

**SERUM PROTEIN REMOVAL FROM SKIM MILK USING POLYMERIC SPIRAL-
WOUND MICROFILTRATION MEMBRANES**

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ABSTRACT

There is interest in membrane fractionation of milk to create novel products with wide-ranging use in food and non-food applications. Various pressure-driven membrane processes are utilized by the dairy industry to create novel food ingredients, including microfiltration (**MF**), ultrafiltration (**UF**), nanofiltration (**NF**), and reverse osmosis (**RO**), in order of decreasing pore size. Microfiltration of skim milk to fractionate casein (**CN**) and serum proteins (**SP**) is the newest application of filtration to dairy fluids. Removing **SP** from skim milk using **MF** creates a micellar **CN** concentrate (**MCC**) that has unique functional properties and can be used as a new ingredient in with unique functional properties food product development. Two primary **MF** membrane materials and configurations are currently used for **SP** removal from skim milk, tubular ceramic and polymeric spiral-wound (**SW**) membranes with pore sizes ranging from 0.1 to 0.5 μm . As with most membrane processes on complex fluids, fouling and flux decline are monitored by the processor to ensure optimal system efficiency. Ceramic membranes, which have been studied more than **SW** membranes, achieve theoretical **SP** removal and achieve high flux (e.g., 54 kg/m^2 per h). Polymeric **SW** **MF** membrane use for **SP** removal has been studied less than ceramic membranes, but they are lower cost and can contain more membrane surface area per unit floor space than ceramic membranes, making them an attractive alternative that warrants further study.

The first objective of our research was to determine the process necessary to create a 95% **SP** reduced **MCC** using a 0.3 μm polyvinylidene fluoride (**PVDF**) polymeric **SW** **MF** at 50°C and compare the efficiency of the **SW** process to theoretical values and to a 0.1 μm ceramic uniform transmembrane pressure (**UTP**) **MF** membrane process. A three-stage, 3.00 \times concentration factor, **MF** diafiltration (i.e., dilute with filtered water) process was employed and

SP removal at each stage was quantified. Permeate flux was low and increased, 14.4, 22.1, 32.6 kg/m² per h, from stage 1 to 3, respectively. Skim SP removal for stage 1 to 3 were 38.6, 20.8, and 10.9%, respectively, and were cumulatively lower than theoretical, 70.3 vs. 97.0%. It was estimated an additional 5 stages (i.e., a total of 8) would be necessary to achieve 95% removal of SP with polymeric SW membranes. Research to improve SP removal from skim milk using PVDF SW MF membranes would allow more efficient and cost effective production of MCC.

Casein in skim milk is the primary foulant of PVDF SW MF membranes. Previously, we observed that flux was higher when the concentration of CN in feed material was lower (i.e., low CF). The second objective of our research was to determine the impact of CF, 3.00, 2.25 and 1.50× on the removal of SP from skim milk during PVDF SW MF at 50°C using a 0.3 μm pore size membrane. Flux increased, 12.8, 15.3, and 19.0 kg/m² per h, with decreasing CF. However, SP removal also decreased, 35.6, 24.3, and 10.6%, as CF decreased from 3.00 to 2.25, and 1.50×, respectively, which was unexpected. The rate of SP removal per unit membrane surface area was relatively constant, 0.036, 0.039, and 0.039 kg/m² per h, among CF. These results led us to believe that the rate at which the concentration of solute near the membrane surface changes during startup is a significant factor in the deposition of foulant on the membrane surface and the foulant changes SP rejection. At low CF, the rate of concentration change and foulant layer formation is slower, which could cause more pore plugging by casein micelles and restrict passage of SP but not solvent (i.e., water) than at high CF. Controlling the startup procedure and foulant deposition onto the membrane could lead to more efficient removal of SP from skim milk using polymeric SW MF membranes.

BIOGRAPHICAL SKETCH

Steven Lawrence Beckman was born in Norfolk, NE in 1985 to parents Marilyn and Bryant Beckman. At the age of five, Steve, his two sisters, Ann and Jill, and his parents moved to Lincoln, NE, where he still calls home. Steve graduated from Lincoln East High School in 2003 with aspirations to attend a university to study science. In the fall of 2003, he matriculated at the University of Nebraska-Lincoln (UNL) in Food Science and Technology. As a Food Science student, he gained additional knowledge by working in the Food Processing Center Dairy Plant at UNL. Steve enjoyed making ice cream, cheese, and other dairy products that were sold on campus. Internships completed during his undergraduate tenure included work as a sensory intern for Wells' Dairy Inc. in Le Mars, IA, and work as a product development intern for Nestlé U.S.A. in Denver, CO. Steve graduated from UNL in December 2007 with a Bachelor's of Science in Food Science and Technology, with eyes towards furthering his education in dairy food science & technology at Cornell University in Ithaca, NY.

Steve matriculated at Cornell in January 2008 into the laboratory of Dr. Dave Barbano to study dairy chemistry. While at Cornell, Steve became engaged (2008) and eventually married (2010) the love of his life, Kelsey. Luckily, he met three friends who shared an affinity for good cheese and together they founded the Cheese Club at Cornell in 2009. After studying filtration processing of milk, and evaluating fluid milk shelf-life extension techniques in Dr. Barbano's laboratory, Steve plans to graduate from Cornell and begin the next chapter in his graduate career by completing a Ph.D. in dairy science at a new school. Future plans include working in the food industry, or in academia as a food science extension agent.

I dedicate this thesis to my family, friends, and mentors who have stood by me during this period of my life. Thank You All.

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LIST OF ABBREVIATIONS

α -LA.....	alpha-lactalbumin
β -LG.....	beta-lactoglobulin
CF.....	Concentration Factor
CN.....	Casein
CN%TP.....	Casein as a Percent of True Protein
Δ P.....	delta-P (change in pressure)
<i>J</i>	Flux
GP.....	Graded Permeability
IR.....	Infrared
MCC.....	Micellar Casein Concentrate
MF.....	Microfiltration
NCN.....	Noncasein Nitrogen
NF.....	Nanofiltration
NPN.....	Nonprotein Nitrogen
OD.....	Optical Density
P_{p_o}	Pressure at the Permeate Outlet
PVDF.....	Polyvinylidene Fluoride
R_A	Resistance due to Adsorption
R_{CP}	Resistance due to Concentration Polarization
R_G	Resistance due to the Gel Layer
R_M	Resistance of the Membrane
RO.....	Reverse Osmosis
R_P	Resistance due to Pore Blocking
R_{p_i}	Retentate Pressure at the Inlet
R_{p_o}	Retentate Pressure at the Outlet
SP.....	Serum Protein
SPC.....	Serum Protein Concentrate
SW.....	Spiral-wound
TMP.....	Transmembrane Pressure
TN.....	Total Nitrogen
TP.....	True Protein
TS.....	Total Solids
UF.....	Ultrafiltration
UHT.....	Ultra High Temperature
UTP.....	Uniform Transmembrane Pressure
WPC.....	Whey Protein Concentrate

CHAPTER ONE

Membrane Filtration and the Removal of Serum Proteins from Milk

Membrane Filtration Introduction

Membrane filtration is a novel tool for food product manufacture. In the past few decades, membrane filtration of milk and dairy fluids has become as common as centrifugal fat separation in a dairy plant. Products created using membrane filtration technologies are revolutionizing the food ingredient uses of dairy products, as demonstrated by the wide spread utilization of whey protein concentrates (**WPC**). The objectives of this chapter are to introduce the reader to membrane filtration and to familiarize them with the technologies used to remove serum proteins (**SP**) from skim milk to produce 95% SP reduced micellar casein concentrate (**MCC**), and to introduce the reader to potential methods for the optimization of SP removal from skim using polymeric spiral-wound (**SW**) microfiltration (**MF**).

Filtration Classification. In essence, a filtration membrane plus associated foulant functions as a barrier of selective pore size for soluble and suspended components within the fluid, allowing only components which are smaller in size than the barrier to pass through (i.e. permeate) the membrane and be separated from the feed material. Anything that does not pass through the barrier stays in the retained portion (i.e., retentate), and becomes concentrated. Cross-flow filtration, where liquid feed flows parallel to the surface of the membrane, is the most used filtration method in the food and dairy industry (Jost and Jelen, 1997; Brans et al., 2004). Other methods of filtration exist (e.g. dead-end filtration), however their use in the dairy and food industries for processing milk and whey is limited (van der Horst and Hanemaaijer, 1990; Belfort et al., 1994; Cheryan, 1998).

Of practical importance in the dairy and food industry are four levels, or processes, of membrane filtration, differentiated based primarily on pore size of the membrane. The four main filtration processes are, in order of decreasing membrane pore size, microfiltration (**MF**), ultrafiltration (**UF**), nanofiltration (**NF**), and reverse osmosis (**RO**). Examples of the sizes of constituents that are rejected by the membrane at each level of filtration are presented in Figure 1.1. Membranes, particularly UF, NF, and RO, may also be classified by their molecular weight cutoff value. Molecular weight cutoff for a membrane is defined by the smallest molecule (by molecular weight) that will have its passage blocked by the membrane. Pressure differential across the membrane acts as the primary driving force during MF, UF, NF, and RO filtration processes, with higher pressure being on the retentate side. This pressure differential is also known as the transmembrane pressure (**TMP**). In addition, osmotic pressure differentials between permeate and retentate play a role in RO filtration, and to limited extent in larger-pore filtration, MF, UF, and NF (Belfort et al., 1994; Cheryan, 1998). Microfiltration, the focus of this research, has the largest pore size, 100 to 10,000 nm (0.1 to 10 μm) of the four processes mentioned here which allows for passage of all but the largest diameter components within the feed to permeate the membrane (Figure 1.1). This chapter will focus on the use of MF in the dairy industry to fractionate proteins from milk.

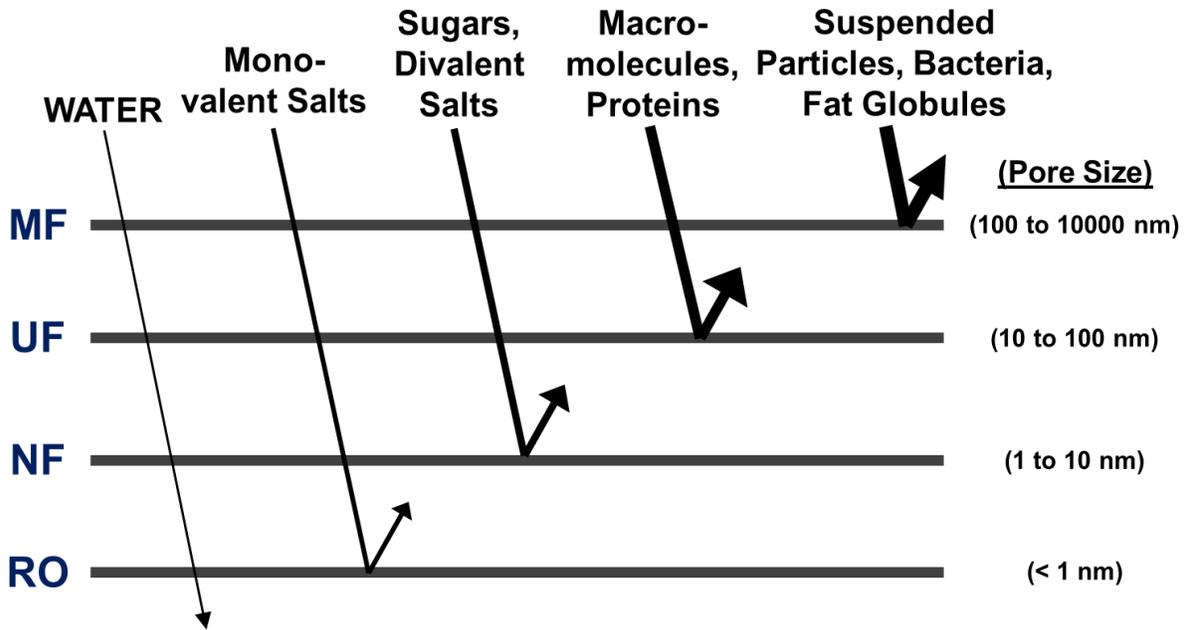


Figure 1.1. Description of the four primary pressure-driven membrane filtration processes used in the food industry. MF = microfiltration; UF = ultrafiltration; NF = nanofiltration; RO = reverse osmosis. (Adapted from Cheryan et al., 1998)

Filtration Process Parameters. During filtration, membrane fouling presents the largest obstacle to maximizing process efficiency. Fouling typically occurs on the surface of the membrane, however many factors are known to lead to fouling (Belfort et al., 1994; Brans et al., 2004). Membrane fouling causes solutes and suspended particles which normally permeate the membrane to be rejected back into the retentate, thus decreasing their removal in the permeate and decreasing overall filtration efficiency. One method to indirectly observe fouling of a membrane is through the measurement of flux. Flux (kg/m^2 per h) is the amount of solute and solvent that passes through the membrane per unit surface area of the membrane per amount of time. Flux decrease during a typical cross-flow filtration process is shown in Figure 1.2. There is an initial rapid decrease in flux at the start of filtration. As filtration progresses, flux decreases at a decreasing rate, eventually stabilizing or decreasing slowly for the remainder of the process. This flux curve can be seen in many dairy and non-dairy filtration processes (Cheryan, 1998; Zulewska et al., 2009). Methods and unique apparatuses to decrease fouling and flux decline

during dairy filtration have been researched (Pouliot and Jelen, 1995; Al-Akoum et al., 2002; Brans et al., 2004). Removing the foulant is commonly achieved by multi-stage chemical cleaning processes (Krack, 1995; Makardij et al., 1999; D'Souza and Mawson, 2005). After cleaning, the membrane flux is measured using purified water to determine if the membrane was thoroughly cleaned and the preprocessing purified water flux was restored prior to additional processing.

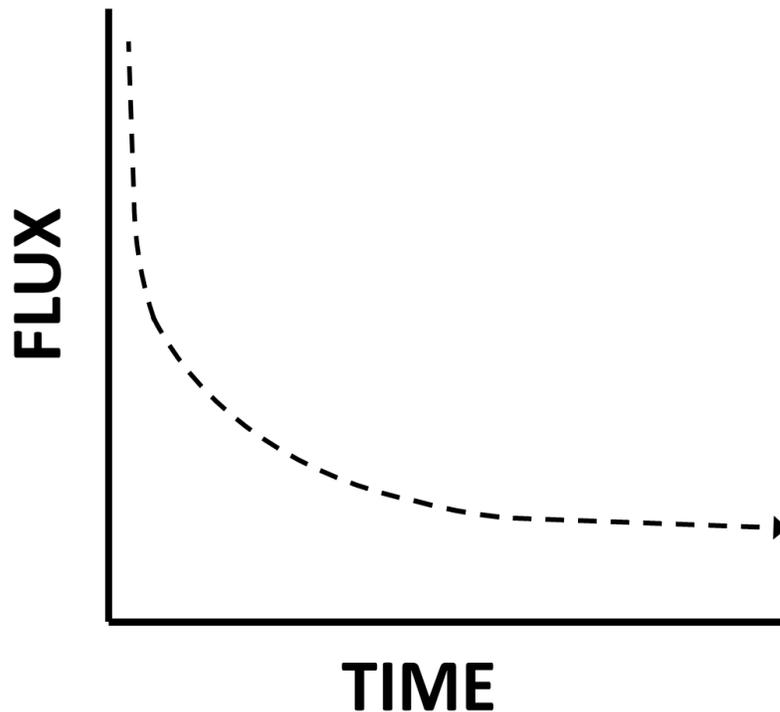


Figure 1.2. Illustration of flux decrease with time that is common in pressure-driven membrane filtration processes of macromolecular solutions.

Transmembrane pressure is the primary driving force during cross-flow filtration of macromolecular solutions. However, there is interplay between TMP (i.e., the driving force) and permeate flux during processing, referred to as critical flux, that exhibits three phases (Field et al., 1995; Brans et al., 2004). Briefly, in the first phase, increasing TMP linearly increases flux due to limited fouling of the membrane. In the second phase, TMP is above a critical pressure,

and an increase in flux is no longer observed, unless a higher cross-flow velocity is used to “sweep” foulant from the membrane surface (Brans et al., 2004). In the final phase, TMP is much greater than the critical pressure, and because of this, fouling at the membrane’s surface is compacted, decreasing flux in a time-dependent manner (Brans et al., 2004). A discussion of TMP and flux in relation to fouling and protein rejection during MF of skim milk will occur later in this chapter.

Membrane Materials and Configuration.

Polymeric Membrane Materials. Membranes used by the dairy industry are constructed of a variety of materials. The most common membrane materials include polymeric and inorganic (i.e., ceramic) compounds. Polymeric membranes are constructed using cellulosic, polyamide, polysulfone, or other polymers such as polyvinylidene fluoride (**PVDF**) (Cheryan, 1998). Ceramic membranes, also referred to as mineral membranes, are constructed using carbon, zirconium, titanium or a combination of these in oxide form (Cheryan, 1998). To be used in dairy and food applications, polymeric or ceramic membranes must be constructed of approved food-grade materials. A benefit of polymeric membranes is that the cost of membrane per m² surface area is less than for ceramic tubular membranes (Renner and El-Salam, 1991), lending to their extensive use. An additional benefit of polymeric membranes is their ability to be highly modified or adapted (e.g., Surface-coated) for application-specific uses (Cheryan, 1998; Mohammad et al., 2012). Choosing the correct membrane material and configuration is based on the intended application, and on fouling propensity and selectivity of the membrane layer (Brans et al., 2004). A discussion of polymeric SW and tubular ceramic membrane construction and configuration follows.

Polymeric membranes are manufactured using a variety of techniques, many of which are proprietary in nature. Different membrane manufacturing techniques create membranes with varied structure and use (Cheryan, 1998; Rahimpour et al., 2009). Phase inversion, stretching, and etching are a few of the methods for manufacturing polymeric membranes (Cheryan, 1998). Polymeric membranes are primarily cast into a flat-sheet, annealed for a time, and then configured into their final shape afterwards (Cheryan, 1998). For example, during phase inversion, a polymer is dissolved in a solvent containing a swelling agent which acts to “open” the structure of the polymer upon casting. This solution is cast, and the solvent is allowed to evaporate at defined rates leaving behind a membrane that has two distinct layers, the actual membrane surface (i.e., pores), and the underlying support structure which is formed by the evaporating swelling agent. The support structure is typically more open to flow than the membrane surface (Cheryan, 1998). The popularity of PVDF in food processing applications is due to its chemical resistance, especially chlorine (i.e., sanitizer), when compared to other polymers (Cheryan, 1998). One issue with un-modified PVDF membranes is their hydrophobicity, which has implications to fouling during dairy filtration processes (Liu et al., 2011). Hydrophilicity and fouling of polymeric membranes will be discussed later in this chapter.

Polymeric Spiral-Wound Membrane Construction. In addition to membrane material, the configuration of the membrane is also very important, and application specific. For dairy UF and MF, the two common configurations include tubular and polymeric spiral-wound (SW) (Brans et al., 2004). Polymeric membranes are manufactured in configurations other than SW (e.g., flat sheet, hollow-fiber, and tubular) (Patel and Reuter, 1985; Merin and Daufin, 1990; Cheryan, 1998), however this review will focus on polymeric SW. Polymeric flat sheet

membranes in a plate-in-frame apparatus have been used for UF of dairy products, but have seen limited large-scale commercial application in dairy (Renner and El-Salam, 1991). Membranes configured in the hollow-fiber design are used for some dairy UF and NF applications, including protein isolation and concentration (Pouliot, 2008). An advantage of the SW configuration is the large amount of membrane surface that can be utilized per unit volume (Saravacos and Kostaropoulos, 2002; Renner and El-Salam, 1991).

Polymeric SW membrane construction is very different from tubular membranes. A schematic of a SW membrane element compared to a tubular element is presented in Figure 1.3. Spiral-wound membranes consist of a leaf of laminated layers that are wrapped around a central permeate collection tube, similar to rolled paper towels around a cardboard tube. The layers inside a leaf of typical SW modules include feed spacers, permeate flow channels, membrane layer, and a cover layer (Figure 1.3). Two membrane sheets, sealed on three sides, enclose a permeate flow channel. The unsealed end of the leaf attaches to the permeate collection tube allowing permeate to flow out of the SW element. A diagram describing the flow of fluid within a SW membrane leaf is shown in Figure 1.4. The feed flows in a cross-flow orientation (i.e., parallel to the permeate tube) across the surface of the membrane through the feed spacer (Figure 1.3), with permeate flowing through membrane pores into the permeate flow spacer. Permeate traverses the leaf to the atmospheric pressure (i.e., low pressure) collection tube, where it exits the element (Figure 1.4). Feed enters on the proximal end of the membrane element, flows across the leaf, and then exits on the distal end of the membrane as a concentrated retentate (Figure 1.4).

One of the most important layers in a SW membrane leaf is the feed spacer (Schwinge et al., 2004a,b). The purpose of a feed spacer in a SW element is to promote turbulence and create

an open structure for the feed to flow across the membrane surface. Feed spacers greatly affect mass transfer and pressure loss as the feed flows across the membrane (Schwinge et al., 2004b). Due to the many sizes and geometries of spacers which exist (Schwinge et al., 2004b), computation fluid dynamic studies have been done (Schwinge et al., 2002; Vrouwenvelder et al., 2010) to understand how the spacers affect membrane performance. Spacers, both feed and permeate, are necessary to keep the flow channels open, especially after the flat leaf is wrapped around the permeate collection tube (Figure 1.4). Open feed spacers allow for turbulent flow and higher retentate cross-flow velocity at or near the membrane surface, increasing mass transfer (Schwinge et al., 2004b). The properties of the membrane layer (e.g., roughness, porosity, pore size) have an impact on filtration efficiency of a polymeric membrane (Reidl et al., 1998; Rahimpour et al., 2009). Additionally, the feed composition and operating conditions used during SW filtration can affect the performance of a polymeric membrane (Schwinge et al., 2004b).

