PRODUCTION OF MICELLAR CASEIN CONCENTRATES USING CERAMIC MICROFILTRATION MEMBRANES: OPTIMAL PROCESS DESIGN AND SYSTEM OPERATION

A Dissertation

Presented to the Faculty of the Graduate School

of Cornell University

In Partial Fulfillment of the Requirements for the Degree of

Doctor of Philosophy

by

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January 2015

PRODUCTION OF MICELLAR CASEIN CONCENTRATES USING CERAMIC MICROFILTRATION MEMBRANES: OPTIMAL PROCESS DESIGN AND SYSTEM OPERATION

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Cornell University 2015

Microfiltration of skim milk using 0.1 µm ceramic membranes can separate micellar casein from serum protein. Both the micellar casein and serum proteins may be valuable food ingredients. To improve the commercial viability of the microfiltration process, the system should be designed and operated to minimize fixed (e.g., membrane area) and variable (i.e., energy) costs. As a first step, to determine what factors were important in process design, a theoretical model for the production of a micellar casein concentrate was developed. From the theoretical model it was determined that the use of ultrafiltration of skim milk prior to microfiltration could reduce the membrane area required. Additionally, it was found that the increasing following factors: number of stages, flux, and recirculation loop protein concentration further decreased the required membrane area. Finally, if the microfiltration feed was ultrafiltered skim milk, it was found that the optimal microfiltration feed protein concentration was 5.4% protein for a 5-stage process.

The next step was to evaluate the performance of ceramic graded permeability membranes with 3 mm and 4 mm channel diameters, by determining the limiting flux and serum protein removal at 8, 9 and 10% protein in the recirculation loop. The

microfiltration feed was an ultrafiltered skim milk. The limiting flux decreased by approximately 24% as the recirculation loop protein concentration was increased from 8% to 10% for both the 3 mm and 4 mm channel diameter membranes. At each protein concentration the limiting flux was about 20% higher with the 4 mm compared to 3 mm channel diameter membranes. Additionally, the serum protein removal factor was higher on the 4 mm than 3 mm channel diameter membranes.

Finally, the impact of increasing the temperature of microfiltration above 50^{o}C on membrane fouling and serum protein removal was determined. Increasing the temperature up to 65^{o}C did not cause any detectable membrane fouling. Increasing the temperature of microfiltration decreased serum protein removal. However, higher temperature also decreased casein concentration in the permeate. Based on this work, it may be feasible to increase the temperature of microfiltration and possibly the microfiltration flux.

BIOGRAPHICAL SKETCH

Emily's education began inauspiciously. As an illegal immigrant she struggled with her homework and had to attend Saturday school, but she persevered. By the time she had graduated as a valedictorian from Chico Senior High she had realized that hard work could compensate for what she lacked in native intelligence (though there was no fix for her personality). With no marketable skills she decided to head to university, where she obtained her B.S. in Chemical/Biochemical Engineering from the University of California at Davis. Having no particular knowledge of food science or dairy (besides her love of butter), Emily began her career at Hilmar Cheese Company as a Research Engineer. Intoxicated by the rock-and-roll like lifestyle of the Dairy Industry, Emily decided to continue her education, leaving to earn a M.S. in Food Science from Cornell University. After receiving her M.S. she decided to stay on for a Ph.D., in part to achieve her lifelong dream of being the oldest graduate student in the department. Her hobbies include: hiking, traveling and judging other people. Always a dreamer, Emily's goals are to travel to all 50 U.S. states and save enough money to retire.

To my grandparents, Dick and Jeanne Sorenson, whose hard work and sacrifice were always an inspiration

ACKNOWLEDGMENTS

This research would not have been possible without the help of O-AT-KA milk products cooperative (Batavia, NY) (with particular thanks to Mac McCampbell) and the assistance of Pall Corporation (Cortland, NY). I'd also like to thank fellow graduate student Michael Adams for all his help conducting the research presented in this dissertation. A special thanks to my Special Committee chair Dave Barbano and committee members: Dennis Miller and Andrew Novakovic.

Thanks to the New York State Milk Promotion Board (Albany, NY) and the Northeast Dairy Foods Research Center (Ithaca, NY) for partial funding of this research. Additional thanks for the technical assistance of Michelle Bilotta, Sara Bova, Chassidy Coon, from the Department of Food Science at Cornell University and the staff of the Cornell Dairy Plant. Their hard work was greatly and sincerely appreciated.

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LIST OF ABBREVIATIONS

ALA	α-lactalbumin
ANOVA	Analysis of variance
BLG	β-lactoglobulin
CD	Channel diameter
CF	Concentration factor
CN	Casein
DF	Diafiltration factor
DUR	Diluted ultrafiltration retentate
GLM	Generalized linear model
GMP	Glycomacro peptide
GP	Graded permeability
IR	Infrared spectrophotometer
MCC	Micellar casein concentrate
MF	Microfiltration
MPC	Milk protein concentrate
NCN	Non casein nitrogen
NPN	Non protein nitrogen
PVDF	Polyvinylidine fluoride
RE	Reynold's number
RO	Reverse osmosis
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide electrophoresis
SM	Skim milk
SP	Serum protein
TMP	Transmembrane pressure
ΔP	Longitudinal pressure drop

TN	Total nitrogen
TP	True protein
UF	Ultrafiltration
UTP	Uniform transmembrane pressure

LIST OF SYMBOLS

μ	viscosity
γ	shear rate
τ	shear stress
ρ	density

CHAPTER 1

Introduction: Microfiltration of Skim Milk to Separate Serum Protein from Micellar Casein

Microfiltration (**MF**) can be used to fractionate casein (**CN**) and serum protein (**SP**) from skim milk (**SM**) to produce higher value dairy products. The choice of the feed, the MF membranes, as well as the operating conditions for the MF process can have an impact both on product quality and cost of the process. This review will focus on the MF of SM to produce micellar CN concentrates (**MCC**) and SP concentrates. Specifically, commercially available MF membranes and their performance will be reviewed as well as factors in process design and operation that could have an impact on the MF membrane area required to produce an MCC.

Skim Milk Composition

SM consists of approximately 3.2% true protein (**TP**) by weight. The main classes of proteins in SM are CN (2.6%) and SP (0.63%) (Walstra et al., 1999). In SM the CN are present in large aggregates called CN micelles roughly 40 to 300 nm in diameter (Walstra, 1990). Proteins that are in solution in the serum phase of SM are the SP. Two proteins: β-Lactoglobulin (**BLG**) (0.32%) (18,000 Da) and α-Lactalbumin (**ALA**) (0.12%) (14,000 Da) account for approximately 80% of the SP in SM (Farrell et al., 2004). Other SP include bovine serum albumin (BSA) (0.03%) and immunoglobulins (0.07%) (Walstra et al., 1999). Major non-protein components of SM include lactose (5%) and minerals (0.7%) (Walstra et al., 1999). SM contains approximately 120 mg calcium/100 g, with about 70% of the calcium associated with the CN micelles (Walstra et al., 1999).

The different components of SM have different degrees of heat stability. CN exhibits excellent heat stability allowing heating to over 100^{o}C for extended periods of time before coagulation is seen (Fox and Morrissey, 1977). The SP are much less heat stable than CN with complete denaturation with heating at 90^{o}C for 10 min (Fox and Morrissey, 1977). Heating can also cause lactose to react with primary amines (such as lysine), generating Maillard reaction products which can change the flavor, color and nutritional value of SM (Walstra et al., 1999).

There is a large size difference between CN micelles and the SP in SM. CN micelles have diameters in the 40 to 300 nm range while SP have diameters in the 3 to 6 nm range (Walstra et al., 1999). Because of the 10 to 100 fold difference in size between the CN micelles and SP, MF membranes which typically retain particles > 100 nm in diameter (Cheryan, 1998) can be used to separate SP from CN micelles. In the MF of SM to separate SP from CN, other soluble components of SM such as lactose and serum phase minerals are removed along with the SP. Any larger particles in SM such as fat globules (100 to 10,000 nm) or bacteria (1,000 nm) (Walstra et al., 1999) will be concentrated along with the CN micelles.

Filtration Processes in the Dairy Industry

The use of filtration in the dairy industry has been reviewed by Merin and Daufin (1990), Hassan et al. (2006) and Pouliot (2008). Both UF and MF membranes are used in the dairy industry. UF membranes typically have pore sizes in the 0.1 to 500 nm range and retain both SP and CN (Pouliot, 2008). UF can be used to standardize the TP concentration in milk for cheese making (Jonsson and Tragardh, 1990) or in fluid milk (Quinones et al., 1997). Another widely used UF application is

to concentrate the whey proteins (ALA and BLG) in cheese whey producing whey protein concentrates (Jonsson and Tragardh, 1990). Finally, UF of SM can be used to manufacture milk protein concentrates (MPC). MPC are widely used in the U.S. (US Trades Commission, 2006) for a variety of applications. Possible uses include, yogurt (Mistry, 2002) and cheese products (Henning et al., 2006).

MF in the dairy industry can be used to remove bacteria from SM or fractionate the SP from the CN micelles depending on the membranes used. MF using ceramic membranes with a 1.4 μm pore size have been found to reduce the bacterial concentration in SM by 2 to 3 log and extend the shelf life beyond that of pasteurized milk (Hoffman et al., 2006; Elwell and Barbano, 2006; Caplan and Barbano, 2013). This review focuses on the use of MF membranes with smaller pore sizes (0.1 to 0.2 μm) to separate the SP from the CN in SM (Fauquant et al., 1988). In MF separating micellar CN from SP, both the permeate and retentate from the process could be of commercial interest.

Products of Skim Milk Microfiltration

Micellar Casein Concentrate. The retentate from the MF of SM consists mainly of micellar CN and is an MCC. The MCC would have a lower concentration of lactose, SP, NPN and serum phase minerals compared to SM (on a protein basis). The MCC is expected to exhibit increased heat stability since the concentration of heat labile components (lactose and SP) has been reduced. Additionally, MCC have shown excellent microbiological stability under refrigeration. Amelia and Barbano (2013) found that an MCC concentrated to 18% protein and pasteurized immediately before

storage, still had bacterial counts under 20,000 CFU after 16 weeks at 4°C. The increased shelf life was attributed to the reduction in NPN and lactose (substrates critical for bacterial growth) in the MCC. The excellent shelf-life means that liquid MCC could be stored as a liquid and used directly in products-as opposed to producing a dry MCC that would have to be rehydrated before use. MCC also exhibits unique rheological properties. At temperatures below 22°C, Amelia and Barbano (2013) found that at 18% protein the MCC was a solid, which melted upon heating above 22°C.

MCC could be used in a variety of applications including cheese making and beverage formulations. Garem et al. (2000) found that using a dried MCC with a 40% SP reduction increased the yield of mozzarella cheese. The removal of some of the SP before drying the MCC reduced the heat induced association of BLG with κ-CN and the reconstituted MCC had excellent rennetability (Garem et al., 2000). Neocleous et al. (2002) found that using MF retentates with a concentration factor (**CF**) of 1.26X to 1.82X increased the cheddar cheese yield, but not the yield efficiency (the actual yield divided by the theoretical yield). The advantage of using a concentrated MCC was that per a given cheese vat more cheese could be produced (Neocleous et al., 2002). Papadatos et al. (2003) found that using an MCC would increase cheese making revenues in most months. The high heat stability of MCCs may also make them suitable for other food applications such as shelf stable beverages. Sauer and Moraru (2012) found that MCC could be retorted or undergo ultra high temperature treatment without aggregation or coagulation if the pH was adjusted to > 6.9.

Serum Protein Concentrates. In the production of MCC the permeate from MF contains SP. This SP could be further purified via UF to produce SP concentrates (34 to 80% TP) or SP isolates (> 80% TP). The SP concentrates could be used as an alternative to whey protein concentrates. SP concentrates have a different composition when compared to whey protein concentrates, and may have sensory and functional advantages when compared to whey protein concentrates.

In comparing SP concentrates and whey protein concentrates (both at 80% TP) made with the same batch of SM, the SP concentrates had much lower levels of fat (0.49%) and NPN (2.34%) compared to the whey protein concentrate (7.63% fat and 7.22% NPN) (Evans et al., 2010). The difference in NPN was due to the presence of glycomacropeptide (**GMP**) (produced during cheese making) in whey protein concentrates (Evans et al., 2010). The difference in fat concentration can also be explained by the differences in production methods. For the SP concentrates, the fat remaining in SM will not pass through the membrane with the SP, while for whey protein concentrate: any fat remaining in the whey after separation will be concentrated by the UF process (Evans et al., 2010). The SP concentrates in Evans et al. (2010) also exhibited increased clarity in solution compared to the whey protein concentrates, probably due to their lower fat concentration. The increased clarity would be an advantage in beverages where a clear formulation was desired. Besides the increased clarity, SP concentrates exhibit better foaming and gelation properties when compared to whey protein concentrates (80% SP concentrates and whey protein concentrates) (Luck et al., 2013). Foams made with SP concentrates had higher overrun and yield stress compared to whey protein concentrates, though there was

significant variation in the properties of whey protein concentrates from different suppliers (Luck et al., 2013). In a sensory comparison of 80% SP to 80% whey protein concentrates, SP concentrates had lower aroma intensity, sweet aromatic, cereal and astringency characteristics (Evans et al., 2010). However, in a product formulation the 80% SP concentrates had a detectable free fatty acid flavor (a negative) that the 80% whey protein concentrate lacked (Evans et al., 2010).

Membranes for the Microfiltration of Skim Milk

A number of MF membranes have been used to successfully separate SP from micellar CN in SM. These membranes include polymeric spiral-wound membranes with pore sizes in the 0.3 µm to 0.5 µm range and tubular ceramic membranes with pore sizes in the 0.1 µm to 0.2 µm range. Polymeric spiral-wound membranes are typically an order of magnitude cheaper than tubular ceramic membranes, but also have a shorter useful life (3 yrs compared to 10 yrs) (Cheryan, 1998).

Polymeric Sprial-Wound Membranes. Polymeric spiral-wound membranes typically operate with cross-flow velocities in the 0.5 to 1 m/s range (Cheryan, 1998). The spiral-wound configuration allows for a larger amount of surface area per membrane volume when compared to tubular ceramic membranes (Cheryan, 1998). Because of their lower cost researchers have explored using spiral-wound polymeric membranes to separate SP from micellar CN. Lawrence et al. (2008) used 0.3 µm and 0.5 µm polyvinylidine fluoride (**PVDF**) spiral-wound membranes to remove SP from micellar CN. They found that while the membranes retained almost all of the CN there was also a high retention of BLG. Beckman et al. (2010) used 0.3 µm PVDF

membranes and found that a 3-stage 3X process only removed 70% of the SP compared to a theoretical removal of 97%. Zulewska and Barbano (2013) found that the rejection of SP by a PVDF spiral-wound membrane was caused not by the membrane itself, but a membrane fouling layer containing CN.

Tubular Ceramic Membranes. Tubular ceramic membranes typically operate at much higher cross-flow velocities than spiral-wound membranes (2 to 6 m/s) (Cheryan, 1998). The permeate outlet pressure can be controlled so that cross-flow velocity and average TMP can be varied independently. At high cross-flow velocities there is a large pressure drop from the retentate inlet to the retentate outlet (ΔP). The large pressure drop along the length of the membrane results in a TMP at the membrane inlet that is much higher than the TMP at the membrane outlet (Cheryan, 1998). The large TMP gradient results in a higher flux through the membrane at the inlet end compared to the outlet end. Gesan et al. (1993) found that for the MF of whey, uneven TMP (and presumably flux) led to increased membrane fouling. Piry et al. (2008) found that SP transmission varied along the length of the membrane in the MF of SM from, 38% transmission at the membrane inlet to 87% transmission at the membrane outlet, due to the uneven TMP causing increased membrane fouling at the inlet end of the membrane. In order to maintain a high cross-flow velocity (large ΔP), while at the same time minimizing the flux gradient along the length of the membrane several ceramic membrane configurations have been developed.

Uniform Transmembrane Pressure System. The first method developed to achieve uniform flux under conditions of high cross-flow velocity was the uniform

transmembrane pressure (**UTP**) system (Holm et al., 1990). In the UTP system permeate is pumped in a co-current direction on the permeate side of the membrane creating a longitudinal pressure drop on the permeate side that matches the pressure drop on the retentate side. A disadvantage to the UTP system is the requirement for a permeate recirculation pump which increases both the fixed and operating (energy) costs for a MF system.

There has not been much published regarding the performance of the same membranes run with or without the UTP system. Pafylias et al. (1996) compared the flux for a system microfiltering SM to remove bacteria with and without the UTP system. Running the membranes in a UTP system resulted in a flux of 900 L/m^2 per h compared to 400 L/m^2 per h without the UTP system. In contrast in looking at the MF of SM to separate SP from micellar CN Vadi and Rizvi (2001) found that the flux was slightly higher on a non-UTP system compared to a UTP system-up to a CF of 4X.

Graded Permeability Membrane. A second technique developed to overcome uneven flux along the length of the membrane was the graded permeability (**GP**) membrane manufactured by Membralox (Pall Corp., East Hills, NY). The GP membranes create a uniform flux by having a longitudinal resistance gradient (Garcera et al., 2002). The membranes are designed with the highest resistance to permeation at the membrane inlet end, which decreases towards the membrane outlet. In GP membranes the graded resistance is achieved by changing the resistance at the exterior surface of the outermost support layer of the membrane (Garcera et al., 2002). The resistance gradient is designed to match a specific pressure drop from the retentate

inlet to outlet (Garcera et al., 2002). The use of GP membranes is more economical than a UTP system, because a permeate recirculation pump is not required to create a uniform flux along the length of the membrane.

GP membranes are available with 3 mm or 4 mm channel diameter (**CD**) (Sondhi et al., 2003). The advantage of the 3 mm membranes is that they have 46% more surface area per stick compared to the 4 mm CD membranes, however their performance in regards to SP removal and flux for the MF of SM has not been compared.

Isoflux Membrane. Isoflux membranes (TAMI, Nyons, France) also create a uniform permeate flux by varying the membrane resistance along the length of the membrane (removing the requirement for a permeate recirculation pump). The Isoflux membranes achieve the resistance gradient by modifying the thickness of the separating layer on the interior surface of the membrane channels (Grangeon et al., 2002). The Isoflux membrane resistance gradient was also designed to match a specific longitudinal pressure drop.

Comparison of Membrane Performance. The different MF membranes available for the separation of micellar CN and SP do not exhibit the same performance. Zulewska et al. (2009) found that in a 1 stage 3X MF process: 0.1µm GP membranes (4 mm CD) removed 61% of the SP, a 0.1 µm ceramic UTP system removed 64% of the SP and a 0.3 µm polymeric (PVDF) spiral-wound system removed 39% of the SP from SM. In a separate experiment Adams and Barbano (2013) found that 0.14 µm Isoflux membranes only removed 40% of the SP from SM

in 1 stage running at a 3X CF. Both the UTP and GP systems were found to have a higher SP removal than PVDF spiral-wound and ceramic Isoflux membranes (Zulewska et al., 2009; Adams and Barbano, 2013). The performance of the GP and UTP systems were similar with SP removal close to theoretical (Zulewska et al., 2009). The GP system has the advantage that a permeate recirculation pump is not required, which would result in less energy use during operation of a GP system.

Optimization of Skim Milk Microfiltration

For a MF process to separate CN micelles from SP in SM, the process needs to be designed to minimize cost of equipment (pumps, valves, membrane area etc.). Parameters that could impact the required membrane area include: choice of MF feed, number of stages used in the process, the protein concentration in the recirculation loop and flux that the system can operate at.

Microfiltration Feed. The composition and characteristics of the MF feed could have an impact on the MF flux and the amount of diafiltration water required. There could be advantages to feeding a MF process producing an MCC with UF SM that had part of the lactose removed from SM. If the target MCC composition required a greater percentage of lactose removal than SP removal, then use of UF prior to MF to remove the extra lactose would reduce the required MF membrane area. This reduction in MF membrane area for a MF system using ceramic membranes could offset the cost of the UF system, since polymeric UF membranes are typically about a tenth the cost of ceramic MF membranes (Cheryan, 1998) and are less costly to operate.

Removing lactose and soluble minerals prior to MF may also increase the MF flux. Removing lactose by UF prior to MF would reduce the viscosity of the MF permeate. Morrison and Mackay (2001) measured the viscosity of lactose solutions and found that viscosity increased as lactose concentration increased. According to Darcy's law the flux is proportional to the TMP and inversely proportional to the permeate viscosity (Belfort et al., 1994). Given Darcy's law, as permeate viscosity decreases the flux at a given TMP should increase.

Number of Microfiltration Stages. The number of MF stages with diafiltration has an impact on the amount of diafiltration water required, MF permeate produced and the membrane area required (Cheryan, 1998). As the number of stages increase the amount of diafiltration water required decreases (Cheryan, 1998). Krstic et al. (2004) found that a UF process that continually concentrated the feed while adding diafiltration water minimized water consumption. The process described by Krstic et al. (2004) was a batch process, when compared to a continuous multi-stage UF process the water consumption was comparable to a six stage process and much less than a 2 stage process. A simplified example that illustrates how increasing the number of stages can decrease the amount of MF permeate that has to be removed is shown in Figure 1.1. The amount of permeate removed for a 1 stage and 2 stage MF process with SM as the feed is compared in Figure 1.1. The same percentage of SP is removed and the MCC produced with either 1 or 2 stages has the same composition, as shown in Figure 1.1. However, twice the mass of permeate has to be removed when only 1 stage is used compared to a 2 stage process.

Theoretical Optimization. Several models have been developed to optimize the design of MF systems. Cross (2002) outlined an approach to filtration process design and optimization. Cross (2002) proposes as a starting point, experimental data on flux and rejection as a function of processing parameters such as concentration, cross-flow velocity and TMP. Singh and Cheryan (1998) looked at the process design of a ceramic MF system to clarify hydrolyzed corn starch. As a 1st step, the steady state flux as a function of CF was determined (Singh and Cheryan, 1998). Systems with 2 to 5 stages were explored, with a total CF of 100X with the CF for each stage as a variable that could be changed to minimize the required membrane area of the system. Including fixed and variable costs it was determined that although the required membrane area decreased with the number of stages, 2-stages was the optimal configuration. The savings in membrane area for adding more than 2 stages was less

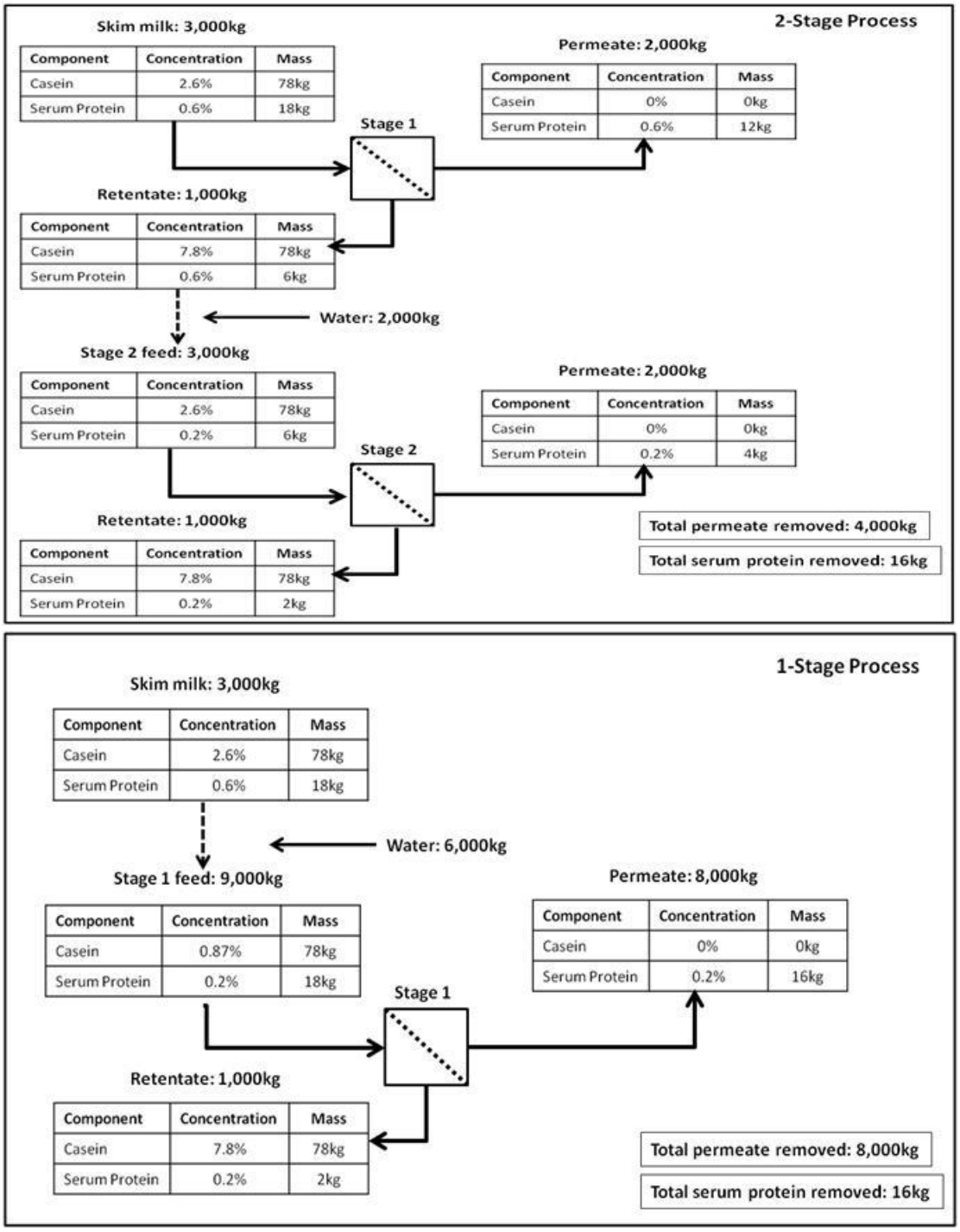

Figure 1.1. Comparison of mass permeate removed between a 2 stage and 1 stage microfiltration process, where the maximum retentate casein concentration is 7.8%. Both systems remove 16 kg of serum protein and have the same membrane rejection characteristics.

than the increased cost incurred by adding an additional stage (control valves, pipes and fittings) (Singh and Cheryan, 1998). Both Cross (2002) and Singh and Cheryan (1998) found theoretical modeling to optimize a MF process to be a useful tool; however, they both required experimental data to determine how the parameters in the model such as CF impacted flux and membrane rejection characteristics.

There have not been theoretical models developed, for the use of MF to produce an MCC. A model that allowed the impact of the MF feed, number of stages and recirculation loop TP concentration to be explored could provide valuable information for the design of later experiments.

Factors Influencing Limiting Flux in the Microfiltration of Skim Milk

The MF flux is an important parameter, the higher the flux the system can operate at, the less membrane area required. As mentioned above, the flux as a function of operating parameters needs to be determined experimentally and is critical for the development of theoretical models. In MF there are two important fluxes: the critical flux and limiting flux, shown in Figure 1.2. Critical flux is the flux below which no membrane fouling occurs; this is often taken as point where flux no longer increases linearly with increasing TMP (Bacchin et al., 2006). As flux is increased above the critical flux membrane fouling increases and the increase in flux with increasing TMP is no longer linear. Limiting flux is the point at which increasing the TMP no longer increases the flux, as shown in Figure 1.2. Methods used to determine critical flux can also be used to determine limiting flux. The simplest method is to measure the TMP profile as flux is increased, to determine limiting flux (Bacchin et

al., 2006). In this method TMP is increased in steps with the flux measured at each step, until the flux can no longer be increased. The flux TMP profile method is simple compared to other methods, though it is not very sensitive for determination of critical flux (Bacchin et al., 2006). Another important flux is the sustainable flux. A sustainable flux is a flux that could be maintained for a production run with low levels of fouling and would be between the limiting and critical fluxes (Bacchin et al., 2006).

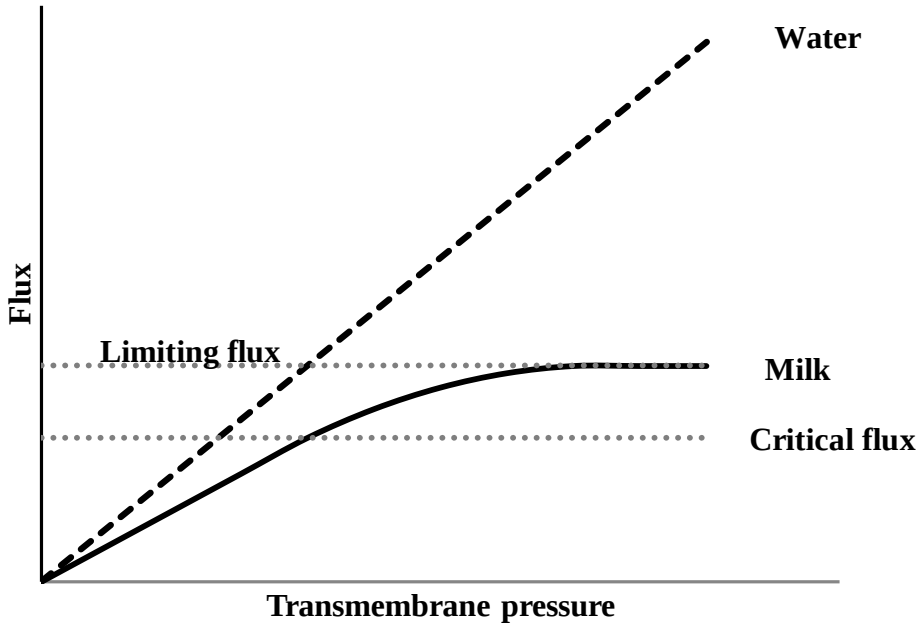

Figure 1.2. Flux as a function of transmembrane pressure.

Fluid Mechanics Principles for the Microfiltration of Skim Milk. The cross-flow velocity at which the MF system operates at can have a large impact on the performance of the MF system, including the maximum flux the system can achieve. In the literature flux is often presented as a function of other values related to cross-flow velocity, such as shear rate and shear stress. A brief summary of fluid mechanics

is presented below underscoring the relationship of cross-flow velocity to shear stress and shear rate as well as turbulent and laminar flow.

Laminar and Turbulent Flow. The Reynold's number (**Re**) is defined in Equation 1.1. In laminar flow (Re below approximately 2,100) the fluid moves only in the direction of fluid flow compared to turbulent flow (Re > 2,100) where the flow is chaotic with mixing and fluid movement perpendicular to the direction of fluid flow (Denn, 1980). Turbulent flow typically improves the flux during MF, as the turbulence helps remove particles that are accumulating on the membrane surface (Cheryan, 1998).

In cross-flow MF using tubular ceramic membranes operation is typically in the turbulent regime. For SM at 50°C and a cross-flow velocity of 5 m/s on membranes with 4 mm CD, the Re is approximately 15,000, however as retentate viscosity increases the Re will decrease. At a CF of 6X for MF SM Solanki and Rizvi (2001) found a viscosity of 0.01 Pa*s which would lead to an approximate Re of 2,000. Additionally, under the same flow conditions (5 m/s) and 1X CF decreasing the CD from 4 mm to 3 mm decreases the Re to 11,250.

$$\textbf{(1.1)}\quad RE = \frac{density\ x\ crossflow\ velocity\ x\ channel\ diameter}{viscosity}$$

Shear Rate and Shear Stress. Shear rate (γ) has units of (1/time) and is the gradient of velocity in a fluid (Denn, 1980). For laminar flow in a pipe the shear rate at the wall is shown in Equation 1.2 (Belfort et al., 1994). There is no simple equation for the calculation of shear rate at the wall for turbulent flow, but shear rate will

increase with increasing cross-flow velocity (at constant CD) and decreasing CD (at constant cross-flow velocity). In MF operation shear rate can be increased by increasing cross-flow velocity.

$$\textbf{(1.2)} \quad \text{Shear rate } (\gamma) = \frac{8 \times \text{cross-flow velocity}}{\text{channel diameter}}$$

Shear stress (τ) has units of pressure (force divided by area) and is the force per unit area required to maintain a given shear rate. Gesan-Guiziou et al. (1999) used Equation 1.3 to calculate shear stress at the wall for the MF of SM. In Equation 1.3, ΔP is the longitudinal pressure drop.

$$\textbf{(1.3)} \quad \text{Shear stress } (\tau) = \frac{\Delta P \times \text{channel diameter}}{4 \times \text{membrane length}}$$

Shear stress is related to shear-rate as shown in Equation 1.4 below for Newtonian fluids (Cheryan, 1998), which is in turn influenced by cross-flow velocity (Equation 1.2). In the operation of a MF unit it is the cross-flow velocity that is controlled directly by changing the operating frequency of the retentate recirculation pump. Functionally, increasing the cross-flow velocity is the same as increasing the shear-rate and shear-stress.

$$\textbf{(1.4)} \quad \text{Shear stress } (\tau) = \text{viscosity} \times \text{shear rate } (\gamma)$$

Predicting Limiting Flux. The limiting flux is the point at which the transport of particles towards the membrane (because of the flux) exceeds the transport of particles away from the membrane. Models have been developed to

predict limiting flux based on the type of particle transport (away from the membrane) that is thought to be occurring. In Belfort et al. (1994) 4 different models are presented: Brownian diffusion (1.5), shear-induced diffusion (1.6), inertial lift (1.7) and surface transport (1.8). All of the models predict increasing flux with increasing shear rate (γ), additionally all but the surface transport model predict decreasing limiting flux as viscosity (μ) increases (Belfort et al., 1994). In experiments limiting fluxes are found to be much higher than the fluxes predicted by Brownian diffusion and lower than the fluxes predicted by surface transport (Belfort et al., 1994). Samuelsson et al. (1997a) in the MF of SM using ceramic membranes found that the shear induced diffusion (Equation 1.6) model provided the best prediction of limiting flux. In the shear induced diffusion model (Equation 1.6) increasing the CN micelle size (a) increases the lift away from the surface of the membrane. The 4 models provide a framework to estimate the impact of changing various operating parameters, but are not robust enough to replace experiments for the determination of limiting fluxes (Cheryan, 1998).

