Production efficiency of micellar casein concentrate using polymeric spiral-wound microfiltration membranes¹

S. L. Beckman,* J. Zulewska,† M. Newbold,* and D. M. Barbano*²

*Northeast Dairy Foods Research Center, Department of Food Science, Cornell University, Ithaca, NY 14853

†Faculty of Food Sciences, University of Warmia and Mazury, Olsztyn, Poland

ABSTRACT

Most current research has focused on using ceramic microfiltration (MF) membranes for micellar casein concentrate production, but little research has focused on the use of polymeric spiral-wound (SW) MF membranes. A method for the production of a serum protein (SP)-reduced micellar casein concentrate using SW MF was compared with a ceramic MF membrane. Pasteurized (79°C, 18s) skim milk (1,100 kg) was microfiltered at 50°C [about 3× concentration] using a 0.3-μm polyvinylidene fluoride spiral-wound membrane, bleed-and-feed, 3-stage process, using 2 diafiltration stages, where the retentate was diluted 1:2 with reverse osmosis water. Skim milk, permeate, and retentate were analyzed for SP content, and the reduction of SP from skim milk was determined. Theoretically, 68% of the SP content of skim milk can be removed using a single-stage 3× MF. If 2 subsequent water diafiltration stages are used, an additional 22% and 7% of the SP can be removed, respectively, giving a total SP removal of 97%. Removal of SP greater than 95% has been achieved using a 0.1-μm pore size ceramic uniform transmembrane pressure (UTP) MF membrane after a 3-stage MF with diafiltration process. One stage of MF plus 2 stages of diafiltration of 50°C skim milk using a polyvinylidene fluoride polymeric SW 0.3-μm membrane yielded a total SP reduction of only 70.3% (stages 1, 2, and 3: 38.6, 20.8, and 10.9%, respectively). The SP removal rate for the polymeric SW MF membrane was lower in all 3 stages of processing (stages 1, 2, and 3: 0.05, 0.04, and 0.03 kg/m² per hour, respectively) than that of the comparable ceramic UTP MF membrane (stages 1, 2, and 3: 0.30, 0.11, and 0.06 kg/m² per hour, respectively), indicating that SW MF is less efficient at removing SP from 50°C skim milk than the ceramic UTP system. To estimate

the number of steps required for the SW system to reach 95% SP removal, the third-stage SP removal rate (27.4% of the starting material SP content) was used to extrapolate that an additional 5 water diafiltration stages would be necessary, for a total of 8 stages, to remove 95% of the SP from skim milk. The 8-plus stages necessary to remove >95% SP for the SW MF membrane would create more permeate and a lengthier process than required with ceramic membranes.

Key words: microfiltration, serum protein, spiral-wound membrane

INTRODUCTION

Micellar casein concentrate (MCC) produced from microfiltration (MF) is a concentrated liquid colloidal suspension consisting mainly of CN in micellar form, lactose, minerals, and a minor amount of serum proteins (SP). The amount of SP removed from the skim milk is usually in the range of 60 to 95% (wt/wt) of the original skim milk SP content, depending on the desired final composition and end use of the MCC product. A potential use of SP-reduced MCC produced by MF is the standardization of cheese milk before cheese making (Papdatos et al., 2003; Nelson and Barbano, 2005; Govindasamy-Lucey et al., 2007); it also has potential applications where concentrated milk proteins occur as ingredients such as in nutritional meal-replacement products, whipped topping, and coffee whiteners. Currently, 2 MF membrane types are being evaluated for their efficiency of SP removal from skim milk: ceramic and polymeric SW membranes. Much of the pilot-scale research on MF of skim milk has focused on using ceramic membranes. Because of the higher capital costs associated with operating ceramic membranes, a less-expensive alternative membrane type is sought.

A potential alternative to ceramic MF membranes is polymeric spiral-wound (SW) membranes. Polymeric SW filtration is most commonly used in low-fouling applications such as water treatment and water reclamation (Schwinge et al., 2004). Most research on SW membranes centers around their application in nano- and ultrafiltration of biological suspensions (Schwinge

Received March 14, 2010.

Accepted June 1, 2010.

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, the Northeast Dairy Foods Research Center, or the University of Warmia and Mazury.

²Corresponding author: dmb37@cornell.edu

et al., 2004). Pearce (2007) compared polymeric membranes offered by different membrane manufacturers and found that all polymeric MF membranes offered were polyvinylidene fluoride (PVDF), whereas UF membranes were offered in both polyethersulfone and PVDF, with polyethersulfone predominating. The SW membranes have high surface area and relatively low cost compared with ceramic membranes. Zulewska et al. (2009) compared the performance of 3 MF membrane types—ceramic uniform transmembrane pressure (UTP), ceramic graded permeability, and polymeric SW—for removal of SP from skim milk and found SP removal of 64 and 61% for the ceramic UTP and graded permeability membranes, respectively, compared with 38% SP removal for the SW MF membrane when using a single-stage 3× bleed-and-feed MF process. Theoretically, 68% SP removal can be achieved during single-stage 3× bleed-and-feed MF processing. Lawrence et al. (2008) found that a SW MF membrane retained 78 to 99% of the main SP, β -LG, under varying transmembrane pressures, with higher transmembrane pressures giving higher rejection. Hurt et al. (2010) demonstrated that with a 0.1- μ m pore size ceramic UTP membrane, greater than 95% of the original SP in pasteurized skim milk could be removed using a 3-stage MF with diafiltration process. Our objective was to determine the process necessary to create a 95% SP-reduced MCC using a 0.3- μ m PVDF polymeric SW MF at 50°C and to compare the efficiency of the SW process to theoretical values and to a 0.1- μ m ceramic UTP MF membrane process.