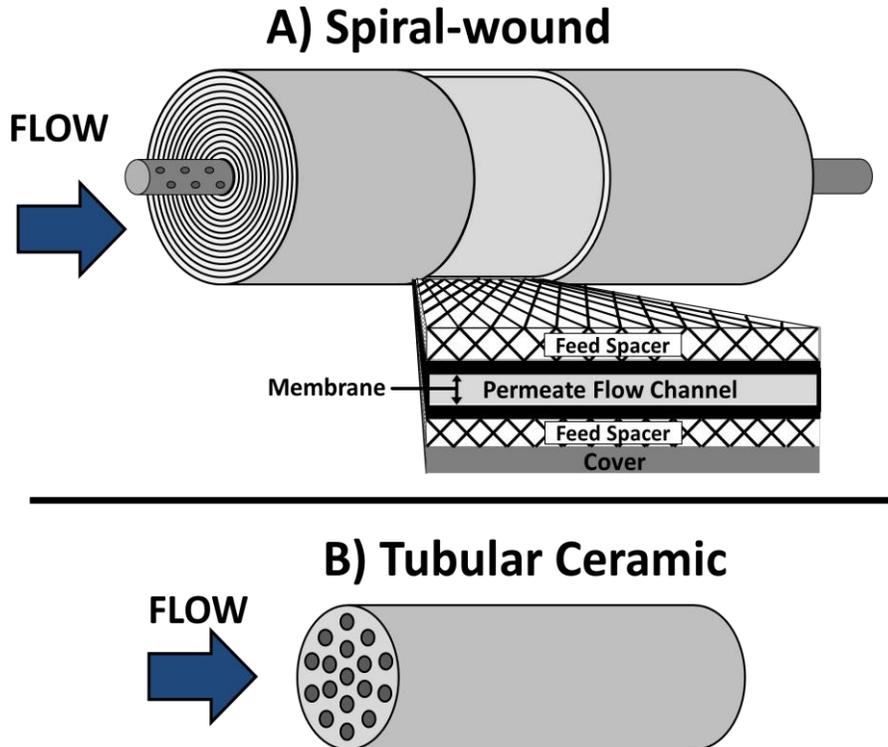


Figure 1.3. Diagram of common filtration membrane configurations used in the dairy industry, including A) polymeric spiral-wound, and B) tubular ceramic. The spiral-wound membrane has been cut away to show details of a leaf. (Adapted from Schwinge et al., 2004b)

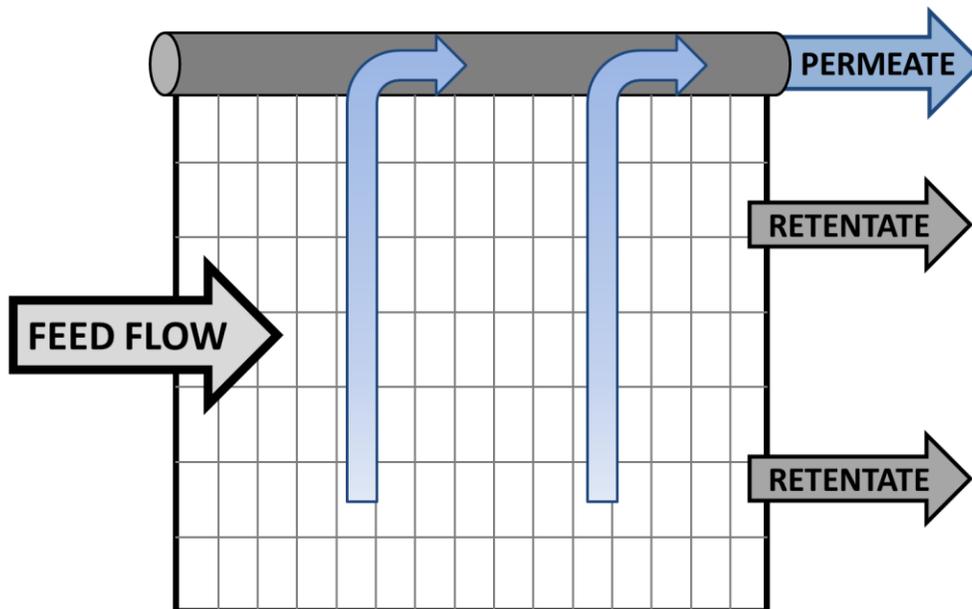


Figure 1.4. Example of fluid flow inside of spiral-wound filtration membrane during cross-flow filtration. Diagram shows an un-wound membrane leaf. Adapted from Schwinge et al., (2004b).

Properties of Ceramic Membranes. Ceramic membranes are constructed of minerals (e.g., titanium, zirconium), as opposed to organic polymers, and are manufactured using such techniques as particle dispersion and slip casting, phase separation and leaching, thin-film etching, and track-etching (Cheryan, 1998). Similar to polymeric membranes, ceramic membrane elements contain two layers, a thin top layer which acts as the membrane, and a more porous underlying support structure. The membrane layer is located on the interior of the open flow channels within the ceramic element (Figures 1.3 and 1.5), and can be layered onto the support after the baking step (Cheryan, 1998). The more open support layer is not always manufactured using the same material as the membrane layer (Cheryan, 1998). Additionally, altering the manufacture of a ceramic element to change the deposition of the membrane surface layer (Grangeon et al., 2002) and the macroporous support layer (Garcera and Toujas, 2002) have been used to enhance membrane element performance. Controlling the deposition of the membrane layer onto the support layer and careful sintering allows for ceramic membranes to have a narrow separating layer pore size distribution, which is highly desired during MF (Saboya and Maubois, 2000, Baruah et al., 2006). Surface coatings can also be applied to the exterior surface of the ceramic membrane element to modify the hydraulic resistance of the membrane from inlet to outlet end of the membrane element (Garcera and Toujas, 2002).

During cross-flow filtration, fluid flows through channels within each element (Figure 1.5). Solvent and unrejected solutes pass through the membrane into the macroporous support layer, and ultimately flow radially out of the membrane element. Many ceramic elements, or sticks, may be included in a membrane module, increasing the amount of membrane surface area (Cheryan, 1998). Manufacturers can alter the shape, size, and number of flow channels within each ceramic element in an effort to maximize membrane surface area and flow characteristics

(Cheryan, 1998). Unlike the polymeric SW membrane configuration, the tubular channel design of ceramic membranes allows for higher cross-flow velocities to be achieved during filtration which can reduce concentration polarization driven fouling (Brans et al., 2004; Karasu et al., 2010). A drawback to tubular ceramic membranes, however, is their fragility when being handled. A ceramic element may crack or break causing the user to replace the element, which is expensive (Cheryan, 1998).

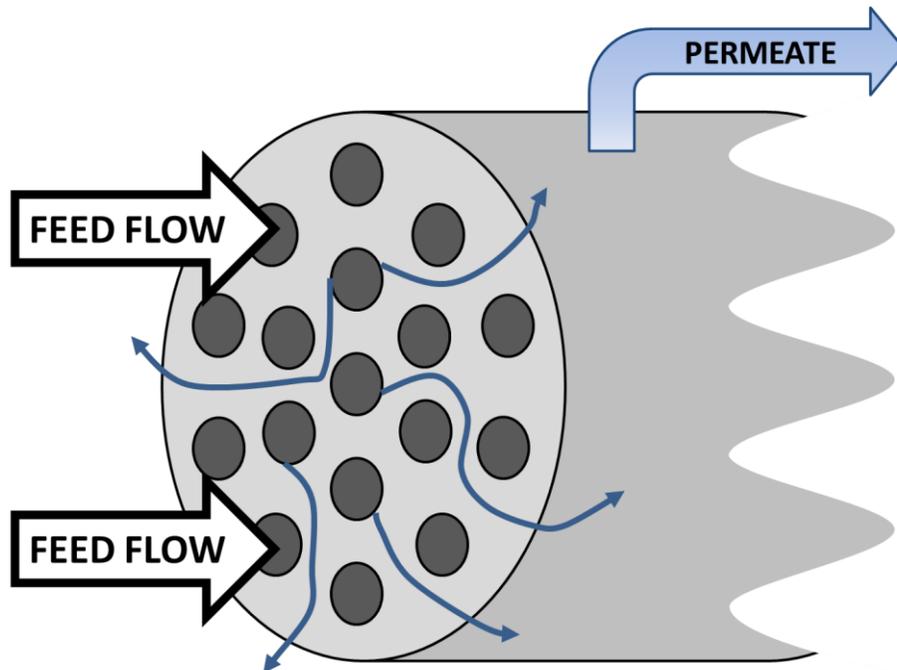


Figure 1.5. Example of fluid flow inside of a tubular ceramic filtration membrane during cross-flow filtration. Permeate (blue) flows from inside the membrane channels through the support layer (medium gray) and out of the membrane element.

Membrane Filtration in the Dairy Industry.

Whey Processing and Bacteria Removal. Membrane filtration in the dairy industry has been used since the 1970's, and is most commonly used for the treatment of cheese whey and other dairy "waste" streams (Renner and El-Salam, 1991). Although many membrane filtration processes have been developed for use in the dairy industry (Pouliot, 2008), two common uses for membrane filtration of dairy products include WPC manufacture using UF, and bacteria

removal from dairy streams using MF (Guerra et al., 1997; Mohammad et al., 2012; García et al., 2012). The UF membranes for whey processing are typically polymeric elements in a SW configuration, although other configurations are utilized (Pouliot, 2008). Liquid whey from the cheese vat contains proteins (e.g., β -LG and α -LA), lactose, fat, and minerals (Renner and El-Salam, 1991). Because whey was considered a waste product of cheese manufacture, UF methods to increase the value of the components within cheese whey have been developed. Ultrafiltration of cheese whey concentrates the valuable proteins in the retentate, and allows lactose, minerals, and water to be collected in permeate. The UF permeate can be filtered using smaller pore size membranes (e.g., NF) to remove milk salts from lactose (Cuartas-Uribe, et al., 2009) which can be used in food and pharmaceutical products, making use of most of the constituents originally available in cheese whey.

Newer technologies, such as “large-pore” MF are being utilized to reduce the bacteria and spore counts in dairy products (Guerra et al., 1997; Brans et al., 2004; Garcia et al., 2012) to create milks and dairy products with extended shelf-life (Elwell and Barbano, 2006). Using larger MF pores (e.g. 0.8 to 1.4 μm) allows milk constituents to permeate the membrane, but retain the relatively large diameter bacteria and spores in the retentate. Elwell and Barbano, (2006) used MF to create pasteurized milks with a shelf-life of > 92 d of 2.0°C storage. A commercialized version of MF to reduce bacteria in milk is called the Bactocatch system, and was proposed by Holm et al., (1986). Bacterial removal from other dairy fluids (e.g., cheese brine and whey) using MF is also commonly practiced in the dairy industry (Rosenberg, 1995). In addition to removing bacteria from milk, somatic cells are also removed from milk during large-pore MF (Saboya and Maubois, 2000; Elwell and Barbano, 2006). If somatic cells, an indicator of a milk quality and a source of unwanted proteolytic and lipolytic enzymes in milk,

are removed during fluid milk processing by MF, then the resulting milks may have better sensory properties (i.e., less bitterness and rancidity) than milks with high somatic cell counts (Ma et al., 2000). However, Elwell and Barbano (2006) observed that even after removing somatic cells from milk using large-pore MF, there was still proteolysis occurring in the somatic cell-free pasteurized skim milk. It was hypothesized that membrane-rejected somatic cells were destroyed by high shear forces in the recirculation loop within the system (Elwell and Barbano, 2006), and that the proteolysis in the somatic cell-free pasteurized milk was caused by plasmin, which is a heat-stable enzyme found naturally in milk. As is often the case in dairy, starting with low somatic cell count milk before product manufacture is desired. High somatic cell counts in milk (e.g., $> 2.0 \times 10^6$ cells/mL) have a significant direct effect on proteolysis within the milk (Saeman et al., 1988).

Microfiltration of Skim Milk. More recent developments for the use of MF in the dairy industry include protein fractionation of skim milk (Lawrence et al., 2008; Zulewska et al., 2009), and the use of MF retentates in standardizing cheese milk prior to cheese making (Nelson and Barbano 2005; Govindasamy-Lucey et al., 2007). Milk contains casein (CN) proteins, in micelles, and SP (i.e., native whey proteins) which are of different diameters (Brans et al., 2004; Walstra et al., 2006) and can be physically separated based on size using a MF membrane. Hurt et al. (2010) demonstrated that a 95% SP reduced MCC could be produced using tubular ceramic MF. From the permeate portion, a milk SP isolate (i.e., $> 90\%$ of solids as protein) can be produced (Hurt and Barbano, 2010). Retentates from MF of skim milk can also be used to standardize the protein content of cheese milk prior to cheese making (Nelson and Barbano, 2005), which was proposed as an economically viable option for cheese makers (Papadatos et al., 2003). When MF of skim milk is done at cold temperatures (4°C), retentates and permeates with

different protein profiles can be obtained (van Hekken and Holsinger, 2000) due to the migration of hydrophobic proteins out of the micelle structure at low temperatures and passage through the MF membrane into the permeate (Davies and Law, 1983). However, at lower temperatures the flux will be much lower than at higher temperatures (Fritsch and Moraru, 2008; Lawrence et al., 2008). Applications of the concentrates and powders from milk MF are few as of today, but emerging research (Evans et al., 2009; Evans et al., 2010, Beliciu et al., 2012) indicates that these products have great potential in both food and non-food applications.

The properties MF products derived from skim milk (e.g., MCC and SPC) are being investigated (Evans et al., 2010; Sauer and Moraru, 2012; Beliciu et al., 2012, Campbell et al., 2013). Due to the removal of heat-labile SP which contain free sulfhydryl groups (i.e., S-H bonds), MCC with low (95% reduced) SP content are very heat stable and can be used in new and unique applications (Sauer and Moraru, 2012; Beliciu et al., 2012). Even after retorting (116°C, 21 min) or ultra-high temperature (UHT) (142°C, 3 s) treatments, MCC (8% casein, pH > 7) was stable (Sauer and Moraru, 2012) indicating that it could successfully be used in shelf-stable milk protein beverage products. Serum protein concentrates exhibit better sensory qualities than comparable WPC (Evans et al., 2009; Evans et al., 2010), primarily because fat is removed by skim MF during manufacture of SPC. When fat is present in protein concentrates, especially WPC, lipid oxidation flavors predominate and cause the product to have a decreased sensory appeal (Whitson et al., 2010). In addition to lower fat content, reconstituted 80% SPC is clearer than reconstituted 80% WPC, which is opaque (Evans et al., 2010). A clearer product, with better or similar sensory profiles would be highly desired by companies trying to develop new products that contain milk proteins.

Serum Protein Removal from Skim Milk

Factors Affecting Serum Protein Removal. Serum proteins constitute approximately 20% of the total protein in bovine milk, whereas CN represent the remaining 80% (Walstra et al., 2006). As indicated previously, removal of SP from skim milk is desired to improve the heat stability of MCC and MF retentate products. Additionally, high SP content MF permeates can be used as replacements for traditional WPC products due to the SPC having superior sensory and functional properties (Evans et al., 2009; Evans et al., 2010). Hurt and Barbano, (2010) reviewed the processing factors that affected SP removal from skim milk during MF. The main factors which affect SP removal from skim include skim protein concentration, concentration and diafiltration factors, SP removal factor (i.e., SP rejection factor), and heat treatment of the skim milk prior to filtration (Hurt and Barbano, 2010). It has been reported that the rejection of SP by polymeric SW membranes can be high (ca. 67% rejection) (Lawrence et al., 2008). With near-theoretical SP removal from skim milk (Hurt et al., 2010), SP rejection in tubular ceramic membranes is close to 0%. Rejection of SP during polymeric SW MF has a large role in SP removal, and will be discussed in a subsequent section of this chapter.

Even though SP is present in skim milk (ca. 0.60%), it must be able to permeate the MF membrane to be removed during filtration. When milk is subjected to high heat treatments such as high-temperature short-time pasteurization (72°C, 15 s), some heat-labile SP (i.e., β -LG) will denature and complex with κ -casein on the surface of the CN micelle (Sawyer, 1969). This bonding causes an increase in the apparent CN as a percentage of SP as measured by Kjeldahl analysis (Lynch et al., 1998; Hurt et al., 2010). The CN-SP complex is not able to permeate an MF membrane and is retained in the retentate, decreasing actual SP removal. It is understood that chemical and processing differences can influence the amount of SP removed from skim milk, but what about the membrane? Typically two membrane types, tubular ceramic and

polymeric SW, are used to MF skim milk for SP removal. A comparison of the two membrane types follows in the next sections.

Use of Ceramic Membranes. The use of tubular ceramic MF membranes to remove SP from milk has been reported (Samuelsson et al., 1997; Vadi and Rizvi, 2001; Hurt et al., 2010). Advantages to using tubular ceramic MF membranes compared to polymeric membranes are their longer usable life spans, their resistance to cleaning chemicals, and their thermal stability (Cheryan, 1998; Baruah et al., 2006), however tubular ceramic membranes are much more expensive than polymeric membranes and that must be balanced against the needs for any application. Ceramic MF membranes have an additional advantage over SW membrane in that they can be operated with a uniform TMP (**UTP**) across the length of the membrane. The UTP concept, introduced in a patent by Sandblom (1978), involves co-current flows of retentate and permeate on each respective side of the membrane. The co-current flow of permeate and retentate allows for a low UTP throughout the membrane module, which as discussed earlier can minimize fouling and flux decreases during filtration. Tubular ceramic membranes operating in a UTP configuration are efficient at removing SP from skim milk (Hurt et al., 2010). Zulewska et al. (2009) and Hurt et al. (2010) reported a near-theoretical (ca. 65%) one-stage removal of SP from skim milk during 50°C MF at a CF of 3.00× using a ceramic UTP membrane. In that system, this equated to a SP removal rate of 0.30 kg/m² per h for the first stage. After three stages of MF and diafiltration, 98% of the SP originally present in the skim milk was removed (Hurt et al., 2010), which was close to theoretical. However, the UTP process requires additional energy input and cost to recirculate the permeate on the permeate side of the membrane to achieve UTP which reduced membrane fouling. Therefore, to try to eliminate the cost of permeate pumping a new design of ceramic membrane was developed to achieve different

hydraulic resistance of the membrane from the inlet to outlet end and achieve reduced fouling as with UTP.

Two types of membranes were developed accomplish the control of fouling that is achieved by UTP without an additional pump, including graded permeability (GP) (Garcera and Toujas, 2002) and Isoflux (Grangeon et al., 2002) ceramic membranes. Both membranes rely on alterations of either the support layer (GP; Garcera and Toujas, 2002) or the membrane surface layer (Isoflux; Grangeon et al., 2002). The GP membrane utilizes a coating on the exterior surface (more thick at the inlet end than the outlet end of the membrane stick) of the ceramic macroporous support structure that produces higher resistance to hydraulic flow through the membrane at the inlet end versus the outlet end of the membrane. The Isoflux membrane contains a decreasing thickness of the membrane selective layer on the inside of the flow channels from the entrance to the exit of the ceramic element. Both GP and Isoflux alterations occur during membrane manufacture and attempt to achieve more resistance to flow of permeate at the inlet vs. outlet end of the membrane. An increase in resistance to permeate flow through the membrane near the inlet is meant to achieve the control of membrane fouling achieved with a UTP (i.e., constant pressure drop) system without the need for an additional pump. Without GP or Isoflux technologies, the TMP at the inlet end of the membrane would be very high, causing fouling and process inefficiency. Of the two membrane types, GP membranes are much more efficient at removing SP from skim milk than Isoflux, and GP SP removal is similar to the ceramic UTP process (Hurt et al., 2010; Adams and Barbano, 2013; J. Zulewska, University of Warmia and Mazury, Olstyn, Poland, unpublished data). Using 3-stage, 3.00× concentration factor (CF) MF of skim milk, ceramic UTP, GP, and Isoflux membranes remove 98.3 (Hurt et al., 2010), 96.5 (J. Zulewska, University of Warmia and Mazury, Olstyn, Poland, unpublished

data), 70.2% (Adams and Barbano, 2013) of the SP originally present. It was postulated that the Isoflux membrane SP removal was lower than GP because the modified membrane selective layer in the Isoflux caused a higher amount of SP rejection than the modified support layer in the GP membrane. The Isoflux membrane also has a different flow channel construction than the circular GP flow channels (Adams and Barbano, 2013), which could cause differences in flow behavior, potentially reducing SP removal.

Use of Polymeric SW Membranes. The use of polymeric SW MF membranes for removing SP from skim milk has not been researched as extensively as with tubular ceramic membranes. An advantage that polymeric SW MF membranes have over tubular ceramic membranes is their low cost per unit of membrane surface area (i.e., capital cost) (Cheryan, 1998). Polymeric SW membranes also allow a processor to install more membrane surface area per unit floor space within their plant. Polymeric membranes, however, are not as robust against cleaning chemicals commonly used to clean filtration membranes, and thus exhibit shorter life span than their ceramic counterparts (Cheryan, 1998). Due to the low cost per unit membrane surface area, and the ability to have large amounts of membrane surface area in a given floor space, more research on polymeric SW MF membrane use by the dairy industry is needed.