(1.5) $\text{Flux} = c \times \gamma^{0.33} \times a^{-0.67} \times \Phi_b^{-0.33} \times L^{-0.33} \times \mu^{-1}$

(1.6) $\text{Flux} = c \times \gamma^{1} \times a^{1.33} \times \Phi_b^{-0.33} \times L^{-0.33} \times \mu^{-0.33}$

(1.7) $\text{Flux} = c \times \gamma^{2} \times a^{3} \times \mu^{-1}$

(1.8) $\text{Flux} = c \times \gamma^{1} \times a^{1}$

a = particle size; c = constant; L = membrane length; γ = shear rate; Φ_b = particle volume fraction; μ = viscosity.

It should be noted that CD and cross-flow velocity do not appear explicitly in Equations 1.5 through 1.8 above. However, these terms are implicit in shear rate (γ) which depends on cross-flow velocity and CD as shown in Equation 1.1 for laminar flow.

Cross-Flow Velocity: Impact on Limiting Flux. A number of researchers have found that increasing the cross-flow velocity (shear stress) can increase the limiting flux. Samuelsson et al. (1997b) measured limiting flux for the MF of SM to separate SP from CN using tubular ceramic membranes. The limiting flux was determined by increasing the TMP in steps and measuring the flux at each TMP. The experiment was conducted at 15°C and 55°C, a CF of 1.15X and a variety of cross-flow velocities up to 8 m/s. The highest limiting flux was found at the highest temperature (55°C) and cross-flow velocity (8 m/s) and was 145 L/m^2 per h (Samuelsson et al., 1997b).

Gesan-Guiziou et al. (1999) also determined the limiting flux for the MF of SM to separate SP from CN. A 0.1µm tubular ceramic membrane in a UTP system operated at 50°C and a 2X CF was used. A cyclical method was used, of increasing TMP or flux at constant shear stress (cross-flow velocity) and of decreasing shear stress at constant TMP or flux. It was found that increasing shear stress increased the limiting flux with a limiting flux of 75 L/m^2 per h at shear stress of 100 Pa. Shear stress was increased by increasing the cross-flow velocity.

Le Berre and Daufin (1996) using a 0.1µm ceramic UTP system operating at a 2X CF found that there was a critical value of the ratio of flux to shear stress. If the MF system was operated below this critical value there was a slow increase in

membrane resistance due to fouling and the MF could be operated for long times. Gesan-Guiziou et al. (1999) also found that there was a critical flux to shear ratio that predicted whether the MF run would operate stably with minimal fouling. These findings are consistent with the various models presented for prediction of limiting flux (Equations 1.5 to 1.8) where limiting flux is a function of shear rate (and shear stress).

Protein Concentration: Impact on Limiting Flux. As the concentration of protein in the retentate increases the limiting flux is expected to decrease, however, there is a dearth of research on the limiting flux as a function of TP concentration for the MF of SM. The proposed models to predict limiting flux (Equations 1.5 to 1.8 above) indicate that as the viscosity of the retentate increases the limiting flux should decrease. Both Solanki and Rizvi (2001) and Sauer et al. (2012) found that the viscosity of micellar CN solutions increased exponentially with increasing CN concentration. Vadi and Rizvi (2001) used a 0.2 μm ceramic UTP system to continually concentrate SM from 1X to 10X at constant TMP. During the run, the flux decreased from approximately 108 kg/m^2 per h at a CF of 1X to 18 kg/m^2 per h at a CF of 7X.

Temperature: Impact on Limiting Flux. In the literature, MF of SM has usually been carried out at around 50^{o}C (Gesan-Guiziou et al. 1999, Zulewska et al., 2009, Vadi and Rizvi, 2001). There has not been research on the MF of SM at temperatures above 55^{o}C. The temperature of MF would have an impact on retentate and permeate viscosity, with lower viscosity at higher temperatures (Sauer et al.,

2012). Samuelsson et al. (1997a) looked at the impact of operating the MF unit at 15°C and 55°C and found that while the shear-induced diffusion model provided the closest fit of the 4 models mentioned above, a better fit was achieved with the empirical relation: flux = 6.94×10^{-10} m/s * Re, where Re is the Reynolds number (Equation 1.1). The limiting flux at both 15°C and 55°C followed the same empirical relationship, indicating that temperature did not have an impact beyond changes in density and viscosity. Based on the work of Samuelsson et al. (1997a) and consistent with Equations 1.5 to 1.8 decreasing the retentate viscosity by increasing the temperature would be expected to increase the limiting flux.

There is a concern that increasing the temperature much above 50°C would lead to the precipitation of calcium salts on to the surface of the membrane causing a decrease in limiting flux. Work with simulated SM ultrafiltrate solutions has shown that even at 55°C there is precipitation of calcium phosphate (Spanos et al., 2007). If calcium precipitation is an issue, the use of a UF SM with a large portion of the soluble minerals removed as a MF feed may be a feasible method to allow the operation of MF at an increased temperature. However, increasing the temperature could also impact the SP and SP removal. At temperatures above approximately 65°C BLG begins to denature and can associate with the CN micelles (Long et al., 1963). BLG associated with the CN micelles could not be removed by MF.

Channel Diameter: Impact on Limiting Flux. As mentioned above GP membranes are available with 3 mm and 4 mm CD. The limiting flux may be a

function of the CD. There is no research looking at flux and SP removal as a function of CD for ceramic GP membranes.

Levy and Earle (1994) found that increasing the CD from 0.5 mm to 0.8 mm for flat sheet polymeric membranes with spacers increased the flux by over 100%. In contrast Cheryan (1998) claims that decreasing the CD may increase flux because of increased shear rate. For systems operating at a constant cross-flow velocity decreasing the CD would increase the shear rate which would be expected to increase the limiting flux as seen in Equations 1.5 to 1.8. However, for membranes operating at the same ΔP the smaller CD membranes are expected to have a lower cross-flow velocity (due to increased frictional losses). The change in shear rate would depend on the relative change in CD and cross-flow velocity. It should be noted that calculation of shear rate by Equation 1.2 above assumes laminar flow, and is not applicable to the turbulent flows typically found in MF with tubular ceramic membranes.

Membrane Selectivity and Limiting Flux

The MF membrane can impact SP removal as discussed above. Additionally, the flux at which an MF system operates at could impact fouling of the membrane, which could influence the passage of SP through the membrane. Samuelsson et al. (1997) found for the MF of SM with 0.14µm ceramic membranes that the concentration of protein in the permeate decreased with increasing flux and the decrease was greater at slower cross-flow velocities. At a cross-flow velocity of 6 m/s at 55^{o}C the decrease in TP in the permeate was approximately 0.1% TP (from 0.67 to 0.60% TP) (Samuelsson et al., 1997). Gesan-Guiziou et al. (1999) operating a 0.1µm

ceramic UTP system on SM at various fluxes found that SP transmission decreased by about 20% as the flux was increased to the limiting flux. Both Gesan-Guiziou et al. (1999) and Samuelsson et al. (1997) attribute the decrease in protein transmission to the build-up of a fouling layer on the membrane surface.

Research Objectives

The cross-flow velocity, CF (protein concentration) and temperature at which an MF system is operated at could impact the limiting flux. Additionally, the flux at which the MF system operates at could have an impact on SP removal and CN retention. Currently, there is no information available on limiting flux and SP removal for 3 mm and 4 mm CD GP MF membranes as a function of TP concentration in the recirculation loop.

The research objectives were:

1) To develop a theoretical model to determine the impact of: type of MF process feed, number of stages, recirculation loop protein concentration and flux on the MF membrane area required to produce a 95% SP reduced MCC. This work would help identify the critical variables to be explored in later research.
2) To determine the limiting flux and SP removal at 8, 9 and 10% TP in the recirculation loop using 0.1 μm GP membranes with both 3 mm and 4 mm CD. In this work the MF feed was a diluted milk protein concentrate (MPC).

3) To determine the impact of operating a 0.1µm ceramic UTP MF unit at temperatures of 50, 55, 60 and 65ºC on membrane fouling and SP removal from SM with and without removal of low molecular weight soluble milk components by UF prior to MF at a flux of 54 kg/m^2 per h. One possible way to increase flux is to increase the temperature of MF. However, with SM as a feed there was concern that increasing the temperature would cause calcium phosphate precipitation and severe membrane fouling.

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CHAPTER 2

Factors that influence the membrane area of a multi-stage microfiltration process required to produce a micellar casein concentrate

ABSTRACT

The objective of the work reported in this paper was to develop a theoretical model to determine the impact of: type of microfiltration (**MF**) process feed, number of stages and flux on the minimization of the MF membrane area required to produce a 95% serum protein (**SP**) reduced micellar casein concentrate (**MCC**). The MF feed, number of stages and flux were all factors that had an impact on the MF membrane area and should be taken into consideration when designing a MF system to produce a 95% SP reduced MCC. Feeding the MF process with a diluted ultrafiltration (**UF**) retentate (**DUR**) diluted to the protein concentration of skim milk (**SM**), as opposed to SM reduced the required membrane area by 36% for a 5-stage process. When DUR was the MF feed, there was an optimal feed protein concentration that depended on the number of MF stages. The DUR protein concentration that minimized the required MF membrane area was: 2.47%, 3.85%, 4.77% and 5.41% for a 2, 3, 4 or 5 stage MF process respectively. For a 5 stage process increasing the protein concentration of the feed from 3.2 to 5.4% decreased the required MF membrane area by 10%. It was also found that as the number of stages increased from 2 to 5 the required MF membrane area decreased by 39%, when the MF feed was DUR at the optimal feed protein concentration. Finally, increasing the flux from 50 to 60 kg/m^2 per h decreased the required MF membrane area by 17% when the MF feed was DUR at the optimal MF

feed protein concentration. Overall, using DUR as a feed for the MF reduced the amount of MF membrane area required to make a 95% SP reduced MCC.

INTRODUCTION

Microfiltration of Skim Milk

Microfiltration (**MF**) can be used to remove serum protein (**SP**) and lactose from the micellar casein (**CN**) in skim milk (**SM**). The micellar CN is retained by the MF membranes and concentrated in the retentate while SP, lactose, non protein nitrogen (**NPN**) and serum phase minerals pass through the membrane into the permeate. Both ceramic (Fauquant et al., 1988, Zulewska et al., 2009, Adams and Barbano, 2013) and polymeric MF membranes (Lawrence et al., 2008, Beckman et al., 2010) have been used to MF SM. The type of membrane has been found to have an impact on the SP removal efficiency. Zulewska et al. (2009) compared 2 types of ceramic MF membranes to a polymeric spiral-wound membrane. Zulewska et al. (2009) found that the ceramic membranes in a 1 stage system operating at a concentration factor (**CF**) of 3X removed 64% and 61% of the SP, which was close to the theoretical removal of 69% (Hurt and Barbano, 2010), the percentage of SP removed by the polymeric membranes was significantly less at 39%.

The retentate from MF is a micellar CN concentrate (**MCC**) that could be used in multiple applications, including formulation of shelf-stable nutritional beverages. For nutritional beverage applications involving high heat treatment the large reduction in the heat labile components in MCC (SP and lactose) may be critical. The sensory properties of fresh liquid MCC retentates may be superior to other dried CN ingredients (i.e., rennet CN, sodium and calcium caseinates). The composition of MCC with respect to SP and lactose concentration as well as protein concentration will depend on the MF process and membrane equipment. The permeate from MF

will consist mainly of SP and lactose. Further processing of the MF permeate by ultrafiltration (**UF**) to concentrate the SP would produce SP concentrates. These SP concentrates could be used in applications similar to whey protein concentrates and in new applications in protein fortification where their clarity relative to whey protein concentrates (Luck et al., 2013) would be an advantage.

Microfiltration Process Design

In designing a multi-stage MF process to produce an MCC, the number of stages, retentate protein concentration and the flux at which the system will operate at all have to be specified. These parameters could have an impact on the overall MF membrane area required and the cost of the system. A processor considering installing an MF system to produce MCC, may already be using UF to produce milk protein concentrates (**MPC**). In this case, there will be the possibility of feeding the MF process with UF SM (MPC) as opposed to SM. Because the UF process will remove lactose, an MCC produced from UF SM would be expected to have a lower concentration of lactose compared to an MCC produced with SM using the same MF process.

For a MF process designed to produce an MCC a main objective would be to produce an MCC meeting customer specifications while minimizing the cost of the system, including the cost of required diafiltration water. In the current work MF membrane area was used as a proxy for system cost, while the amount of MF permeate produced (and diafiltration water) was also calculated. To determine the relationship between the process design parameters and required MF membrane area a theoretical MF model was developed where the impact of: MF process feed, number

of stages and flux on MF membrane area could be determined. The objective of the work reported in this paper was to develop a theoretical model to determine the impact of: type of MF process feed, number of stages and flux on the minimization of the MF membrane area required to produce a 95% SP reduced MCC.

MATERIALS AND METHODS

Micellar Casein Concentrate Composition

The goal of the theoretical MF process was to produce an MCC with a reduced concentration of SP and lactose. The MF process would also reduce the concentration of other serum phase components of SM such as NPN and ash in the MCC, but the concentration of these components in the final MCC was not specified. The target MCC composition is shown in Table 2.1. The target MCC protein concentration was 9% with at least 95% of the SP and 98.8% of the lactose removed. The target MCC composition was somewhat arbitrary, but input from retorted milk based beverage processors indicated that it was desirable to remove a large amount of the heat labile SP, as well as to have a high final protein concentration. Additionally, a very low level of lactose in the MCC was desired so that the beverages produced using this protein ingredient could be labeled lactose free.

Table 2.1. Micellar casein concentrate (MCC) target composition (% by weight) and percent reduction of lactose and serum protein compared to skim milk.

Component	Concentration in MCC (% by weight)	Percent reduction (compared to skim milk)
Protein	9.00	--
Serum protein	0.098	95
Lactose	0.20	98.8

Model Development

A theoretical model was developed using Excel 2007 (Microsoft, Redmond, WA) to determine the composition and mass of the retentate and permeate produced from each stage of a MF process that could consist from 2 to 5 stages. The retentate from the final stage was the MCC. The model was based on previous work by Hurt and Barbano (2010). It was assumed that each MF stage was a continuous feed-and-bleed system (with the composition of the material in the recirculation loop equal to the composition of the retentate removed from that stage) with water dilution between stages. The composition used for the SM feeding the first MF stage is shown in Table 2.1. A mass of 1,000 kg of SM was used in the model as the initial MF feed.

The CF and diafiltration factor (DF) determined the mass and composition of the retentates and permeates produced as shown in Figure 2.1. The CF was the mass of MF feed for a stage divided by the mass of retentate produced in that stage. The DF determined how much water was added to the retentate from the previous stage to arrive at the feed for the current stage. The DF was the mass of MF feed from the current stage divided by the mass of retentate from the previous stage.

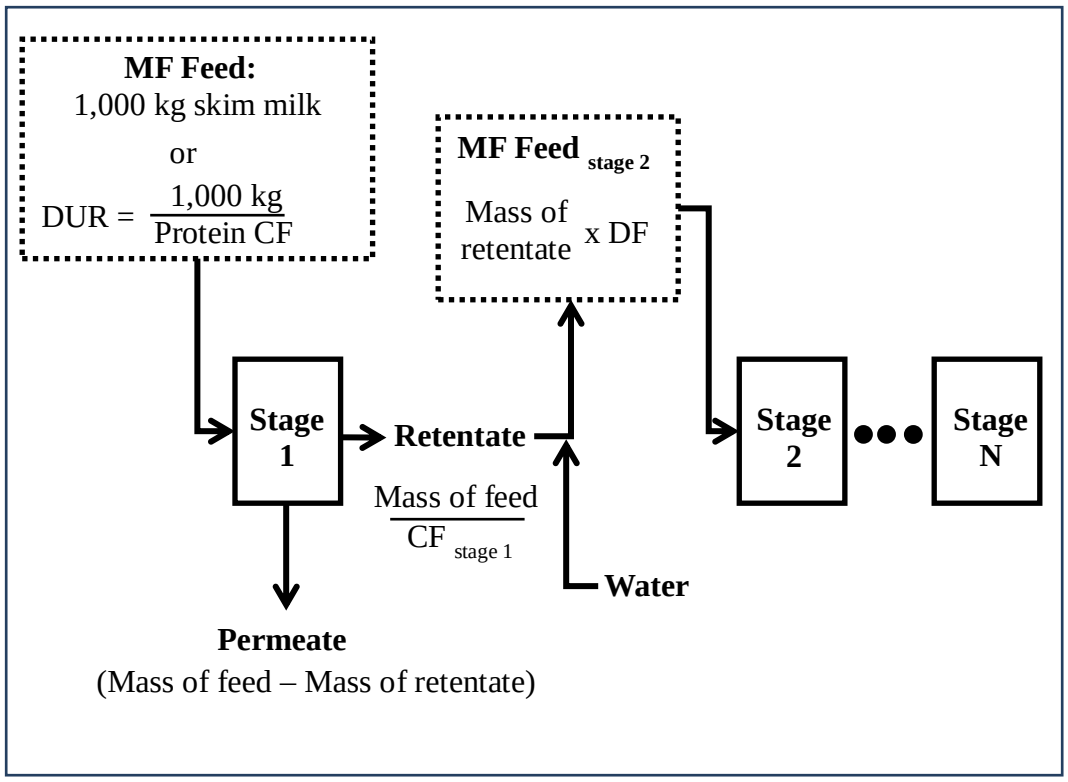

Figure 2.1. Mass balance basis of the theoretical model developed for the production of a micellar casein concentrate by microfiltration (MF). The initial feed was either skim milk (SM) or diluted ultrafiltration retentate (DUR). The mass of retentate, permeate and MF feed for the subsequent stage was determined by the concentration factor (CF) and diafiltration factor (DF).

Model Assumptions. As in the research by Hurt and Barbano (2010), it was assumed that 2/3 of the ash in the SM was associated with the CN micelles and could not be removed by MF, this assumption was necessary to calculate the concentration of components in the serum phase of SM. Assumptions were also made regarding the transmission of components through the membrane. It was assumed that the removal factors for SP and lactose were 1. A removal factor of 1 indicated that the component was not rejected by the membrane. The removal factor was equal to the concentration of the component in the permeate divided by the concentration of that component in the serum phase of the MF feed. The concentration of SP in the serum phase (% by

weight) was calculated using Equation 2.1. Additionally, it was assumed that the MF membranes retained all of the CN (the concentration of CN in the permeate was 0%). The assumptions on the transmission characteristics of the MF membrane are a best case scenario. However, previous research has found that 0.1 μm ceramic membranes with 4 mm channel diameters in a uniform transmembrane pressure system performs with near theoretical SP removal for a 3X process (67% for 1 stage compared to a theoretical removal of 68%) (Hurt et al., 2010). The SP removal reported for ceramic membranes is higher than the reported SP removal for polymeric membranes (Beckman et al., 2010).

(2.1) $$[SP]_{\text{serum phase}} = \frac{[SP]_{feed}}{1 - \frac{[\text{CN}]_{feed}}{100} - \frac{2}{3}\frac{[\text{Ash}]_{feed}}{100}}$$

Factors that Could Impact the Required Microfiltration Membrane Area

Ultrafiltration of Skim Milk. The possibility of ultrafiltering SM prior to MF to remove lactose was included in the analysis. SM that had been UF (with or without diafiltration) would be an MPC, in this work it was assumed that the MPC had a protein concentration of 12%. The MPC would have to be diluted prior to MF to produce the MF feed: a diluted UF retentate (**DUR**), however it did not have to necessarily be diluted to the protein concentration in the original SM prior to MF. In this work it was assumed that UF would remove 76% of the lactose, NPN and soluble ash (where the soluble ash was assumed to be 1/3 of the ash in SM) from SM and would have the composition shown in Table 2.2. A 76% reduction in lactose was chosen, because an additional 95% removal of lactose from the UF SM (MPC) would

result in a 98.8% lactose removal compared to SM, which was the target lactose removal for the MCC. In the model it was assumed that the rejection characteristics for lactose and SP by the MF membranes were the same, so the required 95% reduction in SP would also reduce the lactose content by 95%.

Ultrafiltered Skim Milk Protein Concentration Factor. If the MF feed was a DUR, the protein concentration of the DUR feeding the MF process was another factor that could impact the required MF membrane area. A DUR protein CF was added to the model. The protein concentration in the MF feed was equal to the DUR protein CF multiplied by the protein concentration of SM (3.2%). With a DUR protein CF of 1 the MF feed would have the same protein concentration as SM, while with a DUR protein CF of 2 the MF feed would have twice the protein concentration of SM. The concentration of lactose, NPN and ash in the DUR was modified so that their concentration equaled the DUR protein CF times the DUR composition shown in Table 2.2. This kept the ratio of lactose, NPN and ash to protein in the DUR constant. If the MF feed was DUR, the mass of MF feed was modified so that the total mass of protein feeding the process was constant. For example if the DUR protein CF was 2 the mass of MF feed would be 500 kg opposed to 1,000 kg. In this way the mass of MCC produced was constant and did not depend on the DUR protein CF.

Number of Microfiltration Stages. The model allowed the MF process to include from 2 to 5 stages. A stage was defined as a MF unit having the same MF feed and producing retentate of the same composition. Water dilution of the retentates took place between stages, to produce the feed for the subsequent stage.

Table 2.2. Composition (% by weight) of the microfiltration (MF) feed, skim milk (SM) or diluted ultrafiltration retentate (DUR) and the starting milk protein concentrate (MPC).

MF Feed	Protein	Casein	Serum protein	Lactose	Non protein nitrogen	Ash
SM	3.20	2.623	0.577	4.85	0.190	0.729
DUR	3.20	2.623	0.577	1.164	0.046	0.544
MPC	12.00	9.836	2.164	4.365	0.171	2.04

Microfiltration Flux. The flux that the MF process operates at would also impact the required membrane area. The flux would not have an impact on the amount of MF permeate that had to be removed, but rather the time required to remove it. A default flux of 54 kg/m^2 per h was chosen for the flux as previous research on the MF of SM had shown that a uniform transmembrane pressure MF system with 0.1 µm ceramic membranes with 4 mm channel diameters could operated for extended periods of time at this flux when the feed was SM and the process operated at a CF of 3X (Zulewska et al., 2009). The impact of increasing the flux up to 80 kg/m^2 per h on required MF membrane area was also calculated.

Minimization of Microfiltration Permeate

Excel 2007 Solver (Microsoft, Redmond, WA) was used to minimize the total mass of MF permeate produced by the MF process in the theoretical model. The total mass of permeate was equal to the sum of the mass of permeate produced in each stage. The mass of MF permeate was minimized by changing the CF and DF for each stage. If the MF feed was DUR the DUR protein CF could be changed as well to minimize the mass of MF permeate.

Model Constraints. In minimizing the mass of permeate in the theoretical model, 4 constraints were imposed as shown in Table 2.3. The first constraint limited

the concentration of protein in the recirculation loop in all but the last stage to ≤ 8.6%. Previous research had indicated that a uniform transmembrane pressure system with ceramic 0.1 μm membranes could operate at a flux of 54 kg/m^2 per h with retentate protein concentrations in the 8.6 to 9% range (Zulewska et al., 2009). A maximum protein concentration of 8.6% was chosen as opposed to 9% in all but the final stage, so that the process would have a built in safety factor. It is acknowledged that there would likely be variation in the retentate protein concentration and having a slightly lower maximum retentate protein concentration in all but the final stage would allow the MF process to operate consistently despite this variation. Constraints 2, 3 and 4 shown in Table 2.3 ensure that the final retentate composition achieved the target MCC composition as shown in Table 2.1.

The total number of stages in the MF process could be 2, 3, 4 or 5. As shown in Table 2.3, there was one less DF variable than CF variable, because the final MF retentate was the MCC and not diluted with water. Additionally, all the CF and DF were constrained to be greater than or equal to 1 to model an actual MF process where MF permeate would be removed in each stage and diafiltration water would be added between stages.

Table 2.3. Objective function and constraints used to minimize the amount of microfiltration (MF) permeate produced by a MF process producing a micellar casein concentrate (MCC). The MF feed could be: skim milk or diluted ultrafiltration retentate (DUR).

Objective function:	Minimize $\sum_{Stage\ i}^{Stage\ n}$ Mass of Permeate$_i$	i = stages 1 to N
Subject to:		
1)	Retentate protein$_i \leq$ 8.6%	i = stages 1 to N-1
2)	Retentate protein$_N$ = 9%	
3)	MCC serum protein concentration $\leq$ 0.098%	
4)	MCC lactose concentration $\leq$ 0.2%	
Variables:		
	DUR protein concentration factor	
	Concentration factor$_i$	i = stages 1 to N
	Diafiltration factor$_k$	k = stages 2 to N
	Where: concentration factor$_i$ and diafiltration factor$_k \geq 1$	
	N = 2, 3, 4 or 5 stages	

Converting Mass of Permeate to Required Membrane Area. Solving the model subjected to the constraints shown in Table 2.3 provided the CF and DF that produced the minimal mass of MF permeate for the entire MF process. Up until this point flux did not play a role in the model. The mass of permeate was converted to membrane area by specifying a mass of SM to be processed in a given period of time at a specific flux. For this work it was assumed that 150,000 kg of SM (or the protein equivalent) was to be processed in 18 h. Finally, unless otherwise specified a flux of 54 kg/m^2 per h was used to calculate the required MF membrane area. The equation used to convert mass of permeate to membrane area is shown in Equation 2.2 below.

(2.2) $$\text{Membrane area } (\text{m}^2) = \frac{150{,}000kg \times Mass \text{ of permeate (kg)}}{1{,}000kg \times Flux(\frac{kg}{m^2 h}) \times 18h}$$

RESULTS AND DISCUSSION

Binding Constraints

One of our goals was to determine the minimum amount of membrane area needed to process a give mass of milk in a given time. When the mass of MF permeate produced by the MF process was minimized using the model described above, some of the constraints (shown in Table 2.3) were always binding (the value of the constraint at the optimal solution was equal to the right hand side of the constraint). The protein concentration of the retentate was always at the maximum allowable concentration (8.6% in all but the final stage) in the optimal solution. If the retentate was only concentrated to 7% protein in a stage when it could go to 8.6% protein, then that stage would remove less SP and lactose than it could have (less permeate is being removed in that stage). The additional mass of SP and lactose would have to be removed in later stages. Because there is diafiltration between stages, to remove this mass of SP and lactose in the next stage would require a removal of an even larger mass of permeate and require more membrane surface area.

When the MF feed was SM the MCC SP constraint (constraint 3 in Table 2.3) was non-binding. The target MCC composition required at least a 95% removal of SP and a 98.8% removal of lactose. When the feed was SM, to achieve 98.8% lactose removal, 98.8% of the SP was removed as well. In contrast when the MF feed was DUR, by design a 95% removal of SP and lactose would produce an MCC meeting the target specification and both the SP and lactose constraints for the MCC were binding (although redundant).

Ultrafiltration of Skim Milk

The MF membrane area required (and mass of MF permeate removed) to produce an MCC from SM compared to DUR is shown in Figure 2.2, for 2, 3, 4 or 5 stages. In Figure 2.2 the DUR protein concentration was the same as SM (3.2%). The feed, retentate and permeate masses and compositions when the MF feed was SM or DUR at the protein concentration of SM are shown in Tables 2.4, 2.5, 2.6 and 2.7. For a 2-stage MF process, feeding the MF process with a DUR reduced the required MF membrane area (Figure 2.2) by 71% (from 1080m^2 to 312m^2) and for a 5-stage process the required MF membrane area (Figure 2.2) was reduced by 36% (from 315 m^2 to 202 m^2).

Feeding the MF process with DUR reduced the required MF membrane area, because the target MCC required a larger reduction in lactose than SP as shown in Table 2.1. UF reduced the amount of lactose in the MF feed to a level where a MF process to remove 95% of the SP from the feed would result in an MCC that met the lactose removal target specification. As shown in Tables 2.4, 2.5, 2.6 and 2.7 the MCC produced starting from SM has a lower concentration of SP than when the starting material was DUR. The use of an MPC with greater than 76% of the lactose removed would not further reduce the required MF membrane area because the MF process would still be required to remove 95% of the SP.

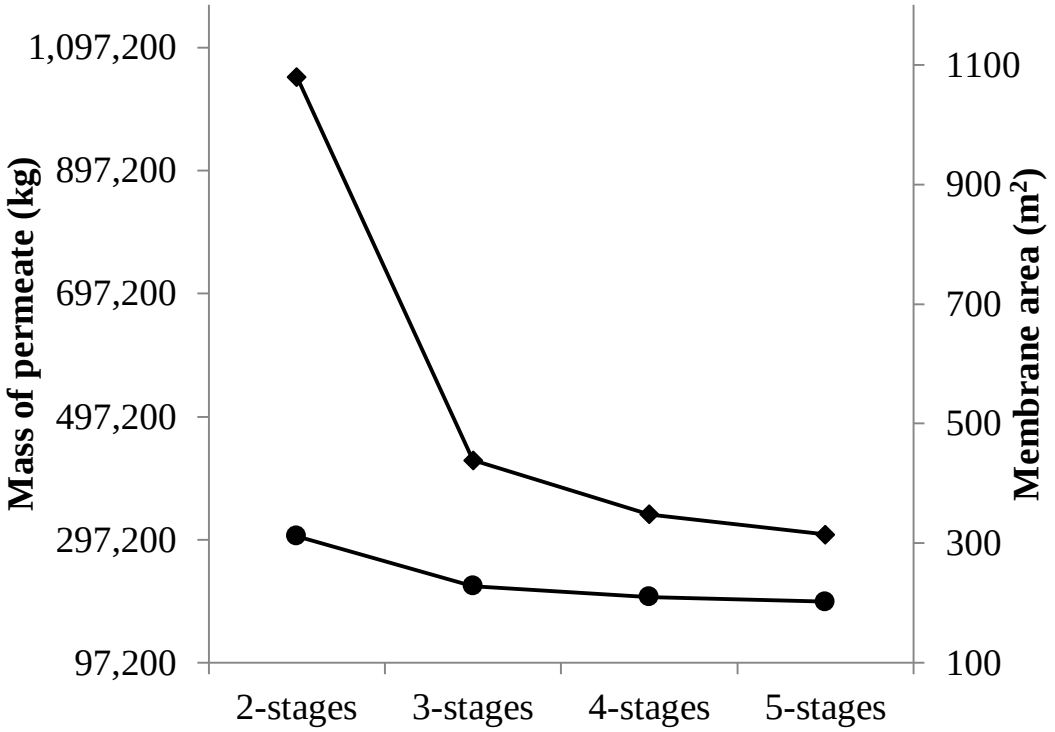

Figure 2.2. Theoretical microfiltration (MF) membrane area and mass of MF permeate produced in a MF process producing a micellar casein concentrate from either (♦) skim milk or (●) diluted ultrafiltration retentate (DUR) (with a 76% reduction in lactose) for 2, 3, 4 or 5 stages. The protein concentration of the DUR feeding the MF process was the same as skim milk.

An UF system to remove lactose from SM prior to MF would be an additional cost. However, polymeric spiral-wound UF systems are less expensive than ceramic MF systems (Cheryan, 1998). The savings achieved by reducing the required MF membrane area could offset the cost of the UF system if no UF system was already available in the factory.

Diluted Ultrafiltration Retentate Protein Concentration Factor. The DUR MF feed would be produced by diluting a fresh liquid MPC, and the protein concentration of this DUR was an additional variable in the model. The DUR protein concentration had an impact on the required MF membrane area and mass of permeate produced for a 2, 3, 4 or 5-stage MF process as shown in Figure 2.3. The DUR protein

CF that minimized the required membrane area was 0.77, 1.20, 1.49 and 1.69 for 2, 3, 4 or 5-stages respectively which corresponds to MF feed protein concentrations of: 2.47%, 3.85%, 4.77% and 5.41% respectively as shown in Tables 2.8 and 2.9. For a 5-stage MF process, increasing the DUR protein concentration from 3.2 to 5.41% reduced the required MF membrane area from 202 m^2 to 182 m^2.

