feed) with temperature was small.

As temperature increased, a nonlinear (i.e., temperature by temperature interaction) decrease in TN and TP in the permeate was noted (Figure 1 and Table 7). The decrease in TP (Table 7) as temperature increased had a slight dependence of feed type ($P < 0.05$; significant temperature by feed interaction). As seen in Figure 1,

Table 7. Permeate composition: ANOVA df and type III sum of squares to determine the effect of feed type (feed), replicate (rep), and temperature (temp) on the composition of microfiltration permeates and serum protein (SP) removal¹

Model term	df	Ca	P	TN	NPN	TP	SP removal
Whole model	—	128,329*	512,385*	0.069*	0.14*	0.0814*	457.65*
Whole plot							
Rep	2	309.25	43.75	0.002	0.0003	0.0009	41.26*
Feed	1	127,022*	511,730*	0.03*	0.14*	0.04*	0.982
Rep $\times$ feed ²	2	54.25	8.58	0.002*	0.0001	0.0017*	1.091
Sub plot							
Temp	1	943.8*	49.43	0.028*	NS	0.0308*	340.71*
Feed $\times$ temp	1	NS	561.47*	NS	NS	0.001*	NS
Rep $\times$ temp	2	NS	NS	NS	NS	NS	NS
Temp $\times$ temp	1	NS	NS	0.007*	NS	0.0073*	84.07*
Temp $\times$ feed $\times$ rep	2	NS	NS	NS	NS	NS	NS
Temp $\times$ temp $\times$ rep	2	NS	NS	NS	NS	NS	NS
Temp $\times$ temp $\times$ feed	1	NS	NS	NS	NS	NS	NS
Temp $\times$ temp $\times$ rep $\times$ feed	2	NS	NS	NS	NS	NS	NS
Reduced model df		6	7	7	5	8	7
Reduced error df		17	16	16	18	15	16
R ²		>0.99	>0.99	0.96	>0.99	0.96	0.92

¹Temperature was transformed to a mean centered continuous variable. TN = total nitrogen $\times$ 6.38, NPN = nonprotein nitrogen $\times$ 6.38, TP = true protein (TN minus NPN), SP = serum protein.

²Used as whole-plot error term for rep and feed.

* $P < 0.05$.

Table 8. Microfiltration processing pressures: mean ($n = 3$) inlet and outlet pressures for the microfiltration of skim milk (SM) and diluted ultrafiltration retentate (DUR) at 50, 55, 60, and 65°C

Feed	Temperature (°C)	Inlet pressure (kPa)			Outlet pressure (kPa)			Δ TMP ² (kPa)
		Retentate	Permeate	TMP ¹	Retentate	Permeate	TMP ¹	
SM	50	390.1	378.1	38.3	236.7	216.0	15.0	23.3
	55	385.9	374.9	37.3	236.5	218.3	12.6	24.7
	60	377.3	372.3	31.3	236.7	222.9	8.2	23.1
	65	372.3	368.1	30.6	236.7	226.3	4.7	25.9
	Mean	381.4 ^a	373.4 ^a	34.4 ^a	236.7 ^a	220.8 ^a	10.1 ^a	24.3 ^a
DUR	50	379.7	366.9	35.1	231.7	214.3	12.7	22.4
	55	374.5	363.9	32.9	232.3	218.3	9.4	23.5
	60	367.7	359.9	30.1	233.0	221.1	7.2	22.9
	65	362.1	357.3	27.1	232.7	225.0	3.0	24.1
	Mean	371.0 ^b	362.0 ^b	31.3 ^a	232.4 ^a	219.7 ^a	8.07 ^a	23.2 ^a

^{a,b}Means in the same column for each feed not sharing a common superscript are different ($P < 0.05$).

¹TMP = transmembrane pressure.

² Δ TMP = TMP at the inlet minus TMP at the outlet.

the largest decrease in TP concentration in the permeate occurred when the temperature was increased from 60 to 65°C and the decrease in TP was larger when the MF feed was DUR than when the feed was skim milk. The decrease in TP concentration in the permeate as temperature increased could be due to several factors, including membrane fouling, β -LG denaturation and association with CN micelles at higher temperatures, or a decrease in CN concentration in the permeate.