Research conducted using polymeric SW MF membranes on skim milk commonly show very low flux at 50°C, 16 kg/m² per h (Zulewska et al., 2009), which is significantly lower than the flux for tubular ceramic UTP membranes, 54 kg/m² per h (Zulewska et al., 2009; Hurt et al., 2010). Higher fluxes (e.g., 30 kg/m² per h) in SW membranes can be achieved by increasing MF temperature to ca. 50°C, and by decreasing the CF below 3.00× (Lawrence et al., 2008). The difference in permeate flux between SW and tubular ceramic MF membranes can be partially attributed to the low cross-flow velocities in SW configurations. Lawrence et al. (2008) and

Karasu et al., (2010) calculated that cross-flow velocities in SW and flat-sheet PVDF membranes used for skim milk MF were 0.4 and 0.5 m/s, respectively. Tubular ceramic UTP membrane cross-flow velocities approach 7.0 m/s (Zulewska et al., 2009; Hurt et al., 2010) due to the open channel design of the membrane element. Higher cross flow velocities in SW polymeric membrane systems are not feasible because the high pressures that would be created will physically damage the membrane structure.

Flux in SW MF membranes may be lower than those observed in ceramic membranes, but of interest to us is how well SW MF removes SP from skim milk. Lawrence et al., (2009) reported a 67% rejection (i.e., 22% removal) of the major SP (i.e., β -LG) when MF skim milk at 10°C using a SW membrane. In a different study of SW MF of skim milk at 50°C, Zulewska et al. (2009) observed a SP removal of ca. 39% during single-stage processing at 3.00× CF, which equates to a 43% rejection of SP by the membrane. A 43% rejection rate is still high, but it is better than 67% and demonstrates that SW MF membranes still have the potential to be used for SP removal from skim milk in some applications. It was not clear from these papers if the membrane structure was causing the high rejection of SP or if the foulant that accumulates on the surface of the polymeric SW membrane was causing the rejection of SP. When creating high CN content retentates (i.e., MCC), the goal is to remove at least 95% of the SP that was originally present in the skim milk. Multiple stages of filtration and diafiltration would be necessary to remove at least 95% of the SP from skim milk using SW MF (Adams and Barbano, 2013).

Fouling of SW Microfiltration Membranes

Causes of SW Membrane Fouling. Polymeric SW membrane fouling during dairy filtration can be due to many factors including feed composition, membrane chemistry and

construction, processing conditions, and others. Fouling, in this discussion, means the deposition of material onto the membrane that cannot be easily removed with flushing or without stopping the system and chemically cleaning the membrane. While not technically fouling, concentration polarization can decrease flux and change selectivity of the membrane (Brans et al., 2004), but is usually reversible and removed by flushing or adjusting process controls (Cheryan, 1998). Concentration polarization is defined as a dynamic accumulation of retained solids at the membrane surface due to the balance between convective transport toward the membrane (driven by TMP) and the rate of back diffusion into the bulk flow away from the membrane (driven by cross-flow velocity and turbulent flow) (Cheryan, 1998). Once a concentration polarization layer has formed, it can become compacted by increases in TMP, resulting in a cake-layer formation at the membrane surface which can impede passage of solutes and decrease flux. Concentration polarization increases the possibility of interaction between accumulated solids and the membrane, leading to membrane fouling, however the membrane itself does not influence concentration polarization (Marshall et al., 1993).

Irreversible fouling of a membrane during filtration occurs through four main methods: adsorption, pore blocking, cake layer formation, and depth fouling (Brans et al., 2004). Adsorption fouling, in polymeric membranes, is the interaction between solutes and the membrane through secondary intermolecular forces (e.g., dipole-dipole interaction, hydrogen bonding, and van der Waals' forces), and can occur on the membrane surface or inside the pores (van der Horst, 1995). The work of Tong et al. (1988) showed that protein (e.g., SP) adsorption onto hydrophobic polymeric membranes can occur under static conditions independent of concentration polarization formed by a TMP. Adsorption at the surface can lead to cake layer formation caused by interaction between built up materials and can cause sections of the

membrane to become covered in a gel-layer, including pores. Pore blocking occurs when material which has a larger diameter than the pore size blocks the entrance to pores, reducing flux through that pore. If the material that is blocking the pore becomes lodged further into the pore, then depth fouling results. Pore blocking and depth fouling cause a decrease in the number of effective pores, and can lead to rejection of the passage of solutes through the membrane.

The composition of the material being filtered and the membrane construction material can play large roles in the fouling of a polymeric membrane. Proteins in milk, which readily adsorb onto polymeric membranes, are considered to be the primary foulant material present during filtration (Marshall and Daufin, 1995; van der Horst, 1995). Protein-protein and protein-polymer interactions are known causes of irreversible fouling of SW membranes during filtration of dairy fluids (Belfort et al., 1994; James et al., 2003). The interaction between milk proteins (i.e., SP) and hydrophobic polymeric membranes (e.g., polyethersulfone and PVDF) occurs very rapidly by adsorption and without any TMP (Tong et al., 1988). According to van der Horst and Hanemaaijer, (1990), the protein-polymer interaction may have more of an impact during polymeric SW UF than SW MF due to UF membranes having smaller pores. A monolayer of adsorbed proteins inside the pores of a MF membrane would cause less resistance to flow than similar sized protein adsorption inside pores of a UF membrane (van der Horst and Hanemaaijer, 1990). Therefore it thought that in MF membranes, hydrophobicity does not play as large a role as does membrane structure and configuration (van der Horst and Hanemaaijer, 1990).

In an effort to determine which components of skim milk cause flux decline and SP rejection during polymeric PVDF SW MF (0.3 μm), Zulewska and Barbano, (2013) created CN-free skim milk (i.e., MF permeate) using ceramic UTP MF, and then filtered it through a clean polymeric PVDF SW MF membrane. Flux for CN free skim milk was 80 kg/m^2 per h, whereas

flux on skim milk that was used to produce the CN free skim milk was 17 kg/m^2 per h. They also reported that there was little resistance to passage of SP through the membrane when filtering CN-free skim milk, as opposed to higher resistance during skim MF. Overall, Zulewska and Barbano, (2013) concluded that the presence of CN in skim milk was the primary cause for flux decline and low SP removal during SW MF using a PVDF ($0.3 \mu\text{m}$) membrane.

Membrane configuration plays a role in fouling during polymeric SW filtration. Spacers in SW membranes are used to help keep layers separated, and to promote turbulent flow containing eddies (Schwinge et al., 2004a,b). Turbulent flow and eddies promote the transport of concentrated material from the membrane surface to the bulk-flow feed, decreasing concentration polarization and fouling. A drawback to mesh spacers is their obstruction to the direct flow of feed material, which causes a significant pressure drop from the inlet of the membrane to the outlet (Schwinge et al., 2004b; Lawrence et al., 2008). This pressure loss can lead to poor hydrodynamic conditions on the surface of the membrane causing foulants to congregate and cause a reduction in flux. The reduction in flux, in addition to a layer of material at the membrane surface, will cause a reduction in permeation of desired components (e.g., SP from skim milk). Another drawback to mesh spacers that is often overlooked is the reduction in usable membrane surface area caused by mesh spacer-membrane contact throughout the module. Schock and Miquel, (1987) were the first to incorporate this metric into a description of the potential for fouling and pressure loss in SW modules. Depending on the spacer thickness and geometry, the volume which a spacer can remove from the total volume in a flow channel can be significant, and must be accounted for (Schwinge et al., 2004a). Even the curvature of the SW membrane element could impact the shear-stress on each side of a feed spacer, potentially

changing colloidal distribution of materials on different sides of the curve and influencing mass transport (Li and Tung, 2008).

Processing factors such as start-up procedure and operational parameters (e.g., TMP, cross-flow velocity) can influence the flux and fouling of SW membranes. Saboya and Maubois (2000) stated that the starting procedure used for MF of milk should be carefully controlled to minimize rapid fouling. The rapid fouling by protein, and subsequent accumulation of material at the surface of the membrane can influence immediately the efficiency of filtration and reduce the flux. As the filtration progresses a dynamic membrane may be formed by the layer of built-up material (James et al., 2003) which can change the selectivity and efficiency of the membrane. Operational parameters during the run can influence the fouling and flux during polymeric SW filtration. The critical flux ratio, as mentioned earlier, is partially determined by TMP and cross-flow velocity. Pressure forces material towards the membrane surface, while cross-flow velocity helps to keep material from accumulating on the surface, as concentration polarization driven layer.

Modeling Fouling in SW MF. Described earlier, fouling in membrane filtration is indirectly measured by observing flux during processing. In polymeric UF processes, most of the flux decline is caused by rapid adsorption fouling of the membrane surface and pores (Tong et al., 1988). In polymeric MF, adsorption of proteins within and onto the membrane occurs, but the major flux decline has been attributed to protein-protein interactions on the surface of the membrane, pore blocking, and subsequent concentration polarization (James et al., 2003). The TMP driven flux decline (i.e., fouling) in membrane filtration, which occurs after TMP pressure independent adsorption fouling, proceeds via two main avenues, an initial concentration polarization caused by the increase in quantity of material at the membrane surface, and

irreversible fouling of the membrane. Modeling flux and fouling in cross-flow membrane filtration is complex, and the models are sometimes obtained using model systems which do not represent complex fluids such as milk (van der Horst, 1995). Many models that try to encompass the decline in flux and increase in fouling during cross-flow filtration center around Darcy's law (equation):

$$J = \frac{\text{TMP}}{(\mathbf{R_M} \times \boldsymbol{\mu})}$$

where J is permeate flux, TMP is transmembrane pressure, R_M is the resistance of the membrane, and μ is the viscosity of the fluid permeating the membrane (Marshall and Daufin, 1995). This model is simplistic and does not directly account for concentration polarization or different forms of fouling including pore plugging, adsorption onto the membrane, or the formation of a “gel-layer” on top of the membrane surface after long filtration times. These resistances to flux are commonly found in dairy fluid filtration due to the wide range of particle diameters and the heterogeneity of the solution (van der Horst, 1995; Brans et al., 2004; Brião and Tavares, 2012). To address these shortcomings, flux models that incorporate the multiple resistances to flow have been developed. The most commonly utilized model to describe flux in complex fluid systems like milk is the resistance-in-series model (Suki et al., 1984; van der Horst, 1995):

$$J = \frac{\text{TMP}}{(\mathbf{R_M} + \mathbf{R_A} + \mathbf{R_P} + \mathbf{R_G} + \mathbf{R_{CP}})}$$

where J is permeate flux, R_M is the resistance of the clean membrane, R_A is resistance caused by adhesion, R_P is resistance from pore blocking, R_G is resistance caused by the gel layer, and R_{CP} is resistance caused by concentration polarization. This model allows for a more precise calculation of the flux during cross-flow filtration using SW membranes on milk because it

accounts for the possible resistances that are commonly observed during milk filtration operations (van der Horst, 1995). Unlike other models, resistance due to concentration polarization is incorporated into the resistance-in-series model. Although concentration polarization is not technically fouling (Belfort et al., 1994), filtration of complex macromolecule solutions (e.g., milk) involves the buildup of material at the membrane surface due to movement in that direction caused by TMP. This buildup must be accounted for when calculating flux, as the concentration polarization layer plays a role in the membrane selectivity and efficiency (van der Horst, 1995; James et al., 2003).

Decreasing SW MF Membrane Fouling. Decreasing fouling of polymeric SW MF membranes during milk filtration may increase their SP removal efficiency, and help polymeric MF membranes become more widely used in the dairy industry. Strategies to decrease the fouling of polymeric SW MF membranes include physical alterations (e.g., changing spacer design) of the module (Schwinge et al., 2004a) or improving or altering the surface characteristics of polymeric membranes (Rahimpour et al., 2009; Liu et al., 2011), and non-membrane alterations such as pre-treating or altering the feed material (Pouliot and Jelen, 1995). Brans et al. (2004) outlines multiple methods to enhance membrane performance, including high cross-flow velocities, turbulence promoters, air slugs, back-pulsing, and vibrating or rotating disks and modules. Of these methods, however, few are applicable to SW membranes. Cross-flow velocity in SW membranes is primarily dictated by spacers (Schwinge et al., 2004b), which can only be altered to a limited degree, short of changing the configuration of the module completely to a non-SW design. Back-pulsing or back-flushing (which is common in hollow fiber membrane designs) is not recommended for SW membranes due to the membranes' laminate-style construction becoming unstable under rapid fluctuations of pressure. Increasing

the cross-flow velocity is not recommended for similar reasons as for back-pulsing, because delamination and deformation of the membrane could result from the increased pressure. Physically altering the SW membrane to achieve higher flux and lower fouling during filtration is difficult and may not be cost efficient.

An additional strategy to potentially minimize fouling, increase flux, and remove more SP from milk could be decreasing the CF used during SW MF. Mentioned earlier, Lawrence et al., (2008) observed higher permeate flux for skim milk at lower CF (e.g., 2.00×) than at higher CF (e.g., 3.00×). Decreasing the CF increases flux by decreasing the rate of concentration polarization and gel-layer buildup at the membrane surface. Coupling a higher flux with less obstruction of the pores could allow the MF membrane (e.g., 0.3 μm pore size) to be the selective layer (as intended) and increase SP passage. It is known that CN is the primary foulant causing flux decline and low SP passage into permeate during PVDF SW MF of skim milk (Zulewska and Barbano, 2013). A lower concentration of CN in the retentate might decrease fouling and favor less SP rejection. Altering the feed, either by lowering CF or pre-treating it, appears to be the most viable option for enhancing SP removal from skim milk during polymeric SW MF.

The two objectives of our research on SP removal from skim milk were, 1) to determine the process necessary to create a 95% SP reduced MCC using a 0.3 μm PVDF polymeric SW MF at 50°C and compare the efficiency of the SW process to theoretical values and to a 0.1 μm ceramic UTP MF membrane process, and 2) to determine the impact of concentration factor (CF) on the removal of SP from skim milk during MF at 50°C using a 0.3 μm pore size polymeric polyvinylidene fluoride SW membrane.

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CHAPTER TWO

Production Efficiency of Micellar Casein Concentrate Using Polymeric Spiral-wound Microfiltration Membranes¹

ABSTRACT

Most current research has focused on using ceramic microfiltration (MF) membranes for micellar casein concentrate (MCC) production, but little research has focused on the use of polymeric spiral-wound (SW) MF membranes. A method for the production of a serum protein (SP) reduced MCC using SW MF was compared to a ceramic MF membrane. Pasteurized (79°C, 18s) skim milk (1100 kg) was microfiltered at 50°C [about 3X concentration] using a 0.3 µm polyvinylidene fluoride (PVDF) spiral-wound membrane bleed-and-feed 3-stage process, using 2 diafiltration stages, where the retentate was diluted 1:2 with reverse osmosis water. Skim milk, permeate and retentate were analyzed for serum protein content, and the reduction of serum protein from skim milk was determined. Theoretically, 68% of the SP content of skim milk can be removed using a single stage 3X MF. If two subsequent water diafiltration stages are used, an additional 22% and 7% of the SP can be removed, respectively, giving a total SP removal of 97%. A SP removal greater than 95% has been achieved using a 0.1 µm pore size ceramic uniform transmembrane pressure (UTP) MF membrane after a 3-stage MF with diafiltration process. One stage of MF plus two stages of diafiltration of 50°C skim milk using a PVDF polymeric SW 0.3 µm membrane yielded a total SP reduction of only 70.3% (stage 1, 2, and 3: 38.6%, 20.8%, and 10.9%, respectively). The SP removal rate for the polymeric SW MF membrane was lower in all three stages of processing (stage 1, 2, and 3: 0.05, 0.04, and 0.03 kg/m² per hour, respectively) than that of the comparable ceramic UTP MF membrane (stage 1, 2, and 3: 0.30, 0.11, and 0.06 kg/m² per hour, respectively), indicating that SW MF is less

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efficient at removing SP from 50°C skim milk than the ceramic UTP system. To estimate the number of steps required for the SW system to reach 95% SP removal, the third stage SP removal rate (27.4% of the starting material SP content) was used to extrapolate that an additional five water diafiltration stages would be necessary for a total of eight stages to remove 95% of the SP from skim milk. The eight-plus stages necessary to remove > 95% SP for the SW MF membrane would create more permeate and a lengthier process than with ceramic membranes

KEYWORDS: microfiltration, serum protein removal, spiral-wound membrane

INTRODUCTION

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

A potential alternative to ceramic MF membranes is polymeric spiral-wound (SW) membranes. Polymeric SW filtration is most commonly used in low fouling applications such as water treatment and water reclamation (Schwinge et al., 2004). Most research on SW membranes centers around their application in nano- and ultra-filtration of biological suspensions (Schwinge et al., 2004). The SW membranes have high surface area and relatively low cost compared to ceramic membranes. Zulewska et al. (2009) compared the performance of three MF membrane types, ceramic (UTP), ceramic graded permeability, and polymeric SW for removal of SP from skim milk and found SP removal of 64 and 61% for the ceramic UTP and graded permeability membranes, respectively, compared to 38% SP removal for the SW MF membrane when using a single-stage 3X bleed-and-feed MF process. Theoretically, a 68% SP removal can be achieved during single stage 3X bleed-and-feed MF processing. Lawrence et al. (2008) found that a SW MF membrane retained 78 to 99% of the main SP, β -LG, under varying transmembrane pressures, with higher transmembrane pressures giving higher rejection. Hurt et al., 2010 demonstrated that with a 0.1 μm pore size ceramic UTP membrane, greater than 95% of the original SP in pasteurized skim milk could be removed using a 3-stage MF with diafiltration process. Our objective was to determine the process necessary to create a 95% SP reduced MCC using a 0.3 μm PVDF polymeric SW MF at 50°C and compare the efficiency of the SW process to theoretical values and to a 0.1 μm ceramic UTP MF membrane process.

MATERIALS AND METHODS

Experimental Design and Statistical Analysis

One lot of bovine milk (approximately 1,151 kg) was separated in the Cornell University dairy plant at 4°C using a Model 590 Air Tight Centrifuge, DeLaval Co., Chicago, IL. Raw skim milk was pasteurized with a plate heat exchanger consisting of three sections: regeneration,

heating, and cooling (Model P13-RCF, Alfa-Laval, Lund, Sweden) at 79°C and a holding time of 18 s. The milk was cooled to 4°C and stored at $\leq 4^\circ\text{C}$ until processing. The processing for each replicate was done in series over a 3-d period: stage 1, stage 2 and stage 3, respectively. During stage 1, the pasteurized skim milk was MF using a polymeric SW membrane using a 3X bleed-and-feed process at 50°C. Retentate was cooled to 4°C and held overnight. On the second day (stage 2), the retentate from stage 1 was diluted by weight (1 part retentate and 2 parts water) with pasteurized reverse osmosis (**RO**) water and diafiltered at 50°C. On the third day (stage 3), the retentate from stage 2 was diluted (as described for stage 2) with pasteurized RO water and diafiltered at 50°C for a second time. The experiment was replicated 3 times.

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

Microfiltration System

Skim milk (approximately 1,151 kg) was processed at 50°C with a polymeric polyvinylidene fluoride (**PVDF**) SW membrane (model FG7838-OS0x-S, 0.3 μm polyvinylidene fluoride, Parker-Hannifin, Process Advanced Filtration Division, Tell City, IN) with a nominal pore size of about 0.3 μm and surface area of 20.5 m^2 . Membrane diameter was 198 mm, spacer thickness was 1.09 mm, and length was 96.5 cm. The membrane was placed in a stainless steel housing (length 1.3 m) and was mounted horizontally in the membrane system. The SW MF system had a feed pump (Model 0, Cherry Burrell, Little Falls, NY) and retentate recirculation pump (Model GHH-30, G&H Products Corp., Kenosha, WI). A 1,150 L cheese vat (Model

DLHD8SSS, Kusel Equipment Co., Watertown, WI) was used as the feed tank and was connected directly to the feed pump. Digital pressure gauges (Model PI2094, IFM Efector Inc., Exton, PA) were used to monitor the retentate pressure outlet (R_{p_o}) and retentate pressure inlet (R_{p_i}). Permeate pressure outlet (P_{p_o}) was assumed to be 0 kPa due to the permeate exit tube being open to the atmosphere. Retentate bleed flow and recirculation flow (L/min) were measured with magnetic volumetric flow meters (model AE202MH, Yokogawa Electronic Corp., Japan; and Model AM204DH, Yokogawa Electronic Corp., Japan, respectively). Permeate and retentate exiting the SW MF unit were collected during the run and weighed (kg) into separate holding tanks. A schematic diagram of the SW MF unit used in the experiment is shown in Figure 2.1.