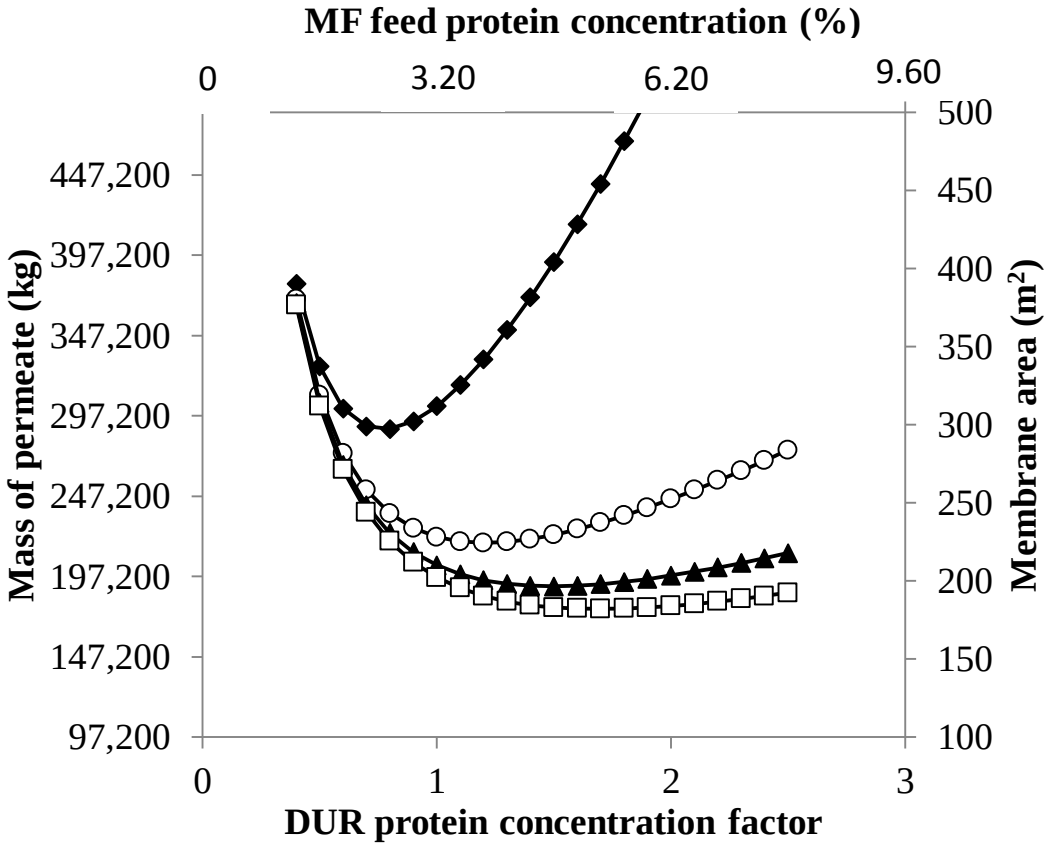

Figure 2.3. Theoretical microfiltration (MF) membrane area required and mass of permeate produced as a function of the diluted ultrafiltration retentate (DUR) concentration factor of the MF feed. The number of MF stages used for the micellar casein concentrate production were: 2 (♦), 3(○), 4(▲) or 5(□). The starting MF feed was DUR.

Table 2.4. Skim milk as the microfiltration (MF) process feed: Composition and mass of MF feeds, retentates and permeates for each stage of a 2 or 3-stage, with concentration factors (CF).

	2-Stage process		3-Stage process		
Feed-skim milk	**Stage 1**	**Stage 2**	**Stage 1**	**Stage 2**	**Stage 3**
Diafiltration water (kg)		943,133		160,115	159,896
Mass (kg)	150,000	991,940	150,000	208,922	206,270
True protein (%)	3.20	0.42	3.20	2.01	1.93
Casein (%)	2.62	0.40	2.62	1.88	1.91
Serum protein (%)	0.58	0.03	0.58	0.13	0.03
Lactose (%)	4.85	0.22	4.85	1.06	0.22
Non protein nitrogen (%)	0.19	0.01	0.19	0.04	0.01
Retentate					
MF CF	3.07	22.63	3.07	4.51	4.71
Mass (kg)	48,807	43,833	48,807	46,374	43,833
True protein (%)	8.60	9.00	8.60	8.60	9.00
Casein (%)	8.06	8.98	8.06	8.48	8.98
Serum protein (%)	0.54	0.02	0.54	0.12	0.02
Lactose (%)	4.53	0.20	4.53	0.97	0.20
Non protein nitrogen (%)	0.18	0.01	0.18	0.04	0.01
Permeate					
Mass (kg)	101,193	948,107	101,193	162,547	162,438
True protein (%)	0.60	0.03	0.60	0.13	0.03
Casein (%)	0.00	0.00	0.00	0.00	0.00
Serum protein (%)	0.60	0.03	0.60	0.13	0.03
Lactose (%)	5.01	0.22	5.01	1.08	0.22
Non protein nitrogen (%)	0.20	0.01	0.20	0.04	0.01

Table 2.5. Skim milk as the microfiltration (MF) process feed: Composition and mass of MF feeds, retentates and permeates for each stage of a 4 or 5-stage MF process, with concentration factors (CF).

	4-Stage process				5-Stage process				
Feed-skim milk	**Stage 1**	**Stage 2**	**Stage 3**	**Stage 4**	**Stage 1**	**Stage 2**	**Stage 3**	**Stage 4**	**Stage 5**
Diafiltration water (kg)		77,558	77,565	77,279		49,966	50,074	49,830	49,851
Mass (kg)	150,000	126,365	124,374	123,395	150,000	98,773	97,203	96,202	95,882
True protein (%)	3.20	3.32	3.24	3.21	3.20	4.25	4.17	4.15	4.13
Casein (%)	2.62	3.11	3.16	3.19	2.62	3.98	4.05	4.09	4.10
Serum protein (%)	0.58	0.21	0.07	0.03	0.58	0.27	0.12	0.06	0.03
Lactose (%)	4.85	1.75	0.62	0.21	4.85	2.24	1.03	0.47	0.21
Non protein nitrogen (%)	0.19	0.07	0.02	0.01	0.19	0.09	0.04	0.02	0.01
Retentate									
MF CF	3.07	2.70	2.70	2.82	3.07	2.10	2.10	2.09	2.19
Mass (kg)	48,807	46,809	46,117	43,832	48,807	47,129	46,372	46,031	43,832
True protein (%)	8.60	8.60	8.60	9.00	8.60	8.60	8.60	8.60	9.00
Casein (%)	8.06	8.41	8.53	8.98	8.06	8.35	8.48	8.55	8.98
Serum protein (%)	0.54	0.19	0.07	0.02	0.54	0.25	0.12	0.05	0.02
Lactose (%)	4.53	1.63	0.57	0.20	4.53	2.12	0.97	0.44	0.20
Non protein nitrogen (%)	0.18	0.06	0.02	0.01	0.18	0.08	0.04	0.02	0.01
Permeate									
Mass (kg)	101,193	79,557	78,257	79,563	101,193	51,643	50,832	50,171	52,050
True protein (%)	0.60	0.22	0.08	0.03	0.60	0.28	0.13	0.06	0.03
Casein (%)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Serum protein (%)	0.60	0.22	0.08	0.03	0.60	0.28	0.13	0.06	0.03
Lactose (%)	5.01	1.82	0.64	0.22	5.01	2.35	1.08	0.49	0.22
Non protein nitrogen (%)	0.20	0.07	0.03	0.01	0.20	0.09	0.04	0.02	0.01

Table 2.6. Diluted ultrafiltration retentate (DUR) at the protein concentration of skim milk as the microfiltration (MF) process feed: Composition and mass of MF feeds, retentates and permeates for each stage of a 2 or 3-stage MF process, with concentration factors (CF).

	2-Stage process		3-Stage process		
Feed-DUR	**Stage 1**	**Stage 2**	**Stage 1**	**Stage 2**	**Stage 3**
DUR protein CF	1		1		
MPC (12% protein) (kg)	40,000		40,000		
Diafiltration water (kg)	110,000	197,696	110,000	58,063	58,014
Mass (kg)	150,000	246,512	150,000	106,880	105,038
True protein (%)	3.20	1.70	3.20	3.93	3.85
Casein (%)	2.62	1.60	2.62	3.68	3.75
Serum protein (%)	0.58	0.11	0.58	0.25	0.10
Lactose (%)	1.16	0.22	1.16	0.50	0.21
Non protein nitrogen (%)	0.05	0.01	0.05	0.02	0.01
Retentate					
CF	3.07	5.58	3.07	2.27	2.38
Mass (kg)	48,816	44,198	48,816	47,024	44,198
True protein (%)	8.60	9.00	8.60	8.60	9.00
Casein (%)	8.06	8.90	8.06	8.37	8.90
Serum protein (%)	0.54	0.10	0.54	0.23	0.10
Lactose (%)	1.09	0.20	1.09	0.47	0.20
Non protein nitrogen (%)	0.04	0.01	0.04	0.02	0.01
Permeate					
Mass (kg)	101,184	202,314	101,184	59,856	60,841
True protein (%)	0.59	0.11	0.59	0.26	0.11
Casein (%)	0.00	0.00	0.00	0.00	0.00
Serum protein (%)	0.59	0.11	0.59	0.26	0.11
Lactose (%)	1.20	0.22	1.20	0.52	0.22
Non protein nitrogen (%)	0.05	0.01	0.05	0.02	0.01

Table 2.7. Diluted ultrafiltration retentate (DUR) at the protein concentration of skim milk as the microfiltration (MF) process feed: Composition and mass of MF feeds, retentates and permeates for each stage of a 4 or 5-stage MF process, with concentration factors (CF).

	4-Stage process				5-Stage process				
Feed-DUR	**Stage 1**	**Stage 2**	**Stage 3**	**Stage 4**	**Stage 1**	**Stage 2**	**Stage 3**	**Stage 4**	**Stage 5**
DUR protein CF	1				1				
MPC (12% protein) (kg)	40,000				40,000				
Diafiltration water (kg)	110,000	32,835	32,840	32,842	110,000	22,719	22,744	22,794	22,741
Mass (kg)	150,000	81,652	80,297	79,543	150,000	71,535	70,472	69,819	69,313
True protein (%)	3.20	5.14	5.08	5.05	3.20	5.87	5.82	5.79	5.78
Casein (%)	2.62	4.82	4.90	4.95	2.62	5.50	5.58	5.64	5.68
Serum protein (%)	0.58	0.32	0.18	.10	0.58	0.37	0.24	0.16	0.10
Lactose (%)	1.16	0.65	0.37	0.21	1.16	0.74	0.49	0.32	0.21
Non protein nitrogen (%)	0.05	0.03	0.01	0.01	0.05	0.03	0.02	0.01	0.01
Retentate									
CF	3.07	1.72	1.72	1.80	3.07	1.50	1.50	1.50	1.57
Mass (kg)	48,816	47,458	46,701	44,198	48,816	47,728	47,025	46,572	44,198
True protein (%)	8.60	8.60	8.60	9.00	8.60	8.60	8.60	8.60	9.00
Casein (%)	8.06	8.29	8.42	8.90	8.06	8.24	8.37	8.45	8.90
Serum protein (%)	0.54	0.31	0.18	0.10	0.54	0.36	0.23	0.15	0.10
Lactose (%)	1.09	0.62	0.35	0.20	1.09	0.72	0.47	0.31	0.20
Non protein nitrogen (%)	0.04	0.02	0.01	0.01	0.04	0.03	0.02	0.01	0.01
Permeate									
Mass (kg)	101,184	34,194	33,596	35,346	101,184	23,807	23,447	23,248	25,115
True protein (%)	0.59	0.34	0.19	0.11	0.59	0.39	0.26	0.17	0.11
Casein (%)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Serum protein (%)	0.59	0.34	0.19	0.11	0.59	0.39	0.26	0.17	0.11
Lactose (%)	1.20	0.69	0.39	0.22	1.20	0.79	0.52	0.34	0.22
Non protein nitrogen (%)	0.05	0.03	0.02	0.01	0.05	0.03	0.02	0.01	0.01

Table 2.8. Diluted ultrafiltration retentate (DUR) at the protein concentration that minimized the microfiltration (MF) membrane area as the MF process feed: Composition and mass of MF feeds, retentates and permeates for each stage of a 2 or 3-stage MF process, with concentration factors (CF).

	2-Stage process		3-Stage process		
Feed-DUR	**Stage 1**	**Stage 2**	**Stage 1**	**Stage 2**	**Stage 3**
DUR protein CF	0.77		1.20		
MPC (12% protein) (kg)	40,000		40,000		
Diafiltration water (kg)	154,446	138,658	84,674	68,888	68,847
Mass (kg)	194,446	186,717	124,674	118,409	116,010
True protein (%)	2.47	2.21	3.85	3.60	3.50
Casein (%)	2.02	2.11	3.16	3.32	3.39
Serum protein (%)	0.45	0.11	0.69	0.27	0.10
Lactose (%)	0.90	0.21	1.40	0.55	0.21
Non protein nitrogen (%)	0.04	0.01	0.05	0.02	0.01
Retentate					
CF	4.05	4.22	2.52	2.51	2.62
Mass (kg)	48,059	44,198	49,521	47,163	44,198
True protein (%)	8.60	9.00	8.60	8.60	9.00
Casein (%)	8.19	8.90	7.95	8.34	8.90
Serum protein (%)	0.41	0.10	0.65	0.26	0.10
Lactose (%)	0.83	0.20	1.32	0.52	0.20
Non protein nitrogen (%)	0.04	0.01	0.05	0.02	0.01
Permeate					
Mass (kg)	146,387	142,519	75,152	71,246	71,812
True protein (%)	0.46	0.11	0.72	0.28	0.11
Casein (%)	0.00	0.00	0.00	0.00	0.00
Serum protein (%)	0.46	0.11	0.72	0.28	0.11
Lactose (%)	0.92	0.22	1.45	0.57	0.22
Non protein nitrogen (%)	0.04	0.01	0.06	0.02	0.01

Table 2.9. Diluted ultrafiltration retentate (DUR) at the protein concentration that minimized the microfiltration (MF) membrane area as the MF process feed: Composition and mass of MF feeds, retentates and permeates for each stage of a 4 or 5-stage MF process, with concentration factors (CF).

	4-Stage process				5-Stage process				
Feed-DUR	**Stage 1**	**Stage 2**	**Stage 3**	**Stage 4**	**Stage 1**	**Stage 2**	**Stage 3**	**Stage 4**	**Stage 5**
DUR protein CF	1.49				1.69				
MPC (12% protein) (kg)	40,000				40,000				
Diafiltration water (kg)	60,715	44,902	44,898	44,896	48,757	33,113	33,124	33,162	33,167
Mass (kg)	100,715	95,472	92,957	91,752	88,757	84,460	81,979	80,635	79,872
True protein (%)	4.77	4.56	4.45	4.39	5.41	5.23	5.13	5.06	5.03
Casein (%)	3.91	4.12	4.23	4.29	4.43	4.66	4.80	4.88	4.93
Serum protein (%)	0.86	0.43	0.21	0.10	0.98	0.57	0.33	0.18	0.10
Lactose (%)	1.73	0.88	0.43	0.21	1.97	1.15	0.66	0.37	0.21
Non protein nitrogen (%)	0.07	0.03	0.02	0.01	0.08	0.04	0.03	0.01	0.01
Retentate									
CF	1.99	1.99	1.98	2.08	1.73	1.73	1.73	1.73	1.81
Mass (kg)	50,570	48,058	46,856	44,198	51,347	48,855	47,473	46,705	44,198
True protein (%)	8.60	8.60	8.60	9.00	8.60	8.60	8.60	8.60	9.00
Casein (%)	7.78	8.19	8.40	8.90	7.66	8.05	8.29	8.42	8.90
Serum protein (%)	0.82	0.41	0.20	0.10	0.94	0.55	0.31	0.18	0.10
Lactose (%)	1.65	0.83	0.41	0.20	1.89	1.10	0.63	0.36	0.20
Non protein nitrogen (%)	0.06	0.03	0.02	0.01	0.07	0.04	0.02	0.01	0.01
Permeate									
Mass (kg)	50,145	47,414	46,101	47,554	37,411	35,605	34,507	33,930	35,675
True protein (%)	0.90	0.46	0.22	0.11	1.03	0.60	0.34	0.19	0.11
Casein (%)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Serum protein (%)	0.90	0.46	0.22	0.11	1.03	0.60	0.34	0.19	0.11
Lactose (%)	1.81	0.92	0.45	0.22	2.07	1.21	0.70	0.39	0.22
Non protein nitrogen (%)	0.07	0.04	0.02	0.01	0.08	0.05	0.03	0.02	0.01

As the protein concentration in the MF feed (DUR) was decreased, the required MF membrane area began to converge, regardless of the number of stages as shown in Figure 2.3. This is because, it was possible to dilute the DUR so that the concentration of SP was less than 0.098%, at this point no diafiltration would be required and the feed would only have to be concentrated to 9% protein making the number of MF stages irrelevant, but requiring more membrane surface area.

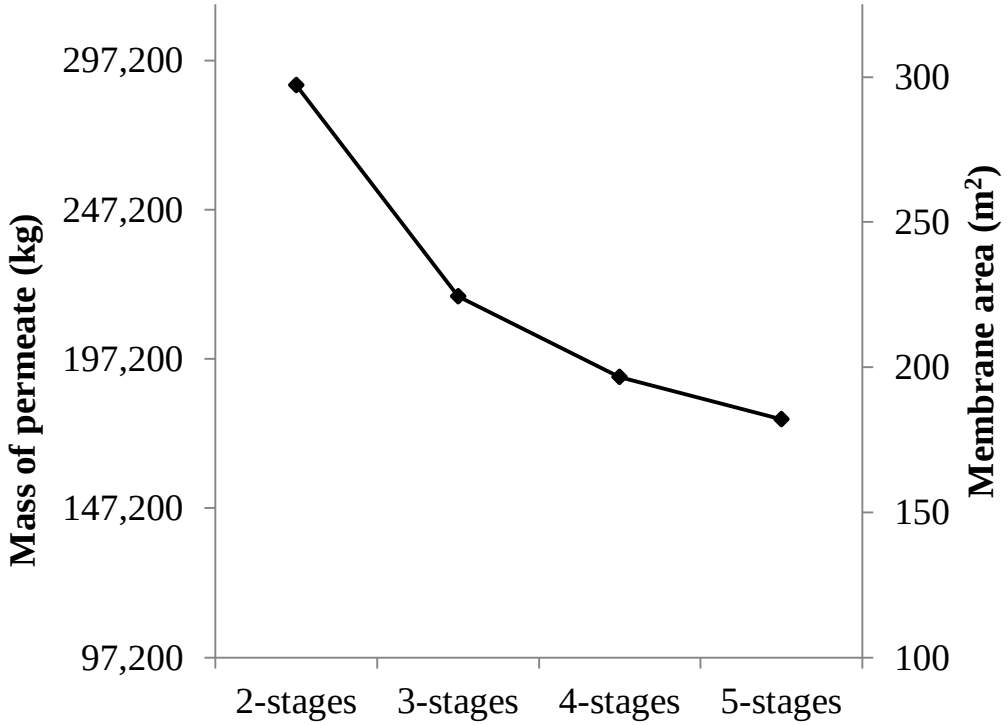

Figure 2.4. Theoretical microfiltration (MF) membrane area and mass of permeate produced in a 2, 3, 4 or 5 stage MF process producing a micellar casein concentrate. The MF feed was diluted ultrafiltration retentate (DUR) with a protein concentration of: 2.47%, 3.85%, 4.77% and 5.41% for a 2, 3, 4 or 5 stage MF process respectively.

Number of Microfiltration Stages

As the number of MF stages was increased from 2 to 5 the required membrane area decreased by 39% from 297 m^2 to 182 m^2, as shown in Figure 2.4. In Figure 2.4 it

was assumed the MF feed was DUR and the DUR protein concentration was at the optimal value for each number of stages (2.47%, 3.85%, 4.77% and 5.4% protein for a 2, 3, 4 or 5-stage MF process respectively). Increasing the number of stages from 2 to 3 resulted in a 24% decrease in required MF membrane area (and in the mass of MF permeate removed), while increasing the number of stages from 4 to 5 only resulted in a 7% decrease in required membrane area.

Additional advantages of increasing the number of MF stages include the reduction in diafiltration water that was required by the process as shown in Tables 2.8 and 2.9 and the decreased mass of MF permeate as shown in Figure 2.4. There will be some cost associated with providing the required diafiltration water. Reducing the mass of MF permeate also increased the average concentration of SP in the permeate (Tables 2.8 and 2.9), which would reduce the concentration required to produce SP concentrates.

Increasing the number of stage beyond 5 would continue to decrease the required membrane area; however, the additional reduction in membrane area for each additional stage would also continue to decrease. At some point, the costs associated with the addition of an extra stage (i.e., pumps, controls, and piping) would be larger than the savings in membrane area realized for the addition of that stage. Additionally, membrane area is not a continuous variable and there will be a finite membrane area that can be accommodated in each module in a stage.

Microfiltration Flux

The impact of flux on the required MF membrane area is shown in Figure 2.5, increasing the flux from 50 to 60 kg/m^2 per h decreased the required membrane area

by 17% regardless of the number of MF stages (from 197m^2 to 164m^2 for a 5-stage MF process). It was assumed that the MF feed was DUR diluted to the optimal protein concentration. The flux did not impact the amount of MF permeate that had to be removed (or diafiltration water required), but as shown in Equation 2.1 and Figure 2.5 it did impact the MF membrane area required. In this work it was assumed that the MF process could operate at a flux of 50, 60, 70 or 80 kg/m^2 per h for extended periods of time without membrane fouling that would lead to a reduction in flux or changes in the transmission of SP or lactose through the membrane. Factors, particularly properties of the feed material that may influence flux could have a large impact on the amount membrane area required in an MF system.

Realistically, given a retentate protein concentration and membrane system there will be a limiting flux, which the MF system will not be able to operate above. A number of factors could impact the limiting flux including: protein concentration (Vadi and Rizvi, 2001), temperature and shear stress at the surface of the membrane (Samuelsson et al., 1997). Further research will be required to determine the limiting flux at different MF recirculation loop protein concentrations.

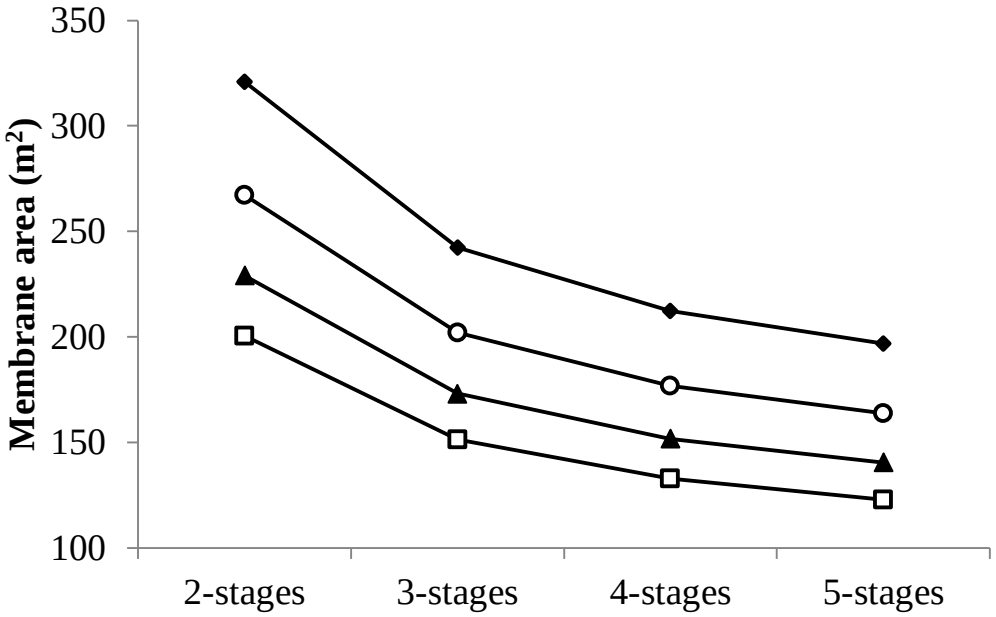

Figure 2.5. Theoretical microfiltration (MF) membrane area required for a process containing 2, 3, 4 or 5 MF stages, operating at a flux of (♦) 50, (○) 60, (▲) 70 or (□) 80 kg/m^2 per h. The MF feed was a diluted ultrafiltration retentate (DUR) protein concentration of: 2.47%, 3.85%, 4.77% and 5.41% for a 2, 3, 4 or 5 stage MF process respectively.

Sensitivity Analysis

The constraints used to minimize the required membrane area are shown in Table 2.3. Analysis was performed to determine how sensitive the mass of MF permeate removed and required MF membrane area was to changes in the various constraints. To perform the analysis a 5-stage MF process was used where the MF feed was DUR diluted to the optimal protein concentration. A ± 5% change in each of the constraints was analyzed; the changes in each of the constraints are shown in Table 2.10.

Table 2.10. Sensitivity analysis, 5% change in the constraints on maximum retentate protein concentration in stages 1, 2, 3 and 4, final micellar casein concentrate (MCC) protein concentration and MCC lactose and serum protein concentration. All values are given as percent on a weight basis.

Constraint	5% increase	5% decrease
Maximum retentate protein concentration in stages 1 to 4	9.03%	8.17%
MCC protein concentration	9.45%	8.55%

Retentate Protein Concentration in Stages 1 to 4. As shown in Figure 2.6, increasing the allowable retentate protein concentration in stages 1 to 4 by 5%, from 8.6% to 9.03% decreased the required MF membrane area from 182 m^2 to 174 m^2; in contrast decreasing the allowable protein concentration from 8.6% to 8.17% increased the required MF membrane area from 182 m^2 to 191 m^2. While the theoretical model indicates that it would be desirable to increase the allowable retentate protein concentration, the impact of this decision on flux and SP transmission would have to be determined.

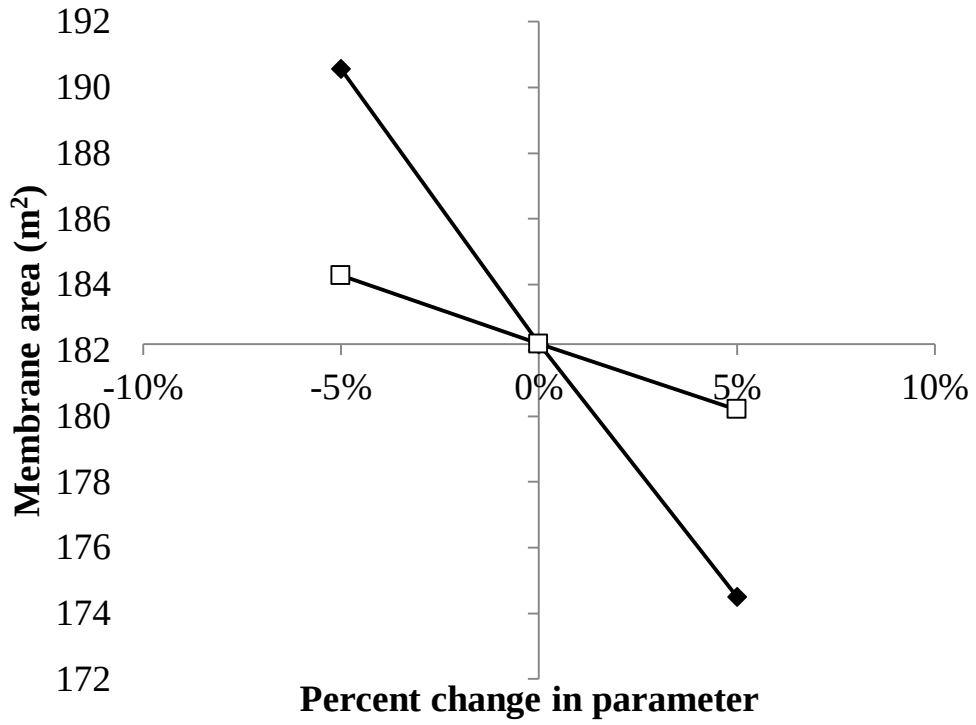

Figure 2.6. Percent change in required membrane area when the maximum allowable (♦) retentate protein concentration was changed by ± 5%, the target (□) micellar casein concentrate protein concentration was changed by ± 5%

Micellar Casein Concentrate Protein Concentration. Increasing the MCC protein concentration from 9.00% to 9.45% decreased the required MF membrane area from 182 m^2 to 180 m^2, while decreasing the target MCC protein concentration from 9.00% to 8.55% increased the required membrane area from 182 m^2 to 184 m^2 as shown in Figure 2.6. Increasing the maximum protein concentration for 4 stages (stages 1 to 4) had a larger impact than increasing the maximum protein concentration for the final stage (Figure 2.6).

CONCLUSIONS

The MF feed, number of stages and flux were all factors that had an impact on the MF membrane area and should be taken into consideration when designing a MF

system to produce a 95% SP reduced MCC. Feeding the MF process with DUR diluted with water to the protein concentration of SM, as opposed to SM reduced the required membrane area by 36% for a 5-stage process. When DUR was the MF feed, there was an optimal feed protein concentration that depended on the number of MF stages. The DUR protein concentration that minimized the required MF membrane area was: 2.47%, 3.85%, 4.77% and 5.41% for a 2, 3, 4 or 5 stage MF process respectively. For a 5 stage process increasing the protein concentration of the feed from 3.2 to 5.4% decreased the required MF membrane area by 10%. It was also found that as the number of stages increased from 2 to 5 the required MF membrane area decreased by 39%, when the MF feed was DUR at the optimal feed protein concentration. Finally, it was found that increasing the flux from 50 to 60 kg/m^2 per h decreased the required MF membrane area by 17% when the MF feed was DUR at the optimal MF feed protein concentration. Overall, using water diluted UF retentate of SM could reduce the amount of ceramic MF membrane area required to make a 95% SP reduced MCC.

ACKNOWLEDGMENTS

The authors thank the Northeast Dairy Foods Research Center and New York State Milk Production Board (Albany, NY) for partial funding of this research.

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CHAPTER 3

Microfiltration: impact of retentate protein concentration on limiting flux and serum protein removal with 4 mm channel ceramic microfiltration membranes.

ABSTRACT

The objective of our study was to determine if the limiting flux and serum protein (**SP**) removal were different at 8, 9 or 10% true protein (**TP**) in the microfiltration (**MF**) retentate recirculation loop using 0.1µm ceramic graded permeability membranes with 4 mm channel diameters operated at 50°C, using a diluted milk protein concentrate with 85% protein on a total solids basis (**MPC85**) as the MF feed. The limiting flux for the MF of diluted MPC85 was determined at 3 TP concentrations in the recirculation loop (8, 9, and 10%). The experiment was replicated 3 times for a total of 9 runs. On the morning of each run MPC85 was diluted with reverse osmosis (**RO**) water to a MF feed TP concentration of 5.4%. In all runs the starting flux was 55 kg/m^2 per h, the flux was increased in steps until the limiting flux was reached. The minimum flux increase was 10 kg/m^2 per h. The limiting flux decreased as TP concentration in the recirculation loop increased. The limiting flux was: 154 ± 0.3, 133 ± 0.7 and 117 ± 3.3 kg/m^2 per h at recirculation loop TP concentrations of 8.2 ± 0.07, 9.2 ± 0.04 and 10.2 ± 0.09% respectively. No impact of recirculation loop TP concentration on the SP removal factor was detected. However, the SP removal factor decreased from 0.80 ± 0.02 to 0.75 ± 0.02 as flux was increased from the starting flux of 55 kg/m^2 per h to the limiting flux, with a similar decrease seen at all recirculation loop TP concentrations.

INTRODUCTION

Micellar Casein Concentrate

Microfiltration (**MF**) can be used to separate micellar casein (**CN**) from serum protein (**SP**) and other serum phase components in skim milk (**SM**). The micellar CN is retained by the membrane, while SP passes through the membrane into the permeate. The retentate is a micellar CN concentrate (**MCC**), while the SP in the permeate can be further concentrated by ultrafiltration (**UF**) to produce SP concentrates. The SP concentrates produced from the MF of SM have been found to have lower fat and NPN concentration when compared to whey protein concentrates (at equivalent protein concentrations) and better foaming and gelling properties (Luck et al., 2013).

The percent reduction in SP and other serum phase compounds such as lactose and non protein nitrogen (**NPN**) in the MCC depends on the process (number of stages, concentration factor (**CF**) and diafiltration factor). The MCC could have a number of commercial uses, including in cheese making to increase yield (Papadatos et al., 2003). Additionally, MCC could be used in the formulation of high protein shelf stable beverages. MCC has a lower concentration of heat labile components SP and lactose and has excellent heat stability under retort conditions (Sauer and Moraru, 2012).

Microfiltration Feed

If the target reduction (on a percent basis) in lactose is greater than the target SP reduction for the MCC, SM can be UF prior to MF to remove the extra lactose and reduce the required MF membrane area (as detailed in Chapter 2). The UF retentate

will be a milk protein concentrate (**MPC**) which will have had a percentage of lactose, soluble minerals, and NPN removed and a true protein (**TP**) concentration greater than that of SM. MPC are often classified based on their TP concentration, for example an MPC85 would be an MPC with 85% TP on a total solids basis. The United States Trade Commission (2004) estimated a global MPC production of 100,000 MT in 2002. Liquid MPC directly from a UF system could be diluted with water to a target TP concentration for use as an MF feed. The characteristics of an MF feed material might have an impact on MF system performance. Jimenez-Lopez et al. (2008) found that the aqueous phase minerals of milk played a role in membrane fouling. Less MF fouling was seen with lower concentrations of serum phase minerals.