Effect of Feed Type and Temperature on Fouling

The MF retentate inlet and outlet pressures at each temperature are shown in Table 8. The retentate inlet pressure was lower ($P < 0.05$) for DUR feed than for skim milk feed (Table 9). Although the pressures differed between skim milk and DUR feeds, the decrease

($P < 0.05$) in retentate inlet pressures that occurred with increasing temperature was similar for both feed types and no feed by temperature interaction (Table 9) was detected ($P > 0.05$). As temperature increased the recirculation pump frequency was decreased (Tables 2 and 3) and, as a consequence, the pressure at the retentate inlet decreased (Table 8). The pressure at the retentate outlet remained unchanged ($P > 0.05$) as temperature increased (Tables 8 and 9).

On the permeate side of the membrane, the inlet pressure was higher ($P < 0.05$) for the skim milk feed than the DUR feed (Tables 8 and 9), as expected due to the similar pressure difference between skim milk and DUR on the retentate side of the membrane. The decrease in permeate inlet pressure as temperature increased ($P < 0.05$; Tables 8 and 9) was in part due to adjustments made to the permeate recirculation rate to maintain a constant Δ TMP. If no changes in processing parameters (Table 2) were made, increasing the MF temperature would have caused the Δ TMP to decrease (i.e., the TMP at the inlet would have decreased more than the TMP at the outlet). The goal was to operate at a Δ TMP of 25 ± 3 kPa. The permeate recirculation rate was decreased by restricting the permeate recirculation flow (using a diaphragm valve in the permeate recirculation loop) as temperature increased to increase the Δ TMP. This caused a decrease in the permeate pressure at the inlet, increasing TMP inlet and Δ TMP. A consistent Δ TMP was achieved (Tables 8 and 9) and Δ TMP was not a function of feed type or temperature ($P > 0.05$).

If increasing the temperature was causing membrane fouling, then we would expect that the TMP required to maintain a flux of 54 kg/m^2 per hour would increase. In our study, the TMP at both the inlet and outlet decreased ($P < 0.05$) as temperature increased (Tables

Figure 1. Permeate composition: mean ($n = 3$) $\pm$ 1 SD for true protein concentration in the permeate [skim milk (■) and diluted ultrafiltration retentate (●)].

Table 9. Microfiltration processing pressures: ANOVA df and type III sum of squares to determine the effect of feed type (feed), replicate (rep), and temperature (temp) on the permeate and retentate pressures at the inlet and outlet ends of the membrane¹

Model term	df	Inlet			Outlet			Δ TMP ¹
		Retentate	Permeate	TMP ¹	Retentate	Permeate	TMP ¹	
Whole model	—	1,814*	1,218*	367.6*	202.6*	415.2*	420.8*	NS
Whole plot								
Rep	2	4.4	133.0*	23.5	39.5	32.5	10.8	NS
Feed	1	666.9*	780.4*	58.8	107.5	7.4	27.0	NS
Rep $\times$ feed ²	2	41.2*	1.0	58.9*	55.5*	9.3*	60.1*	NS
Subplot								
Temp	1	1,113*	319.4*	234.9*	NS	368.7*	329.6*	NS
Feed $\times$ temp	1	NS	NS	NS	NS	NS	NS	NS
Rep $\times$ temp	2	22.0 *	NS	NS	NS	NS	NS	NS
Temp $\times$ temp	1	NS	NS	NS	NS	NS	NS	NS
Temp $\times$ feed $\times$ rep	2	NS	NS	NS	NS	NS	NS	NS
Temp $\times$ temp $\times$ rep	2	NS	NS	NS	NS	NS	NS	NS
Temp $\times$ temp $\times$ feed	1	NS	NS	NS	NS	NS	NS	NS
Temp $\times$ temp $\times$ rep $\times$ feed	2	NS	NS	NS	NS	NS	NS	NS
Reduced model df		8	6	6	5	6	6	—
Reduced error df		15	17	17	18	17	17	—
R ²		0.98	0.99	0.89	0.97	0.96	0.96	—

¹Temperature was transformed to a mean centered continuous variable. TMP = transmembrane pressure, Δ TMP = TMP at the inlet minus TMP at the outlet.

²Used as whole-plot error term for rep and feed.

* $P < 0.05$.