MATERIALS AND METHODS

Experimental Design and Statistical Analysis

One lot of bovine milk (approximately 1,151 kg) was separated in the Cornell University dairy plant (Ithaca, NY) at 4°C using a model 590 Air Tight Centrifuge (DeLaval Co., Chicago, IL). Raw skim milk was pasteurized with a plate heat exchanger consisting of 3 sections: regeneration, heating, and cooling (model P13-RCF, Alfa-Laval, Lund, Sweden) at 79°C and a holding time of 18 s. The milk was cooled to 4°C and stored at $\leq 4^\circ\text{C}$ until processing. The processing for each replicate was done in series over a 3-d period: stage 1, stage 2, and stage 3, respectively. During stage 1, the pasteurized skim milk was MF using a polymeric SW membrane using a 3× bleed-and-feed process at 50°C. Retentate was cooled to 4°C and held overnight. On the second day (stage 2), the retentate from stage 1 was diluted by weight (1 part retentate and 2 parts water) with pasteurized reverse osmosis (RO) water and diafiltered at 50°C. On the third day (stage 3), the

retentate from stage 2 was diluted (as described for stage 2) with pasteurized RO water and diafiltered at 50°C for a second time. The experiment was replicated 3 times.

To determine if the stage of processing made a significant difference on the removal of SP from the skim milk, statistical analysis of the data was done using Proc GLM of SAS (version 8.02, SAS Institute Inc., Cary, NC). The GLM was dependent variable = stage of processing + replicate + error. The GLM for comparing SP removal and membrane type was SP removal = type membrane + error.

MF System

Skim milk (approximately 1,151 kg) was processed at 50°C with a polymeric PVDF spiral-wound membrane (model FG7838-OS0x-S, 0.3- μ m polyvinylidene fluoride, Parker-Hannifin, Process Advanced Filtration Division, Tell City, IN) with a nominal pore size of about 0.3 μ m and surface area of 20.5 m². Membrane diameter was 198 mm, spacer thickness was 1.09 mm, and length was 96.5 cm. The membrane was placed in a stainless steel housing (length 1.3 m) and mounted horizontally in the membrane system. The SW MF system had a feed pump (model 0, Cherry Burrell, Little Falls, NY) and retentate recirculation pump (model GHH-30, G&H Products Corp., Kenosha, WI). A 1,150-L cheese vat (model DLHD8SSS, Kusel Equipment Co., Watertown, WI) was used as the feed tank and was connected directly to the feed pump. Digital pressure gauges (model PI2094, IFM Efector Inc., Exton, PA) were used to monitor the retentate pressure outlet (R_{p_o}) and retentate pressure inlet (R_{p_i}). Permeate pressure outlet (P_{p_o}) was assumed to be 0 kPa because the permeate exit tube was open to the atmosphere. Retentate bleed flow and recirculation flow (L/min) were measured with magnetic volumetric flow meters (model AE202MH and model AM204DH, respectively; Yokogawa Electronic Corp., Tokyo, Japan). Permeate and retentate exiting the SW MF unit were collected during the run and weighed (kg) into separate holding tanks. A schematic diagram of the SW MF unit used in the experiment is shown in Figure 1.

Initial Cleaning

Immediately before processing skim milk, the SW MF system was cleaned (short clean). First, the soak solution (0.26% solution of Ultrasil MP; Ecolab Inc., Food and Beverage Division, St Paul, MN) was drained from the system and the system was flushed with RO water until the water exiting the system was at neutral pH. The R_{p_i} and R_{p_o} were set by adjusting the speed

Figure 1. The polymeric spiral-wound microfiltration (SW MF) system used for microfiltration and diafiltration of 50°C skim milk. During processing, the temperature (T_c), retentate pressure inlet (R_{pi}), retentate pressure outlet (R_{po}), recirculation flow rate (L/min; recirculation flow), and retentate bleed flow (L/min; retentate bleed flow) were measured every 10 min. Retentate (R) and permeate (P) were collected and weighed continuously during processing.

of the retentate recirculation pump with the retentate and permeate outlets fully open. The membrane was cleaned for 20 min at 131 kPa R_{pi} and 61 kPa R_{po} with a combination of Ultrasil 110 liquid alkaline membrane cleaner (0.39% vol/vol) and XY-12 liquid sanitizer (0.15% vol/vol; Ecolab Inc.) diluted in 50°C RO water to a pH of 11.2 to 11.4. The operating conditions were set such that the average ΔP [$\Delta P = (R_{pi} + R_{po})/2 - P_p$] was <100 kPa. After the alkaline cleaning cycle was completed, the membrane system was drained and flushed with 50°C RO water until a neutral pH was obtained. The membrane was cooled to <24°C and sanitized with a solution of Ultrasil 110 liquid alkaline membrane cleaner (0.39% vol/vol) and XY-12 liquid sanitizer (0.15% vol/vol) to achieve a pH 11.2 to 11.4 and a chlorine level of 150 to 180 ppm in RO water. This solution was circulated through the membrane for 10 min at 131 kPa R_{pi} and no permeate back pressure. The membrane was drained and flushed with 25°C RO water to neutral pH. The starting water flux before milk was determined using 25°C RO water with only the feed pump running, the permeate outlet fully open, and a R_{pi} of 35 kPa adjusted by partially opening the retentate bleed valve with recirculation loop closed (Figure 1). The clean water flux (typically about 37 kg/m² per hour) was calculated based on the weight of permeate collected in 30 s and the total membrane surface area. After determining the starting water flux before milk, the MF system was drained of all liquids.

Milk Filtration and Diafiltration

Skim milk and diluted retentate were processed using the procedure outlined below. In stage 1, skim milk was heated to 50°C and then MF. For stages 2 and 3, retentate from the previous stage was diluted (1 part retentate and 2 parts RO water) back to the original weight of skim milk from stage 1 (about 1,150 kg). Before processing, skim milk or diluted retentate was heated to 50°C and held at that temperature for the duration of processing. The SW MF system was started as follows: the feed pump was turned on with the permeate exit valve closed. When skim milk or diluted retentate started exiting the retentate outlet, the retentate recirculation loop shut-off valve was opened to allow milk to fill the recirculation loop. Then, the recirculation pump was turned on and the permeate exit valve was opened slowly to balance the pressures and flows of retentate and permeate to achieve a ΔP <100 kPa. Throughout the run, the retentate removal rate was adjusted to maintain a 3× concentration factor (CF). The CF [permeate (kg) + retentate (kg)]/retentate (kg)] was monitored every 10 min by collecting permeate and retentate for 30 s into buckets and weighing each. Flux (kg/m² per h) was determined every 10 min using the weight of permeate collected in 30 s and the surface area of the membrane. Samples of permeate and the retentate were collected every 10 min for analysis by infrared spectrophotometer (Lactoscope FTIR, Delta Instruments, Drachten, the Netherlands)