Initial cleaning

Immediately prior to processing skim milk, the SW MF system was cleaned (short clean). First, the soak solution (0.26% solution of Ultrasil MP, Ecolab Inc., Food and Beverage Division, St Paul, MN) was drained from the system and then the system was flushed with RO water until the water exiting the system was at neutral pH. The R_{p_i} and R_{p_o} were set by adjusting the speed of the retentate recirculation pump with the retentate and permeate outlets fully open. The membrane was cleaned for 20 min at 131 kPa R_{p_i} and R_{p_o} of 61 kPa with a combination of Ultrasil 110, liquid alkaline membrane cleaner (0.39% v/v), XY-12, liquid sanitizer (0.15% v/v) (Ecolab Inc., Food and Beverage Division, St Paul, MN) diluted in 50°C

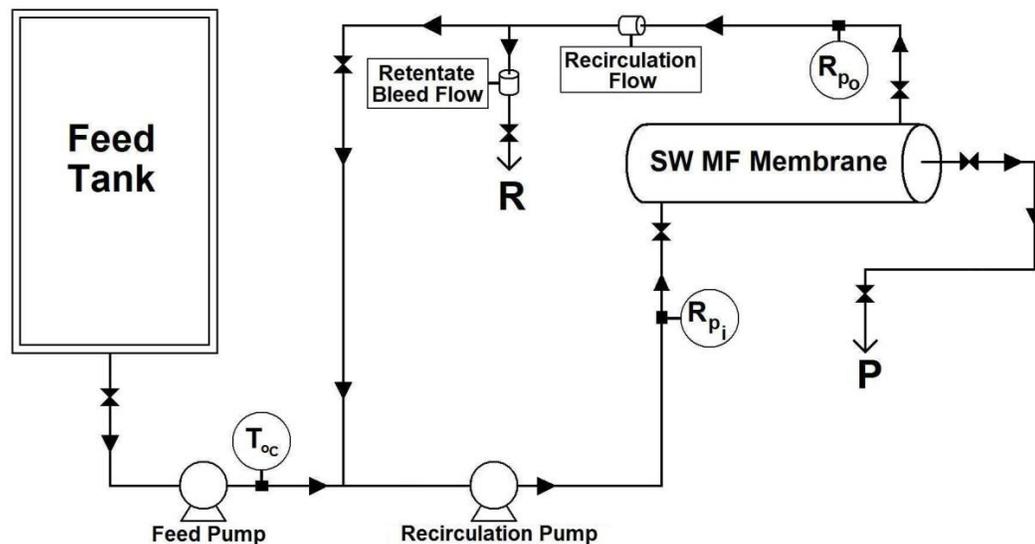


Figure 2.1. The polymeric spiral-wound microfiltration (SW MF) system used for microfiltration and diafiltration of 50°C skim milk. During processing, the temperature (T_{oc}), retentate pressure inlet (R_{pi}), retentate pressure outlet (R_{po}), recirculation flow rate (L/min; recirculation flow), and retentate bleed flow (L/min; retentate bleed flow) were measured every ten minutes. Retentate (R) and permeate (P) were collected and weighed continuously during processing.

RO water to a pH of 11.2 to 11.4. The operating conditions were set such that the average ΔP ($\{[(R_{pi} - R_{po})/2] + R_{po}\} - P_{po}$) was <100 kPa. After the alkaline cleaning cycle was completed, the membrane system was drained and flushed with 50°C RO water until neutral pH was obtained. The membrane was cooled to $< 24^\circ\text{C}$ and sanitized with a solution of Ultrasil 110, liquid alkaline membrane cleaner (0.39% v/v), XY-12 liquid sanitizer (0.15% v/v) to achieve a pH 11.2 to 11.4 and a chlorine level of 150 to 180 ppm in RO water. This solution was circulated through the membrane for 10 min at 131 kPa R_{pi} and no permeate back pressure. The membrane was drained and flushed with 25°C RO water to neutral pH. The starting water flux before milk was determined using 25°C RO water with only the feed pump running, the permeate outlet fully open and a R_{pi} of 35 kPa adjusted by partially opening the retentate bleed valve with recirculation loop closed (Figure 2.1). The clean water flux (typically about 37 kg/m² per h) was

calculated based on the weight of permeate collected in 30 s and the total membrane surface area. After determining the starting water flux before milk, the MF system was drained of all liquids.

Milk Filtration and Diafiltration

Skim milk and diluted retentate were processed using the procedure outlined below. In stage 1, skim milk was heated to 50°C then MF. For stages 2 and 3, retentate from the previous stage was diluted (1 part retentate and 2 parts RO water) back to the original weight of skim milk from stage 1 (about 1,150 kg). Before processing, skim milk or diluted retentate was heated to 50°C and held at that temperature for the duration of processing. The SW MF system was started as follows: the feed pump was turned on with the permeate exit valve closed. When skim milk, or diluted retentate, started exiting the retentate outlet, the retentate recirculation loop shut-off valve was opened to allow milk to fill the recirculation loop. Then, the recirculation pump was turned on and the permeate exit valve was opened slowly to balance the pressures and flows of retentate and permeate to achieve a ΔP less than 100 kPa. Throughout the run, retentate removal rate was adjusted to maintain a 3X CF. The concentration factor (CF) [permeate (kg) + retentate (kg)] / retentate (kg) was monitored every 10 min by collecting permeate and retentate for 30 s into buckets and weighing each. Flux (kg/m² per h) was determined every 10 min using the weight of permeate collected in 30 s and the surface area of the membrane. Samples of permeate and the retentate were collected every 10 min for analysis by infrared spectrophotometer (Lactoscope FTIR, Delta Instruments, The Netherlands) to monitor their composition. At the end of the MF run for each stage, all retentate and all permeate from the processing run was weighed to the nearest 0.01 kg (model I5S, Ohaus Corporation, Pine Brook, NJ), mixed, and sampled. A final CF was calculated at the end of the run using the final weights of permeate and retentate.

After weighing and sampling, the collected permeate was discarded. The small amount of unprocessed skim milk or diluted retentate remaining in the feed vat at the end of the run was weighed and subtracted out of the starting material weight. The pH of the retentates was measured with an electrode (model Electrolyte 9823, Mettler Toledo, Columbus, OH) that was standardized at pH 4.06 and 6.97 at 50°C (pH 4.00 and 7.00 Standard Buffer Solutions, Fisher Scientific, Fair Lawn, NJ). The retentate was stored overnight in a water-cooled tank (Hinged Lidded-Water Cooled 110 gal, Pfaudler, Rochester, NY).

Post-Process Cleaning

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

Division, St Paul, MN) in 50°C RO water for 30 min. The pH of the acid cleaning solution was 2.0 to 2.2. After the 30 min wash, the membrane was flushed to a neutral pH with 50°C RO water. The membrane was then cleaned for 30 min with a combination of Ultrasil 110, liquid alkaline membrane cleaner (0.39% v/v), and XY-12, liquid sanitizer (0.15% v/v) in 50°C RO water. The pH of the alkaline cleaning solution was 11.2 to 11.4 and the chlorine level was 150 to 180 ppm. After the 30 min clean, the membrane was flushed to a neutral pH with 50°C RO water. When the rinse water pH was neutral, the system was flushed with RO water at 25°C and the final clean water flux was determined by operating only the feed pump with the permeate outlet fully open and a R_{p_i} of 34.5 kPa adjusted by the retentate bleed valve and with the recirculation loop closed. The flux (kg/m^2 per h) was calculated based on the weight of permeate collected in 30 s and the membrane surface area. The ending flux after cleaning was about 36.0, 36.0 and 36.7 kg/m^2 per h for the three stages, respectively. After the ending flux was determined, the membrane was sanitized with a solution of Ultrasil 110, liquid alkaline membrane cleaner (0.39% v/v) and XY-12, liquid sanitizer (0.15 % v/v) in RO water ($< 24^\circ\text{C}$) at pH 11.2 to 11.4 and a chlorine level of 150 to 180 ppm. This solution was circulated through the membrane for 10 min at R_{p_i} of 131 kPa and no permeate back pressure. The membrane was then flushed with room temperature RO water to a neutral pH. After a neutral pH was obtained, a storage solution of Ultrasil MP, soak solution, (0.26% v/v, Ecolab Inc., Food and Beverage Division, St Paul, MN) and room temperature RO water were combined to achieve a pH 3.5 to 3.9, and this solution was circulated through the membrane for 10 min. After 10 min, the pumps were shut off and all the valves on the membrane housing were closed so that the soak solution could stay in contact with the membrane until the next processing day.

Chemical and Instrumental Analyses

Skim milk, permeate and retentate collected during processing, final permeate, and final retentate were analyzed using an infrared spectrophotometer (**IR**) (Lactoscope FTIR, Delta Instruments, The Netherlands) for fat, lactose and true protein content (Kaylegian et al., 2006). Skim milk, final retentate, final permeate and retentate hold-up was analyzed for total N (**TN**), noncasein N (**NCN**), and nonprotein nitrogen (**NPN**) content using Kjeldahl methods (AOAC 2000; method 991.20, 33.2.11; method 998.05, 33.2.64; and method 991.21, 33.2.12, respectively). Total Solids (**TS**) was measured by forced air oven drying (AOAC, 2000; method 990.20, 33.2.44). True protein (**TP**) was calculated by subtracting NPN from TN and then multiplying by 6.38, CN was calculated by subtracting the NCN from TN and multiplying by 6.38, and SP content was calculated by subtracting NPN from NCN and multiplying by 6.38. The SP content in the permeate portion of the skim milk (expressed as a percentage) was calculated by dividing SP in milk by SP in permeate portion of the milk multiplied by 100, where permeate portion of milk is considered as 100 kg minus the weight of fat plus CN in 100 kg of milk. All samples were analyzed fresh.

Serum Protein Removal

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

SDS-PAGE

Samples of pasteurized skim milk, final retentate, and final permeate from all 3 MF stages were analyzed for protein profiles using SDS-PAGE electrophoresis. A 10 to 20% polyacrylamide gradient gel was used to determine the relative proportion of protein types. Skim milk samples were prepared by diluting 0.1 mL of skim milk into 0.9 mL of electrophoresis sample buffer containing 10mM Tris-HCL pH 6.8, 1.0% SDS, 20% glycerol, and bromophenol blue tracking dye. Retentate samples were prepared by diluting 0.1 mL of retentate into 2.9 mL of sample buffer and stored at -20°C. Permeate 0.1, 0.3 and 0.54 mL was diluted into 0.9, 0.7 and 0.46 mL of sample buffer for stages 1, 2 and 3, respectively and stored at -20°C. Immediately before loading on and electrophoresis gel, the diluted samples in glass vials (Target DP™ Vials C4000-1W, National Scientific Company, Rockwood, TN) sealed with DP Blue Cap (C4000-51B, National Scientific Company, Rockwood, TN) were thawed at room temperature and then heated to 100°C in a steam chamber for 3 min. The prepared retentates and milk samples were loaded, 10.5, 12.5, 14.0 and 8.5 µL, for stages 1, 2, 3, and milk respectively, onto an SDS-PAGE gel. Permeate samples (25 µL) were loaded onto an SDS-PAGE gel. Each gel consisted of 15 lanes, ten lanes contained a sample, and five lanes were left open. An example of the sample layout on a gel can be seen in Figure 2.2. One gel represented either the permeate or retentate samples from one of the three replicates of this experiment. Permeate or retentate samples from each stage were run in triplicate (3 slots) on a gel, utilizing an open lane between adjacent groups of three lanes. The procedure of Verdi et al. (1987) was used for running, staining and destaining the gels. The gels were scanned with a USB GS 800 Densitometer using Quantity One 1-D Analysis Software (BIO-RAD Laboratories, Inc., Hercules, CA) to obtain relative proportions of individual proteins within in each sample. The loading of the samples on

the SDS-PAGE gel was chosen to achieve a maximum optical density (**OD**) of the predominant protein in each sample in the range of 1.0 to 1.4 OD to not exceed the linear range of response of the detector. A milk sample was run on each gel as a reference to verify that proper resolution of milk proteins occurred and also as a check for consistency of quantitative analysis of the same sample from gel to gel. The background was adjusted separately for each lane using the rolling disk method of subtraction to obtain a flat base on the pop-up trace. The adjust band function of the software was used with brackets to set the leading and trailing edge for each band as visually observed on the image of the gel, not the beginning and end of the peak in the pop-up trace. If a band was not horizontal, then the tracking line for the lane was adjusted to transect the band perpendicularly. An example of the layout a gel for analysis of permeate samples from one replicate of this experiment, with one lane used for a skim milk reference sample, can be seen in Figure 2.2. The bands in the skim lane have been labeled for easy identification of the individual proteins. For all gels, the relative amount of CN in a sample was quantified by adding the CN1 through CN6 bands in a lane (Figure 2.2), and the SP was quantified by adding SP1 through SP4 bands (Figure 2.2). Once totals of the CN and SP bands from each lane were quantified, the relative proportion could be established. To quantify the proportion of β -LG and α -LA as a percentage of β -LG + α -LA, bands SP3 (β -LG) and SP4 (α -LA) were used (Figure 2.2).

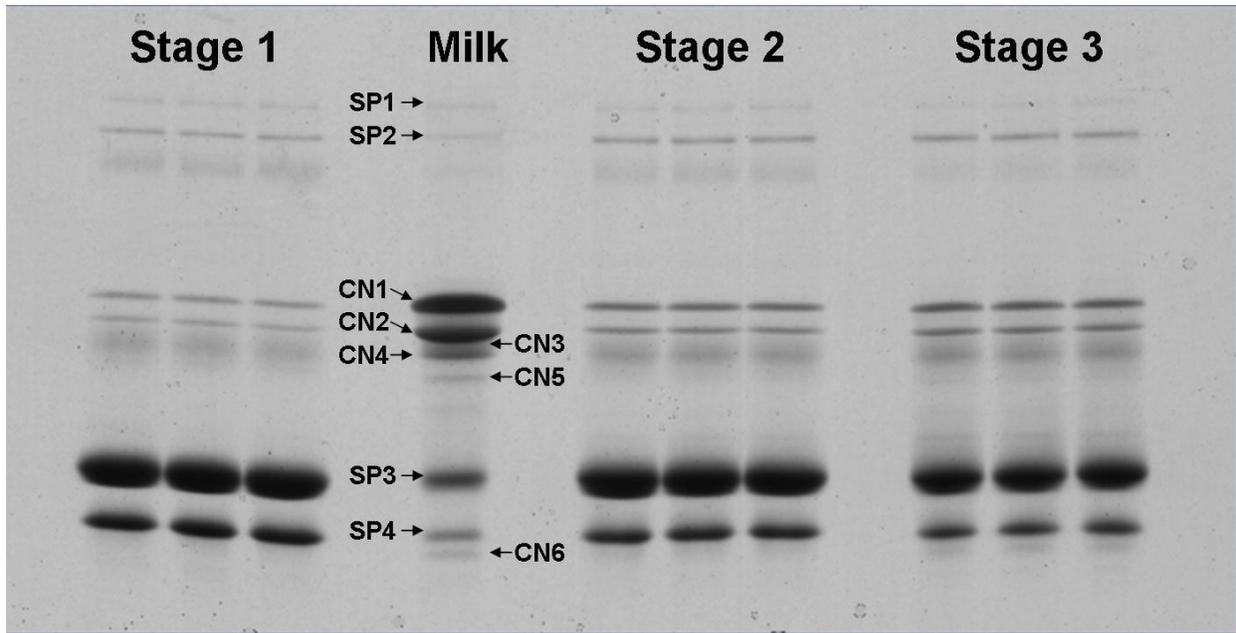


Figure 2.2. Sodium dodecylsulfate-PAGE of permeate samples collected after each stage of processing during a 3-stage microfiltration with water diafiltration (stage 2 and stage 3). Skim milk from the replicate was used as a reference sample. Bands in skim milk are identified on the gel: SP1 and SP2 = BSA, lactoferrin, lactoperoxidase, and high molecular weight soluble protein aggregates (Jovanovic et al., 2007), CN1 = α_s -CN (combination of α_{s1} and α_{s2} -CN), CN2 = β -casein, CN3 = proteolysis products of casein, CN4 = κ -casein, CN5 = proteolysis products of casein, SP3 = β -LG, SP4 = α -LA, CN6 = proteolysis products of casein.

RESULTS AND DISCUSSION

Processing

Mean ($n = 3$) operational parameters for the 3 stages of the MF process are reported in Table 2.1. The ΔP did not vary ($P > 0.05$) among stages because it was our goal to maintain a ΔP of 100 kPa for all three stages. Retentate and permeate bleed rates were adjusted to maintain a 3X CF and both bleed rates increased ($P < 0.05$) with stage (Table 2.1) due to the fact that the unrestricted permeate flux at a ΔP of 100 kPa increased with stage (Table 2.2). The R_{p_i} increased ($P < 0.05$) with stage and the R_{p_o} decreased ($P < 0.05$) with stage (Table 2.1) ($P < 0.05$), which was expected because retentate bleed rate was adjusted to maintain a target 100 kPa ΔP during processing. The R_{p_i} and R_{p_o} values observed in stage 1 were similar to those reported

by Zulewska et al. (2009) for a single stage SW MF of skim milk. Concentration factor did not vary ($P > 0.05$) with stage of processing (Table 2.1). The CF for all three stages were below the 3X target and only in stage three was close to 3X.

Table 2.1. Mean ($n = 3$) operational parameters recorded during the three stages of a microfiltration with diafiltration of skim milk using a 0.3 μ m polyvinylidene fluoride spiral-wound membrane

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

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

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

Average ($n = 3$) total time of processing was 133, 83 and 55 min, for stage 1, stage 2 and stage 3, respectively. Average flux (kg/m² per h) for milk processing increased ($P < 0.05$) with stage of processing (Table 2.2). All three stages exhibited an initial decline in flux during the first 30 min of milk processing (Figure 2.3). This initial decline in flux can be attributed to the transition from water to milk and the gradual decrease in water content of the liquid in the MF recirculation loop. Due to the initial transition from water to milk the average flux calculations were done using values starting at 30 min to the end of the run, instead of using values from time zero.

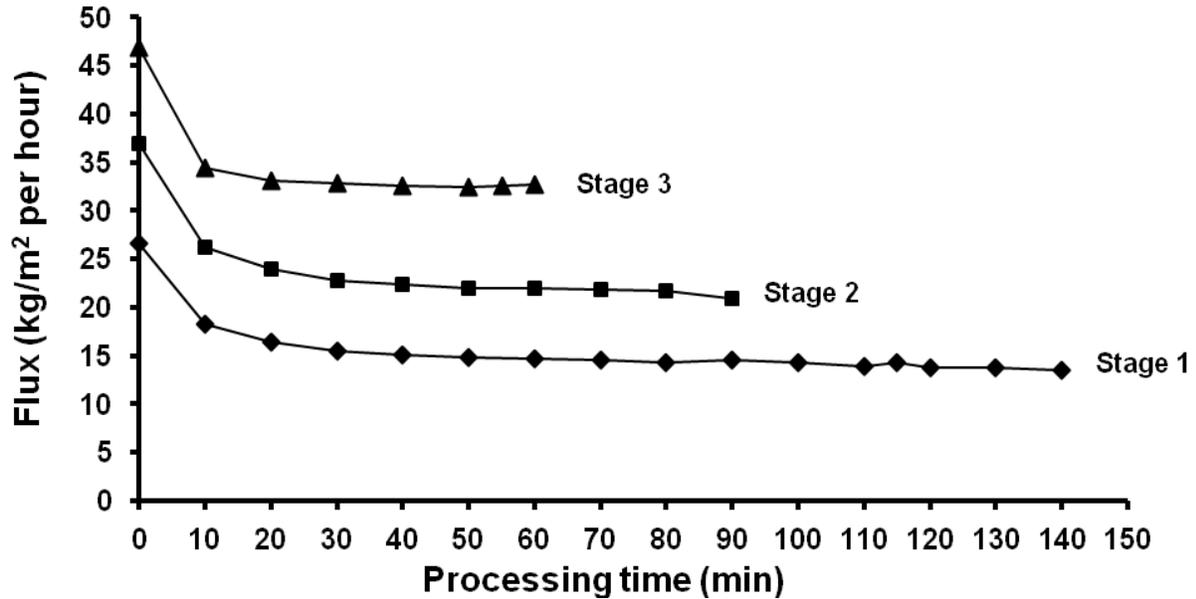


Figure 2.3. Mean ($n = 3$) flux for stage 1 (\blacklozenge), stage 2 (\blacksquare), and stage 3 (\blacktriangle) when using a 0.3- μm polyvinylidene fluoride spiral-wound microfiltration with water diafiltration (stages 2 and 3) of skim milk at 50°C.

Water flux before and after running milk or diluted retentate are shown in Table 2.2. No difference in starting water flux before milk processing was detected ($P > 0.05$) with stage of processing showing that the resistance of the membrane to water flow was consistent prior to each run. No difference in the water flux was detected when comparing flux before milk processing and the ending flux after the final cleaning for any stage of processing (Table 2.2). This indicates that the long clean performed after milk processing adequately removed foulant. Average ($n = 3$) fouled water flux increased ($P < 0.05$) from the first stage (7.10 kg/m² per h) to the third stage (13.94 kg/m² per h). The increased flux on milk with increasing stage and the increased water flux for the fouled membrane (Table 2.2) meant that there was progressively less membrane fouling across the 3 stages. The explanation of this behavior of flux will be given in the context of retentate composition in the next section.

Table 2.2. Mean (n = 3) flux by stage of processing during a 3-stage microfiltration with water diafiltration (stages 2 and 3) using a 0.3µm polyvinylidene fluoride spiral-wound membrane

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

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

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

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

³Fouled water flux = water flux of membrane after processing

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

Composition

Skim Milk. The mean (n = 3) composition of the pasteurized skim milk (Table 2.3) used in the SW MF was typical except that the CN as a percentage of TP (**CN%TP**) was higher than in our previous work on MF. The CN%TP for the 3 replicates in the current study were 83.4, 84.2, and 83.2% respectively, while the mean CN%TP for the studies by Zulewska et al. (2009) and Hurt et al. (2010) were 82.4 and 82.4%, respectively. The higher CN%TP in the current study was because of the higher temperature and longer hold time used to pasteurize the skim milk (79°C, 18 s) than the 72°C for 16 s used in the previous studies. High heat treatment of milk causes disulfide bond formation between the heat labile β-LG and the κ-CN located on the exterior of the CN micelle (Donato and Guyomarc'h, 2009). Heat denaturation of SP causes an over-estimation of the amount of CN in milk when analyzed using Kjeldahl analysis (Lynch et al., 1998).