Gradient Permeability Microfiltration Membranes

There are a variety of MF membranes that can be used to separate micellar CN and SP. The research reported in the current paper will focus on the use of graded permeability (GP) ceramic membranes (manufactured by Pall Corporation, Cortland, NY). The membranes are available in configurations with 3 mm and 4 mm channel diameters. GP membranes are tubular ceramic membranes that attempt to achieve a constant flux along the length of the membrane by building in a resistance gradient along the outside support layer of the membrane (Garcera and Toujas, 2002). The resistance gradient was designed for operation at a constant longitudinal pressure drop (**ΔP**), and due to this constraint, the MF system was operated at a constant ΔP in the current study. When operating the system at a constant ΔP the cross-flow velocity will depend on the density and viscosity of the retentate in the recirculation loop (Denn, 1980).

Factors That Impact Limiting Flux.

The flux at which a MF process operates at will have an impact on the MF membrane area required to process a fixed mass of feed in a fixed time. Less membrane area will be required if the system operates at a higher flux (As detailed in Chapter 2). The limiting flux is the maximum flux that can be achieved by increasing the transmembrane pressure (**TMP**) (Bacchin et al., 2006). A sustainable flux is a flux that the system can maintain for extended periods of time, such as for an entire production day. Once limiting flux has been determined, sustainable flux can be estimated.

Research on the MF of SM has found that limiting flux increases with increasing temperature and cross-flow velocity while it decreases with recirculation loop TP concentration. Samuelsson et al. (1997) found that increasing both temperature and cross-flow velocity increased the limiting flux, with a limiting flux of 145 L/m^2 per h at a temperature of 55^oC and a cross-flow velocity of 8 m/s (for the MF of SM with a recirculation loop TP concentration around 3.6%). Samuelsson et al. (1997) also reported that the increase in limiting flux was proportional to the increase in cross-flow velocity. Gesan-Guiziou et al. (1999) also reported that the limiting flux increased as shear-stress (cross-flow velocity) increased for the MF of SM (recirculation loop TP concentration around 5.8%) with limiting fluxes in the 80 to 90 L/m^2 per h range (at 50^oC). Increasing the concentration of TP in the recirculation loop is expected to decrease the limiting flux (Cheryan, 1998). Though not explicitly determining limiting flux, Vadi and Rizvi (2001) continuously concentrated SM and

found that the flux decreased as the recirculation loop TP concentration increased (the flux dropped from 120 kg/m^2 per h at a **CF** of 1X to 30 kg/m^2 per h at 6X).

Limiting Flux and Serum Protein Passage Through the Membrane.

Zulewska et al. (2009) reported that for the MF of SM (with a TP concentration in the recirculation loop of 8.3%) using 0.1 µm GP (4 mm channel diameter) membranes that SP passage through the membrane to be close to expected given that the concentration of SP in the permeate was similar to the concentration of SP in the feed (both 0.56%), although SP passage was not corrected for any possible CN in the permeate. In the same experiment using a uniform transmembrane pressure (**UTP**) system with 0.1 µm ceramic membranes with 4 mm channel diameters with a recirculation loop TP concentration of 8.7%, Zulewska et al. (2009) found a similar SP removal to the GP system (64% for the UTP compared to 61% for the GP system for a 3X CF). In Gesan-Guiziou et al. (1999) TP passage through the membrane decreased as flux increased, they found that the transmission of BLG and ALA dropped to around 60% at the limiting flux compared to an initial transmission of 100%. No systematic studies of the impact of protein concentration in the MF recirculation loop on limiting flux and SP removal have been reported. The objective of our study was to determine if the limiting flux and SP removal were different at 8, 9 or 10% TP in the MF retentate recirculation loop using 0.1µm ceramic GP membranes with 4 mm channel diameters operated at 50^{o}C, using a diluted MPC85 as the MF feed.

MATERIALS AND METHODS

Experimental Design and Statistical Analysis

The limiting flux for the MF of diluted MPC85 was determined at 3 TP concentrations in the recirculation loop (8, 9, and 10%) on 0.1µm GP membranes with 4 mm channel diameters. The experiment was replicated 3 times for a total of 9 runs. On the morning of each run MPC85 was diluted with RO water to a MF feed TP concentration of 5.4%. In all runs the starting flux was 55 kg/m^2 per h, the flux was increased in steps until the limiting flux was reached. The minimum flux increase was 10 kg/m^2 per h. Due to production scheduling of MPC85 supplied by O-AT-KA milk cooperative (Batavia, NY), the same batch of MPC85 was used in multiple replicates for the 4 mm channel diameter membranes (2 batches of MPC85 were used for the 3 experimental replicates).

All data were analyzed by ANOVA using the Proc GLM (general linear model) procedure of SAS (SAS version 8.02, 1999-2001, SAS Institute Inc., Cary, NC). The data was analyzed at the starting and limiting flux using a GLM where the dependent variable = target TP + error. With target TP (a class variable with 3 levels: 8%, 9% or 10%) being the target TP concentration in the recirculation loop.

To determine the impact that flux and recirculation loop TP concentration had on membrane performance a split-plot model was used with target TP as the whole plot term and flux and flux by target TP interaction as the sub plot terms. Flux was a categorical variable (starting flux or limiting flux). The GLM was: dependent variable = Target TP + Replicate(Target TP) + Flux + Target TP*Flux + error. The type III mean squares for Replicate(Target TP) was used as the denominator in the F-test to

test the significance of the whole-plot term (Target TP). The type III mean square error for the model was used as the denominator in the F-test to test the significance of the sub plot terms. Target TP was nested in Replicate because there were only two different batches of MPC85 and that did not correspond to replicate. The impact of TMP on measured flux was determined using the following GLM: Measured flux = Target TP + Replicate (Target TP) + TMP + TMP*Target TP + TMP*Replicate(Target TP) + TMP*TMP + TMP*TMP*Target TP + TMP*TMP*Replicate(Target TP). Where TMP was a mean centered continuous variable.

Preparation of Microfiltration Feed

The MF feed was an MPC85 with 85.5 ± 0.3% TP on a total solids basis, provided by O-AT-KA Milk Products Cooperative, Inc. (Batavia, NY). Liquid MPC85 at approximately 12.34 ± 0.04% TP determined using an infrared spectrophotometer (**IR**) (Milkoscan, Foss, Hillerod, Denmark) was stored at < 4^{o}C until use. On each day MPC85 (325 ± 2 kg) was diluted to 5.4% TP as determined with by IR, with hot RO water (390 ± 4 kg) (70^{o}C) in a separate jacketed MF product feed tank. The diluted MPC85 was brought to a final temperature of 50^{o}C in the MF product feed tank.

Microfiltration System

A 0.1µm ceramic GP Membralox module with 4 mm channel diameters (7 sticks with 19 channels per stick and a membrane area of 1.7 m^2) (EP1940GL0.1µA, alumina, Pall Corp, Cortland, NY) was used in this work. A pilot MF system shown in Figure 3.1 was used (Model CF 1000, Pall Corp., Cortland, NY) consisting of a 7.5 HP feed pump (LKH 10, Alpha Laval, Lund Sweden) that determined the pressure at

the outlet end of the membrane on the retentate side (Pro) and a 20 HP recirculation pump (LKH25, Alpha Laval, Lund Sweden) that determined the ΔP (Pro-Pri). The location of the 3 pressure measurements are shown in Figure 3.1**,** the pressures were determined using pressure transmitters (Cerabar M-PMP, Endress+Hauser, Greenwood, IN). Electromagnetic flow meters were used to measure the recirculation rate (Promag 53, Endress+Hauser, Greenwood, IN) and permeate and retentate removal rates (Promag H, Endress+Hauser, Greenwood, IN). The permeate and retentate removal rates were controlled by air actuated diaphragm valves (Gemu Type 650, Atlanta, GA). The MF rig also contained an onboard feed tank (350L). The chilled water flow rate, feeding a shell and tube heat exchanger (Enerquip, LLC. Medford, WI) on the recirculation loop was used to control the temperature of the system.

The flux, CF, ΔP and outlet pressure on the retentate side (Pro) were controlled during the run by an onboard computer (1500P, Allen-Bradley, Milwaukee, WI). The flux was controlled by opening and closing the permeate removal valve. The CF was

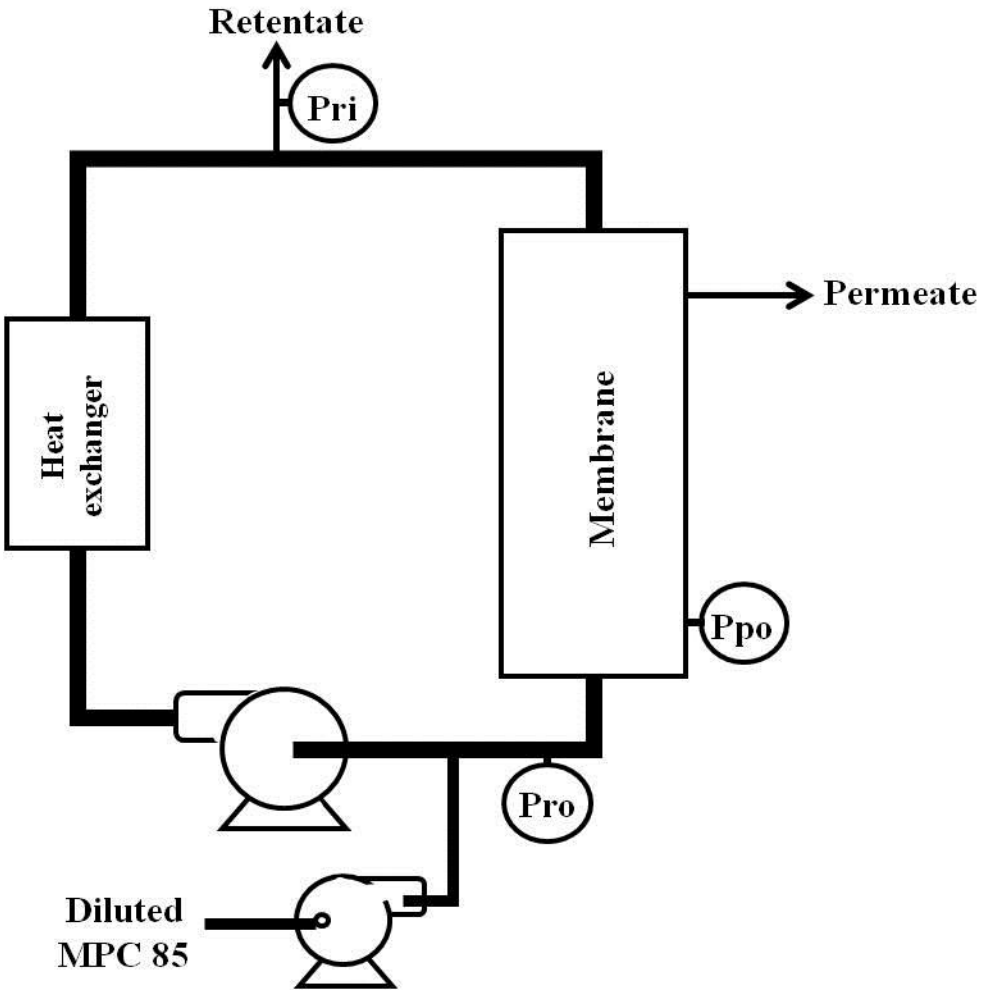

Figure 3.1. Diagram of the pilot microfiltration unit showing the location of the pressure measurements. Pressure on the inlet end of the membrane on the retentate side (Pri), pressure on the outlet end of the membrane on the retentate side (Pro) and pressure on the outlet end of the membrane on the permeate side (Ppo)

controlled by opening and closing the retentate removal valve. The ΔP was controlled by increasing or decreasing the recirculation pump speed, and the retentate outlet pressure (Pro) was controlled by modifying the feed pump speed. TMP was calculated as: The average of the pressure at the inlet and outlet end of the membrane on the

retentate side minus the permeate pressure at the outlet end of the membrane [(Pro+Pri)/2 – Ppo].

Microfiltration Cleaning

Immediately after each run the MF system was cleaned. The system was rinsed with approximately 350 L of water (30°C). The retentate removal rate was approximately 600 L/h and the permeate removal rate was approximately 180 L/h during the rinse. After the system was rinsed, the fouled water flux was measured as follows: the recirculation pump was turned off and the retentate valve was completely shut, the permeate valve was then opened to 95% and the permeate removal rate recorded as well as the pressures at the retentate inlet and outlet (Pri, Pro) and the permeate pressure (Ppo). After the fouled water flux was measured the recirculation pump was turned on and the retentate valve opened and the permeate valve set to a removal rate of approximately 180 L/h. 12 L of an alkaline membrane cleaner, Ultrasil 25 (Ecolab Inc., Food and Beverage Division, St Paul, MN) was added to the clean in place (CIP) feed tank filled with 320 L of water (3.75% vol/vol). The alkaline cleaner was recirculated at 50°C for 30 min. In previous research the caustic clean took place at 80°C, while in this work, a mechanical issue meant that we were not able to produce 80°C hot water for the caustic clean and the cleaning step took place at 50°C. After 30 min the system was rinsed with approximately 350 L of water **(**50°C). The clean water flux was measured (50°C) after the rinse using the same method used to determine the fouled water flux. After the clean water flux, the recirculation pump was turned on and the retentate valve opened and 12 L of acid membrane cleaner, Ultrasil 76 (Ecolab Inc., Food and Beverage Division, St Paul, MN) was added to the CIP tank filled with

320 L of water (3.75% vol/vol). The acid was recirculated at 50°C for 10 min. The MF system was stored in this acid solution until the next run.

Microfiltration Operation

On the day of the processing run the MF membranes-which were stored in acid overnight, were rinsed with approximately 400 L of water at approximately 50°C. The CIP tank was then filled with water and the system allowed to heat up to 50°C by recirculating the retentate and permeate back into the CIP tank. When the system was at 50°C the clean water flux was measured using the method described above. The average clean water flux for the 4 mm membranes was 363 ± 1 kg/m^2 per h. After the clean water flux was measured, the feed pump was set so that the pressure at the outlet end of the membrane on the retentate side (Pro) was 230 kPa. The recirculation rate was then increased by increasing the recirculation pump speed until the ΔP was 220 kPa. The permeate removal rate was set to achieve a flux of 55 kg/m^2 per h and the retentate removal rate was set to achieve the target CF (approximately 1.6X, 1.8X and 2.0X for target recirculation loop TP concentrations of 8%, 9% and 10% respectively).

Start-up. After the system was heated to 50°C and the flux and CF were set, the valve from the CIP tank (containing water) was closed while simultaneously the valve to the MF product feed tank (with the diluted MPC85) was opened. In order to flush the system, approximately 302 ± 12 kg of permeate and retentate were collected, weighed and discarded. Once the concentration of TP in the retentate was within 10% of the target TP concentration as determined by IR analysis of the retentate, the retentate and permeate were recycled to the MF product feed tank.

Determination of the Limiting Flux. After the retentate and permeate were recycled to the MF product feed tank, the system was operated at a flux of 55 kg/m^2 per h for 1h. Permeate and retentate removal rates were measured by weight every 15 min. Pressures, temperature and the recirculation rate were also recorded every 15 min. After 1 h at 55 kg/m^2 per h the flux was increased. The flux steps are shown in Table 3.1 and depended on the retentate TP concentration. The flux was increased in steps with a 1 h stabilization period after each increase. At some point the target flux could not be achieved and further increasing the TMP did not lead to an increase in flux. The limiting flux was taken as the last flux that the system was able to operate at in a stable manner (constant TMP) for 1 h. The goal was to have each run last approximately 5 h and to have the target fluxes spaced closer together as the limiting flux was approached.

Table 3.1. Target fluxes for the 8, 9 and 10% retentate true protein (TP) concentrations on the 4 mm channel diameter ceramic graded permeability membranes when using diluted 85% milk protein concentrate as a feed.

Target TP concentration	Flux (kg/m^2 per h)					
8%	55	85	115	145	155	165
9%	55	85	115	125	135	145
10%	55	85	95	105	115	125

Samples of retentate and permeate were taken after the 1 h stabilization period at each flux and frozen for later chemical analysis. The TP concentration of the retentate and permeates was monitored during the run by IR analysis every 15 min. Based on the results from the IR, the CF was adjusted to maintain the target TP concentration.

Chemical Analyses

A sample of the MF feed (diluted MPC85) was taken before each run and samples of the permeate and retentate were taken after 1h at each flux. The samples were frozen (-40^{o}C) until analysis. The MF feed (diluted MPC85) was analyzed for TS, fat and lactose, using forced air oven drying (AOAC, 2000; method 990.20; 33.2.44), ether extraction (AOAC 2000; method 989.05; 33.2.26) and enzymatic lactose (AOAC 2000; method 984.15; 33.2.67, Lynch et al. 2007) respectively. The diluted MPC85, retentates and permeates were analyzed for total nitrogen (**TN**), and NPN by Kjeldahl (AOAC, 2000; method 991.20; 33.2.11), and (AOAC, 2000; method 991.21; 33.2.12), respectively. Noncasein nitrogen (**NCN**) content of the diluted MPC85 was determined using Kjeldahl (AOAC, 2000; method 998.05; 33.2.64), modified by using 5.5 mL of acetic acid (10% vol/vol) and 5.5 mL of sodium acetate (1N) instead of 1 mL of each, to ensure that a final filtrate pH of less than 4.6 was achieved and that all of the CN was precipitated. TP 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.

Viscosity of the retentates was measured at each flux step. The viscosity was measured at 50°C using a Brookfield viscometer (Model: DV2TLVTJO) with a UL adapter for low viscosity fluids (Brookfield Engineering Laboratories, Inc., Middleboro, MA). A water bath was heated to 50°C and this water was recirculated through the jacketed sample cup using a peristaltic pump. The retentate samples were heated in a water bath at 50°C, then approximately 16 mL was placed in the sample cup. The viscosity was measured at 60 rpm (shear rate of 73 1/s) for 50 s. The recorded viscosity was the average viscosity measured in Pa*s during the last 30 s of the measurement.

Calculation of the Serum Protein Removal Factor

The SP removal factor was the ratio of SP removed in the permeate to the theoretical SP removal. A SP removal factor of 1 would mean that the membrane was not rejecting any SP. Theoretical SP removal (in kg) was equal to the mass of permeate multiplied by the concentration of SP in the permeate portion of the MF feed. The concentration of SP in the permeate portion of the MF feed was the mass of SP in the feed divided by the mass of feed less the mass of CN in the feed. The actual SP removed in the permeate (in kg) was the mass of permeate multiplied by the concentration of TP in the permeate. It was assumed that all of the TP in the permeate was SP. Because mass of permeate appears in both the numerator and denominator the SP removal factor was independent of permeate mass.

RESULTS

Microfiltration Feed Composition

The average composition of the diluted MPC85 used as the MF feed is shown in Table 3.2 for each target TP concentration. Because the MPC85 was produced using ultrafiltration with diafiltration to remove a large portion of the lactose and NPN the concentration of these components in the diluted MPC85 was lower than in SM (typical SM anhydrous lactose concentration: 4.75% and NPN x 6.38: 0.18%).

MPC85 (12.35% TP) was diluted with RO water to produce a MF feed with 5.4% TP as determined using IR analysis calibrated for testing milk in the range of 2 to 4.5% TP. As shown in Table 3.2, the TP concentration measured by Kjeldahl was higher than 5.4%, but no difference among the 3 different target TP concentrations was detected ($P > 0.05$). CN as a percentage of TP is shown in Table 3.2**.** There was a trend ($P = 0.06$) towards a higher CN as a percentage of TP in the diluted MPC85 compared to samples of pasteurized milk taken from the same production facility, which had an average (n=3) CN as a percentage of TP of 82.7 ± 0.4%. It is possible that the slightly higher CN as a percentage of TP in the MF feed was due to some SP being lost during the UF process. Barbano et al. (1988) reported that UF of whole milk produced permeate with a TP concentration in the permeate of 0.25 g/L (most of which was ALA).

Table 3.2. Mean (n=3) composition of the diluted 85% milk protein concentrates used as the microfiltration feed for the 3 target recirculation loop true protein (**TP**) concentrations. All values are given in percent by weight.

Target TP concentration	Microfiltration feed composition									
	TS[1]	Lactose[1]	Fat	TN[1]	NPN[1]	NCN[1]	TP[1]	SP[1]	CN[1]	CN%TP[1]
8%	6.48	0.24	0.08	5.56	0.03	0.92	5.53	0.89	4.65	83.96
9%	6.49	0.23	0.08	5.63	0.04	0.89	5.59	0.86	4.74	84.71
10%	6.43	0.26	0.08	5.50	0.03	0.94	5.47	0.90	4.57	83.49
SE	0.04	0.02	0.002	0.06	0.009	0.02	0.06	0.03	0.07	0.57
R squared	0.15	0.23	0.05	0.25	0.03	0.27	0.26	0.21	0.31	0.28

Values in columns were not different ($P > 0.05$).

[1] TS = total solids, anhydrous lactose, TN = total nitrogen x 6.38, NPN = nonprotein nitrogen x 6.38, NCN = noncasein nitrogen x 6.38, TP = true protein, (TN minus NPN), SP = serum protein, (NCN minus NPN), CN = casein, (TN minus NCN), CN%TP = 100 times CN divided by TP.

Retentate Composition

The average retentate TN, NPN and TP concentrations are shown in Table 3.3, at both the starting and limiting flux for the 3 target recirculation loop TP concentrations. To determine limiting flux as a function of recirculation loop TP concentration it was important to control the TP concentration, both as the flux was increased during a run and between replicates. The TP concentration was well controlled at the 3 recirculation loop TP levels with no detectable impact of flux on TP concentration in the recirculation loop ($P > 0.05$) (Table 3.4**)**. The NPN concentration in the retentate increased slightly (Tables 3.3 and 3.4) from the beginning to the end of the run from 0.05 ± 0.003% to 0.06 ± 0.005%, but no impact of retentate TP concentration on this increase was detected and the magnitude of increase was small.

Permeate Composition

The TN, NPN and TP content of the MF permeates at the starting and limiting fluxes at each target recirculation loop TP concentration are shown in Table 3.3. No difference in TN, NPN and TP among target recirculation loop TP concentrations was detected ($P > 0.05$). As shown in Tables 4.3 and 4.4 the TN, and TP concentration in the permeate decreased ($P < 0.05$) as flux increased and NPN increased (Table 3.4), but these changes did not depend on target recirculation loop TP concentration.

Table 3.3. Mean (n=3) composition of the microfiltration retentates and permeates produced at the 3 target recirculation loop true protein (TP) concentrations: 8, 9, and 10% at the starting and limiting flux using diluted 85% milk protein concentrate as a feed material for a 4 mm ceramic membrane.

	Target TP	Starting flux			Limiting flux		
	concentration	TN[1]	NPN[1]	TP[1]	TN[1]	NPN[1]	TP[1]
Retentate	8%	8.25[c]	0.042	8.22[c]	8.25[c]	0.059	8.19[c]
	9%	9.24[b]	0.047	9.19[b]	9.23[b]	0.069	9.16[b]
	10%	10.12[a]	0.048	10.07[a]	10.23[a]	0.067	10.16[a]
	SE	0.10	0.007	0.09	0.07	0.009	0.07
	R squared	0.97	0.08	0.97	0.98	0.11	0.99
Permeate	8%	0.79	0.037	0.75	0.75	0.048	0.70
	9%	0.75	0.036	0.72	0.70	0.041	0.66
	10%	0.79	0.036	0.75	0.76	0.041	0.72
	SE	0.03	0.005	0.03	0.03	0.004	0.03
	R squared	0.16	0.009	0.12	0.32	0.25	0.26

[a - c]Means in the same column not sharing a common superscript differ ($P < 0.05$).
[1]TN = total nitrogen x 6.38, NPN = nonprotein nitrogen x 6.38, TP = true protein, (TN minus NPN).

Table 3.4. ANOVA df and type III sum of squares microfiltration retentate and permeate composition produced at the 3 target recirculation loop true protein (TP) concentrations: 8, 9, and 10% at the starting and limiting flux.

		Retentate			Permeate		
Model term	df	TN[1]	NPN[1]	TP[1]	TN[1]	NPN[1]	TP[1]
Whole model		11.29*	0.004*	11.18*	0.042*	0.001*	0.052*
Target TP	2	11.07*	0.0002	10.98*	0.008	0.00007	0.008
Rep (target TP)[2]	6	0.20	0.0019*	0.18	0.025*	0.00068*	0.033*
Sub plot							
Flux	1	0.005	0.0016*	0.0008	0.0078*	0.00021*	0.011*
Target TP *flux	2	0.013	0.00002	0.014	0.0006	0.00003	0.0007
R squared		>0.99	0.94	>0.99	0.97	0.93	0.97

[1] TN = total nitrogen, NPN = non protein nitrogen, TP = true protein [TN minus NPN]
[2] Used as error term to test significance of whole plot term (target TP)
P < 0.05

Microfiltration Process Parameters

Cross-Flow Velocity and Longitudinal Pressure Drop. The MF system recirculation rate was controlled to maintain a constant ΔP. As shown in Tables 3.5

and 3.6**,** the ΔP averaged 220 kPa and no difference between target recirculation loop TP concentrations was detected ($P > 0.05$). A consequence of operating the MF at a constant ΔP was that the cross-flow velocity decreased as recirculation loop TP concentration increased (Table 3.5). This is consistent with fluid mechanics, which predicts that for turbulent flow as viscosity and density increase the cross-flow velocity will decrease when ΔP is kept constant (Denn, 1980).

Transmembrane Pressure and Flux. No difference in TMP was detected among the 3 target recirculation loop TP concentrations at the starting or limiting flux ($P > 0.05$) as shown in Table 3.5. As flux increased the TMP increased ($P < 0.05$) (Figure 3.2 and Table 3.7**)** for the 3 target recirculation loop TP concentrations. The increase in flux with increasing TMP was dependent on the recirculation loop TP concentration (i.e., significant TMP*target TP interaction) (Figure 3.2 and Table 3.7) and the flux versus TMP curves increasingly diverged for the different target TP as TMP increased (Figure 3.2).

Limiting Flux. Although no difference in TMP among the target recirculation loop TP concentrations was detected at the limiting flux (Table 3.5), the limiting flux increased ($P < 0.05$) as recirculation loop TP concentration decreased as shown in Table 3.5 and Figure 3.2**.** The significant flux by TP interaction for measured flux (Table 3.7) is a reflection of the same starting flux for all 3 target TP concentrations, but a different limiting flux.

Table 3.5. Mean (n=3) longitudinal pressure drop (ΔP), cross-flow velocity and transmembrane pressure (TMP) at the starting flux of 55 kg/m^2 per h and limiting flux at 8, 9, and 10% target true protein (TP) concentration in the recirculation loop when using diluted 85% milk protein concentrate as a feed material for a 4 mm ceramic microfiltration membrane.

Target TP concentration	Starting flux				Limiting flux			
	Measured flux (kg/m^2 per h)	ΔP (kPa)	Cross-flow velocity (m/s)	TMP (kPa)	Measured flux (kg/m^2 per h)	ΔP (kPa)	Cross-flow velocity (m/s)	TMP (kPa)
8%	55.76	220	7.10[a]	64.92	154[a]	220	7.08[a]	194.57
9%	54.58	220	7.03[b]	66.24	133[b]	220	6.98[b]	192.20
10%	53.78	221	6.92[c]	68.32	117[c]	220	6.88[c]	183.36
SE	1.45	0.14	0.01	1.28	1.94	0.25	0.007	11.88
R squared	0.14	0.34	0.95	0.38	0.97	0.32	0.98	0.08

[a - c] Means within the same column not sharing a common superscript are different ($P < 0.05$).

Table 3.6. ANOVA df and type III sum of squares with measured flux, longitudinal pressure drop (ΔP), cross-flow velocity and serum protein removal factor as the dependent variables at the 3 target recirculation loop true protein (TP) concentrations: 8, 9, and 10% at the starting and limiting flux.

Model term	df	Measured flux (kg/m^2 per h)	ΔP (kPa)	Cross-flow velocity (m/s)	Serum protein removal factor[2]
Whole model		30,967*	N.S.	0.112*	531.72*
Target TP	2	1,174*	N.S.	0.104*	7.25
Rep (target TP)[1]	6	73.49	N.S.	0.002	392.42*
Sub plot					
Flux	1	28,769*	N.S.	0.005*	122.83*
Target TP *flux	2	950*	N.S.	0.0007	9.21
R squared		>0.99	--	0.99	0.97

[1] Used as error term to test significance of whole plot term (Target TP)

[2]Serum protein removal factor = true protein in the permeate divided by serum protein concentration in the permeate portion of the microfiltration feed.

*$P < 0.05$

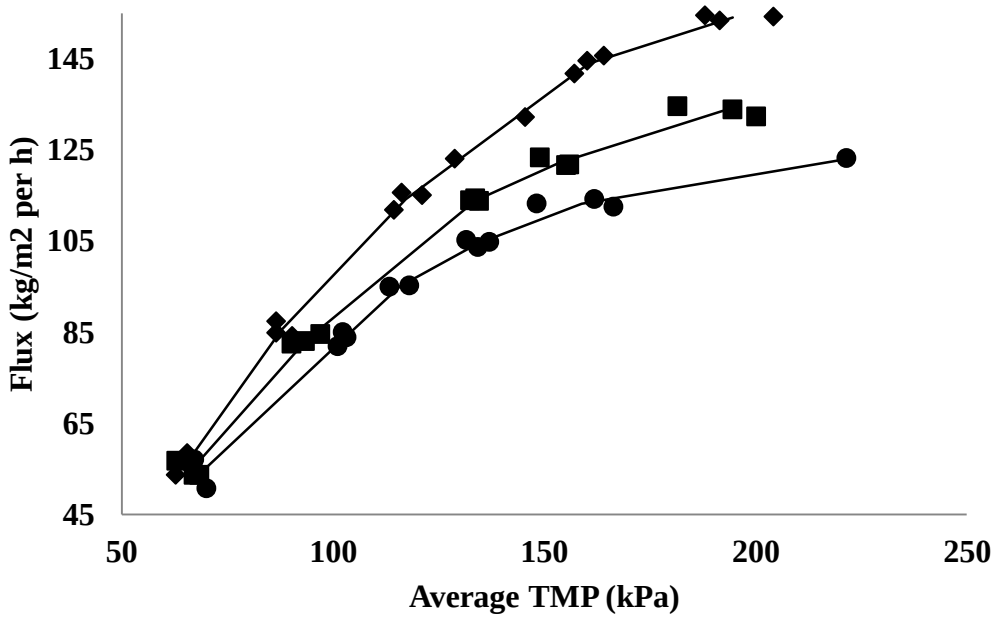

Figure 3.2. Plot of flux versus average transmembrane pressure (TMP) for target retentate protein concentrations of 8% (◆), 9% (■) and 10% (●) for 4 mm ceramic graded permeability membranes using a diluted 85% milk protein concentrate feed . Data from all 3 replicates shown.

Table 3.7. ANOVA df and type III sum of squares for measured flux as the dependent variable at the 3 target recirculation loop true protein (TP) concentrations: 8, 9, and 10%. With mean centered transmembrane pressure (TMP) and target TP as independent variables.

Model term	df	Measured flux
Whole model		43,829*
Target TP	2	1,608*
Rep (target TP)[1]	6	29.78*
Sub plot		
TMP	1	28,205*
TMP* Target TP	2	587*
TMP*Replicate(target TP)	6	95.11*
TMP*TMP	1	1,732*
TMP*TMP*target TP	2	4.74
TMP*TMP*Replicate (target TP)	6	22.46
R squared		>0.99

[1] Used as error term to test significance of whole plot term (target TP)
*$P < 0.05$

The relationship between TMP and flux was explained by Darcy's law shown in Equation 3.1**,** where viscosity used was permeate viscosity**.** The resistance in Equation 3.1 is the total resistance and includes the membrane resistance, resistance caused by membrane fouling and concentration polarization. Initially the increase in flux with TMP was close to linear, which indicated that there was not much of a change in total resistance (Figure 3.2). However, the change in flux with changing TMP depended on the recirculation loop TP concentration (Table 3.7). This may indicate different levels of concentration polarization depending on the TP concentration in the recirculation loop (or the cross-flow velocity which was a function of TP concentration in our work).

As shown in Figure 3.2, eventually larger increases in TMP were required to continue increasing the flux, according to Equation 3.1 this indicates an increase in membrane resistance-likely due to membrane fouling. Eventually increasing the TMP no longer increased the flux indicating that the limiting flux had been reached (Figure 3.2).

(3.1) $$\text{flux} = \frac{\text{TMP}}{\text{viscosity} \times \text{resistance}}$$

Serum Protein Removal

The SP removal factors are shown in Table 3.8 at the starting and limiting fluxes for the 3 target recirculation loop TP concentrations. No difference in SP removal factor due to recirculation loop TP concentration was detected ($P > 0.05$) at the starting or limiting flux. The SP removal factor decreased ($P < 0.05$) as the flux was increased to the limiting flux (Tables 3.8 and 3.6). However, no difference in the

decrease in SP removal at the 3 different target TP concentrations was detected ($P > 0.05$). As discussed above, as the flux approached the limiting flux, there was evidence of membrane fouling (increasing resistance) which likely changed the rejection characteristics of the membrane and reduced SP removal. This is consistent with the work of Gesan-Guiziou et al. (1999) where SP removal also decreased as flux was increased for the MF of SM.