to monitor their composition. At the end of the MF run for each stage, all retentate and all permeate from the processing run was weighed to the nearest 0.01 kg (model I5S scale, Ohaus Corporation, Pine Brook, NJ), mixed, and sampled. A final CF was calculated at the end of the run using the final weights of permeate and retentate. After weighing and sampling, the collected permeate was discarded. The small amount of unprocessed skim milk or diluted retentate remaining in the feed vat at the end of the run was weighed and subtracted from the starting material weight. The pH of the retentates was measured with an electrode (model Electrolyte 9823, Mettler Toledo, Columbus, OH) that was standardized at pH 4.06 and 6.97 at 50°C (pH 4.00 and 7.00 standard buffer solutions, Fisher Scientific, Fair Lawn, NJ). The retentate was stored overnight in a water-cooled tank (Hinged Lidded-Water Cooled 110 gal, Pfaudler, Rochester, NY).

Post-Process Cleaning

After processing, the SW MF system was cleaned (long clean) as follows: first, the MF system was flushed with 150 L of 50°C RO water at 158 kPa R_{p_i} and 68 kPa R_{p_o} , with no back pressure on the permeate side. During a second rinse using 150 L of 25°C RO water, the recirculation pump was turned off and the inlet pressure was adjusted to 34.5 kPa by adjusting the retentate outlet valve. The fouled water flux (kg/m^2 per hour) was calculated based on the weight of permeate collected in 30 s and the membrane area and was typically about 19.4, 26.0, and 36.8% of the clean water flux for stages 1, 2, and 3, respectively. After determination of the fouled water flux, the membrane was cleaned for 30 min with a combination of Ultrasil 110 liquid alkaline membrane cleaner (0.39% vol/vol), and Ultrasil 01 liquid high surfactant cleaner (0.08% vol/vol, Ecolab Inc.) in 50°C RO water at 158 kPa R_{p_i} and 68 kPa R_{p_o} , with no back pressure on the permeate side. These inlet and outlet pressures were used for all cleaning procedures unless otherwise indicated. The pH of the alkaline cleaning solution was 11.2 to 11.4. After the 30-min clean, the membrane was flushed to a neutral pH with 50°C RO water. The membrane was then cleaned with Ultrasil 76 liquid acid cleaner (0.15% vol/vol, Ecolab Inc.) in 50°C RO water for 30 min. The pH of the acid cleaning solution was 2.0 to 2.2. After the 30-min wash, the membrane was flushed to a neutral pH with 50°C RO water. The membrane was then cleaned for 30 min with a combination of Ultrasil 110 liquid alkaline membrane cleaner (0.39% vol/vol), and XY-12 liquid sanitizer (0.15% vol/vol) in 50°C RO water. The pH of the alkaline cleaning solution was 11.2 to 11.4 and the chlorine level was 150 to 180 ppm.

After the 30-min clean, the membrane was flushed to a neutral pH with 50°C RO water. When the rinse water pH was neutral, the system was flushed with RO water at 25°C and the final clean water flux was determined by operating only the feed pump with the permeate outlet fully open and a R_{p_i} of 34.5 kPa adjusted by the retentate bleed valve and with the recirculation loop closed. The flux (kg/m^2 per hour) was calculated based on the weight of permeate collected in 30 s and the membrane surface area. The ending flux after cleaning was about 36.0, 36.0, and 36.7 kg/m^2 per hour for the 3 stages, respectively. After the ending flux was determined, the membrane was sanitized with a solution of Ultrasil 110 liquid alkaline membrane cleaner (0.39% vol/vol) and XY-12 liquid sanitizer (0.15% vol/vol) in RO water (<24°C) at pH 11.2 to 11.4 and a chlorine level of 150 to 180 ppm. This solution was circulated through the membrane for 10 min at 131 kPa R_{p_i} and no permeate back pressure. The membrane was then flushed with room temperature RO water to a neutral pH. After a neutral pH was obtained, a storage solution of Ultrasil MP soak solution (0.26% vol/vol, Ecolab Inc.) and room temperature RO water were combined to achieve a pH 3.5 to 3.9, and this solution was circulated through the membrane for 10 min. After 10 min, the pumps were shut off and all the valves on the membrane housing were closed so that the soak solution could stay in contact with the membrane until the next processing day.

Chemical and Instrumental Analyses

Skim milk, permeate, and retentate collected during processing, final permeate, and final retentate were analyzed using an infrared spectrophotometer (Lactoscope FTIR, Delta Instruments) for fat, lactose, and true protein content (Kaylegian et al., 2006). Skim milk, final retentate, final permeate, and retentate hold-up were analyzed for total N (TN), noncasein N (NCN), and NPN content using Kjeldahl methods (AOAC 2000; methods 991.20, 33.2.11; 998.05, 33.2.64; and 991.21, 33.2.12, respectively). Total solids were measured by forced-air oven drying (AOAC, 2000; method 990.20, 33.2.44). True protein (TP) was calculated by subtracting NPN from TN and then 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 SP content in the permeate portion of the skim milk (expressed as a percentage) was calculated by dividing SP in milk by SP in permeate portion of the milk multiplied by 100, where the permeate portion of milk is considered as 100 kg minus the weight of fat plus CN in 100 kg of milk. All samples were analyzed fresh.

SP Removal

The SP removal for each stage was calculated using Kjeldahl data and processing data. The SP removal was calculated by dividing the mass of SP (kg) in the permeate of each stage by the SP (kg) in the skim milk. The SP in permeates and SP in skim milk were calculated from the TN, NPN, and NCN values obtained from Kjeldahl analysis for each of those sample types. Weights of the samples were obtained during processing.