Table 2.3. Mean (n = 3) composition (% by weight) of the pasteurized skim milk

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

¹TS = total solids; TN = total nitrogen × 6.38; NCN = noncasein nitrogen × 6.38; NPN = nonprotein nitrogen × 6.38; TP = true protein, TN – NPN; casein = TN – NCN; serum proteins = TP – casein; CN%TP = casein as a percentage of TP

Retentate. The mean (n = 3) composition of retentates produced during each of 3 stages using polymeric SW MF with water diafiltration is presented in Table 2.4. Total solids, TN, NCN, NPN, and SP decreased ($P < 0.05$) with stage. True protein content of the retentate decreased ($P < 0.05$) with increasing stages. Equations to predict the expected TP content retentate after each stage of a 3-stage 3X MF with 2 water diafiltration stages were developed by Hurt and Barbano (2009). Using those equations and the skim milk TP reported in Table 2.3, expected values for TP in retentate after each stage are 8.18, 7.81 and 7.65%, for stages 1 through 3, respectively. These expected values for TP in retentate are higher than the actual TP in retentate observed (Table 2.4). The lower observed TP values in the present study were due to over-dilution of retentate with RO water for diafiltration prior to stages 2 and 3. Over-dilution stemmed from our inability to achieve the target 3X CF (Table 2.1) during any of the three stages. The RO water for diafiltration was added (by weight) assuming a 3X CF was achieved in the previous stages. As demonstrated in the modeling by Hurt and Barbano (2010), a CF < 3X and a dilution factor = 3X caused the TP and CN concentrations in retentates in latter diafiltration stages to be lower than expected. Casein content in retentate decreased ($P < 0.05$) with stage during the 3-stage SW MF with diafiltration (Table 2.4). Theoretically, the CN content of the retentate should remain constant (CF times percent CN in skim milk) if the CF and

dilution factor are both controlled. Accurate control of the CF could be achieved by adding volumetric flow meters and flow control valves on the feed inlet, permeate outlet and retentate outlet areas of the membrane. The flow meters could be monitored and the control valves adjusted accordingly to change the instantaneous CF. The progressively lower than expected CN concentration in the MF feed from stage to stage (Table 2.4) explains the progressively higher mean flux in the second and third stages (Table 2.2 and Figure 2.3). Figure 2.4 gives a visual representation of the increase in total protein concentration in retentate during MF for each stage measured by infrared spectroscopy. The initial increase in protein content during the first 10 min was caused by switching of feed material from water to milk (stage 1) or to diluted retentate (stage 2 and stage 3). This large increase in protein and CN content during the beginning of the run for each stage (Figure 2.4) corresponds to the large decrease in flux (Figure 2.3) for each stage during the first 10 min of operation. The mean ($n = 3$) pH of retentates produced by each of the three stages of MF with diafiltration is presented in Table 2.4. The pH of the retentate increased ($P < 0.05$) with stage and is consistent with similar results for MF retentates produced with ceramic membranes that were reported by Hurt et al. (2010).

Table 2.4. Mean (n = 3) composition of retentates (% by weight) and pH by stage of processing during a 3-stage microfiltration with water diafiltration (stages 2 and 3) using a 0.3- μm polyvinylidene fluoride spiral-wound membrane

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

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

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

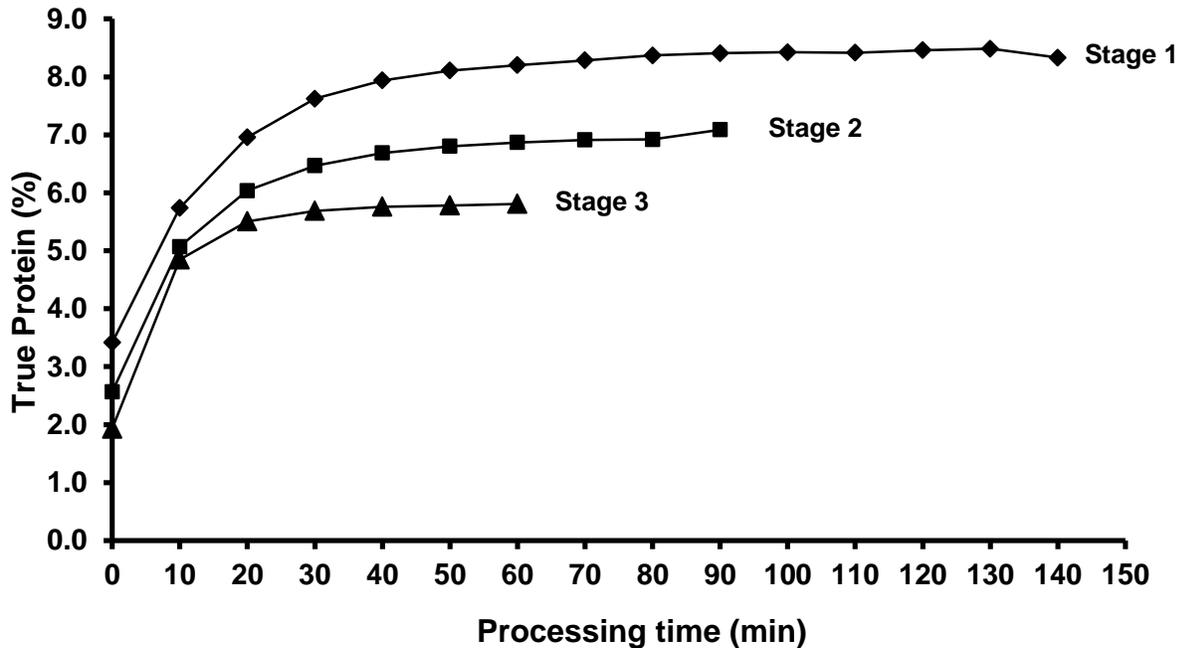


Figure 2.4. True protein (% by weight) in the retentate as a function of time of processing (min) for a 3-stage microfiltration with diafiltration of skim milk at 50°C using a 3.0- μm polymeric spiral-wound membrane during stage 1(\blacklozenge), stage 2(\blacksquare), and stage 3(\blacktriangle). True protein content measured by infrared spectroscopy.

Permeate. The mean ($n = 3$) composition of permeate obtained from each MF stage is presented in Table 2.5. Total Solids, TN, NCN, NPN, and TP in permeate decreased ($P < 0.05$) with stage but the decreases would have been smaller if the concentration and diafiltration dilution factors were matched at 3X. Hurt et al., 2010 reported higher TN and TP in permeate from all 3 stages when using ceramic UTP membranes to remove SP from skim milk than those reported in Table 2.5 for SW polymeric membranes. Thus, a lower SP removal from skim milk when using polymeric SW membranes may be expected compared to ceramic membranes. Casein, as measured by Kjeldahl, was present at about 0.017% in permeate from all 3 stages (Table 2.5). The presence of CN in the permeates was confirmed by SDS-PAGE (Figure 2.2). We could not detect a difference ($P > 0.05$) in the amount of CN in permeate among stages (Table 2.5). The CN as percentage of true protein determined by SDS-PAGE of permeates (Table 2.6) increased ($P < 0.05$) with each successive stage due to a fixed concentration of CN passing through the membrane for each stage while the concentration of SP in the permeate was decreasing. In addition, the relative proportion of β -LG to α -LA was also increasing ($P < 0.05$) with increasing MF stage (Table 2.6). It is interesting to note that we also observed increasing flux with increasing stage (Table 2.2 and Figure 2.3) which indicated that fouling and hydraulic resistance of the membrane plus foulant was progressively decreasing from one stage to next under the processing conditions in our study. If the membrane plus the foulant were determining the resistance to passage of SP, particularly β -LG, then as fouling decreased with increasing stage, β -LG became an increasing percentage of the SP in the permeate (Table 2.6). Therefore, at lower concentration of CN in the retentate within recirculation loop there was less fouling and higher rate of passage β -LG into permeate. It is interesting to note that the nominal pore size of the PVDF membranes used in this study was 0.3 μm , which is larger than the CN micelles but

the fact is very little CN passed through the membranes into permeate. This further supports the role of the foulant in determining the final selectivity to passage of SP through the polymeric membrane. With respect to maximization of the percentage removal of SP from skim milk, the optimum CF for operation of PVDF SW membranes may be lower than 3X at 50°C.

Table 2.5. Mean (n = 3) composition (% by weight) of permeates by stage of processing produced during a 3-stage microfiltration with water diafiltration (stages 2 and 3) using a 0.3- μ m polyvinylidene fluoride spiral-wound membrane

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

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

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

Table 2.6. Mean (n = 3) relative proportions of casein and serum proteins and the relative percentage of β -LG and α -LA as a percentage of their sum in permeate produced during a 3-stage spiral-wound microfiltration with water diafiltration (stages 2 and 3) process measured by densitometry analysis of the SDS-PAGE gels

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

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

SP Removal

The mean (n = 3) SP removal from 50°C skim milk using SW MF with water diafiltration is shown in Table 2.7. Theoretically, when using a 3X CF 68, 22 and 7% of the original SP

should be removed from skim milk during the 1st, 2nd and 3rd stage respectively, regardless of membrane type (Hurt and Barbano, 2010). After 3 stages of MF with diafiltration, the theoretical cumulative removal should be 97%. The SW MF process with diafiltration at 50°C described in our study resulted in 38.6, 20.8 and 10.9% SP removal from skim milk for the 1st, 2nd and 3rd stages, respectively. The SP removal from skim milk in the first stage of SW MF, 38.56%, was lower ($P < 0.05$) than what was found for a ceramic UTP MF system, 64.83%, using a similar 3-stage, 3X process (Table 2.7). The ceramic UTP system exhibited removal rates close to theoretical (Table 2.7). We calculated (using 27.4% SP removal rate during third-stage operation) that an additional five RO water diafiltration stages would be needed to remove 95% of the SP from milk, thus bringing the total stages to eight to achieve what has been achieved in 3 stages with ceramic membranes (Nelson and Barbano, 2005; Hurt et al., 2010). Hurt and Barbano (2010) evaluated the parameters that can cause low SP removal rate when microfiltering skim milk and reported that the two most important factors influencing SP removal were heat denaturation of SP prior to MF and rejection of SP passage through the membrane by either the membrane or a combination of the membrane plus foulant. Cumulative removal of SP from 50°C skim milk using SW MF with diafiltration was 70.3% of the original nonheat denatured SP content of the skim (Table 2.7). The relative percentage SP removal was lower ($P < 0.05$) with the SW polymeric PVDF membrane system (70.3%) than reported (98.26%) by Hurt et al., 2010 for a similar process using a ceramic UTP system. In both studies, the amount of SP that was heat denatured and bound to CN micelles was not counted in the amount of SP in the skim milk as SP that was available for removal by the MF process when the estimation of removal was based on Kjeldahl analysis of skim milk and permeates. Therefore, the difference in heat treatments of the milk between the two studies would not influence the

estimation of relative percent removal of SP from skim milk. The fact that the calculated relative percentage of SP removal was not influenced by the heat denatured SP was confirmed by comparison of the mean percent SP removal in the first stage in the present study (i.e., 38.56%) with the SP removal (38.62%) reported by Zulewska et al. (2009) for a 3X MF of skim pasteurized at 72°C for 16 s.

Table 2.7. Mean (n = 3) percentage serum protein (SP) removal for each stage as percentage of SP in starting skim milk for a spiral-wound microfiltration with water diafiltration (stages 2 and 3) process and a ceramic uniform transmembrane pressure (UTP) process as determined by Kjeldahl analysis

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

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

¹ Assuming no rejection of serum proteins and complete rejection of casein

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

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

A difference in heat denaturation of SP would influence relative proportion of SP retained in the retentate (as measured by SDS-PAGE) and the comparison of the kg of SP removed per meter squared per hour between the two studies. If there was no selective blockage of the passage of β -LG vs. α -LA into permeate, the ratio of β -LG to α -LA in the retentate would be the same in the retentates from all stages and the ratio β -LG to α -LA would be the same as in the original skim milk. As shown in Table 2.8, the proportion of β -LG to α -LA increased with stage and was higher than the original skim milk, indicating there was more resistance to passage of β -LG through the membrane. This could be due to a combination of both the β -LG bound to CN

micelles due to heat denaturation of β -LG and blockage of passage of some β -LG through the membrane. The removal of SP expressed as kg SP removed per m^2/h was much lower for PVDF membranes (about 0.05 vs. 0.31 kg/m^2 per h in the first stage) than for UTP ceramic membranes (Table 2.9). Part of the lower SP removal in kg/m^2 per h for the PVDF membranes was due to the higher heat, but based on the information presented by Hurt and Barbano (2010), the reduction in kg/m^2 per h due to the higher heat treatment of the milk in the current study would be about 0.03 to 0.04 kg/m^2 per h and this a very small part of the observed difference (i.e., about 0.25 kg/m^2 per h in the first stage) between the two systems (Table 2.9). Why was the relative removal of SP by the polymeric SW system lower than the ceramic system? It appears that the PVDF polymeric membrane in combination with the foulant was blocking the passage of SP through the membrane. The relative contribution of the membrane versus the foulant needs to be determined.

Table 2.8. Mean ($n = 3$) relative proportions of casein and serum proteins and the relative percentage of β -LG and α -LA as a percentage of their sum in skim milk and retentates produced during a 3-stage spiral-wound microfiltration with water diafiltration (stages 2 and 3) process measured by densitometry analysis of the SDS-PAGE gels

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

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

Table 2.9. Comparison of the mean ($n = 3$) kilograms of serum protein (SP) removed per hour per meter squared of membrane for each stage between spiral-wound microfiltration (SW MF) and ceramic uniform transmembrane pressure (UTP) microfiltration systems

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

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

¹Data from Hurt et al. (2010).

CONCLUSIONS

Theoretically, 68% of the SP content of skim milk can be removed using a single stage 3X MF. If two subsequent water diafiltration stages are used, an additional 22% and 7% of the SP can be removed, respectively, giving a total SP removal of 97%. A SP removal greater than 95% has been achieved using a 0.1 μm pore size ceramic UTP MF membrane (Hurt et al., 2010) after a 3-stage MF with diafiltration process. One stage of MF plus two stages of diafiltration of 50°C skim milk using a PVDF polymeric SW 0.3 μm membrane yielded a total SP reduction of only 70.3% (stage 1, 2, and 3: 38.6%, 20.8%, and 10.9%, respectively). The SP removal rate for the polymeric SW MF membrane was lower in all three stages of processing (stage 1, 2, and 3: 0.05, 0.04, and 0.03 kg/m² per h, respectively) than that of the comparable ceramic UTP MF membrane (stage 1, 2, and 3: 0.30, 0.11, and 0.06 kg/m² per hour, respectively), indicating that SW MF is less efficient at removing SP from 50°C skim milk than the ceramic UTP system. To estimate the number of steps required for the SW system to reach 95% SP removal, the third stage SP removal rate (27.4% of the starting material SP content) was used to extrapolate that an additional five water diafiltration stages would be necessary for a total of eight stages to remove

95% of the SP from skim milk. The eight-plus stages necessary to remove > 95% SP for the SW MF membrane would create more permeate and a lengthier process than with ceramic membranes.

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CHAPTER THREE

Impact of microfiltration concentration factor on serum protein removal from skim milk using polymeric spiral-wound membranes.

ABSTRACT

The objective of this experiment was to determine the impact of concentration factor (**CF**) on the removal of serum protein (**SP**) from skim milk during microfiltration (**MF**) at 50°C using a 0.3 µm pore size polymeric polyvinylidene fluoride (**PVDF**) spiral wound (**SW**) membrane. Pasteurized (72°C, 16 s) skim milk was MF (50°C) at three CF, 1.50, 2.25, and 3.00×, each on a separate day of processing starting with skim milk. Two phases of MF were used at each CF, with an initial startup-stabilization phase (40 min, full recycle mode) to achieve desired CF, followed by a steady-state phase (90 min, feed-and-bleed with recycle) where data was collected. The experiment was replicated three times, and SP removal from skim milk was quantified at each CF. System pressures, flow rates, CF, and fluxes were monitored during the 90 min run. Permeate flux increased (12.8 15.3, and 19.0 kg/m² per h) with decreasing CF from 3.00 to 1.50×, while fouled water flux did not differ among CF, indicating that the effect of membrane fouling on hydraulic resistance of the membrane was similar at all CF. However, the CF used when MF skim milk (50°C) with a 0.3 µm polymeric SW PVDF membrane did affect the percentage of SP removed. As CF increased from 1.50 to 3.00×, the percentage of SP removed from skim milk increased from 10.56 to 35.57%, in a single stage bleed-and-feed MF system. Percentage SP removal from skim milk was lower than theoretical. Rejection of SP during MF of skim milk with SW PVDF membranes was caused by fouling of the membrane, not by the membrane itself and differences in the foulant characteristic among CF influenced SP rejection more than it influenced hydraulic resistance. We hypothesize that differences in the conditions

near the surface of the membrane and within the pores during the first few minutes of processing, when casein micelles pass through the membrane, influenced the rejection of SP because more pore size narrowing and plugging occurred at low CF than at high CF due to a slower rate of gel layer formation at low CF. It is possible that percentage removal of SP from skim milk at 50°C could be improved by optimization of the membrane pore size, feed solution composition and concentration, and controlling the rate of formation of concentration polarization derived gel layer at the surface of the membrane during the first few minutes of processing.

KEYWORDS: microfiltration, concentration factor, serum protein, spiral-wound membrane

INTRODUCTION

Cross-flow microfiltration (**MF**) of skim milk is a pressure driven membrane separation that can be used to separate serum proteins (**SP**) from casein (**CN**) proteins in skim milk. Pore sizes of membranes used for MF range from 0.1 to 5.0 μm , depending on the application (Cheryan, 1998). Casein micelles, 0.02 to 0.40 μm diameter, are in colloidal suspension in milk, and are approximately 100 times larger than SP, 0.003 to 0.010 μm , which are soluble in milk (Walstra et al., 2006). Retentates produced during MF of skim milk using a membrane with a pore size of 0.1 to 0.3 μm contain higher concentrations of CN micelles and other suspended particles which are larger than the maximum membrane pore size. The CN-rich retentates, often called micellar casein concentrates (**MCC**), can be used in myriad of products including cheese (Nelson and Barbano, 2005), or other food and beverage products.

Membrane materials commonly used to MF skim milk to create MCC include ceramic (i.e. inorganic) and polymeric organic materials. Ceramic MF membranes used for the production of MCC have been studied (Zulewska et al., 2009; Hurt et al., 2010), and the advantages of their use versus polymeric MF membranes include longer life, and greater

resistance to cleaning chemicals and higher temperatures (Cheryan, 1998). The most common configuration of polymeric membranes used by the dairy industry in North America is spiral-wound (**SW**) but other configurations (e.g., tubular, hollow fiber and flat sheet) are possible. Polymeric SW MF membranes have the advantage of being less expensive to purchase (Cheryan, 1998), and SW membrane technology is familiar to the dairy industry, as it has been used since the 1970's for ultrafiltration of cheese whey (Maubois, 1980). Most published research on MCC manufacture using MF has focused on ceramic membranes (Saboya and Maubois, 2000; Zulewska et al., 2009; Hurt et al., 2010) because of their recognized ability to separate SP from CN in skim milk. Ceramic membranes have a narrower pore size distribution than polymeric membranes (Brans et al., 2004), which gives ceramic membranes an enhanced ability to retain particles with diameters greater than a given pore size.

What are the current factors limiting the implementation of production of MCC and milk serum protein concentrates (**SPC**)? There is not history of use of these two protein ingredients prepared from skim in food formulations, so experimentation and product formulation experience is needed to establish any functionality advantage of these ingredients in food and beverage applications, however their potential looks good, e.g., use of SPC for fortification of low pH clear shelf-stable beverages and use of MCC in retorted shelf-stable high protein nutritional beverages (Beliciu et al., 2012; Sauer and Moraru, 2012). Sensory and functionality comparisons of SPC and whey protein concentrate (**WPC**) have been reported (Evans et al., 2009; Evans et al., 2010, Jervis et al., 2012). Clearly, the cost of ceramic membranes and the energy use are both high and that has been a cost concern that has made dairy processors cautious about investing in this technology even when their flux of $\geq 54 \text{ kg/m}^2$ per h is attractive (Hurt et al., 2010). The cost of polymer MF membrane systems is much lower than ceramic

systems (Cheryan, 1998), but their propensity to foul and exhibit low flux compared to ceramic MF membranes (Zulewska et al., 2009) is a concern and reduces their cost advantage. Low permeate flux, 6 to 17 kg/m² per h, during SW MF of skim milk have been reported (Lawrence et al., 2008; Zulewska et al., 2009; Beckman et al., 2010). Fouling of ceramic membranes during skim milk MF is minimized by high cross-flow velocities (e.g., 5 to 7 m/s) and a uniform transmembrane pressure (**TMP**) (Saboya and Maubois, 2000). Not all configurations of ceramic membranes produce the same percent SP removal, and a recent study of Isoflux ceramic membranes reported a substantially lower (ca. 70%) SP removal for a 3-stage, 3.00× process at 50°C (Adams and Barbano, 2013). Spiral-wound MF membranes operate at lower (e.g., < 2 m/s) cross-flow velocities (Lawrence et al., 2008) and typically do not operate with a uniform TMP (Zulewska et al., 2009; Beckman et al., 2010), leading to much lower flux than ceramic membranes.

One of the largest disadvantages of polymeric SW MF membranes is the low efficiency of SP removal from skim milk. Zulewska et al. (2009) reported single-stage SP removal rates from skim milk at 50°C at 3.00× concentration factor (**CF**) of 64, 61, and 39% for ceramic uniform TMP (**UTP**), ceramic graded-permeability (**GP**), and SW polymeric membranes, respectively. For a SW MF system, Beckman et al. (2010) reported about a 70% SP removal from skim milk in a 3-stage 3.00× process at 50°C compared with a SP removal of about 98% with UTP (Hurt et al., 2010) and 97% with graded permeability ceramic membranes (J. Zulewska, Faculty of Food Science, University of Warmia and Mazury, Olsztyn, Poland 10-719, personal communication). In the report by Beckman et al. 2010, it was observed that flux increased with decreasing CF and we hypothesize that SP removal with SW membranes may be

higher when the SW system is operated at lower CF, higher flux, and with less concentration polarization fouling.