Previous research using 4 mm channel diameter GP membranes reported a SP removal factor close to 1 (Zulewska et al., 2009) while in our work a SP removal factor of 0.8 was found at the starting flux. This could be due to the different cleaning protocol in the current study. In the previous work the caustic clean took place at 80°C as opposed to 50°C in the current study and this may have impacted post cleaning performance. Additional differences between the studies that might have caused the difference are the use of a diluted MPC85 as the MF feed, or the higher concentration of SP in the permeate compared to SM.

Table 3.8. Mean (n=3) serum protein (SP) removal factor at a flux of 55 kg/m^2 per h and the limiting flux for at the target true protein (TP) recirculation loop concentrations of 8, 9 and 10% when using diluted 85% milk protein concentrate as a feed material for 4 mm ceramic MF membranes.

Target TP concentration	SP removal factor[1] at a flux of 55 kg/m^2 per h	SP removal factor[1] at the limiting flux
8%	0.81	0.75
9%	0.80	0.73
10%	0.80	0.77
SE	0.030	0.037
R squared	0.01	0.06

Means within columns did not differ ($P > 0.05$).
[1]SP removal factor = true protein in the permeate divided by SP concentration in the permeate portion of the microfiltration feed.

DISCUSSION

Predicting the Limiting Flux

In order to predict the limiting flux a number of theoretical equations have been developed (Belfort et al., 1994), however Samuelsson et al. (1997) found that none of them were able to predict the limiting flux for the MF of SM. Samuelsson et al. (1997) developed an empirical model for the limiting flux for the MF of SM using ceramic membranes shown in Equation 3.2, where density times cross-flow velocity times channel diameter divided by retentate viscosity is the dimensionless Reynold's number.

(3.2) $$\text{Limiting flux} = 0.0025 \ \frac{L}{m^2 h} x \frac{density \text{ x cross-flow velocity x channel diameter}}{\text{Retentate viscosity}}$$

Equation 3.2 was used to calculate the predicted flux shown in Table 3.9. The retentate viscosities were measured in this work, while the densities were estimated using an equation developed by Stepp and Smith (1991). The calculated Reynold's numbers at each recirculation loop TP concentration are also provided in Table 4.9.

Equation 3.2, underestimated the limiting flux found in our research (Table 3.9). There could be a number of reasons Equation 3.2 underestimated the limiting flux in our experiment, including that the MF feed in this work was diluted MPC85 as opposed to SM and the use of a different membrane system (channel diameter, length etc.). The impact of the MF feed (SM or MPC85) on limiting flux warrants further investigation. In preliminary work using SM as the MF feed a limiting flux of approximately 90 kg/m^2 per h (at a TP concentration of 8.6%) was found, which is

much lower than the limiting flux found when diluted MPC85 was the MF feed (Figure 3.2).

A plot of Reynold's number versus the limiting flux appeared linear and the slope that best fit the data points was determined using Excel 2007 (Microsoft, Redmond, WA). A better correlation between the limiting flux determined in our research and Reynold's number was found when the constant in Equation 3.2 was replaced with 0.00764 kg/m^2 per h (R squared = 0.94) (Table 3.9). However, the predictive utility of this relationship needs to be validated. In our research cross-flow velocity, viscosity and density were all a function of the recirculation loop TP concentration and were not varied independently and only one channel diameter was used.

Table 3.9. Prediction of limiting flux.

Target TP concentration	Viscosity[1] (Pa*s)	Density[2] (kg/m^3)	Reynold's number[3]	Limiting flux (kg/m^2 per h)	Samuelsson[4] Predicted limiting flux (kg/m^2 per h)	Predicted limiting flux[5] (kg/m^2 per h)
8%	0.00147[c]	1,036[c]	19,922[a]	154[a]	50	152
9%	0.00163[b]	1,039[b]	17,824[b]	133[b]	45	136
10%	0.00190[a]	1,043[a]	15,116[c]	117[c]	38	116
SE	0.00002	0.25	233	1.94	--	--
R squared	0.97	0.98	0.97	0.97	--	--

[1]Viscosity measured at 50^{o}C

[2]Density calculated using equation of Stepp and Smith (1991): (Density = 1,034.72 - 0.39*temperature (^{o}C) + 1.69*TN + 0.10*TN^2). TN = total nitrogen.

[3]Reynold's number calculated as: density*cross-flow velocity*channel diameter/ viscosity.

[4]Predicted using the equation developed by Samuelsson et al. (1997) where: flux = 0.0025 L/m^2 per h*Reynold's number

[5]Predicted using equation: flux = 0.00764 kg/m^2 per h *Reynold's number, which was the slope that provided the best fit between limiting flux and Reynold's number.

Sustainable Flux

The limiting flux provides an upper bound on flux. A sustainable flux would be a flux that could be maintained for a long period of time, such as a production run. A plot of scaled flux (measured flux divided by the limiting flux at each recirculation loop TP concentration) versus TMP is shown in Figure 3.3. Regardless of the target recirculation loop TP there appears to be a similar relationship between scaled flux and TMP. Figure 3.3 implies that the limiting flux could be estimated for a TP concentration by operating the system at a specific TMP (which corresponds to a percentage of the limiting flux) and measuring the flux. The data in Figure 3.3 also suggests that a sustainable flux may be achieved by operating at a specific TMP which corresponds to a percentage of the limiting flux. This is consistent with a report by Bacchin (2004) that the critical flux (below which no membrane fouling occurs) was 2/3 of the limiting flux. We found (data not reported) in 2 runs using the 4 mm channel diameter GP membranes, with a recirculation loop TP concentration of 10%, that a flux of 100 kg/m^2 per h (85% of the limiting flux) could be maintained for over 10 h with an average TMP of approximately 130 kPa.

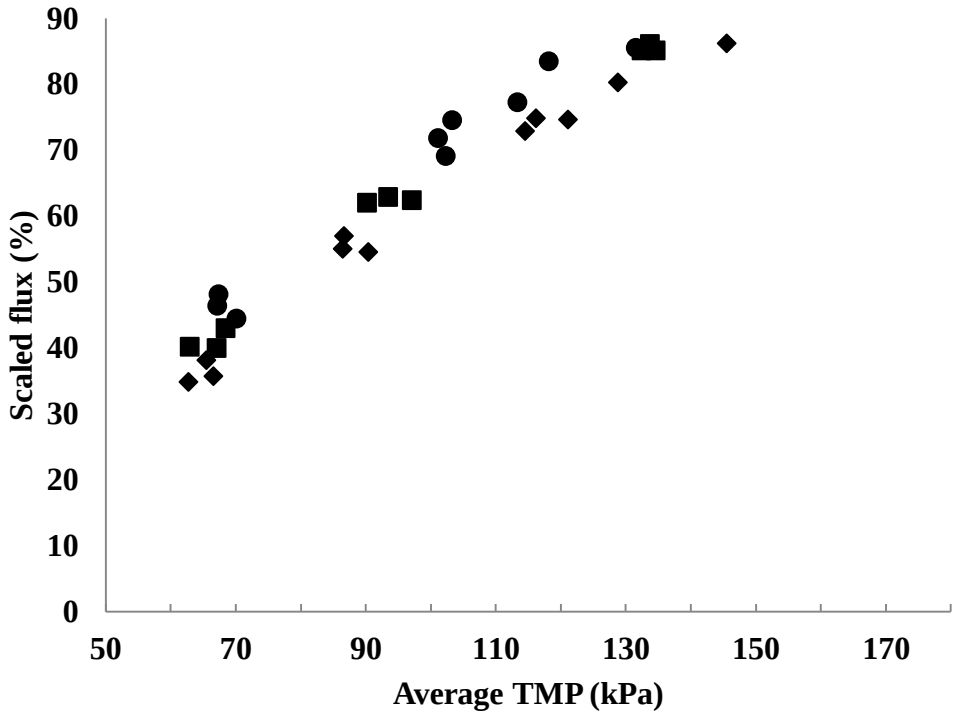

Figure 3.3. Plot of scaled flux versus average transmembrane pressure (TMP) for target retentate protein concentrations of 8% (◆), 9% (■) and 10% (●) for 4 mm ceramic graded permeability membranes using a diluted 85% milk protein concentrate feed . Data from all 3 replicates shown.

CONCLUSIONS

The limiting flux decreased as TP concentration in the recirculation loop increased. The limiting flux was: 154 ± 0.3, 133 ± 0.7 and 117 ± 3.3 kg/m^2 per h at recirculation loop TP concentrations of 8.2 ± 0.07, 9.2 ± 0.04 and 10.2 ± 0.09% respectively. No impact of recirculation loop TP concentration on the SP removal factor was detected ($P > 0.05$). However, the SP removal factor decreased from 0.80 ± 0.02 to 0.75 ± 0.02 as flux was increased from the starting flux of 55 kg/m^2 per h to the limiting flux, with a similar decrease seen at all recirculation loop TP concentrations.

ACKNOWLEDGMENTS

The authors thank the Northeast Dairy Foods Research Center (Ithaca, NY) and New York State Milk Production Board (Albany, NY) for partial funding of this research. The authors would also like to thank O-AT-KA Milk Products Cooperative (Batavia, NY) and Pall Corporation (Cortland, NY) for their support of this research. Additionally, the technical assistance of Michelle Bilotta, Sara Bova and Chassidy Coon from the Department of Food Science at Cornell University was greatly appreciated.

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CHAPTER 4

Microfiltration: Impact of channel diameter on limiting flux and serum protein removal

ABSTRACT

Our objective was to determine the limiting flux and serum protein (SP) removal at 8, 9 and 10% true protein (TP) in the retentate recirculation loop using 0.1 µm ceramic graded permeability (GP) microfiltration (MF) membranes with 3 mm channel diameters (CD). An additional objective was to compare the limiting flux and SP removal between 0.1 µm ceramic GP membranes with 3 mm CD and previous research using 4 mm CD membranes. The MF system was operated at 50°C, using a diluted milk protein concentrate with 85% protein on a total solids basis (MPC85) as the MF feed. The limiting flux for the MF of diluted MPC85 was determined at 8, 9, and 10% TP concentration in the recirculation loop. The experiment using the 3 mm CD membranes was replicated 3 times for a total of 9 runs. On the morning of each run MPC85 was diluted with reverse osmosis water to a MF feed TP concentration of 5.4%. In all runs the starting flux was 55 kg/m^2 per h, the flux was then increased in steps until the limiting flux was reached. For the 3 mm CD membranes the limiting flux was: 128 ± 0.3, 109 ± 4 and 97 ± 0.5 kg/m^2 per h at recirculation loop TP concentrations of 8.1 ± 0.07, 9.2 ± 0.04 and 10.2 ± 0.03% respectively. For the 3 mm CD membranes increasing the flux from the starting to the limiting flux decreased the SP removal factor from 0.72 ± 0.02 to 0.67 ± 0.01, however no difference in SP removal factor among the target recirculation loop TP concentrations was detected.

The limiting flux at each recirculation loop target TP concentration was lower for the 3 mm CD membranes compared to the 4 mm CD membranes. The differences in limiting fluxes between the 3 mm and 4 mm CD membranes were explained in part by the difference in cross-flow velocity (5.5 ± 0.03 m/s and 7.0 ± 0.03 m/s for the 3 mm and 4 mm CD membranes respectively). The SP removal factor was also lower for the 3 mm CD membranes compared to the 4 mm CD membranes, indicating that more membrane fouling may have occurred in the 3 mm versus 4 mm CD membranes.

INTRODUCTION

Microfiltration (**MF**) has been used to remove serum protein (**SP**) and other low molecular weight components (i.e. lactose and non protein nitrogen (**NPN**)) from skim milk (Fauquant et al., 1988; Zulewska et al., 2009) or a milk protein concentrate (**MPC**) (Chapter 3). As a feed material for MF, MPC will have had a large amount of lactose, soluble minerals and NPN removed prior to MF compared to skim milk. The use of a diluted MPC as an MF feed would produce a micellar casein concentrate (**MCC**) with a lower lactose and NPN concentration compared with the use of skim milk as the MF feed. An MCC would have a low concentration of heat labile components such as SP and lactose and may be suitable for the formulation of high protein shelf-stable beverages. Both the membranes used for MF and the operating conditions (including flux) could impact the MCC composition and the MF membrane area required to produce the MCC.

In MF there are 3 important fluxes: critical, limiting, and sustainable flux. The critical flux is the flux at which membrane fouling begins to occur (Bacchin et al., 2006). Below the critical flux there is a linear relationship between flux and increasing transmembrane pressure (**TMP**) and as flux exceeds the critical flux the membrane starts to foul and the relationship between flux and TMP is no longer linear. The limiting flux is the highest flux that can be achieved by increasing the TMP (Bacchin et al., 2006). The critical and limiting fluxes are shown in Figure 4.1. The third important flux is the sustainable flux. A sustainable flux is a flux that the system can operate at for extended periods of time, such as a production run (Bacchin et al.,

2006). The sustainable flux would fall somewhere between the critical and limiting fluxes, where the rate of membrane fouling is low (Bacchin et al., 2006).

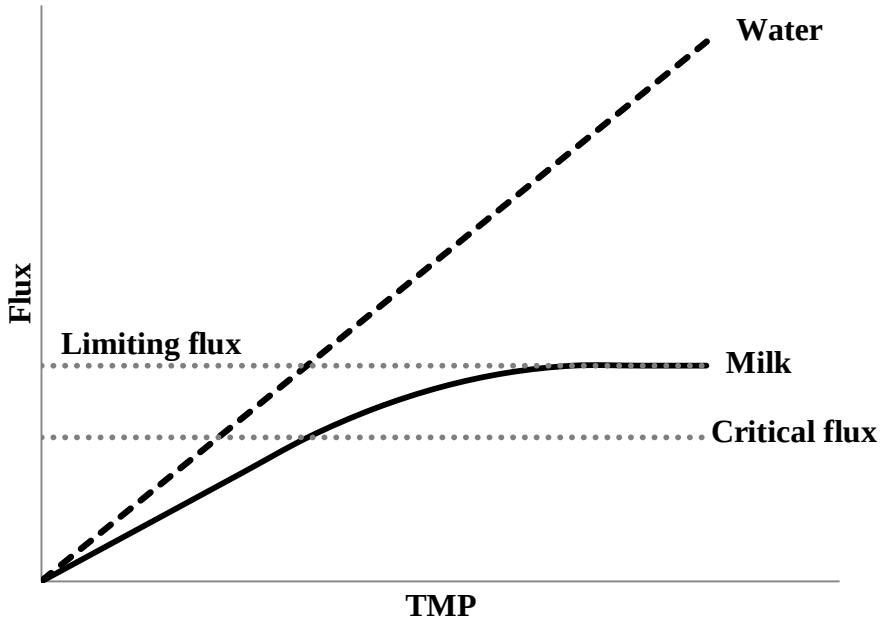

Figure 4.1. Limiting flux and critical flux on a graph of flux as a function of transmembrane pressure (TMP).

The MF membranes used to produce an MCC could impact both the SP removal and the overall flux and performance of the MF system. Previous work has compared the flux and SP removal of ceramic versus polymeric membranes (Zulewska et al., 2009). In general, flux and SP removal are lower with polymeric membranes (Zulewska et al., 2009) than ceramic membranes. Different types of ceramic membranes have been used to MF milk. Ceramic membranes typically operate at high cross-flow velocities (2 to 6 m/s) (Cheryan, 1998). A large longitudinal pressure drop (**ΔP**) on the retentate side of the membrane is required to achieve high cross-flow velocities. A high cross-flow velocity results in a TMP at the inlet end of the

membrane that is much larger than the TMP at the outlet end of the membrane which could result in higher fluxes at the inlet end of the membrane and increased membrane fouling at the inlet end.

There have been several methods developed to create a uniform flux along the length of ceramic membranes. In the uniform transmembrane pressure (**UTP**) system a permeate recirculation pump is used to produce co-current flow of permeate in parallel to the retentate, that produces a gradient of back pressure on the permeate side of the membrane, this creates a pressure drop on the permeate side of the membrane that matches the pressure drop on the retentate side of the membrane (Holm et al., 1990). Another method is to manufacture the membranes with a resistance gradient so that the flux is constant along the length of the membrane even with a large ΔP. Two commercially available ceramic membranes with a resistance gradient are the graded permeability (**GP**) membranes (Pall Corp., Cortland NY), which have the resistance gradient on the outside of the support layer (Gracera and Toujas, 2002) and Isoflux membranes (TAMI, Nyons, France), which has the resistance gradient built into the separating layer of the membrane (Grangeon et al., 2002).

Zulewska et al. (2009) reported that in a 1 stage 3X MF process 64% of the SP was removed in a UTP system (0.1 µm, 4 mm channel diameter (**CD**)) and 61% of the SP was removed using GP membranes (0.1 µm 4 mm CD). Isoflux membranes have been reported to remove less SP (40%) than the UTP or GP membranes (Adams and Barbano, 2013). While SP removal was similar for the UTP and GP systems, the GP

system does not require a permeate recirculation pump and a system with GP membranes would have both a lower fixed and operating cost.

GP membranes come in several configurations. GP membranes are available with both 3 mm and 4 mm CD (Sondhi et al., 2003). The GP membranes are designed to operate at a specific ΔP (Garcera and Toujas, 2002).The 3 mm CD membranes have a greater surface area (46%) per stick compared to 4 mm CD membranes. The limiting flux and SP removal factor for 4 mm CD membranes at 8, 9 and 10% target true protein (**TP**) concentrations in the recirculation loop were reported in Chapter 3, however, there is little information on the performance of 3 mm CD membranes for the production of an MCC using diluted MPC as a feed material.

Limiting flux and SP removal could be a function of the MF membrane CD. The limiting flux is a function of the back transport of molecules away from the surface of the membrane (Belfort et al., 1994). A number of factors could impact the back transport of molecules including viscosity, particle size, concentration and shear rate at the surface of the membrane (Belfort et al., 1994). From the literature it is not clear what impact CD will have on membrane fouling and thus limiting flux or SP removal factor. In a review by Belfort et al. (1994), four models for the prediction of limiting flux were presented. CD does not appear explicitly in any of the models, but in all of the models increasing the shear rate at the wall was predicted to increase limiting flux.

The shear rate at the wall could be a function of CD. RE is defined in Equation 1. For laminar flow (REs < 2,100 (Denn, 1980)) shear rate at the wall is proportional

to cross-flow velocity and inversely proportional to CD (Belfort et al., 1994) and at a constant cross-flow velocity decreasing the CD would increase the shear rate and be expected to increase the limiting flux. However, for tubular ceramic membranes the flow is usually turbulent and the cross-flow velocity is in the range of 2 to 6 m/s. In Chapter 3 it is reported that for 4 mm CD ceramic GP membranes the Reynold's numbers (**RE**) were greater than 15,000, indicating turbulent flow. There is not a simple relationship for shear rate as a function of cross-flow velocity and CD for turbulent flow. If decreasing the CD increases the shear rate at the wall, according to Belfort et al. (1994) the limiting flux should increase.

$$(\mathbf{4.1})\ \mathrm{Re} = \frac{\text{density}\left(\frac{\text{kg}}{m^3}\right) \times \text{cross-flow velocity}\left(\frac{\text{m}}{s}\right) \times \text{CD(m)}}{\text{retentate viscosity(Pa s)}}$$

In contrast, Samuelsson et al. (1997) found that limiting flux was a linear function of the RE as also shown in Chapter 3, which predicts that at a constant cross-flow velocity decreasing the CD would decrease the limiting flux. However, in both Samuelsson et al. (1997) and Chapter 3 CD was a constant, so the linear relationship between limiting flux and RE has not been validated for membranes with different CD. An additional complication arising from the use of GP membranes is that in operating at a constant ΔP, cross-flow velocity will depend on the CD, viscosity, and density (e.g., protein concentration) of the retentate (Denn, 1980). Our objective was to determine the limiting flux and SP removal at 8, 9 and 10% TP in the recirculation loop using 0.1 μm ceramic GP membranes with 3 mm CD. An additional objective

was to compare the limiting flux and SP removal between 0.1 µm ceramic GP membranes with 3 mm and 4 mm CD.

MATERIALS AND METHODS

Experimental Design and Statistical Analysis

The limiting flux for the MF of diluted MPC85 (MPC with 85% TP as a percentage of total solids (**TS**)) was determined at 3 TP concentrations in the recirculation loop (8, 9, and 10%) on 0.1 µm GP membranes with 3 mm CD. The experiment was replicated 3 times. On the morning of each processing run MPC85 was diluted with reverse osmosis water to a MF feed TP concentration of 5.4%. In all processing runs the starting flux was 55 kg/m^2 per h and the flux was increased in steps until the limiting flux was reached. The minimum flux increase was 10 kg/m^2 per h. For the 3 mm CD membranes a different batch of MPC85 was used for each replicate. In Chapter 3 the limiting flux and SP removal at 8, 9 and 10% TP using 4 mm CD membranes (0.1 µm ceramic GP) with a ΔP of 220 kPa was reported. The experiments performed using the 4 mm CD membranes were completed first with different batches of MPC85. The performance of the 3 mm CD membranes are compared to the previously reported performance of the 4 mm CD membranes in the current paper.

All data were analyzed by ANOVA using the Proc GLM (general linear model) procedure of SAS (SAS version 8.02, 1999-2001, SAS Institute Inc., Cary, NC). For the 3 mm CD membranes, the data was analyzed at the starting and limiting flux using a GLM where the dependent variable = target TP + error. The target TP

concentration in the recirculation loop was a class variable with 3 levels: 8%, 9% or 10%.

To determine the impact of CD, recirculation loop TP concentration (target TP) and flux had on membrane performance (i.e., measured flux, cross-flow velocity and SP removal factor) a split-plot model was used with CD and target TP as the whole plot terms and flux with interactions as the sub plot terms. Flux was a categorical variable (starting flux or limiting flux). The GLM was: dependent variable = CD + Target TP + CD*Target TP + Flux + Target TP*Flux + CD*Flux + CD*Target TP*Flux + error. There is no separate term for replicate in the model because all 3 replicates on the 4 mm CD membranes were performed using different batches of MPC85 at a different time than the 3 replicates on the 3 mm CD membranes, so there is no relationship between replicates of the same number among the 2 experiments. The type III mean squares for CD*Target TP was used as the denominator in the F-test to test the significance of the whole-plot terms (CD, target TP). The type III mean square error for the model was used as the denominator in the F-test to test the significance of the sub plot terms.

Preparation of Microfiltration Feed

For MF processing with the 3 mm CD membranes, the MF feed was a fresh liquid MPC85 with 86 ± 0.2% TP on a total solids basis, provided by O-AT-KA Milk Products Cooperative, Inc. (Batavia, NY). The liquid MPC85 at approximately 12.35 ± 0.07% TP determined using an infrared spectrophotometer (**IR**) (Milkoscan, Foss, Hillerod, Denmark) was stored at < 4°C until use. On each day MPC85 (277 ± 12 kg

at 4°C) was diluted to 5.4% TP as determined with by IR, with hot (70°C) RO water (322 ± 12 kg) in a separate jacketed MF product feed tank. The diluted MPC85 was heated to a final temperature of 50°C in the MF product feed tank.

Microfiltration System

A 0.1 µm ceramic GP Membralox module with 3 mm CD (7 sticks with 37 channels per stick and a membrane area of 2.5 m^2) (EP3730GL0.1µA, alumina, Pall Corp, Cortland, NY) was used. The membranes with 4 mm CD had 7 sticks with 19 channels per stick for a membrane area of 1.7 m^2 (EP1940GL0.1µA, alumina, Pall Corp, Cortland, NY). A pilot MF system (Model CF 1000, Pall Corp., Cortland, NY) as described in Chapter 3 was used.

The flux, concentration factor (CF), ΔP and outlet pressure on the retentate side (Pro) were controlled during the processing run. The flux was controlled by opening and closing the permeate removal valve. The CF was controlled by opening and closing the retentate removal valve. The ΔP was controlled to 220 kPa by increasing or decreasing the recirculation pump speed, and the retentate outlet pressure (Pro) was controlled by modifying the feed pump speed. TMP was calculated as: The average of the pressure at the retentate inlet (Pri) and outlet end (Pro) of the membrane on the retentate side minus the permeate pressure at the outlet (Ppo) end of the membrane [(Pro+Pri)/2 – Ppo].

Microfiltration Operation

On each day of processing the MF system was rinsed as described in Chapter 3. Next, the clean water flux was measured at 50°C by turning off the recirculation pump (with the feed pump running), closing the retentate removal valve and opening the permeate removal valve to 95% of full open. The average clean water flux for the 3 mm membranes was 280 ± 4 kg/m^2 per h which was less than the clean water flux for 4 mm CD membranes (363 ± 1 kg/m^2 per h) reported in Chapter 3. The difference in clean water flux with CD was, at least in part, due to the difference in membrane area and membrane resistance. If the membranes contributed no resistance to fluid flow, in the module a maximum "permeate" removal rate would be achieved under the conditions at which the clean water flux was measured. The flux (removal rate divided by membrane area) would be lower (by about 46%) for the 3 mm membranes because of the larger surface area of the 3 mm CD than the 4 mm membranes. The clean water flux on the 3 mm CD membranes was only about 30% less than on the 4 mm membranes. This was probably because the 3 mm CD membranes have more membrane area per stick and thus less support material and resistance to flow compared to the 4 mm CD membranes.

Start-up. The system was started-up as described in Chapter 3, the pressure at the outlet end of the membrane on the retentate side was set to 230 kPa and the ΔP was set to 220 kPa. The flux was set to 55 kg/m^2 per h and the CF set to achieve the target recirculation loop protein concentrations. To flush the MF system, approximately 298 ± 13 kg of permeate and retentate were collected, weighed and

discarded while feeding the system with diluted MPC85 at 5.4% TP. Once the concentration of TP in the retentate in the recirculation loop was within 10% of the target TP concentration as determined by IR analysis of the retentate, the retentate and permeate were recycled to the MF product feed tank. The start-up took about 60 min.

Determination of Limiting Flux. After the retentate and permeate were recycled to the MF product feed tank, the system was operated at a flux of 55 kg/m^2 per h for 1 h. Permeate and retentate removal rates were measured by weight every 15 min. Pressures, temperature and the recirculation rate were also recorded every 15 min. After 1 h at 55 kg/m^2 per h the flux was increased. The flux was increased in steps (the minimum flux step was 10 kg/m^2 per h) with a 1 h stabilization period after each increase. At some point the target flux could not be achieved and further increasing the TMP did not produce an increase in flux. The limiting flux was taken as the last flux that the system was able to operate at stably (constant TMP) for 1 h.

Samples of retentate and permeate (65 mL per container) were taken after the 1 h stabilization period at each flux and frozen (in an insulted cooler using dry ice) for later chemical analysis. The TP concentration of the retentate and permeates was monitored during the run every 15 min by IR analysis. Based on the results from the IR, the CF was adjusted to maintain the target TP concentration. Immediately after each run the MF system was cleaned as described in Chapter 3.

Chemical Analyses

A sample of the MF feed (diluted MPC85) was taken before each run and samples of the permeate and retentate were taken at each flux. The samples were

frozen (-40°C) until analysis. The MF feed (diluted MPC85) was analyzed for TS, fat and lactose, using forced air oven drying (AOAC, 2000; method 990.20; 33.2.44), ether extraction (AOAC 2000; method 989.05; 33.2.26) and enzymatic lactose (AOAC 2000; method 984.15; 33.2.67, Lynch et al. 2007) respectively. The diluted MPC85, retentates and permeates were analyzed for total nitrogen (**TN**), and NPN by Kjeldahl (AOAC, 2000; method 991.20; 33.2.11), and (AOAC, 2000; method 991.21; 33.2.12), respectively. Noncasein nitrogen (**NCN**) content of the diluted MPC85 was determined using Kjeldahl (AOAC, 2000; method 998.05; 33.2.64), modified by using 5.5 mL of acetic acid (10% vol/vol) and 5.5 mL of sodium acetate (1N) instead of 1 mL of each, to ensure that a final filtrate pH of less than 4.6 was achieved and that all of the casein (**CN**) was precipitated. TP 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.

Viscosity of the retentates was measured at each flux. The viscosity was measured at 50°C using a Brookfield viscometer (Model: LV-DV2T) with a UL adapter for low viscosity fluids (Brookfield Engineering Laboratories, Inc., Middleboro, MA). A water bath was heated to 50°C and this water was recirculated through the jacketed sample cup using a peristaltic pump. The retentate samples were heated in a water bath at 50°C, then approximately 16 mL was placed in the sample cup. The viscosity was measured at 60 rpm (shear rate of 73 1/s) for 50 s. The recorded viscosity was the average viscosity measured in Pa*s during the last 30 s of the measurement.

Calculation of the Serum Protein Removal Factor

The SP removal factor was the ratio of SP removed in the permeate to the theoretical SP removal. A SP removal factor of 1 would mean that the membrane was not rejecting any SP. Theoretical SP removal (in kg) was equal to the mass of permeate multiplied by the concentration of SP in the permeate portion of the MF feed. The concentration of SP in the permeate portion of the MF feed was the mass of SP in the feed divided by the mass of feed less the mass of CN in the feed. The actual SP removed in the permeate (in kg) was the mass of permeate multiplied by the concentration of TP in the permeate. It was assumed that all of the TP in the permeate was SP. Because mass of permeate appears in both the numerator and denominator the SP removal factor was independent of permeate mass.

RESULTS

3 mm Channel Diameter Membranes

Microfiltration Feed Composition. The composition of the MF feed for the 3 mm CD membranes at the 3 target recirculation loop TP concentrations is shown in Table 4.1. The MPC85 was diluted based on IR analysis results to achieve the same TP concentration in the diluted MPC for all 3 target recirculation loop TP concentrations. No differences in the composition of the MF feed among the 8, 9 and 10% target TP concentrations were detected. In Chapter 3 it was reported that the same result was achieved in an experiment with 4 mm membranes.

Retentate and Permeate Composition. The CF was controlled during each processing run to achieve the target recirculation loop TP concentration. The average

retentate TN, NPN and TP at the starting and limiting flux are shown in Table 4.2. The TN and TP concentrations in the retentate in the recirculation loop were different, as expected, among the 3 target TP concentrations ($P < 0.05$) at both the starting and limiting flux. The TN, NPN and TP concentrations in the permeates are shown in Table 4.2. No difference in TN, TP or NPN concentration in the permeate was detected ($P > 0.05$) among the 3 target recirculation loop TP concentrations at either the starting or limiting flux.

Microfiltration Process Parameters. The measured flux, ΔP, cross flow velocity, and TMP are shown in Table 4.3. The MF system was operated to maintain a constant ΔP. As shown in Table 4.3, no difference in ΔP among the 3 target TP concentrations was detected at either the starting or limiting flux. Because the system

Table 4.1. Mean (n=3) composition of the diluted milk protein concentrate used as the microfiltration (MF) feed for the 3 mm channel diameter (CD) membranes all values are given in percent by weight for 8, 9 and 10% recirculation loop target true protein (TP) concentrations and the average MF feed composition on the 3 mm and 4 mm CD membranes.

Target TP concentration	TS[1]	Lactose[1]	Fat[1]	TN[1]	NPN[1]	NCN[1]	TP[1]	SP[1]	CN[1]	CN%TP[1]
8%	6.51	0.28	0.084	5.65	0.042	0.98	5.61	0.94	4.67	83.30
9%	6.52	0.28	0.084	5.69	0.043	0.98	5.65	0.94	4.71	83.42
10%	6.46	0.27	0.084	5.59	0.040	0.97	5.55	0.93	4.62	83.27
SE	0.03	0.01	0.004	0.05	0.003	0.04	0.05	0.04	0.08	0.82
R squared	0.26	0.005	0.002	0.28	0.05	0.007	0.28	0.004	0.10	0.003
CD										
3 mm	6.50	0.28	0.084	5.64	0.04	0.98	5.60	0.93	4.67	83.33
4 mm	6.47	0.24	0.080	5.57	0.03	0.92	5.53	0.88	4.65	84.05
SE	0.02	0.008	0.002	0.03	0.003	0.02	0.03	0.02	0.04	0.39
R squared	0.25	0.41	0.14	0.37	0.18	0.30	0.37	0.23	0.20	0.17

Means in the same column within target TP or CD do not differ ($P > 0.05$).

[1] TS = total solids, anhydrous lactose, TN = total nitrogen x 6.38, NPN = nonprotein nitrogen x 6.38, NCN = noncasein nitrogen x 6.38, TP = true protein, (TN minus NPN), SP = serum protein, (NCN minus NPN), CN = casein, (TN minus NCN), CN%TP = 100 times CN divided by TP.

Table 4.2. Mean (n=3) composition of the microfiltration retentates and permeates for ceramic microfiltration membranes with 3 mm channel diameter (CD) for recirculation loop target true protein (TP) concentrations of 8, 9, and 10% at the starting or limiting flux and the average (n = 9) retentate TP concentration for the 4 mm CD membranes.