SDS-PAGE

Samples of pasteurized skim milk, final retentate, and final permeate from all 3 MF stages were analyzed for protein profiles using SDS-PAGE. A 10 to 20% polyacrylamide gradient gel was used to determine the relative proportion of protein types. Skim milk samples were prepared by diluting 0.1 mL of skim milk in 0.9 mL of electrophoresis sample buffer containing 10 mM Tris-HCl, pH 6.8, 1.0% SDS, 20% glycerol, and bromophenol blue tracking dye. Retentate samples were prepared by diluting 0.1 mL of retentate into 2.9 mL of sample buffer and stored at -20°C . Permeate aliquots of 0.1, 0.3, and 0.54 mL were diluted into 0.9, 0.7, and 0.46 mL of sample buffer for stages 1, 2, and 3, respectively, and stored at -20°C . Immediately before loading on the electrophoresis gel, the diluted samples in glass vials (Target DP Vials C4000-1W, National Scientific Company, Rockwood, TN) sealed with DP Blue Caps (C4000-51B, National Scientific Company) were thawed at room temperature and then heated to 100°C in a steam chamber for 3 min. The prepared retentates and milk samples were loaded at 10.5, 12.5, 14.0, and 8.5 μL , for stages 1, 2, 3, and milk, respectively, onto an SDS-PAGE gel. Permeate samples (25 μL) were loaded onto an SDS-PAGE gel. Each gel consisted of 15 lanes: 10 lanes contained a sample and 5 lanes were left open. An example of the sample layout on a gel can be seen in Figure 2. One gel represented either the permeate or retentate samples from 1 of the 3 replicates of this experiment. Permeate or retentate samples from each stage were run in triplicate (3 slots) on a gel, utilizing an open lane between adjacent groups of 3 lanes. The procedure of Verdi et al. (1987) was used for running, staining, and destaining the gels. The gels were scanned with a USB GS 800 Densitometer using Quantity One 1-D Analysis software (Bio-Rad Laboratories Inc., Hercules, CA) to obtain relative proportions of individual proteins within each sample. The loading of the samples on the SDS-PAGE gel was chosen to achieve a maximum optical density of the predominant protein in each sample in the range of 1.0 to 1.4 optical density to

not exceed the linear range of response of the detector. A milk sample was run on each gel as a reference to verify that proper resolution of milk proteins occurred and to check for consistency of quantitative analysis of the same sample 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 adjust band function of the 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 the beginning and end of the peak in the pop-up trace. If a band was not horizontal, then the tracking line for the lane was adjusted to transect the band perpendicularly. An example of the layout of a gel for analysis of permeate samples from one replicate of this experiment, with one lane used for a skim milk reference sample, can be seen in Figure 2. The bands in the skim lane have been labeled for easy identification of the individual proteins. For all gels, the relative amount of CN in a sample was quantified by adding the CN1 through CN6 bands in a lane (Figure 2), and the SP was quantified by adding SP1 through SP4 bands (Figure 2). Once totals of the CN and SP bands from each lane were quantified, the relative proportion could be established. To quantify the proportion of β -LG and α -LA as a percentage of β -LG + α -LA, bands SP3 (β -LG) and SP4 (α -LA) were used (Figure 2).

RESULTS AND DISCUSSION

Processing

Mean ($n = 3$) operational parameters for the 3 stages of the MF process are reported in Table 1. The ΔP did not vary ($P > 0.05$) among stages because it was our goal to maintain a ΔP of 100 kPa for all 3 stages. Retentate and permeate bleed rates were adjusted to maintain a $3\times$ CF, and both bleed rates increased ($P < 0.05$) with stage (Table 1) because the unrestricted permeate flux at a ΔP of 100 kPa increased with stage (Table 2). The R_{p_i} increased ($P < 0.05$) with stage and the R_{p_o} decreased ($P < 0.05$) with stage (Table 1; $P < 0.05$), which was expected because the retentate bleed rate was adjusted to maintain a target ΔP of 100 kPa during processing. The R_{p_i} and R_{p_o} values observed in stage 1 were similar to those reported by Zulewska et al. (2009) for a single-stage SW MF of skim milk. Concentration factor did not vary ($P > 0.05$) with stage of processing (Table 1). The CF for all 3 stages were below the $3\times$ target and only in stage 3 was close to $3\times$.

Average ($n = 3$) total time of processing was 133, 83, and 55 min for stages 1, 2, and 3, respectively. Average flux (kg/m^2 per hour) for milk processing increased ($P < 0.05$) with stage of processing (Table 2). All 3 stages

Table 1. Mean ($n = 3$) operational parameters recorded during the 3 stages of a microfiltration with diafiltration of skim milk using a 0.3- μm polyvinylidene fluoride spiral-wound membrane

Stage	Operational parameters ¹						
	ΔP (kPa)	Retentate bleed flow (L/min)	Permeate bleed flow (kg/min)	Recirculation flow (L/min)	R_{p_i} (kPa)	R_{p_o} (kPa)	Concentration factor
Stage 1	99.9 ^a	2.8 ^c	5.4 ^c	237.9 ^c	132.4 ^b	67.3 ^a	2.88 ^a
Stage 2	99.7 ^a	4.3 ^b	8.3 ^b	248.6 ^b	136.2 ^a	63.1 ^b	2.87 ^a
Stage 3	100.0 ^a	5.6 ^a	12.0 ^a	256.5 ^a	139.4 ^a	60.6 ^c	2.98 ^a
SE	0.4	0.2	0.1	1.3	0.9	0.2	0.02
R ²	0.69	0.96	>0.99	0.96	0.90	>0.99	0.82

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

¹ $\Delta P = \{[(R_{p_i} - R_{p_o})/2] + R_{p_o}\} - P_{p_o}$, where R_{p_i} = retentate pressure inlet, R_{p_o} = retentate pressure outlet, P_{p_o} = permeate pressure outlet.

exhibited an initial decline in flux during the first 30 min of milk processing (Figure 3). This initial decline in flux can be attributed to the transition from water to milk and the gradual decrease in water content of the liquid in the MF recirculation loop. Because of the initial transition from water to milk, the average flux calculations were done using values starting at 30 min to the end of the run, instead of using values from time zero.

