MICROFILTRATION OF SKIM MILK TO SEPARATE MICELLAR CASEIN FROM SERUM PROTEIN: THEORETICAL INFLUENCE OF FIVE FACTORS AND PERFORMANCE OF A MICROFILTRATION UNIT

A Thesis

Presented to the Faculty of the Graduate School

of Cornell University

In Partial Fulfillment of the Requirements for the Degree of

Master of Science

by

Emily Elizabeth Hurt

May 2010

ABSTRACT

The production of serum protein **(SP)** and micellar casein **(CN)** from skim milk can be accomplished using microfiltration **(MF)**. There are potential commercial applications for both the SP and micellar CN. Our 1st objective was to demonstrate the impact of: skim milk composition, heat treatment of skim milk, concentration factor (**CF**) and diafiltration factor (**DF**), control of CF and DF, and SP rejection of membrane on the performance of a MF system designed to process skim milk and separate CN from SP. A mathematical model of a skim milk MF process was developed and used to predict the effect of the 5 factors on retentate and permeate composition, SP removal, and micellar CN concentrate **(MCC)** and milk SP isolate **(MSPI)** yield for a 3 stage process. When skim milk TP increased from 3.2 to 3.8%, the yield of MCC and MSPI increased by 19% and 18%, respectively. Increased heat treatment (72.9 to 85.2^{o}C) of skim milk caused CN as a percentage of TP in skim milk as measured by Kjeldahl analysis to increase from 81.97 to 85.94% and the yield of MSPI to decrease 22%, while the 3rd stage cumulative SP removal decreased from 96.96 to 70.08%. A CF and DF of 2X gave a 3rd stage retentate TP concentration of 5.38% compared to 13.13% for a CF and DF of 5X. Variation in control of the balance between CF and DF (unequal CF and DF) caused either an increase or decrease in TP concentration in the retentate across stages depending if CF was greater than DF (increasing TP in retentate) or CF was less than DF (decreasing TP in retentate). An increased rejection of SP by the membrane from a SP removal factor of 1 to 0.6 caused a reduction in MSPI yield by 17%, 3rd stage cumulative SP removal decreased from 96.96 to 79.74%. Within the ranges of the 5 factors studied, the TP content of the 3rd stage retentate was strongly impacted by the target CF and DF and variation in skim milk composition. Cumulative SP removal was strongly impacted by the heat treatment of skim milk, SP removal factor, and target CF and DF. The MCC and

MSPI yield was most strongly impacted by initial skim milk composition. MSPI yield was also impacted by the heat treatment of milk and SP removal factor.

Our 2nd research objective was to determine the efficiency of SP removal for a 3X continuous feed and bleed uniform transmembrane pressure **(UTP)** system with 0.1 μm ceramic membranes, when processing pasteurized skim milk at 50°C with two stages of water diafiltration (for a total of 3 stages). For each of 4 replicates about 1100kg of skim milk was pasteurized (72°C, 16s) and processed at 3X through the UTP MF system. Retentate from stage 1 was diluted with reverse osmosis water back to a 1X concentration and again processed through the MF system (stage 2) to a 3X concentration. The retentate from stage 2 was diluted with reverse osmosis water back to a 1X concentration, before running through the MF system at 3X for a total of 3 stages. Theoretically, from the 1st part of our research a 3-stage 3X MF process could remove 97% of the SP from skim milk. The total SP removal in this experiment was 98.27 ± 2.25%, when SP removal was calculated using the mass of SP removed in the permeate of each stage.

BIOGRAPHICAL SKETCH

Emily Hurt was born on January 16th 1979 in Chico, California as the first child to Christina and Raymond Hurt. Later Emily welcomed a sister Jessica and a brother Timothy. Possessing an unpleasant personality and a homely appearance it was clear from an early age that Emily would have to make her fortune using her mind, thus she put her energy into excelling at school. After graduating as a valedictorian from Chico Senior High School in 1997, Emily attended the University of California at Davis. She graduated with high honors in 2002 with a degree in Chemical/Biochemical Engineering. Along with her natural aptitude the five years of study to become an engineer solidified her enjoyment in solving technical problems, and her fear of human emotion. After graduation Emily began work as a research engineer in the dairy industry, where she learned the realities and technical challenges of large scale production. Her job focused on the purification of lactose from cheese whey, which exposed her to most of the processing steps found in the dairy industry, and peaked her interest in pursuing her own research. After five years she decided that a return to college for an advanced degree was in her best interest, to expand her opportunities in the dairy industry. As for hobbies Emily enjoys traveling, harboring an unnatural obsession with Alexander Hamilton, and sleeping. Someday she hopes to own many cats and have the neighborhood children think her a witch.

I dedicate this work to my parents Raymond and Christina Hurt.

ACKNOWLEDGMENTS

Thanks to the New York State Milk Promotion Board (Albany, NY) and the Northeast Dairy Foods Research Center for partial funding of this research.

On a personal note there are numerous people, without whom this work would not have been possible. I find it hard to choose the right words to convey the true depth of my appreciation, so please accept my apology if any feel they have been inadequately recognized. First a sincere thanks to Dave Barbano, for his guidance and invaluable input, it would be impossible to overstate the impact he has had on this work, and my development as a researcher. The Barbano laboratory group also deserves recognition, most of the work requiring a team effort, so a very hearty thanks to: Maureen Chapman, Chassidy Coon, Mark Newbold, Jessica Mallozzi, and Karen Wojciechowski, for their sample analysis, technical support and help with pilot plant operation. I truly appreciated all of their generous help and support. Additional thanks to my other Special Committee member Ashim Datta, and fellow researchers Justyna Zulewska and Steve Beckman.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

β-LG ..... **β-Lactoglobulin**

CF ..... **Concentration factor**

CN ..... **Casein**

CN%TP ..... **Casein as a percentage of true protein**

DF ..... **Diafiltration factor**

GP ..... **Graded permeability**

MCC ..... **Micellar casein concentrate**

MF ..... **Microfiltration**

MSPI ..... **Milk serum protein isolate**

NCN ..... **Non casein nitrogen**

NPN ..... **Non protein nitrogen**

SDS-PAGE ..... **Sodium dodecyl sulfate polyacrylamide gel electrophoresis**

SP ..... **Serum protein**

TN ..... **Total nitrogen**

TP ..... **True protein**

TS ..... **Total solids**

UTP ..... **Uniform transmembrane pressure**

WPC ..... **Whey protein concentrate**

Chapter 1

Introduction: Microfiltration to Separate Micellar Casein from Serum Protein

Milk Composition and Component Uses

Composition. Bovine milk is a complex biological fluid containing about 3.3% crude protein of which about 0.2% is nonprotein nitrogen (Walstra et al., 1999 p4). The true protein in milk can be further divided into two main classes; caseins **(CN)**, that make up about 80% of the protein in milk are defined as phospho-proteins that precipitate from raw skim milk at a pH of 4.6 at 20^{o}C (Jenness et al. 1956, Eigel et al. 1984), milk serum proteins **(SP)** make up the remaining 20% of protein in bovine milk (Walstra et al., 1999 p15). There are 4 main types of CN in milk; α_{s1}-CN, α_{s2}-CN, β-CN and κ-CN. The κ-CN is unique in that it is a glycoprotein (contains attached carbohydrate groups) (Walstra et al., 1999 p 86-90). During cheese making chymosin cleaves κ-CN releasing glycomacropeptide (**GMP**) into the whey (El Salam et al., 1996). Serum proteins consist mainly of β-lactoglobulin, α-lactalbumin, immunoglobulins, and bovine serum albumin with concentrations in milk of roughly 0.3, 0.1, 0.07 and 0.03% respectively (Walstra et al. 1999 p 8). The CN in milk are associated into micelles, the molecular weights of CN are in the 20 to 25kDa range, while the average molecular weight of CN micelles is much larger at 5x10^{5}kDa (Fox, 2001). Casein micelles are very heat stable, with micelles remaining intact at temperatures as high as 140^{o}C (Walstra et al., 1999 p133). Serum proteins on the other hand are not as heat stable with the denaturation of SP occurring with temperatures in the range of 60 to 100^{o}C, though the exact temperature of denaturation is a function of pH and protein (de Wit and Klarenbeck, 1984). At temperatures in the 100-150^{o}C

range irreversible chemical changes occur in SP, such as cystein breakdown, and maillard reactions with lactose (de Wit and Klarenbeck, 1984).

Whey protein products. Whey proteins are purified from the whey produced as a byproduct in cheese making. Whey protein concentrates (**WPC)**, which are produced by the ultrafiltration of whey can be from 34 to 85% protein (Fox, 2001) and have a high nutritional value (de Wit, 1998). Concentrates of whey proteins consist mainly of SP; however they also include GMP cleaved from κ-CN during cheese making. The GMP can make up 15 to 25% of the protein in whey (El Salam et al., 1996). Whey protein concentrates also contain milk fat, minerals and lactose. Whey protein concentrates can be used to replace eggs in baked goods, as emulsifiers, as gelling agents to increase yields in processed meats and to increase nutritional value of foods (de Wit, 1998).

Serum protein products. Microfiltration **(MF)** provides a new method for separating micellar CN from SP that provides some functional and sensory advantages in the products manufactured by this approach. In this method, first developed in the 1990's (Pouliot, 2008), MF is used to separate CN micelles from SP starting with skim milk. A MF membrane is chosen that retains CN micelles while allowing SP to pass through. This separation is feasible due to the 10 to 100 fold radius size difference between CN micelles and SP (Walstra et al., 1999 p6). The permeate from MF contains SP as well as lactose and minerals, further purification using ultrafiltration can be used to increase the concentration of SP on a dry basis.

Evans et al. (2009) found that 34% SP purified from the permeates of skim milk MF were free of GMP (compared to 34% WPC that contained 1.45% GMP on a dry basis) and contained less fat, 0.25% fat on a dry basis compared to 1.93% fat for the 34% WPC. Research on the functional properties of SP as compared to whey proteins has indicated that SP have better gelling and foaming properties (Britten and

Pouliot, 1996). Comparison of sensory properties has shown that 34% SP concentrates lack diacetyl flavors present in 34% WPC (Evans et al., 2009) making SP better candidates for protein fortification of foods, such as beverages.

Casein products. As a food ingredient, purified CN has found a variety of uses in dairy and non-dairy applications. Caseins are typically prepared by either rennet or acid precipitation. Caseinates are produced by the alkaline neutralization of acid precipitated CN (Huffman and Harper, 1999). In 2001, it was estimated that 250,000 metric tons of caseinates were produced each year (Fox, 2001). Renneted CN is commonly used as an ingredient in cheese analogues. Caseinates find use in synthetic whipping creams, cream liqueurs, and as an emulsifier in coffee whiteners (Huffman and Harper, 1999). Non-food applications of caseinates include use in adhesives, inks, paints and as paper coatings (Audic et al., 2003).

A newer CN product is micellar CN, purified from skim milk by MF in the same process that separates SP from micellar CN. Purified CN remains in its micellar form, which has applications in cheesemaking for increasing curd firmness and reducing coagulation time (Maubois, 2002). Micellar CN could also be used to increase cheese yields and enhance profits in cheesemaking (Papadatos et al., 2003). Micellar CN might also be an advantageous starting material for the further purification of specific CN such as β-CN or GMP (Maubois, 2002). Neocleous et al. (2002) found that using milk concentrated to 1.26 to 1.82X by MF in cheddar cheese making, increased yields, mainly attributable to increased CN recovery. In an economic analysis Papadatos et al. (2003) found that MF of milk would increase revenues in 30 of the 36 months modeled for both cheddar and mozzarella cheese manufacture under the prevailing milk and product pricing conditions at that time.

Microfiltration Membrane Types and Modes of Operation

Microfiltration can be used to separate CN micelles from SP, producing a SP containing filtrate and a micellar casein concentrate that could be of increasing economic importance. To better understand the use of MF for the production of CN micelles and SP, MF in general will be discussed, focusing on membrane types and modes of operation.

Membrane types. Within the category of MF there is a choice between ceramic and polymeric membranes. Ceramic membranes have been the most widely studied for the separation of micellar CN from SP. Ceramic membranes are usually tubular in form consisting of an inorganic macro-porous support and a thin filtration layer. Both the support and filtration layer are usually mineral oxides such as zirconium, titanium and aluminum oxides (Benfer et al., 2004). Ceramic membranes have good chemical and physical stability with a typical pH range of 0.5 to 13 and operation up to 125^{o}C (Cheryan, 1998 p66), and cross-flow velocities can be on the order of 5m/s. It should be noted that ceramic membranes can crack if subjected to a rapid temperature or pressure changes (Cheryan, 1998 p66).

An alternative to ceramic membranes is polymeric membranes. Polymeric membranes can come in many geometries, such as flat sheet, tubular and spiral-wound (Cheryan, 1998 p178-210). Spiral-wound polymeric membranes have the advantage of having greater surface area per length of membrane compared to other geometries. Spiral-wound membranes can telescope when subject to high (greater than1.4 bar) pressure drops along the length of the membrane, this limits the cross-flow velocity achievable to approximately 0.5 m/s (Cheryan 1998 p220). Polymeric membranes in general cannot be subject to permeate backpressure (Cheryan 1998 p 274). Common materials for polymeric MF membranes include polyvinyldeneflouride (PVDF) and polyethersulfone (PES) (Belfort et al, 1994).

Operation. In cross-flow (also called tangential flow) filtration, the feed is pumped across the surface of the membrane, the retentate is the material that is retained by the membrane, while the permeate is the material that passes through the membrane (Van Der Horst and Hanemaaijer, 1990). The driving force in cross-flow filtration is the pressure difference from the retentate to permeate side of the membrane. Typically MF systems are operated at a specific concentration factor **(CF)**, which is the ratio of feed mass to retentate mass. For example a CF of 3 means that for every 3kg of feed 1kg of retentate is obtained. There are several methods of system operation to achieve a specified CF. The first is by using a batch mode, the entirety of the retentate is recirculated back to the feed tank, with permeate being removed until the desired CF is achieved, a second method is often called "feed and bleed", in this mode of operation there is an internal retentate recirculation loop and the ratio of retentate being bled out and permeate is set for the desired CF, and the feed rate is enough to match the removal of retentate plus permeate. Flux can be modeled as being proportional to the pressure drop across the membrane and inversely proportional to the resistance of the system (this can include resistance due to the membrane and fouling) (Cheryan, 1998 p132). Membranes are also characterized by their rejection. If a membrane does not reject a certain compound its concentration in the permeate will equal its concentration in the permeate portion of the feed, if a membrane completely rejects a compound it will have a concentration of 0% in the permeate. Typically membrane rejections are not 0% or 100%, but fall somewhere in between. The total amount of material that can be removed in 1-stage of filtration is a function of both the membrane rejection and CF (Cheryan, 1998 p302).

Membrane Fouling

Adoption of MF technologies by industry is highly dependent on cost. Cost of a MF system in turn depends on the average flux and rejection characteristics of the system. Fouling caused by the build up of macromolecules, colloidal and dissolved on the membrane surface, reduces flux and can change the rejection characteristics of the membrane (Sachdeva and Buchheim, 1997; Marshal et al., 1997). On an industrial scale this means that to remove a certain kilogram per hour of SP, more membrane area is needed, increasing the cost of the system. Belfort et al. (1994) describes the major stages of fouling. The first stage that occurs rapidly is the adsorption of macromolecules on to the membrane, followed by build up and compaction of a cake made up of suspended and dissolved solutes. The adsorption of foulants on a membrane is dependent on membrane material, Belfort et al., (1994) provides a table that lists typical protein adsorption capacities of different membrane materials. PVDF has a protein binding capacity of 150 mg/cm^2, while a modified PVDF that is more hydrophilic and has a protein binding capacity of only 4 mg/cm^2. Ceramic membranes are hydrophilic with an approximate protein adsorption is $14mg/cm^2$ (Caric et al., 2000). Most of the research done indicates that adsorption occurs as a monolayer on the membrane (including surface area of pores), and that hydrophilic membranes tend to adsorb less protein (Belfort et al., 1994). The buildup of the cake layer on the surface of the membrane is dependent on flow characteristics and concentration polarization. Concentration polarization is a critical factor in MF fouling. Near the surface of a membrane the concentration of retained solutes is higher since the solvent and other solutes are permeating the membrane, the increased concentration of retained solutes can build up on the membrane (Belfort et al., 1994). Concentration polarization cannot be eliminated, but it can be reduced by increasing the back

transport of macromolecules from the membrane surface by increasing cross-flow velocity (Belfort et al., 1994).

For both polymeric and ceramic MF membranes the cross-flow velocity and flux impact the fouling of a MF system, this relationship has been researched extensively. In the filtration of solutions devoid of suspended particles the end flux of the system is well predicted by equations that treat the diffusion of particles away from the membrane as controlled by Brownian diffusion, however the actual flux achieved in MF has been found to be much higher than predicted (Belfort et al., 1994). There have been several explanations for this contradiction, Green and Belfort (1980) hypothesized that for colloidal solutions (e.g., skim milk) inertial lift (caused by cross-flow) at the membrane surface lifts particles off the membrane and if this lift offsets the permeate velocity no deposition will occur. Another explanation is that shear induced diffusion increases back transport away from the membrane reducing concentration polarization (Belfort et al., 1994). Samuelsson et al. (1997) found that for the MF of skim milk using a tubular ceramic membrane; the shear induced diffusion model provided the closest match to experimental results. Le Berre and Daufin (1996) developed the concept of a critical ratio of flux and shear stress at the wall for the MF of skim milk, where flux is a measure of convection towards the membrane, and shear stress is a measure of erosion at the membrane surface. Le Berre and Daufin (1996) found a critical ratio for flux to shear stress of 1.0 L/hr per m^2 per Pa (the pressure is a measure of the difference between the retentate inlet and outlet), if the system operated below this critical ratio then long run times with high SP transmission and slow increase in fouling occurred, operation above this critical ratio led to rapid fouling and a decrease in SP transmission. Le Berre and Daufin (1996) determined their critical ratio for the MF of skim milk using a uniform transmembrane pressure system with 0.1µm ceramic membranes, operating at a concentration factor

of 2X at 50^{o}C. As mentioned earlier tubular ceramic membranes can operate at higher cross-flow velocities than spiral-wound polymeric, this gives a system with ceramic membranes more flexibility in operating conditions to control fouling.

Uniform transmembrane pressure. There have also been MF hardware developments to reduce fouling. Operation of tubular ceramic membranes in an MF system with only a feed and retentate recirculation pump has a major drawback. There will be a large pressure decrease from the inlet to outlet end of the retentate side of the membrane while the pressure on of the permeate side will be relatively constant. This means that the flux at the inlet of the membrane will be much higher than at the outlet and there will be accelerated fouling at the inlet end of the membrane. This makes it impossible to operate at the most efficient flux over the entire length of the membrane. To get around the problems caused by non-constant flux along the length of a ceramic membrane, a uniform transmembrane pressure **(UTP)** system was developed. The UTP system was patented by Sandblom (1978). In this system there is an additional pump that recirculates the permeate on the permeate side of the membrane, in a co-current direction to the retentate flow. This causes a pressure drop on the permeate side of the membrane from permeate inlet to outlet that mirrors the pressure drop on the retentate side of the membrane from retentate inlet to outlet. This causes the pressure difference (i.e., transmembrane pressure) between the permeate and retentate sides of the membrane to be roughly constant along the length of the membrane resulting in a more constant flux along the length of the membrane. Holm et al. (1990) patented the above process with the inclusion of beads on the permeate side of the membrane, increasing the pressure drop on the permeate side for a given flow rate, which reduces pumping costs while maintaining low transmembrane pressure along the full length of the membrane. Pafylias et al. (1996) compared the MF of milk to remove bacteria using 1.4µm ceramic membranes in a UTP system to the same

membranes in a non-UTP system. They found that using the UTP system resulted in average fluxes 2 times greater than the non-UTP system.

Graded permeability membranes. A recent development in ceramic membranes has been the production of ceramic membranes modified to allow a constant flux along the length of the membrane, the advantage being a flux profile similar to a UTP system without the requirement of the permeate recirculation pump. Two patented methods to accomplish this are: 1st by Gracera et al. (2002) who developed a membrane with a decreasing hydraulic resistance in the support layer from the inlet (higher resistance) to the outlet (lower resistance), the 2nd by Grangeon et al. (2002) who developed a ceramic membrane with a decreasing thickness of the selective membrane layer instead of the ceramic support material. In either method the gradient chosen is specific to the flow rate and viscosity of the material being filtered (Grangeon et al., 2002). In both cases the change in resistance from inlet to outlet of the membrane is meant to compensate for differences in transmembrane pressure along the length of the membrane and produce a constant flux profile along the length of the membrane. These membranes are often called graded permeability **(GP)** membranes. Zulewska et al. (2009) found that a 0.1 μm ceramic GP system rejected more SP than a similar UTP system (61.04 and 64.4% SP removal in one stage at 3X, respectively).

Microfiltration in the Dairy Industry

Microfiltration in the dairy industry has been reviewed by Merin and Daufin (1990), Saboya and Maubois (2000) and Pouliot (2008). Uses for MF in the dairy industry include: defatting whey, removing bacteria from skim milk, separating fat from milk, cheese brine purification, and separation of CN micelles from SP in skim milk. Both Saboya and Maubois (2000) and Pouliot (2008) mark the development of

ceramic membranes in the 1980's as a major breakthrough allowing increased use of MF in the dairy industry. Ceramic membranes allowed the invention of the UTP system, which is widely used for bacterial removal from milk (Saboya and Maubois, 2000).

Microfiltration for production of SP and micellar CN. Although MF can be used for a variety of purposes within the dairy industry, the focus of our research will be on the use of MF to separate micellar CN from SP. Separation of micellar CN from SP using MF is a more recent development with the first published research coming in around 1988 (Faquant et al., 1988).

The viability of using MF to separate SP from micellar CN depends on the efficiency of separation, as well as the average flux. An ideal system would retain 100% of the CN and 0% of the SP. The efficiency of removal could be impacted by many factors; the membrane type, the flux to shear ratio, the concentration factor used, and the temperature of operation.

Zulewska et al. (2009) compared a ceramic (0.1µm) UTP system to a ceramic GP (0.1µm) membrane and a spiral-wound polymeric membrane (PVDF 0.3 µm). Using bleed-and-feed MF systems at a concentration factor of 3X operated at 50^{o}C, they found that 64.40% of the SP was removed in one pass for by a single stage UTP system compared to 61.04% and 38.62% for the GP ceramic and polymeric (PVDF) spiral-wound membranes, respectively. The low SP removal for the spiral-wound membranes is supported by research conducted by Lawrence et al. (2008) that found PVDF membranes of the same pore size rejected 78% of the β-lactoglobulin.