8 and 9), with the decrease being similar for both feed types (nonsignificant feed by temperature interaction). The decrease in TMP at the inlet and outlet as temperature increased (Figure 2) was likely due to the decreased viscosity of the permeate as temperature increased. It does not appear (based on TMP) that increasing the temperature of MF from 50 to 65°C caused fouling of the MF membranes with either skim milk or DUR as a feed type. However, slight fouling caused by increased temperature cannot be ruled out; as the decreased permeate viscosity could mask changes in the membrane's resistance. In addition in these experiments, the MF system was only operated at each temperature for 1 h and a slow accumulation of a fouling layer could occur with a longer time of processing at the higher temperature.

Percentage SP Removal

SP Removal Calculated Using Kjeldahl Data.

The calculated SP removal is shown in Table 10. This calculation is based on SP in the MF feed as measured using Kjeldahl analysis (NCN minus NPN) and the concentration of TP in the permeate as measured using Kjeldahl analysis (TN minus NPN). This assumed that all of the TP in the permeate was SP (i.e., no CN passage through the membrane into permeate). All permeates from the 2 feed types and different processing temperatures were clear based on visual examination. The percentage of SP removal did not depend ($P > 0.05$)

on MF feed type (Table 7). As temperature increased, a nonlinear (significant temperature by temperature term) decrease ($P < 0.05$) in SP removal was observed (Tables 10 and 7). The decrease in SP removal as temperature increased mirrored the decreasing TP concentration in the permeate (Figure 1) as temperature increased. For each MF feed type, the percentage SP removal was relatively constant until 65°C (Table 10). No difference ($P > 0.05$) in the decrease in SP removal as temperature increased was detected between feeds (i.e., no interaction between feed and temperature) for

Figure 2. Mean ($n = 3$) ± 1 SD for transmembrane pressure at the inlet [skim milk (■) and diluted ultrafiltration retentate (●)] and transmembrane pressure at the outlet [skim milk (□) and diluted ultrafiltration retentate (○)].

Table 10. Percentage of serum protein removal: Mean ($n = 3$) serum protein removal (%) from microfiltration of skim milk (SM) and diluted ultrafiltration retentate (DUR) at 50, 55, 60, and 65°C

Feed	Temperature (°C)	Serum protein removal
SM	50	66.39
	55	65.49
	60	63.50
	65	57.76
	Mean	63.29 ^a
DUR	50	67.47
	55	66.50
	60	65.43
	65	54.84
	Mean	63.56 ^a

^aMeans in the same column for each feed did not differ ($P > 0.05$).

SP removal (Table 7), indicating that temperature had the same effect regardless of MF feed type. To determine if the decrease in SP removal was due to β -LG denaturation and association with CN at higher temperatures, or perhaps to changes in CN concentration in the permeate, both permeate and retentate samples were analyzed using SDS-PAGE.

SDS-PAGE Analysis of the Retentates. The DUR retentates had a higher ($P < 0.05$) proportion of SP than skim milk retentates (Tables 11 and 12; which was consistent with the trend toward a lower CN as a percentage of TP seen in the DUR feeds in Table 1). The proportion of SP in the retentate increased ($P < 0.05$) as temperature increased. This is consistent with the hypothesis that some of the decrease in TP in the permeates as temperature increased was caused by increased membrane rejection of SP. The DUR retentates also had a higher ($P < 0.05$) proportion of CN hydrolysis products than skim milk retentates, which is probably a consequence of the additional processing that the DUR underwent (the UF process) and proteolysis of CN that occurred during longer processing times at 50°C than for the skim milk feed. No effect of

feed type or temperature on β -LG-to- α -LA ratio was detected ($P > 0.05$), indicating that higher temperature was not causing a change in association of β -LG with CN micelles.

SDS-PAGE Analysis of the Permeates. An image of the SDS-PAGE gel with the permeate from MF with both feed types (at 50 and 65°C) is shown in Figure 3. Permeate from a skim milk feed had a slightly higher ($P < 0.05$) β -LG-to- α -LA ratio than permeate from a DUR feed (Tables 11 and 12), but no change in the ratio as temperature increased was detected ($P > 0.05$) for either feed type. If β -LG was associating with CN micelles at higher temperatures, we would have expected the ratio of β -LG to α -LA to decrease as temperature increased. From the SDS-PAGE analysis of the permeates, it does not appear that β -LG was associating with CN micelles at the higher MF processing temperatures in our study.