Water flux values before and after running milk or diluted retentate are shown in Table 2. No difference in starting water flux before milk processing was detected ($P > 0.05$) with stage of processing, showing that the resistance of the membrane to water flow was consistent before each run. No difference in water flux was detected when comparing flux before milk process-

ing and the ending flux after the final cleaning for any stage of processing (Table 2). This indicates that the long clean performed after milk processing adequately removed foulant. Average ($n = 3$) fouled water flux increased ($P < 0.05$) from the first stage (7.10 kg/m² per hour) to the third stage (13.94 kg/m² per hour). The increased flux on milk with increasing stage and the increased water flux for the fouled membrane (Table 2) meant that there was progressively less membrane fouling across the 3 stages. The explanation of this behavior of flux will be given in the context of retentate composition in the next section.

Composition

Skim Milk. The mean ($n = 3$) composition of the pasteurized skim milk (Table 3) used in the SW MF

Figure 2. Sodium dodecyl sulfate-PAGE of permeate samples collected after each stage of processing during a 3-stage microfiltration with water diafiltration (stage 2 and stage 3). Skim milk from the replicate was used as a reference sample. Bands in skim milk are identified on the gel: SP1 and SP2 = BSA, lactoferrin, lactoperoxidase; CN1 = α_s -CN (combination of α_{s1} - and α_{s2} -CN); CN2 = β -casein; CN3 = proteolysis products of casein; CN4 = κ -casein; CN5 = proteolysis products of casein; SP3 = β -LG; SP4 = α -LA; CN6 = proteolysis products of casein.

Table 2. Mean ($n = 3$) flux by stage of processing during a 3-stage microfiltration with water diafiltration (stages 2 and 3) using a 0.3- μm polyvinylidene fluoride spiral-wound membrane

Stage of processing	Milk flux ¹ (kg/m ² per h)	Starting water flux before milk ² (kg/m ² per h)	Fouled water flux ³ (kg/m ² per h)	Ending water flux after cleaning ⁴ (kg/m ² per h)
Stage 1	14.4 ^c	36.6 ^a	7.1 ^b	35.9 ^a
Stage 2	22.1 ^b	37.9 ^a	9.9 ^{ab}	36.0 ^a
Stage 3	32.6 ^a	37.4 ^a	13.9 ^a	36.7 ^a
SE	0.3	0.9	1.4	1.6
R ²	>0.99	0.47	0.89	0.35

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

¹Milk flux = average flux during processing skim milk ($t = 30$ min to end of processing).

²Starting water flux before milk = water flux of clean membrane before processing.

³Fouled water flux = water flux of membrane after processing.

⁴Ending water flux after cleaning = water flux measured after cleaning of the membrane.

was typical except that the CN as a percentage of TP (CN%TP) was higher than in our previous work on MF. The CN%TP for the 3 replicates in the current study were 83.4, 84.2, and 83.2% respectively, whereas the mean CN%TP for the studies by Zulewska et al. (2009) and Hurt et al. (2010) were 82.4 and 82.4%, respectively. The higher CN%TP in the current study was because of the higher temperature and longer hold time used to pasteurize the skim milk (79°C, 18 s) than that used in the previous studies (72°C, 16 s). High heat treatment of milk causes disulfide bond formation between the heat-labile β -LG and the κ -CN located on the exterior of the CN micelle (Donato and Guyomarc'h, 2009). Heat denaturation of SP causes an overestimation of the amount of CN in milk when analyzed using Kjeldahl analysis (Lynch et al., 1998).

Retentate. The mean ($n = 3$) composition of retentates produced during each of 3 stages using polymeric SW MF with water diafiltration is presented in Table 4. Total solids, TN, NCN, NPN, and SP decreased ($P < 0.05$) with stage. True protein content of the retentate decreased ($P < 0.05$) with increasing stages. Equations to predict the expected TP content retentate after each stage of a 3-stage $3 \times$ MF with 2 water diafiltration stages were developed by Hurt and Barbano (2010). Using those equations and the skim milk TP reported in Table 3, expected values for TP in retentate after each stage are 8.18, 7.81, and 7.65% for stages 1 through 3, respectively. These expected values for TP in retentate are higher than the actual TP in retentate observed (Table 4). The lower observed TP values in the present study were due to overdilution of retentate with RO wa-

Figure 3. Mean ($n = 3$) flux for stage 1 ($\diamond$), stage 2 ($\blacksquare$), and stage 3 ($\blacktriangle$) when using a 0.3- μm polyvinylidene fluoride spiral-wound microfiltration with water diafiltration (stages 2 and 3) of skim milk at 50°C.

Table 3. Mean ($n = 3$) composition (% by weight) of the pasteurized skim milk

Item	Composition ¹							CN%TP
	TS	TN	NCN	NPN	TP	Casein	Serum proteins	
Skim milk	9.17	3.29	0.68	0.17	3.11	2.60	0.51	83.6
SD	0.03	0.01	0.03	0.01	0.02	0.03	0.01	0.53

¹TS = total solids; TN = total nitrogen $\times 6.38$; NCN = noncasein nitrogen $\times 6.38$; NPN = nonprotein nitrogen $\times 6.38$; TP = true protein, TN - NPN; casein = TN - NCN; serum proteins = TP - casein; CN%TP = casein as a percentage of TP.