From the work of Zulewska et al. (2009) it is clear that membrane type and flow system type have a large impact on production of micellar CN and SP, another factor that appears to be important is operating conditions. Sachdeva and Buchheim (1997) looked at SP removal as a function of initial permeation rate (rates between

250 and 62.5 L/m^2 per hour) of a 0.1 µm ceramic UTP system. They found that higher initial flux lead to lower SP removal. This is consistent with the lower transmission found by Le Berre and Daufin (1996) as flux to shear rate ratio increased above the critical level. Vadi and Rizvi (2001) used a 0.2 µm ceramic UTP system operated in batch mode to concentrate skim milk to CF of 10. They found average flux decreased as concentration factor increased, they did not measure SP rejection at the various CF, but at a CF 8, 63% of the SP was removed. The SP removal of 63% found in Vadi and Rizvi (2001) for a CF of 8 was less than the 64.4% SP removal for a CF of 3 found by Zulewska et al. (2009), both for a UTP system, even though Vadi and Rizvi (2001) used a larger pore size membrane (0.2µm compared to 0.1µm). The large difference in removal is probably an indication of the importance of initial permeation rates in the overall system performance and the cumulative effect of membrane fouling during a batch run with continually increasing concentration factor. In Vadi and Rizvi, permeation rate was uncontrolled with a large initial permeation rate (in the range of 108 to 126 kg/m^2 per h) which decreased as CF increased. It is likely that the flux to shear rate exceeded critical values at startup leading to cake build up at the filter surface which progressively changed rejection characteristics of the membrane plus foulant. On the other hand in Zulewska et al. (2009) the UTP system permeation rate was controlled at 53 L/m^2 per hr for the entire run. The research indicates that the permeation rates (flux) chosen at a given cross-flow velocity have 2 related effects. First is an increase in hydraulic resistance caused by membrane fouling if the ratio of flux to shear exceeds the critical value. The 2nd related effect of flux on membrane performance is an increase in SP rejection as fouling of the membrane increases.

The CF at which filtration is performed is another parameter that can influence both fouling and SP removal. In the review by Saboya and Maubois (2000) a CF 3 to 4 was given as typical for micellar CN and SP separation using MF, but no justification

was given. In the work of Vadi and Risvi (2001) the viscosity of the micellar CN enriched retentates was measured at various CF. As CF increased from 2 to 4 to 10 the viscosity increased from approximately 2 to 3.5 to 12 centipoise and flux decreased with increasing viscosity. The choice of CF appears to be a balance between controlling fouling of the membrane and SP rejection and optimal use of the membrane.

The majority of research into using MF to separate CN from SP has been performed at 50^{o}C (Maubois, 2002). There has, however, been some research using low temperatures (< 7^{o}C) to MF skim milk to separate CN and SP. The advantage of using cold temperatures would be for the MF of raw milk (Govindasamy-Lucey et al. 2007), or to separate β-CN (van Hekken and Holsinger, 2000). The β-CN dissociates from CN micelles at 4^{o}C (Davies and Law, 1983), MF could then be used to produce a permeate containing SP enriched in β-CN compared to α-CN (van Hekken and Holsinger, 2000). A major drawback to MF at low temperature is low permeate fluxes. Permeate flux is highly dependent on temperature, in a study looking at the MF of skim milk to reduce bacterial load Beolchini et al. (2005) found that a 10^{o}C decrease in temperature from 40^{o}C to 30^{o}C decreased flux from 850 to 650 L/m^{2} per hr when running at a transmembrane pressure of 0.6 bar for a 1.4μm ceramic membrane system (non-UTP).

Multi-stage process. Theoretically, a single stage 3X MF system could remove 68% of the SP and lactose from skim milk (Nelson and Barbano, 2005). However, there could be instances were more SP and lactose removal would be required. Casein micelles are very heat stable (Holt, 1992 p.133), however, both lactose and SP undergo changes when exposed to high heat (Walstra et al., 1999 p 28-29). There may be applications for a micellar CN concentrate where a majority of both the heat labile components (SP and lactose) of skim milk have been removed.

To remove additional SP and lactose a multiple stage MF system could be used with dilution of the retentate between stages. Nelson and Barbano (2005) looked at the SP removal of a 3-stage 3X MF process using a 0.1µm ceramic UTP system. Their goal was to produce a retentate with a mineral and lactose composition similar to milk, but reduced in SP for use in cheese making. To accomplish this, they diluted the retentate between stages with the permeate from the ultrafiltration of the MF permeate from stage 1. They found that the 3-stage process, with stages 2 and 3 being a diafiltration with UF permeate, removed 95% of the SP, with the results slightly confounded by the presence of a low concentration of SP in the ultrafiltration permeate.

Comparing actual SP removed in each stage to theoretical or expected removal was very useful in accessing performance of the MF system in the research of Nelson and Barbano (2005). In a 3-stage MF process to separate CN from SP there are many choices in regards to operating parameters and equipment to be made, such as the CF for each stage, UTP vs GP, and the type of membrane (e.g., ceramic vs. polymeric) to use. These choices would influence the performance of the system, including SP removal and product yields, however there is a lack of published research on how such variables impact the process. In addition, there is little research looking at the effectiveness of CF control in a multistage system using water diafiltration and the effect this can have on performance, as well as variation in the starting skim milk composition. It would be useful to calculate on a theoretical basis expected SP removal and SP and micellar CN yields for a variety of conditions. These theoretical calculations would be useful in accessing the performance of multi-stage MF process to separate SP from micellar CN.

Zulewska et al. (2009) showed that a 0.1µm ceramic UTP MF system was the most efficient at removing SP when compared to a GP and polymeric spiral-wound

membranes. It would be expected that the 0.1µm ceramic UTP system would also be very effective at removing SP in a 3-stage process with water dilution between stages, if the casein micelles maintain their integrity when diluted with water at 50^{o}C in a soluble milk salts depleted environment. However, there is no published research on the total amount of SP that can be removed in a 3-stage 3X UTP MF with 0.1µm ceramic membranes with water dilution between stages.

The objectives of our research were, 1st to examine on a theoretical basis the various factors that could influence the yield and composition of the micellar CN and SP separated using a multiple stage MF process. The 2nd objective of our research was to determine the SP removal from skim milk using a 3-stage 3X UTP MF system with 0.1µm ceramic membranes operating at 50^{o}C, with water dilution between stages.

REFERENCES

Audic, J-L., B. Chaufer and G. Daufin. 2003. Non-food applications of milk components and dairy co-products: A review. Lait 83:417-438.

Belfort, G., Davis, R.H. and A.L. Zydney. 1994. The behavior of suspensions and macromolecular solutions in crossflow microfiltration. J. Membrane Sci. 96:1-58.

Benfer, S., P. Arki and G. Tomandl. 2004. Ceramic membranes for filtration applications- preparation and characterization. Advanced Engineering Materials 6(7): 495-500.

Beolchini, F., S. Cimini, L. Mosca, F. Veglio and D. Barba. 2005. Microfiltration of bovine and ovine milk for the reduction of microbial content: effect of some operating conditions on permeate flux and microbial reduction. Separation Science and Technology 40(4): 757-772.

Britten, M. and Y. Pouliot. 1996. Characterization of whey protein isolate obtained from milk microfiltration permeate. Lait 76:255-265.

Caric, M.D., S.D. Milanovic, D.M. Krstic, M.N. Tekic. 2000. Fouling of inorganic membranes by adsorption of whey proteins. J. Membrane Sci. 165:83-88.

Cheryan M. 1998. Ultrafiltration and microfiltration handbook. Technomic Publishing Company, Inc., Lancaster, PA, USA.

Davies, D.T and A.J.R. Law. 1983. Variation in the protein composition of bovine CN micelles and serum casein in relation to micellar size and temperature. J. Dairy Research 50:67-75.

de Wit, J.N. and G. Klarenbeck. 1984. Effects of various heat treatments on structure and solubility of whey proteins. J. Dairy Sci. 67:2701-2710.

de Wit, J.N. 1998. Nutritional and functional characteristics of whey proteins in food products. J. Dairy Sci. 81:597-608.

Eigel, W.N., J.E. Butler and C.H. Ernstrom. 1983. Nomenclature of cow's milk 5th revision. J. Dairy Sci. 67:1599-1631.

El Salam, M.H.A., S. El Shibiny and W. Buchheim. 1996. Characteristics and potential uses of the casein macropeptide. International Dairy Journal 6(4):327-341.

Evans, J. J. Zulewska, M. Newbold, M.A. Drake and D.M. Barbano. 2009. Comparison of composition, sensory and volatile components of thirty-four percent whey protein and milk serum protein concentrates. J. Dairy Sci. 92:4773-4791.

Faquant, J., J.L. Maubois and A. Pierre. 1988. Microfiltration du lait sur membrane minerale. Technique Laitiere 1028:21-23.

Fox, P.F. 2001. Milk proteins as food ingredients. Int. J. Dairy Technol. 54 :41-55.

Graceran, D. and E. Toujas. 2002. Graded permeability macroporous support for crossflow filtration. Societe Ceramiques Techniques, assignee. USPat. No. 6,375,014B1.

Grangeon, A., P. Lescoche, T. Fleischmann and B. Ruschel, inventors. 2002. Cross-flow filter membrane and method of manufacturing it. Technologies Advancees and Membranes Industrielles (Societe Anonyme), assignee. USPat. No. 6,499,606B1.

Green, G. and G. Belfort 1980. Fouling of ultrafiltration membranes: Lateral migration and particle trajectory. Desalination 35: 129-147.

Govindasamy-Lucey, S., J. J. Jaeggi, M. E. Johnson, T. Wang, and J. A. Lucey. 2007. Use of Cold Microfiltration Retentates Produced with Polymeric Membranes for Standardization of Milks for Manufacture of Pizza Cheese. J Dairy Sci. 90: 4552-4568.

Holt, C. 1992. Structure and stability of bovine casein micelles. Advances in Protein Chemistry 43: 64-151.

Holm, S., R. Malmberg and K. Svensson, inventors. 1990. Arrangement in membrane filter. Alfa-Laval AB, assignee. US Pat. No. 4,906,362.

Huffman, L.M. and W.J. Harper. 1999. Symposium: Marketing dairy value through technology, maximizing the value of milk through separation technologies. J. Dairy Sci. 82:2238-2244.

Jenness, R. B.C. Larson, T.L. Mcmeekin. 1956. Nomenclature of the proteins of bovine milk. J. Dairy Sci. 39:536-541.

Lawrence, N.D., S.E. Kentish, A.J. O'Conner, A.R. Barber and G.W. Stevens. 2008. Microfiltration of skim milk using polymeric membranes for casein concentrate manufacture. Separation and purification technology 60:237-244.

Le Berre, O., and G. Daufin. 1996. Skimmilk crossflow microfiltration performance versus permeation flux to wall shear stress ratio. J. Membrane Sci. 117:261-270.

Marshall, A.D., P.A. Munro and G. Tragardh. 1997. Influence of permeate flux on fouling during the microfiltration of β-lactoglobulin solutions under cross-flow conditions. J. Membrane Sci. 130:23-30.

Maubois, J.L. 2002. Membrane microfiltration: a tool for a new approach in dairy technology. Aust. J. Dairy Technol. 57:92-96.

Merin and G. Daufin. 1990. Crossflow microfiltration in the dairy industry: state-of-the-art. Lait 70:281-291.

Nelson, B.K., and D.M. Barbano. 2005. A microfiltration process to maximize removal of serum protein from skim milk before cheese making. J. Dairy Sci. 88:1891-1900.

Neocleous, M., D.M. Barbano and M.A. Rudan. 2002. Impact of low concentration factor microfiltration on milk component recovery and cheddar cheese yield. J. Dairy Sci. 85: 2415-2424.

Pafylias, I., M. Cheryan, M.A. Mehaia and N. Saglam. 1996. Microfiltration of milk with ceramic membranes. Food Research International 29(2): 141-146.

Papadatos, A., M. Neocleous, A.M. Berger, and D.M. Barbano. 2003. Economic feasibility of microfiltration of milk prior to cheesemaking. J. Dairy Sci. 86:1564-1577.

Pouliot, Y. 2008. Membrane processes in dairy technology-From a simple idea to worldwide panacea. Int. Dairy J. 18: 735-740.

Saboya, L.V. and J. Maubois. 2000. Current developments of microfiltration technology in the dairy industry. Lait 80:541-553.

Sachdeva, S. and W. Buchheim. 1997. Separation of native casein and whey proteins during crossflow microfiltration of skim milk. The Aust. J. of Dairy Technol. 52: 92-97.

Samuelsson, G., I.H. Huisman, G. Tragardh and M. Paulsson. 1997. Predicting limiting flux of skim milk in crossflow microfiltration. J. Membrane Sci. 129:277-281.

Sandblom R.M., inventor. 1978. Filtering process. Alfa-Laval AB, assignee. US Pat. No. 4,105,547.

Vadi P.K. and S.S.H. Rizvi. 2001. Experimental evaluation of a uniform transmembrane pressure crossflow microfiltration unit for the concentration of micellar casein from skim milk. J. Membrane Sci. 189:69-82.

Van Hekken, D.L. and V.H. Holsinger. 2000. Use of cold microfiltration to produce unique β-casein enriched milk gels. Lait 80:69-76.

Van Der Horst, H.C. and J.H. Hanemaaijer. 1990. Cross-flow microfiltration in the food industry. State of the art. Desalination 77:235-258.

Walstra, P., T.J. Geurts, A. Noomen, A. Jallema, M.A.J.S. van Boekel. 1999. Dairy Technology: Principles of Milk Properties and Processes. Marcel Dekker, Inc. New York, NY.

Zulewska, J., M. Newbold, D.M. Barbano. 2009. Efficiency of serum protein removal from skim milk with ceramic and polymeric membranes at 50^{o}C. J. Dairy Sci. 92:1361-1377.

Chapter 2

Processing Factors that Influence Casein and Serum Protein Separation by Microfiltration*

ABSTRACT

Our objective was to demonstrate the impact of various processing factors on the performance of a microfiltration (**MF**) system designed to process skim milk and separate casein (**CN**) from serum proteins (**SP**). A mathematical model of a skim milk MF process was developed with 3 stages, and an additional 4th finishing stage was added to standardize the retentate to 9% true protein (**TP**) and allow calculation of yield of liquid 9% TP micellar CN concentrate (**MCC**) and milk SP isolate (**MSPI**) (90% SP on a dry basis). The model was used to predict the effect of 5 factors**:** skim milk composition, heat treatment of skim milk, concentration factor (**CF**) and diafiltration factor (**DF**), control of CF and DF, and SP rejection of membrane on retentate and permeate composition, SP removal, and MCC and MSPI yield. When skim milk TP concentration increased from 3.2 to 3.8%, the TP concentration in the 3rd stage retentate increased from 7.92 to 9.40%, and the yield MCC from 1000 kg of skim milk increased from 293 to 348 kg and yield of MSPI increased from 6.24 to 7.38 kg. Increased heat treatment (72.9 to 85.2^{o}C) of skim milk caused CN as a percentage of TP content of skim milk as measured by Kjeldahl analysis to increase from 81.97 to 85.94% and the yield of MSPI decreased from 6.24 to 4.86 kg, while the 3rd stage cumulative SP removal decreased from 96.96 to 70.08%. A CF and DF of 2X gave a 3rd stage retentate TP concentration of 5.38% compared to 13.13% for a CF

---

* Hurt E.E. and D.M. Barbano. Processing factors that influence casein and serum protein separation by microfiltration. Submitted for publication.

and DF of 5X with the 3rd stage cumulative SP removal increasing from 88.66 to 99.47%, respectively. Variation in control of the balance between CF and DF (instead of an equal CF and DF) caused either a progressive increase or decrease in TP concentration in the retentate across stages depending on whether CF was greater than DF (increasing TP in retentate) or CF was less than DF (decreasing TP in retentate). An increased rejection of SP by the membrane from a SP removal factor of 1 to 0.6 caused a reduction in MSPI yield from 6.24 to 5.19 kg per 1000 kg of skim milk, 3rd stage cumulative SP removal decreased from 96.96 to 79.74%. Within the ranges of the 5 factors studied, the TP content of the 3rd stage retentate was most strongly impacted by the target CF and DF and variation in skim milk composition. Cumulative SP removal was most strongly impacted by the heat treatment of skim milk, the SP removal factor, and the target CF and DF. The MCC yield was most strongly impacted by initial skim milk composition. MSPI yield was strongly impacted by skim milk composition, whereas the heat treatment of milk and SP removal factor also had a large impact.

Key words: microfiltration, serum protein, micellar casein concentrate.

INTRODUCTION

Microfiltration (**MF**) can be used to fractionate skim milk into a micellar casein concentrate (**MCC**) and milk serum protein isolate (**MSPI**). Skim milk is processed with an MF system which retains casein (**CN**) and allows serum proteins (**SP**) to pass through the membrane. A 1-stage system will only remove a portion of the SP from the skim milk, if higher levels of SP removal from the MCC are desired, then additional MF diafiltration stages are required, with dilution of the retentate from the previous stage with water or UF permeate (Nelson and Barbano, 2005) for

diafiltration. Papadatos et al. (2003) found that in 30 of 36 months the use of a skim milk MF retentate increased net revenues for the production of cheddar and low moisture part skim mozzarella compared to conventional cheesemaking. The MCC could also have applications outside cheesemaking, in a variety of dairy and non-dairy beverage applications. The SP purified from the skim milk has enhanced functionality when compared to similar products made from cheese whey (Britten and Pouliot, 1996).

Whether on a research or industrial scale, efficient production of retentate and permeate products at target composition will be important. There are multiple factors that influence both the composition of MCC and the efficiency of the MF system. These factors include the initial skim milk composition, the heat treatment history of the skim milk; the concentration factor (**CF**) used for each stage of the MF, the control of the CF and diafiltration factors (**DF**), and the SP rejection characteristics of the membrane used in the MF process.

Milk composition can vary due to breed, genetics, nutrition, season, stage of lactation, and health status of the cow (Laben, 1963). A milk processing plant will experience variation in incoming milk composition. Regional and seasonal variations in milk composition can be large (Barbano, 1990). This variation in milk composition would cause variation in the composition of MF retentates and permeates unless other processing steps (e.g., ultrafiltration) are taken to protein standardize the skim milk prior to MF (Quinones et al., 1997, 1998).

The temperature and time of heat treatment of skim milk is another factor that can influence the performance of an MF process of skim milk to separate CN and SP. Heat treatment of milk is known to cause β-LG to form disulfide bonds with κ-casein on the surface of CN micelles (Sawyer, 1969). This causes an increase in the size of CN micelles, and an apparent increase in CN as a percentage of TP (**CN%TP**) as

measured by Kjeldahl (Lynch et al. 1998). The β-LG bound to the micelles is incorrectly counted as CN in the Rowland based fractionation (Rowland, 1938) Kjeldahl noncasein nitrogen method (AOAC, 2000; method number 998.05; 33.2.64), however the increase in measured CN%TP in milk as a function of increasing heat treatment is a useful quantitative index of the extent of heat denaturation of SP. Casein bound β-LG due to heat induced interactions cannot pass through an MF membrane and this will reduce the yield of MSPI. Ma et al. (2000) reported that CN as a percentage of crude protein in commercial samples of pasteurized fluid milk increased 2.81% to 5.56% over the level in raw milk due to pasteurization with an average increase of 3.79%. Therefore, pasteurization of skim milk prior to MF may reduce the removal of SP proteins.

The target CF will influence the performance of the MF system. The higher the CF, the higher the SP removal for each stage possibly resulting in fewer stages. However, there will be practical limits to the extent that CF can be increased. As CF increases, the concentration of CN in the retentate will increase and at some point the viscosity of the retentate at the membrane surface will be such that the membranes will foul extensively, reducing permeate and SP removal. Vadi and Rizvi (2001) reported that as skim milk was concentrated by MF the apparent viscosity of the retentate at 50^{o}C increased from less than 2 cP for skim milk to approximately 3cP for 4X retentate. While the target CF and DF may both be 3X, in practice there will be some variation in ability to achieve these targets. Variation or limitations in the ability of the system to accurately control CF and DF could also be a cause of variation in performance of an MF system and variation in product composition.

Different types of MF membranes (e.g., polymeric versus ceramic) could have different SP removal factors, which would affect end product compositions and yield of MSPI. A wide range of MF systems have been used to separate micellar CN from

SP and there is also a wide range of reported removal (rejection) factors for SP. Nelson and Barbano (2005) found SP removal was close to theoretical indicating a removal factor close to 1. Zulewska et al. (2009) found a SP removal factor of 0.99 for a ceramic uniform transmembrane pressure system and 0.66 for a polymeric spiral wound system. The influence of the factors mentioned above are not well defined and their relative importance may not be clear to potential users of MF technology for separation of CN and SP.

The objective of our research was to demonstrate how variation in skim milk composition, different heat treatments of skim milk (i.e., denaturation of serum protein), different target MF concentration factors, variation in control of MF CF and DF, and differences in degree of SP removal by the membrane influenced the expected composition of retentate (MCC), permeate (i.e., SP yield) and SP removal when produced using a 3-stage MF process designed to separate SP from micellar CN.

MATERIALS AND METHODS

Processing Factors Studied

Five different factors were explored in our study: 1) the effect of variation in skim milk composition that could reflect within or between day, or seasonal variation; 2) the effect of differing heat treatments of skim milk as they cause heat denaturation of SP resulting in binding of SP to CN micelles; 3) the effect of the choice of different MF CF and DF; 4) the effect of lack of exact control of CF and DF, specifically cases where CF does not equal DF; and 5) the effect of different retention factors by the MF membrane for SP. For each of these 5 factors, the affect on retentate composition, permeate composition, and overall SP removal was calculated for each MF stage. Additionally, yields of a MCC and MSPI were calculated, assuming that liquid MCC contained 9% true protein (**TP**) and that dried MSPI was 90% SP. These 5 factors are

process control issues commonly encountered during MF processing of skim milk to create a SP reduced MCC and an MSPI.

Model Development

A model was developed using Excel 2007 (Microsoft – Redmond, WA), to calculate the composition of retentate and permeate produced in each stage of a 3-stage bleed and feed MF processing of skim milk. The model was independent of the type of MF system used and established theoretical values that should be achieved given well-defined assumptions. For each stage there was a feed, retentate, and permeate. Input and default values for model calculations are shown in Table 2.1. All concentrations are expressed as percent on a weight basis. The CF is the ratio of feed mass to retentate mass. The DF is the sum of mass of water added and mass of retentate divided by mass of retentate, where the DF for each stage used the mass of retentate produced in the previous stage.

Table 2.1. Default skim milk composition and model inputs for component removal factors, concentration factors (CF) and diafiltration factors (DF).

Skim milk composition (% by weight)		**Model factors**		
Casein	2.623	Removal factor:	Ash	1
Serum protein	0.577		Lactose	1
Ash	0.729		NPN	1
NPN	0.190		Serum protein	1
Lactose	4.850		Casein	0
		CF:	Stage 1	3.0
			Stage 2	3.0
			Stage 3	3.0
		DF:	Stage 2	3.0
			Stage 3	3.0

The solute removal factors, a required model input, are the ratio of the solute's concentration in the MF permeate to its concentration in the MF permeate portion of the feed. A removal factor of 1 would mean that the solute is not retained by the membrane. It was necessary to use the solute concentration in the permeate portion of skim milk because skim milk contains CN micelles and the dry mass of CN takes up a portion of the mass of skim milk. As a result, other soluble milk components exist in solution in the MF permeate portion of skim milk at a higher concentration than indicated by analysis of the skim milk. If a solute in the MF permeate portion of skim milk was not retained by the membrane, then its concentration in the MF permeate would be equal to its concentration in the permeate portion of the MF feed solution.