		3 mm CD						4 mm CD
	Target TP concentration	Starting Flux			Limiting Flux			TP[1]
		TN[1]	NPN[1]	TP[1]	TN[1]	NPN[1]	TP[1]	
Retentate	8%	8.25[c]	0.049	8.21[c]	8.18[c]	0.068	8.11[c]	8.21[c]
	9%	9.18[b]	0.051	9.13[b]	9.24[b]	0.066	9.18[b]	9.18[b]
	10%	10.22[a]	0.041	10.18[a]	10.25[a]	0.062	10.19[a]	10.12[a]
	SE	0.02	0.005	0.02	0.05	0.006	0.05	0.05
	R squared	>0.99	0.29	>0.99	>0.99	0.07	>0.99	0.98
Permeate	8%	0.74	0.043	0.70	0.70	0.045	0.66	
	9%	0.75	0.044	0.71	0.70	0.040	0.66	
	10%	0.75	0.034	0.72	0.69	0.040	0.65	
	SE	0.03	0.009	0.03	0.02	0.006	0.02	
	R squared	0.01	0.12	0.05	0.02	0.08	0.03	

[a - c]Means in the same column within retentate or permeate not sharing a common superscript are different ($P < 0.05$).

[1] TN = total nitrogen x 6.38, NPN = nonprotein nitrogen x 6.38, TP = true protein, (TN – NPN).

Table 4.3. Mean (n=3) longitudinal pressure drop (ΔP), cross-flow velocity and transmembrane pressure (TMP) at the starting flux of 55 kg/m^2 per h and limiting flux for the 3 mm channel diameter (CD) membranes at 8, 9, and 10% target true protein (TP) in the recirculation loop.

	Target TP concentration	Flux (kg/m^2 per h)	ΔP (kPa)	Cross-flow velocity (m/s)	TMP (kPa)
Starting flux	8%	54.78	220	5.57^{a}	75.05 ^{b}
	9%	55.80	220	5.50^{b}	78.40 ^{ab}
	10%	54.70	220	5.40^{c}	84.81^{a}
	SE	0.47	0.14	0.007	1.50
	R squared	0.36	0.13	0.98	0.78
Limiting flux	8%	128^{a}	220	5.57^{a}	228.77
	9%	109^{b}	220	5.46^{b}	217.14
	10%	97^{c}	220	5.38^{c}	250.58
	SE	2.10	0.17	0.006	16.25
	R squared	0.95	0.02	0.99	0.27

$^{a-c}$ Means in the same column within starting flux or limiting flux not sharing a common superscript are different at either the starting or limiting flux ($P < 0.05$).

was operated at a constant ΔP the cross-flow velocity decreased ($P < 0.05$) as the TP concentration in the recirculation loop increased at both the starting and limiting flux (Table 4.3). Cross-flow velocity was also found to decrease as recirculation loop TP concentration increased when 4 mm CD membranes were operated at a ΔP of 220 kPa (Chapter 3).

As shown in Table 4.3, no differences in the starting flux of 55 kg/m^2 per h among the target recirculation loop TP concentrations were detected; however TMP increased ($P < 0.05$) with increasing recirculation loop TP concentration at the starting flux. This may indicate that at the starting flux there was some resistance to permeate flow through the membrane that depended on the recirculation loop TP concentration. This is in contrast to what was found with 4 mm CD membranes, where no difference in TMP at the starting flux was detected among the 3 target TP concentrations (Chapter 3). At the limiting flux (which depended on the recirculation loop TP

concentration) no difference in TMP between the target recirculation loop TP concentrations was detected ($P > 0.05$) for the 3 mm CD membranes, but overall TMP was much higher at the limiting than at the starting flux. This is similar to the 4 mm CD membranes, where no difference in the TMP at the limiting flux was detected among target TP concentrations (Chapter 3).

Limiting Flux and Serum Protein Removal Factor. The limiting flux decreased as the TP concentration in the recirculation loop increased as shown in Table 4.3. The limiting flux will depend on the balance between transport of molecules towards the membrane due to flux and the back transport of molecules away from the surface of the membrane due to turbulent flow created by the high cross-flow velocity of the retentate. The back transport of CN away from the membrane is predicted to increase with increasing cross-flow velocity and decrease with increasing retentate viscosity (Belfort et al., 1994). The dependence of limiting flux on TP concentration in the retentate (Table 4.3) is consistent with the back transport away from the membrane being dependent on the cross-flow velocity (which decreased as TP concentration increased) and the TP concentration (and viscosity) in the recirculation loop.

The SP removal factors are shown in Table 4.4. No differences ($P > 0.05$) in the SP removal factor among target TP concentrations at either the starting or limiting fluxes were detected. There were differences ($P < 0.05$) in TMP required to achieve the starting flux at the different target recirculation loop TP concentrations (Table 4.3), indicating a resistance that depended on the recirculation loop TP concentration, but

this did not appear to impact the rejection characteristics of the membrane (Table 4.4). No differences ($P > 0.05$) in SP removal factors at the limiting flux were detected for the 3 recirculation loop TP concentrations indicating that the extent of membrane fouling that impacted the rejection characteristics of the membrane were similar for all target TP concentrations at the limiting flux with 3 mm membranes.

Table 4.4. Mean (n=3) serum protein (SP) removal factor at the starting and limiting flux for the 3 mm channel diameter (CD) ceramic membranes at 8, 9 and 10% recirculation loop target true protein (TP) concentrations.

Target TP concentration	SP removal factor at the starting flux	SP removal factor at the limiting flux
8%	0.71	0.67
9%	0.72	0.68
10%	0.74	0.67
SE	0.03	0.02
R squared	0.06	0.02

Means in the same column do not differ ($P > 0.05$).

Comparison of 3 mm and 4 mm Channel Diameter Membranes

Microfiltration Feed Composition. The average (n = 9) MF feed compositions (diluted MPC 85) for the 3 mm and 4 mm CD membranes are shown in Table 4.1. The 9 processing runs on the 4 mm CD membranes were performed in a previous month and the batches of MPC85 used were different from the batches of MPC85 used for the 3 mm CD membranes. The dilution of MPC85 concentrate with water was controlled using an infrared milk analyzer in attempt to achieve the same starting composition at all times. No difference among the MF feed compositions (i.e., TP, SP, CN and CN as a percentage of TP) for the 3 mm and 4 mm CD membranes was detected ($P > 0.05$).

Table 4.5. ANOVA df, type III sum of squares (SS) with: retentate true protein (TP) concentration, measured flux, longitudinal pressure drop (ΔP), cross-flow velocity, average transmembrane pressure (TMP) and serum protein removal factor as the dependent variables, for the 3 mm and 4 mm channel diameter (CD) membranes at target TP recirculation loop concentrations of 8, 9 and 10%.

		Retentate TP concentration	Measured flux	ΔP	Cross-flow velocity	TMP	Serum protein removal factor
Model term	df	SS	SS	SS	SS	SS	SS
Whole Model		23.35*	47,760*	1.55	21.01*	181,884*	0.079*
Whole plot							
CD	1	<0.001	1,188.13*	N.S.	20.80*	6,817	0.054*
Target TP	2	23.33*	1,879.74*	N.S.	0.197*	436.85	0.0009
CD x Target TP[1]	2	0.022	26.24	N.S.	0.0003	944.44	0.0006
Sub plot							
Flux	1	--	41,722.4*	N.S.	0.007*	171,768*	0.0235*
Flux x CD	1	--	1,268.00*	N.S.	--	1,918*	--
Flux x Target TP	2	--	1,675.63*	N.S.	0.002*	--	--
Flux x CD x Target TP	2	--	--	N.S.	--	--	--
model df		5	9	--	8	7	6
error df		30	26	--	27	28	29
R squared		0.99	>0.99	0.39	>0.99	0.96	0.54

[1] Used as error term for whole plot variables: CD and target TP

*$P < 0.05$

Retentate True Protein Concentration. As presented above for the 3 mm and as reported in Chapter 3 for the 4 mm CD membranes, the limiting flux depended on the TP concentration in the recirculation loop. The target recirculation loop TP concentrations were the same for the work with the 3 mm and 4 mm CD membranes (i.e., 8, 9 and 10%). The measured TP concentrations in the recirculation loop for the 4 mm CD membranes are shown in Table 4.2. No difference ($P > 0.05$) in recirculation loop TP concentrations (Table 4.2) between the 3 mm and 4 mm CD (CD) membranes was detected (Table 4.5). Additionally, no influence of flux on retentate TP concentration was detected ($P > 0.05$) indicating that the recirculation loop TP concentration was well controlled during processing as flux was increased.

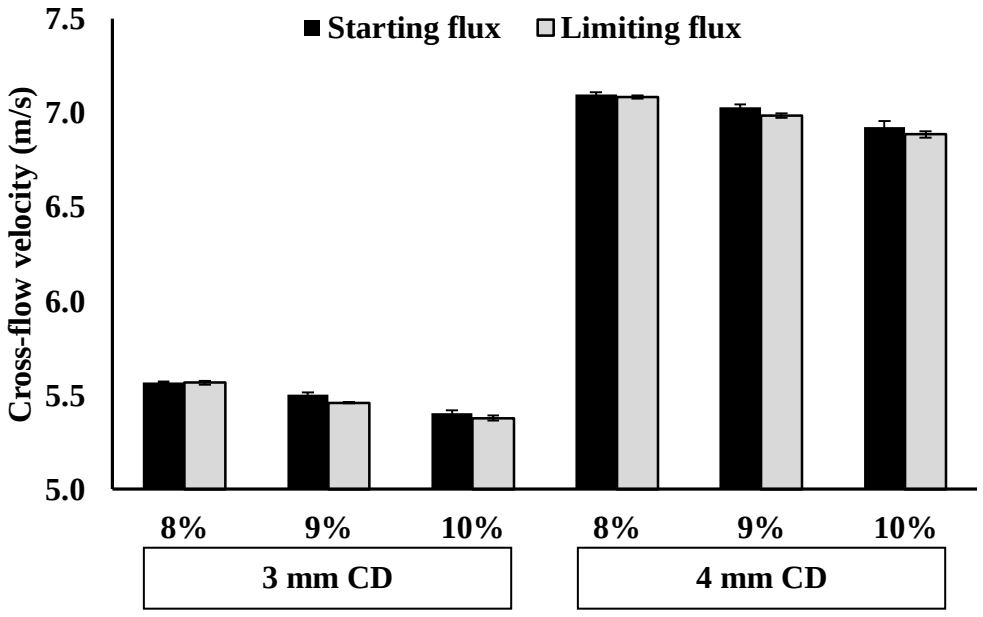

Figure 4.2. Mean (n = 3) cross-flow velocity (with standard deviation) at the starting and limiting flux for the 3 mm and 4 mm channel diameter (CD) membranes at 8%, 9% and 10% recirculation loop target true protein (TP) concentration.

Processing Parameters. For both the 3 mm and 4 mm CD membranes the ΔP was set to 220 kPa. As shown in Table 4.5, ΔP did not depend on target recirculation loop TP concentration, CD or flux. Because the system was operated at a constant ΔP the cross-flow velocity depended on both the membrane CD ($P < 0.05$, Table 4.5) and recirculation loop TP concentration ($P < 0.05$, Table 4.5) as shown in Figure 4.2. The retentate cross-flow velocity was much lower ($P < 0.05$) for 3 mm CD than 4 mm CD membranes (about 5.5 versus, 7.0 m/s, respectively). Lower cross-flow velocity may result in more membrane fouling and reduced passage of SP through the MF membrane. The membrane CD had a much larger impact on cross-flow velocity than differences in recirculation loop TP concentration as shown in Figure 4.2 and the higher type III sum of squares for the CD model term than target TP (Table 4.5). The cross-flow velocity tended to decrease as the run progressed from starting to limiting flux, but the magnitude was small (0.03 ± 0.005 m/s), and explains the significant flux term in Table 4.5.

There was a trend for TMP to depend ($P = 0.06$) on CD and there was an effect ($P < 0.05$) of flux and a flux by CD interaction (TMP pressure increased as flux was increased). The TMP at the starting flux, which was 55 kg/m^2 per h on both the 3 mm and 4 mm CD membranes, was higher for the 3 mm membranes than the 4 mm membranes (Figure 4.3). At the limiting flux there was overlap in the required TMP as shown in Figure 4.3, with a trend towards a higher TMP with the 3 mm CD membranes compared to the 4 mm CD membranes.

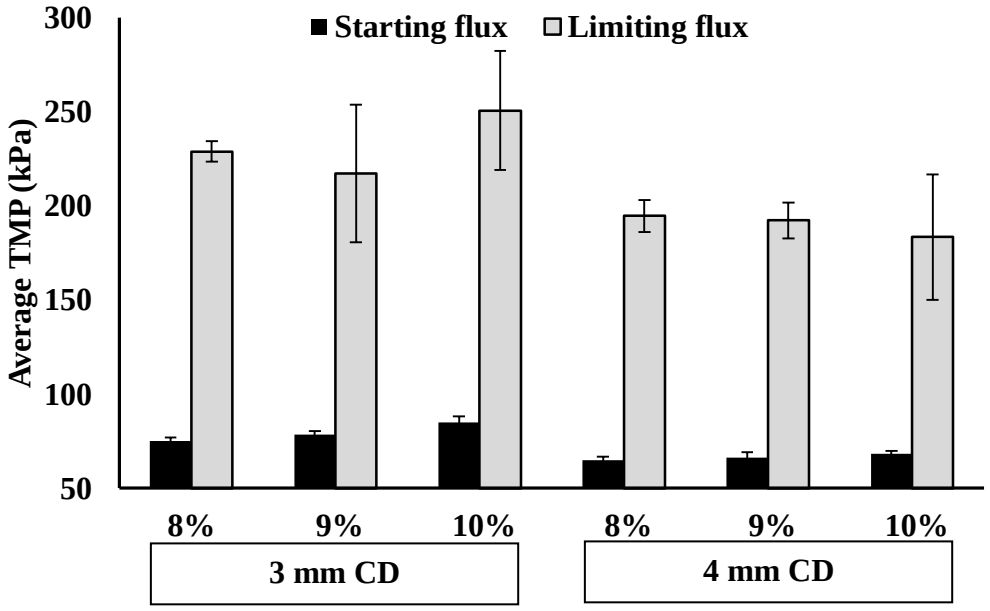

Figure 4.3. Mean (n = 3) Transmembrane pressure (TMP) (with standard deviation) at the starting and limiting flux for the 3 mm and 4 mm channel diameter (CD) membranes at 8% , 9% and 10% recirculation loop target true protein (TP) concentration.

Limiting Flux. The limiting flux for the target recirculation loop TP concentrations for both the 3 mm and 4 mm membranes are shown in Table 4.6. The limiting flux was a function of both recirculation loop TP concentration and CD (Table 4.5). At each TP concentration in the retentate recirculation loop the limiting flux was higher ($P < 0.05$) on the 4 mm CD membranes than the 3 mm membranes ($P < 0.05$) (Table 4.6). The difference between the limiting fluxes explain the significant flux by TP and flux by CD interactions for measured flux in Table 4.5. For all processing runs the starting flux was 55 kg/ m^2 per h, while the limiting flux however depended both on the TP concentration and the CD. The difference in limiting flux between the 3 mm and 4 mm CD membranes was probably in part due to the

difference in cross-flow velocity (Figure 4.2). To operate the 3 mm CD membranes at the same cross-flow velocity as the 4 mm CD membranes would have required a much larger ΔP and this would exceed the membrane manufacturers' design parameters for the membrane.

Table 4.6. Limiting flux and permeate removal rate per module for the 3 mm channel diameter (CD) membranes (2.5 m^2) and the 4 mm CD membranes (1.7 m^2) at 8, 9 and 10% recirculation loop target true protein (TP) concentrations.

		Target TP concentration		
CD		8%	9%	10%
3 mm	Limiting flux (kg/m^2 per h)	128[b]	109[b]	97[b]
4 mm	Limiting flux (kg/m^2 per h)	154[a]	133[a]	117[a]
	SE	0.35	2.58	2.34
	R squared	>0.99	0.92	0.90
3 mm	Permeate removal rate (kg/h)	320[a]	273[a]	242[a]
4 mm	Permeate removal rate (kg/h)	262[b]	227[b]	198[b]
	SE	0.75	6.40	4.03
	R squared	>0.99	0.87	0.94

[a - b]Means in the same column within limiting flux or permeate removal rate not sharing a common superscript are different for either the limiting flux or the permeate removal rate ($P < 0.05$).

Serum Protein Removal. The SP removal decreased as flux increased for both the 3 mm and 4 mm CD membranes as shown in Figure 4.4 and Table 4.5. The SP removal factor was lower ($P < 0.05$) for the 3 mm membranes than the 4 mm CD membranes (Figure 4.4 and Table 4.5). However, the decrease in SP removal with flux was similar among the 3 mm and 4 mm CD membranes (no significant interactions as shown in Table 4.5). The lower SP removal factor for the 3 mm membranes may indicate that even at the starting flux there is increased membrane fouling due to lower cross-flow velocity that changed the rejection characteristics of the membrane.

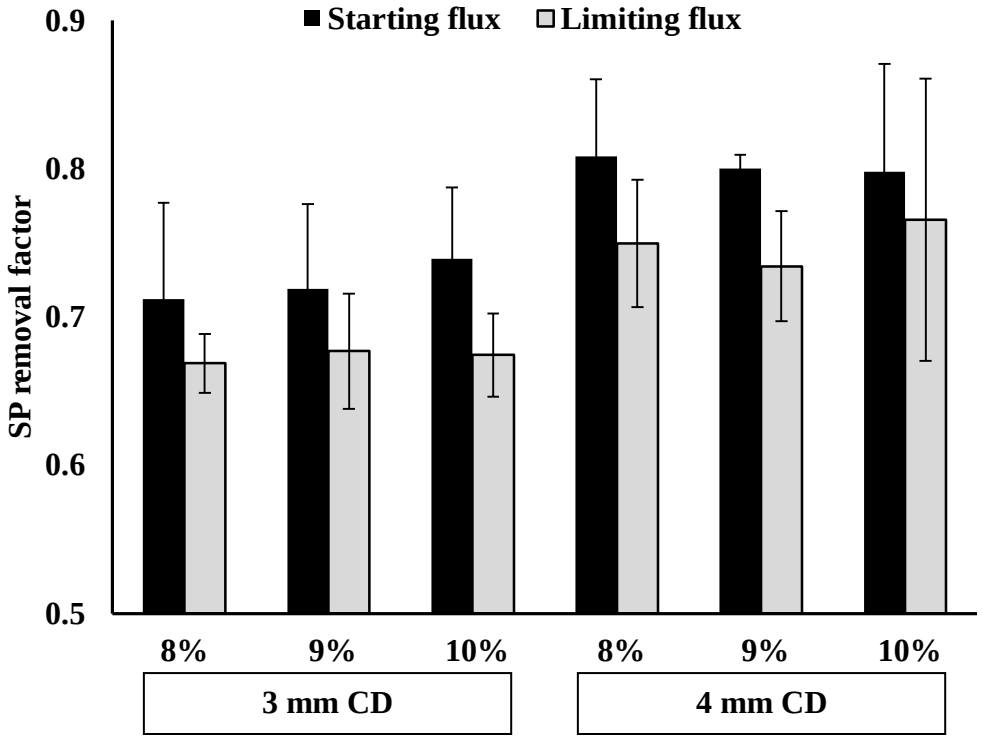

Figure 4.4. Mean (n=3) serum protein (SP) removal factor at the starting and limiting flux for the 3 mm and 4 mm channel diameter (CD) membranes at 8%, 9% and 10% recirculation loop target true protein (TP) concentration.

DISCUSSION

Impact of Channel Diameter on Limiting Flux

In Chapter 3 it was found that limiting flux could be predicted as a linear function of the RE (Equation 4.1) for the MF of diluted MPC85 on 4 mm CD membranes. However, CD was a constant in Chapter 3. The measured viscosities, calculated densities and REs for the 3 mm CD membranes are shown in Table 4.7. For the 3 mm CD membranes the limiting flux predicted using Equation 4.2 was lower than the measured limiting flux, for example at an 8% target TP Equation 4.2 would predict a limiting flux of 91 kg/m^2 per h, which was lower than the measured limiting flux of 128 ± 0.3 kg/m^2 per h. The limiting fluxes as a function of RE are shown in

Figure 4.5 for the 3 mm and 4 mm CD membranes. The RE does not explain the difference in limiting flux between the 3 mm and 4 mm CD membranes, as shown in Figure 4.5.

(**4.2**) Limiting flux = $0.00764 \frac{kg}{m^2 h} \times$ Re

Table 4.7. Density, viscosity and Reynold's number at the limiting flux for target true protein (TP) recirculation loop concentrations of 8, 9, and 10% on the 3 mm channel diameter (CD) ceramic membranes.

Target TP concentration	Viscosity[1] (Pa*s)	Density[2] (kg/m^3)	Reynold's number[3]
8%	0.0014^c	$1{,}036^c$	$12{,}014^a$
9%	0.0017^b	$1{,}040^b$	$10{,}162^b$
10%	0.0019^a	$1{,}043^a$	$8{,}764^c$
SE	0.00001	0.18	110
R squared	>0.99	>0.99	0.99

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

[1]Viscosity measured at 50°C

[2]Density calculated using equation of Stepp and Smith (1991): (Density = 1,034.72 - 0.39*temperature (°C) + 1.69*TN + 0.10*TN^2). TN (%) = total nitrogen*6.38.

[3]Reynold's number calculated as: density (kg/m^3)*cross-flow velocity (m/s)*CD(m)/ viscosity (Pa*s).

If CD was removed from the calculation of RE (Equation 4.1) and replaced by membrane length (1.02 m for both membranes) in order to maintain the dimensionless nature of the RE, a better correlation for limiting flux was achieved (Figure 4.6), where limiting flux could be predicted using Equation 4.3. In Equation 4.3 the slope that provided the best fit to the observed limiting flux was determined by minimizing the sum of the squared difference between predicted and measured limiting flux. It

appears that the limiting flux was well explained by the cross-flow velocity and retentate TP concentration (which impacted density and viscosity) and not the CD.

(**4.3**)

$$\text{Limiting flux} = 3.07\times10^{-5}\,\frac{\text{kg}}{m^2 h}\times\,\frac{\text{density}\times\text{cross-flow velocity}\times\text{membrane length}}{\text{retentate viscosity}}$$

The limiting flux depends on the back transport of molecules away from the surface of the membrane, which is increased as shear rate at the membrane surface increases (Belfort et al., 1994). For laminar flow the shear rate is proportional to the cross-flow velocity and inversely proportional to the CD (Belfort et al., 1994). As shown in Figure 4.5 for both the 3 mm and 4 mm CD membranes the RE >> 2,100 indicating turbulent flow (Denn, 1980). It may be that CD did not have a large impact on the shear rate near the wall because the flow was turbulent for both CD. However, in this work, cross-flow velocity was not an independent variable, and was a function of both CD and target TP concentration (Figure 4.2), so additional research would be required to verify whether or not CD had an independent impact on limiting flux.

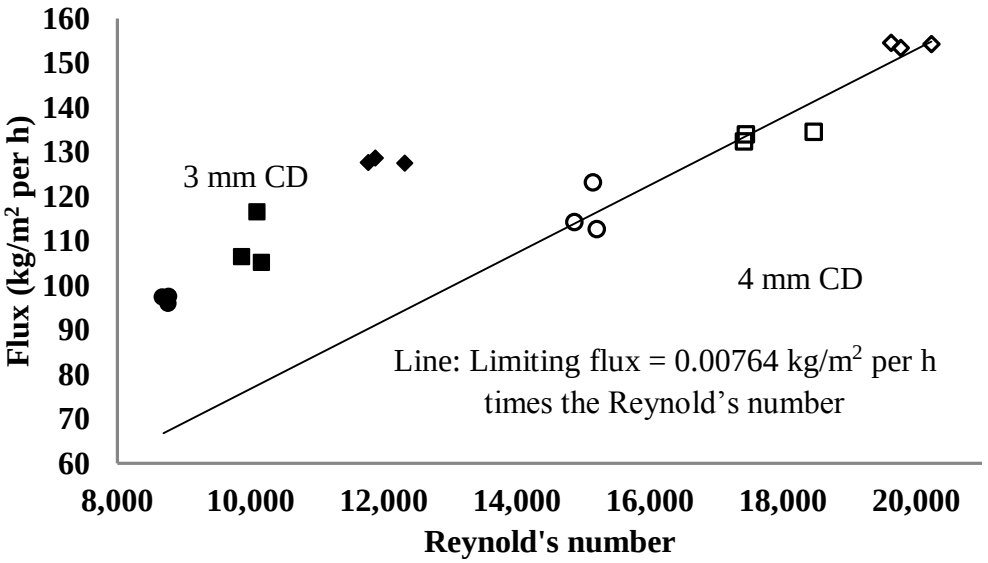

Figure 4.5. Limiting flux as a function of Reynold's number for 4 mm channel diameter (CD) membranes at 8% (◊), 9% (□) and 10% (○) recirculation loop target true protein (TP) concentration and the 3 mm CD membranes at 8% (◆), 9% (■) and 10% (●) recirculation loop target TP concentration.

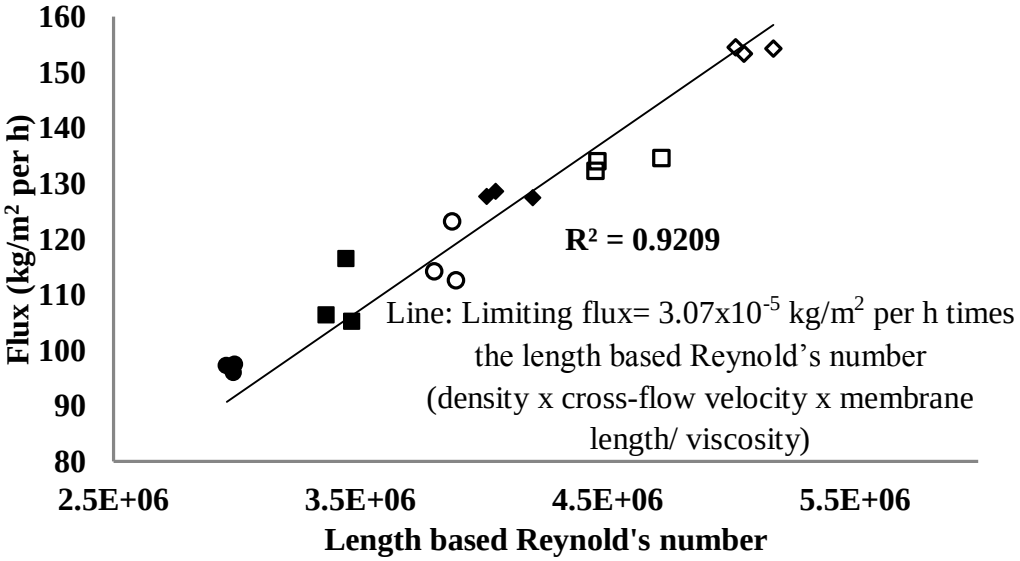

Figure 4.6. Limiting flux as a function of length dependent Reynold's number for 4 mm channel diameter (CD) membranes at 8% (◊), 9% (□) and 10% (○) recirculation loop protein concentration and the 3 mm CD membranes at 8% (◆), 9% (■) and 10% (●) recirculation loop true protein (TP) concentration

Sustainable Flux

The sustainable flux is a flux that can be maintained for extended periods of time, and where the rate of membrane fouling is slow (Bacchin et al., 2006). The flux versus TMP profiles for both the 3 mm and 4 mm CD membranes are shown in Figure 4.7. The flux versus TMP curves begin to level off after a TMP of approximately 150 kPa, indicating that membrane fouling was increasing at TMP higher than 150 kPa (Figure 4.7).

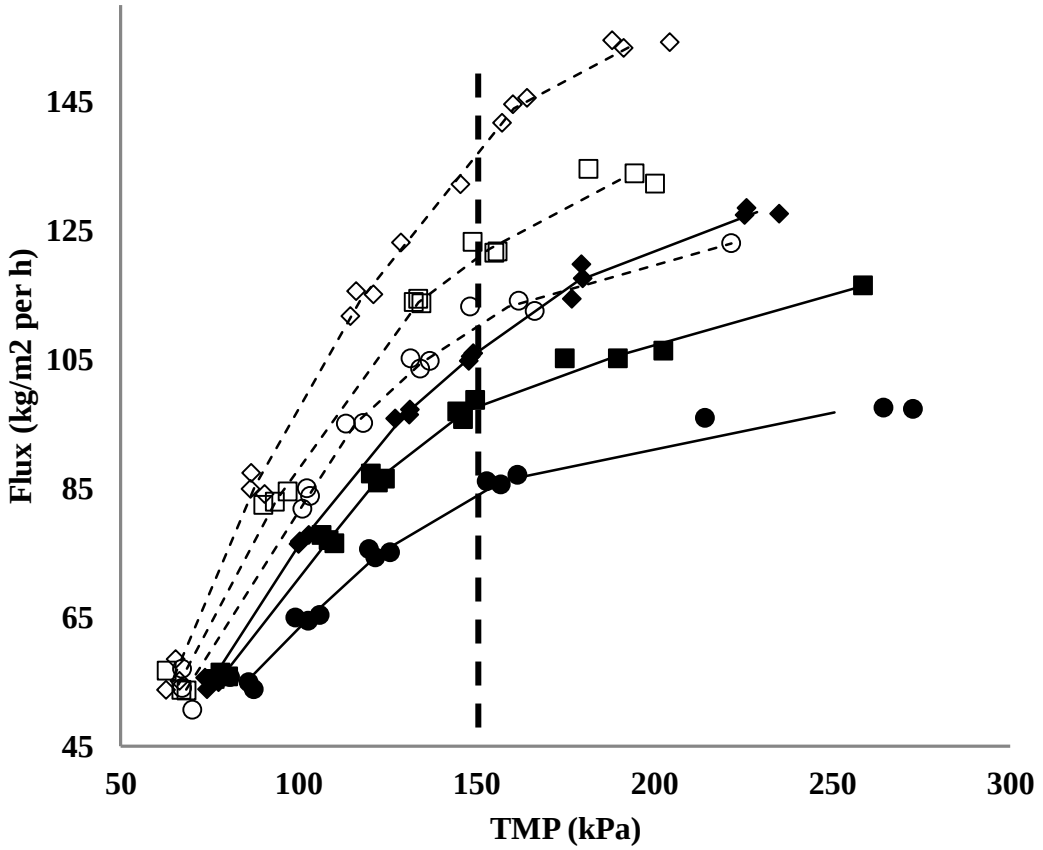

Figure 4.7. Flux versus transmembrane pressure profiles for 4 mm channel diameter (CD) membranes at 8% (◊), 9% (□) and 10% (○) recirculation loop protein concentration and the 3 mm CD membranes at 8% (◆), 9% (■) and 10% (●) recirculation loop true protein (TP) concentration.

The relationship between TMP, limiting and sustainable flux in Figure 4.7 is obscured by the difference in the limiting flux among the CD and target recirculation loop TP concentrations. To better explore the relationship between TMP and flux, flux was scaled (100 times measured flux divided by limiting flux) as shown in Figure 4.8. Regardless of the CD or recirculation loop protein concentration, it appears that a TMP corresponds to a percentage of the limiting flux. A TMP of approximately 150 kPa corresponded to roughly 80 to 85 percent of the limiting flux, independent of the target TP concentration or membrane CD. The concept that a sustainable flux would be a fraction of the limiting flux is consistent with a report by Bacchin (2004) indicating that critical flux was a fraction of the limiting flux. Although, it would need to be validated, Figures 4.7 and 4.8 suggests that 150 kPa may be a practical TMP that corresponds to a sustainable flux for both the 3 mm and 4 mm CD membranes at a variety of recirculation loop protein concentrations.

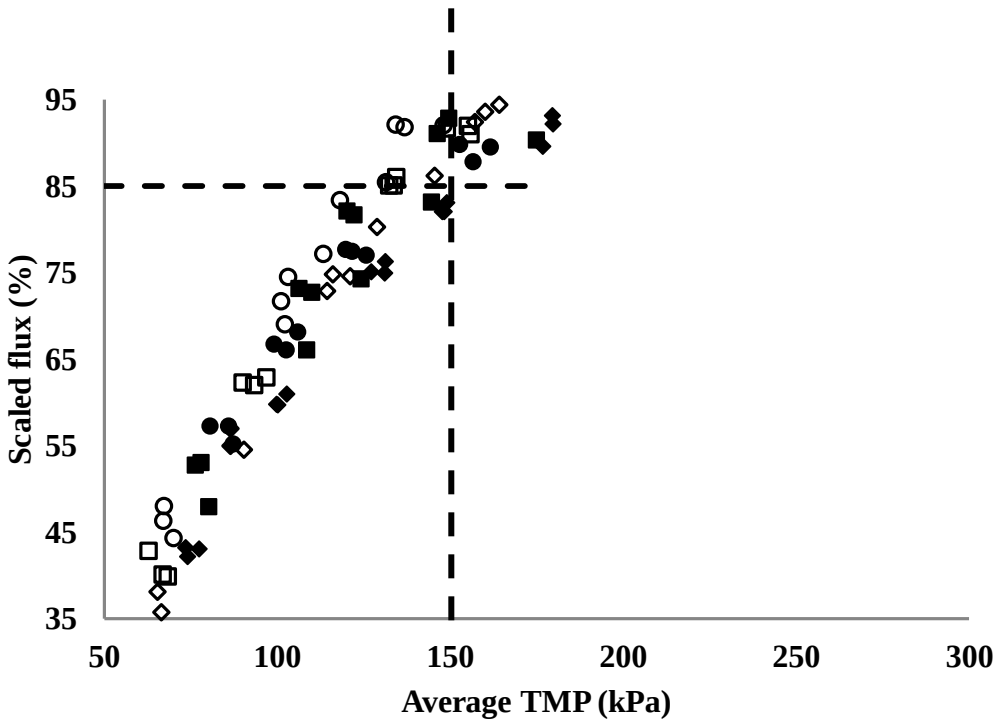

Figure 4.8. Scaled flux as a function of transmembrane pressure (TMP) for the 4 mm channel diameter (CD) membranes at 8% (◊), 9% (□) and 10% (○) recirculation loop protein concentration and the 3 mm CD membranes at 8% (◆), 9% (■) and 10% (●) recirculation loop protein concentration.