ter for diafiltration before stages 2 and 3. Overdilution stemmed from our inability to achieve the target $3\times$ CF (Table 1) during any of the 3 stages. The RO water for diafiltration was added (by weight) assuming a $3\times$ CF was achieved in the previous stages. As demonstrated in the modeling by Hurt and Barbano (2010), a CF $<3\times$ and a dilution factor = $3\times$ caused the TP and CN concentrations in retentates in later diafiltration stages to be lower than expected. Casein content in retentate decreased ($P < 0.05$) with stage during the 3-stage SW MF with diafiltration (Table 4). Theoretically, the CN content of the retentate should remain constant (CF $\times$ % CN in skim milk) if the CF and dilution factor are both controlled. Accurate control of the CF could be achieved by adding volumetric flow meters and flow control valves on the feed inlet, permeate outlet, and retentate outlet areas of the membrane for each stage. The flow meters could be monitored and the control valves adjusted continuously accordingly to change the concentration factor. The progressively lower than expected CN concentration in the MF feed from stage to stage (Table 4) explains the progressively higher mean flux in the second and third stages (Table 2 and Figure 3). Figure 4 gives a visual representation of the increase in total protein concentration in retentate during MF

for each stage measured by infrared spectroscopy. The initial increase in protein content during the first 10 min was caused by switching of feed material from water to milk (stage 1) or to diluted retentate (stage 2 and stage 3). This large increase in protein and CN content during the beginning of the run for each stage (Figure 4) corresponds to the large decrease in flux (Figure 3) for each stage during the first 10 min of operation. The mean ($n = 3$) pH of retentates produced by each of the 3 stages of MF with diafiltration is presented in Table 4. The pH of the retentate increased ($P < 0.05$) with stage and is consistent with similar results for MF retentates produced with ceramic membranes that were reported by Hurt et al. (2010).

Permeate. The mean ($n = 3$) composition of permeate obtained from each MF stage is presented in Table 5. Total solids, TN, NCN, NPN, and TP in permeate decreased ($P < 0.05$) with stage but the decreases would have been smaller if the concentration and diafiltration dilution factors were matched at $3\times$. Hurt et al. (2010) reported higher TN and TP in permeate from all 3 stages when using ceramic UTP membranes to remove SP from skim milk than those reported in Table 5 for SW polymeric membranes. Thus, a lower SP removal from skim milk when using polymeric SW membranes

Table 4. Mean ($n = 3$) composition (% by weight) and pH of retentates by stage of processing during a 3-stage microfiltration with water diafiltration (stages 2 and 3) using a 0.3- μ m polyvinylidene fluoride spiral-wound membrane

Stage of processing	Composition ¹								pH
	TS	TN	NCN	NPN	TP	Casein	Serum proteins	CN%TP	
Stage 1	13.90 ^a	7.75 ^a	1.13 ^a	0.17 ^a	7.58 ^a	6.62 ^a	0.96 ^a	87.3 ^a	6.49 ^c
Stage 2	8.86 ^b	6.35 ^b	0.59 ^b	0.08 ^b	6.27 ^b	5.76 ^b	0.51 ^b	91.9 ^b	6.80 ^b
Stage 3	6.87 ^c	5.59 ^c	0.36 ^c	0.04 ^c	5.55 ^c	5.22 ^c	0.32 ^c	94.2 ^c	6.96 ^a
SE	0.07	0.05	0.006	0.002	0.05	0.05	0.007	0.14	0.008
R ²	>0.99	>0.99	>0.99	>0.99	>0.99	>0.99	>0.99	>0.99	>0.99

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

¹TS = total solids; TN = total nitrogen $\times 6.38$; NCN = noncasein nitrogen $\times 6.38$; NPN = nonprotein nitrogen $\times 6.38$; TP = true protein, TN - NPN; casein = TN - NCN; serum proteins = TP - casein; CN%TP = casein as a percentage of TP.

Figure 4. True protein (% by weight) in the retentate as a function of time of processing (min) for a 3-stage microfiltration with diafiltration of skim milk at 50°C using a 0.3- μ m polymeric spiral-wound membrane during stage 1 (♦), stage 2 (■), and stage 3(▲). True protein content measured by infrared spectroscopy.

may be expected compared with that achieved with ceramic membranes. Casein, as measured by Kjeldahl, was present at about 0.017% in permeate from all 3 stages (Table 5). The presence of CN in the permeates was confirmed by SDS-PAGE (Figure 2). We could not detect a difference ($P > 0.05$) in the amount of CN in permeate among stages (Table 5). The CN as percentage of true protein determined by SDS-PAGE of permeates (Table 6) increased ($P < 0.05$) with each successive stage because of a fixed concentration of CN passing through the membrane for each stage while the concentration of SP in the permeate was decreasing. In addition, the relative proportion of β -LG to α -LA was also increasing ($P < 0.05$) with increasing MF stage (Table 6). It is interesting to note that we also observed increasing flux with increasing stage (Table 2 and Figure 3), which indicated that fouling and hydraulic resistance of the membrane plus foulant was progressively decreasing from one stage to next under the processing conditions in our study. If the membrane plus the foulant were determining the resistance to passage of SP, particularly β -LG, then as fouling decreased with increasing stage, β -LG would become an increasing percentage of the SP in the permeate (Table 6). Therefore, at a lower concentration of CN in the retentate within the recirculation loop, there was less fouling and a higher rate of passage β -LG into permeate. It is interesting to note that the nominal pore size of the PVDF membranes used in this study was 0.3 μ m, which is larger than the CN casein micelles; but, in fact, very little CN passed through

the membranes into permeate. This further supports the role of the foulant in determining the final selectivity to passage of SP through the polymeric membrane. With respect to maximizing the percentage removal of SP from skim milk, the optimum CF for operation of PVDF SW membranes may be lower than 3 $\times$ at 50°C.

SP Removal

The mean ($n = 3$) SP removal from 50°C skim milk using SW MF with water diafiltration is shown in Table 7. Theoretically, when using a 3 $\times$ CF, 68, 22, and 7% of the original SP should be removed from skim milk during the first, second and third stages, respectively, regardless of membrane type (Hurt and Barbano, 2010). After 3 stages of MF with diafiltration, the theoretical cumulative removal should be 97%. The SW MF process with diafiltration at 50°C described in our study resulted in 38.6, 20.8, and 10.9% SP removal from skim milk for the first, second and third stages, respectively. The SP removal from skim milk in the first stage of SW MF, 38.56%, was lower ($P < 0.05$) than what was found for a ceramic UTP MF system, 64.83%, using a similar 3-stage, 3 $\times$ process (Table 7). The ceramic UTP system exhibited removal rates close to theoretical (Table 7). We calculated (using 27.4% SP removal rate during third-stage operation) that an additional 5 RO water diafiltration stages would be needed to remove 95% of the SP from milk, thus bringing the total stages to 8 to achieve what has been achieved in 3 stages with