Assumptions. Several assumptions were made regarding the MF feed material and partitioning of milk components during MF. It was assumed that the initial skim milk had 0% fat. For each scenario, it was assumed that 100% of CN was rejected (i.e. concentration of CN in the permeate was 0%). The ash in skim milk was calculated using the empirical equation: Ash = 0.0596*[TP] + 0.5379 (Kaylegian et al., 2006). Approximately two thirds of the calcium and phosphate content of milk was assumed to be bound to the CN micelles (Jenness, 1959).

Permeate portion calculations. The concentration of SP, lactose and nonprotein nitrogen (**NPN**) in the MF permeate portion was calculated as the mass of SP, lactose, NPN divided by the mass of the permeate portion. The mass of the permeate portion in the feed solution was the total mass minus the mass of CN plus the mass of ash associated with the CN micelles. The equation for serum protein is $[SP]_{serumphase} = [SP]_{bulk} * mass_{feed}/(mass_{feed} - mass_{feed} * [CN]_{feed} / 100 - mass_{skimmilk} * 2/3 [ash]_{skimmilk}/100)$. For lactose and NPN the same equation was used replacing the concentration of SP with the concentration of lactose or NPN.

The concentration of ash in the permeate portion for the initial MF feed was calculated as for SP, lactose, and NPN except that the ash not associated with the CN micelles was assumed to be one third of the total ash. For subsequent stages the permeate portion ash in the feed was calculated as the remaining soluble ash divided by the mass of the permeate portion (the same denominator as the equation for SP shown above).

Composition calculations for retentate, permeate and feed. The concentration of SP, lactose, NPN and ash in the MF permeate was calculated as their concentration in the permeate portion of the feed times the removal factor. The concentration of CN in the permeate was assumed to be 0%.The concentration of CN, SP, NPN, lactose, and ash in the retentate was calculated as the mass of each milk component in the feed

minus its mass in the permeate, with the difference divided by the total mass of retentate. This equation can also be expressed in terms of CF for each: $[SP]_{retentate} = CF * [SP]_{feed} - (CF - 1) * [SP]_{permeate}$.

The feed for the first stage was skim milk with the default composition, model factors for component removal, and CF and DF values shown in Table 2.1. For the 2nd and 3rd stages, the feed composition was calculated from the retentate composition of the previous stage and the DF as follows for SP in the 2nd stage: $[SP]_{feedstage2} = [SP]_{retentatestage1} / DF_{stage2}$. True protein concentration in the feed was calculated as the sum of the SP and CN concentrations.

Cumulative SP removal calculation. The cumulative SP removal for each stage was calculated as the mass of SP in the skim milk minus the mass of SP remaining in the retentate with this sum divided by the mass of SP in the skim milk and the total multiplied by 100. In terms of CF the above reduces to equations for each stage shown below:

Stage 1: % Removal $= 100*(1-[SP]_{retentate}/([SP]_{milk}*CF_{stage1}))$

Stage 2: % Removal $= 100*(1-([SP]_{retentate}/[SP]_{milk})*DF_{stage2}/(CF_{stage1}*CF_{stage2}))$

Stage 3: % Removal $= 100*(1-([SP]_{retentate}/[SP]_{milk}) * (DF_{stage2}*DF_{stage3}) / (CF_{stage1}*CF_{stage2}*CF_{stage3}))$

Yield of MCC and MSPI. The processing end products were assumed to be a liquid MCC with 9% TP and a dried MSPI containing 90% SP. A finishing 4th MF stage would be required in most cases to bring the TP content of the retentate to 9%, if after the 3rd stage the retentate TP was greater than 9%, then water would be added to bring the TP concentration down to 9%. A basis of 1000 kg of starting skim milk was used to calculate theoretical yield and the 4th stage was an MF stage with the same removal characteristics as earlier stages. There was no water added to the retentate at the start of the 4th stage, but the calculations for concentration in the permeate and

retentate were the same as for earlier stages (DF equaled 1). The permeate to be removed in the 4th stage was found by iteration to achieve 9% TP in the retentate. The yield of MSPI includes SP removed in the 4th finishing MF stage.

Definition of Parameters Studied

Influence of skim milk composition. Four different skim milk compositions were used for estimating the effects of variation in milk composition on MF (Table 2.2). The model factors were kept constant and were the default factors shown in Table 2.1. Four concentrations of TP were chosen with CN%TP kept constant at 81.97%. Ash content of each skim milk was calculated as described in Kaylegian et al. (2006). Lactose and NPN were assumed not to vary among the 4 milks. To determine the influence of skim milk composition on a MF process several model outputs were calculated. The effect on retentate composition was determined by comparing the composition of the 3rd stage retentates, and the TP concentration of in the retentates for each 3 stages with different skim milks. The variation in SP content of the permeates from 3 stages and SP removal as skim milk composition changed was also determined. Finally, the yield of MCC and MSPI from 1000 kg of skim milk with the 4 different TP levels was calculated.

Influence of heat treatment of skim milk. Heat treatment of skim milk increases the apparent CN%TP as measured by Kjeldahl analysis, due to β-LG and α-LA binding to κ-casein. A preliminary experiment was conducted with 2% fat milk to demonstrate the magnitude of change in CN%TP at various pasteurization temperatures at a constant holding time of 25 s in a tubular pasteurizer and these values were used to help define the range of CN%TP used in the modeling. Raw milk was cold separated into cream and skim fractions with a DeLaval separator (Model 590; Poughkeepsie, NY) and then raw skim milk and cream were blended to

make 2% fat milk. Approximately 18.9L of 2% fat raw milk were added to a jacketed steam kettle, heated to 60°C, and then homogenized with 2 passes through a 2-stage Gaulin APV (Model 200 E; Everett, MA) homogenizer with the first stage pressure set a 13,790 kPa and the second stage at 3,448 kPa. After homogenization, the milk was pasteurized at approximately: 72.9, 77.2, 79.9 or 85.2°C for 25 s in a tubular pasteurizer, as described by Ma and Barbano (2003). Milk samples after heating and homogenization prior to pasteurization and after pasteurization were analyzed by the Kjeldahl method for TN, NCN, and NPN using the following Kjeldahl methods (AOAC, 2000; method number 991.20; 33.2.11), (AOAC, 2000; method number 998.05; 33.2.64), and (AOAC, 2000; method number 991.21; 33.2.12), respectively.

Table 2.2. Skim milk compositions (% by weight) used to determine the impact of variation in skim milk composition on MF performance.

Milk component	% by weight			
True protein	3.200	3.400	3.600	3.800
Casein	2.623	2.787	2.951	3.115
Serum protein	0.577	0.613	0.649	0.685
NPN	0.190	0.190	0.190	0.190
CN % TP[1]	81.97	81.97	81.97	81.97
Lactose	4.850	4.850	4.850	4.850
Ash	0.729	0.741	0.752	0.764

[1]CN%TP = Casein as a percentage of true protein

The heat induced binding of SP to CN micelles reduces the amount of SP that can be removed from skim milk. The model factors and the skim milk composition were the default factors shown in Table 2.1, except that the amounts of CN and SP were was modified to give different CN%TP values shown in Table 2.3. To determine

the influence of heat treatment of skim milk on SP removal by the MF process, the SP bound to CN in the MF retentates is presented as SP and the CN that would be seen by SDS PAGE, not as the "casein + heat denatured whey protein" that would be measured by using the Kjeldahl methods. The effect on 3rd stage retentate composition and the TP concentration in the retentates for the individual stages with different CN%TP in skim milk was determined. The variation in SP content in the MF permeates from 3 stages and SP removal as CN%TP in skim milk changed were calculated. Finally, the yields of MCC and MSPI from 1000 kg of skim milk were determined.

Influence of CF. The input skim milk composition and removal factors were kept constant as shown in Table 2.1. Paired CF and DF of 2, 3, 4 and 5 were used to estimate the impact of CF selection on MF performance. The influence of CF and DF was calculated for a 5-stage MF process with the same outputs calculated for retentates, permeates and SP removal as for the influence of skim milk composition. Final yield of MCC and MSPI was not calculated, because at 5 stages the yield would not be comparable to other calculated yields and with the retentates standardized to 9% TP the MCC yields would be nearly identical.

Table 2.3. Milk composition (% by weight) used to determine the impact of different degrees of heat treatments (i.e., increased CN%TP[1]) on microfiltration performance.

Milk component	% by weight			
True protein	3.2	3.2	3.2	3.2
Casein	2.623	2.68	2.72	2.75
Serum protein	0.577	0.52	0.48	0.45
NPN	0.19	0.19	0.19	0.19
CN % TP	81.97	83.75	85.00	85.94
Lactose	4.85	4.85	4.85	4.85
Ash	0.729	0.729	0.729	0.729

[1]CN%TP = Casein as a percentage of true protein that would be expected as measured by Kjeldahl that increases as a result of SP being counted as if it was casein.

Influence of variation in control of CF and DF. The input skim milk composition and removal factors were kept constant as shown in Table 2.1. The combinations of CF and DF used are shown in Table 2.4. In practice it would be difficult to exactly control CF and DF in real time during processing and in practice they could vary within and between days causing variation in composition of retentates and permeates. The influence of CF and DF was calculated for a 3-stage MF process with the same outputs determined for retentates, permeates and SP removal as were determined for the influence of skim milk composition.

Table 2.4. Combinations of concentration factor (CF) and diafiltration factor (DF) used to determine the impact of variation in control of CF and DF on microfiltration performance.

	Stage	CF3.0 & DF3.0	CF3.1 &DF3.0	CF2.9 &DF3.0	CF3.0 &DF3.1	CF3.0 &DF2.9
CF:	1	3.0	3.1	2.9	3.0	3.0
	2	3.0	3.1	2.9	3.0	3.0
	3	3.0	3.1	2.9	3.0	3.0
DF:	2	3.0	3.0	3.0	3.1	2.9
	3	3.0	3.0	3.0	3.1	2.9

Influence of SP rejection by the membrane. The input skim milk composition and the default model factors were kept constant, as shown in Table 2.1, except the SP removal factor was changed from 1 to 0.6. A removal factor of 0.6 corresponds to a classical rejection coefficient of 0.65 (1 minus the concentration of SP in the permeate divided by the concentration of SP in the retentate). The influence of CF and DF was calculated for a 3-stage MF process with the same outputs for retentates, permeates and SP removal that were used to determine the influence of skim milk composition. In addition, 3rd stage cumulative SP removal as a function of SP rejection was plotted for each stage.

RESULTS

Influence of Skim Milk Composition.

Retentate. The protein content of skim milk to be processed by MF may vary within and between days in a typical milk processing factory. The TP concentration in the retentate for each stage of MF increased as TP content of skim milk increased (Figure 2.1). The TP content of the MF retentate for each of 3 stages as a function of TP content of skim milk can be predicted by using the following 3 linear equations: stage 1: $TP_{retentate} = 2.601*(TP_{skim\ milk}) + 0.0859$; stage 2: $TP_{retentate} = 2.4898*(TP_{skim\ milk}) + 0.0701$; stage 3: $TP_{retentate} = 2.4578*(TP_{skim\ milk}) + 0.00566$, when the model factors in Table 2.1 were applied . These equations could be used in a factory to provide a point of reference on expected protein content of MF retentate from each stage when the protein content of skim milk varies. The expected compositions of final MF retentates produced from milks with 4 different TP contents are shown in Table 2.5 using the assumptions in Table 2.1 and the milk compositions in Table 2.2. The TP and CN content of the final 3rd stage retentates increased with increasing TP content of the starting skim milk. The increase in CN content is expected to increase retentate viscosity, as reported by Vadi and Rizvi (2001). Increasing casein content of the MF retentate may increase concentration polarization driven fouling of the MF system. From a practical perspective, the SP, NPN, CN%TP, lactose, and NPN contents of the 3rd stage MF retentate are not influenced by variation in the TP content of the starting skim milk (Table 2.5). Ash concentration in the 3rd stage MF retentate increased with increasing TP in the skim milk (Table 2.5), as expected because CN concentration in the 3rd stage MF retentate increased and the CN carried bound minerals with it.

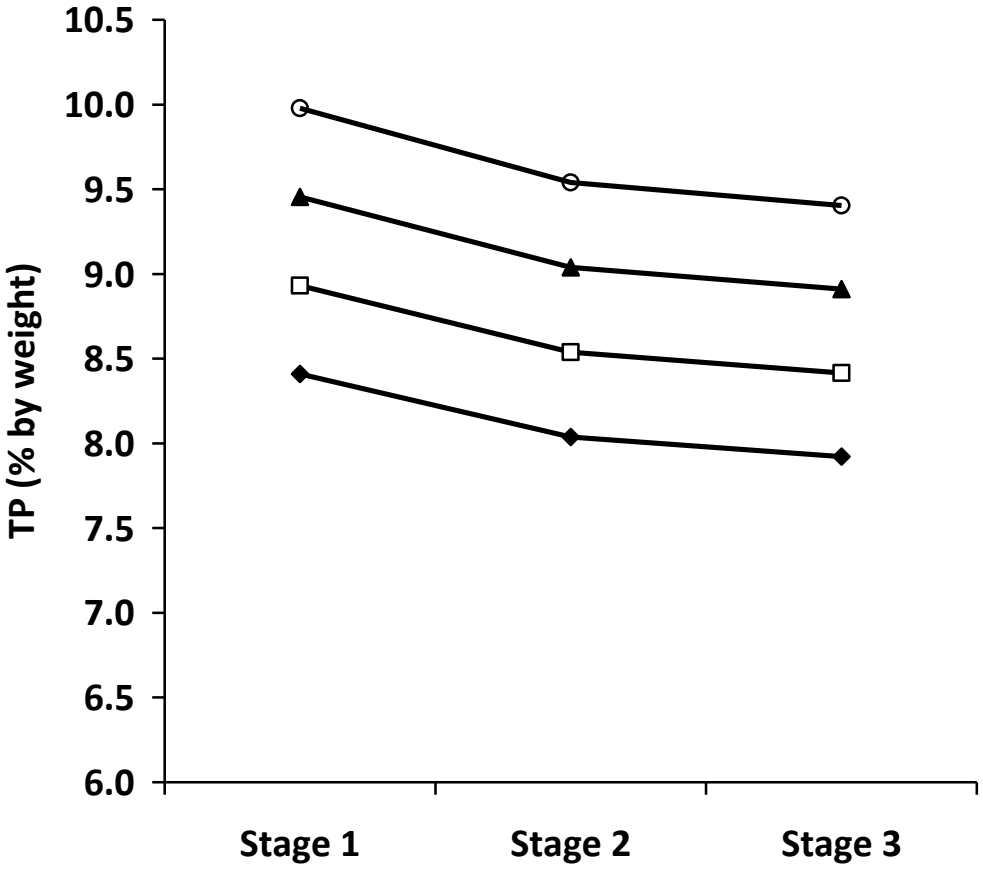

Figure 2.1. The effect of variation in true protein (TP) content of skim milk, (♦) 3.2% TP, (□) 3.4% TP, (▲) 3.6% TP, and (○) 3.8% TP, on the TP concentration in the retentate in each stage of a 3-stage microfiltration process with water diafiltration (stages 2 and 3) with the model factors from Table 2.1 and the skim milk compositions from Table 2.2

Table 2.5. Composition of retentate (% by weight) produced in 3rd stage of a 3X microfiltration process with water diafiltration (stages 2 and 3), with different true protein levels in the starting skim milk, model factors from Table 2.1 and skim milk compositions from Table 2.2.

	True protein in skim milk (% by weight)			
	3.2	3.4	3.6	3.8
Milk component	Retentate composition (% by weight)			
True protein	7.9215	8.4159	8.9101	9.4046
Casein	7.8690	8.3607	8.8524	9.3444
Serum protein	0.0525	0.0552	0.0577	0.0602
NPN	0.0173	0.0171	0.0169	0.0167
CN %TP	99.34	99.34	99.35	99.36
Lactose	0.4417	0.4365	0.4313	0.4262
Ash	1.4583	1.4806	1.5028	1.5251

[1]CN%TP = Casein as a percentage of true protein.

Permeate. Higher TP concentration in the skim milk (Table 2.2) led to a higher concentration of SP in the MF permeates from each stage (Figure 2.2). This was due to the higher SP concentration in the skim milk (Table 2.2) and higher concentration of SP in the permeate portion of MF feed when the skim milk had higher TP concentrations. Irrespective of starting skim milk TP, there was a large decrease in SP in the permeate between stages 1 and 2 (Figure 2.2). A large amount of SP was removed in the 1st stage and when the 1st stage MF retentate was diluted with water for diafiltration, the concentration of SP remaining in the permeate portion of the 2nd stage feed and the resulting MF permeate for the 2nd stage was reduced greatly.

Serum protein removal. Cumulative SP removal by MF increased gradually as TP content of the skim milk increased (Table 2.6), but the change was very small. Skim milk with a higher concentration of TP also had a higher concentration of SP and CN (Table 2.2). The higher concentration of SP in the skim milk did not affect cumulative SP removal, but the higher CN content of the skim milk did by increasing the concentration of SP in the permeate portion of the MF feed.

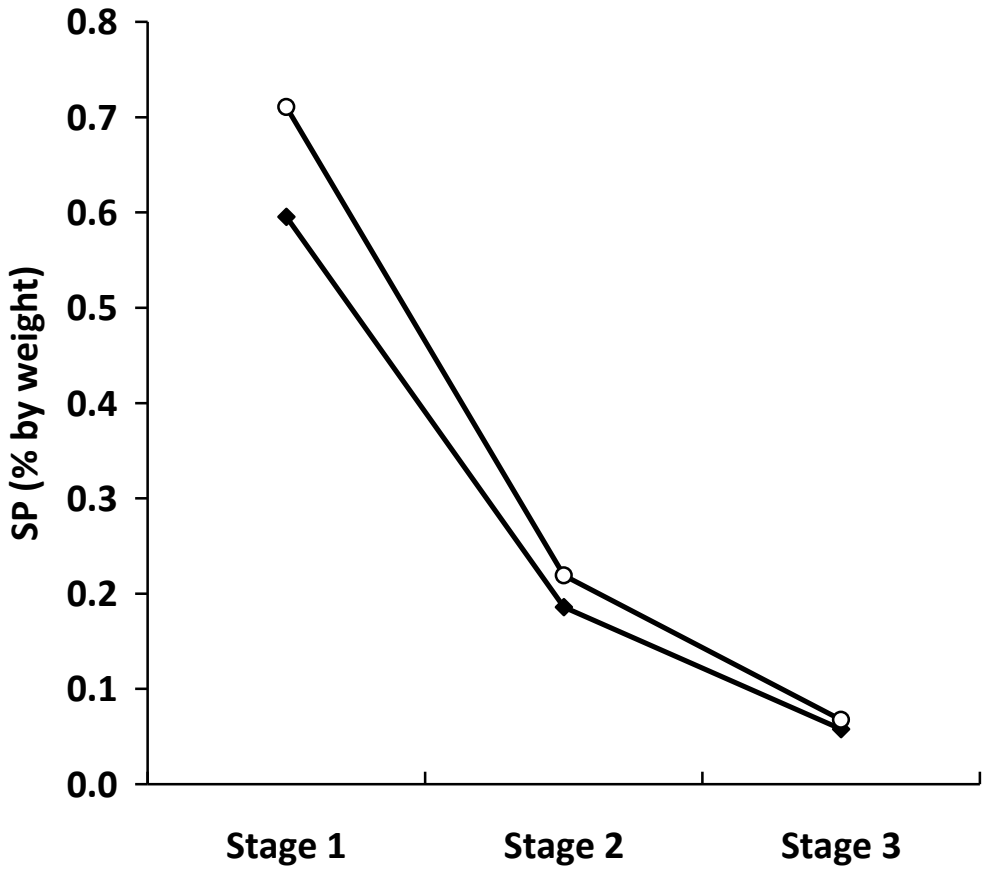

Figure 2.2. The effect of variation in true protein content of skim milk (♦) 3.2% TP, and (○) 3.8% TP, on serum protein (SP) concentration in the permeate in each stage of a 3-stage microfiltration process with water diafiltation (stages 2 and 3) with the model factors from Table 2.1 and the skim milk compositions from Table 2.2.

Table 2.6. Effect of skim milk compositon on cumulative serum protein (SP) removal in a 3-stage 3X microfiltration (MF) process with water diafiltration (stages 2 and 3) using the model factors from Table 2.1 and the skim milk compositions from Table 2.2.

	True protein in skim milk (% by weight)			
	3.2	3.4	3.6	3.8
MF stage	Cumulative SP removal (%)			
1	68.80	68.93	69.05	69.17
2	90.27	90.35	90.42	90.49
3	96.96	97.00	97.03	97.07

MCC and MSPI yield. The yield of MCC and MSPI were expected to increase with increasing concentration of CN and SP, respectively, in the milk and they did (Table 2.7). The goal of the MF process was to produce a MCC that contained 9% TP. The TP content of the final 3rd stage retentate was lower than 9% for starting skim milk TP of 3.2, 3.4, and 3.6% and therefore a 4th stage was required to remove additional permeate to achieve the final target concentration of 9% TP in the MCC, while the final TP content of the 3rd stage retentate (i.e., MCC) was higher than 9% TP when starting with a skim milk containing 3.8% TP and a 4th stage was not required (Table 2.7). The liquid yield of MCC from skim milk containing 3.8% TP was incrementally higher because the 3rd stage retentate the protein content had to be standardized down by the addition of water (Table 2.7). The 3 lower levels of TP in the skim milk required more permeate removal in a 4th stage MF finishing step to produce an MCC with 9% TP and the result resulting yield of MCC was lower, because there was less TP in the skim milk (Table 2.7). The total SP removal from MCC in Table 2.7 was slightly higher when the starting skim milk had lower TP,

because more permeate (which contains SP) had to be removed in the 4th finishing stage to increase the TP in the MCC to 9%.

While the TP concentration of skim milk affected the SP removal and lactose, NPN and ash content of the retentates produced during MF, the main impact of different skim milk composition was on the TP content of the retentates produced by MF and yield of MCC and MSPI (Table 2.7). In addition to yield, lower TP concentration in skim milk required more processing to produce the desired liquid MCC containing 9% TP. If a 9% protein standardized MCC product need to be produced, then either the protein content of the skim milk would have to be increased (possibly by UF) prior to MF, the CF and DF of the MF process would have to be adjusted in response to changing incoming skim milk composition, or an extra filtration finishing step would be required.