Factors to Consider in Choice of Membranes and Operating Conditions

Recirculation Loop Protein Concentration. The choice of recirculation loop TP concentration will impact both the amount of permeate that will need to be removed and the sustainable flux that the system can operate at. To produce an MCC with a specific reduction in SP or lactose, increasing the recirculation loop TP concentration will decrease the amount of MF permeate that has to be removed (Chapter 2), however as shown in this present study, increasing the recirculation TP concentration will decrease the sustainable and limiting flux (Table 4.6).

Channel Diameter. Increasing CD increased ($P < 0.05$) both the limiting flux (Tables 4.5 and 4.6) and the SP removal (Figure 4.6 and Table 4.5). Because of the

lower SP removal factor, to remove the same amount of SP with the 3 mm membranes more permeate would have to be removed **(**either by adding more diafiltration water or increasing the CF**)** than with the 4 mm CD membranes. Even though the limiting fluxes were lower for the 3 mm CD membranes as shown in Table 4.6, the permeate removal rate per stainless steel module was larger for the 3 mm CD membranes because of the increased surface area per module. The surface area per module could also impact process operation. At start-up water has to be flushed from the system, and the concentration of TP in the recirculation loop has to be increased to the target concentration by the removal of permeate. A higher removal rate per module would reduce the time required to reach steady state but the risk of going too high in protein too fast and fouling out the system would increase as well. If an increase in retentate recirculation loop TP concentration (above the target TP concentration) caused the membranes to foul to the extent that caused SP passage and flux to decreased to an unacceptable level, then termination of the run and a full clean of the system would be required. The potential for variation in the recirculation loop TP concentrations should be factored into the target recirculation loop TP concentration and flux for the process, to avoid short and variable length production runs. This may be more of a concern for a system with 3 mm CD membranes than 4 mm CD membranes because of the greater membrane area per module.

Number of Recirculation Loops. Each recirculation loop in a MF process would require a recirculation pump, valves and control systems regardless of the membrane area per loop. If each module requires a 220 kPa pressure drop, the number of modules per recirculation loop will also be constrained by the ability of the

recirculation pump to provide the necessary pressure drop. In designing a multi-stage ceramic MF system, minimization of the number of recirculation loops or modules may be more important than the minimization of membrane area from the perspective the hardware cost and daily operational expense. Increasing the number of recirculation loops will also increase the dead volume of the system. As the dead volume of the MF process increases, the potential loss of product at both the start and end of the run increases. At start-up, as water is flushed from the system, the retentate TP concentrations will be below their target concentrations and a decision would have to be made about where to send the retentate (i.e. to the drain, recycle to feed tank). At the end of the run all recirculation loops would be filled with retentate, some of which may be recovered in a flush step. Operational procedures at both the start and end of the run would need to be implemented to minimize TP loss.

CONCLUSIONS

For the 3 mm CD membranes the limiting flux was: 128 ± 0.3, 109 ± 4 and 97 ± 0.5 kg/m^2 per h at recirculation loop TP concentrations of 8.1 ± 0.07, 9.2 ± 0.04 and 10.2 ± 0.03% respectively. For the 3 mm CD membranes increasing the flux from the starting to the limiting flux decreased the SP removal factor from 0.72 ± 0.02 to 0.67 ± 0.01, however no difference in SP removal factor among the target recirculation loop TP concentrations was detected ($P > 0.05$). The limiting flux at each recirculation loop target TP concentration was lower ($P < 0.05$) for the 3 mm CD membranes compared to the 4 mm CD membranes. The differences in limiting fluxes between the 3 mm and 4 mm CD membranes were explained in part by the difference in cross-flow velocity (5.5 ± 0.03 m/s and 7.0 ± 0.03 m/s for the 3 mm and 4 mm CD membranes

respectively). The SP removal factor was also lower for the 3 mm CD membranes compared to the 4 mm CD membranes ($P < 0.05$), indicating that more membrane fouling may have occurred in the 3 mm versus 4 mm CD membranes.

ACKNOWLEDGMENTS

The authors thank the Northeast Dairy Foods Research Center (Ithaca, NY) and New York State Milk Production Board (Albany, NY) for partial funding of this research. The authors would also like to thank O-AT-KA Milk Products Cooperative (Batavia, NY) and Pall Corporation (Cortland, NY) for their support of this research. Additionally, the technical assistance of Michelle Bilotta, Sara Bova and Chassidy Coon from the Department of Food Science at Cornell University was greatly appreciated.

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CHAPTER 5

Microfiltration of skim milk and modified skim milk using a 0.1µm ceramic uniform transmembrane pressure system at temperatures of 50, 55, 60 and 65ºC

ABSTRACT

Increasing the temperature of microfiltration (**MF**) to > 50ºC 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 impact of operating a 0.1µm ceramic uniform transmembrane pressure MF unit at temperatures of 50, 55, 60 and 65ºC 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**) prior to MF at a flux of 54 kg/m^2 per h. 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±2% of the lactose, 15±2% of the calcium and 38±1% of the 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/m^2 per h and a concentration factor of 3X and the system run in recycle mode. The temperature of MF was increased in 5ºC steps from 50 to 65ºC, with a 1h stabilization period after each increase. During the run transmembrane pressure (**TMP**) was monitored and permeate and retentate samples were taken and analyzed to determine if there were any changes in SP,

calcium or phosphorus passage through the membrane. Increasing temperature of MF from 50 to 65°C at a flux of 54 kg/m^2 per h 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 TMP. Increasing the temperature to 65°C only caused a slight reduction in calcium concentration in the permeate (11 ± 3%) that was similar between the 2 MF feed types. Increasing processing temperature did reduce the percentage of SP removal by the process, but the increased temperature also caused a decrease in casein (**CN**) contamination in the permeate.

INTRODUCTION

Much of the research using microfiltration (**MF**) to separate serum protein (**SP**) and micellar casein (**CN**) in skim milk (**SM**) has been conducted at temperatures of 50°C to 55°C.While some research has looked at MF at lower temperatures (15°C) (Lawrence et al., 2008 and Samuelsson et al., 1997) temperatures above 55°C have not been explored. There are several possible advantages for operating at higher temperatures. 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 (**CN**). 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, there are possible disadvantages that 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 regards to temperature. Spiral-wound polymeric membranes typically have a 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^{o}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 SM ultrafiltrate (containing the mineral concentration of the permeate phase of SM, but no protein) at 55^{o}C, precipitation of calcium phosphate was seen and the amount precipitated increased as temperature increased (Spanos et al., 2007).

In SM it is not as clear if increasing the temperature in the range of 55^{o}C to 65^{o}C would cause calcium phosphate precipitation. Heating of milk at 60^{o}C for 1h caused a roughly a 30% decrease in the concentration of calcium and phosphorus in the permeate portion of milk isolated by ultrafiltration (**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 SM prior to 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^{o}C to 110^{o}C, 50 to 70% of the foulant was protein (30 to 40% mineral) and it was not until temperatures above 110^{o}C that 70 to 80% of the foulant was mineral (Bansal and Chen, 2006).

Increasing the temperature of MF from 50 to 65^{o}C might also cause denaturation of SP (β-lactoglobulin (**BLG**) and α-lactalbumin (**ALA**)). Long et al. (1963) found that only 3.4% of BLG associated with κ-CN after 20 min at 65^{o}C. Any

BLG covalently associated with the CN micelles would not be removed by MF. If BLG was covalently associated with κ-CN at higher temperatures, then the yield of SP in the permeate would be reduced. BLG and ALA have also been found to form aggregates with each other when heated at 75°C (Dalgleish 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 non-micellar 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 by MF.

The impact 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 there is no change in membrane resistance 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-Guiziou et al. (1999) found that as membrane resistance (fouling) increased transmission of SP decreased during the MF of SM.

If operation at elevated temperatures is not feasible with SM 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 SM prior to MF to minimize the impact of heat on fouling of MF membranes at temperatures higher than 50ºC. Our objective was to determine the impact of operating a 0.1µm ceramic UTP MF unit at temperatures of 50, 55, 60 and 65ºC on membrane fouling and SP removal from SM with and without removal of low molecular weight soluble milk components by UF prior to MF at a flux of 54 kg/m^2 per h.

MATERIALS AND METHODS

Experimental Design and Statistical Analysis

Each replicate consisted of 4 days of processing. On the first day approximately 1,000 kg of raw SM was pasteurized at 72ºC for 16 s, cooled to 4ºC and stored overnight. The SM was split into 2 portions. The first portion of milk was UF on the second day to reduce the low molecular weight solutes in SM by approximately 90%, the UF retentate was then diluted with reverse osmosis (**RO**) water to achieve the protein concentration of original SM. The second portion of SM was MF directly with no prior UF. In this way the impact 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 SM were microfiltered, respectively. The MF was operated at a concentration factor (**CF**) of 3X 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 65ºC. 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, 1999-2001, SAS Institute Inc., Cary, NC). A split plot model was used. Replicate and type of MF feed (i.e., DUR or SM) 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*replicate + temperature + temperature*feed + temperature*replicate +temperature*feed*replicate + temperature*temperature + temperature*temperature*feed + temperature*temperature*replicate + temperature*temperature*feed*replicate.

For the analysis of retentate and permeate composition measured by SDS-PAGE**,** only the 50^{o}C and 65^{o}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*replicate + temperature + temperature*feed + temperature*replicate. The type III mean sum of squares for feed*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 non-significant terms in the model were discarded one by one (if they did not appear in significant

terms) and the model was re-run 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 4ºC using a Model 372 Air Tight Centrifuge, (DeLaval Co., Chicago, IL), if the fat content of the SM contained more than 0.15% as measured using an infrared spectrophotometer (**IR**) (Lactoscope FTIR, Delta Instruments, Drachten, The Netherlands) the milk was re-separated before pasteurization. Raw SM 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 SM was cooled to 4°C and stored at ≤ 4°C until processing. The pasteurized SM was split into 2 portions; one portion was as ultrafiltered prior to MF as described below, the other portion was MF as SM.

Diluted UF Retentate. On the second day of processing about 540 kg of the pasteurized SM was UF to remove approximately 90% of the lactose and soluble minerals. The UF system was run in batch recirculation mode using a PES spiral wound UF membrane (Model 3838, GEA NIRO Inc., Hudson, WI; nominal molecular weight cutoff: 10,000 Da, surface area: 13.6 m^2). Before processing, the UF membrane was cleaned using the following procedure: first, the soak solution (0.26% vol/vol, Ultrasil MP, Ecolab Inc., Food and Beverage Division, St. Paul, MN) was flushed from the system until the flush water was at neutral pH. The membrane was then washed for 20 min at 276 kPa inlet pressure and no permeate back pressure with a combination of Ultrasil 110 liquid alkaline membrane cleaner (0.40% vol/vol,

Ecolab, Inc.), and XY-12 liquid sanitizer (0.15% vol/vol, Ecolab Inc.) diluted in 50°C RO water at a pH of 11.0 to 11.4. After the wash cycle was completed, the membrane system was flushed with 50°C RO water until neutral pH was obtained. The membrane was cooled below 24°C and sanitized with a solution of Ultrasil 110 liquid alkaline membrane cleaner (0.40% vol/vol) and XY-12 liquid sanitizer (0.15% vol/vol) at pH 11.0 to 11.4 and a chlorine level of 150 to 180 ppm. This solution was recirculated through the membrane for 10 min with 276 kPa of inlet pressure and no permeate back pressure. 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/m^2 per h) was measured by weight by collecting permeate for 30 s.

The SM was heated to 51°C before being transferred to the UF feed tank. The UF feed pump was started and approximately 20L 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, there was 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, Delta Instruments, Drachten, The Netherlands) to monitor retentate and permeate composition. The UF feed tank could only hold approximately 315 kg of SM, so as permeate was collected the remaining SM was added to the UF feed tank. When a CF of 2X had been achieved, the second stage (first diafiltration step) began. RO water

heated to 50ºC was 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 2X, when a 2X 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 the ratio of lactose to protein by IR (Lactoscope FTIR, Delta Instruments, Drachten, The Netherlands) > 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 SM as measured by IR. This diluted UF retentate then had the approximate protein concentration of SM, but with a greatly reduced lactose and soluble mineral concentration. The DUR was chilled to ≤ 4ºC and stored at ≤ 4ºC overnight.

Immediately after processing the UF system was cleaned. First, the UF system was rinsed with two 70 L portions of 50°C RO water at 276 kPa retentate inlet pressure and 124 kPa retentate outlet pressure with no permeate back pressure. During the second rinse, the recirculation pump was turned off and the inlet pressure was adjusted to 172 kPa to determine the fouled water flux, which was, on average 20% of the initial clean water flux (8.3 vs. 41.6 L/m^2 per h). Next, the membrane was washed for 30 min at 50ºC with a combination of Ultrasil 110 liquid alkaline membrane cleaner (0.40% vol/vol, pH 11.0 to 11.4) and Ultrasil 01 liquid high-surfactant cleaner

(0.08% vol/vol, Ecolab Inc.) at 276 kPa inlet pressure and 124 kPa retentate outlet pressure. These inlet and outlet pressures were used throughout all cleaning procedures. After the 30 min wash, the membrane was flushed to a neutral pH with 50°C RO water and then washed with a 50°C aqueous Ultrasil 76 liquid acid cleaner (0.30% vol/ vol, pH 1.9 to 2.2, Ecolab Inc.) for 30 min followed by a flush to a neutral pH with 50°C RO water. The membrane was then washed for 30 min at 50°C with a combination of Ultrasil 110 liquid alkaline membrane cleaner (0.40% vol/ vol, pH 11.0 to 11.4) and XY-12 liquid sanitizer (0.15% vol/ vol, chlorine 150 to 180 ppm) and flushed to a neutral pH with 50°C RO water. When the rinse water pH was neutral, the clean water flux (typically about 39.6 L/m^2 per h) was determined by operating only the feed pump with an inlet pressure of 172 kPa. After the clean water flux was determined, the membrane was cooled below 24°C with a 10 min recirculation (276 kPa inlet pressure) of a 24°C solution of Ultrasil 110 liquid alkaline membrane cleaner (0.40% vol/vol, pH 11.0 to 11.4) and XY-12 liquid sanitizer (0.15% vol/vol, 150 to 180 ppm chlorine). The membrane was then flushed with room temperature RO water to a neutral pH, followed by a 10 min recirculation of an Ultrasil MP soak solution (0.26% vol/vol, pH 3.5 to 4.0; Ecolab Inc.) that remained in the system until the next processing run.

Microfiltration Operation

A pilot scale UTP MF system (Tetra Alcross M7, TetraPak Filtration Systems, Aarhus, Denmark) equipped with a ceramic Membralox (EP1940GL0.1μA, 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 channel diameter. The permeate section of the stainless steel module was filled with polymeric beads (3.72 to 3.78 mm 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 as follows: with 50^{o}C RO water in the system and only the feed pump turned on, the retentate and permeate outlet valves were 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 ($\Delta TMP = TMP_i - TMP_o$) of 25 ± 3 kPa for a membrane length of 1.02m. 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°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. The following conditions were applied during the flux measurement: the retentate removal outlet valve was closed, and permeate outlet valve was fully open and only the feed pump running.

Processing: Diluted UF Retentate. The DUR (about 320 kg) was processed at a 3X CF (a 3X CF being 2 kg permeate removed for every 1 kg retentate) at 50, 55, 60 and 65°C using the UTP MF system described above. 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 there was a transition from water to DUR with all the pumps running, the retentate recirculation rate was approximately 648 L/min with a linear velocity of approximately 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 3X CF. If the ΔTMP 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 ΔTMP 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 1h. The flux (kg/m² per h) 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/m^2 per h was met. For example if the permeate removal rate was such that the flux was 54 kg/m^2 per h, but the CF was off target, then the retentate removal rate would be adjusted to achieve a 3X CF. Samples of permeate and retentate were taken every 15 min for analysis using an IR (Lactoscope FTIR, Delta Instruments, Drachten, The Netherlands) to monitor retentate and permeate composition. After 1h 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 regime used at 50°C. After 1 h at 55°C, a 15 min collection of retenate and permeate was done and composite samples were taken, as described previously. This procedure was repeated for 60°C and 65°C temperatures. During the entire run, as temperatures increased the diaphragm valve was adjusted as necessary to maintain a ΔTMP of 25 ± 3kPa. 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 above. SM (about 335 kg) was processed at a 3X CF at 50, 55, 60 and 65°C using the UTP MF system described above, using the same operating conditions and parameters as for the DUR. To flush the 50°C water out of the system with SM at the beginning of the process, about 116 kg of 50°C SM 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 L/h and 120 L/h, respectively. The MF system was flushed until no SM 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/m^2 per h). 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 L/h 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 post run 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 post-run clean water fluxes were close to pre-run clean water flux (about 860 to 830 L/m^2 per h). 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 L/h 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 SM, DUR, permeate, and retentate collected during processing were analyzed using an IR (Lactoscope FTIR, Delta Instruments) for fat, lactose and true protein content (Kaylegian et al., 2006). The MF feeds (DUR and SM) were analyzed for TS, fat and anhydrous lactose, using forced air oven drying (AOAC, 2000; method 990.20; 33.2.44), ether extraction (AOAC 2000; method 989.05; 33.2.26) and enzymatic lactose (AOAC 2000; method 984.15; 33.2.67) respectively. The DUR, SM, retentates and permeates were analyzed for total N (**TN**), and non protein nitrogen (**NPN**) content by Kjeldahl (AOAC, 2000; method 991.20; 33.2.11),

and (AOAC, 2000; method 991.21; 33.2.12), respectively. Noncasein nitrogen (**NCN**) content of DUR, SM and retentates was determined using a Kjeldahl method (AOAC, 2000; method 998.05; 33.2.64) modified in that 5.5mL of acetic acid (10% vol/vol) was added instead of 1 mL and 5.5 mL of sodium acetate (1N) instead of 1mL, to ensure that all of the CN was precipitated at the higher protein concentrations found in the retentates. True protein (**TP**) 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, SM, MF retentates and MF permeates were measured at Dairy One Forage Analysis Laboratory (Ithaca, NY) using a Thermo IRIS Advantage HX Inductively Coupled Plasma (ICP) Radial Spectrometer (Waltham, MA). The samples were prepared by predigesting a 5 g sample with 8 mL nitric acid and 2 mL hydrochloric acid for 15 min at 20^{o}C. The samples were then heated to 190^{o}C and held for 15 min using microwave digestion (CEM Microwave Accelerated Reaction System (Mathews, NC) with MarsXpress Temperature Control using 50 mL calibrated Xpress Teflon PFA vessels with Kevlar/fiberglass insulating sleeves) the samples were then diluted to 50 mL using a buffer consisting of 1.5N HNO3 and 0.5N 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 SM and DUR at 50 and 65^{o}C, the MF feeds (SM and DUR) were also analyzed. 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 10mM Tris-HCl pH 6.8, 1.0% SDS, 20% glycerol, and 0.02% bromophenol blue tracking dye and 50mM 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 (OD) of the predominant protein in the sample in the range of 1.0 to 1.4 OD. 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.

Serum Protein Removal Calculation

Percentage SP removal was calculated at each temperature using the following formula: 100 times 2/3 times the SP concentration in the permeate divided by the SP concentration in the MF feed. The value 2/3 is related to the CF, for each 1 kg of MF feed 2/3 kg of permeate is removed. For this calculation it was assumed that the CF remained constant at 3X.

RESULTS

Composition of Microfiltration Feeds

The composition of the SM and DUR prepared by UF of SM used as MF feeds is shown in Table 5.1. The UF process was expected to remove the low molecular weight components in the serum phase of SM 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 SM (Table 5.1). The concentration of lactose was reduced by 89.2 ± 0.2%. As expected, the concentration of fat in the MF feeds were similar ($P > 0.05$).

No difference in calcium concentration was ($P = 0.07$) among feeds was detected, but in each replicate the calcium concentration in the DUR was lower than in the SM, the phosphorus concentration was lower ($P < 0.05$) in the DUR than in the SM. The trend (P = 0.07) for reduction in calcium averaged 15 ± 2% and the

Table 5.1. Microfiltration feed composition: Skim milk (SM) and diluted ultrafiltration retentate (DUR) microfiltration feed composition for each of the 3 replicates. All values are percent by weight except, calcium and phosphorus which are given as mg per kg.

SM	TS[1]	Fat	Lactose	Ca[1]	P[1]	TN[1]	NPN[1]	NCN[1]	TP[1]	CN[1]	SP[1]	CN%TP[1]
Replicate 1	9.23	0.17	4.77	1063	1002	3.30	0.20	0.78	3.10	2.51	0.59	81.07
Replicate 2	9.10	0.10	4.77	1070	1016	3.30	0.19	0.77	3.11	2.53	0.58	81.31
Replicate 3	9.13	0.07	4.80	1015	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]	1049[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 ($P < 0.05$).

[1]TS: total solids, Ca: calcium (mg/kg), P: phosphorus (mg/kg), TN = total nitrogen x 6.38, NCN = noncasein nitrogen x 6.38, NPN = nonprotein nitrogen x 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 CN divided by TP.

reduction in phosphorus averaged 38 ± 1% for the DUR. The total reduction in calcium and phosphorus was less than the reduction in lactose, because only about 1/3 of the calcium and phosphorus in milk is soluble in the serum phase of SM. 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 concentration in the DUR was higher ($P < 0.05$) than the SM as shown in Table 5.1 and 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 5.1).

Microfiltration Process Control Parameters

Parameters Controlled During Microfiltration. For consistent operation, 4 processing parameters were controlled (Table 5.2). Flux was maintained at 54 kg/m^2 per h by controlling the permeate removal rate. The CF was set at 3X and controlled by changing the retentate removal rate. The retentate recirculation rate was kept constant at 648L/min by decreasing the retentate recirculation pump frequency using an inverter as temperature increased. Finally, the ΔTMP was controlled to 25 ± 3 kPa by using a diaphragm valve in the permeate recirculation loop to change the permeate recirculation rate.

Impact of Microfiltration Feed Type. The mean values for process control parameters for DUR and SM at each temperature are shown in Table 5.2. No effect of

the MF feed type on flux, CF, retentate recirculation rate or recirculation pump frequency was detected, as shown in Table 5.3.

Table 5.2. 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 h)	CF	Recirculation rate (L/min)	Recirculation pump frequency (Hz)
SM	50	53.5	3.04	647.2	59.0
SM	55	53.8	3.02	648.8	58.7
SM	60	54.2	3.00	648.2	58.0
SM	65	54.7	2.98	646.4	57.7
SM mean		54.0[a]	3.01[a]	647.6[a]	58.35[a]
DUR	50	53.0	3.03	647.5	58.6
DUR	55	53.2	3.03	648.3	58.2
DUR	60	54.0	3.04	647.4	57.7
DUR	65	54.5	3.05	647.9	57.3
DUR mean		53.7[a]	3.04[a]	647.8[a]	57.95[a]

[a-b] Means in the same column for each feed not sharing a common superscript are different ($P < 0.05$).

Impact of Microfiltration Temperature. During each run, the temperature was increased in 5°C steps. The actual temperatures averaged 50 ± < 0.1°C, 54.9 ± 0.2°C, 60.1 ± 0.5°C and 64.8 ± 0.6°C and did not vary with replicate or feed ($P > 0.05$). As temperature increased there was a slight increase in flux (Table 5.2). The increase in flux was similar for both feeds as confirmed by the fact that the feed by temperature interaction in Table 5.3 was not significant. If the permeate removal rate had not been controlled, the increase in flux as temperature increased would have been greater.

Table 5.3. Microfiltration processing control: ANOVA df and type III sum of squares to determine the impact of feed type (feed), replicate (rep) and temperature (temp) on flux, concentration factor (CF), recirculation rate and recirculation pump frequency. Temperature was transformed to a mean centered continuous variable.

Model term	df	Flux	CF	Recirculation rate	Recirculation pump frequency
Whole model	--	7.359*	0.054*	N.S.	6.865*
Whole plot					
Rep	2	0.356	0.0002	N.S.	0.207
Feed	1	0.641	0.0016	N.S.	0.961
Rep x feed[1]	2	0.272	0.0076*	N.S.	0.185*
Sub plot					
Temp	1	6.055*	0.0007	N.S.	5.627*
Feed x temp	1	N.S.	0.0078*	N.S.	N.S.
Rep x temp	2	N.S.	0.0093*	N.S.	N.S.
Temp x temp	1	N.S.	0.00004	N.S.	N.S.
Temp x feed x rep	2	N.S.	0.0020*	N.S.	N.S.
Temp x temp x rep	2	N.S.	0.0054*	N.S.	N.S.
Temp x temp x feed	1	N.S.	N.S.	N.S.	N.S.
Temp x temp x rep x feed	2	N.S.	0.0133*	N.S.	N.S.
Reduced model df		6	17	--	6
Reduced error df		17	6	--	17
R squared		0.72	0.98	--	0.96

*P-value < 0.05.

[1] used as whole plot error term for rep and feed.

Recirculation rate was independent ($P > 0.05$) of feed type and temperature (Tables 5.2 and 5.3). 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 5.2 and 5.3. 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

Impact of Microfiltration Feed Type. No impact of feed type ($P > 0.05$) on calcium, TN, NCN, TP, and CN concentration (Table 5.4) was detected, as shown in Table 5.5. The reason a difference in calcium concentration between SM and DUR was not detected was probably because much of the calcium in SM and DUR was associated with the CN micelles and CN concentration was similar between feeds ($P > 0.05$). Feed type had an impact ($P < 0.05$) with phosophorous and NPN concentrations being lower (consistent with the MF feed composition in Table 5.1) and SP being higher in the DUR retentate (Table 5.4). There was a feed type by temperature interaction ($P < 0.05$) with NCN (and SP) concentration in the MF retentates, with the NCN (and SP) content of the SM retentate increasing with temperature and the reverse happening for the DUR retentate.

Impact of Microfiltration Temperature. Temperature had a non-linear effect on calcium, phosphorus, TN, NCN, TP and CN concentration (Table 5.4), as shown by the significant temperature by temperature interactions (Table 5.5). From Table 5.4, it appears that initially as temperature increased the concentrations of calcium, phosphorus, TN, NCN, TP and CN decrease and then increase again as temperature continues to increase. NPN increases slightly with temperature ($P < 0.05$) as shown in Tables 5.4 and 5.5. There was a feed type by linear and feed type by quadratic temperature interaction on SP concentration with SP concentration in the SM 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 membranes. The change in TP, TN, NCN, SP and CN with temperature could be a result of changes in CF, rejection characteristics of the membranes, and the impact of heat on SP and their classification in the Kjeldahl analysis.

Table 5.4. 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°C.

Feed	Temperature (°C)	mg per kg		% by weight					
		Ca[1]	P[1]	TN[1]	NPN[1]	NCN[1]	TP[1]	CN[1]	SP[1]
SM	50	2919	2191	8.79	0.17	0.95	8.62	7.84	0.78
SM	55	2843	2137	8.52	0.18	0.97	8.34	7.56	0.78
SM	60	2832	2159	8.44	0.19	0.98	8.26	7.46	0.80
SM	65	2949	2187	8.74	0.19	1.02	8.55	7.72	0.84
SM mean		2886[a]	2166[b]	8.62[a]	0.18[a]	0.98[a]	8.44[a]	7.64[a]	0.80[b]
DUR	50	2792	1773	8.72	0.06	1.03	8.66	7.69	0.97
DUR	55	2648	1708	8.49	0.07	1.06	8.43	7.44	0.99
DUR	60	2711	1727	8.64	0.06	1.04	8.58	7.60	0.98
DUR	65	2753	1750	8.82	0.07	0.99	8.75	7.83	0.92
DUR mean		2726[a]	1739 [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 ($P < 0.05$).

[1]Ca: calcium (mg/kg), P: phosphorus (mg/kg), TN = total nitrogen x 6.38, NCN = noncasein nitrogen x 6.38, NPN = nonprotein nitrogen x 6.38, TP = true protein, (TN minus NPN), CN = casein, (TN minus NCN), SP = serum protein, (NCN minus NPN), SP/TP = SP divided by TP.

Table 5.5. Retentate composition: ANOVA df and type III sum of squares to determine the impact of feed type (feed), replicate (rep) and temperature (temp) on the concentration of calcium (Ca), phosphorus (P) true protein (TP), casein (CN) and serum protein (SP) in microfiltration retentates. Temperature was transformed to a mean centered continuous variable.

Model term	df	Ca[1]	P[1]	TN[1]	NPN[1]	NCN[1]	TP[1]	CN[1]	SP[1]
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 x feed[2]	2	67,386*	36,357*	0.802*	0.0002	0.01*	0.800*	0.645*	0.01*
Sub plot									
Temp	1	121	83.74	0.002	0.0005*	0.001*	0.0003	0.0002	<0.0001
Feed x temp	1	N.S.	N.S.	N.S.	N.S.	0.01*	N.S.	N.S.	0.009*
Rep x temp	2	N.S.	6,830*	0.211*	N.S.	N.S.	0.210*	0.217*	N.S.
Temp x temp	1	50,215*	11,403*	0.312*	N.S.	0.0007*	0.322*	0.351*	0.0003
Temp x feed x rep	2	38,581*	11,460*	0.324*	N.S.	N.S.	0.329*	0.372*	N.S.
Temp x temp x rep	2	26,103*	11,484*	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Temp x temp x feed	1	N.S.	N.S.	N.S.	N.S.	0.005*	N.S.	N.S.	0.006*
Temp x temp x rep x feed	2	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Reduced model df		14	14	12	6	9	12	12	9
Reduced error df		9	9	11	17	14	11	11	14
R squared		0.97	>0.99	0.90	0.99	0.98	0.90	0.90	>0.99

*P-value < 0.05.

[1]Ca: calcium (mg/kg), P: phosphorus (mg/kg), TN = total nitrogen x 6.38, NCN = noncasein nitrogen x 6.38, NPN = nonprotein nitrogen x 6.38, TP = true protein, (TN minus NPN), CN = casein, (TN minus NCN), SP = serum protein, (NCN minus NPN).

[2] used as whole plot error term for rep and feed.

Permeate Composition

Impact of Microfiltration 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 SM (Tables 5.6 and 5.7). Because DUR had some calcium and phosphorus removed by UF, a lower concentration of calcium and phosphorus in permeate from DUR than permeate from SM was expected. TN and NPN were both lower ($P < 0.05$) in DUR permeate than SM permeate (Tables 5.6 and 5.7), but the TP in was higher ($P < 0.05$) in the DUR permeate than the SM permeate. The DUR permeate was expected to have a lower concentration of NPN, because DUR feed had a lower concentration of NPN (Table 5.1). 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 5.1).

Table 5.6. Permeate composition: Mean (n=12) composition of permeate from microfiltration of skim milk (SM) and diluted ultrafiltration retentate (DUR).

	mg per kg		% by weight		
Feed	Calcium	Phosphorus	TN[1]	NPN[1]	TP[1]
SM	254[a]	401[a]	0.76[a]	0.20[b]	0.56[a]
DUR	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 uppercase superscript are different ($P < 0.05$).

TN = total nitrogen x 6.38, NPN = nonprotein nitrogen x 6.38, TP = true protein, (TN minus NPN).

Impact of Microfiltration Temperature. Calcium concentration in the permeate decreased ($P < 0.05$) as temperature increased (Figure 5.1 and Table 5.7). The feed by temperature interaction term was not significant (Table 5.7), indicating that the decrease in calcium in the permeate with increase in temperature did not

depend on the feed type (Figure 5.1). If increasing the temperature of MF to 65ºC when SM 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 SM with increasing temperature than from DUR). If this had happened, then the temperature by feed interaction in Table 5.7 for calcium would have been significant. There did not appear to be calcium phosphate precipitation as temperature increased for either DUR or SM 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 SM was the feed (Figure 5.2), as shown by the significant feed by temperature interaction (Table 5.7), but the magnitude of the decrease in phosphorus (in permeate from DUR feed) with temperature was small.

As temperature increased, there was a non-linear (i.e., temperature by temperature interaction) decrease in TN and TP in the permeate (Figure 5.3 and Table 5.7). The decrease in TP (Table 5.7) as temperature increased had a slight dependence of feed type ($P < 0.05$) (significant temperature by feed interaction). As seen in Figure 5.3, the largest decrease in TP concentration in the permeate occurred when the temperature was increased from 60 to 65ºC and the decrease in TP was larger when the MF feed was DUR than when the feed was SM. The decrease in TP concentration in the permeate as temperature increased could be due to several factors including:

membrane fouling, BLG denaturation and association with CN micelles at higher temperatures or a decrease in CN concentration in the permeate.

Table 5.7. Permeate composition: ANOVA df and type III sum of squares to determine the impact of feed type (feed), replicate (rep) and temperature (temp) on the concentration of calcium (Ca), phosphorus (P) and true protein (TP) in microfiltration permeates. Temperature was transformed to a mean centered continuous variable.