Increasing the SP removal performance of polymeric SW MF would improve the chances of wide spread implementation of this technology in the dairy industry and potentially increase the utilization of milk proteins in foods. An improved understanding of fouling of SW MF membranes and development of methods to improve polymeric membrane performance for separation of CN and SP from skim milk are needed. Our objective was to determine the effect of different MF CF, 1.50, 2.25, and 3.00 \times , on SP removal from skim milk, and fouling of polymeric polyvinylidene fluoride (**PVDF**) SW MF 0.3 μm membranes at 50 $^{\circ}\text{C}$.

MATERIALS AND METHODS

Experimental Design and Statistical Analysis

On day 1 of processing, raw whole milk was centrifugally (4 $^{\circ}\text{C}$) separated into skim and cream. The skim was pasteurized (72 $^{\circ}\text{C}$, 16 s) and stored at 4 $^{\circ}\text{C}$. On day 2, 3, and 4, the pasteurized skim milk was MF at 50 $^{\circ}\text{C}$ using a 0.3 μm nominal pore size polymeric PVDF SW membrane, using one of three CF, 1.50, 2.25, or 3.00 \times , on each of the days. On each day of filtration there were two phases of the MF processing run: startup-stabilization (about 40 min) and steady-state processing phase (about 90 min), each using skim as the feed material. Flux ($\text{kg permeate}/\text{m}^2$ per h) and CF [(permeate (kg) + retentate (kg)) / retentate (kg)] were measured at 5 min intervals during the startup-stabilization until the desired CF was achieved. The steady-state processing phase lasted 90 min during which flux and CF were measured every 5 min (2.25 and 3.00 \times CF) or 2.5 min (1.50 \times CF). Samples of permeate and retentate were taken during the run to determine the amount of SP being removed. The experiment was replicated in 3 different weeks using different lots of whole milk. The order of the CF among days 2, 3, and 4 was rotated

from one replicate to the next to achieve a balanced design with respect to the day on which each CF was run.

To determine if the CF used during MF of skim milk at 50°C influenced the observed operational parameters, mean flux, and composition of the composite permeates and retentates, statistical analysis of the data was done using Proc GLM of SAS (Version 9.3, 2011, SAS Institute, Cary, NC). The GLM was: dependent variable = target CF + replicate + error, with target CF and replicate as category variables. Milk permeate fluxes, protein contents of permeates, and protein contents of retentates measured throughout the steady-state processing were analyzed for change over time using the data analysis tool-pack regression function in Microsoft Excel (Excel 2010, Microsoft Corp., Redmond, WA) to determine if the slope of the relationship with time was different ($P < 0.05$) than zero.

Separation and Pasteurization of Milk

On the first day of each replicate, one lot of raw whole milk (approximately 2,145 kg) from the Cornell University Dairy was separated (model 372 Airtight, DeLaval Separator Co., Poughkeepsie, NY) into skim and cream at 4°C. The raw skim milk was pasteurized with a plate heat exchanger consisting of 3 sections: regeneration, heating, and cooling (model 080-S, AGC Engineering, Manassas, VA) at an average of $72.5 \pm 0.1^\circ\text{C}$ and a holding time of 16 s. The pasteurized skim milk was pumped into a jacketed stainless steel tank where it was held without agitation at $< 4^\circ\text{C}$ for use during the subsequent three processing days. Approximately 560 kg of skim milk to be used each day for MF was pumped out of the tank after agitating the tank for 10 min. A sample of pasteurized skim milk was taken on day 1 for chemical analysis by agitating the skim milk in the large jacketed tank for ≥ 10 min and using a sanitized sampling dipper.

Microfiltration of Skim Milk

A diagram of the SW MF system used in this experiment is shown in Figure 3.1. On day 1 in each of the 3 processing weeks, the MF system was cleaned using the “long-clean” procedure used by Beckman et al. (2010). Before MF of skim milk on days 2, 3, and 4, the membrane was sanitized and a starting water flux before milk processing was determined, following the “short clean” procedure from Beckman et al. (2010). The membrane was drained of storage solution, flushed and rinsed with < 24°C reverse osmosis (**RO**) water, and then sanitized with a solution of Ultrasil 110 (EcoLab Inc., St. Paul, MN) liquid alkaline membrane cleaner (0.39% vol/vol) and XY-12 (EcoLab Inc., St. Paul, MN) liquid sanitizer (0.15% vol/vol) in RO water (< 24°C) to a pH of 11.2 to 11.4 and a chlorine level of 150 to 180 ppm. The sanitizer was circulated through the MF system for 10 min before being drained and flushed out with < 24°C RO water to neutral pH. A starting water flux was measured (Beckman et al., 2010), and the MF system was then stopped, valves closed, and left standing with RO water filling the system until the system was ready to be fed with 50°C skim milk.

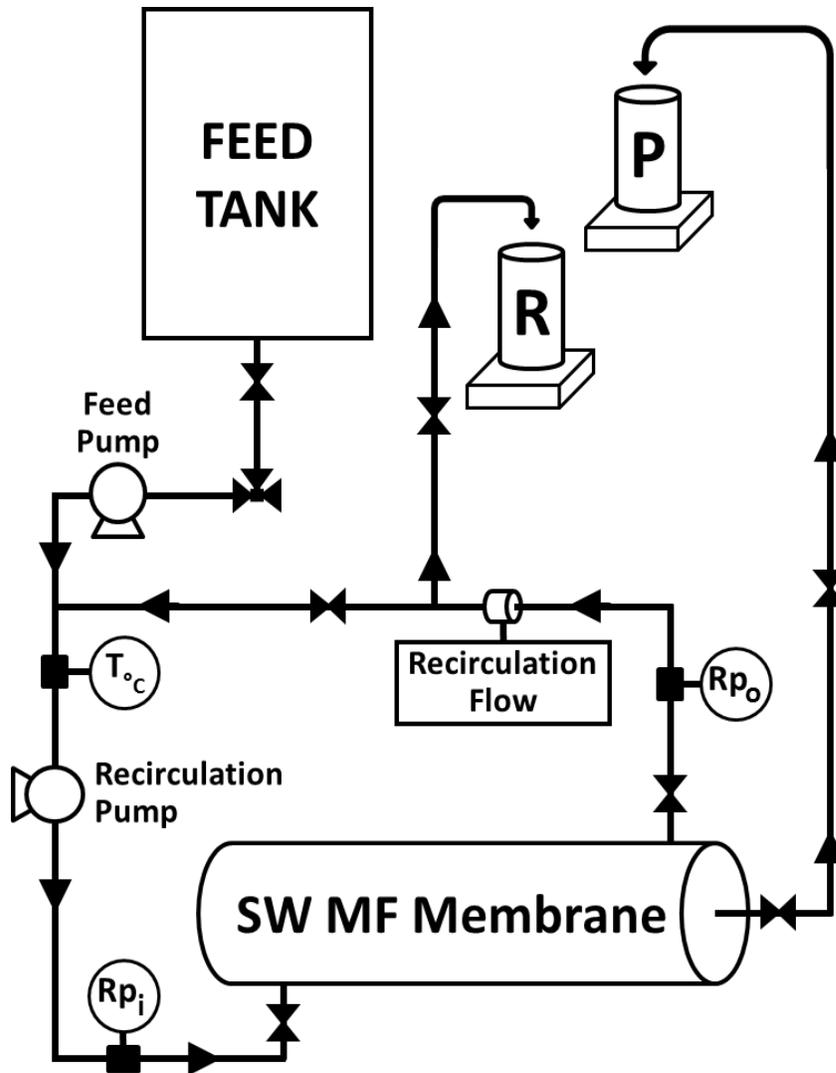


Figure 3.1. The polyvinylidene fluoride spiral-wound microfiltration (SW MF) system used during the microfiltration of skim milk (50°C) at three concentration factors, 1.50×, 2.25×, and 3.00×. Measurements in the system during processing included temperature (T_c), retentate pressure inlet (Rp_i), retentate pressure outlet (Rp_o), and recirculation flow rate (Recirculation Flow; L/min). Retentate (R) and Permeate (P) were collected and weighed during processing.

Microfiltration of skim milk for each replicate occurred sequentially on three days, using a different CF on each day, 1.50, 2.25, and 3.00×, respectively. For each CF with a day, there were two phases of processing, startup-stabilization (about 40 min) and steady-state processing phases (about 90 min). Each phase began with skim milk heated to 50°C. On the first day of

MF, approximately 260 kg of 4°C pasteurized skim milk was quantitatively transferred into a feed vat (Figure 3.1) and was heated to 50°C using a pump and plate heat-exchanger. The MF system was full of water and air was pushed out of the system with water. The SW MF startup-stabilization phase was as follows: the permeate exit valve was closed, retentate bleed valve opened, recirculation loop closed at the return line to the recirculation pump (Figure 3.1), and the feed pump was turned on, feeding the retentate side of the membrane with 50°C skim milk, clearing the RO water out, and not allowing any permeation of the membrane. After skim milk exited the retentate hose, the valve in the recirculation loop was opened and the recirculation pump was turned on to set the R_{p_i} at the correct pressure for the run, as established with water prior to skim milk. Next, the permeate exit valve was slowly opened to allow system pressures to balance and to begin MF. The retentate bleed valve was adjusted to give the correct CF, and the system pressures were maintained. Flux and CF measurements commenced once permeate exited the system. The 50°C skim milk was MF using a polymeric SW MF membrane (model FG7838-OSx-S, 0.3 μm pore size PVDF, 20.5 m^2 surface area, 1.09 mm spacer, 96.5 cm length \times 19.8 cm diameter, Parker-Hannifin, Process Advanced Filtration Division, Tell City, IN) at the target CF for 40 min in total recirculation mode, with a measurement of flux and CF every 5 min. The purpose of startup-stabilization phase was to flush the water out of the system and to achieve a retentate composition to near steady-state before the steady-state processing phase. Permeate and retentate were recirculated to the feed vat for 40 min, then allowed to flow to the drain to reduce the volume of skim in the feed vat to near zero. Next, more pasteurized skim milk (approximately 300 kg) at 50°C was weighed into to the feed vat and MF at the target CF for the steady-state processing phase without stopping the system. During the steady-state processing phase (90 min), flux and CF were measured, along with collection of permeate and retentate

samples, approximately every 2.5 min for $CF = 1.50\times$ and 5 min for $CF = 2.25$ and $3.00\times$. The different interval of measurement for $CF = 1.50\times$ vs. $CF = 2.25$ and $3.00\times$ was due to the high rate of flow of permeate and retentate exiting the system at lower CF. After weighing and sampling the collected permeate and retentate, both portions were added back to the feed vat to maintain constant feed level and composition throughout the run. At the end of the 90 min steady-state processing phase, the MF system was drained, shut down, and cleaned to prepare for day 2 of MF. At the end of the run each day, the membrane was given a “long-clean”, as described by Beckman et al. (2010). A fouled water flux and ending water flux after cleaning were obtained during the long-clean to determine the extent of fouling on the membrane and to ensure the water permeability of the membrane was restored to the pre-run condition. Microfiltration using the other two CF, 2.25 and $3.00\times$ occurred on day 3 and day 4 of the replicate, respectively. The processing procedures used during those MF were the same as with $CF = 1.50\times$. In subsequent replicates, the day on which each CF was run was changed so that each CF was carried out on day 1, 2, and 3 only once during the three replicates.

Pressures (kPa) in the MF system (Figure 3.1), measured by digital pressure gauges (model PI2094, IFM Efector Inc., Exton, PA), were recorded each time the flux was measured, and included: retentate pressure at the inlet (Rp_i), and retentate pressure at the outlet (Rp_o). Permeate pressure at the outlet (Pp_o) was not measured and was assumed to be 0 kPa because the permeate exit tube was open to the atmosphere. The difference in pressure (ΔP) between the retentate inlet and retentate outlet [$\Delta P = [Rp_i + Rp_o]/2$] of the membrane was kept constant at 100 kPa (14.5 p.s.i.) for all CF used. A digital magnetic mass flow meter (model AM204DH, Yokogawa Electronic Corp., Tokyo, Japan) was used to measure the recirculation loop flow rate (L/min).

Analyses of Milk, Retentate, and Permeate

Retentate and permeate samples collected at 5 min (2.25 and 3.00× CF) or 2.5 min (1.50× CF) intervals during the steady-state processing phase were combined into a weighted composite retentate and permeate sample, respectively. For example, the calculated mass of liquid permeate added to the composite from each fraction of permeate collected was based on the mass (kg) of the permeate fraction collected during the 5 or 2.5 min intervals divided by the total mass (kg) of permeate collected during the entire 90 min steady-state processing phase. Composite retentate samples were created using the same method. Composites were created so that representative samples of steady-state phase permeate and retentate could be analyzed for SP removal.

Pasteurized skim milk, composite retentate and composite permeate were analyzed for protein, fat, and total solids content. Protein content (%) was measured using total N (**TN**), noncasein N (**NCN**), and nonprotein N (**NPN**) content by Kjeldahl methods (AOAC 2000; methods 991.2, 33.2.11; 998.05, 33.2.64; and 991.21, 33.2.12, respectively). Fat content was measured by Mojonnier ether extraction (AOAC 2000; method 989.05, 33.2.26). Total solids (**TS**) were measured using the forced-air oven drying method (AOAC 2000; method 990.20, 33.2.44). True protein (**TP**) was calculated by subtracting NPN from TN and multiplying the difference by 6.38. Casein (**CN**) content was calculated by subtracting NCN from TN and multiplying the difference by 6.38; and SP content was calculated by subtracting NPN from NCN and multiplying the difference by 6.38. Serum protein in the permeate portion of the skim milk (%) was calculated by dividing SP in milk by SP in the permeate portion of the skim milk multiplied by 100, where the permeate portion of skim milk is considered: 100 kg minus weight of fat plus CN in 100 kg of milk. The permeate portion of skim milk represents only the

components of milk that would be available to flow freely through the membrane. Serum protein removal (%) from skim was calculated by dividing SP (kg) in composite permeate by SP (kg) in skim and multiplying by 100%, using the Kjeldahl nitrogen analyses above and recorded processing data.

Pasteurized skim milk, composite retentate, composite permeate, and individual fractions of retentate and permeate from the data collection stage of processing were all analyzed using an infrared (**IR**) spectrophotometer (Lactoscope FTIR, Delta Instruments, Drachten, the Netherlands) for fat, lactose, and protein content (Kaylegian et al., 2006). To reduce the impact of any day-to-day instrument variation, all IR measurements were analyzed in one batch on the same day (d = 4) of the replicate. Hunter color value L (lightness) of composite permeate was analyzed (25°C) using a MacBeth Color-Eye (model 2020, Kollmorgen Instrument Corp., Newburgh, NY) to determine if there were differences in opacity of permeate among CF which might reflect differences in CN passage through the membrane.

RESULTS AND DISCUSSION

Milk Processing Parameters and Flux

Operational Parameters. The average (n = 3) SW MF operational parameters recorded during steady-state processing of skim milk are shown in Table 3.1. No differences in the ΔP (i.e., pressure drop from inlet to outlet of the SW MF membrane), the R_{p_i} and R_{p_o} among the three CF were detected ($P > 0.05$) (Table 3.1). The ΔP were similar to values reported by Zulewska et al. (2009) and Beckman et al. (2010) for MF of skim (50°C) at CF 3.00 \times using the same membrane system and membrane. However, the average R_{p_i} reported by Zulewska et al. (2009) and Beckman et al. (2010), 131.3 and 132.4 kPa, respectively, were higher than what we observed in all three CF used (Table 3.1). A higher R_{p_i} while maintaining a similar ΔP (about

100 kPa) required lower R_{p_o} values, according to the equation for calculating ΔP [$\Delta P = [R_{p_i} + R_{p_o}]/2$], which was what occurred in the previous work of Zulewska et al. (2009) and Beckman et al. (2010). The average difference between R_{p_i} and R_{p_o} was smaller in the present study, 30.2 kPa, than for Zulewska et al. (2009), 70.7 kPa, or Beckman et al. (2010), 65.1 kPa. A smaller difference between the TMP from the inlet side of the membrane and the outlet side of the membrane is desirable. Lawrence et al. (2008) demonstrated that as TMP increases, the rejection of all milk proteins increases when MF skim using polymeric SW membranes. This smaller difference between R_{p_i} and R_{p_o} in the present study versus the previous studies was achieved by reducing the pipe inner-diameter from 35 mm to 22 mm (i.e., increasing resistance to recirculation loop flow) between the recirculation loop flow sensor and the recirculation pump (Figure 3.1).

Table 3.1. Mean ($n = 3$) operational parameters recorded during microfiltration of skim milk at three concentration factors (CF) using a 0.3 μm pore size polyvinylidene fluoride spiral-wound membrane at 50°C.

CF	Operational parameters ¹						
	ΔP (kPa)	R_{p_i} (kPa)	R_{p_o} (kPa)	Retentate removal (kg/min)	Permeate removal (kg/min)	Recirculation flow (L/min)	Measured concentration factor
1.50×	100.6 ^a	115.8 ^a	85.3 ^a	13.2 ^a	6.5 ^a	178.0 ^a	1.49 ^c
2.25×	100.5 ^a	115.6 ^a	85.3 ^a	4.1 ^b	5.1 ^b	168.7 ^b	2.25 ^b
3.00×	100.5 ^a	115.5 ^a	85.5 ^a	2.1 ^c	4.2 ^c	163.9 ^c	2.97 ^a
SE	0.1	0.5	0.4	0.1	0.1	0.4	0.004
R-square	0.72	0.78	0.89	>0.99	>0.99	0.99	>0.99

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

¹ $\Delta P = [(R_{p_i} + R_{p_o})/2]$, R_{p_i} = retentate pressure at the inlet of the membrane, R_{p_o} = retentate pressure at the outlet of the membrane

Average ($n = 3$) retentate and permeate removal rates (kg/min) decreased ($P < 0.05$) with increasing CF (Table 3.1). Retentate removal rate decreased from 13.2 kg/min at 1.50× to 2.1 kg/min at 3.00×, and permeate removal rate decreased from 6.5 kg/min at 1.50× to 4.2 kg/min at 3.00×. The retentate removal rate was expected to show a larger decrease than permeate removal

when processing at higher CF because the CF was controlled by adjusting only retentate removal rate. Retentate recirculation flow (L/min) decreased ($P < 0.05$) with increasing CF (Table 3.1). All recirculation flows in this experiment (Table 3.1) were lower than those reported by Zulewska et al. (2009) and Beckman et al. (2010) during MF of skim, 239 and 238 L/min, respectively. The lower recirculation rates observed in the current study were caused by the smaller diameter piping being used in the retentate recirculation loop located after the retentate outlet and before the recirculation pump, as described above. Average ($n = 3$) measured CF were different ($P < 0.05$) but were near the target CF of 1.50, 2.25, and 3.00 \times , respectively (Table 3.1). The CF measured during steady-state processing were near the target values because the CF were set and stabilized during the startup phase of processing.

Flux. Average ($n = 3$) milk permeate flux, starting water flux, fouled water flux, and ending water flux are shown in Table 3.2. Average milk permeate flux decreased ($P < 0.05$) with increasing CF (Table 3.2). After 10 min into steady-state processing, flux at 1.50, 2.25, and 3.00 \times CF, decreased ($P < 0.05$) 0.16, increased ($P < 0.05$) 0.15, and decreased ($P < 0.05$) 0.48 kg/m² per h, respectively (Figure 3.2). The changes with time at 1.50 and 2.25 \times CF were very small, however the size of the decrease in flux at 3.00 \times over many hours would be of practical importance for a factory. Mean milk permeate flux at CF 3.00 \times (Table 3.2, 12.81 kg/m²) was slightly lower than those reported by Zulewska et al. (2009), 16.2 kg/m² per h, and Beckman et al. (2010), 14.4 kg/m² per h, for MF of skim milk (50°C) at CF = 3.00 \times using a polymeric SW MF, but all of these are much lower than values (i.e., 54.2 kg/m² per h) reported by Hurt et al. (2010) for ceramic uniform TMP membranes. Higher fluxes during MF of skim milk using ceramic membranes than with polymeric SW membranes have been attributed to reduced protein fouling of the membrane resulting from the high cross-flow velocities (e.g. 6 to 8 m/s) and lower

TMP that are achieved in ceramic MF processes (Lawrence et al., 2008). Less fouling (i.e., lower resistance) of the membrane plus foulant and low permeate viscosity (e.g., at low CF) are expected to achieve higher flux according to Darcy's law. Permeate flux is directly proportional to the TMP and inversely proportional to the dynamic viscosity of the fluid permeating the membrane and overall hydraulic resistance (Marshall and Daufin, 1995; Le Berre and Daufin, 1996).

Table 3.2. Mean (n = 3) flux by concentration factor (CF) during skim milk microfiltration and cleaning using a 0.3 μm polyvinylidene fluoride spiral-wound membrane.

CF	Milk permeate flux ¹ (kg/m ² per h)	Starting water flux before milk ² (kg/m ² per h)	Fouled water flux ³ (kg/m ² per h)	Ending water flux after cleaning ⁴ (kg/m ² per h)
1.50×	18.97 ^a	33.54 ^a	21.12 ^a	35.13 ^a
2.25×	15.33 ^b	31.96 ^a	18.90 ^a	35.25 ^a
3.00×	12.81 ^c	33.41 ^a	18.68 ^a	34.88 ^a
SE	0.08	0.98	0.79	0.36
R-square	>0.99	0.42	0.70	0.45

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

¹Milk permeate flux = average permeate flux during processing skim milk (t = 90 min).