Table 2.7. Protein content of the 3rd stage retentate, yields of liquid MCC[1] standardized to 9% true protein (TP) with a 4th stage finishing step and dry solids yield of MSPI[2], and total percentage serum protein (SP) removal for a 3-stage 3X microfiltration process with water diafiltration (stages 2 and 3) with 1000 kg of the different skim milk compositions from Table 2.2.

	True protein in skim milk (% by weight)			
	3.2	3.4	3.6	3.8
	Yield			
Skim milk (kg)	1000	1000	1000	1000
3rd stage TP (% by weight)	7.92	8.42	8.91	9.40
4th stage permeate to remove (kg)	40.22	21.78	3.37	-15.09
Yield liquid MCC[1] (9%TP) (kg)	293	312	330	348
Yield dry MSPI[2] (90%) (kg)	6.24	6.62	7.00	7.38
Total SP removal (%)	97.36	97.21	97.07	96.92

[1]MCC = micellar casein concentrate
[2]MSPI = milk serum protein isolate

Influence of Heat Treatment of Skim Milk

Selection of the time and temperature of heat treatment of skim milk prior to MF may influence the ability of the process to remove SP from skim milk. A preliminary experiment demonstrated that the CN%TP (measured using Kjeldahl analysis) increased as the temperature of pasteurization increased (Figure 2.3) due to denaturation and binding of SP to CN micelles. The SP that was bound to CN micelles will not pass through an MF membrane into the permeate and will be expected to decrease the yield of removed SP. A quadratic equation fits the observed data well (Figure 2.3) with an R^2 of 0.974. A hypothetical increase in CN%TP with increasing temperature of pasteurization (as shown in Table 2.3) means that the concentration of SP soluble in the permeate portion of the skim milk will decrease (Table 2.3) and thus it would be expected that the removal of SP from skim milk will decrease with increasing heat treatment.

Retentate. The increase in apparent CN%TP in skim milk caused by increased pasteurization temperature (Table 2.3) increased the TP content of the retentates produced by MF (Figure 2.4). Unlike the increase in TP content of MF retentate due to increased TP concentration in skim milk in Figure 2.1, the increase in TP concentration of the MF retentates was caused by increased retention of bound SP due to heat denaturation of SP and not increased CN content in the skim milk. Thus, the proportion of CN and SP contained in the retentates in Figures 2.1 and 2.4 would be different at the same TP concentration and their functionality may be different.

Skim milk with increased apparent CN%TP due to heat denaturation of SP produced 3rd stage MF retentates with much higher levels (0.05% to 0.43%) of SP (Table 2.8) because some of the SP was bound to CN and could not be removed by MF. The CN%TP in the retentates, not including the heat denatured SP, decreased with increasing heat treatment (Table 2.8).

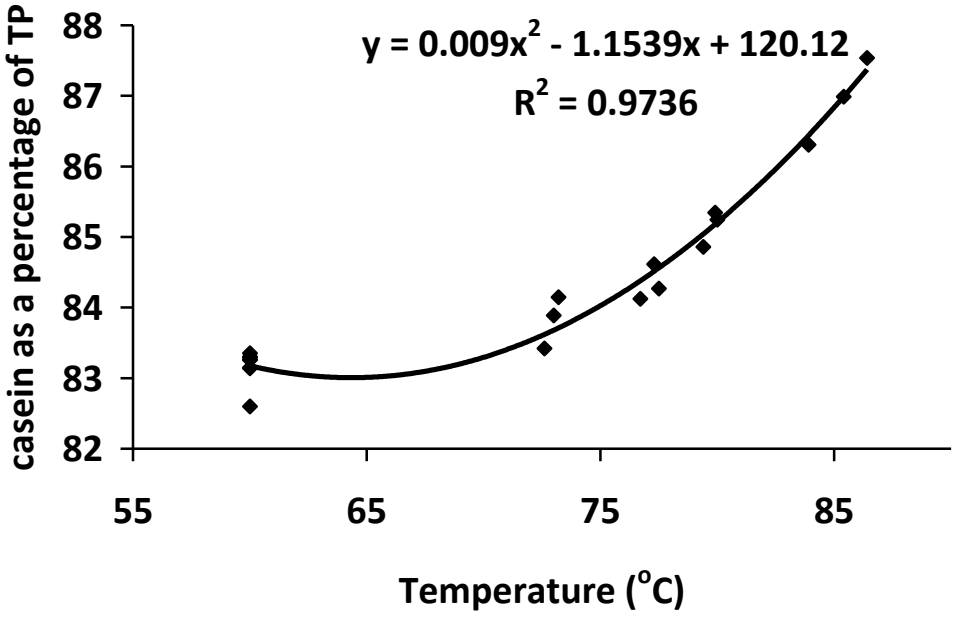

Figure 2.3. Casein as a percentage of true protein (as measured by Kjeldahl analysis) as a function of pasteurization temperature with a hold time of 25

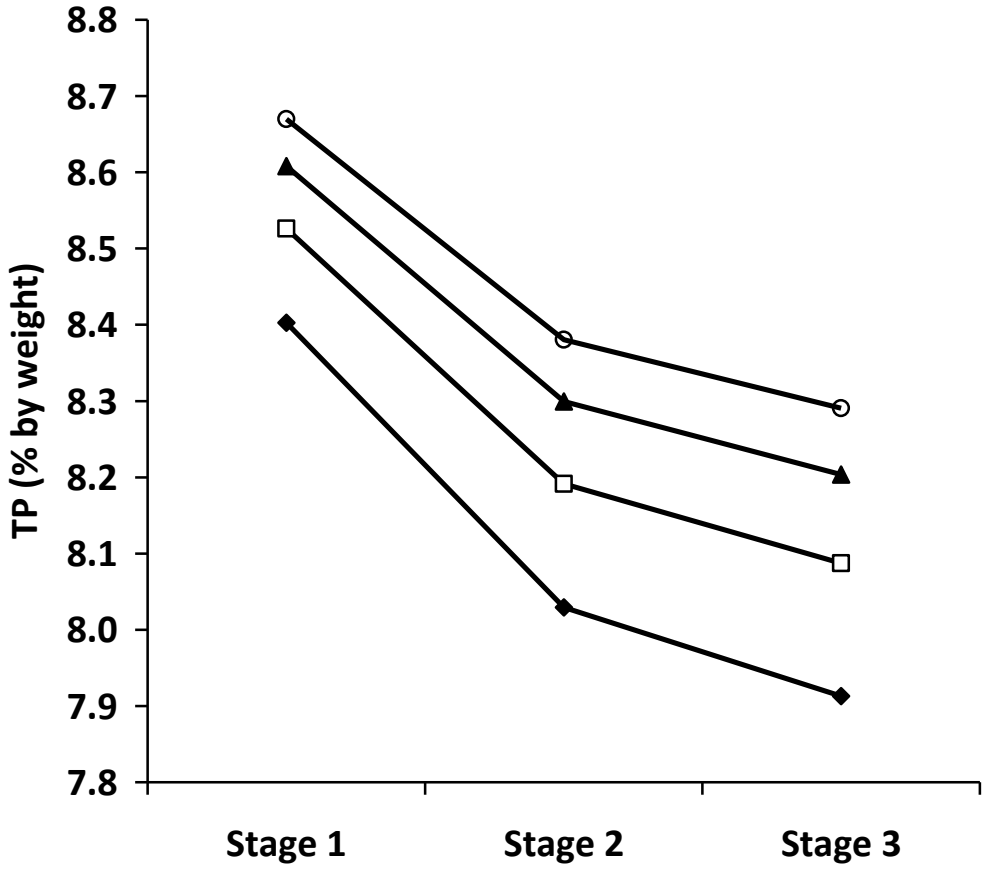

Figure 2.4. True protein (TP) in the retentate (% by weight) in each stage of a 3-stage microfiltration process with water diafiltration (stages 2 and 3) with increasing levels of casein as a percent of TP (CN%TP) (♦) 81.97CN%TP, (□) 83.75 CN%TP , (▲) 85.00 CN%TP, and (○) 85.94 CN%TP, due to heat denaturation of serum protein when starting with skim milk containing 3.2% TP and 81.97% CN%TP. Model factors from Table 2.1, skim milk composition from Table 2.3.

Table 2.8. Composition of retentate (% by weight) produced in 3rd stage of a 3X microfiltration process with water diafiltration (stage 2 and 3) where initial skim milk contained 3.2% true protein and that skim milk had different levels of heat treatment that caused apparent CN%TP[2] to increase.

	Casein as a percentage of true protein in skim milk (Kjeldahl)			
	81.97	83.75[1]	85.00[1]	85.94[1]
Milk component	Retentate composition (% by weight)			
True protein	7.9215	8.0871	8.2034	8.2906
Casein	7.8690	7.869	7.869	7.869
Serum protein	0.0525	0.2272	0.3434	0.4306
NPN	0.0173	0.0172	0.0172	0.0172
CN%TP[2]	99.34	97.30	95.92	94.91
Lactose	0.4417	0.4399	0.4387	0.4387
Ash	1.4583	1.4578	1.4578	1.4578

[1] Estimated higher casein as a percentage to TP caused by heat treatment.
[2]CN%TP = Casein as a percentage of true protein in the retentate was calculated to not include heat denatured SP.

Permeate. The concentration of SP in the MF permeate decreased with increasing heat denaturation of SP and increased apparent CN%TP in the skim milk (Figure 2.5), because the concentration of SP in the permeate portion of the MF feed had decreased as more of the SP was bound to CN. The decreased SP content of MF permeate was largest in the first stage of the MF system.

Serum protein removal. Cumulative SP removal decreased as heat treatment of the milk and CN%TP increased in the skim milk (Figure 2.6), because larger and larger amounts of SP were bound to CN and could not be removed by MF. The impact was large, with the percentage SP removal in the final MCC decreasing from 97.36% to about 75.85% for apparent CN%TP of 81.97 and 85.94% respectively (Table 2.9).

MCC and MSPI yield. Increased apparent CN%TP in skim milk caused by heat treatment led to an increased yield and changed protein composition of MCC, but a decreased yield of MSPI when starting from the same skim milk (Table 2.9). When the apparent CN%TP increased from 81.97 to 83.75% the MCC yield increased by approximately 2%, but the MSPI yield decreased by about 10%. When heat denaturation of SP increased producing a change in CN%TP from 81.97 to 85.94%, the decrease in yield of MSPI was about 22%. There is a very large impact on the yield of MSPI due to pasteurization time and temperature and therefore it will be important to minimize heat denaturation of SP prior to MF to maximize the economic performance of the process.

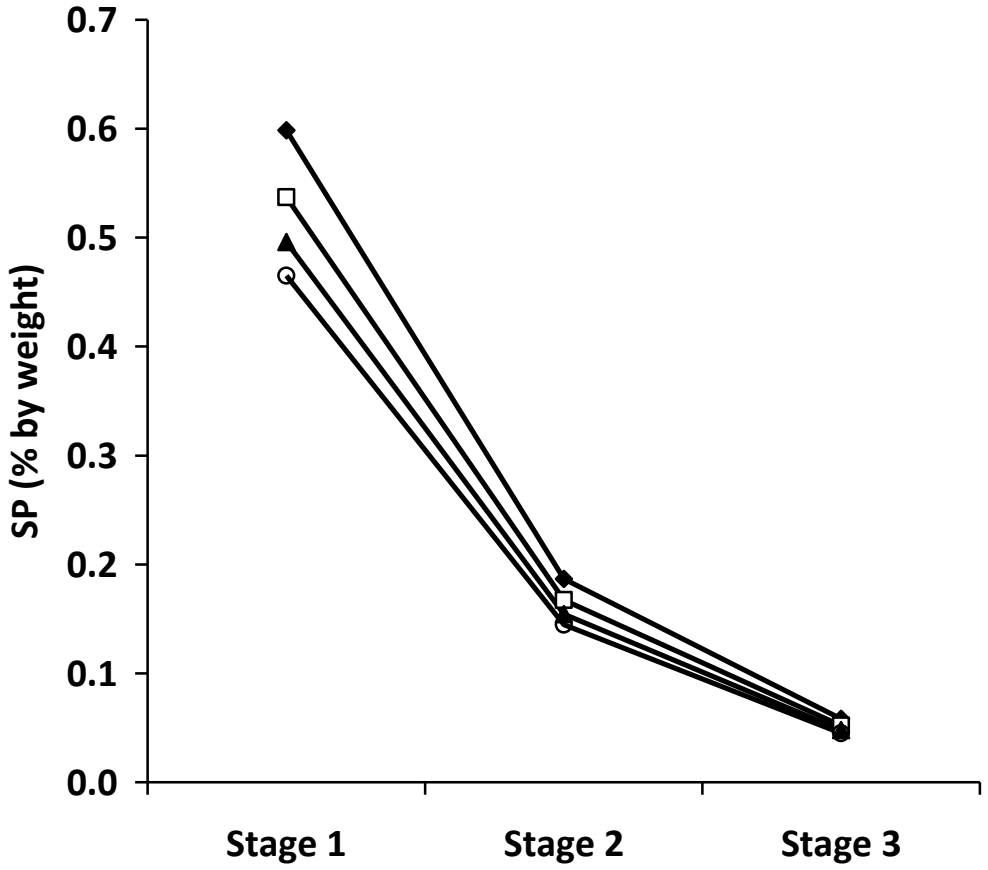

Figure 2.5. Serum protein (SP) (% by weight) in the permeate of each stage of a 3-stage 3X microfiltration process with water diafiltration (stages 2 and 3), where casein as a percentage of true protein (CN%TP) in the skim milk varied, (♦) 81.97 CN%TP, (□) 83.75 CN%TP, (▲) 85.00 CN%TP and (○) 85.94 CN%TP. Model factors from Table 2.1, skim milk composition from Table 2.3.

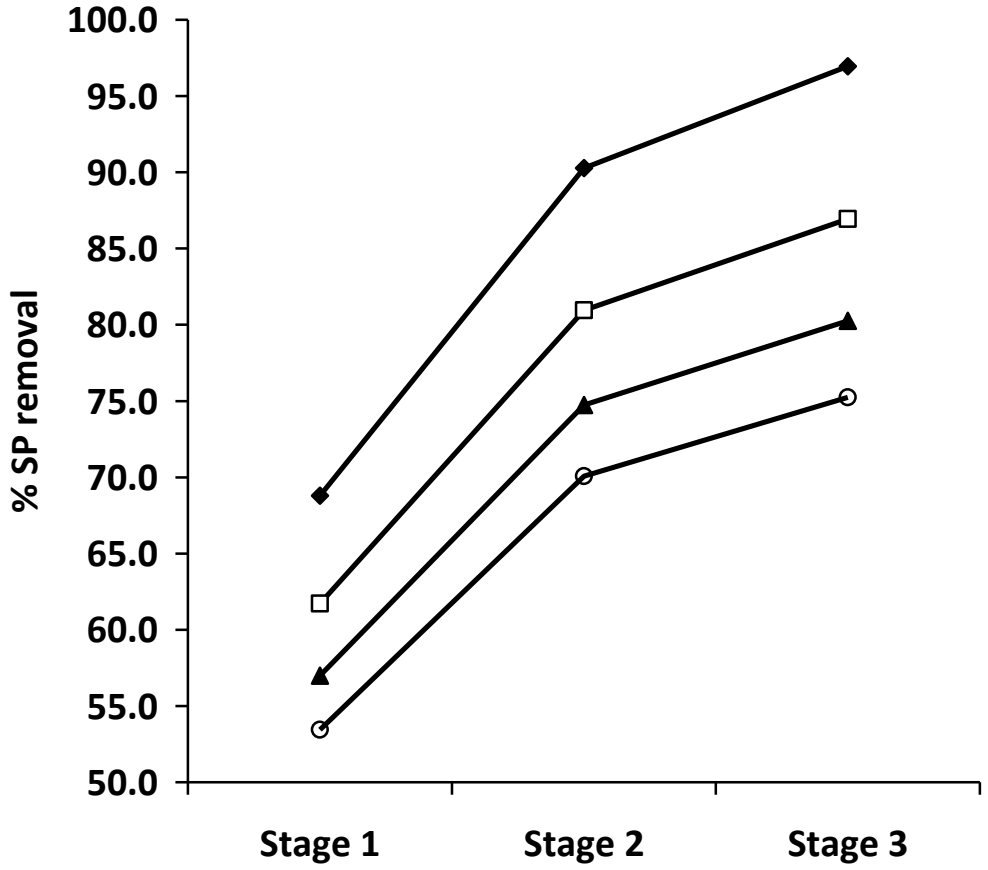

Figure 2.6. Cumulative serum protein (SP) removal (%) for each stage of a 3-stage 3X microfiltration process with water diafiltration (stages 2 and 3), where casein as a percentage of true protein (CN%TP) in the skim milk varied, (♦) 81.97 CN%TP, (□) 83.75 CN%TP, (▲) 85.00 CN%TP and (○) 85.94 CN%TP. Model factors from Table 2.1, skim milk composition from Table 2.3.

Table 2.9. Protein content of the 3rd stage retentate, yields of liquid MCC[1] standardized to 9% true protein (TP) with a 4th stage finishing step and dry solids yield of MSPI[2], and total percentage serum protein (SP) removal for a 3-stage microfiltration process with water diafiltration (stages 2 and 3), model factors from Table 2.1, with different casein as a percentage of true protein starting with 1000 kg of the skim milks with the compositions from Table 2.3.

	Casein as a percentage of true protein in skim milk (Kjeldahl)			
	81.97	83.75	85.00	85.94
	Yield			
Skim milk (kg)	1000	1000	1000	1000
3rd stage TP (% by weight)	7.92	8.09	8.20	8.29
4th stage permeate to remove (kg)	40.22	34.00	29.67	26.40
Yield liquid MCC[1] (9%TP) (kg)	293	299	304	307
Yield dry MSPI[2] (90%) (kg)	6.24	5.62	5.19	4.86
Total SP removal (%)	97.36	87.70	80.92	75.85

[1]MCC = micellar casein concentrate
[2]MSPI = milk serum protein isolate

Influence of Target Concentration Factor

Retentate. When MF milk fractionation process is being designed for a factory, the CF and DF factors under which the process will be operated may influence the efficiency of the process and the amount of membrane area required to process a particular volume of milk and the composition of the products produced. As expected, the TP concentration in the MF retentates from each stage increased with increasing target CF (Figure 2.7). The large increase in protein and CN concentration in both the final retentate (Table 2.10) and within each stage (Table 2.7) as CF and DF factor increased would probably require a different design of the membrane modules (i.e., more open retentate flow channels and more energy for pumping) to cope with the

higher protein concentrations in the retentates at CF of 4X and 5X. As CF and DF increased, the 3rd stage retentate had lower concentrations of SP, lactose and NPN. Ash content of the 3rd stage retentate increased when CF and DF were increased, because a majority of ash is bound to CN and CN concentration increased.

Permeate. The SP concentration in the MF permeate for the 1st stage was independent of CF (Figure 2.8) because the SP concentration in the permeate portion of the skim milk feed was the same for all CF. However, the volume of permeate portion remaining in the retentate at the end of the first stage was lower with increasing CF. Therefore when the retentate from the first stage was diluted back to the original volume of skim with water, there was a much lower SP concentration in the permeate from the 2nd stage and the SP concentration in the permeate decreased with increasing CF. After the 2nd stage, the difference in SP concentration in the permeate among the different concentration factors became smaller (Figure 2.8).

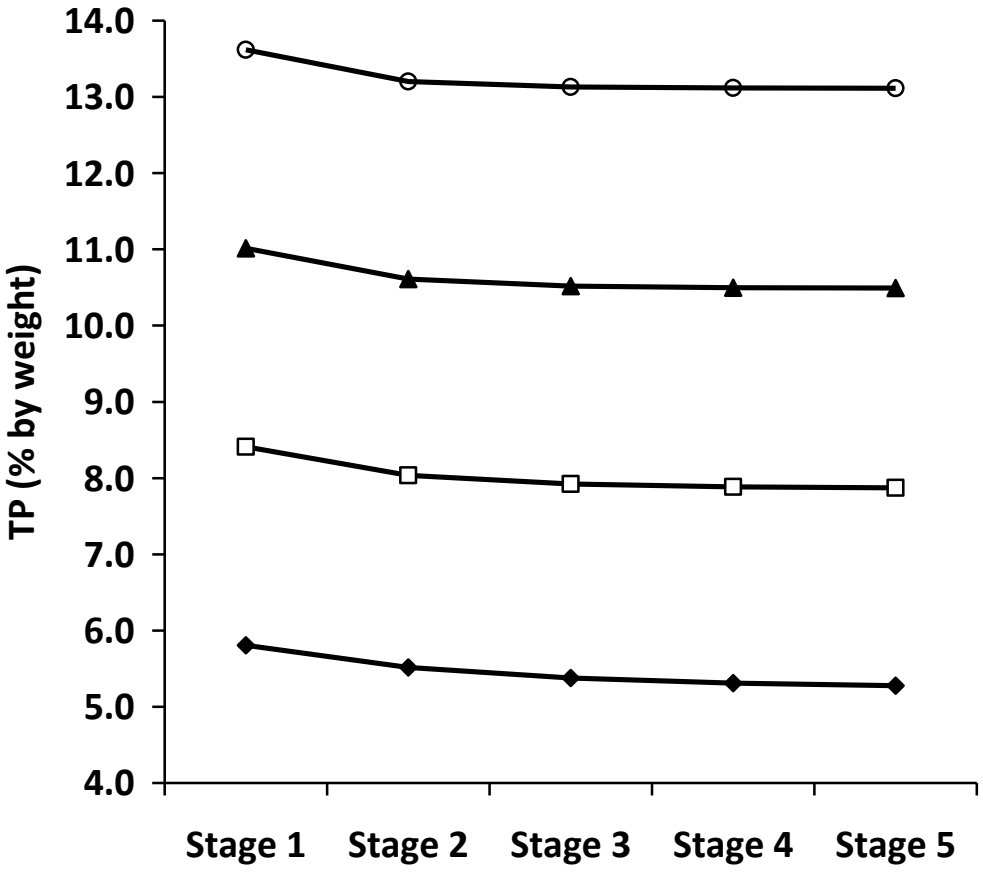

Figure 2.7. Effect of target microfiltration concentration factors (CF) and diafiltration factors (DF) (♦) CF2&DF2, (□) CF3&DF3, (▲) CF4&DF4, and (○) CF5&DF5, on true protein (TP) concentration of retentate in each stage of a 5-stage microfiltration process with water diafiltration at stage 2 and higher with skim milk composition and component removal factors from Table 2.1.

Table 2.10. Composition of retentate (% by weight) produced in 3rd stage of a microfiltration process (with water diafiltration after stage 1) with skim milk composition and component removal factors from Table 2.1 and different target concentration factors (CF) and diafiltration factors (DF).