Table 5. Mean ($n = 3$) composition (% by weight) of permeates by stage of processing produced during a 3-stage microfiltration with water diafiltration (stages 2 and 3) using a 0.3- μm polyvinylidene fluoride spiral-wound membrane

Stage of processing	Composition ¹					
	TS	TN	NCN	NPN	TP	Casein
Stage 1	6.18 ^a	0.51 ^a	0.49 ^a	0.17 ^a	0.34 ^a	0.018 ^a
Stage 2	2.02 ^b	0.26 ^b	0.24 ^b	0.06 ^b	0.20 ^b	0.017 ^a
Stage 3	0.70 ^c	0.14 ^c	0.12 ^c	0.02 ^c	0.11 ^c	0.017 ^a
SE	0.009	0.007	0.008	0.006	0.005	0.002
R ²	>0.99	>0.99	>0.99	0.99	>0.99	0.64

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

¹TS = total solids; TN = total nitrogen $\times 6.38$; NCN = noncasein nitrogen $\times 6.38$; NPN = nonprotein nitrogen $\times 6.38$; TP = true protein, TN - NPN; casein = TN - NCN.

ceramic membranes (Nelson and Barbano, 2005; Hurt et al., 2010). Hurt and Barbano (2010) evaluated the parameters that can cause low SP removal rate when microfiltering skim milk and reported that the 2 most important factors influencing SP removal were heat denaturation of SP before MF and rejection of SP passage through the membrane by either the membrane or a combination of the membrane plus foulant. Cumulative removal of SP from 50°C skim milk using SW MF with diafiltration was 70.3% of the original non-heat-denatured SP content of the skim (Table 7). The relative percentage SP removal was lower ($P < 0.05$) with the SW polymeric PVDF membrane system (70.3%) than reported (98.26%) by Hurt et al. (2010) for a similar process using a ceramic UTP system. In both studies, the amount of SP that was heat denatured and bound to CN micelles was not counted in the amount of SP in the skim milk as SP that was available for removal by the MF process when the estimation of removal was based on Kjeldahl analysis of skim milk and permeates. Therefore, the difference in heat treatments of the milk between the 2 studies would not influence the estimation of relative percentage removal of SP from skim milk. The fact that the calculated relative percentage of SP removal was not influenced by the heat-denatured

SP was confirmed by comparison of the mean percentage SP removal in the first stage in the present study (i.e., 38.56%) with the SP removal (38.62%) reported by Zulewska et al. (2009) for a 3 $\times$ MF of skim pasteurized at 72°C for 16 s.

A difference in heat denaturation of SP would influence the relative proportion of SP retained in the retentate (as measured by SDS-PAGE) and the comparison of the kilograms of SP removed per meter squared per hour between the 2 studies. If there was no selective blockage of the passage of β -LG versus α -LA into permeate, the ratio of β -LG to α -LA in the retentate would be the same in the retentates from all stages and the ratio β -LG to α -LA would be the same as in the original skim milk. As shown in Table 8, the proportion of β -LG to α -LA increased with stage and was higher than that in the original skim milk, indicating there was more resistance to passage of β -LG through the membrane. This could be due to a combination of both the β -LG bound to CN micelles due to heat denaturation of β -LG and blockage of passage of some β -LG through the membrane. The removal of SP expressed as kilograms of SP removed per meter squared per hour was much lower for PVDF membranes (about 0.05 vs. 0.31 kg/m² per h in the first stage) than for

Table 6. Mean ($n = 3$) relative proportions of casein and serum proteins and the relative percentage of β -LG and α -LA as a percentage of their sum in permeate produced during a 3-stage spiral-wound microfiltration with water diafiltration (stages 2 and 3) process measured by densitometry analysis of the SDS-PAGE gels

Product	% of protein		% of total (β -LG plus α -LA)	
	Casein	Serum proteins	β -LG	α -LA
Permeate stage 1	3.80 ^c	96.20 ^a	71.91 ^c	28.09 ^a
Permeate stage 2	9.74 ^b	90.26 ^b	77.66 ^b	22.34 ^b
Permeate stage 3	16.10 ^a	83.90 ^c	79.97 ^a	20.03 ^c
SE	0.22	0.22	0.14	0.14
R ²	0.99	0.99	0.99	0.99

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

Table 7. Mean ($n = 3$) percentage serum protein (SP) removal for each stage as percentage of SP in starting skim milk for a spiral-wound microfiltration with water diafiltration (stages 2 and 3) process and a ceramic uniform transmembrane pressure (UTP) process as determined by Kjeldahl analysis

Stage of processing	Theoretical SP removal ¹	Milk SP removal			
		Spiral wound	Spiral wound cumulative	Ceramic ²	Ceramic cumulative ³
Stage 1	68	38.6 ^a	38.6 ^c	64.8 ^a	64.8 ^c
Stage 2	22	20.8 ^b	59.3 ^b	22.9 ^b	87.8 ^b
Stage 3	7	10.9 ^c	70.3 ^a	10.5 ^c	98.3 ^a
SE		0.75	0.40	0.19	0.40
R ²		0.99	>0.99	>0.99	>0.99

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

¹Assuming no rejection of SP and complete rejection of casein.

²SP removal from starting milk for ceramic UTP microfiltration ($n = 4$); data from Hurt et al. (2010).

³Cumulative SP removal for a ceramic UTP microfiltration ($n = 4$); data from Hurt et al. (2010).

UTP ceramic membranes (Table 9). Part of the lower SP removal for the PVDF membranes was due to the higher heat, but based on the information presented by Hurt and Barbano (2010), the reduction due to the higher heat treatment of the milk in the current study would be about 0.03 to 0.04 kg/m² per h, and this is a very small part of the observed difference (i.e., about 0.25 kg/m² per h in the first stage) between the 2 systems (Table 9). Why was the relative removal of SP by the polymeric SW system lower than that with the ceramic system? It appears that the PVDF polymeric membrane in combination with the foulant was blocking the passage of SP through the membrane. The relative contribution of the membrane versus the foulant needs to be determined.