Model term	df	Ca[1]	P[1]	TN[1]	NPN[1]	TP[1]
Whole model	--	128,329*	512,385*	0.069*	0.14*	0.0814*
Whole plot						
Rep	2	309.25	43.75	0.002	0.0003	0.0009
Feed	1	127,022*	511,730*	0.03*	0.14*	0.04*
Rep x feed[2]	2	54.25	8.58	0.002*	0.0001	0.0017*
Sub plot						
Temp	1	943.8*	49.43	0.028*	N.S.	0.0308*
Feed x temp	1	N.S.	561.47*	N.S.	N.S.	0.001*
Rep x temp	2	N.S.	N.S.	N.S.	N.S.	N.S.
Temp x temp	1	N.S.	N.S.	0.007*	N.S.	0.0073*
Temp x feed x rep	2	N.S.	N.S.	N.S.	N.S.	N.S.
Temp x temp x rep	2	N.S.	N.S.	N.S.	N.S.	N.S.
Temp x temp x feed	1	N.S.	N.S.	N.S.	N.S.	N.S.
Temp x temp x rep x feed	2	N.S.	N.S.	N.S.	N.S.	N.S.
Reduced model df		6	7	7	5	8
Reduced error df		17	16	16	18	15
R squared		>0.99	>0.99	0.96	>0.99	0.96

*P-value < 0.05.

[1]Ca: calcium (mg/kg), P: phosphorus (mg/kg), TN = total nitrogen x 6.38, NPN = nonprotein nitrogen x 6.38, TP = true protein, (TN minus NPN).

[2] used as whole plot error term for rep and feed.

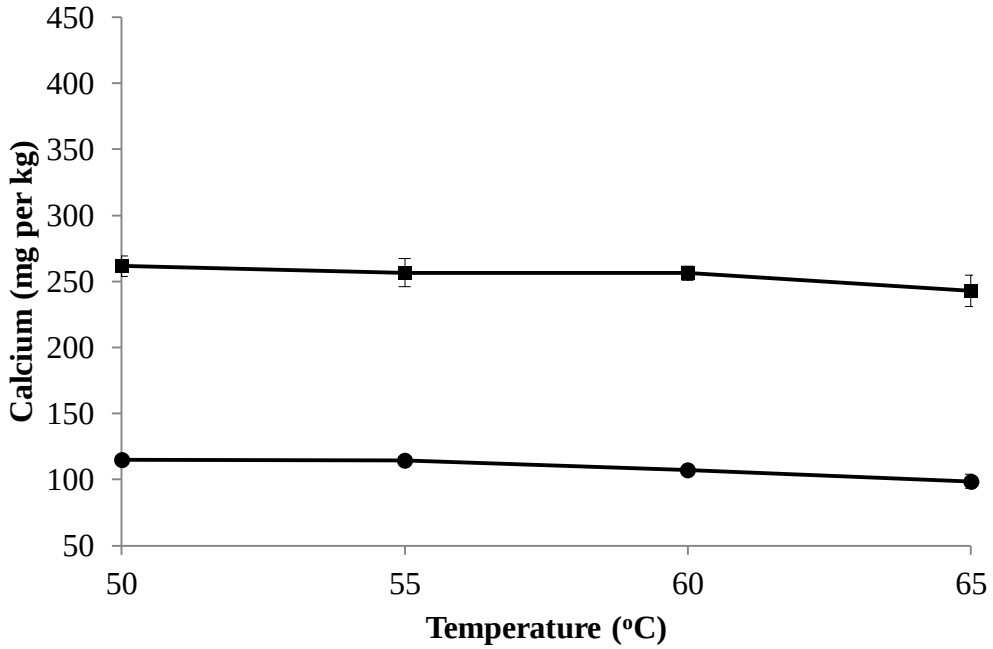

Figure 5.1. Permeate composition: mean (n=3) calcium concentration in the permeate (skim milk (■) and diluted ultrafiltration retentate (DUR) (●))

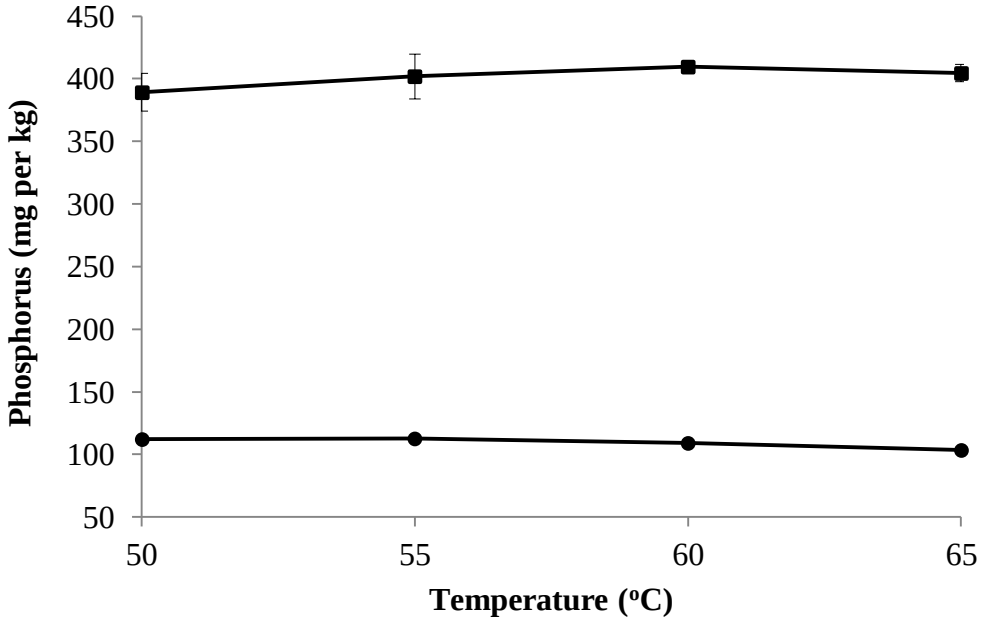

Figure 5.2. Permeate composition: mean (n=3) phosphorus concentration in the permeate (skim milk (■) and diluted ultrafiltration retentate (DUR) (●))

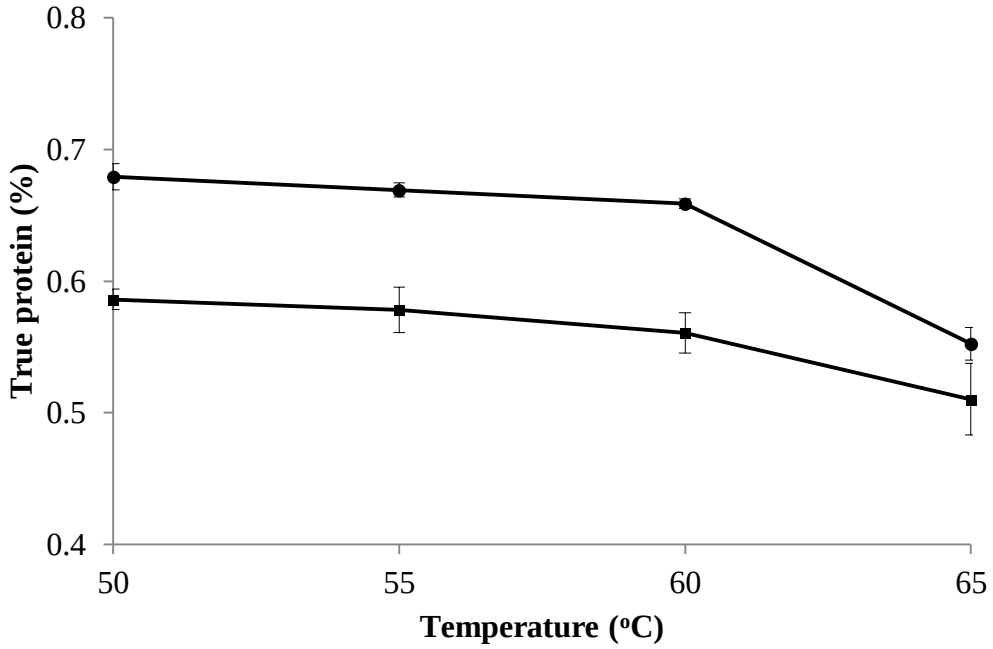

Figure 5.3. Permeate composition: mean (n=3) true protein concentration in the permeate (skim milk (■) and diluted ultrafiltration retentate (DUR) (●))

Table 5.8. 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)			ΔTMP[2] (kPa)
		retentate	permeate	TMP[1]	retentate	permeate	TMP[1]	
SM	50	390.1	378.1	38.5	236.7	216.0	15.0	23.3
SM	55	385.9	374.9	37.3	236.5	218.3	12.6	24.7
SM	60	377.3	372.3	31.3	236.7	222.9	8.2	23.1
SM	65	372.3	368.1	30.6	236.7	226.3	4.7	25.9
SM 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
DUR	55	374.5	363.9	32.9	232.3	218.3	9.4	23.5
DUR	60	367.7	359.9	30.1	233.0	221.1	7.2	22.9
DUR	65	362.1	357.3	27.1	232.7	225.0	3.0	24.1
DUR mean		371.0[b]	362.0[b]	31.3 [a]	232.4[a]	219.7 [a]	8.07[a]	23.2[a]

[1]TMP= transmembrane pressure.

[2] ΔTMP = TMP at the inlet minus TMP at the outlet.

[a-b]Means in the same column for each feed not sharing a common superscript are different ($P < 0.05$).

Impact of Feed Type and Temperature on Fouling

The MF retentate inlet and outlet pressures at each temperature are shown in Table 5.8. The retentate inlet pressure was lower ($P < 0.05$) for DUR feed than for SM feed (Table 5.9). Although the pressures differed between SM 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 5.9) was detected ($P > 0.05$). As temperature increased the recirculation pump frequency was decreased (Tables 5.2 and 5.3), and as a consequence the pressure at the retentate inlet decreased (Table 5.8). The pressure at the retentate outlet remained unchanged ($P > 0.05$) as temperature increased (Tables 5.8 and 5.9).

On the permeate side of the membrane, the inlet pressure was higher ($P <0.05$) for the SM feed than the DUR feed (Tables 5.8 and 5.9), as expected due to the similar pressure difference between SM and DUR on the retentate side of the membrane. The decrease in permeate inlet pressure as temperature increased ($P<0.05$) (Table 5.8 and 5.9), was in part due to adjustments made to the permeate recirculation rate to maintain a constant ΔTMP. If no changes in processing parameters (Table 5.2) were made, increasing the MF temperature would have caused the ΔTMP 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 ΔTMP 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 ΔTMP. This caused a decrease in the permeate pressure at the inlet, increasing TMP inlet and

Table 5.9. Microfiltration processing pressures: ANOVA df and type III sum of squares to determine the impact of feed type (feed), replicate (rep) and temperature (temp) on the permeate and retentate pressures at the inlet and outlet ends of the membrane. Temperature was transformed to a mean centered continuous variable.

		Inlet			Outlet			ΔTMP[1]
Model term	df	Retentate	Permeate	TMP[1]	Retentate	Permeate	TMP[1]	
Whole model	--	1814*	1218*	367.6*	202.6*	415.2*	420.8*	N.S.
Whole plot								
Rep	2	4.4	133.0 *	23.5	39.5	32.5	10.8	N.S.
Feed	1	666.9 *	780.4 *	58.8	107.5	7.4	27.0	N.S.
Rep x feed[2]	2	41.2*	1.0	58.9*	55.5*	9.3*	60.1*	N.S.
Sub plot								
Temp	1	1113*	319.4*	234.9*	N.S.	368.7*	329.6*	N.S.
Feed x temp	1	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Rep x temp	2	22.0 *	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Temp x temp	1	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Temp x feed x rep	2	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Temp x temp x rep	2	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Temp x temp x feed	1	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Temp x temp x rep x feed	2	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Reduced model df		8	6	6	5	6	6	--
Reduced error df		15	17	17	18	17	17	--
R squared		0.98	0.99	0.89	0.97	0.96	0.96	--

*P-value < 0.05.

[1]TMP= transmembrane pressure, ΔTMP = TMP at the inlet minus TMP at the outlet.

[2] used as whole plot error term for rep and feed.

ΔTMP. A consistent ΔTMP was achieved (Tables 2.8 and 2.9) and ΔTMP was not a function of feed type or temperature ($P > 0.05$).

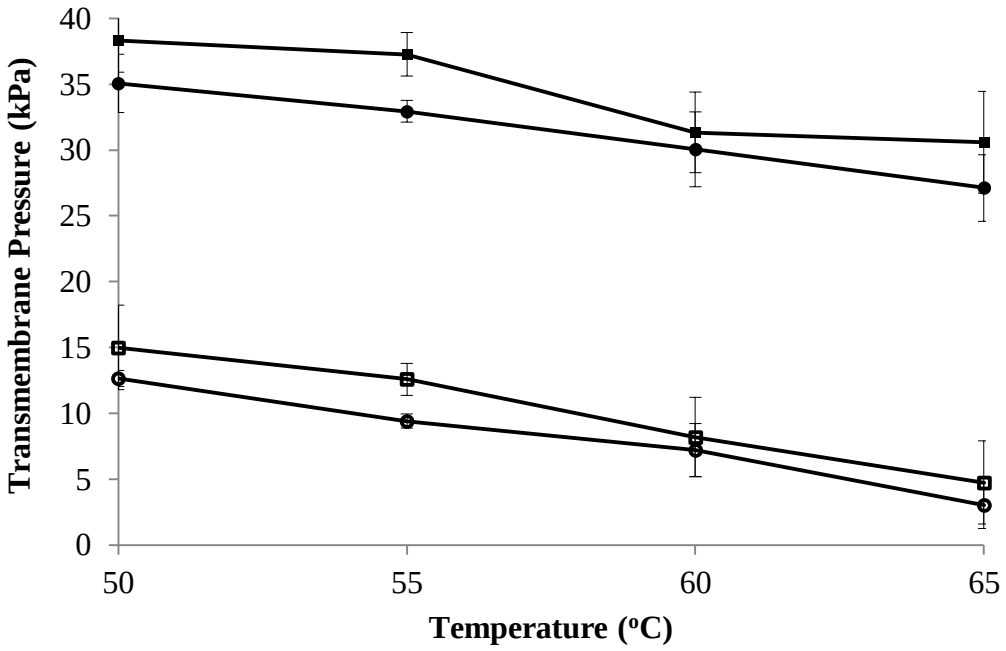

Figure 5.4. Mean (n=3) transmembrane pressure at the inlet (skim milk (■) and diluted ultrafiltration retentate (DUR) (●)) and transmembrane pressure at the outlet (skim milk (□) and DUR (○))

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 h would increase. In this work the TMP at both the inlet and outlet decreased ($P < 0.05$) as temperature increased (Tables 5.8 and 5.9) with the decrease being similar for both feed types (non-significant feed by temperature interaction). The decrease in TMP at the inlet and outlet as temperature increased (Figure 5.4) 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 65ºC caused more fouling of the MF membranes with either SM 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 Serum Protein Removal

Serum Protein Removal Calculated Using Kjeldahl Data. The calculated SP removal is shown in Table 5.10. 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 assumes that all of the TP in the permeate is 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 5.11). As temperature increased, there was a non-linear (significant temperature by temperature term) decrease ($P < 0.05$) in SP removal (Tables 5.10 and 5.11). The decrease in SP removal as temperature increased mirrors the decreasing TP concentration in the permeate (Figure 5.3) as temperature increased. For each MF feed type, the percentage SP removal is relatively constant until 65°C (Table 5.10). 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 SP removal (Table 5.11) indicating that temperature had the same impact regardless of MF feed type.

To determine if the decrease in SP removal was due to BLG 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.

Table 5.10. 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, 65°C.

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

[a-b]Means in the same column for each feed not sharing a common superscript are different ($P < 0.05$).

Table 5.11. Percentage of serum protein removal: ANOVA df and type III sum of squares for serum protein removal as a function of feed type (feed), replicate (rep) and temperature (temp). Temperature was transformed to a mean centered continuous variable.

Model term	df	Serum protein removal
Whole model	--	457.65*
Whole plot		
Rep	1	41.26*
Feed	2	0.982
Rep x feed[1]	2	1.091
Sub plot		
Temp	1	340.71*
Feed x temp	2	N.S.
Rep x temp	1	N.S.
Temp x temp	2	84.07*
Temp x temp x rep	2	N.S.
Temp x temp x rep	1	N.S.
Temp x temp x feed		N.S.
Temp x rep x feed	2	N.S.
Reduced model df		7
Reduced error df		16
R squared		0.92

*P-value < 0.05.
[1] used as whole plot error term for rep and feed.

SDS-PAGE Analysis of the Retentates. The DUR retentates had a higher (P < 0.05) proportion of SP than SM retentates (Table 5.12 and 5.13) (which was consistent with the trend towards a lower CN as a percentage of TP seen in the DUR feeds in Table 5.1). 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 SM 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 time at 50°C than for the SM feed. No effect of feed type or temperature on BLG to ALA ratio was detected ($P > 0.05$) indicating that higher temperature was not causing a change in association of BLG with CN micelles.

Table 5.12. Retentate SDS-PAGE: Mean (n=3) serum protein (SP) as a percentage of protein in the retentates from microfiltration with skim milk (SM) and diluted ultrafiltration retentate (DUR) as feeds at 50 and 65°C.

Feed	Temperature (°C)	SP (% of protein)	Casein hydrolysis product	β-lactoglobulin/ α-lactalbumin
SM	50	9.38	3.23	5.22
SM	65	12.36	3.73	5.07
SM mean		10.87[b]	3.48[b]	5.15[a]
DUR	50	13.35	7.08	5.13
DUR	65	16.75	7.65	5.62
DUR mean		15.05[a]	7.37[a]	5.37[a]

[a-b]Means in the same column for each feed not sharing a common superscript are different ($P < 0.05$).

Table 5.13. Retentate SDS-PAGE: ANOVA df and type III sum of squares to determine the impact of microfiltration feed type (feed), replicate (rep) and temperature (temp) on the relative proportion of α-lactalbumin and β-lactoglobulin, casein hydrolysis products and ratio of β-lactoglobulin to α-lactalbumin (as determined by SDS-PAGE) in the microfiltration retentates.

Model term	df	SP (% of protein)	Casein hydrolysis product	β-lactoglobulin/ α-lactalbumin
Whole model	--	90.49*	51.49*	N.S.
Whole plot				
Rep	2	4.18	3.99	N.S.
Feed	1	52.50*	45.24*	N.S.
Rep x feed	2	1.14	1.40*	N.S.
Sub plot				
Temp	1	30.40*	0.85*	N.S.
Feed x temp	1	N.S.	N.S.	N.S.
Temp x rep	2	2.27*	N.S.	N.S.
Reduced model df		8	6	--
Reduced error df		3	5	--
R squared		>0.99	>0.99	--

*P-value < 0.05.

[1] used as whole plot error term for rep and feed.

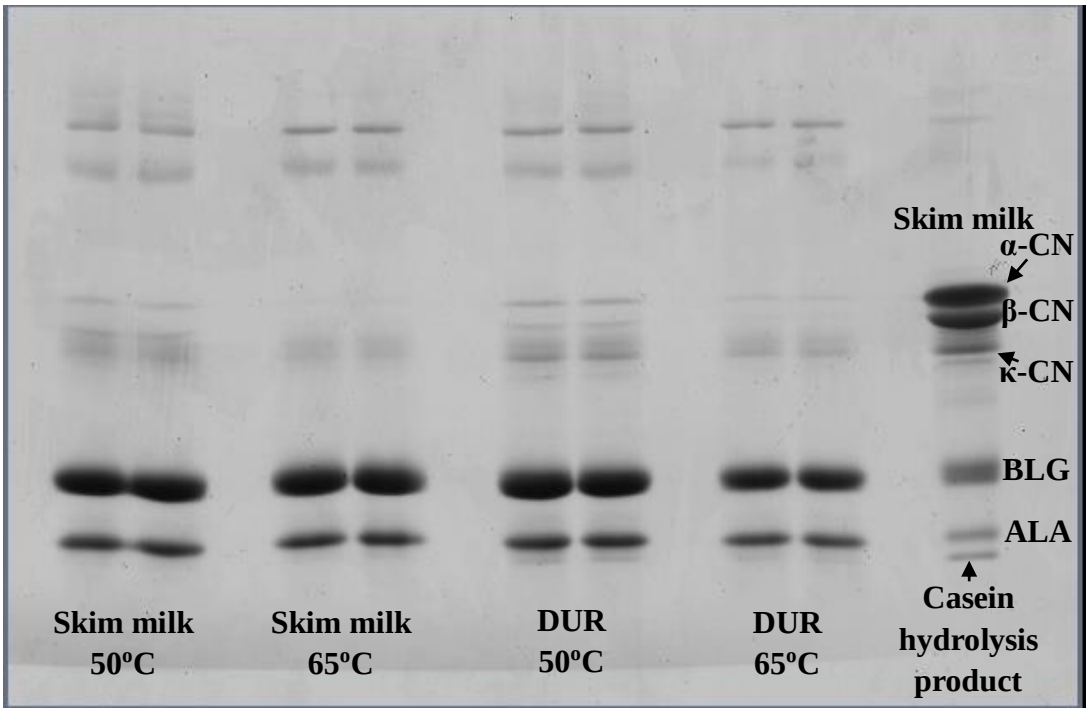

Figure 5.5. SDS PAGE gel image of the microfiltration permeates at 50ºC and 65ºC with both the skim milk and diluted ultrafiltration retentates (DUR) used as the microfiltration feed
α-CN = α-casein ,β-CN = β-casein ,κ-CN = κ-casein, BLG = β-lactoglobulin, ALA = α-lactalbumin

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 65ºC) is shown in Figure 5.5. Permeate from a SM feed had a slightly higher ($P < 0.05$) BLG to ALA ratio than permeate from a DUR feed (Tables 5.14 and 5.15), but no change in the ratio as temperature increased was detected ($P > 0$ .05) for either feed type. If BLG was associating with CN micelles at higher temperatures, we would have expected the ratio of BLG to ALA to decrease as temperature increased. From the SDS-PAGE analysis of the permeates it does not appear that BLG was associating with CN micelles at the higher MF processing temperatures in this study.

The proportion of CN in the permeate from SM 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 there was a trend ($P = 0.07$) for a lower proportion of CN in the permeates from SM compared to DUR (Tables 5.14 and 5.15). 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. 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 SPs 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 SP removal would be larger at 50°C than 65°C, because the relative proportion of CN in the permeate decreased as temperature increased. Therefore, the actual decrease in percentage SP removal with increasing temperature was not as large as indicated in Table 5.10.

Table 5.14. Permeate SDS-PAGE: Mean (n=3) casein (CN as a percentage of protein) and ratio of β-lacotglobulin divided by α-lactalbumin in the permeates from microfiltration with skim milk (SM) and diluted ultrafiltration retentate (DUR) as feeds at 50 and 65ºC.

Feed	Temperature (ºC)	CN (% of protein)	β-lactoglobulin/ α-lactalbumin
SM	50	5.37	3.15
SM	65	2.53	3.14
SM mean		3.95[a]	3.14[a]
DUR	50	9.32	3.13
DUR	65	6.25	2.93
DUR mean		7.78[a]	3.03[b]

[a-b]Means in the same column for each feed not sharing a common superscript are different ($P < 0.05$).

Table 5.15. Permeate SDS-PAGE: ANOVA df and type III sum of squares to determine the impact of microfiltration feed type (feed), replicate (rep) and temperature (temp) on the ratio of β-lactoglobulin to α-lactalbumin and casein (as determined by SDS-PAGE) in the microfiltration permeates.

Model term	df	Casein (% of protein)	β-lactoglobulin/ α-lactalbumin
Whole model	--	100.1*	0.80*
Whole plot			
Rep	2	23.0	0.76*
Feed	1	44.1	0.04*
Rep x feed[1]	2	6.9	0.001
Sub plot			
Temp	1	26.1*	N.S.
Feed x temp	1	N.S.	N.S.
Temp x rep	2	N.S.	N.S.
Reduced model df		6	5
Reduced error df		5	6
R squared		0.96	0.89

*P-value < 0.05.

[1] used as whole plot error term for rep and feed.

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 Kjeldhal analysis and the relative quantity of CN as determined by SDS-PAGE analysis. Using SDS-PAGE analysis of the permeates for a rough estimation of the concentration of CN in the permeate at 50°C was approximately 0.03 ± 0.005% and 0.06 ± 0.02% for SM and DUR MF feeds, respectively. Because the decrease in TP in the permeates was 0.08 ± 0.02% and 0.13 ± 0.01% for SM 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°C to 65°C). So although CN concentration in the permeates is decreasing the concentration of SP in the permeates is probably decreasing 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 SM that has been UF to reduce the concentration of soluble calcium and phosphorus (a DUR). In this work two MF feeds were used: a SM and a DUR with 15 ± 2% of the calcium and 38 ± 1% of the phosphorus removed.

It was found that increasing the temperature of MF from 50 to 65ºC decreased the TMP required to maintain a flux of 54 kg/m^2 per h regardless of whether the MF feed was SM 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 65ºC 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 SM and processing with DUR at higher temperatures would result in lower levels of calcium phosphate precipitation. However, there was only a slight decrease in calcium concentration in the permeate (11 ± 3%) as temperature increased to 65ºC and a similar decrease was seen with both feed types (Figure 5.1). Additionally, the concentration of phosphorus in the permeate did not decrease as temperature increased when the MF feed was SM (a slight decrease was seen when DUR was the MF feed). Calcium phosphate precipitation does not appear to cause membrane fouling when operating a MF process at temperatures up to 65ºC. 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 65ºC (with a similar decrease found for both SM 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 BLG association with CN micelles at higher temperatures, but a change in the ratio of BLG to ALA 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 temperate of MF from 50 to 65°C when using 0.1µm ceramic membrane in a UTP process at a flux of 54 kg/m^2 per h did not produce a large increase in membrane fouling, when using either SM 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 SM may provide benefits in controlling microbial growth by using higher operating temperatures during long processing times 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 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 was greatly appreciated.

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CHAPTER 6

Conclusions and future work

Microfiltration (**MF**) can be used to separate the micellar casein (**CN**) from serum protein (**SP**) in skim milk (**SM**). The micellar CN would remain in the MF retentate and be a micellar CN concentrate (**MCC**) and part of the SP would be removed in the permeate. More filtration and diaflitration stages in the process will increase the percentage of total SP removal with the goal of removing about 95% of the SP. The MF permeate could be further processed using ultrafiltration (**UF**) to produce SP concentrates (**SPC**). Both the MCC and SPC could be commercially valuable products. A MF process to produce a MCC should be designed and operated to minimize the cost of MCC production. A number of variables and operating parameters could have an impact on the cost of a MF process.

The objective of this research was to improve the commercial feasibility of a MF process designed to produce a MCC by reducing fixed and variable costs. As a first step a theoretical model was developed to identify the factors that could impact the MF membrane surface area need to process a target mass of milk in a fixed period of time. The MF feed, number of stages and flux were all factors that had an impact on the MF membrane area and should be taken into consideration when designing a MF system to produce a 95% SP reduced MCC. Feeding the MF process with a diluted UF retentate (**DUR**) diluted to the true protein (**TP**) concentration of skim milk (**SM**), as opposed to SM reduced the membrane area required to product a 95% SP reduced MCC by 36% for a 5-stage process. When DUR was the MF feed, there was an optimal feed TP

concentration that depended on the number of MF stages. The TP concentration in the DUR that minimized the required MF membrane area was: 2.47%, 3.85%, 4.77% and 5.41% for a 2, 3, 4 or 5 stage MF process respectively. For a 5 stage process increasing the TP concentration of the diluted DUR feed from 3.2 to 5.4% decreased the required MF membrane area by 10%. It was also found that as the number of stages increased from 2 to 5 the required MF membrane area decreased by 39%, when the MF feed was a DUR at the optimal feed TP concentration. Finally, increasing the flux from 50 to 60 kg/m^2 per h decreased the required MF membrane area by 17% when the MF feed was DUR at the optimal MF feed TP concentration.

The recirculation loop TP concentration was a factor that was found to have an impact on the required membrane area in the theoretical work, with increasing TP concentration reducing the required membrane area (at a constant flux). However, the recirculation loop TP concentration would also have an impact on the flux that the system could operate at, with higher fluxes possible at lower TP concentrations. The optimal target recirculation loop TP concentration would depend on the relationship between flux and TP concentration in the recirculation loop assuming that SP removal did not change with increasing flux or TP concentration. Additionally, membrane channel diameter (**CD**) could have an impact on limiting flux and SP removal.

In the next experiments the limiting flux as a function of recirculation loop TP concentration was determined using 0.1 µm ceramic graded permeability (**GP**) membranes with either 3 mm or 4 mm CD with a diluted milk protein concentrate (**MPC**) as the MF feed. For the 4 mm CD membranes the limiting flux was: 154 ± 0.3,

133 ± 0.7 and 117 ± 3.3 kg/m^2 per h at recirculation loop TP concentrations of 8.2 ± 0.07, 9.2 ± 0.04 and 10.2 ± 0.09% respectively. No impact of recirculation loop TP concentration on the SP removal factor was detected ($P > 0.05$). However, the SP removal factor decreased from 0.80 ± 0.02 to 0.75 ± 0.02 as flux was increased from the starting flux of 55 kg/m^2 per h to the limiting flux, with a similar decrease seen at all recirculation loop TP concentrations.

The limiting flux was lower ($P < 0.05$) at each target TP concentration on the 3 mm than the 4 mm CD membranes which averaged: 128 ± 0.3, 109 ± 4, 97 ± 0.5 kg/m^2 per h at 8.1 ± 0.07, 9.2 ± 0.04 and 10.2 ± 0.03% TP in the recirculation loop for 3 mm membranes, respectively. The SP removal factor was also lower for the 3 mm CD membranes ($P < 0.05$) than the 4 mm CD, decreasing from 0.72 ± 0.02 to 0.67 ± 0.01 as the flux increased from the starting to the limiting flux. As with the 4 mm CD membranes, no impact of the recirculation loop TP concentration on the SP removal factor was detected ($P > 0.05$). Although the limiting flux was lower for the 3 mm CD membranes at all recirculation loop TP concentrations. However, because the 3 mm CD membranes had 46% more membrane area per module the permeate removal rate per module was higher for the 3 mm CD membrane. A MF system designed using 4 mm CD membranes would require less membrane area than a system with 3 mm CD membranes, but a greater number of modules would be required for the 4 mm system than the 3 mm system.

Finally, as found in the theoretical work, increasing the flux decreased the required membrane area. One possible way of increasing the flux could be to increase

the temperature of MF. However, with SM there was a concern that increasing the temperature of MF might cause calcium precipitation and membrane fouling. Our objective was to determine the impact of operating a 0.1 µm ceramic uniform transmembrane pressure (**UTP**) 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 UF prior to MF at a flux of 54 kg/m^2 per h. For each replicate 1,000 kg of pasteurized SM was split into 2 batches. One batch was ultrafiltered (with diafiltration) to remove an average of 89 ± 2% of the lactose, 15 ± 2% of the calcium and 38 ± 1% of the phosphorus. The retentate from UF was diluted back to the TP concentration of SM, creating a DUR. On subsequent days both the DUR and SM were run on the MF unit with the flux maintained at 54 kg/m^2 per h and a concentration factor of 3X 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 (**TMP**) was monitored and permeate and retentate samples were taken and analyzed to determine if there were any changes in SP, calcium or phosphorus passage through the membrane. Increasing temperature of MF from 50 to 65°C at a flux of 54 kg/m^2 per h did not produce a large increase in membrane fouling, measured by changes in TMP, when using either SM or a DUR as the MF feed. Increasing the temperature to 65°C only caused a slight reduction in calcium concentration in the permeate (11 ± 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 CN contamination in the permeate.

These findings open up interesting avenues for further research. The next step in exploring the impact of temperature on flux is to determine the limiting flux as a function of MF temperature and to examine whether microbiological quality of the MCC is improved by operation at temperatures higher than 50°C. From practical experience in operating a MF system, as the limiting flux is approached, the TMP required to maintain a flux becomes very sensitive to changes in temperature. It is hypothesized that increasing the temperature of MF even by 5°C could lead to substantial increases in the limiting flux.

In determining the limiting flux on the 3 mm and 4 mm CD membranes, several questions arose, which could be topics for further research. The limiting fluxes found when diluted MPC was the MF feed were much higher than expected from preliminary work using SM as the MF feed. Additional research could confirm that this is indeed the case and explore what factors might be causing the higher limiting flux. Another avenue of research could focus on the impact of CD on limiting flux. In the determination of the limiting flux on the 3 mm and 4 mm CD membranes, it was found that CD did not appear to have an impact on limiting flux. In the experiments determining the limiting flux, cross-flow velocity was a function of CD, and the impact of CD could not be completely separated from the impact of cross-flow velocity. Determining the limiting flux at a constant cross-flow velocity on the 3 mm and 4 mm membranes would provide insight into whether CD had an independent impact on limiting flux.

Also, since it was found that limiting flux was higher with increasing CD, investigation of a larger CD diameter MF membrane (i.e., CD = 6 mm) might allow

higher recirculation loop TP concentration. A higher recirculation loop TP concentration could be useful as an MF finishing stage to produce a MCC with up to 13% TP that would allow more flexibility in high protein beverage formulation.