The proportion of CN in the permeate from skim milk was in the same range as that reported in earlier work (Zulewska et al., 2009). The percentage of CN in the permeates decreased ($P < 0.05$) as temperature increased, and a trend ($P = 0.07$) for a lower proportion of CN in the permeates from skim milk compared with DUR was noted (Tables 11 and 12). The relative decrease in CN in the permeate as temperature increased to 65°C was probably caused by CN migration back into the CN micelles. The β -CN concentration in the serum phase of milk is known to decrease as temperature increases (Rose, 1968).

The decrease in the relative proportion of CN as temperature increased indicates that the purity of the SP in the permeate was increasing with increasing temperature. Additionally, the SP removal calculated using Kjeldahl analysis was overestimating the percentage SP removal because the calculation assumed that there was no CN in the permeate. The error in the calculated percentage of SP removal would be larger at 50 than 65°C because the relative proportion of CN in the permeate

Table 11. Retentate and permeate SDS-PAGE: mean ($n = 3$) serum protein (SP) as a percentage of protein, CN hydrolysis products, and ratio of β -LG to α -LA in the retentates and CN as a percentage of protein and ratio of β -LG to α -LA in the permeates from microfiltration with skim milk (SM) and diluted ultrafiltration retentate (DUR) feeds at 50 and 65°C

Feed	Temperature (°C)	Retentate			Permeate	
		SP ¹	Casein hydrolysis product	β -LG/ α -LA	CN ¹	β -LG/ α -LA
SM	50	9.38	3.23	5.22	5.37	3.15
	65	12.36	3.73	5.07	2.53	3.14
	Mean	10.87 ^b	3.48 ^b	5.15 ^a	3.95 ^a	3.14 ^a
DUR	50	13.35	7.08	5.13	9.32	3.13
	65	16.75	7.65	5.62	6.25	2.93
	Mean	15.05 ^a	7.37 ^a	5.37 ^a	7.78 ^a	3.03 ^b

^{a,b}Means in the same column for each feed not sharing a common superscript are different ($P < 0.05$).

¹SP = serum protein as a percentage of protein, CN = casein as a percentage of protein.

Table 12. Retentate and permeate SDS-PAGE: ANOVA df and type III sum of squares to determine the effect of microfiltration feed type (feed), replicate (rep), and temperature (temp) on the relative proportion of serum protein (SP), casein (CN), casein hydrolysis products, and ratio of β -LG to α -LA (as determined by SDS-PAGE) in the microfiltration retentates and permeates

Model term	df	Retentate			Permeate	
		SP ¹	CN hydrolysis product	β -LG/ α -LA	CN ¹	β -LG/ α -LA
Whole model	—	90.49*	51.49*	NS	100.1*	0.80*
Whole plot						
Rep	2	4.18	3.99	NS	23.0	0.76*
Feed	1	52.50*	45.24*	NS	44.1	0.04*
Rep $\times$ feed ²	2	1.14	1.40*	NS	6.9	0.001
Subplot						
Temp	1	30.40*	0.85*	NS	26.1*	NS
Feed $\times$ temp	1	NS	NS	NS	NS	NS
Temp $\times$ rep	2	2.27*	NS	NS	NS	NS
Reduced model df	8		6	—	6	5
Reduced error df	3		5	—	5	6
R ²		>0.99	>0.99	—	0.96	0.89

¹SP = Serum protein as a percentage of protein, CN = casein as a percentage of protein.

²Used as whole-plot error term for rep and feed.

* $P < 0.05$.

decreased as temperature increased. Therefore, the actual decrease in percentage SP removal with increasing temperature was not as large as indicated in Table 10.