CONCLUSIONS

Theoretically, 68% of the SP content of skim milk can be removed using a single-stage 3× MF. If 2 subsequent water diafiltration stages are used, an additional 22 and 7% of the SP can be removed, respectively, giv-

ing a total SP removal of 97%. Removal of SP greater than 95% has been achieved using a 0.1-μm pore size ceramic UTP MF membrane (Hurt et al., 2010) after a 3-stage MF with diafiltration process. One stage of MF plus 2 stages of diafiltration of 50°C skim milk using a PVDF polymeric SW 0.3-μm membrane yielded a total SP reduction of only 70.3% (stages 1, 2, and 3: 38.6, 20.8, and 10.9%, respectively). The SP removal rate for the polymeric SW MF membrane was lower in all 3 stages of processing (stages 1, 2, and 3: 0.05, 0.04, and 0.03 kg/m² per hour, respectively) than that of the comparable ceramic UTP MF membrane (stages 1, 2, and 3: 0.30, 0.11, and 0.06 kg/m² per hour, respectively), indicating that SW MF is less efficient at removing SP from 50°C skim milk than the ceramic UTP system. To estimate the number of steps required for the SW system to reach 95% SP removal, the third-stage SP removal rate (27.4% of the starting material SP content) was used to extrapolate that an additional 5 water diafiltration stages would be necessary, for a total of 8 stages to remove 95% of the SP from skim milk. The 8-plus stages necessary to remove >95% SP

Table 8. Mean ($n = 3$) relative proportions of casein and serum proteins and the relative percentage of β-LG and α-LA as a percentage of their sum in skim milk and retentates produced during a 3-stage spiral-wound microfiltration with water diafiltration (stages 2 and 3) process measured by densitometry analysis of the SDS-PAGE gels

Product	% of total protein		% of total (β-LG plus α-LA)	
	Casein	Serum proteins	β-LG	α-LA
Skim milk	80.80 ^d	19.20 ^a	80.30 ^d	19.70 ^a
Retentate stage 1	89.17 ^c	10.83 ^b	86.89 ^c	13.11 ^b
Retentate stage 2	93.96 ^b	6.04 ^c	91.54 ^b	8.46 ^c
Retentate stage 3	95.90 ^a	4.10 ^d	93.43 ^a	6.57 ^d
SE	0.21	0.21	0.44	0.44
R ²	>0.99	>0.99	0.95	0.95

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

Table 9. Comparison of the mean ($n = 3$) kilograms of serum proteins (SP) removed per hour per meter squared of membrane for each stage between spiral-wound microfiltration (SW MF) and ceramic uniform transmembrane pressure (UTP) microfiltration systems

Stage of processing	SP removed (kg/m ² per h)	
	SW MF	Ceramic UTP ¹
Stage 1	0.05 ^a	0.30 ^a
Stage 2	0.04 ^b	0.11 ^b
Stage 3	0.03 ^c	0.06 ^c
SE	0.001	0.003
R ²	0.95	>0.99

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

¹Data from Hurt et al. (2010).

for the SW MF membrane would create more permeate and a lengthier process than a process using ceramic membranes

REFERENCES

- AOAC. 2000. Official Methods of Analysis. 17th ed. Association of Official Analytical Chemists, Gaithersburg, MD.
- Donato, L., and F. Guyomarc'h. 2009. Formation and properties of the whey protein/ κ -casein complexes in heated skim milk—A review. *J. Dairy Sci. Technol.* 89:3–29.
- Govindasamy-Lucey, S., J. J. Jaeggi, M. E. Johnson, T. Wang, and J. A. Lucey. 2007. Use of cold microfiltration retentates produced with polymeric membranes for standardization of milks for manufacture of pizza cheese. *J. Dairy Sci.* 90:4552–4568.
- Hurt, E. E., and D. M. Barbano. 2010. Processing factors that influence casein and serum protein separation by microfiltration. *J. Dairy Sci.* 93:(JDS 3121; accepted, update at pagination).
- Hurt, E. E., J. Zulewska, M. W. Newbold, and D. M. Barbano. 2010. Production of micellar casein concentrate with a 3-stage UTP ceramic membrane process at 50°C. *J. Dairy Sci.* 93:(JDS 3169; in review, update at pagination).
- Kaylegian, K. E., G. E. Houghton, J. M. Lynch, J. R. Fleming, and D. M. Barbano. 2006. Calibration of infrared milk analyzers: Modified milk versus producer milk. *J. Dairy Sci.* 89:2817–2832.
- Lawrence, N. D., S. E. Kentish, A. J. O'Connor, A. R. Barber, and G. W. Stevens. 2008. Microfiltration of skim milk using polymeric membranes for casein concentrate manufacture. *Separ. Purif. Tech.* 60:237–244.
- Lynch, J. M., D. M. Barbano, and J. R. Fleming. 1998. Indirect and direct determination of the casein content of milk by Kjeldahl nitrogen analysis: Collaborative study. *J. AOAC Int.* 81:763–774.
- Nelson, B. K., and D. M. Barbano. 2005. A microfiltration process to maximize removal of serum proteins from skim milk before cheese making. *J. Dairy Sci.* 88:1891–1900.
- Papadatos, A., M. Neoclous, A. M. Berger, and D. M. Barbano. 2003. Economic feasibility evaluation of microfiltration of milk prior to cheesemaking. *J. Dairy Sci.* 86:1564–1577.
- Pearce, G. 2007. Introduction to membranes: Manufacturers' comparison: Part 1. *Filtration-Separation* 44:35–37.
- Schwinge, J., P. R. Neal, D. E. Wiley, D. F. Fletcher, and A. G. Fane. 2004. Spiral wound modules and spacers: Review and analysis. *J. Membr. Sci.* 242:129–153.
- Verdi, R. J., D. M. Barbano, and M. E. Dellavalle. 1987. Variability in true protein, casein, nonprotein nitrogen, and proteolysis in high and low somatic cell count milks. *J. Dairy Sci.* 70:230–242.
- Zulewska, J., M. W. Newbold, and D. M. Barbano. 2009. Efficiency of serum protein removal from skim milk with ceramic and polymeric membranes at 50°C. *J. Dairy Sci.* 92:1361–1377.