	CF and DF			
Milk	CF2&DF2	CF3&DF3	CF4&DF4	CF5&DF5
component	Retentate composition (% by weight)			
True protein	5.3768	7.9215	10.5186	13.1303
Casein	5.2460	7.8690	10.4920	13.1150
Serum protein	0.1308	0.0525	0.0266	0.0153
NPN	0.0431	0.0173	0.0088	0.0050
CN % TP[1]	97.57	99.34	99.75	99.88
Lactose	1.0995	0.4417	0.2237	0.1285
Ash	1.0146	1.4583	1.9246	2.3974

[1]CN%TP = Casein as a percentage of true protein

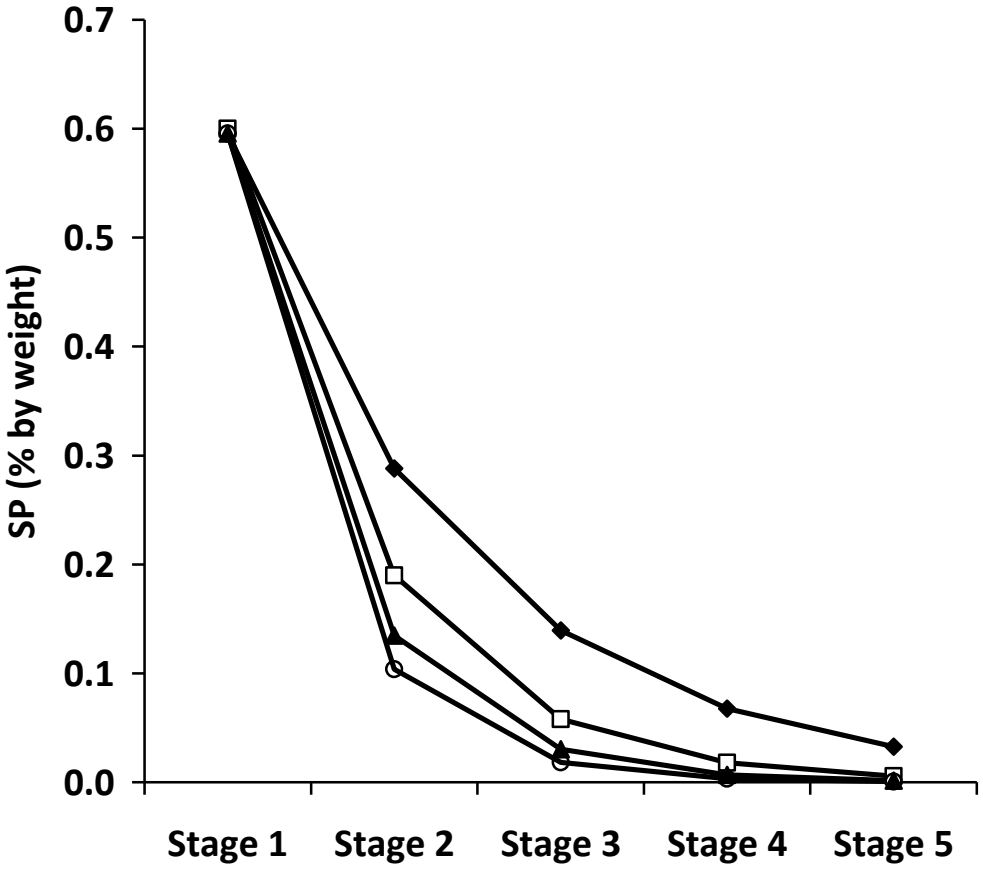

Figure 2.8. Effect of target concentration factors (CF) and diafiltration factors (DF) (♦) CF2&DF2, (□) CF3&DF3, (▲) CF4&DF4, and (○) CF5&DF5, on serum protein (SP) concentration in the permeate for each stage of a 5-stage microfiltration process with water diafiltration at stage 2 and higher with skim milk composition and component removal factors from Table 2.1.

Serum protein removal. Cumulative SP removal for each stage of the MF process increased as target CF and DF increased, with the greatest increase in removal occurring when CF and DF went from 2 to 3X (Figure 2.9). With a CF and DF of 3 there was more SP removed in 3 stages than in 5 stages with a CF and DF of 2X (Figure 2.9). The largest difference in percent SP removal due to difference in CF was in the first stage, with about 82% SP removal in the first stage and greater than 95% SP removal in 2 stages at a CF and DF of 5X. This would be one less stage than running with a CF and DF of 3X to achieve at least a 95% SP removal from skim milk. However, for each type of MF membrane there will likely be a maximum concentration of CN concentration in the retentate and exceeding this concentration would lead to rapid fouling of the membranes. Extensive membrane fouling that might occur at higher CF might also increase the rejection of SP by the membrane and reduce SP removal. This possible effect was not included in our theoretical calculations or in Figure 2.9.

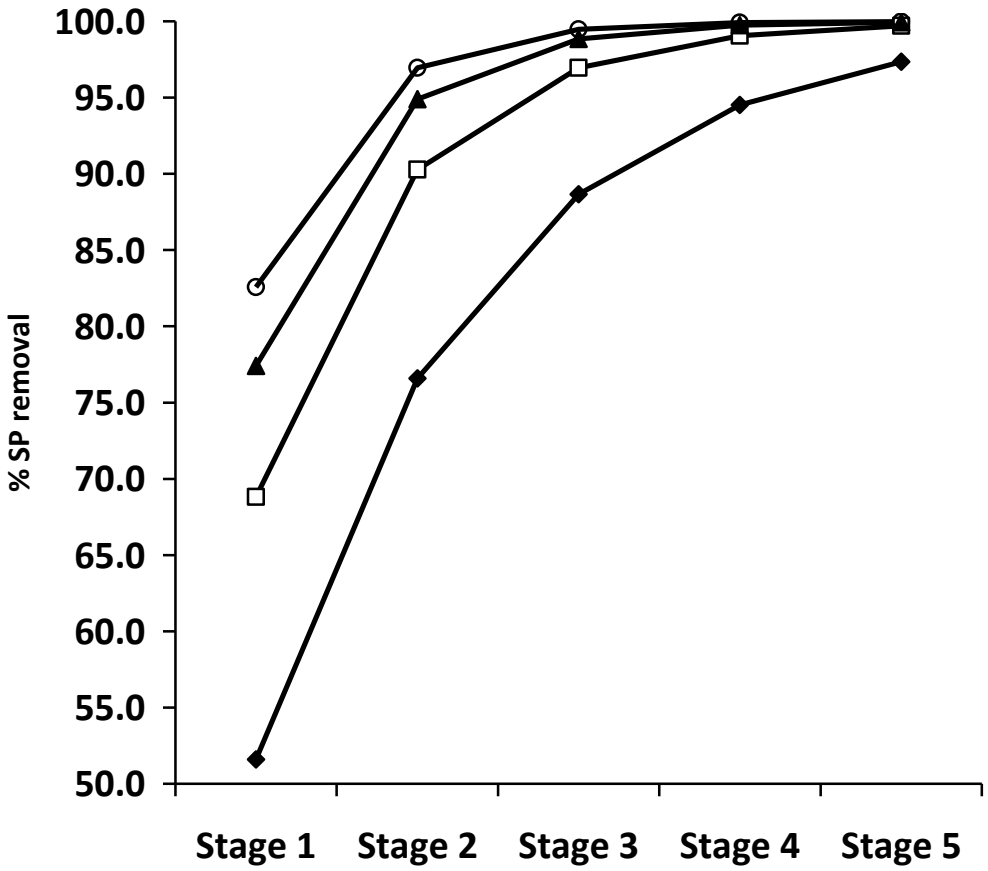

Figure 2.9. Effect of target concentration factors (CF) and diafiltration factors (DF) (♦) CF2&DF2, (□) CF3&DF3, (▲) CF4&DF4, and (○) CF5&DF5, on cumulative serum protein (SP) removal for a 5-stage microfiltration system with water diafiltration at stage 2 and higher with skim milk composition and component removal factors from Table 2.1.

Influence of Control of Concentration and Diafiltration Factors

Retentate. During normal operation of a multistage MF and diafiltration system, it is very difficult to control the CF and DF exactly in real time throughout the day. When the CF equaled the DF (i.e., a balanced scenario) the TP concentration of the MF retentates decreased gradually from stage to stage, because SP was removed in the permeate (Figure 2.10). The balanced scenario is the same as the values for the 3.2% TP in skim milk in Tables 2, 5 and Figure 2.1. When the DF was less than the

CF, then the TP concentration in the MF retentates increased compared to the balanced scenario (Figure 2.10) and the opposite happened when DF was greater than CF (TP concentration decreased) (Figure 2.10). There was a slightly larger effect of changing the CF than the DF because there was 1 more concentration than diafiltration step in the total 3-stage process. If it was necessary to produce a MCC (i.e., final retentate) to a specific target TP or CN concentration, then accurate control of CF and DF would be necessary or a final finishing process step might be necessary to standardize MCC composition. The variation in components other than TP and CN in the final retentate was expected to be small (Table 11).

Permeate. The CF and DF in this range had a very small effect on the permeate composition from each stage (Table 12), while these same variations had a large impact on retentate composition (Table 11).

Serum protein removal. When the CF was greater than the DF, percentage SP removal increased compared to the balanced scenario, while SP removal decreased when CF was less than DF in comparison with the balanced scenario (Table 13), however the impact of the differences in the balance in CF and DF on SP removal were very small compared to other factors.

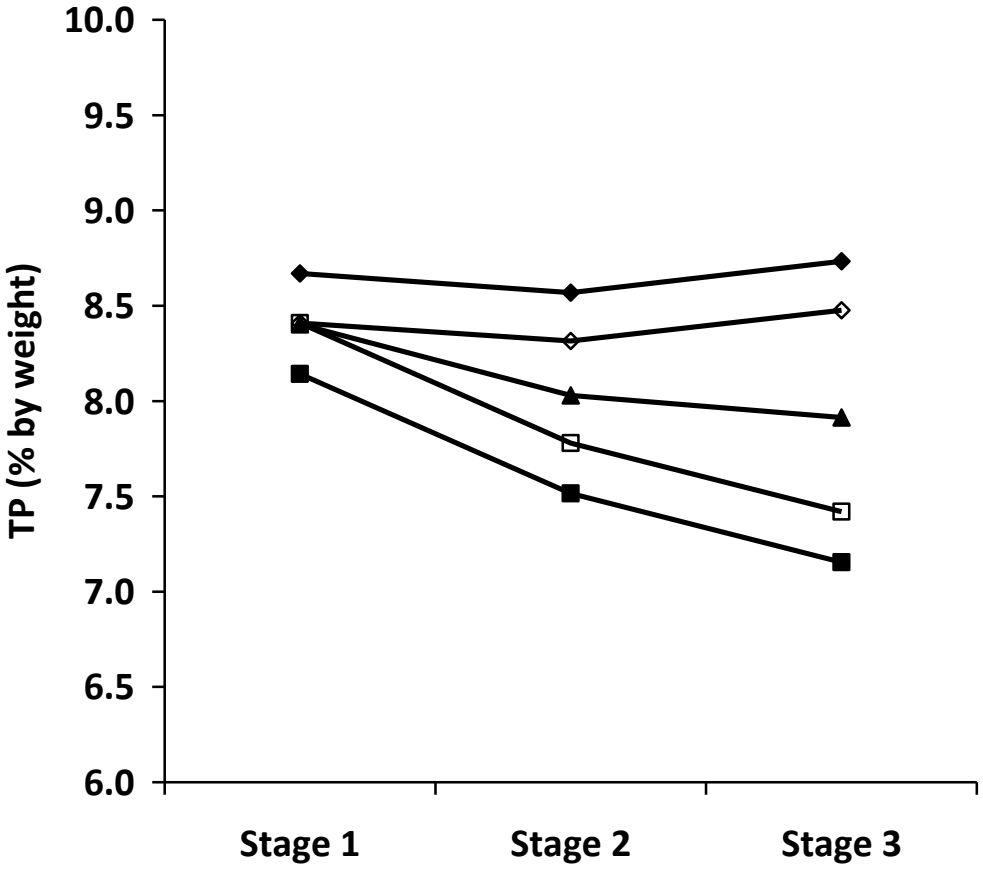

Figure 2.10. Effect of concentration factors (CF) and diafiltration factors (DF) (♦) CF3.1&DF3.0, (■) CF2.9&DF3.0, (▲) CF3.0&DF3.0, (◊) CF3.0&DF2.9, (□) CF3.0&DF3.1, on true protein (TP) concentration of retentate in each stage of a 3-stage microfiltration process with water diafiltration (stages 2 and 3) with skim milk composition and component removal factors from Table 2.1.

Table 2.11. Composition of retentate (% by weight) produced in 3rd stage of a microfiltration process with skim milk composition and component removal factors from Table 2.1 and variable concentration factors (CF) and diafiltration factors (DF) from Table 2.4.

	CF and DF				
	CF3.0& DF3.0	CF3.1& DF3.0	CF2.9& DF3.0	CF3.0& DF3.1	CF3.0& DF2.9
Milk component	Retentate composition (% by weight)				
True protein	7.9215	8.7340	7.1615	7.4190	8.4769
Casein	7.8690	8.6824	7.1080	7.3695	8.4210
Serum protein	0.0525	0.0516	0.0534	0.0495	0.0558
NPN	0.0173	0.0170	0.0176	0.0163	0.0184
CN % TP[1]	99.34	99.41	99.25	99.33	99.34
Lactose	0.4417	0.4338	0.4492	0.4164	0.4691
Ash	1.4583	1.6062	1.3199	1.3658	1.5604

[1]CN%TP = casein as a percentage of true protein.

Table 2.12. Effect of concentration factors (CF) and diafiltration factors (DF) on serum protein (SP) concentration in the permeate for each stage of a 3-stage microfiltration (MF) process with skim milk composition and component removal factors from Table 2.1 and CF and DF from Table 2.4.

	CF and DF		
	CF3.0&DF3.0	CF3.1&DF3.0	CF2.9&DF3.0
MF stage	SP (% by weight)		
1	0.599	0.599	0.599
2	0.187	0.186	0.187
3	0.058	0.058	0.059

Table 2.13. Effect of concentration factors (CF) and diafiltration factors (DF) on cumulative serum protein removal for a 3-stage microfiltration (MF) with skim milk composition and component removal factors from Table 2.1 and CF and DF from Table 2.4.

	CF and DF				
	CF3.0& DF3.0	CF3.1& DF3.0	CF2.9& DF3.0	CF3.0& DF3.1	CF3.0& DF2.9
MF stage	Cumulative SP removal (%)				
1	68.80	69.91	67.62	68.80	68.80
2	90.27	90.97	89.49	90.25	90.29
3	96.96	97.30	96.58	96.94	96.99

MCC and MSPI yield. The main affect of CF and DF on yield was on permeate removal required in the finishing stage (i.e., 4th stage) to produce an MCC with a 9% TP (Table 14). There is only a small effect on the yield of MSPI (Table 14).

Influence of Serum Protein Removal Factors

Differences in SP removal factors reflect a difference in resistance of the MF membrane to passage of SP through the membrane. In some cases this resistance may be due to inherent characteristics of the membrane, resistance caused by foulant on the membrane, or a combination of both. In the example provided below, 2 SP removal factors were used, 1 and 0.6. A factor < 1 indicates resistance to passage of SP. The removal factor of 1 is similar to reported performance (Zulewska, et. al., 2009) of a pilot-scale ceramic MF membranes when processing skim milk in a 3-stage, 3X MF system at 50^{o}C and the 0.6 is similar to reported performance for a pilot-scale polymeric SW MF system (Zulewska et al., 2009).

Retentate. As the membrane rejected more SP, the TP concentration in the MF retentate for each stage increased (Figure 2.11). The lactose, NPN and ash content of the 3rd stage MF retentate was unchanged in lactose, NPN and ash content when SP removal factors changed (Table 15) because the removal factors for these components were assumed to be 1. There is no information in the literature to indicate if the removal factors for these components change when SP removal factor decreases. The CN%TP in the 3rd stage retentate decreased as the removal factor decreased, because SP concentration in the 3rd stage MF retentate increased (Table 15).

Permeate. The concentration of SP in the permeate was higher in stages 2 and 3 of the MF process for the 0.6 removal factor than for a removal factor of 1 (Figure 2.12), because with a removal factor of 1 a larger amount of SP was removed in the 1st stage so the concentration was lower in the MF permeate of the 2nd and 3rd stages.

Table 2.14. Protein content of the 3rd stage retentate, yields of liquid MCC[1] standardized to 9% true protein (TP) with a 4th stage finishing step and dry solids yield of MSPI[2], and total percentage serum protein (SP) removal for a 3-stage microfiltration process with water diafiltration (stages 2 and 3) and concentration factors (CF) and diafiltration factors (DF) from Table 2.4, starting with 1000 kg of 3.2% TP skim milk from Table 2.1.

	CF and DF				
	CF3.0& DF3.0	CF3.1& DF3.0	CF2.9& DF3.0	CF3.0& DF3.1	CF3.0& DF2.9
	Yield				
Skim milk (kg)	1000	1000	1000	1000	1000
3rd stage TP (% by weight)	7.92	8.73	7.16	7.42	8.48
4th stage permeate to remove (kg)	40.22	9.00	75.87	62.91	18.23
Yield liquid MCC[1] (9%TP) (kg)	293	293	293	293	293
Yield dry MSPI[2] (90%) (kg)	6.24	6.24	6.24	6.25	6.23
Total SP removal (%)	97.36	97.39	97.34	97.53	97.18

[1]MCC = micellar casein concentrate
[2]MSPI = milk serum protein isolate

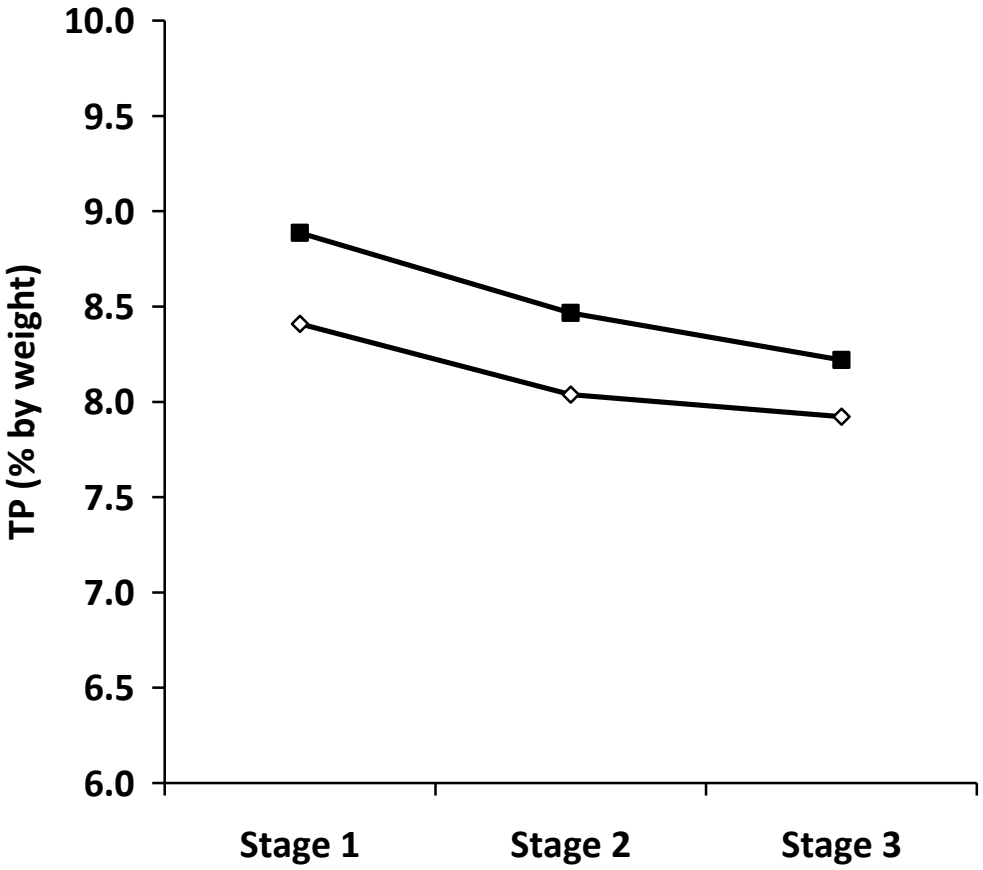

Figure 2.11. Effect of serum protein removal factor (R), (◊) R1, (■) R0.6, on true protein (TP) concentration in retentate of each stage of a 3-stage microfiltration process with water diafiltration (stages 2 and 3) with skim milk and concentration and diafiltration factors from Table 2.2

Table 2.15. Composition of retentate (% by weight) produced in 3rd stage of a 3X microfiltration process with water diafiltration (stages 2 and 3), with different serum protein (SP) removal factors with skim milk composition from Table 2.1.

	SP removal factor	
	1	0.6
Milk component	Retentate composition	
True protein	7.9215	8.2194
Casein	7.8690	7.8690
Serum protein	0.0525	0.3504
NPN	0.0173	0.0173
CN%TP[1]	99.34	95.74
Lactose	0.4417	0.4417
Ash	1.4583	1.4583

[1]CN%TP = casein as a percentage of true protein

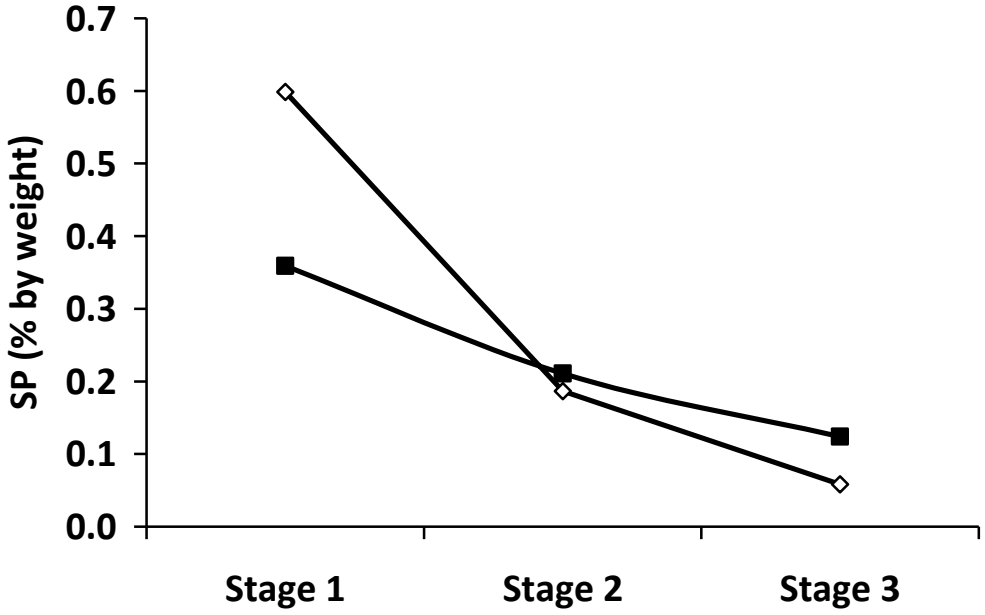

Figure 2.12. Effect of serum protein (SP) removal factors (R), (◊) R1, (■) R0.6, on SP composition (% by weight) of permeate in each stage of a 3-stage microfiltration with water diafiltration (stages 2 and 3) process with skim milk composition and concentration and diafiltration factors from Table 2.1.