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

³Fouled water flux = water flux of membrane after processing

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

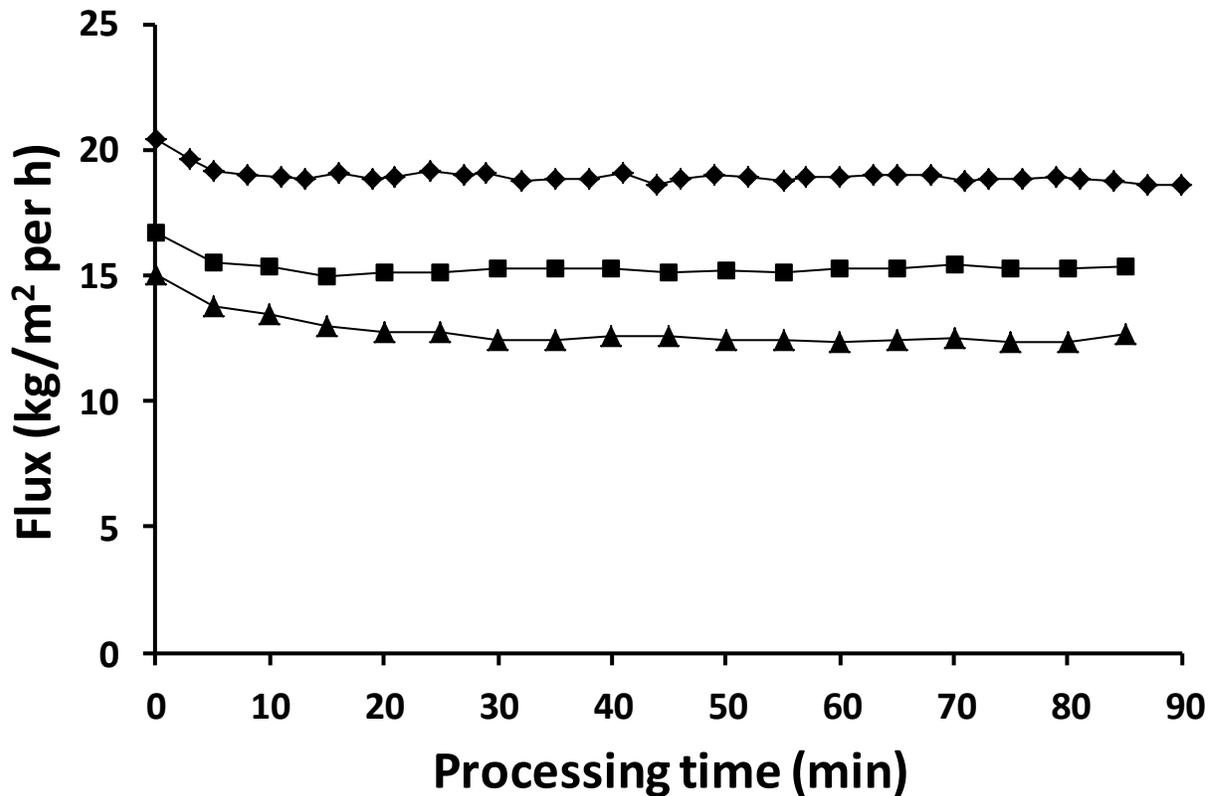


Figure 3.2. Average ($n = 3$) flux (kg/m^2 per h) during polyvinylidene fluoride spiral-wound microfiltration of skim milk (50°C) using three concentration factors, (◆) 1.50 \times , (■) 2.25 \times , and (▲) 3.00 \times .

No difference in both clean-membrane fluxes (starting water flux and ending water flux) were detected ($P > 0.05$) among CF (Table 3.2). Ending water fluxes were similar to starting water fluxes which meant that membrane cleaning restored membrane permeability in preparation for each run. No difference in fouled water flux after processing was detected ($P > 0.05$) among CF (Table 3.2) indicating that hydraulic resistance of the fouled membrane was consistent among the CF used. Fouled water flux at all three CF in this experiment (Table 3.2, 18.68 kg/m^2 per h) were higher than values reported by Zulewska et al. (2009), 11.5 kg/m^2 per h, and Beckman et al. (2010), 13.9 kg/m^2 per h, using the same SW MF system and membrane. The degree of fouling (see equation below), which is an indication of the fouling of the membrane

$$\text{Degree of Fouling} = 1 - \left[\frac{\text{Fouled Water Flux}}{\text{Clean Membrane Water Flux}} \right] \times 100\%$$

that is only reversed by cleaning (degree of fouling of 100% = total blockage), was in the range of 40 to 46% in this study (average = $44.2 \pm 2.2\%$), which is lower than the 80% reported by Beckman et al. (2010), using the same system and membrane. The only difference in the membrane system between the current study (at 3.00×) and the previous study by Beckman et al. (2010) was a slower recirculation rate (164 vs. 238 kg/min), lower retentate removal rate (2.1 vs. 2.8 kg/min) and a smaller difference between the membrane inlet and outlet pressures (30 vs. 65 kPa); all caused by the smaller diameter piping in the recirculation loop used in this study. This resulted in a lower TMP in the current study (TMP inlet, 116 vs. 132 kPa) and apparently less hydraulic resistance due to the deposited foulant layer. A lower fouled water flux indicates more hydraulic resistance due to fouling of the membrane, which is caused by protein-protein and protein-polymer interaction with the membrane (James et al., 2003). Adsorption of protein to hydrophobic polymeric membranes occurs rapidly on contact of milk with the membrane, and it occurs without any TMP (Tong et al., 1988; Rudan, 1990; Bowen and Gan, 1991). The higher fouled water fluxes observed in this experiment (Table 3.2) compared to Beckman et al. (2010), result from decreased fouling that can only be removed by cleaning the membrane. The lower degree of fouling observed in the present study vs. Beckman et al. (2010) may be due to differences in system start up procedures, different feed material compositions, lower TMP at the inlet, or a combination of these. Less fouling in polymeric PVDF membranes due to lower TMP has been reported (Grandison et al., 2000). As stated above, decreased differences between R_{p_i} and R_{p_o} (i.e., decreased TMP over the length of the membrane) were caused by the constriction in the retentate recirculation pipe, which was not done in the previous studies on our system.

Composition

Skim. Average ($n = 3$) composition of pasteurized ($72.5 \pm 0.1^\circ\text{C}$, 16 s) skim milk used in this experiment is in Table 3.3. The skim composition is similar to the skim used by Beckman et al. (2010) and Hurt et al. (2010). Casein as a percentage of TP (**CN%TP**) in skim milk was lower in this experiment (Table 3.3), than was reported by Beckman et al. (2010). The lower CN%TP in this experiment was caused by the lower pasteurization temperature (72.5°C for 16 s vs. 79°C for 19 s, respectively) in the current study than in the previous study (Beckman et al., 2010). Pasteurization of skim milk can lead to disulfide bonding between SP and CN micelles (Sawyer, 1969) and a subsequent increase in the apparent CN%TP as determined by Kjeldahl analysis (Lynch et al., 1998). If SP becomes bound to CN, a decrease in SP removal from skim milk would be observed as described by Hurt and Barbano, (2010). In the present study we used a pasteurization temperature very close to minimum (72.5°C , 16 s) pasteurization conditions (FDA, 2011) to minimize SP bonding to CN.

Table 3.3. Mean (n = 3) composition (% by weight) of the pasteurized skim milk

Item	Composition ¹									
	TS	Lactose	Fat	TN	NCN	NPN	TP	Casein	Serum proteins	CN%TP
Skim Milk	9.17	4.74	0.10	3.29	0.77	0.17	3.12	2.52	0.59	80.95
SE	0.02	0.004	0.02	0.01	0.01	0.01	0.01	0.01	0.003	0.13

¹TS = total solids; Lactose = lactose content measured by infrared spectroscopy; Fat = fat content measured by ether extraction; TN = total nitrogen × 6.38; NCN = noncasein nitrogen × 6.38; NPN = nonprotein nitrogen × 6.38; TP = true protein, TN – NPN; casein = TN – NCN; serum proteins = TP – casein; CN%TP = casein as a percentage of TP

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Table 3.4. Mean (n = 3) composition of composite retentates (% by weight) by concentration factor (CF) during microfiltration of skim milk (50°C) using a 0.3 µm polyvinylidene fluoride spiral-wound membrane.

CF	Composition (%) ¹									
	TS	Lactose	Fat	TN	NCN	NPN	TP	Casein	Serum proteins	CN%TP
1.50×	10.63 ^c	4.68 ^a	0.14 ^c	4.67 ^c	0.98 ^b	0.183 ^a	4.48 ^c	3.68 ^c	0.80 ^c	82.14 ^c
2.25×	12.56 ^b	4.57 ^b	0.21 ^b	6.51 ^b	1.16 ^a	0.170 ^a	6.34 ^b	5.35 ^b	0.99 ^b	84.42 ^b
3.00×	14.06 ^a	4.47 ^c	0.26 ^a	7.87 ^a	1.20 ^a	0.166 ^a	7.71 ^a	6.67 ^a	1.03 ^a	86.58 ^a
SE	0.03	0.003	0.01	0.01	0.01	0.003	0.02	0.02	0.01	0.19
R-square	>0.99	>0.99	0.97	>0.99	0.98	0.87	>0.99	>0.99	0.98	0.99

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

¹TS = Total Solids; Lactose = lactose measured by infrared spectroscopy; Fat = fat content measured by ether extraction; TN = total nitrogen × 6.38; NCN = noncasein nitrogen × 6.38; NPN = nonprotein nitrogen × 6.38; TP = true protein, TN – NPN; casein = TN – NCN; serum proteins = TP – casein; CN%TP = casein as a percentage of TP.

Retentate. The mean ($n = 3$) composition of composite retentates produced during skim milk MF (50°C) at three CF are shown in Table 3.4. Total solids, fat, TN, TP, CN, SP and CN%TP increased ($P < 0.05$) with increasing CF (Table 3.4). Lactose was the only component that decreased ($P < 0.05$) with increasing CF (Table 3.4). A decrease in lactose in retentate with increasing CF was expected as the CF increased from 1.50 to 3.00× due the displacement effect of the increase in concentration of protein and bound minerals. Fat and CN in the retentate were expected to increase with increasing CF because fat globules and CN micelles are rejected by the 0.3 µm pore size membrane. Total solids in retentates were expected to increase with increasing CF due to the increasing protein and fat content as CF increased (Table 3.4). Serum protein in retentate was expected to increase with increasing CF because some SP was being rejected by the membrane, as reported previously using this same system (Zulewska et al., 2009; Beckman et al., 2010). The increasing true protein as a function of CF can be seen in Figure 3.3. Even after 40 min of system startup and stabilization, protein content (Figure 3.3) in 3.00× retentate did not reach steady-state until approximately 30 min into steady-state processing (70 min total) which agrees with the previous report by Beckman et al. (2010) for skim milk MF at 3.00×. It was approximately 0 and 10 min of processing during the steady-state phase at 1.50 and 2.25×, respectively for the retentate to reach consistent protein content (Figure 3.3). Protein in the retentate at 1.50, 2.25, and 3.00× CF reached maximum values at the end of the steady-state phase of 4.57, 6.48, and 8.01% (Figure 3.3), respectively, and were 98, 92, and 86% of the expected TP in the retentate at 1.50, 2.25, and 3.00× CF, respectively. The CN%TP increased ($P < 0.05$) with increasing CF (Table 3.4). A higher CN%TP with increasing CF was expected because as CF increases, there is more removal of SP. No difference in the NPN content of retentates was detected ($P < 0.05$) among CF.

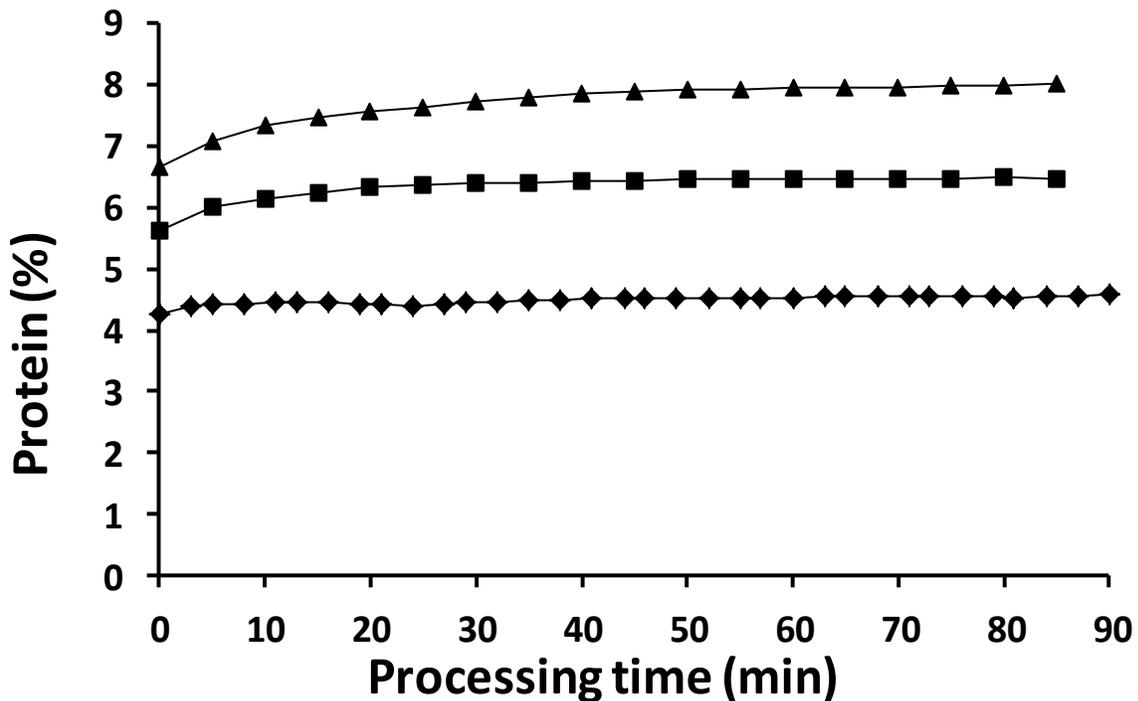


Figure 3.3. Average ($n = 3$) protein content (%) in the retentate during polyvinylidene fluoride spiral-wound microfiltration of skim milk (50°C) using three concentration factors, (\blacklozenge) 1.50 \times , (\blacksquare) 2.25 \times , and (\blacktriangle) 3.00 \times . Protein content was measured by infrared spectroscopy.

Permeate. The mean ($n = 3$) composition of composite permeates produced during skim milk MF (50°C) at three CF are shown in Table 3.5. In permeates, TS, TN, NCN, TP, and SP increased ($P < 0.05$) with increasing CF (Table 3.5). No differences in lactose, fat, NPN, CN, and CN%TP were detected ($P > 0.05$) among composite permeates produced at the three different CF (Table 3.5). The composition of 3.00 \times permeate was similar to the composition of 3.00 \times MF permeate from skim created by Zulewska et al. (2009) and Beckman et al. (2010) using the same SW membrane system. Fat contents of permeates were low (Table 3.5) due to the inability of fat globules to pass through the membrane. If there was no rejection of lactose or SP by the membrane, then we would expect both lactose and SP concentration in permeate to remain constant with increasing CF, and the expected concentration of SP in the MF permeate

Table 3.5. Mean (n = 3) composition of composite permeates (% by weight) by concentration factor (CF) during microfiltration of skim milk (50°C) using a 0.3 µm polyvinylidene fluoride spiral-wound membrane.

CF	Composition (%) ¹									
	TS	Lactose	Fat	TN	NCN	NPN	TP	Casein	Serum proteins	CN%TP
1.50×	6.03 ^c	4.90 ^a	0.0008 ^a	0.36 ^c	0.36 ^c	0.166 ^a	0.20 ^c	0.008 ^a	0.19 ^c	4.13 ^a
2.25×	6.14 ^b	4.90 ^a	0.0012 ^a	0.44 ^b	0.44 ^b	0.176 ^a	0.27 ^b	0.007 ^a	0.26 ^b	2.60 ^a
3.00×	6.19 ^a	4.90 ^a	0.0005 ^a	0.51 ^a	0.49 ^a	0.172 ^a	0.33 ^a	0.015 ^a	0.32 ^a	4.44 ^a
SE	0.005	0.005	0.0008	0.003	0.003	0.007	0.006	0.004	0.008	1.51
R-square	0.99	0.54	0.35	>0.99	>0.99	0.61	0.98	0.36	0.97	0.24

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

¹TS = Total Solids; Lactose = lactose measured by infrared spectroscopy; Fat = fat content measured by ether extraction; TN = total nitrogen × 6.38; NCN = noncasein nitrogen × 6.38; NPN = nonprotein nitrogen × 6.38; TP = true protein, TN – NPN; casein = TN – NCN; serum proteins = TP – casein; CN%TP = casein as a percentage of TP.

would be slightly higher than the SP concentration in the original skim milk (Table 3.3, i.e., 0.59%) as calculated by the SP in the permeate portion. In previous work with ceramic membranes (Zulewska et al., 2009; Hurt et al., 2010), the SP concentration in the MF permeate was very close to this theoretical value. This was not the case in the present study, concentration of SP in the MF permeate at all CF (Table 3.5) were well below theoretical values (Hurt and Barbano, 2010). However, lactose, but not SP concentration, in the permeate remained constant among the three CF used (Table 3.5). The increase in SP in the permeate with increasing CF could be caused by a lower rejection of SP by the foulant layer at different CF due to a difference in the porosity of the foulant layer. It was reported by Zulewska and Barbano, (2013) that there was little rejection of SP by the same PVDF membrane in our system if MF permeate produced with a ceramic membrane system was MF with the PVDF membrane starting from a clean membrane without any fouling of the membrane by skim milk. An increase in SP concentration in the permeate (i.e., less rejection of SP passage) with increasing CF (Table 3.5) indicated that (1) SP rejection by the foulant layer decreased with increasing CF, (2) SP rejection by the membrane decreased with increasing CF, or (3) SP rejection by the membrane and foulant layer did not change with CF, but concentration of SP near the surface of the membrane increased with increasing CF due to SP rejection producing a higher concentration of SP in the permeate portion of the recirculating retentate, allowing more SP to permeate the membrane. A higher CF during skim milk MF was expected to lead to more concentration polarization fouling near the surface of the membrane, which might be expected to decrease fouled water flux and increase SP rejection. This was not the case. As shown in Table 3.2, no difference ($P > 0.05$) between the fouled water flux was detected among the three CF, indicating the resistance to water flow through the membrane by the combination of the membrane and the foulant layer that can only

be removed by cleaning was not different among the three different CF. However, passage of SP through the membrane plus foulant layer increased with increasing CF, as reflected by the increase in SP in the MF permeate with increasing CF (Table 3.5). If the higher concentration of SP in the MF permeate with increasing CF was solely due to the higher concentration of SP in the retentate in the recirculation loop, then one would not expect the percentage removal of SP from the original skim milk to differ among CF. This point will be addressed later in the section of the discussion on SP removal.

Casein contents of the composite permeates were low for all CF (Table 3.5), which was supported by the color analysis of the permeates, which detected no difference ($P < 0.05$) in L-values (lightness/opacity), 20.31 ± 0.03 , 20.66 ± 0.21 , and 20.52 ± 0.08 , among 1.50, 2.25, and 3.00× permeates, respectively. The concentration of true protein (%) in permeates at 1.50, 2.25, and 3.00× CF for the 90 min duration of the steady-state phase of MF is shown in Figure 3.4. From 10 to 90 min during steady-state processing, protein in permeate at 1.50, 2.25, and 3.00× CF, decreased ($P < 0.05$) 0.022, 0.034, and 0.035% per h, respectively (Figure 3.4). Decreasing protein in permeate during MF at all CF can be attributed to the fouling of the SW MF membrane due to concentration polarization driven fouling, which progressively blocked SP passage into permeate with time at all CF.

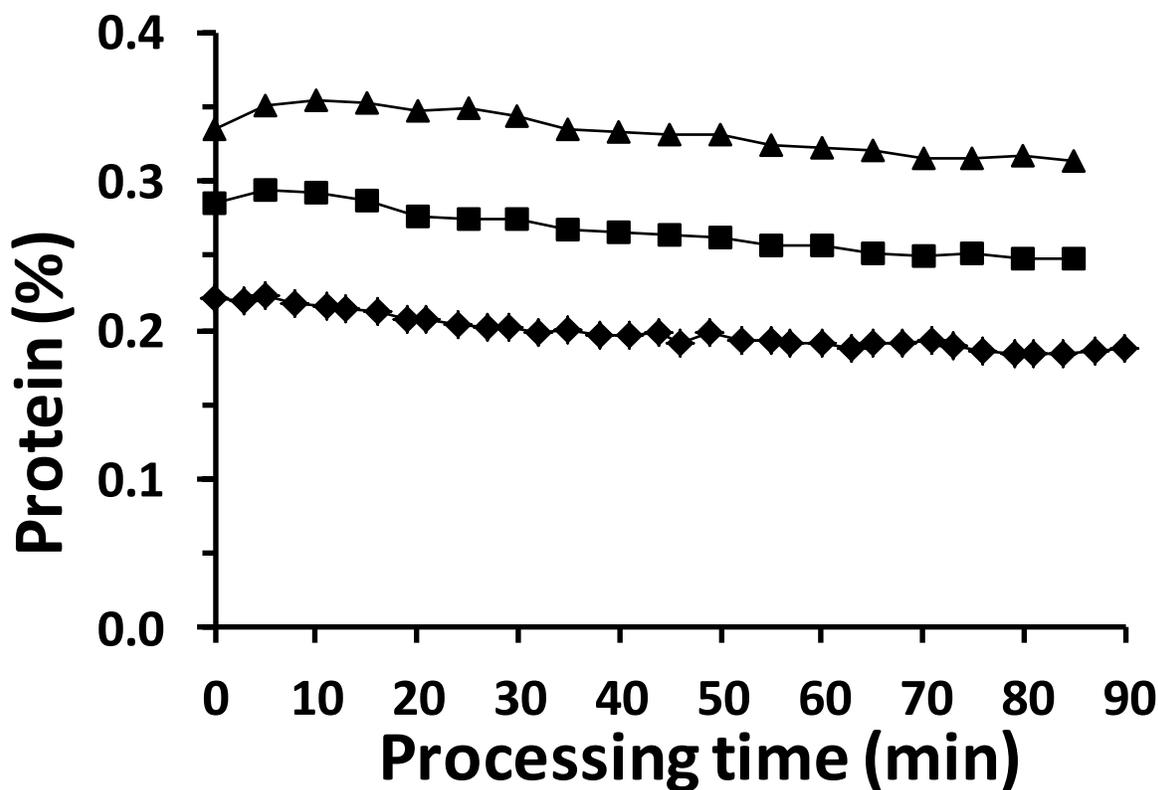


Figure 3.4. Average ($n = 3$) protein content (%) in the permeate during polyvinylidene fluoride spiral-wound microfiltration of skim milk (50°C) using three concentration factors, (◆) 1.50 \times , (■) 2.25 \times , and (▲) 3.00 \times . Protein content was measured by infrared spectroscopy.