The decrease in CN concentration does not account for all of the TP decrease as temperature increased to 65°C. The concentration of CN in the permeate was estimated using the TP concentration of the permeates from Kjeldahl analysis and the relative quantity of CN as determined by SDS-PAGE analysis. Using SDS-PAGE analysis of the permeates for a rough es-

timination of the concentration of CN in the permeate at 50°C was approximately 0.03 ± 0.005 and $0.06 \pm 0.02\%$ for skim milk and DUR MF feeds, respectively. Because the decrease in TP in the permeates was 0.08 ± 0.02 and $0.13 \pm 0.01\%$ for skim milk and DUR MF feeds, respectively, at 65°C, there was not enough CN in the permeates to account for the total TP decrease (as temperature of MF increased from 50 to 65°C). Thus, although CN concentration in the permeates decreased, the concentration of SP in the permeates probably

Figure 3. The SDS-PAGE gel image of the microfiltration permeates at 50 and 65°C with both the skim milk and diluted ultrafiltration retentates (DUR) used as the microfiltration feed. BLG = β -lactoglobulin, ALA = α -lactalbumin.

decreased as well. This could be a sign of membrane fouling that is changing the rejection characteristics of the membrane.

DISCUSSION

Increasing the temperature of MF may allow for operation at higher fluxes and reduce bacterial growth during MF. However, there was a concern that operating at higher temperatures could cause calcium phosphate precipitation that would lead to membrane fouling. An additional concern was that operation at temperatures above 50°C might cause SP to denature (and be covalently bound to the CN micelles) reducing SP removal. If mineral precipitation was an issue, MF at higher temperatures may be possible with skim milk that has been UF to reduce the concentration of soluble calcium and phosphorus (DUR). In our study, 2 MF feeds were used: a skim milk and a DUR with a trend toward lower calcium concentration ($P = 0.07$) and a reduced phosphorus concentration ($P < 0.05$).

It was found that increasing the temperature of MF from 50 to 65°C decreased the TMP required to maintain a flux of 54 kg/m² per hour regardless of whether the MF feed was skim milk or DUR. If severe membrane fouling was occurring, the TMP would have had to increase to maintain a constant flux. The TMP decrease as temperature was increased to 65°C was similar for both feed types, and did not indicate membrane fouling.

It was thought that increasing the temperature of MF might cause calcium phosphate precipitation in skim milk and processing with DUR at higher temperatures would result in lower levels of calcium phosphate precipitation. However, only a slight decrease in calcium concentration in the permeate ($11 \pm 3\%$) was observed as temperature increased to 65°C; a similar decrease was seen with both feed types (Table 6). Additionally, the concentration of phosphorus in the permeate did not decrease as temperature increased when the MF feed was skim milk (a slight decrease was seen when DUR was the MF feed). Calcium phosphate precipitation does not appear to cause membrane fouling when operating an MF process at temperatures up to 65°C. As other researchers have found, SP may have prevented or reduced calcium precipitation (Brule et al., 1978).

Increasing the temperature of MF did cause changes in the permeate protein concentration. The SP removal decreased as temperature of MF increased to 65°C (with a similar decrease found for both skim milk and DUR). Part of the decrease in SP removal was caused by a decrease in the relative proportion of CN in the permeate, but could not account for the total decrease in SP removal. The decrease in concentration of CN in

the permeate may have been due to CN migration back into the micelle at higher temperatures (Rose, 1968). The decrease in SP could be due to β -LG association with CN micelles at higher temperatures, but a change in the ratio of β -LG to α -LA in the permeate was not detected. Membrane fouling that changed the rejection characteristics of the membrane as temperature increased to 65°C might also account for the decrease in SP in the permeate; if so, this fouling was not detected as changes in TMP.

CONCLUSIONS

Increasing temperature of MF from 50 to 65°C when using 0.1- μ m ceramic membrane in a UTP process at a flux of 54 kg/m² per hour did not produce a large increase in membrane fouling, when using either skim milk or a DUR as a feed material, due to either an increase in calcium phosphate precipitation or heat denaturation of milk SP. Increasing processing temperature did cause a reduction in the percentage of SP removal by the process, but the increased temperature also caused a decrease in CN contamination in the permeate. Thus, increasing MF processing temperature from 50 to 65°C for separation of CN from SP in skim milk may provide processing benefits without causing a major fouling problem or may allow operation at a higher flux.

ACKNOWLEDGMENTS

The authors thank the Northeast Dairy Foods Research Center and New York State Milk Production Board (Albany) for partial funding of this research. Additionally, the technical assistance of Michelle Bilotta, Sara Bova, and Chassidy Coon from the Department of Food Science at Cornell University (Ithaca) and the staff of the Cornell Dairy Plant was greatly appreciated.

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