Serum protein removal. Cumulative percentage of SP removal increased as the SP removal factor increased (Figure 2.13). A membrane with a rejection factor of 0.8 would achieve approximately the same SP removal in 2 stages, as would be achieved in 3 stages by a membrane with a SP removal factor of 0.6 (Figure 2.13). Heat denaturation of milk serum proteins by pasteurization prior to MF had a similar impact on SP removal as operating an MF system with a lower SP removal factor. When apparent CN%TP was 83.75% (Table 2.3) cumulative percentage SP removal after 3 stages was 86.94% and this SP removal corresponds to a SP removal factor (caused by rejection of SP by the membrane) of between 0.7 and 0.8 for skim milk with a CN% TP of 81.97%.

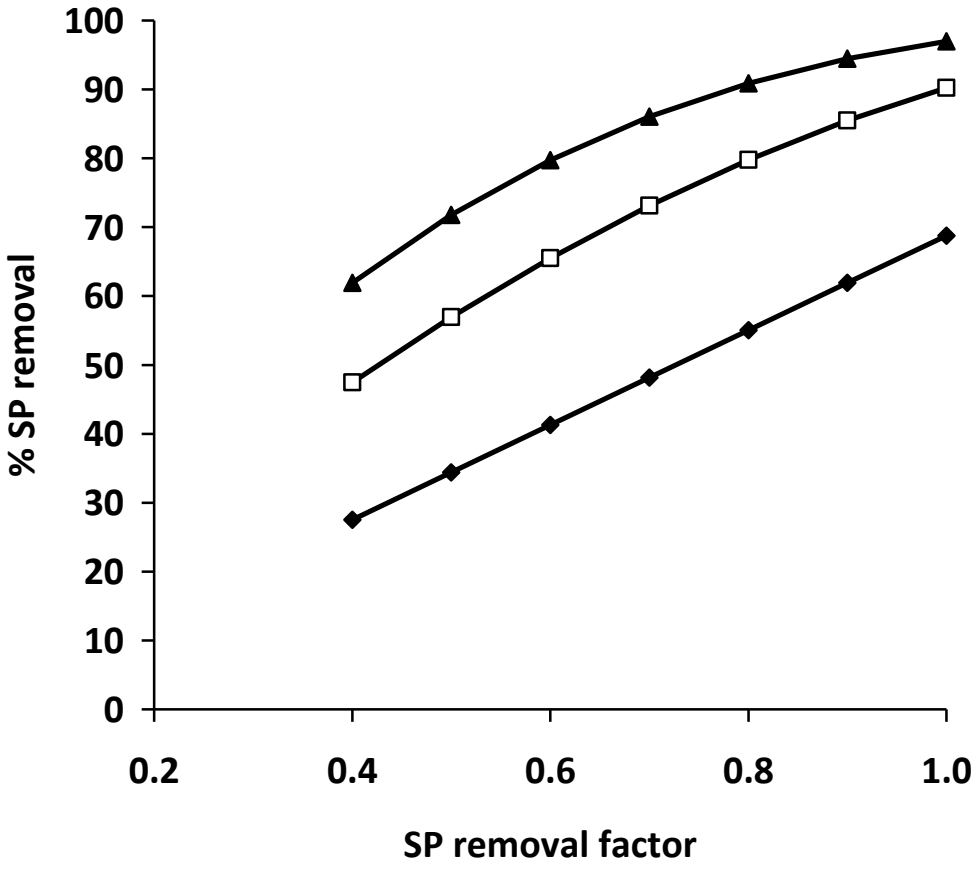

Figure 2.13. Effect of serum protein (SP) removal factor on cumulative SP removal for each stage, (♦) stage 1, (□) stage 2, (▲) stage 3, of a 3-stage microfiltraion process with water diafiltration (stages 2 and 3) with skim milk composition and concentration and diafiltration factors from Table 2.1.

MCC and MSPI yield. The dry yield of MSPI decreased and the liquid MCC yield standardized to 9% TP increased as SP removal factors decreased, because more of the SP was retained in the retentate (Table 16). This resulted in a decreasing CN%TP and increasing SP content of the final retentate (i.e., MCC) and may produce an MCC with different functionality than one with lower SP content (Table 15). Two MCC with the same CN%TP, but one caused by heat denaturation induced binding of SP to CN micelles during pasteurization (Table 8) versus one of the same CN%TP due

to rejection of undenatured SP by the MF membrane (Table 15) may have different functionality even though their yields and compositions are the same.

Table 2.16. Protein content of the 3rd stage retentate, yields of liquid MCC[1] standardized to 9% true protein (TP) with a 4th stage finishing step and dry solids yield of MSPI[2], and total percentage serum protein (SP) removal for a 3-stage 3X microfiltration process with water diafiltration (stages 2 and 3) with different SP removal factors starting with 1000 kg of the skim milk with the composition from Table 2.1.

	SP removal factor	
	1	0.6
	Yield	
Skim milk (kg)	1000	1000
3rd stage TP (% by weight)	7.92	8.22
4th stage permeate to remove (kg)	40.22	29.67
Yield liquid MCC[1] (9%TP) (kg)	293	304
Yield dry MSPI[2] (90%) (kg)	6.24	5.19
Total SP removal (%)	97.36	80.93

[1]MCC = micellar casein concentrate
[2]MSPI = milk serum protein isolate

CONCLUSIONS

When skim milk TP concentration increased from 3.2 to 3.8%, the TP concentration in the 3rd stage retentate increased from 7.92 to 9.40%, with the yield of liquid MCC (9%TP) from 1000 kg of skim milk increased from 293 to 348 kg and yield of dried MSPI (90% SP) increasing from 6.24 to 7.38 kg. Increased heat treatment of skim milk (72.9 to 85.2°C) caused skim milk CN%TP as measured by Kjeldahl analysis to increase from 81.97 to 85.94% and the yield of MSPI decreased from 6.24 to 4.86 kg, while the 3rd stage cumulative SP removal decreased from 96.96

to 70.08%. A CF and DF of 2X gave a 3rd stage retentate TP concentration of 5.38% compared to 13.13% for a CF and DF of 5X with the 3rd stage cumulative SP removal increasing from 88.66 to 99.47%, respectively. Variation in control of the balance between CF and DF (instead of an equal CF and DF) caused either a progressive increase or decrease in TP concentration in the retentate across stages depending on whether CF was greater than DF (caused increasing TP in retentate) or CF was less than DF (caused decreasing TP in retentate). An increase in rejection of SP by the membrane from a SP removal factor of 1 to 0.6 caused a reduction in MSPI yield from 6.24 to 5.19 kg per 1000 kg of skim milk, 3rd stage cumulative SP removal decreased from 96.96 to 79.74%.

Within the ranges of the 5 factors studied, the TP content of the 3rd stage retentate was most strongly impacted by the target CF and DF and variation in composition of skim milk. Cumulative SP removal was most strongly impacted by the heat treatment of skim milk, the SP removal factor, and the target CF and DF. MCC yield was most strongly impacted by initial skim milk composition. MSPI yield was also most strongly impacted by composition of skim milk, whereas the heat treatment of milk and SP removal factor also had large impacts.

REFERENCES

AOAC. 2000. Official Methods of Analysis. 17th ed. Association of Official Analytical Chemists, Gaithersburg, MD.

Barbano, D.M. 1990. Seasonal and Regional Variation in Milk Composition in the U.S. Pages 96 to 105 in Proceedings of the 1990 Cornell Nutrition Conference. Cornell University, Ithaca, NY.

Britten, M. and Y. Pouliot. 1996. Characterization of whey protein isolate obtained from milk microfiltration permeate. Lait 76:255-265.

Kaylegian, K. E, J. M. Lynch, G. E., Houghton, J. R., Fleming, and D. M. Barbano. 2006. Modified Versus Producer Milk Calibration: Mid-Infrared Analyzer Performance Validation J. Dairy Sci. 89:2833–2845.

Jenness, R. 1959. Milk Salts. Page 170 in Principles of Dairy Chemistry. S. Patton, ed. John Wiley and Sons Inc. New York, NY.

Laben, R.C. 1963. Factors responsible for variation in milk composition. J. Dairy Sci. 46:1293-1301.

Lynch, J.M., D.M. Barbano, and J.R. Fleming. 1998. Indirect and direct determination of the casein content of milk by Kjeldahl nitrogen analysis: collaborative study. JAOACI 81:763-774.

Ma Y. and D.M. Barbano. 2003. Milk pH as a function of CO_2 concentration, temperature and pressure in a heat exchanger. J. Dairy Sci. 86:3822-3820.

Ma, Y., C. Ryan, D.M. Barbano, D.M. Galton, M.A. Rudan and K.J. Boor. 2000. Effect of somatic cell count on quality and shelf-life of pasteurized fluid milk. J. Dairy Sci. 83:264-274.

Nelson, B.K. and D.M. Barbano. 2005. A microfiltration process to maximize removal of serum proteins from skim milk before cheese making. J. Dairy Sci. 88:1891-1900.

Papadatos, A., M. Neocleous, A.M. Berger, and D.M. Barbano. 2003. Economic feasibility evaluation of microfiltration of milk prior to cheesemaking. J. Dairy Sci. 86:1564-1577.

Quinones, H.J., D.M. Barbano, and L.G. Phillips. 1997. Influence of protein standardization by ultrafiltration on the viscosity, color and sensory properties of skim and 1% milk. J. Dairy Sci. 80:3142-3151.

Quinones, H.J., D.M. Barbano, and L.G. Phillips. 1998. Influence of protein standardization on the viscosity, color, and sensory properties of 2% and 3.3% fat milks. J. Dairy Sci. 81:884-894.

Rowland, S.J. 1938. The determination of the nitrogen distribution in milk.. J. Dairy Res. 9:42-46.

Sawyer, W.H. 1969. Complex between β-lactoglobulin and κ-casein. A review. J. Dairy Sci. 52:1347-1355.

Vadi, P.K. and S.S.H. Rizvi. 2001. Experimental evaluation of a uniform transmembrane pressure crossflow microfiltration unit for the concentration of micellar casein from skim milk. J. Membrane Sci. 189:69-82.

Zulewska, J., M. Newbold, and D.M. Barbano. 2009. Efficiency of serum protein removal from skim milk with ceramic and polymeric membranes at 50^{o}C. J. Dairy Sci. 92:1361-1377.

Chapter 3

Micellar Casein Concentrate Production with a 3X, 3-stage Uniform Transmembrane Pressure Process at 50°C*

ABSTRACT

The production of serum protein **(SP)** and micellar casein from skim milk can be accomplished using microfiltration **(MF)**. There are potential commercial applications for both the SP and micellar casein. Our research objective was to determine the total SP removal and the SP removal for each stage and the composition of retentates and permeates, for a 3X continuous bleed and feed 3-stage uniform transmembrane pressure **(UTP)** system with 0.1 µm ceramic membranes, when processing pasteurized skim milk at 50°C with two stages of water diafiltration. For each of 4 replicates about 1100kg of skim milk was pasteurized (72°C, 16s) and processed at 3X through the UTP MF system. Retentate from stage 1 was cooled to <4°C and stored until the next processing day, when it was diluted with reverse osmosis water back to a 1X concentration and again processed through the MF system (stage 2) to a 3X concentration. The retentate from stage 2 was stored at <4°C, on the next processing day the retentate was diluted with reverse osmosis water back to a 1X concentration, before running through the MF system at 3X for a total of 3-stages. The retentate and permeate from each stage was analyzed for total nitrogen, non-casein nitrogen and non-protein nitrogen using Kjeldahl methods, SDS-PAGE analysis was also performed on the retentates from each stage. Theoretically, a 3-stage 3X MF process could remove 97% of the SP from skim milk, with a cumulative SP removal

* Hurt, E.E., J. Zulewska, M. Newbold and D.M. Barbano. Micellar Casein Concentrate Production with a 3X, 3-stage UTP Ceramic Membrane Process at 50°C. Submitted for publication

of 68 and 90% after the 1st and 2nd stages respectively. The cumulative of SP removal using a 3-stage 3X MF process with a UTP system with 0.01µm ceramic membranes in this experiment was 64.83 ± 0.76, 87.75 ± 1.56 and 98.27 ± 2.25% for the 1st, 2nd and 3rd stages respectively, when SP removal was calculated using the mass of SP removed in the permeate of each stage. Various methods of calculation of SP removal were evaluated. Given the analytical limitation in the various methods for measuring SP removal, calculation of SP removal based on the mass of SP in the skim milk (determined by Kjeldahl) and the mass SP present in all of the permeate produced by the process (determined by Kjeldahl) provided the best estimate of SP removal for a MF process.

Key words: microfiltration, flux, serum protein, protein fractionation.

INTRODUCTION

Casein **(CN)** micelles and serum proteins (**SP**) in skim milk can be separated by microfiltration (**MF**). This separation is possible because of the approximately 10 to 100 fold difference in diameter between CN micelles and SP (Walstra et al. 1999 p 9). A limitation of MF is membrane fouling, which reduces flux and can decrease transmission of SP (Sachdeva and Buchheim, 1997). One technique to minimize fouling is the use of cross-flow (the retentate is pumped tangentially across the surface of the membrane). Increased flux seen in cross-flow filtration can be explained by the decrease in concentration polarization layer and lifting of solute particles away from the membrane surface due to shear at the membrane surface (Belfort et al., 1994). Le Berre and Daufin (1996) characterized the relationship between flux and shear rate at the membrane surface during MF of skim milk to separate CN micelles from SP. They found that there was a critical ratio of flux to shear stress of 1.0 L/ hr per m^2 per Pa, where the pressure is the pressure decrease from the inlet to outlet of the membrane,

when operating a uniform transmembrane pressure (**UTP**) system at a concentration factor **(CF)** of 2X at 50^{o}C and that operating above this ratio led to decreased SP transmission and increased fouling.

In a standard cross-flow MF module there will be a pressure drop along the length of the membrane on the retentate side in the direction of fluid flow, in contrast pressure on the permeate side of the membrane is relatively constant along the length of the membrane. This means the transmembrane pressure (**TMP**) and thus flux at the membrane inlet is higher than the outlet and varies along the length of the membrane. No matter what operating conditions are chosen, parts of the membrane could be operating under non-ideal conditions, leading to excessive fouling congruent with the concept of a critical flux to shear ratio. A solution to this problem was developed by Sandblom (1978), where the permeate was recirculated on the permeate side of the membrane in the same direction as retentate flow. The recirculation of permeate creates a pressure drop on the permeate side of the membrane from inlet to outlet mirroring the pressure drop on the retentate side from inlet to outlet creating UTP along the membrane's length (and uniform flux).

A UTP system requires membranes that are rigid and self supporting, as they must be able to handle back pressure. This rules out the UTP approach for most polymeric membranes, including spiral-wound (Cheryan, 1998 p 274). Tubular ceramic membranes have been used successfully in UTP systems to separate CN micelles from SP (Nelson and Barbano, 2005; Zulewska et al. 2009). Some of the earliest published work was done by Pierre et al. (1992), using 0.2 µm ceramic membranes, and concentrating skim milk to 3X before diafiltering. They found near theoretical transmission of whey proteins. Le Berre and Daufin (1996) found that under optimal operating conditions transmission of SP was 70 to 80% and greater than 99% of the CN was retained using a 0.1µm ceramic UTP system where skim milk was

concentrated to 2X at 50^{o}C. Nelson and Barbano (2005) used a 3-stage 3X UTP MF process with 0.1 µm ceramic membranes, with dilution using UF permeate between stages. They found an overall SP removal after 3-stages of 95%.

Both the micellar CN concentrate and SP separated by MF have the potential to be valuable products. The SP has been further purified by ultrafiltration to produce SP concentrates. SP concentrates lack the glycomacropeptides present in whey protein concentrates and have lower concentration of lipids (Evans et al., 2009). SP isolates exhibit better foaming a gelling properties when compared to whey protein concentrates (Britten and Pouliot, 1996). In addition whey protein concentrates have been found to have diactyl flavors that SP concentrates lacked (Evans et al., 2009).

The micellar CN concentrate could be used to increase cheese yields and revenue (Papadatos et al., 2003) or potentially in food ingredient applications where caseinates are currently used. A single stage UTP MF process with a CF of 3 using 0.1µm ceramic membranes can remove greater than 60% of the SP from the micellar CN (Nelson and Barbano, 2005; Zulewska et al., 2009), however there could be advantages to using multiple stages to remove a greater percentage of SP, soluble minerals and lactose from the micellar CN concentrate. Casein micelles are very heat stable (Holt, 1992 p.133), while whey proteins are not as heat stable and begin denaturing at 70^{o}C (de Wit and Klarenbeck, 1984). Lactose also undergoes thermal degradation including maillard reactions with proteins that can lead to off flavors and browning (Walstra et al., 1999 p 28-29).

Theoretically 97% of the SP should be removed from skim milk in a 3-stage 3X MF process, but the actual removal and yield of micellar CN concentrate can be influenced by a number of operational parameters (Hurt and Barbano, submitted). There has been no published research to determine the actual amount of SP that can be removed in a 3-stage UTP MF process with water diafiltration between stages relative

to theoretical values. Our objective was to determine the total SP removal and the SP removal for each stage for a 3X continuous bleed- and-feed 3-stage UTP system with 0.1 µm ceramic membranes, when processing pasteurized skim milk at 50°C with two stages of water diafiltration.

MATERIALS AND METHODS

Experimental Design and Statistical Analysis

One lot of bovine milk (ca 1099 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 with 3 sections: regeneration, heating, and cooling (Model 080-S, AGC Engineering, Manassas, VA) at 72°C with a holding time of 16 s. Temperature was kept at minimum for pasteurization to minimize denaturation of SP. The milk was cooled to 4°C and stored at ≤4°C until processing. On day 1, pasteurized skim milk was heated to 50°C with a plate heat exchanger (Model A3, DeLaval, Inc., Kansas, MO) and microfiltered using a pilotscale ceramic UTP system in a bleed-and-feed mode to continuously produce a 3X MF retentate and MF permeate at 50°C. The MF retentate was cooled to ≤4°C as it was collected and stored until the next processing day. On the second day, MF retentate from the first day was diluted back to a 1X concentration (2 kg of water for every 1 kg of retentate) with pasteurized RO, heated to 50°C and diafiltered with the ceramic UTP MF system to produce a 3X retentate. On the third day, this diafiltration procedure was repeated to complete a 3-stage process. This process was replicated 4 times starting with different batches of raw milk.

Microfiltration Operation

A pilotscale UTP MF system (Tetra Alcross M7, TetraPak Filtration Systems, Aarhus, Denmark) equipped with a ceramic Membralox (EP1940GL0.1µA, alumina, Pall Corp, Cortland, NY) membranes (pore diameter: 0.1 µm; surface area: 1.7 m^2) and variable area flow meters were used. The membranes in a tubular stainless module consisted of 7 ceramic tubes, 19 channels each with 4 mm channel diameter. The permeate section of the stainless steel module was filled with polymeric beads (3.72 to 3.78 mm diameter) to reduce dead volume, act as buffer for pressure changes and produce a larger pressure decrease from inlet to out on the permeate side of the membrane. The UTP MF system consisted of a feed pump (type LKH 10/110 SSS 1.75 kW), a retentate recirculation pump (type LKH 20/125 SSS 6.3 kW) and a permeate recirculation pump (type LKH 10/130 SSS 2.5 kW) all from Alfa Laval, (Kansas City, MO). The membranes were 1.02 m in length and were mounted vertically in the MF system with permeate and retentate flow co-current from the top to the bottom of the module. Because the membrane was mounted vertically the inlet and outlet gauge pressures had to be corrected for the difference due to the weight of the vertical column of liquid. The correction was measured as follows: with 50^{o}C RO water in the system and only the feed pump turned on, the retentate and permeate outlet valves were closed. Retentate inlet pressure (Rp_i), permeate inlet pressure (Pp_i), retentate outlet pressure (Rp_o), and permeate outlet pressure (Pp_o) were measured under these conditions. A correction factor for calculating transmembrane pressure was calculated for each gauge pressure as follows: the Rp_i gauge pressure correction was Pp_o minus Rp_i, the Rp_o gauge pressure correction was Pp_o minus Rp_o, the Pp_i gauge pressure correction was Pp_o minus Pp_i, and the Pp_o gauge pressure correction was zero. This correction factor was determined at the beginning of each run of each stage. Next retentate and permeate recirculation pumps were turned on and the

retentate bleed flow was set to 45 L/h and the permeate bleed flow was set to 90 L/h. The elevation corrected inlet and outlet pressures were measured and the transmembrane pressure from the retentate to the permeate side of the membrane at retentate inlet (TMP_i) and outlet (TMP_o) ends of the membrane were calculated. The goal was to have a ΔP ($\Delta P = TMP_i - TMP_o$) of 25 ± 3 kPa for a membrane length of 1.02m. A diaphragm valve in the permeate recirculation loop was used to adjust the recirculation flow rate on the permeate side of the membrane. The permeate recirculation flow rate was adjusted with the diaphragm valve until the ΔP was 25 ± 3 kPa.

Cleaning prior to processing. Immediately prior to processing on each day, the MF system was cleaned. Storage solution (0.55% vol/vol solution nitric acid) was flushed out of the system with room temperature RO water until the pH was neutral. The MF flow system was heated with RO water to 80°C and then Ultrasil 25 (Ecolab Inc., Food and Beverage Division, St Paul, MN) liquid alkaline membrane cleaner (1.95 % vol/vol) was added to the water to reach pH 11. The alkaline solution was recirculated for 25 min at a permeate removal rate of approximately 1000 L/h, the retentate removal rate of approximately 160 to 180 L/h, with all pumps running. After cleaning, the membrane system was slowly (< 10^oC per min) cooled to 50^oC with the tubular heat exchanger in the recirculation loop. The MF system was then flushed with RO water (about 300 kg at 30^oC) until neutral pH was reached. The membrane was flushed with 50℃ RO water until the system temperature was 50^oC (about 60 kg) and the initial clean water flux was determined. The following conditions were applied during the flux measurement: the retentate outlet valve was closed, and permeate outlet valve was fully open and only feed pump running.