Serum Protein Removal

Determination of the effect of CF on SP removal from skim milk was a focus of this research. The quantity of skim milk that was MF, the percent SP removal from skim milk, and the rate (kg/m^2 per h) of SP removal from skim milk at each of the three CF are shown in Table 3.6. The quantity of skim milk used for each CF was calculated as the sum of permeate (kg) and retentate (kg) collected during the 90 min steady-state phase of MF. As CF increased, the quantity of skim that was MF with same membrane surface area (i.e., 20.5 m^2) in 90 min decreased by about 68% ($P < 0.05$) (Table 3.6), which was expected due to the lower ($P < 0.05$) removal rates of permeate and retentate with increasing CF (Table 3.1). For comparison, theoretical percent SP removals (assuming no resistance to passage of SP through the MF

membrane) from skim milk using MF at the 3 CF are presented in Table 3.6, using the approach described by Hurt and Barbano, (2010) to calculate theoretical SP removal at different CF. Zulewska et al. (2009) and Beckman et al. (2010) reported SP removals using 3.00× CF SW MF of skim of 38.6 and 38.6%, respectively, which are similar to the value of 35.6% at 3.00× CF in the present study (Table 3.6). The observed percentage SP removal from skim milk decreased ($P < 0.05$) from about 36% to nearly 10% (Table 3.6) with decreasing CF under the conditions used

Table 3.6. Mean (n = 3) quantity of skim used, percentage skim serum protein (SP) removed, and SP removal rate during steady-state phase for a spiral-wound polymeric polyvinylidene fluoride microfiltration (0.3 μm) of skim milk at three concentration factors (CF).

CF	Skim ¹ (kg)	Theoretical SP removal ² (%)	Skim SP removed (%)	Percent of theoretical SP removal (%) ³	SP removal rate (kg/m ² per h)
1.50×	1774.0 ^a	34.4	10.56 ^c	30.69 ^c	0.036 ^a
2.25×	836.5 ^b	57.3	24.33 ^b	42.42 ^b	0.039 ^a
3.00×	572.6 ^c	68.8	35.57 ^a	51.70 ^a	0.039 ^a
SE	8.2		0.79	1.30	0.001
R-square	>0.99		0.99	0.97	0.51

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

¹Skim (kg) = permeate (kg) + retentate (kg) collected during 90 min collection.

²Theoretical SP removal calculated as in Hurt and Barbano, (2010)

³Percent of theoretical SP removal = average skim SP removed at each CF divided by theoretical SP removal

in the current study primarily due to the lower SP content in the permeate (Table 3.5). We observed increased flux ($P < 0.05$) at lower CF (Table 3.2), but SP removal as a percentage of theoretical SP removal unexpectedly decreased with decreasing CF (Table 3.6) due to a lower SP concentration in the permeate at lower CF (Table 3.5). The percent of theoretical SP removal increased ($P < 0.05$) with increasing CF (Table 3.6). What could have caused this unexpected decrease in SP removal at lower CF? We hypothesize that the structure of the concentration polarization driven foulant layer may be different or the interaction of the foulant layer with the

membrane structure may be different depending on the conditions near the membrane during the formation of the foulant layer at start-up.

No difference in the rate of SP removal (kg/m^2 per h) from skim milk was detected ($P > 0.05$) among CF (Table 3.6). This is interesting that there is a constant amount of SP passing through the membrane per m^2 per h, while the amount of water passing through the membrane per m^2 per h is increasing with decreasing CF. Thus, resistance to passage of water decreases with decreasing CF, but the SP removal increases with increasing CF indicating that the rejection characteristics of the foulant layer formed at different CF is different. The SP removal rate at all three CF used (Table 3.6) were similar to the SP removal rate found by Beckman et al. (2010), 0.05 kg/m^2 per h, for a $3.00\times$ CF skim (50°C) SW MF.

Membrane Foulant Role in SP Removal

Foulant Composition. Bovine milk contains fat, protein, lactose, minerals (ex. calcium, phosphate), and other minor constituents. During membrane MF of skim milk, the primary membrane foulants are proteins and milk minerals (Cheryan, 1998). The hydrophilicity of the membrane material is an important consideration when MF solutions with proteins (Cheryan, 1998; Rudan, 1990), as hydrophobic organic membranes, including PVDF, have been known to be rapidly fouled by adsorbed hydrophobic proteins (Tong et al., 1988; Bowen and Gan, 1991). The adsorption of proteins onto the membrane can account for a significant portion of the reduction in flux (van der Horst, 1995, James et al., 2003). In addition to protein adsorption onto membranes, CN micelles may also become lodged into the MF membrane pores, effectively blocking passage of permeate through that pore (Attia et al, 1991). Casein has been demonstrated to be the primary foulant during SW MF of skim milk that restricts passage of SP through a PVDF membrane (Zulewska and Barbano, 2013). We have observed in our

experiments with MF skim at 50°C using 0.3 µm pore size PVDF SW MF membranes (Zulewska et al., 2009; Beckman et al., 2010; Zulewska and Barbano, 2013), including this experiment during the startup-stabilization phase (data not reported), that during the first 5 to 10 min of filtration, the permeate exiting the system is opaque and white in color. This means that CN micelles are passing through the membrane. After 5 min of skim MF, the permeate becomes clear, which indicates that CN micelles pass through the membrane plus foulant until the effective “pore size” decreases and rejects the passage of CN micelles. Protein interaction with the membrane through adsorption and through pore blocking may account for a significant portion of the initial flux decline and increased rejection of SP seen in skim milk MF using polymeric SW membranes (Zulewska et al., 2009; Beckman et al., 2010). Zulewska and Barbano, (2013) demonstrated that when casein-free skim milk (i.e., permeate from MF) was MF using a polymeric PVDF SW membrane, there was little decrease in hydraulic resistance of the membrane and little rejection of SP, indicating that CN is the important foulant causing SP rejection during MF of skim milk using PVDF MF membranes at 50°C to process skim milk.

Formation and Progression of Fouling. Interaction of milk components with a SW polymeric MF membrane surface is thought to occur as a series of steps. Description of the progression of fouling during SW MF of skim using hydrophobic polymeric membranes has been discussed previously (Marshall and Daufin, 1995, Lawrence et al., 2008; Zulewska and Barbano, 2013). From the data in this experiment and the data from Zulewska et al. (2009), Beckman et al. (2010), and Zulewska and Barbano, (2013), we believe a good model to describe fouling during polymeric SW skim MF is the “resistance-in-series” model (Suki et al., 1984; van der Horst, 1995) (as shown below):

$$J = \frac{\text{TMP}}{(\mathbf{R_M} + \mathbf{R_A} + \mathbf{R_P} + \mathbf{R_G} + \mathbf{R_{CP}})}$$

where J = permeate flux, TMP = transmembrane pressure, R_M = resistance due to the membrane, R_A = resistance due to adsorption, R_P = resistance due to pore blocking, R_G = resistance due to precipitation or “gel-layer” formation, and R_{CP} = resistance due to concentration polarization. This model is used to describe flux decline in a membrane as a function of the total membrane resistance applied by the individual factors. This same thinking can be used when considering the rejection characteristics of the foulant, but the rejection characteristics may or may not be directly correlated with the change in flux.

In our study, the TMP at the inlet and outlet of the membrane were the same among CF (Table 3.1), and the resistance of the clean membrane (R_M) did not change among CF (Table 3.2). As discussed earlier, protein adsorption (R_A) and CN infiltration into the membrane pores (R_P) occurred early in the MF of skim milk and likely accounted for the majority of fouling during the first few minutes of MF when the permeate changed in appearance from white to clear and the difference in SP rejection among different CF was created. During the remainder of the 40 min start-up phase of this experiment, these factors (TMP, R_M , R_A , and R_P) remained constant or changed very little while the R_G was mostly developed before steady state processing began. Any further resistance caused by concentration polarization (R_{CP}) continued to progress during the steady state phase. A secondary effect of concentration polarization is the accumulation of retained feed solids at the surface of the membrane during the steady state phase of processing causing the formation of a “gel-layer” (R_G) which produced the gradual decline ($P < 0.05$) in flux at 3.00× CF with time shown in Figure 3.2. As concentration polarization increases during SW MF of skim, so too should R_G .

To help describe how the initial deposition of foulants may affect SP removal during MF, a diagram is presented as Figure 3.5. The SW MF system was started similarly at each CF. However, after the permeate exit valve was opened to allow passage of water and solutes through the MF membrane, the magnitude of restriction of the retentate bleed valve differed among CF. The difference in restriction in retentate removal may have influenced the interaction of milk constituents with the MF membrane as shown in Figure 3.5. To achieve a low retentate removal rate at CF 3.00× (Table 3.1), the retentate removal valve was closed over about a 5 min period until the correct CF was achieved. At CF 3.00×, the increased back-pressure on the retentate side of the membrane caused by rapid closing of retentate removal rate caused a decrease in retentate recirculation flow (Table 3.1). Low recirculation rate, and a rapid increase in large rejected solutes (i.e., CN micelles) at high CF (e.g., 3.00×) (Table 3.4) caused a rapid formation of the concentration polarization (R_G and R_{CP}) gel-layer (Figure 3.5) with minimal time for CN micelle infiltration into the pores of the membrane to cause pore blocking (R_P). It is likely that adsorption (R_P) of small SP was occurring within the pores but had little impact on overall hydraulic resistance or SP rejection (Figure 3.5). In contrast, at CF 1.50×, the retentate removal valve was not closed as completely or as quickly (15 min duration) as at 3.00×, the retentate removal rate was six times faster than at 3.00× (13.2 kg/min versus 2.1 kg/min), the recirculation rate was faster (Table 3.1), and the concentration of rejected solutes (i.e., CN micelles) were lower (Table 3.4) than at 3.00× which would favor less concentration polarization gel layer formation (i.e., lower R_G and R_{CP}) and allow more time for pore blocking (R_P) and increased extent of pore blocking by CN micelles at 1.50× compared to the conditions at 3.00×. Thus, we hypothesize that there was a greater opportunity for CN micelles to become lodged in the membrane pores (Figure 3.5) producing a higher R_P at 1.50× than at 3.00×, effectively reducing

the effective pore size and causing a difference in SP rejection among CF. We hypothesize that differences in pore-plugging (R_p) among CF in the first few minutes of operation may cause relatively large differences in SP rejection by the membrane without causing the same magnitude of differences in hydraulic resistance. Thus, we feel that increased pore plugging at low CF increased the rejection of SP and lowered the removal of SP from skim milk (Table 3.6).

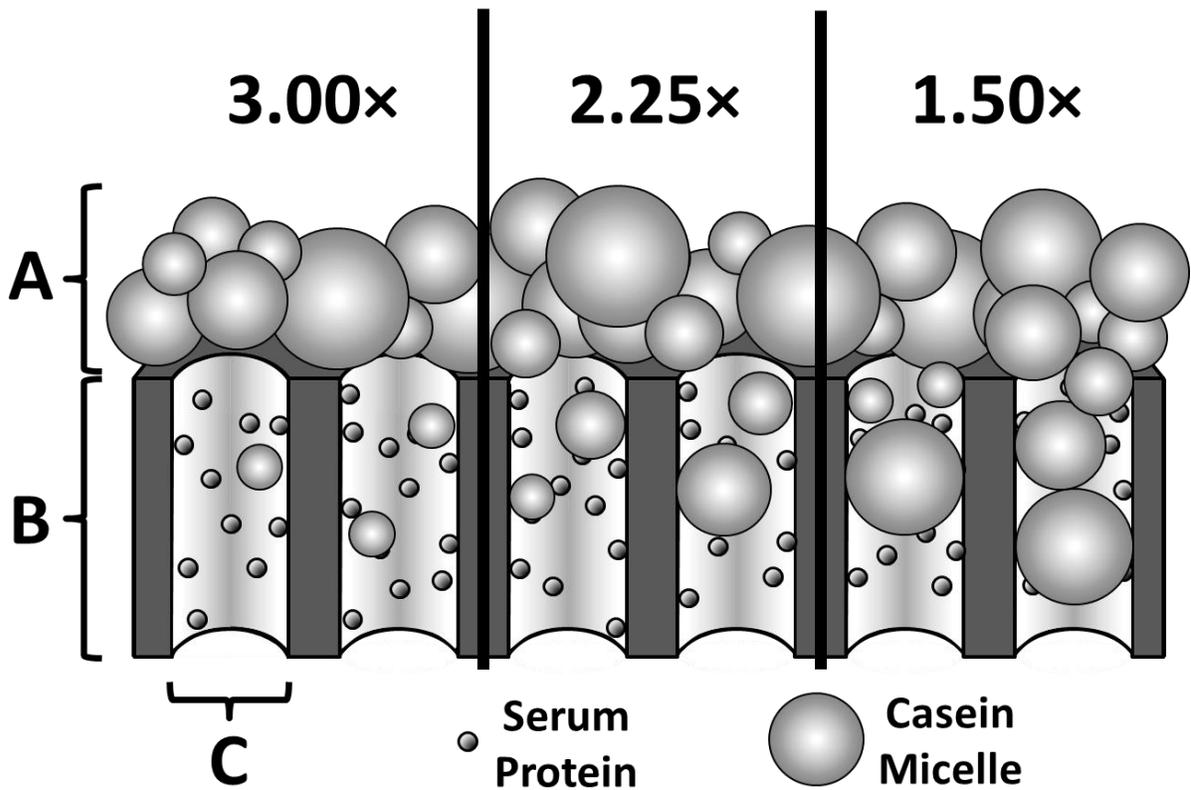


Figure 3.5. The hypothesized fouling of a polymeric spiral-wound microfiltration membrane ($0.3 \mu\text{m}$ pore size) at three concentration factors (1.50, 2.25, and $3.00\times$) by skim milk at 50°C . Casein micelles have formed a gel-layer (A) on the retentate side of the membrane (B), and serum proteins are adhered onto the interior of the membrane pore (C)

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demonstrated that a $0.3 \mu\text{m}$ PVDF SW MF membrane provides little resistance to passage of SP into permeate and that the CN foulant and the characteristics of that foulant were the determinant

of SP removal from skim milk during MF at 50°C. Future studies should focus on controlling the series of events in the formation of the resistances to passage of solutes through the membrane pores not just the resistance to passage of solvent. For example, a strategy of using a smaller membrane pore size in combination with rapidly building the gel layer could actually improve the passage of SP through the combination of membrane plus foulant without having much influence on overall solvent flux. The rapid formation of the gel layer and minimization of pore plugging might be achieved by starting the MF system with a more concentrated feed solution in the very beginning to achieve a rapid formation of a reasonable gel layer while minimizing pore block that may have a larger impact on SP rejection.

CONCLUSIONS

Permeate flux was increased (12.8, 15.3, and 19.0 kg/m² per h) with decreasing CF from 3.00 to 1.50×, while fouled water flux did not differ among CF indicating that the effect of membrane fouling on hydraulic resistance of the membrane was similar at all CF. However, the CF used when MF skim milk (50°C) with a 0.3 μm polymeric SW PVDF membrane did affect the percentage of SP removed. As CF increased, from 1.50, to 3.00×, the percentage of SP removed from skim milk increased from 10.56 to 35.57%, in a single stage bleed and feed MF system. Rejection of SP during MF of skim milk with SW PVDF membranes was caused by fouling of the membrane, not by the membrane itself and differences in the foulant characteristic among CF influenced SP rejection more than it influenced hydraulic resistance. We hypothesize that differences in the conditions near the surface of the membrane and within the pores during the first few minutes of processing, when CN micelles pass through the membrane, influenced the rejection of SP protein because more pore size narrowing and plugging occurred at low CF than at high CF due to a slower rate of gel layer formation at low CF. It is possible that

percentage removal of SP from skim milk at 50°C could be improved by optimization of the membrane pore size, feed solution composition and concentration, and controlling the rate of formation of concentration polarization derived gel layer at the surface of the membrane during the first few minutes of processing.

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CHAPTER FOUR

Conclusions and Future Work

Microfiltration of skim milk can be used to separate casein from milk serum proteins (SP). Different membrane materials and configurations will have different SP removal properties. Other research on tubular ceramic membranes has achieved near theoretical levels of serum proteins from skim milk. Polymeric spiral-wound (SW) membranes offer some advantages over ceramic membranes such as having lower capital cost, and larger membrane surface area available per unit floor space that make them attractive to budget-conscious dairy processors wishing to separate casein and serum proteins in skim milk. Our study focused on determining and improving the efficiency of SP removal from skim milk using SW polymeric MF membranes.

Previous work on ceramic uniform transmembrane pressure (UTP) MF membranes has demonstrated that they are very efficient at removing SP from skim milk, attaining theoretical removal (ca. 98%) after three stages of $3.00\times$ CF MF at 50°C. In the present work, we determined that a PVDF SW 0.3 μm pore size MF of skim milk removed 38.6, 20.8, and 10.9% SP from skim milk during stage 1, 2, and 3, respectively, which is cumulatively lower, 70.3%, than theoretical, 98.0%, and for a comparable ceramic UTP MF, 98.3%. Serum protein removal rates for stages 1 to 3 during SW MF were low, 0.05 to 0.04 kg/m^2 per h, respectively, and were lower than observed for ceramic UTP MF, 0.3 kg/m^2 per h. Permeate flux increased ($P < 0.05$) from 14.4 to 32.6 kg/m^2 per h, for stage 1 to 3, respectively. Flux increased due decreasing ($P < 0.05$) concentration of casein in retentates in the second and third stages which we found was caused by over dilution with water during diafiltration. To reach a target of 95% SP removal from skim milk, we calculated that an additional 5 diafiltration stages would be necessary for this

PVDF SW MF system. These extra diafiltration stages would create additional permeate that would contain low solids (i.e., SP, lactose, and minerals) and be very expensive to process further.

Serum protein removal from skim milk using 0.3 μm PVDF SW MF at 50°C was not as high as for ceramic UTP MF when running at a 3.00 \times concentration factor like that use with ceramic MF membranes. In the second study, we reduced the CF used while MF skim milk with polymeric SW MF membranes to determine if we could increase SP removal to a level near ceramic MF because we expected to less fouling and achieve higher flux. Permeate flux increased ($P < 0.05$) from 12.8 to 19.0 kg/m^2 per h as the CF decreased from 3.00 to 1.50 \times , respectively, as expected. It was thought that increasing flux would increase SP removal into permeate, however that was not what was observed. As CF decreased, 3.00 to 1.50 \times , SP removal from skim also decreased ($P < 0.05$), 35.6 to 10.6%, respectively. Actual SP removal as a percentage of theoretical removal was calculated, and decreased ($P < 0.05$), 51.7, 42.4, and 30.7%, with decreasing CF, 3.00, 2.25, and 1.50 \times . This was counter to our hypothesis that a higher flux (i.e., low CF) would lead to higher SP removal from skim milk. The rate of SP removal (kg SP removed per unit membrane surface area) remained constant ($P > 0.05$) among CF. A constant SP removal rate and constant ($P < 0.05$) ΔP among CF with an increase in SP removal as a % of theoretical as CF increased indicated to the authors that SP rejection differed among CF used. We hypothesized that at low CF (e.g., 1.50 \times), the buildup of the concentration polarization gel-layer was slower, allowing time for large CN micelles to lodge themselves into the pores of the membrane causing more rejection of SP. At high CF (e.g., 3.00 \times), the rapid formation of a gel-layer on the surface of the membrane prevented much of the pore blocking by

CN micelles, and allowed SP to pass through. Thus, in SW MF of skim milk, a dynamic membrane is formed which controls SP passage into the permeate not the membrane itself.

Future research on polymeric SW MF membrane use in the removal of SP from skim milk should focus on identifying the role of pore blocking and foulant deposition on the rejection of SP. Controlling fouling during the startup procedure for the membrane system can influence foulant deposition and the formation of the dynamic membrane by which SP removal is controlled. The fouling during start up would also be influence by the relationship of the membrane pore size to the casein micelle size and determination of the effect of different membrane pore size (i.e., smaller) on SP rejection at 50°C should be done. In addition to startup procedure and membrane material pore size, assessing how the composition of the feed material affects pore blocking and foulant layer deposition would be beneficial. Casein is the primary foulant during SW MF of skim milk. Possibly altering the level of CN in the feed material prior to filtration could change the SP rejection profile of the dynamic membrane. Finally, the use of different pore size polymeric SW MF membranes should be investigated. Because smaller CN micelles can permeate the membrane and block the pores of a 0.3 μm membrane, a smaller pore size (i.e., 0.1 μm) membrane might prevent the pore blocking which may be why we observed lower SP removal at lower concentration factors.