First stage: day 1. Skim milk (about 1099 kg) was processed to approximately a 3X CF at 50°C using a pilot-scale UTP MF system described above. A 3X CF being

2 kg permeate removed for every 1 kg retentate. The system was started on 50°C RO water and there was a transition from water to milk with all the pumps running, the recirculation rate was approximately 644 L per min with a linear velocity of approximately 6.4 m per s. To flush the 50°C water out of the system with milk at the beginning of the process, about 14 kg of retentate and 31 kg of permeate were collected, the weights were recorded and both were discarded (mostly water). After this start up, retentate and permeate were collected continuously and cooled to 4°C as they were collected. Retentate and permeate removal rates were 45 and 90 L/h, respectively. If the ΔP was not 25 ± 3 kPa after switching from water to milk, then the permeate recirculation diaphragm valve was adjusted while processing skim milk to achieve and maintain this transmembrane pressure difference between the outlet and inlet end of the membrane. Typical retentate (Rp_i) and permeate (Pp_i) inlet pressures (without the correction factors) were 419.8 and 387.5 kPa, respectively, and typical retentate (Rp_o) and permeate (Pp_o) outlet pressures were typically 229.8 and 218.8 kPa, respectively. The flux (kg/m² per hour) was measured every 15 min and samples of permeate and the retentate were taken for analysis using an infrared spectrophotometer (Lactoscope FTIR, Delta Instruments, Drachten, The Netherlands) to monitor retentate and permeate composition for process control. At the end of the MF run, the collected retentate and permeate were mixed separately and sampled.

Second stage: day 2. The second stage feed of the 3-stage process was the retentate from the first stage diluted by weight 2 kg pasteurized RO water for every 1 kg retentate (about 320 kg retentate and 640 kg water). This is a diafiltration factor **(DF)** of 3. Retentate and water were mixed before heating to 50°C and processed with MF UTP system using the same operating conditions as described for the first stage. All retentate was collected, cooled, mixed and sampled. Permeate was weighed,

sampled (every 15 min), and discarded. A composite sample of permeate was used for analysis.

Third stage: day 3. The third stage of the 3-stage process was as described for the second stage, with the feed being the retentate from the second stage diluted with RO water (about 274 kg retentate and 549 kg water). The amount of retentate decreased from stage to stage because of the loss of retentate as the dead volume of the system when ending the previous stage. The average total time of processing was about 497 min for the 1st stage, 428 min for the 2nd stage and 343 min for the 3rd stage.

Cleaning after processing. Immediately after processing, 50℃ RO water (about 150 to 200 L) was flushed through the system with all pumps on. The retentate and permeate removal rates were set at approximately 160 L/h and 120 L/h, respectively. The MF system was flushed until no retentate was visible in the flush water on the retentate side. When the water flush was complete, then fouled membrane water flux was determined (retentate outlet valve closed, permeate outlet valve completely open, only the feed pump on with temperature maintained at 50℃). Typically, fouled membrane flux was about 46% of the clean membrane water flux (1065 vs 491 kg/m^2 per hour). Next, the MF flow system was heated with RO water to 80°C. Ultrasil 25, liquid alkaline membrane cleaner (Ecolab Inc.) was added (1.95% vol/vol) to the water to reach pH 11. This solution was recirculated for 25 min with the permeate and retentate exit flows at approximately 1000 L/h and 160 to 180 L/h, respectively, with all pumps on. After cleaning, the membrane system was slowly (< 10^oC per min) cooled to 50^oC with the heat exchanger on retentate recirculation loop. The membrane was then flushed with approximately 30^oC RO water until neutral pH was reached. The MF flow system was heated to 50℃ by flushing with 50℃ RO water and the post run clean water flux was determined. During the flux determination the retentate outlet valve was closed, and permeate outlet valve was fully open with

only the feed pump on and the temperature maintained at 50℃. The post-run clean water fluxes were also close to pre-run clean water flux (i.e., about 1050 to 1070 L/m^2 per hour). After determination of clean water flux a 0.55% vol/vol solution of 70% nitric acid and water was recirculated through the membrane at 50^{o}C for 10 min. Permeate and retentate outlet flows were approximately 1000 L/h and 160 to 180 L/h, respectively. After 10 min of the nitric acid solution recirculation, the permeate and retentate outlet valves were closed and the pumps turned off. The membrane was stored in 0.55% vol/vol nitric acid solution.

Chemical Analyses

Samples of skim milk, permeate, and retentate collected during processing were analyzed using an infrared spectrophotometer (IR) (Lactoscope FTIR, Delta Instruments) for fat, lactose and true protein content (Kaylegian et al., 2006). This was done to quickly monitor the composition of retentate and permeate during the run to detect if the system was running normally.

Skim milk, retentate and permeate for each stage were analyzed for TS, total N (TN), and NPN content using forced air oven drying (AOAC, 2000; method 990.20; 33.2.44), Kjeldahl (AOAC, 2000; method 991.20; 33.2.11), and Kjeldahl (AOAC, 2000; method 991.21; 33.2.12), respectively. Noncasein nitrogen (NCN) content of retentates was determined using Kjeldahl (AOAC, 2000; method 998.05; 33.2.64). True protein (TP) was calculated by subtracting NPN from TN and multiplying by 6.38, CN was calculated by subtracting the NCN from TN and multiplying by 6.38, and SP content was calculated by subtracting NPN from NCN and multiplying by 6.38. The SP content in the permeate portion of the skim milk (expressed as a percentage) was calculated by dividing mass of SP in 1 kg of milk by the permeate

portion of the milk multiplied by 100, where permeate portion of milk is 1 kg minus the weight of CN in 1 kg of skim milk.

Serum protein removal estimation using Kjeldahl analysis of permeates. The SP removal for each stage was estimated using Kjeldahl analysis (TN and NPN) of permeates. SP removal equaled the percentage of SP in the original skim milk removed in each stage. It was calculated by dividing the mass of TP (TP concentration was calculated from TN and NPN concentrations obtained by Kjeldahl analysis of the permeates, and mass of TP was calculated by multiplying the concentration of TP by the mass of permeate) in the permeate of each stage by the mass of SP in the starting skim milk times 100.

Theoretical values for removal were calculated using the above equations assuming that CN was 100% retained and that the concentration of SP in the permeate equaled the concentration of SP in the permeate phase of skim milk or water diluted retentate feed and that the CF and DF were both exactly 3.

Serum protein removal estimation using Kjeldahl analysis of retentates. For each stage TN, NCN and NPN concentrations obtained by Kjeldahl analysis of retentates were used to calculate SP and CN concentration in retentates. SP removal based on Kjeldahl analysis of retentates was then calculated as: 100 times the ratio of SP to CN in retentate subtracted from the SP to CN ratio in skim milk, with the result divided by the ratio of SP to CN in skim milk.

Particle Size Analysis of Skim Milk and Retentate

Particle size distribution was measured using a Mastersizer 2000 with a Hydro 2000-S liquid sample dispersion unit (pump speed 2250 rpm) with software version 5.40 (Malvern Instruments, Westborough, MA). A combination of a red (633 nm) and blue laser (466 nm) were used. The sample material refractive index was set at

1.458 and an absorption value of 0.00001. The blue light refractive index for fat was set at 1.460 with an absorption value of 0.00001. The dispersant (water) refractive index was set at 1.33. The density of the particulate material was set at 0.902 g/cm^3. The general purpose predictive model type was used, with the particle shape set to spherical. Size range of particles to be detected was 0.020 to 2000 μm. The obscuration limits were set from 7 to 9% to achieve a consistent amount of sample loading and to minimize the risk of multiple light scattering. The sample and dispersant temperatures were between 22 to 24^{o}C. Background and sample measurement time was 5 seconds and 5000 snaps. There were 3 measurement cycles with no delay between measurements. The average of the 3 cycles was reported. The majority of samples had residual values for the statistical model that were between 0.4 to 1 %, with occasional samples having residuals between 1 and 2 %. The volume mean diameter [d(4,3)] and the diameter below which 90% of the casein was contained [d(0.9)] were reported.

Color Analysis of Retentate

Hunter L, a, b values for the permeates were determined in duplicate with a MacBeth Color-Eye spectrophotometer (Model 2020; Kollmorgen Instruments, Corp., Newburgh, NY) with Optiview software from the same company. Hunter values were computed from the diffuse reflectance of light in the 360 to 740 nm range, at 20-nm intervals, based on illuminant A. The measurements were done at 23 to 25^{o}C (Quinones et al., 1997). The retentates for color analysis were taken from the complete mixed batch of collected retentate at the end of each stage of processing.

SDS-PAGE Electrophoresis

A 10 to 20% polyacrylamide gradient was used to determine the relative proportion of protein types in retentates and permeates from 3 MF systems. Retentate samples (0.1 mL) were diluted with sample buffer (0.9 mL) consisting of 10mM Tris-HCl pH 6.8, 1.0% SDS, 20% glycerol, and 0.02% bromophenol blue tracking dye and 50mM dithiothreitol and stored frozen (– 17^{o}C) in glass vials (Target DP^{TM} Vials C4000-1W, National Scientific Company, Rockwood, TN) sealed with DP Blue Cap (C4000-51B, National Scientific Company) . Diluted samples were thawed, heated to 100^{o}C in a steam chamber, and held at 100^{o}C for 3 min and then cooled to about 25^{o}C. Retentates and milks were loaded 10 and 8.5 μL, respectively, onto an SDS-PAGE gel (Verdi et al., 1987), and the procedure of Verdi et al. (1987) was used for running, staining and destaining the gels. Gels were scanned with USB GS 800 Densitometer using Quantity 1 1-D Analysis software (BIO-RAD Laboratories, Inc., Hercules, CA) to obtain a relative protein composition of samples. Loading of the samples was chosen to achieve an optical density (OD) of the predominant protein in the sample in the range of 1.0 to 1.4 OD. A milk sample was run on each gel as a reference for proper resolution of milk proteins and a check for consistency of quantitative analysis from gel to gel. The background was adjusted separately for each lane using the rolling disk method of subtraction to obtain a flat base on the pop-up trace. The line that defined each lane was adjusted using the lane tool function (add, adjust anchors) in the software so that the lane line crossed each band at the center. The adjust band function of the software was used with brackets to set the leading and trailing edge for each band as visually observed on the image of the gel, not based on the based on the beginning and end of the peak in the pop-up trace. The bracket width was set to include the full width of all bands.

Serum Protein Removal Estimation Using SDS-PAGE

To calculate relative percent SP removal using SDS-PAGE results, first the SP as a percentage of CN for each lane was calculated, which was the sum of the relative density of all SP bands divided by the sum of relative density of the CN bands times 100. Calculation of SP reduction was SP as a percentage of CN in the retentate subtracted from SP as a percentage of CN in skim milk divided by SP as a percentage of CN in skim milk then the total times 100. The bands corresponding to SP and CN are shown and labeled in Figure 3.1.

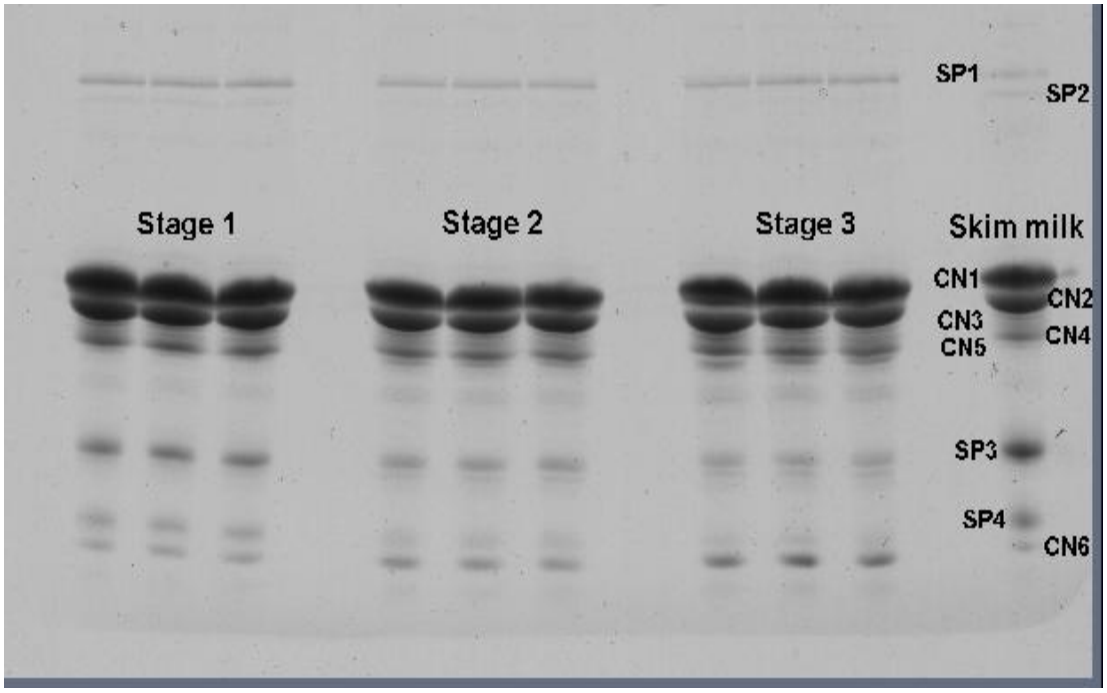

Figure 3.1. The proteins in skim milk and the microfiltration retentates produced in each stage using SDS-PAGE. Bands in skim milk are identified on the gel: SP1, SP2 = serum proteins, CN1 = α_s-CN (combination of α_{s1} and α_{s2}-CN), CN2 = β-casein; CN4 = κ-casein; CN3 = proteolysis products of casein, CN5 = proteolysis products of casein, SP3 = β-LG; SP4 = α-LA and CN6 = proteolysis products of casein.

RESULTS

Processing

Operational parameters. No differences in the TMP at the inlet and outlet (Table 3.1) were detected among stages ($P > 0.05$). The TMP pressure at the membrane outlet end was always greater than 0 indicating no reverse flow of permeate back into the retentate side at the outlet. The TMP at the inlet was 27 kPa higher than at the outlet and was within the operational parameter of 25 ± 3kPa. Flux averaged 54.2 kg/m^2 per hour and no differences were detected in flux ($P > 0.05$) over the 3 stages and 4 replicates. Flux was under direct control and did not decrease during the run.

Concentration factor. Concentration factors for each stage were higher than 3X as shown in Table 3.1. Dilution of the retentate between stages was independent of actual CF and 2 kg of RO water per 1 kg retentate was always added. An average flux of 54.2 kg/m^2 per hour corresponds to 92.14 kg per hour of permeate removal for this system. The retentate removal to get the CF in Table 3.1 would have had to have been < 42 kg per hour. Both permeate and retentate bleed rates were set using volumetric flow meters. No correction in the flow measurement for retentate was made for increase in viscosity as CF increased and that may explain why we were not able to control CF to exactly 3X during processing.

Table 3.1. Mean (n = 4) transmembrane pressure (TMP) at the membrane inlet and outlet, flux, and concentration factors for each stage of the 3-stage uniform transmembrane ceramic microfiltration (MF) system operational parameters

MF stage	TMP inlet (kPa)	TMP outlet (kPa)	Flux (kg/m^2 per hour)	Concentration factor
Stage 1	42	15	53.96	3.20^{b}
Stage 2	42	15	53.96	3.20^{b}
Stage 3	42	16	54.62	3.30^{a}
SE	0.59	0.83	0.189	0.018
R^2	0.28	0.06	0.89	0.89

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

Composition and Color

Skim milk composition. Composition of the protein portion of the 4 batches of skim milk used in the study was very similar (Table 3.2). Heat treatment of milk can increase the apparent CN concentration in milk (Lynch et al. 1998). This is caused by SP denaturing and becoming associated with the CN micelle, specifically β-LG forms disulfide bonds with κ-CN. This bound β-LG is measured as CN when using Kjeldahl analysis. In Ma et al. 2000, it was reported that in raw milk CN as a percentage of TP was 82.32% and that after pasteurization (74^{o}C for 34 s) CN as a percentage of TP increased by 2.89%. In our study the pasteurization temperature was lower than that used by Ma et al. 2000 and the amount of CN as a percentage of TP in Table 3.2 indicates that the milk had not undergone excessive heat treatment in the present study and was similar to that of raw milk.

Table 3.2. Mean (n = 4) composition of pasteurized skim milk (% by weight).

Pasteurized skim milk	TN[1]	NCN[2]	NPN[3]	TP[4]	Casein[5]	Serum protein[6]	Serum protein in permeate portion of skim milk	% Casein in TP
Replicate 1	3.33	0.74	0.18	3.15	2.59	0.56	0.57	82.13
Replicate 2	3.39	0.74	0.18	3.21	2.65	0.56	0.58	82.66
Replicate 3	3.34	0.73	0.16	3.18	2.61	0.57	0.59	82.09
Replicate 4	3.30	0.73	0.19	3.11	2.57	0.54	0.55	82.63
Mean	3.34	0.73	0.18	3.16	2.60	0.56	0.57	82.38
SD	0.04	0.01	0.01	0.04	0.03	0.01	0.01	0.31

[1] TN = total nitrogen x 6.38.
[2] NCN = noncasein nitrogen x 6.38.
[3] NPN = nonprotein nitrogen x 6.38.
[4] TP = true protein, (TN - NPN)
[5] Casein = TN – NCN
[6] Serum proteins = TP - casein

Permeate composition. The TS, TN and NPN concentration in permeate (Table 3.3) decreased with increasing stage ($P < 0.05$). This was primarily due to the retentate being diluted with water before stages 2 and 3. Permeate from the first stage (Table 3.3) had a TP concentration similar to the SP concentration in the permeate portion (Table 3.2) of skim milk (0.58 vs 0.57, respectively). Theoretically using the same calculation method as Hurt and Barbano (submitted), a 3X MF process starting with skim milk containing 3.16% TP should have a concentration of SP in permeate of 0.58, 0.18 and 0.06% for the 1st 2nd and 3rd stages, respectively. The actual permeate contained 0.58, 0.25 and 0.14% TP in each stage, respectively. The higher than theoretical SP concentrations observed for the 2nd and 3rd stages was in part due to TP

in the permeate being measured instead of SP. Another contribution to higher TP concentration in permeate compared to predicted TP concentration in permeate for the 2nd and 3rd stages was SP rejection by the membrane. If the membrane rejects SP, the concentration of SP in permeate of later stages is expected to be higher than when the membrane does not reject SP (Hurt and Barbano, submitted).

Table 3.3. Mean (n = 4) composition (% by weight) of permeates from each stage of the 3-stage uniform transmembrane ceramic microfiltration (MF) system.

MF stage	Total solids	Lactose	TN[1]	NPN[2]	TP[3]
Stage 1	6.53[a]	5.01[a]	0.76[a]	0.18[a]	0.58[a]
Stage 2	2.09[b]	1.62[b]	0.31[b]	0.06[b]	0.25[b]
Stage 3	0.73[c]	0.61[c]	0.17[c]	0.03[c]	0.14[c]
SE	0.01	0.009	0.003	0.003	0.002
R^2	>0.99	>0.99	>0.99	>0.99	>0.99

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

Retentate composition. Based on the paper by Hurt and Barbano (submitted), if both the CF and water DF were 3 given the mean skim milk composition used in the present study (3.16% TP), then concentration of TP in the retentate would decrease slightly in each stage from 8.32 to 7.96 to 7.85% for each of the 3-stages, respectively due to the removal of SP. An increase in TP concentration in retentate was predicted if the CF was greater than the water DF by Hurt and Barbano (submitted) and a CF of 3.1 and water DF of 3.0 would lead to increasing TP concentration in the retentate of 8.67, 8.57 and 8.73% for each stage, respectively. The TP concentration in the retentate for each stage shown in Table 3.4 indicates no change in TP concentration

from stage 1 to 2 and an increase in stage 3, which was consistent with a scenario where CF was greater than water DF.

The TS, lactose, TN, NCN, NPN, TP, CN and SP concentrations in retentate (Table 3.4) decreased with increasing stage ($P < 0.05$). This decrease was expected because the retentate was diluted with water after stage 1 and 2, and TS, NPN and SP are removed in permeate. However based on the theoretical calculations from Hurt and Barbano (submitted), if the membrane did not reject SP, then the concentration of SP in the 3rd stage retentate should be around 0.1%, not the 0.4% SP measured in the 3rd stage retentate (Table 3.4). This was due to the fact that measured concentration of SP was overestimated in MF retentates and CN was underestimated in the MF retentates when using the Kjeldahl NCN method that was designed for analysis of milk not retentates, as reported previously by Nelson and Barbano, 2005. NCN must be measured to determine both SP and CN using Kjeldahl analysis. Measured NCN content of a 3X MF retentate will be erroneously high, which leads to an overestimated SP concentration [(NCN-NPN)*6.38] and underestimated CN concentration [(TN-NCN)*6.38] in MF retentates when the NCN method designed for milk is used directly on retentate samples. An improved NCN sample preparation procedure for the Kjeldahl analysis that is designed for retentates is needed.

Table 3.4. Mean (n = 4) composition (% by weight) of the retentates from each stage of the 3-stage uniform transmembrane ceramic microfiltration (MF) system.

MF stage	Total solids	Lactose (IR)	TN[1]	NCN[2]	NPN[3]	TP[4]	Casein[5]	Serum proteins[6]
Stage 1	15.05[a]	4.50[a]	8.85[ab]	0.95[a]	0.17[a]	8.67[b]	7.90[b]	0.78 [a]
Stage 2	11.41[b]	1.38[b]	8.68[b]	0.56[b]	0.07[b]	8.61[b]	8.13[b]	0.49[b]
Stage 3	10.80[c]	0.40[c]	9.12[a]	0.45[c]	0.04[c]	9.08[a]	8.68[a]	0.40[c]
SE	0.097	0.006	0.086	0.017	0.003	0.086	0.074	0.016
R^2	>0.99	>0.99	0.90	0.99	>0.99	0.90	0.95	0.98

[a - c] Means in the same column not sharing a common superscript are different ($P < 0.05$).
[1] TN = total nitrogen x 6.38.
[2] NCN = noncasein nitrogen x 6.38.
[3] NPN = nonprotein nitrogen x 6.38.
[4] TP = true protein, (TN – NPN)
[5] Casein = TN – NCN
[6] Serum proteins = TP – casein.

Retentate pH. The pH of both the starting materials and retentates (Table 3.5) increased with increasing stage ($P < 0.05$). The starting material for stage 1 was skim milk, for stage 2 it was the MF retentate from stage 1 diluted with 2 parts RO water to 1 part retentate by weight, and for stage 3 it was the MF retentate from stage 2 diluted with 2 parts RO water to 1 part retentate by weight. Milk had a pH of about 6.6 at 50^{o}C, due to the presence of buffers such as citric acid and soluble minerals. Concentration of these buffers in the permeate portion of the retentate is the same as the feed for stage 1 and there was very little different in pH of the starting material and the 3X retentate for stage 1. Before each subsequent stage retentate was diluted with RO water resulting in a slight increase in pH of both the starting material and the retentate from that stage. The pH of the pasteurized RO water used to dilute the retentate was 6.83 and this combined with dilution of the concentration of buffering

salts in 2nd and 3rd stage caused, the pH of the MF retentates to increase ($P < 0.05$) with stage.

Table 3.5. Mean (n = 4) pH values (50°C) of the stage starting material and final retentate from each stage of the 3-stage uniform transmembrane ceramic microfiltration (MF) system.

MF stage	Starting material	Retentate
Stage 1	6.62[c]	6.58[c]
Stage 2	6.82[b]	6.81[b]
Stage 3	7.01[a]	6.97[a]
SE	0.05	0.02
R^2	0.86	0.97

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

Retentate color and particle size. The L-value for retentates (Table 3.6) increased ($P < 0.05$) with increasing stage indicating the retentate was getting whiter, a-values got less negative indicating sample was less green, and b-values got more negative meaning sample was becoming more blue or less yellow ($P < 0.05$). Typical color values for skim, 1% fat and 2% fat milk (Philips et al., 1995) are also shown in Table 3.6. The L-values and a-values of retentates were becoming more similar to 2% fat milk as the number of stages increased, even though the fat content of the 3rd stage MF retentates was typically between 0.2 and 0.3%. The L-value increased with stage because compounds in the permeate portion of the milk that provide a green and yellow color to skim milk are being removed (i.e., MF permeate has a green color). The increase in CN concentration with increasing stage (Table 3.4) would also be expected to increase whiteness of the retentate due to increased light scattering. The particle size distribution (within the limits of the laser light scattering method we used)

did not change between stages. The mean d (0.9) was 0.22µm and the D [4,3] was 0.16µm. We could not detect a change in size of CN micelles due to processing, or changes in the composition of the water phase surrounding the CN micelles. Thus, the MF retentate could be used as a beverage ingredient that will provide some of the desirable appearance of milk containing fat without any non-dairy ingredients that are sometimes used to increase whiteness (Phillips and Barbano, 1997).

Table 3.6. Mean (n = 4) Hunter L, a, b color values for retentates from each stage of the 3-stage uniform transmembrane ceramic microfiltration (MF) system.

MF stage	L-value	a-value	b-value
Stage 1	78.28[c]	-4.57[c]	3.41[a]
Stage 2	79.09[b]	-4.40[b]	0.81[b]
Stage 3	80.07[a]	-3.97[a]	-0.16[c]
SE	0.051	0.018	0.025
R^2	0.99	>0.99	>0.99
Skim milk*	73.77	-6.68	1.13
1% milk*	78.92	-4.54	2.55
2% milk*	81.11	-3.74	2.99

[a - c] Means in the same column not sharing a common superscript are different ($P < 0.05$).
*Data from Phillips et al. 1995.

Serum Protein Removed

The theoretical percentage removal of SP by stage for a 3X, 3-stage MF process is shown in Table 3.7. Removal of SP during MF can be influenced by many factors (Hurt and Barbano, submitted). In the present study, TP content of MF permeate was used as an estimate of SP content with the assumption that there was no

CN in the MF permeate. When TP values for SP concentration in permeate were used, the calculated SP removal by the 3-stage process was 104.31% (Table 3.7). The SDS-PAGE analysis of the stage 1, 2, and 3 retentates indicates that not all SP was removed from the retentates but a progressive reduction with increasing stage in SP content of the retentate is apparent in Figure 1. It also can be seen from the SDS-PAGE analysis of the MF permeate from the first stage (Figure 3.2) that MF permeate contains some CN, so the assumption we made that all the TP in the permeate was SP was incorrect. Therefore, a sensitivity analysis is presented in Table 3.7 to demonstrate how much influence the presence of 0.01, 0.02, and 0.03% CN in MF permeate would have on the expected SP removal by stage and total SP removal. In subsequent work (data not reported), we have found (by Kjeldahl analysis of MF permeates) that the CN content of MF permeate from our ceramic UTP system is typically between 0.02 and 0.03%. Therefore, the SP removal achieved in the current project with a 3X, 3-stage UTP MF process using ceramic membranes was probably between 95 and 98%, as shown in Table 3.7.

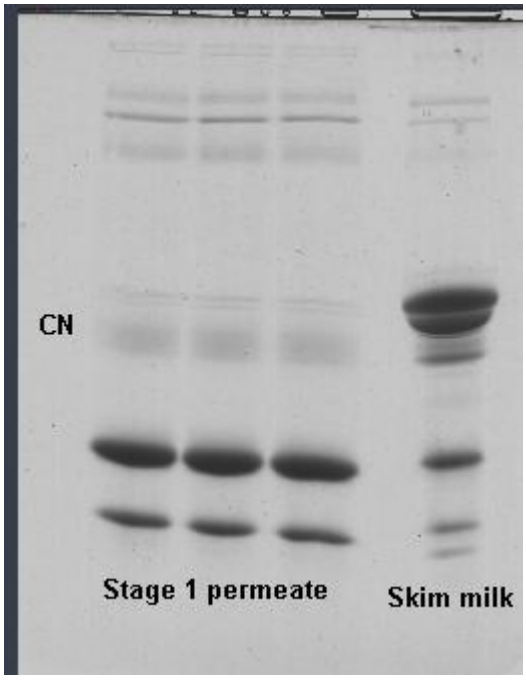

Figure 3.2. An SDS-PAGE gel for separation of proteins in skim milk and the microfiltration permeates produced by ceramic UTP.

Table 3.7. Sensitivity analysis for mean (n = 4) serum protein removal (%) as percent of serum protein (SP) in starting skim milk determined by Kjeldahl analysis from each stage of the 3-stage uniform transmembrane ceramic microfiltration (MF) system, with different assumed amounts of casein (percent by weight) in the permeate.

	Assumed casein in the permeate (% wt/wt)				
	0.00	0.00	0.01	0.02	0.03
MF stage	Theoretical SP removal[1]	SP removal	SP removal	SP removal	SP removal
Stage 1	68	67.15[a]	65.99[a]	64.83[a]	63.66[a]
Stage 2	22	24.92[b]	23.92[b]	22.92[b]	21.92[b]
Stage 3	7	12.24[c]	11.38[c]	10.51[c]	9.65[c]
Total removal	97	104.31	101.29	98.26	95.23
SE	--	0.19	0.19	0.18	0.18
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 (i.e., 0.00% casein)

A measure of the productivity and efficiency of an MF process for removal of SP from skim milk in the context of a manufacturing facility may be best expressed as kg of SP removed per square meter of membrane surface area per hour within each stage. The data from the current study are presented in that form in Table 3.8. The mass SP removal decreased progressively with increasing stage ($P < 0.05$), which was expected. The presence of a 0.02% CN contamination does not have much impact on the estimate of the mass removal of SP per meter square of membrane per hour (Table 3.8).

Table 3.8. Mean (n = 4) kg of serum protein (SP) removed by each stage of the 3-stage uniform transmembrane ceramic microfiltration (MF) system measured by Kjeldahl.

MF stage	SP removed (kg/m^2 per h)	SP removed (kg/m^2 per h) Adjusted for an assumed 0.02% casein in the permeate
Stage 1	0.31^a	0.30^a
Stage 2	0.12^b	0.11^b
Stage 3	0.07^c	0.06^c
SE	0.0037	0.0033
R^2	>0.99	>0.99

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

DISCUSSION

Challenges and Issues in Measuring Serum Protein Removal

Removal of SP can be calculated using several different methods, such as from Kjeldahl analysis of the starting skim milk and the retentates of each stage, from Kjeldahl analysis of starting skim milk and the permeates as shown in Table 3.7, or from SDS-PAGE analysis of the retentates. All of these methods were subject to errors which influenced the calculated SP removal. It is important to understand the specific factors and sources of error that were important for each approach, and how they influenced the calculated SP removal.

Serum protein removal estimated by Kjeldahl analysis of retentates. The SP removal calculated using data obtained from Kjeldahl analysis of the retentates is shown in Table 3.9. The SP removal calculated from data obtained by Kjeldahl analysis of retentates was lower than removal calculated using either Kjeldahl analysis of permeates or SDS-PAGE analysis of retentates. This was expected because the NCN Kjeldahl method designed for milk, when applied to 3X MF retentate, fails to precipitate all the CN in the retentate which leads to an overestimation of NCN and thus SP in retentates.

Table 3.9. Mean ± one standard deviation (n = 4) relative percent SP reduction in microfiltration (MF) retentate by each stage of the 3-stage uniform transmembrane ceramic MF system measured by SDS- PAGE and Kjeldahl.

MF stage	Theoretical cumulative SP reduction	Cumulative SP reduction (SDS-PAGE)	Cumulative SP reduction (Kjeldahl on retentates)	Cumulative SP reduction (Kjeldahl on permeates assuming 0.02% casein in permeates)
Stage 1	68	$72.39^{a} \pm 2.95$	$54.61^{c} \pm 2.75$	$64.83^{b} \pm 0.76$
Stage 2	90	$88.92^{a} \pm 3.50$	$72.36^{b} \pm 0.83$	$87.75^{a} \pm 1.56$
Stage 3	97	$91.33^{b} \pm 3.72$	$78.62^{c} \pm 1.03$	$98.26^{a} \pm 2.25$

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

Serum protein removal estimated by Kjeldahl analysis of permeates.

Calculating SP removal using data from the Kjeldahl analysis of permeates required measurement of the mass of permeate produced in each stage. A source of error associated with this method has to do with mass balance issues. The calculation of SP removal with data obtained from Kjeldahl analysis of the permeates is influenced by loss of feed, retentate and permeate during each of the 3 stages. There is loss at start-up for each stage, shut-down for each stage and between stages. At start-up for each stage some feed material is lost as hold up in the heat exchanger and pump used to bring the feed to 50^{o}C. There is also material lost in the first 14 kg of retentate and 31 kg of permeate discarded, though most of the mass is water in the system at start-up. There is some dilution of the collected retentate and permeate because not all of the water is removed in the discarded fraction. At shut-down the material remaining in the system and feed tank is lost. The loss associated with start-up and shut-down averaged 64 ± 1.70 kg. There is an additional loss averaging 4.40 ± 0.76 kg due to the transfer

of the retentate between stages. The estimate of percentage SP removal by Kjeldahl analysis of permeates was higher ($P < 0.05$) than the estimate of percentage SP removal by Kjeldahl analysis of retentates for all stages (Table 3.9). This was expected give the over estimation of SP in the retentates described in the previous section. In calculating mass based SP removal the denominator for each stage was the SP in the skim milk times the mass of skim milk, the numerator was the mass of permeate times SP concentration in the permeate for that stage. In stages 2 and 3, the feed mass was less than the initial mass of milk, meaning that the mass of permeate from these stages was lower than if no product had been lost and the feed volume for each stage was constant. This would suggest SP removal was underestimated.

There are also analytical issues with the Kjeldahl analysis that could influence calculation of SP removal. The SP concentration of the skim milk was used in the calculation of SP removal for all three stages; any error in the SP measured by Kjeldahl would have a large impact on calculated SP removal. A factor of 6.38 was used to convert nitrogen values into percent protein, it has been found that for β-LG this value was 6.37 and 6.14 for α-LA. (Karman and van Boekel, 1987). However, the calculated percentage SP removal is a ratio of the calculated the SP in the permeate divided by the calculated SP in the skim milk so the nitrogen to protein conversion factor used does not matter when calculating the relative percentage SP reduction. However, the calculation of the mass of SP removed per unit surface are of MF membrane per hour (as in Table 3.8) would be influenced the assumption of a 6.38 Kjeldahl for factor for the SP.

If the skim milk had undergone more heat treatment during pasteurization, then the amount of SP in milk determined with Kjeldahl would be lower because some SP would be associated with the CN micelles (Harland et al., 1952) and counted by the Kjeldahl method as if it was CN (Lynch et al., 1998). Bound SP would not be

removed during MF, and thus not change the estimate of percent SP removal determined using Kjeldahl analysis. However, SDS-PAGE analysis of the retentate would cause dissociation of the CN-SP complex and indicate higher than expected levels of SP in retentate and would be expected to produce lower estimates of SP removal than Kjeldahl.

Serum protein removal estimated by SDS-PAGE analysis of retentates. The SP removal calculated using SDS-PAGE analysis of MF retentates and the starting skim milk are shown in Table 3.9. No use of mass data collected during milk processing was required for this calculation and therefore any errors associated with estimates of masses of skim milk, retentate, or permeate were not an issue in these calculations. The SP removal calculated using this method was subject to several different sources of error than found in the approach using Kjeldahl. The retentates from each stage have a high concentration of CN compared to SP, which means that the CN could be in the non-linear range of detector response due to large amount of CN loaded on the gel in a slot. On the other hand the SP bands could be below the limits of quantification for the detector. The problem of limit of quantification for SP was especially true in later MF stages when the concentration of SP in the retentates was very low. Using SDS-PAGE analysis to calculate SP removal is much less precise than both the Kjeldahl methods.

CONCLUSIONS

Theoretically, a 3-stage 3X MF process could remove 97% of the SP from skim milk, with a cumulative SP removal of 68 and 90% after the 1^{st} and 2^{nd} stages respectively. The cumulative of SP removal using a 3-stage 3X MF process with a UTP system with 0.01µm ceramic membranes in this experiment was 64.83 ± 0.76, 87.75 ± 1.56 and 98.27 ± 2.25% for the 1^{st}, 2^{nd} and 3^{rd} stages respectively, when SP

removal was calculated using the mass of SP removed in the permeate of each stage. Various methods of calculation of SP removal were evaluated. Given the analytical limitation in the various methods for measuring SP removal, calculation of SP removal based on the mass of SP in the skim milk (determined by Kjeldahl) and the mass SP present in all of the permeate produced by the process (determine by Kjeldahl) provided the best estimate of SP removal for a MF process.

REFERENCES

Association of Official Analytical Chemists. 2000. Official Methods of Analysis. 17th ed. AOAC, Gaithersburg, MD.

Belfort, G., Davis, R.H. and A.L. Zydney. 1994. The behavior of suspensions and macromolecular solutions in crossflow microfiltration. J. Membrane Sci. 96:1-58.

Britten, M. and Y. Pouliot. 1996. Characterization of whey protein isolate obtained from milk microfiltration permeate. Lait 76:255-265.

Cheryan M. 1998. Ultrafiltration and microfiltration handbook. Technomic Publishing Company, Inc., Lancaster, PA, USA.

de Wit, J.N. and G. Klarenbeck. 1984. Effects of various heat treatments on structure and solubility of whey proteins. J. Dairy Sci. 67:2701-2710.

Evans, J. J. Zulewska, M. Newbold, M.A. Drake and D.M. Barbano. 2009. Comparison of composition , sensory and volatile components of thirty-four percent whey protein and milk serum protein concentrates. J. Dairy Sci. 92:4773-4791.

Harland, H.A., S.T. Coulter, R. Jenness. 1952. The effect of the various steps in the manufacture on the extent of serum protein denaturation in nonfat dry milk. J. Dairy Sci. 35:363-368.

Holt, C. 1992. Structure and stability of bovine casein micelles. Advances in Protein Chemistry 43: 64-151.

Karman, A.H., M.A.J.S. van Boekel. 1986. Evaluation of the Kjeldahl factor for conversion of the nitrogen-content of milk and milk products to protein content. Netherlands Milk and Dairy J. 40(4): 315-336.

Kaylegian, K.E., G.E. Houghton, J.M. Lynch, J.R. Fleming, and D.M. Barbano. 2006. Calibration of Infrared Milk Analyzers: Modified Milk versus Producer Milk. J. Dairy Sci. 89:2817-2832.

Le Berre, O., and G. Daufin. 1996. Skimmilk crossflow microfiltration performance versus permeation flux to wall shear stress ratio. J. Membrane Sci. 117:261-270.

Lynch, J.M., D.M. Barbano, and J.R. Fleming. 1998. Indirect and direct determination of the casein content of milk by Kjeldahl nitrogen analysis: collaborative study. JAOACI 81:763-774.

Ma, Y., C. Ryan, D.M. Barbano, D.M. Galton, M.A. Rudan, K.J. Boor. 2000. Effects of somatic cell count on quality and shelf-life of pasteurized fluid milk. J. Dairy Sci. 83:264-274.

Nelson, B.K., and D.M. Barbano. 2005. A microfiltration process to maximize removal of serum protein from skim milk before cheese making. J. Dairy Sci. 88:1891-1900.

Papadatos, A., M. Neocleous, A.M. Berger, and D.M. Barbano. 2003. Economic feasibility of microfiltration of milk prior to cheesemaking. J. Dairy Sci. 86:1564-1577.

Phillips, L.G., and D.M. Barbano. 1997. The influence of fat substitutes based on protein and titanium dioxide on the sensory properties of lowfat milks. J. Dairy Sci. 80:2726-2731.

Phillips, L.G., M.L. McGiff, D.M. Barbano, H.T. Lawless. 1995. The influence of fat on the sensory properties, viscosity, and color of low fat milk. J. of Dairy Sci. 18:1258-1266.

Pierre, A., J. Fauquant, Y. Le Graet, M. Piot and J.L. Maubios. 1992. Native micellar casein separation through cross-flow membrane microfiltration. Lait 72(5): 461-474.

Quinones H.J., D.M. Barbano, L.G. Philips. 1998. Influence of protein standardization by ultrafiltration on the viscosity, color, and sensory properties of 2 and 3.3% milks. J. Dairy Sci. 81:884-894.

Sandblom R.M., inventor. 1978. Filtering process. Alfa-Laval AB, assignee. US Pat. No. 4,105,547.

Sachdeva, S. and W. Buchheim. 1997. Separation of native casein and whey proteins during crossflow microfiltration of skim milk. The Aust. J. of Dairy Technol. 52: 92-97.

Verdi R.J., D.M. Barbano, and M.E. Dellavalle. 1987. Variability in true protein, casein, nonprotein nitrogen, and proteolysis in high and low somatic cell count milks. J. Dairy Sci. 70:230-242.

Walstra, P., T.J. Geurts, A. Noomen, A. Jallema, M.A.J.S. van Boekel. 1999. Dairy Technology: Principles of Milk Properties and Processes. Marcel Dekker, Inc. New York, NY.

Zulewska, J., M. Newbold, D.M. Barbano. 2009. Efficiency of serum protein removal from skim milk with ceramic and polymeric membranes at 50^{o}C. J. Dairy Sci. 92:1361-1377.

Chapter 4

Conclusions and Future Work

Microfiltration of skim milk to separate CN micelles from SP has the potential to be a commercially important process. In designing such a process there are multiple factors that could influence the yield of MSPI and MCC, as well as consistence of 3rd stage microfiltration retentate. If the impact of these factors was understood, operating conditions could be modified in order to maintain consistent MCC and MSPI. In order to explore the impact of these factors, a mathematical model of a skim milk MF process was developed with 3-stages, an additional 4th finishing stage was added to standardize the retentate to 9% TP and allow calculation of yield of liquid 9% TP MCC and MSPI (90% SP on a dry basis). The model was used to predict the effect of 5 factors**:** skim milk composition, heat treatment of skim milk, CF and DF, control of CF and DF, and SP rejection of membrane on the retentate and permeate composition, SP removal, and MCC and MSPI yield. Within the ranges of the 5 factors studied, it was found that skim milk composition had a large impact on MCC and MSPI yields, while the heat treatment of skim milk had a large influence on yield of MSPI, and composition of MCC. The CF and DF chosen, as well as the possible mismatch in CF and DF influenced both the yields and composition of MCC and MSPI. Finally the SP rejection of the membrane influenced yield of MSPI and overall SP removal. Overall the work indicated how important the skim milk composition and heat treatment was to yield of MSPI and yield and composition of MCC, as well as how important the control of the CF and DF can be in the production of MCC and MSPI.

Understanding the impact the 5 factors have provides a powerful tool for the design and operation of a MF process to separate SP from CN in skim milk. For example if the TP concentration in the retentate is higher than predicted on a given

day, the problem can be narrowed down to either a control issue with the CF or DF, or an increased TP content of the incoming skim milk. The model also indicates that it would be most efficient in terms of SP removal to run at the largest CF possible while still achieving satisfactory flux and low SP rejection by the membrane.

From the work on theoretical scenarios a 3-stage MF with a 3X CF and DF could remove 97% of the SP from skim milk with a cumulative SP removal of 68 and 90% after the 1st and 2nd stages respectively. In the 2nd part of the research the SP removal for a 3-stage MF process with 0.1µm ceramic membranes in a UTP system was determined. The cumulative SP removal was 64.83 ± 0.76, 87.75 ± 1.56 and 98.27 ± 2.25% for the 1st, 2nd and 3rd stages respectively, when SP removal was calculated using the mass of SP removed in the permeate of each stage. Various methods of calculation of SP removal were evaluated, including SDS-PAGE analysis of the retentates and TN, NCN and NPN analysis of the retentates by Kjeldahl methods. The SDS-PAGE analysis of the retentates to determine SP removal was influence by the non-linearity of response for CN bands and limits of detection for SP bands. Using Kjeldahl analysis of the retentates to calculate SP removal gave erroneously low SP removal, because the method used to determine NCN (SP equals NCN minus NPN) did not precipitate all of the CN, meaning some CN was counted as SP. Given the analytical limitation in the various methods for measuring SP removal, calculation of SP removal based on the mass of SP in the skim milk and the mass SP present in the permeate produced in each stage of the process (where SP concentration was determined by Kjeldahl analysis) provided the best estimate of SP removal for a MF process. It was found that a 3-stage UTP system with 0.1 µm ceramic membranes achieved a removal of SP from skim milk that was close to what would be expected theoretically.

From the results of the research presented in this thesis, possibilities for further work become apparent. The overall goal of further research, building on the research presented here would be to improve the feasibility of commercial adoption of MF to separate CN micelles from SP. Firstly, if SP depleted MCC were to be sold as a product, there needs to be a method to accurately determine SP removal, using only analysis of the product (MCC), and not requiring processing information. The current Kjeldahl NCN method doesn't precipitate 100% of the casein from MCC, meaning that Kjeldahl analysis of the MCC cannot be used to determine SP removal. Modification of the Kjeldahl NCN method to precipitate 100% of the casein seems like a logical step.

Secondly, the theoretical part of this research made it clear that tight control of the CF and DF for each stage was necessary to insure consistent MCC and MSPI, which would be important at an industrial scale. The actual set up and configuration of a large scale multi-stage MF system could impact the ability to control the CF and DF, as well as system performance. The different possible configurations of multi-stage MF process need to be explored, along with the advantages and disadvantages of each set up. As a 1st step the effect of different stage set-ups could be explored theoretically.

An additional area of exploration would be in processing changes to maximize SP removal, or minimize the number of stages required. This would lower the cost of the process, making it more economically attractive. One possible area of exploration would be in using an ultrafiltration stage, before microfiltration to concentrate the skim milk. This step would remove some lactose, reduce the overall volume to be microfiltered, and concentrate the SP so the permeate stream would have a higher SP concentration. Required for this work would be a study of the operating limits of a MF system, such as the maximum CF and permeate removal rate.

The research presented in this thesis shows the importance of skim milk composition and processing parameters for MCC and MSPI composition and yield, further more it was found that a 3-stage MF using a ceramic UTP system (0.1µm ceramic membranes) with a 3X concentration factor and water dilution between stages had a SP removal close to theoretical, making it an ideal candidate for further work. Further work should focus on the actual configuration of a multi-stage MF unit suitable for large scale production of MCC and MSPI, as well as processing changes that could increase performance, reducing cost.

APPENDIX

Diagram of a 3-stage microfiltration process with a concentration and diafiltration factor of 3X: