MICELLAR CASEIN CONCENTRATE AS A NOVEL DAIRY PROTEIN INGREDIENT: SHELF-LIFE STABILITY AND ITS APPLICATION IN THE PRODUCTION OF LOW FAT CHEDDAR CHEESE

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ABSTRACT

Caseins are regarded as one of the principal functional food proteins which provide foaming, emulsifying, and water binding properties in food systems. Most of commercial caseins are produced by destabilizing casein micelles by rennet coagulation or acid precipitation, hence the native casein micelles are disrupted. With the advancement of microfiltration technology, it is possible to separate casein by microfiltration (MF) of skim milk while maintaining its micellar structure. The MF retentate of skim milk is called micellar casein concentrate (MCC). Fresh liquid MCC can be further concentrated by ultrafiltration (UF) to remove excess water, and at the same time remove low molecular weight (MW) compounds which can pass through the UF membrane. The liquid MCC can also be spray dried to produce powder. The MCC is a milk protein ingredient with properties that are different from other available protein ingredients, thus it might serve particular functions that other protein ingredients can't deliver. MCC could be used for high-protein, low-lactose drinks, cheesemaking, including production of low-fat Cheddar cheese.

Our 1st objective was to develop a process to produce a high concentration liquid MCC (18% protein) with a long refrigerated shelf-life. To achieve a long refrigerated shelf-life, the processing of MCC18 was designed to maximize the removal of low MW compounds, e.g. lactose, nonprotein nitrogen (NPN) which can be easily metabolized by microbes, while minimizing the microbial count in the final product. The production of MCC18 was replicated 3 times. Skim milk was ultrafiltered (UF) which removed more than a half of lactose and NPN. The UF skim milk retentate was microfiltered (MF) in 3 stages to remove approximately 95% of the serum protein and further remove lactose and NPN. The final MF retentate was concentrated to 18% protein by UF, then batch pasteurized. The MCC18 was collected immediately in sterile

plastic vials and stored at 4°C. The MCC18 contained 21.75% total solids, 18.27% true protein, 0.31% nonprotein nitrogen and 0.13% lactose. The mean aerobic bacterial and spore counts of MCC18 at day 0 were 2.1 log cfu/mL and 2.3 log cfu/mL, respectively. The MCC18 produced in this study maintained a bacterial count < 20,000 cfu/mL for 16 wk when stored at refrigeration temperature of 4°C. The conversion of skim milk to MCC and its coproducts (serum protein concentrate and lactose concentrate) could be used as an alternative to balance milk production seasonality.

Our 2nd objective was to produce low-fat Cheddar cheese (LFCC) by combining reduced-fat Cheddar cheese (RFCC) made by a fat removal process with MCC to try to achieve texture and flavor characteristics of full-fat Cheddar cheese (FFCC). The production of LFCC was replicated 3 times with a different batch of commercial FFCC, from which RFCC was produced, as an ingredient for LFCC-making. The LFCC was formulated to achieve 6% fat, 28% protein, 1.2% salt by combination of RFCC, MCC powder, water, salt, lactic acid and rennet. The 6% fat target was used to comply with the FDA standard for a low-fat label claim. The pH of the LFCC mixture was adjusted to 5.3 by lactic acid. Rennet was added, followed by pressing and packaging. Chemical and sensory data were analyzed by ANOVA using the Proc GLM of SAS to determine if there were differences on chemical compositions and sensory among different cheeses. Descriptive sensory scores were used to construct a PCA biplot to visualize flavor profile differences among cheeses. The LFCC had 83% less fat, 32% less sodium, higher protein and moisture than FFCC. When the cheese texture was evaluated in the context of a filled-gel model consisting matrix and filler (100% minus % matrix), the LFCC had lower filler volume than FFCC, yet the LFCC had a softer texture than FFCC. The LFCC contained some of the original FFCC cheese matrix that had been disrupted by the fat removal process, and this original

FFCC matrix was embedded in a LFCC matrix formed by the action of rennet on casein from the continuous phase of hydrated MCC. Thus, the texture of the LFCC was desirable and was softer than the FFCC it was made from, whereas commercial RFCC (50% and 75% fat reduction) were firmer than the FFCC. The sulfur flavor in LFCC was closer to FFCC, than commercial RFCC. The LFCC had bitter and grape-tortilla off-flavors which came from the dried MCC ingredient. The commercial RFCC and experimental LFCC made in this study were missing the typical aged Cheddar character (catty, nutty, fruity, brothy, milkfat) found in FFCC.

BIOGRAPHICAL SKETCH

Irma Amelia was born in Jakarta, Indonesia on June 19, 1985 as the third child of Halim Dharmadi and Janny Karlina Kurnia. After finishing high school in her hometown in 2003, she came to the United State to pursue further education. She enrolled in the Food Science program with a minor in Agribusiness Management at Purdue University, West Lafayette, IN. During her junior year, she completed an internship with Sensient Flavours Inc., Indianapolis, IN, in the flavor application department. She was granted a scholarship from General Mills in her senior year to conduct a research study in carbohydrate chemistry. Under the mentorship of Dr. James N. BeMiller, she completed her research study on the preparation and properties of amorphous pregelatinized maize starch. She graduated in May 2007 with highest distinction. She gained industry experience working as a research and development technologist at Kerry Ingredients and Flavours to develop spray dried cheese and other dairy products for various food applications.

With a deepened interest in research pertaining to our food system, she decided to pursue a Master's degree, with focus on dairy processing and chemistry, under the guidance of Dr. David M. Barbano. During the course of her study, she was actively involved in the product development team at Cornell University which competed nationally in the Institute of Food Technology (IFT) annual meetings and won 3rd place in 2011, and 1st place in 2012. Her aspiration is to assist the utilization of agricultural commodities, especially in the developing countries to be more sustainable and affordable in providing wholesome nutritious foods to their people.

I dedicate this work to
my late father, Halim Dharmadi, and my mother, Janny Karlina Kurnia.
Thank you for your love and sacrifice

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LIST OF ABBREVIATIONS

ANOVA .... **Analysis of variance**

CF .... **Concentration factor**

EMC .... **Enzyme modified cheese**

FDM .... **Fat in dry matter**

FFCC .... **Full-fat Cheddar cheese**

GLM .... **General linear model**

GMP .... **Glycomacropeptides**

GP .... **Graded permeability**

HTST .... **Hight temperature short time**

LFCC .... **Low-fat Cheddar cheese**

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

MPC .... **Milk protein concentrate**

MW .... **Molecular weight**

NDM .... **Nonfat dry milk**

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

NPN%TP .... **Non protein nitrogen as a percentage of true protein**

PCA .... **Principal component analysis**

RFCC .... **Reduced-fat Cheddar cheese**

RO .... **Reverse osmosis**

S/M .... **Salt in moisture**

SPC .... **Serum protein concentrate**

SW .... **Spiral-wound**

TA .... **Titratable acidity**

TCA .... **Trichloroacetic acid**

TN .... **Total nitrogen**

TP .... **True protein**

TS .... **Total solids**

UF .... **Ultrafiltration**

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

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

Chapter 1

Introduction: Milk proteins as food ingredients

Properties of Caseins

Milk proteins are valuable components of milk with high nutritional value and desirable functional properties, hence they are utilized as food ingredients. Bovine milk contains ca. 3.5 g total protein per 100 mL, and about 80% of the proteins are caseins that are the group of phosphoproteins that precipitate from milk at pH 4.6 at temperatures > 8°C (Mulvihill and Ennis, 2003). The non-casein proteins are called serum proteins, which include β-lactoglobulin, α-lactalbumin, bovine serum albumen, and immunoglobulins. The addition of rennet causes coagulation of caseins in milk. Caseins lack organized secondary and tertiary structure due to their high proline content, which render them heat stable (Fox and Kelly, 2004). For example, sodium caseinates in water are stable when heated to 140°C for 60 min, whereas serum proteins undergo denaturation and aggregation at temperatures >70°C (Mulvihill and Ennis, 2003). The size of casein micelles is on average 100 nm, which is roughly 100 times larger than serum protein (Walstra et al., 2006). Separation of caseins from serum proteins is feasible by exploiting principal differences between the two proteins (e.g. stability at pH 4.6, sensitivity to rennet, heat stability, particle size).

The production of caseins as food ingredients was initiated in the 1960's in Australia and New Zealand, which coincides with the need of functional ingredients in processed foods (Fox and Kelly, 2004). Previously, casein production was geared toward industrial applications (e.g. plastic, paints, glues). Now caseins are regarded as one of the principal functional food proteins (Fox and Kelly, 2004). The amphiphilic character, open and flexible structures of casein have been utilized in food systems to provide foaming, emulsifying, and water binding ability

(Rollema and Muir 2009). In addition to their functional properties, caseins provide essential amino acids required in the body, such as valine, leucine, isoleucine, phenylalanine, tyrosine and proline (Pritchard and Kailasapathy, 2011). Another nutritional aspect of caseins is that casein micelles contain calcium that is essential for bone health (Walstra et al., 2006). Some of the commercial casein products are rennet caseins, acid caseins, caseinates, co-precipitates , milk protein concentrate. Recent development in ceramic microfiltration (MF) has produced a novel casein ingredient, called micellar casein concentrate (MCC). The production and characteristic of casein products have been reviewed by Fox, 2001; Mulvihill and Ennis, 2003; Rollema and Muir; 2009; Modler, 1985; Augustin et al., 2011.

Various casein products

Rennet caseins. Rennet caseins are obtained by destabilization of casein micelles by chymosin-like proteinase which cleaves Phe_{105}-Met_{106} bond of κ-casein. Upon hydrolysis of κ-casein, the surface charge and steric repulsion which stabilize casein micelles becomes reduced, as a result micelles begin to aggregate into a gel network (curds) in the presence of sufficient ionic calcium. The curds are cooked (60°C) to encourage syneresis, increase firmness, and inactivate the coagulant. They are subsequently washed and dried. The drying process can range from roller drying, to fluidized bed drying, or spray drying. Depending on the drying process, a grinding step might be necessary to produce desired particle-size. The coagulation of casein by rennet takes place at neutral pH, therefore the mineral associated with the casein is retained. Rennet caseins have low solubility in water, but they can be solubilized in water at high pH (> pH 9) and by addition of calcium sequestering salts (e.g. phosphates, citrates)

(Mulvihill and Ennis, 2003). Rennet casein is mainly used in the production of cheese analogues, which includes the addition of polyphosphates (Fox and McSweeney. 1998)

Acid caseins. Acid caseins are obtained by isoelectric precipitation of caseins at pH 4.6 and at this pH, serum proteins remain soluble. The acidification can be achieved by the lactic acid produced by starter cultures or by addition of mineral acids (e.g., HCl, HNO_3, H_2SO_4). The acidification process dissolves the colloidal calcium phosphate in the casein micelles, hence acid caseins have lower mineral content compared to rennet caseins. Similar to rennet caseins, acid caseins are not soluble in water.

Caseinates. Caseinates are obtained by the addition of alkali (e.g. NaOH, NH_4OH, KOH, $Ca(OH)_2$) to acid caseins to reach pH 7. The increase in pH will dissolve the acid caseins and make them water-soluble. The caseinates produce a viscous solution, thus caseinate solutions are limited to about 20% solids for ease of handling during production (Augustin et al., 2011). As a result, the efficiency in the drying process is low. Calcium caseinates behave differently from other caseinates due to the fact that calcium interacts with the phosphoserine residues of the casein (Rollema and Muir, 2009). Calcium caseinates form highly aggregated colloidal dispersions which appears to be milky in appearance, whereas other caseinates form nearly clear to slight opalescence solutions (Rollema and Muir, 2009).

Co-precipitates. Co-precipitates contain casein and serum proteins in denatured form. Co-precipitates are produced by heating skim milk at 90 to 95°C for 30 min so that the majority of serum proteins are denatured and complexed with casein through a disulphide bond between β-lactoglobuin with κ-casein (Modler, 1985; Rollema and Muir, 2009). Upon acidification to pH 4.6 with mineral acid, the serum proteins co-precipitate with caseins. The addition of $CaCl_2$ is often included in the process to recover most of the milk proteins (Rollema and Muir, 2009). The

co-precipiates are washed and dried. Co-precipitates have relatively good solubility and form viscous solutions. Co-precipitates have a higher nutritional value than casein alone, and for this reason it is often used in the infant formulations.

Milk protein concentrate (MPC). Skim milk is converted to MPC using ultrafilration of skim milk to remove lactose and minerals, while retaining both the caseins and serum proteins. Unlike co-precipitates which using chemical modification to isolate milk proteins, MPC is made by physical separation technology which largely maintains the milk protein structure. By using ultrafiltration followed by diafiltration steps, MPC can be concentrated to 85 to 90% protein (dry weight basis). The protein content in milk for cheesemaking and yogurt production can be standardized using MPC in some countries (Fox, 2001). The MPC can be dried or used in a concentrated liquid form. Havea (2006) observed that the solubility of MPC powder decreased with storage time. The insoluble materials in rehydrated MPC was large particles (ca 100 μm) of casein micelles fused together, as observed under a transmission electron microscope. Havea (2006) believed that the casein micelles were fused together via non-covalent linkages (e.g. hydrophobic interaction), because when MPC powder was dissolved in 0.1% SDS solution instead of water, no insoluble materials was observed .

Micellar Casein Concentrate (MCC). It is now possible to separate serum proteins from caseins using microfiltration (MF) membranes without chemical modification of the milk (e.g. acid precipitation or enzyme addition). When skim milk is circulated along a MF membrane with 0.1 to 0.2 μm pore size, caseins and casein-bound minerals are retained by the membrane, while serum proteins, lactose, unbound minerals pass through the membrane. The MF retentate is an enriched milk solution of micellar casein. To achieve a more complete removal of serum proteins and lactose, diafiltration steps can be employed by diluting the MF retentate with RO

water to its original volume and use it as a feed in subsequent MF stages (Nelson and Barbano, 2005). The feasibility of manufacturing MCC has been shown in several studies using various membrane types: polymeric spiral-wound (SW) membrane (Beckman et al., 2010), uniform transmembrane pressure (UTP) ceramic systems (Hurt et al., 2010); graded permeabililty (GP) ceramic membrane (Zulewska et al., 2009), and Isoflux ceramic membrane (Adams, 2012). The types of membrane used in the manufacturing of MCC may affect the efficiency of transmission of serum protein, protein composition of the retentate, the level of residual casein in the permeate, and the cost of process (Zulewska et al., 2009). In a MF process of skim milk at 50°C with 3X concentration factor (CF) in a continuous bleed-and-feed process, the use of GP and UTP membrane systems had higher efficiency of serum protein removal than SW and Isoflux membrane (Zulewska et al., 2009; Adams, 2012). The GP and UTP membranes allow more transmission of serum proteins to the permeate than SW and Isoflux membranes (Zulewska et al., 2009). Thus, the production of MCC using SW and Isoflux membranes would require more processing time (more diafiltration stages) or additional membrane surface area to achieve a similar serum protein removal than GP and UTP membranes.

Theoretically, up to 97% of serum proteins can be removed by 3 stages of MF at 3X CF, assuming no rejection of serum proteins and complete rejection of caseins. Various processing factors can affect the serum protein removal rate (Hurt and Barbano, 2010). Excessive heating of skim milk (85°C) would promote the binding of serum proteins to casein micelles, and reduce the amount of serum proteins that can be removed from skim milk during MF (Hurt and Barbano, 2010). Different membranes have different serum protein removal factors that reflect differences in resistance of the MF membrane to passage of serum proteins through the membrane. With higher rejection of serum proteins, the true protein concentration in the MF

retentate for each stage increased, and the cumulative percentage of serum protein removal decreased (Hurt and Barbano, 2010). Other factors that influence the casein and serum protein separations are the initial composition of milk, and control of CF and amount of diafiltration during processing run (Hurt and Barbano, 2010).

The composition and properties of MCC is different from other available casein products. The main mechanism of producing rennet caseins, acid caseins, caseinates, and co-precipitates is by destabilizing native casein micelles by the addition of enzymes or acid. Hence, the resulting casein products are in a non-micellar form. In comparision, MCC is made by physical separation in which the casein micelles are maintained. The MCC also retains the bound minerals associated with the micelles, whereas in acid caseinates these minerals are solubilized lost into the whey. The MCC maintain the intact structure of the proteins, which is not the case for rennet casein that lost its glycomacropeptides (GMP) of κ-casein. The presence of oligosaccharides in GMP increases the hydrophilicity of the casein (Fox and Kelly, 2004). The structure of caseins in MCC is comparable to the ones in MPC, however the main difference between the two is the presence of serum protein in MPC. The MCC is a milk protein ingredient with properties that are different from other available protein ingredients, thus it might serve particular functions that other ingredients cannot deliver.

Viable forms of MCC

Liquid MCC. Liquid MCC is a fresh product of MF retentate of skim milk. Zulewska et al. (2009) reported the composition of skim milk MF retentate 3X CF (1-stage) at 7.79 to 8.70% true protein, and 84.97 to 86.04% moisture, depending on MF membrane types and system. In a 3-stage MF at 3X CF, the final retentate range from 8.52 to 9.08% true protein and 89.20 to

89.44 % moisture (using various membranes of polymeric spiral-wound, ceramic UTP and Isoflux (Hurt et al., 2010; Adams, 2012). Fresh liquid MCC is high in moisture content and needs to be refrigerated. This condition might add a significant cost when transporting MCC, especially for long distance. The high-cost of transporting refrigerated product might reduce the competitiveness of this product. This liquid form of MCC is suitable when used at the same site where it's being manufactured. For example, a cheese plant that is capable of running MF can produce this form of MCC to be used directly as an ingredient for the cheesemaking.

Concentrated MCC. The high moisture in liquid MCC can be partially removed by using UF membrane. An increase in viscosity as water is removed will limit the amount of water that can be practically removed. When the feed material in UF processing becomes too viscous, fouling will occur followed by a sharp decline in flux. There is an economic benefit of concentrating MCC, because it will decrease the volume of the product during transportation. Producing concentrated MCC may be a good way of storing the valuable casein in skim milk, and at the same time this process produces valuable co-products (e.g. serum protein concentrate and liquid lactose concentrate). The dairy industry often faces milk production seasonality. During the peak season of milk production, the demand for milk does not always match up. As a result, excess milk is converted to storable products e.g. butter, nonfat dry milk (NDM). In the production of NDM, excess skim milk is often transported long distance to a drying facility. This can add a significant cost, in addition to high cost of drying. The production of concentrated MCC would eliminate the cost associated with drying, and transportation cost to a drying facility when a MF system is set up in a milk processing plant in the area of high milk production. Capital investment of installing a MF system is less than building an evaporator and tower dryer, and it requires less space. To improve the competitiveness of concentrated MCC, shelf-life

stability of this product becomes an important factor. During the filtration process, the low molecular weight compounds (e.g. lactose, nonprotein nitrogen), which provide nutrients for microbial growth, can be removed with the permeate. Thus, lowering the amount of low molecular weight compounds could act as a hurdle for microbial growth and prolong the refrigerated shelf-life of concentrated MCC.

Dried MCC. Fresh liquid or concentrated MCC can be spray dried to produce powder MCC, which has longer shelf-life and requires no refrigeration. This form of MCC provides ease of handling, transporting, and storing. However, the MCC powder needs to be reconstituted to be used in food applications which is an extra step in the manufacturing process.

Possible application of MCC

Beverages. A MCC is a milk ingredient with high protein and low lactose content, which makes it suitable for high-protein, low-carbohydrate drink applications (e.g. sport drinks, meal replacement drink). The MCC is very heat stable and can withstand retort process without precipitating. Additionally, MCC is very bland in flavor and can provide improved mouthfeel in the absence of fat.

Cheesemaking. The use of MCC to fortify milk for cheese making, or replace milk altogether, is very logical given the fact that the protein in cheese is mostly casein. Papadatos et al. (2003) demonstrated an increase in net revenue for the cheesemaker when MF is done prior to cheesemaking, as calculated using a nonlinear programming optimization model. The economic benefits of using MF before cheesemaking are achieved by improvement plant efficiency and production of valuable co-products from the MF permeate (Papadatos et al., 2003). In order to remove a high percentage of serum proteins from skim milk while maintaining the same

concentrations of soluble minerals, nonprotein nitrogen and lactose of skim milk, Nelson and Barbano (2005) used the UF permeate of MF permeate of skim milk as a diafiltrant. Caron et al., 1997 observed an increase in gel firmness and coagulation time with milk fortified with protein (4 to 5% final protein) from MCC powder, probably due to higher calcium content that is complexed with casein and retardation of rennet diffusion in higher protein cheesemilk, respectively.

Low-fat cheese. The main components of the fat-free portion of the Cheddar cheese are protein (mainly casein), water, and minerals. The MCC consists of casein micelles, water (before being spray dried), and minerals. In other words, hydrated MCC has a similar composition to that of the fat-free portion of cheese and might be used as a building block to make low-fat Cheddar cheese. St.-Gelais et al. (1998) utilized different protein concentrate powders, e.g diafiltered MF retentate (similar to MCC powder), UF retentate powder, and calcium caseinate powder to fortify milk at 3%, 4%, 5% or 6% casein, and a target casein to fat ratio of 1.61 to produce ca. 45% reduced-fat Cheddar cheese. Cheese yield was higher for milk enriched with diafiltered MF retentate than UF retentate or calcium caseinate, especially at higher protein fortification (5% and 6% casein) (St-Gelais et al., 1998). The composition analysis of the whey from cheese made from milk enriched with calcium caseinate showed high amount of fat, which indicated that the curd did not retain fat well (St-Gelais et al., 1998). No sensory analyses of flavor and texture of these reduced-fat Cheddar cheeses were reported in this study (St-Gelais et al., 1998). The MCC is an ideal ingredient to build the structure of low-fat Cheddar cheese. However, the main problem with production of low-fat Cheddar cheese is the development of atypical Cheddar flavor during aging, and excessive firmness because reduction in fat concomitantly increases the protein content in the final cheese when made using a

conventional cheddar cheesemaking. Thus, the use of MCC in the production of low-fat Cheddar cheese needs to take a non-conventional approach to avoid development of atypical flavor during aging and excessive firmness. Nelson and Barbano (2004) introduced a unique process to produce reduced-fat Cheddar cheese (up to 50% fat reduction). The process developed by Nelson and Barbano (2004) involves tempering shredded full-fat Cheddar cheese (20 to 33°C) to melt the fat and separating the melted fat by centrifugal force. The resulting reduced-fat Cheddar cheese, which requires no aging, has a flavor that is at least as intense as the original full-fat Cheddar cheese, and it has softer texture than the full-fat Cheddar cheese. It's challenging to use the same technique to make low fat Cheddar cheese (at least 82% fat reduction) without concomitantly extracting the water-phase from the cheese, which contains compounds responsible for Cheddar flavor (McGugan et al, 1979). The MCC can be potentially added to this reduced-fat Cheddar cheese to produce low-fat Cheddar cheese.

The objectives of our research were, first to develop a process to produce a high concentration liquid micellar casein concentrate with a long refrigerated shelf-life. The second objective of our research was to develop an alternative process to produce low fat Cheddar cheese using MCC as an ingredient.

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Chapter 2

Production of an 18% Protein Liquid Micellar Casein Concentrate With a Long Refrigerated Shelf-life

ABSTRACT

Our objective was to develop a process to produce a high concentration liquid micellar casein concentrate (18% protein – MCC18) with a long refrigerated shelf-life. The MCC18 is a novel milk protein ingredient produced by fractionating skim milk using the microfiltration. To achieve a long refrigerated shelf-life, the processing of MCC18 was designed to maximize the removal of low molecular weight compounds, e.g. lactose, nonprotein nitrogen (NPN) which can be easily metabolized by microbes, while minimizing the microbial count in the final product. The production of MCC18 was done over a period of 5 d. The experiment was replicated 3 times in different wk with a different batch of raw milk. Raw whole milk was pasteurized and separated to produce skim milk. Skim milk was ultrafiltered (UF) to remove more than half of the lactose and NPN. The UF milk retentate was diluted with water and then microfiltered (MF) in 3 stages to remove approximately 95% of the serum protein and further remove lactose and NPN. The retentate from the last stage of MF was UF to concentrate the protein to 18% and batch pasteurized. MCC18 was collected immediately after processing in sterile plastic vials and stored at 4°C. The average MCC18 contained 21.78% total solids, 18.27% true protein, 0.31% nonprotein nitrogen and 0.13% lactose. The MCC18 at the day of processing contained a mean aerobic bacterial count of 2.1 log cfu/mL and mean aerobic spore count of 2.3 log cfu/mL. The MCC18 formed a solid gel at temperatures < 22°C, but the MCC18 reverted back to a liquid when warmed from 4°C temperature to > 22°C. This provides a unique opportunity in ingredient handling and packaging and eliminates the challenges encountered in reconstitution of dried milk

protein ingredients. The MCC18 produced in this study maintained a bacteria count < 20,000 cfu/mL for 16 wk when stored at refrigeration temperature of 4°C. Further study is needed to determine if there are changes in the organoleptic and functional properties of MCC18. We envision that the conversion of skim milk to MCC and its coproducts (serum protein concentrate and lactose concentrate) could be used as an alternative to production of nonfat dry milk to balance milk production seasonality, specifically the components of skim milk portion.
Key words: micellar casein, microfiltration, shelf-life

INTRODUCTION

Milk production varies throughout the year. During the spring, milk production reaches its peak, whereas the lowest milk production occurs during the fall. This trend has been very consistent from year to year. Because an increase in the milk production is not always in phase with variations in consumption, the dairy industry faces seasonal supply and demand imbalances each year (Weldon et al., 2003). The current strategy to minimize inevitable loss due to milk surplus is by converting excess milk to storable products, such as NDM, cheese, and butter. In the production of NDM, excess skim milk often needs to be transported long distances to a drying facility. The high cost of transportation, evaporation and drying to balance supply and demand, reduces the profitability of the dairy industry. During milk deficit months, extra skim milk solids are needed for many products. A technology to store the high value components in skim milk and using them in the fall when milk production is low could be a lower cost milk-balancing strategy. Therefore, the goal of our study was to develop an alternative for balancing the milk production seasonality, specifically the skim milk portion, by utilizing membrane technology. Skim milk consists of major components such as micellar casein, serum proteins,

and lactose. Refining skim milk into well-defined fractions could lead to a more profitable use of milk components. The individual milk fractions can be used as ingredients that fulfill specific needs that cannot be delivered by milk itself (Huffman and Harper, 1999). The milk protein fraction that is substantially low in lactose can be used to enhance nutritional values or to improve textural properties in food applications (Huffman and Harper, 1999). Further fractionating the major milk proteins, casein and serum protein, could produce protein ingredients that have distinct and enhanced functionalities. Similarly, isolated lactose can be transformed to value added ingredients for food and pharmaceutical applications (Durham, 2009). The isolated lactose from the milk filtration by-product can produce higher purity and yield when compared to the one obtained from cheesemaking whey, because the lactose fractionated directly from skim milk does not contain cheesemaking residuals (rennet, culture, color and lactic acid) (Nelson and Barbano, 2005).

With the advancement of membrane filtration technology, the milk fractionation process has become more technically and economically feasible (Papadatos et al., 2003; Brans et al., 2004). A microfiltration (MF) unit can be installed in a fluid milk processing plant or cheese plant, to separate excess skim milk and produce liquid micellar casein concentrate (MCC), liquid serum protein concentrate (SPC) and liquid lactose concentrate. Liquid MCC is a novel dairy ingredient which is characterized by high protein (mainly casein micelles) and low NPN and lactose contents. The more widely used methods to isolate caseins are isoelectric precipitation and proteolytic coagulation (Mulvihill and Ennis, 2003). Casein isolated by filtration is unique because the protein is in its micellar form and not contaminated with any additives (e.g. acids, alkalis, enzymes), which can affect the flavor profiles of casein.

The feasibility of producing MCC by filtration processes have been demonstrated previously (Saboya and Maubois, 2000; Nelson and Barbano, 2005, Hurt et al., 2010, Beckman et al., 2010). Production and utilization of liquid MCC and SPC membrane concentrates would eliminate the costs associated with drying and would enhance the economic competitiveness of this approach. However to capture this economic advantage, shelf-life stability of liquid protein concentrates becomes an important factor. Shelf-life stability of a product is defined as the time during which the product remains safe and exhibits no organoleptic defects (Muir, 1996). In general, the shelf-life of dairy product is limited by the growth of spoilage bacteria (Muir, 1996). Spoilage bacteria produce enzymes that can degrade milk constituents and cause unacceptable quality. A liquid MCC with a long refrigerated shelf-life could be distributed long distances (with much less volume than original milk), also to be stored for future use when milk production is low and when additional casein is needed for cheese or cultured product production. There has not been any report to date regarding the shelf-life stability of liquid MCC. For the purpose of this study, the end of shelf-life was defined as total bacterial counts >20,000 cfu/ml (>4.3 log cfu/mL). Our objective was to develop a process to produce a high concentration liquid micellar casein concentrate 18% protein (MCC18) with a long refrigerated shelf-life.

MATERIALS AND METHODS

Experimental Design and Statistical Analysis

The production of MCC18 was done over a period of 5 d in the Cornell Pilot Plant. The experiment was replicated 3 times starting with a different batch of raw whole milk each time. All raw whole milk was received from the Cornell University dairy farm. Throughout the

processing, the exposure of the product to open air was minimized by covering any open vats, tanks, milk cans that were being used. This was done to avoid airborne contamination, especially from spores that were ubiquitous in the pilot plant environment. In order to have a long refrigerated shelf-life, the processing of MCC18 was designed to maximize the removal of low molecular weight (MW) compounds (e.g. lactose, NPN) which can be easily metabolized by microbes and used as nutrient sources. The manufacturing process involved UF and multiple stages of MF in which low MW compounds were removed with the permeate. Another strategy to prolong refrigerated shelf-life was to minimize the microbial count in the final product which was achieved through gravity separation (Caplan, 2007) and pasteurization. The final product of pasteurized MCC18 at wk 0 was analyzed for total aerobic bacteria and spores. Over the course of 16 wk, the total aerobic bacteria count was determined weekly to assess the shelf-life stability of MCC18 stored at 4°C. It was important to note that due to the capacity of the processing equipment and limited staffing, the complete manufacturing process required 5 d. However, in a factory setting, it would be a continuous process that would run continuously in the same day. Thus, from a microbial contamination and microbial growth perspective, the processing conditions in our study are probably not as good as those that could be achieved commercially in a process designed for this purpose.

Chemical composition of pasteurized skim milk, MF stage 1 to 3 retentate, and pasteurized MCC 18 at wk 0 was analyzed by ANOVA using the Proc GLM procedures of SAS (version 8.02, SAS Institute Inc., Cary, NC), followed by running the least square means if the F-test for the model was significant (i.e., $P < 0.05$). Bacterial and spore data were log-transformed and used as a response in a GLM model to determine if there were significant differences in log bacterial and spore count in raw milk, gravity separated cream, gravity separated skim milk,

pasteurized gravity separated skim milk, pasteurized skim milk, UF milk retentate, MF stage 1 retentate, MF stage 3 retentate, MCC18 before and after batch pasteurization. Log bacterial count of MCC18 at wk 0 to 16 was analyzed by ANOVA to determine if the effect of replicate and time (wk of storage) were significant. The model was dependent variable (log bacterial count MCC18) = time + replicate + time x replicate + error. Replicate was treated as a categorical variable. Time was treated as a continuous variable and was transformed by mean centering the weeks of storage to minimize distortion of the ANOVA model by multicollinearity (Glantz and Slinker, 2001). Time was transformed as follows: time = wk of storage – [(wk 16 – wk 0)/2].

Manufacturing of MCC 18

The processing overview of MCC18 production is shown in Figure 1. The processing in each day was described in details as follows:

Day 1. Raw whole milk (approximately 1170 kg) was weighted into cone bottom gravity separation tanks and held at 4°C.

Day 2. After 20 h at 4°C, the bottom 90% of the milk in each tank was collected by weight. The gravity separated cream was not used in the present study. The bottom 90% of the milk (gravity separated skim, about 2.2% fat) was then pasteurized at 72°C for 16 s with a plate heat exchanger with 3 sections: regeneration, heating and cooling (model 080-S, AGC Engineering, Manassas, VA), and then separated at 49°C using a cream separator (model 619, DeLaval Co., Chicago, IL). The pasteurized skim milk was ultrafiltered (50°C) using a polyethersulfone spiral-wound UF membrane with a nominal pore size of 10,000 Da (model 3838, GEA Niro Inc., Hudson,WI) in a batch recirculation mode (2.2X concentration factor or CF). The UF system was a two-pump system with a feed pump and a recirculation pump to

maintain cross flow velocity and minimize fouling. Prior to and after UF processing, the UF membrane system was cleaned using the procedures described previously by Evans et al. (2009). The purpose of this UF step was to remove a little more than half of the lactose and NPN from the skim milk prior to MF. The UF retentate inlet pressure was 276 kPa, and the retentate outlet pressure was 103 kPa. The retentate and permeate composition, e.g. protein, lactose, and fat, was analyzed every 15 min for process control using an infrared spectrophotometer (Lactoscope FTIR, Delta Instruments, Drachten, The Netherlands). At the end of the UF processing run, the retentate was diluted with cold reverse osmosis (RO) water (4°C) to decrease the protein content to the level in the original skim milk and followed by storage overnight at 4°C.

Day 3. The RO water diluted UF retentate was heated to 50°C using a plate heat exchanger (Model A3, DeLaval, Inc., Kansas, MO) and then fed into an MF system (Tetra Alcross MFS-7, TetraPak Filtration Systems) with 0.1µm nominal pore diameter ceramic Membralox graded permeability (GP) membrane (model EP1940GL0.1µAGP1020, alumina, Pall Corp.) and 1.7 m^2 surface area. This MF process (MF stage 1) was a continuous bleed and feed. The CF was set to 3X with retentate and permeate removal rates of 60 L/h and 120 L/h, respectively, which produced an MF permeate flux of 70 L/m^2h. The MF retentate was cooled and held at 4°C to be processed the next day. The detailed procedures for the cleaning of the ceramic GP membrane system prior to and after milk processing are provided by Zulewska et al. (2009).

Day 4. The MF retentate from the previous day was diluted with RO water to achieve a target true protein of 2.8%, as measured by an infrared spectrophotometer. It was then heated to 50°C by an AGC plate heat exchanger (model 080-S, AGC Engineering, Manassas, VA) before being microfiltered. This MF diafiltration process (MF stage 2) was a continuous bleed and feed

with a CF of 3X. The retentate and permeate removal rates were set to 70 L/h and 140 L/h, respectively, resulting in an MF permeate flux of 82 L/m^{2}h. The stage 2 MF retentate was mixed with 50°C RO) water (2 kg of RO water per 1 kg of retentate) and used as the feed solution for MF stage 3. The retentate and permeate removal rates at MF stage 3 were maintained at 70 L/h and 140 L/h, respectively, resulting in an MF permeate flux of 81 L/m^{2}h and the final MF retentate was concentrated to 10.5% protein, as measured using an infrared spectrophotometer, in a batch operation process. The sampling of MF stage 3 retentate reported in Table 3 was taken from the feed tank, into which the retentate was collected and mixed with the feed solution. The product was cooled to 4°C and stored to be processed the next day.

Day 5. The MF retentate was heated to 50°C and then UF in a batch operation process. The purpose of this step was primarily to concentrate the protein to about 18%, but it also removed some additional lactose and NPN. The retentate inlet pressure was 276 kPa, and the retentate outlet pressure was 103 kPa. The UF process was stopped when the protein content of the product in the feed tank was 18% as measured using an infrared spectrophotometer. The resulting MCC18 was batch pasteurized at 157°F for 30 min in a stainless steel jacketed steam kettle (Groen DN30, Chicago, IL) to kill vegetative bacterial cells. Liquid MCC18 after batch pasteurization was immediately collected into 48 90-mL plastic snap-top vials (model CPP03CL, Capitol Vial, Inc., Auburn, AL) and stored in a 4°C cooler. Thirty-two filled vials were designated for shelf life study to determine bacterial growth over a 16 wk period, and the rest of the vials were used for chemical analyses.

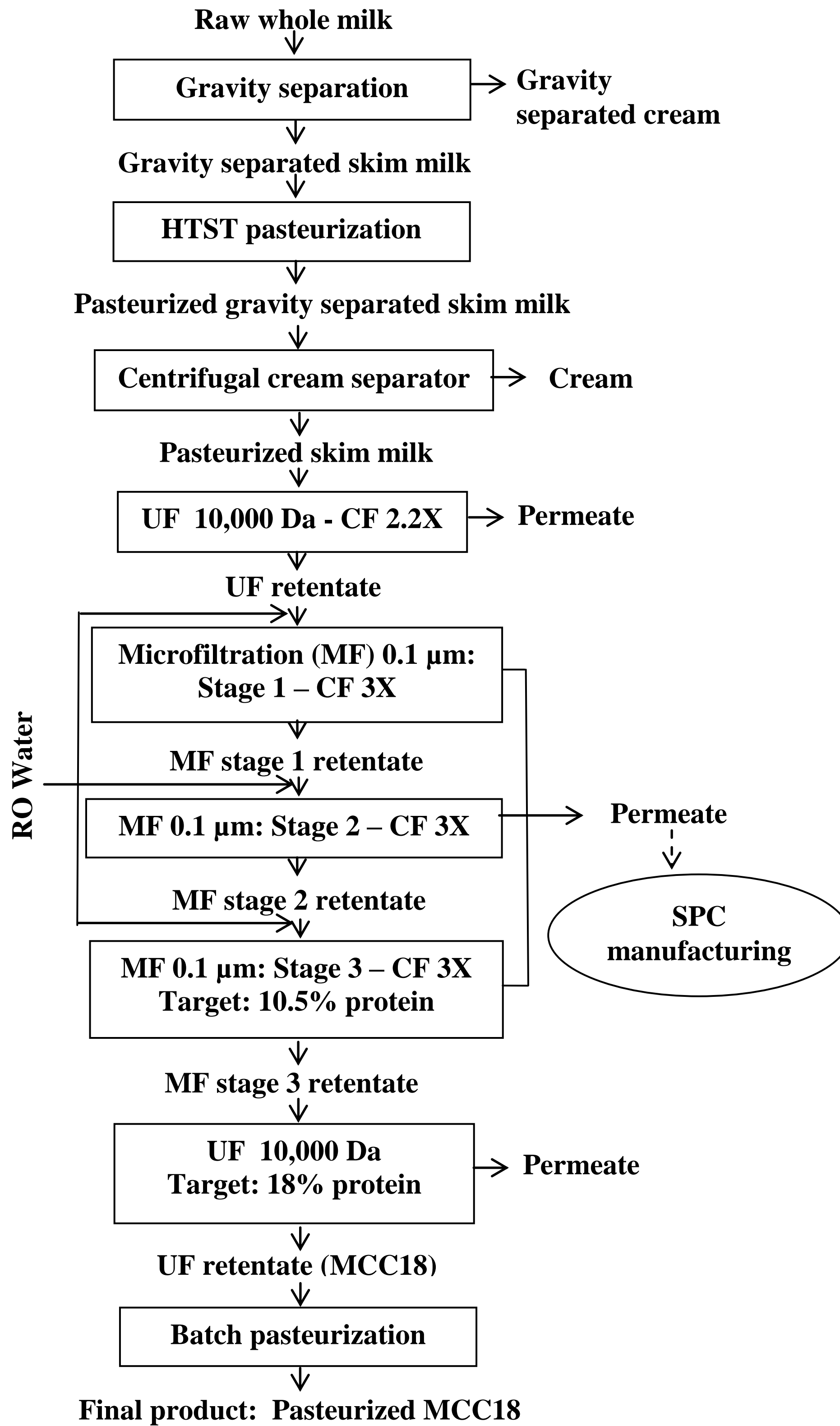

Figure 2.1. Schematic process diagram of liquid MCC18 with a long refrigerated shelf-life.

Chemical Analyses

Samples of pasteurized skim milk, permeate, and retentate at each filtration stage were analyzed in duplicate for TS by drying in a forced-air oven at 100°C for 4 hours (AOAC, 2000; 33.2.44, method 990.20), fat content by ether extraction (AOAC, 2000; 989.05 method 33.2.26), lactose by the enzymatic method (AOAC, 2006.06, method 33.2.67, Lynch et al., 2007) using Megazyme lactose kit # K-LACGAR (Megazyme International Ireland Ltd., Bray, County Wicklow, Ireland). The total N (TN) and NPN were determined in duplicate by the Kjeldahl method (AOAC, 2000; 991.20, method 33.2.11; 991.21, method 33.2.12, respectively). For MCC18, the analyses of TN and NPN were done in triplicate. True protein (TP) was calculated by subtracting NPN from TN. The nitrogen conversion factor used was 6.38. The high protein retentates were diluted with RO water to a protein level similar to milk for TN and NPN analyses.

Microbiological Analyses

Total aerobic bacteria and spores counts were determined for the incoming raw milk, gravity separated cream , gravity separated skim , pasteurized skim milk, UF milk retentate, MF stage 1 retentate, MF stage 3 retentate, and the final product of MCC 18 after batch pasteurization. RO water used in the diafiltration steps was also analyzed for total aerobic bacteria and spores. All the samples were held at 4°C prior to the analysis, which was done on the same day the sample was collected. For the total aerobic bacteria (method 6.040, Wehr and Frank, 2004), samples were collected in sterile 90-mL plastic snap-top vials (model CPP03CL, Capitol Vial, Inc., Auburn, AL). For the total aerobic bacterial spore count (method 8.090, Wehr and Frank, 2004), the samples were collected into sterile 300-mL plastic snap-top vials (model

CPP10LK-CL, Capitol Vial, Inc., Auburn, AL) to hold approximately 200 mL sample. Samples were incubated in a water bath until the temperature reached 80°C and then held for 12 min to germinate the spores. A pilot vial filled with the same sample material was used as a temperature control to avoid contaminating the actual sample when in contact with the thermometer probe. The sample was cooled in an ice bath until the temperature reached at least 35°C before being plated. The 3M Petrifilm Aerobic Count (3M, St.Paul, MN) was used for both aerobic bacteria and spores enumeration. For each sample, serial dilutions were made in a sterile phosphate buffer (Weber Scientific, Hamilton, NJ), and each dilution was plated in quadruplicate. All petrifilms were incubated at 32 ± 1°C for 48 ± 3 h. Petrifilm containing 25 to 250 colonies were counted when determining total aerobic bacteria, and the reported count was rounded to 2 significant digits (method 6.020, Wehr and Frank, 2004). For sequential dilutions that both contained 25 to 250 colonies, the reported count was calculated using the following formula, $N=\sum C/[(1 \times n_1)+(0.1 \times n_2)] \times d$, where N = number of colonies (cfu/mL), $\sum C$ = sum of all colonies on all plates counted, n_1 = number of plates in lower dilution counted, n_2 = number of plates in next higher dilution counted, and d = dilution from which the first counts were obtained (method 6.020, Wehr and Frank, 2004).

Shelf Life Study

Two vials were selected randomly from the population of 32 vials for total aerobic bacteria count each wk, for a period of 16 wk using the 3M Petrifilm Aerobic Count method. After sampling, the remainder of product in that vial was discarded. MCC 18 formed a firm gel at 4°C; thus the vial was tempered in a 45°C water bath for 20 min to liquefy the sample for ease of sampling and plating. For the purpose of this study, the end of shelf-life was defined as total

bacterial counts >20,000 cfu/mL (>4.3 log cfu/mL). The cutoff of 20,000 cfu/mL has been used as the legal limit for the shelf life of pasteurized milk based on the Pasteurized Milk Ordinance (FDA, 2009).

RESULTS AND DISCUSSION

Microbiological Analyses

Microbiological quality of the raw milk was assessed by determining total aerobic bacterial and spore counts. The mean bacterial count of the raw milk from the 3 replicates was 3.8 log cfu/mL (Table 2.1). The spore count in the raw milk was reported as < 1.4 log cfu/mL because it was below the optimal countable range (25 to 250 colonies/plate) for all 3 replicates (Table 2.2). When the raw milk was gravity separated, the fat rose to the top along with bacteria and spores. After 20 h at 4°C, the mean bacterial count in the top 10% of the raw milk by weight (gravity separated cream) was 5.0 log cfu/mL, which was significantly higher ($P < 0.05$) than in the raw milk before gravity separation, whereas the mean spore count in the gravity separated cream was 2.2 log cfu/mL (Tables 2.1 and 2.2, respectively). The gravity separated skim was collected, and the bacteria in this portion was significantly reduced ($P < 0.05$) to an average count of 2.8 log cfu/mL (Table 2.1) when compared to the bacteria in the raw milk. The mean spore count of the gravity separated skim was <1.4 log cfu/mL for all 3 replicates. It is crucial to remove spores originating from raw milk because they are capable of germinating under refrigeration temperature and eventually limit the shelf life of the final product (Fromm and Boor, 2004; Barbano et al., 2006). It was shown in this study that the use of gravity separation in the raw milk removed spores and bacteria from the gravity separated skim portion by concentrating them in the cream portion. This observation has been reported in previous studies,

although the mechanism of this natural process is not understood (Dellagio et al., 1969; Caplan, 2007).

The HTST pasteurization of the gravity separated skim reduced bacterial count to <1.4 log cfu/mL (Table 2.1). The spore count of the pasteurized gravity separated skim was <1.4 log cfu/mL, except in replicate 3 with 1.7 log cfu/mL (Table 2.2). The next steps in processing, which included centrifugal separation, UF and MF, were expected to increase microbial count due to environmental contamination. Because UF and MF membranes used in this study had smaller pore size than the size of microbes (0.4 to 2.0 μm), microbes which survived pasteurization and any microbial contamination introduced during processing and handling were retained by the membrane and concentrated in the same manner as the casein micelles (Brans et al., 2004; Saboya and Maubois, 2000). The microbial load of RO water used in the diafiltration steps was analyzed and found to have < 1.4 log cfu/mL for both total bacterial and spore counts, except in replicate 1 which had a total bacteria count of 1.72 log cfu/mL and total spore count of 1.73 log cfu/mL. As expected, the mean bacterial count showed a significant increase during successive UF and MF process. The mean bacterial count of UF milk retentate, MF stage 1 retentate, MF stage 3 retentate, and MCC 18 before being batch pasteurized were 1.8 log cfu/mL, 2.2 log cfu/mL, 3.3 log cfu/mL, and 3.4 log cfu/mL, respectively (Table 2.1). At the end of MF stage 3, the mean spore count in the retentate was 2.2 log cfu/mL and concentrated to 2.4 log cfu/mL in MCC18 before being batch pasteurized (Table 2.2). The batch pasteurization of MCC18 as the last processing step was conducted to reduce vegetative bacterial cells that were introduced and concentrated during processing and handling. The final MCC18 at wk 0 had mean bacteria count of 2.1 log cfu/mL and mean spore count of 2.3 log cfu/mL.

Table 2.1. Total aerobic bacterial counts in raw whole milk, gravity separated cream, gravity separated skim milk, pasteurized gravity separated skim milk, pasteurized skim milk, UF milk retentate, MF stage 1 retentate, MF stage 3 retentate, MCC18 before and after batch pasteurization for replicate 1, 2, and 3.

	Total aerobic bacteria (log cfu/mL)			
Materials	Replicate 1	Replicate 2	Replicate 3	Mean[2]
Raw whole milk	3.4	4.1	4.0	3.8[b]
Gravity separated cream	4.9	5.1	5.0	5.0[a]
Gravity separated skim milk	2.5	3.0	3.0	2.8[e]
Pasteurized gravity separated skim milk	< 1.4[1]	< 1.4	< 1.4	-
Pasteurized skim milk	< 1.4	< 1.4	1.5	-
UF milk retentate	1.7	1.5	2.1	1.8[h]
MF stage 1 retentate	1.7	1.7	3.2	2.2[f]
MF stage 3 retentate	2.4	3.9	3.6	3.3[d]
MCC18 before batch pasteurization	2.9	3.4	3.8	3.4[c]
Final product – pasteurized MCC18	2.0	1.9	2.3	2.1[g]

[a-h]Means in the same column not sharing a common superscript are different ($P < 0.05$).
[1] The result was below the optimal countable range of 25 to 250 cfu/mL, before being log-transformed.
[2] SE = 0.012

Table 2.2. Total aerobic spore counts in raw whole milk, gravity separated cream, gravity separated skim milk, pasteurized gravity separated skim milk, pasteurized skim milk, UF milk retentate, MF stage 1 retentate, MF stage 3 retentate, MCC18 before batch pasteurization, and pasteurized MCC 18 for replicate 1, 2, and 3.

	Total aerobic spores (log cfu/mL)			
Materials	Replicate 1	Replicate 2	Replicate 3	Mean[2]
Raw whole milk	< 1.4[1]	< 1.4	< 1.4	-
Gravity separated cream	2.0	2.1	2.7	2.2[c]
Gravity separated skim milk	< 1.4	< 1.4	< 1.4	-
Pasteurized gravity separated skim milk	< 1.4	< 1.4	1.7	-
Pasteurized skim milk	< 1.4	< 1.4	1.9	-
UF milk retentate	< 1.4	< 1.4	2.1	
MF stage 1 retentate	< 1.4	1.6	2.2	-
MF stage 3 retentate	2.1	1.9	2.7	2.2[c]
MCC18 before batch pasteurization	2.5	1.9	2.9	2.4[a]
Final product – pasteurized MCC18	2.5	1.8	2.6	2.3[b]

[a-c] Means in the same column not sharing a common superscript are different ($P < 0.05$).
[1] The result was below the optimal countable range of 25 to 250 cfu/mL, before being log-transformed.
[2] SE = 0.017

Chemical Analyses

Pasteurization of the gravity separated skim milk was carried out at 72°C for 16 s to produce minimal heat denaturation of SP and binding of SP to casein which can negatively affect the removal of serum protein (Hurt and Barbano, 2010). The MF stage 3 was initially a bleed and feed process with 3X CF, but at the end of the run, the retentate was concentrated in a batch operation process which accounted for an increase in TS, TN and TP from the MF stage 2 retentate to MF stage 3 retentate (Table 2.3). The MF stage 3 retentate was further concentrated using UF to produce higher TS, TN and TP in the final MCC 18 (Table 2.3). The principal NPN compounds in milk include urea, creatine, creatinine, uric acid, orotic acid, hippuric acid, peptide, ammonia, and α-amino acid (Wolfschoon-Pombo and Klostermeyer, 1981), all of which are small enough in size to pass freely through UF and MF membranes (Saboya and Maubois, 2000). The nitrogen from these low molecular weight compounds is more readily utilized by microbes as nutrient sources compared to the nitrogen from more complex compounds (e.g. intact proteins). Therefore, the removal of NPN during the filtration process was expected to improve the shelf-stability of MCC18. In theory, NPN content in the retentate would be reduced throughout the filtration process. The NPN in MF stage 2 retentate and MF stage 3 retentate was lower than in skim millk ($P < 0.05$) (Table 2.3). The last UF processing step increased the NPN value from 0.12% in the MF stage 3 retentate to 0.27% in the final pasteurized MCC18 (Table 2.3). The increase of NPN from the MF stage 3 retentate to pasteurized MCC18 gave the impression that the NPN was retained by the UF membrane, although it was plausible that the NPN measured in this sample was not the typical low molecular weight nitrogen compounds associated in milk. As an example, phospholipids in milk (e.g. phosphatidylcholine, phosphatidylethanolamine) contain nitrogen (Fox and McSweeney, 1998) that could be soluble

in 12%TCA, hence would be counted as NPN. Phospholipids as part of milk fat globule membrane present in the skim milk were concentrated throughout the UF and MF process, and the phospholipids may have become a substantial fraction of the NPN. When NPN was measured as a percent of true protein (NPN%TP), a significant reduction was seen from the pasteurized skim milk (6.22%) to MF stage 1 retentate (1.6%). Further reduction of NPN%TP in the remainder of the process was not detected. The NPN%TP of the MCC18 was 1.47%, significantly lower than of the skim milk. The mean lactose content was reduced significantly ($P < 0.05$) from 4.74% in the pasteurized skim milk to 0.13% in the final MCC18 (Table 2.3). Lactose serves as a carbon source and provides nutrients for microbial growth. Lowering the amount of lactose could act as a hurdle for microbial growth, thus it can be beneficial to prolong the shelf-life of MCC18.

Table 2.3. Mean (n = 3) composition of pasteurized skim milk, MF stage 1 retentate, MF stage 2 retentate, MF stage 3 retentate, and pasteurized MCC18.

Sample	TS	TN[1]	TN (dwb)[2]	NPN[3]	TP[4]	NPN %TP[5]	Lactose
	%						
Pasteurized skim milk	9.09^{d}	3.25^{d}	35.78^{d}	0.191^{b}	3.06^{d}	6.22^{a}	4.74^{a}
MF stage 1 retentate	11.01^{c}	7.75^{c}	70.40^{c}	$0.122^{b,c}$	7.63^{c}	1.60^{b}	1.93^{b}
MF stage 2 retentate	9.16^{d}	7.40^{c}	80.70^{b}	0.074^{c}	7.32^{c}	1.01^{b}	0.64^{c}
MF stage 3 retentate	13.30^{b}	11.21^{b}	$84.24^{a,b}$	0.117^{c}	11.09^{b}	1.05^{b}	0.19^{d}
Pasteurized MCC18	21.78^{a}	18.58^{a}	85.36^{a}	0.271^{a}	18.27^{a}	1.47^{b}	0.13^{e}
SE	0.251	0.172	0.880	0.015	0.147	0.128	0.0009

[a-e]Means in the same column not sharing a common superscript are different ($P < 0.05$).
[1] TN = total nitrogen x 6.38.
[2] TN (dwb) = TN (dry weight basis).
[3] NPN = nonprotein nitrogen x 6.38.
[4] TP = true protein (TN - NPN) .
[5] NPN%TP = nonprotein nitrogen as a percentage of true protein

Shelf Life Study

The aerobic bacterial count of the pasteurized MCC18 immediately after manufacturing (at wk 0) was determined to be 2.0 log cfu/mL, 1.9 log cfu/mL, and 2.3 log cfu/mL for replicate 1, 2, and 3, respectively (Table 2.1). The mean aerobic bacterial counts of the MCC18 from two vials randomly sampled at each wk were plotted as a function of weeks of storage for replicate 1, 2, and 3 (Figure 2.2). In replicate 2 for 1, 5, and 6 wk of storage, the aerobic bacterial counts of MCC18 between the two vials were greater than 2-log difference. The vial with higher aerobic bacterial count was suspected to have post-processing contamination, e.g. accidentally touching the sample during the open air hand-filling. Therefore, the high count might not have represented the aerobic bacterial count of the actual product. These possible outliers were removed when running the ANOVA test to determine the effect of time (wk of storage), replicate, and time x replicate. No change in the aerobic bacterial count over 16 wk ($P > 0.05$) was detected, however the effect of replicate on the aerobic bacterial count was significant ($P < 0.05$). The interaction effect between time and replicate was not significant ($P > 0.05$) and was removed from the model. With the effect of time being not significant ($P > 0.05$), it was concluded that the bacteria in MCC18 were still in the lag phase and not actively proliferating during 16 wk of storage in 4°C. The spore counts of the pasteurized MCC18 at wk 0 were determined to be 2.5 log cfu/mL, 1.8 log cfu/mL, and 2.6 log cfu/mL for replicate 1, 2, and 3, respectively. There was no indication of spore germination over 16 wk of storage at 4°C. Given the end of shelf life defined as bacterial count over > 4.3 log cfu/mL, MCC18 manufactured in this study was shown to have a microbial shelf-life of at least 16 wk when stored at refrigeration temperature of 4°C.

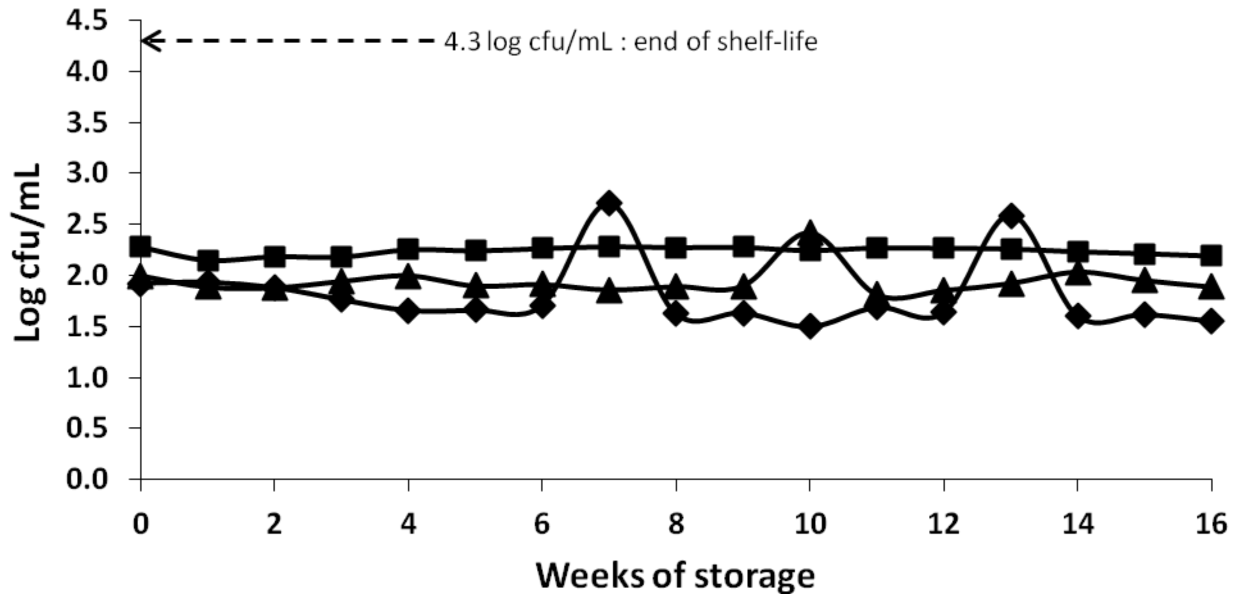

Figure 2.2. Log total bacteria count of MCC18 determined weekly over a 16-week period for replicate 1 (▲), replicate 2 (◆), and replicate 3 (■).

Implication to the dairy industry

The processing method proposed in this study includes, among others, gravity separation and membrane filtration. Gravity separation of milk is an efficient technique to remove spores and thermoduric bacteria. Moreover, gravity separation is a simple technology that can be implemented with minimal additional capital and operating cost. The use of membrane filtration technology has increased in the dairy industry, especially with the advanced development of membrane designs and system configurations which can minimize fouling, improve flux and selectivity, while maintaining chemical and heat stability of the membrane for prolonged use (Saboya and Mauboius, 2000).

The MCC18 is a pourable liquid at room temperature (22°C) or higher, and becomes gel at refrigeration temperature (4°C) or lower (Figure 2.3). With this property, it is important that MCC18 needs to maintain high enough temperature during processing to prevent clogging in the

processing lines. A hot fill process would be ideal for MCC18. The solid consistency of MCC18 during refrigeration storage provides ease of handling and transporting in wide variety of bulk packaging and handling systems.

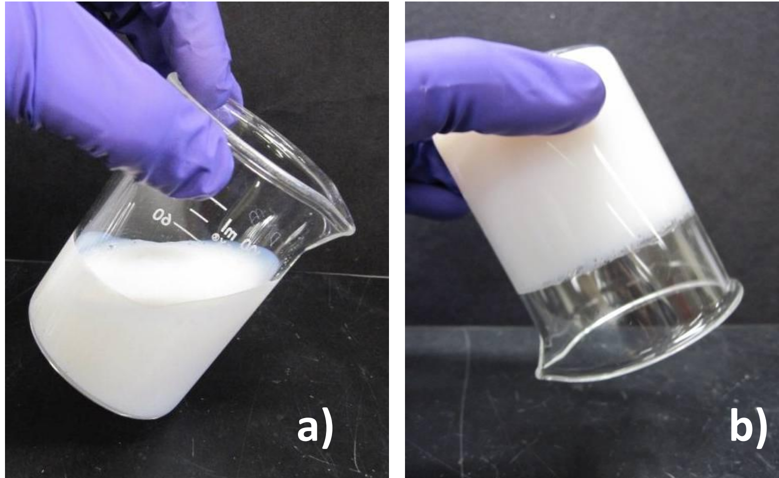

Figure 2.3. Micellar casein concentrate 18% protein exists as liquid form at 22 °C (a) and firm gel at 4°C (b)

The permeate from MCC production contains valuable components which can be processed to produce serum protein concentrate and lactose concentrate. In determining the economic feasibility of skim milk fractionation, the processing of all individual milk fractions (micellar casein concentrate, serum protein concentrate, lactose concentrate) needs to be evaluated. The resulting products of this process are not standard dairy commodity products, instead they are value-added ingredients which can have higher economic value in the market due to their distinct functional properties.

The MCC18 could be used as an ingredient in cheesemaking. Casein is the primary protein that is retained in the cheese curd, while other proteins are lost in the whey. The use of MCC can be beneficial for cheesemaking because it would increase the amount of cheese produced per day, given the same volume of the starting material in a vat. Consequently, the

utilization of the plant capacity will be maximized. Pierre et al. (1992) reported a reduction in coagulation time and an increase in cheese curd firmness when using reconstituted MCC powder as the starting material instead of raw milk. Papadatos et al. (2003) demonstrated that the use of MF milk (rich in casein) in Cheddar cheesemaking resulted in greater net revenue when compared to the one using NDM and condensed milk for fortification. The minimal serum protein present in MCC reduced the detrimental effect of heating on rennet coagulability (Saboya and Maubois, 2000). Given the unique composition of MCC, it is an attractive ingredient to produce purified nutraceutical derivatives from milk proteins or to be used in nutritional drinks that are high in protein and low in carbohydrate.

The MF permeate from MCC manufacturing can be processed to serum protein concentrate (SPC), which is a nutritious and functional dairy protein. In comparison to whey protein concentrate (WPC) derived from the cheesemaking whey, SPC is practically sterile, absent of cheesemaking residuals (milk coagulation enzymes, lactic acid, starter culture), and has minimal amount of fat (Britten and Pouliot, 1996). These composition differences affect sensory and functional properties. SPC has less lipid oxidation products and aroma-active compounds than WPC (Evans et al., 2010). In addition, SPC has a better solubility, foaming and gelling properties than WPC (Britten and Pouliot, 1996; Punidadas and Rizvi; 1998). Lactose recovered from MF milk permeate has higher purity and consequently higher lactose crystal recovery (Nelson and Barbano, 2005). This high quality lactose might be preferred for certain niche markets, such as pharmaceutical and baby formulas.

CONCLUSIONS

A process to produce MCC18 with a long refrigerated microbial shelf-life was developed. The MCC18 formed a solid gel at temperatures < 22°C, but the MCC18 reverts back to a liquid when warmed from 4°C temperature to > 22°C. This provides a unique opportunity in ingredient handling and packaging and eliminates the challenges encountered in reconstitution of dried milk protein ingredients. The MCC18 produced in this study maintained a bacterial count < 20,000 cfu/mL for 16 wk when stored at refrigeration temperature of 4°C. Further study is needed to determine if there are changes in the organoleptic and functional properties of MCC18. We envision that the conversion of skim milk to MCC and its coproducts (SPC and lactose concentrate) could be used as an alternative to production of NDM to balance milk production seasonality, specifically the components of skim milk portion.

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Chapter 3

A New Method for Production of Low-Fat Cheddar Cheese.

ABSTRACT

The objective of our study was to develop an alternative process to produce low-fat Cheddar cheese (LFCC) by combining reduced-fat Cheddar cheese (RFCC) made by a fat removal process with micellar casein concentrate (MCC) to try to achieve texture and flavor characteristics of full-fat Cheddar cheese (FFCC). The production of LFCC was replicated 3 times with a different batch of commercial FFCC, from which RFCC was produced, as an ingredient in LFCC-making. The MCC was produced by ultrafiltration of skim milk, followed by 3 stages of microfiltration, and the final product was spray dried. The LFCC was formulated to achieve 6% fat, 28% protein, 1.2% salt by combination of RFCC, MCC powder, salt, and water. The 6% fat target was used to comply with the FDA standard for a low-fat label claim. The pH of the LFCC mixture was adjusted to 5.3 by lactic acid. Rennet was added, followed by pressing and packaging. Chemical and sensory data were analyzed by ANOVA using the Proc GLM of SAS to determine if there were differences on chemical compositions and sensory among different cheeses. Descriptive sensory scores were used to construct a PCA biplot to visualize flavor profile differences among cheeses. The LFCC had 83% less fat, 32% less sodium, higher protein and moisture than FFCC. When the cheese texture was evaluated in the context of a filled-gel model consisting matrix and filler (100% minus % matrix), the LFCC had lower filler volume than FFCC, yet the LFCC had a softer texture than FFCC. The LFCC contained some of the original FFCC cheese matrix that had been disrupted by the fat removal process, and this

original FFCC matrix was embedded in a LFCC matrix formed by the action of rennet on casein from the continuous phase of hydrated MCC. Thus, the texture of the LFCC was desirable and was softer than the FFCC it was made from, whereas commercial RFCC (50% and 75% fat reduction) were firmer than the FFCC. The sulfur flavor in LFCC was closer to FFCC, than commercial RFCC. The LFCC had bitter and grape-tortilla off-flavors which came from the dried MCC ingredient. The commercial RFCC and LFCC made in this study were missing the typical aged Cheddar character (catty, nutty, fruity, brothy, milkfat) found in FFCC. Future work to improve the flavor of LFCC made by the process described in this study should include the addition of a flavoring ingredient, e.g. enzyme modified cheese, to enhance the aged Cheddar flavors and mask undesirable flavors.

Key words: cheese, low fat, micellar casein concentrate

INTRODUCTION

With a rising prevalence of obesity in the US, individuals are advised to make significant changes in their lifestyle, which includes healthier eating habits. In the Dietary Guidelines for Americans (USDA CNPP, 2010), the recommended fat intake in adults should not be higher than 35% of the total calories. This translates to a maximum of 78 g of fat per day in a 2,000 calorie diet. Although Cheddar cheese is considered a nutrient dense food providing high protein and calcium to our diet, it contributes significantly to dietary fat intake. Cheddar cheese contains 9 g fat per 28 g of serving. A strategy that can be used to achieve dietary fat reduction is by eating smaller amounts of full-fat foods or substitute with a reduced-fat version. To help consumers meeting their dietary guidelines, the cheese industry strives to provide a healthier option that is reduced in fat. The FDA regulation mandates that food products claimed as 'low-fat' must not contain more than 3 g of fat per reference amount (50 g), whereas 'reduced-fat' labeling can be used for food that is 25% less fat of the regular version (CFR 21 [101.62b]; FDA-DHHS, 2002).

Is it easy to make a good quality reduced-fat Cheddar cheese (RFCC)? It's technically challenging to produce RFCC with flavor and texture comparable to full-fat Cheddar cheese (FFCC). Extensive reviews about reduced- and low-fat cheese are available (Drake and Swanson, 1995; Mistry, 2001; Banks, 2004; Johnson et al. 2009); all of which reported poor flavor and texture on reduced- and low-fat cheese. Some of the flavors defects mentioned were meaty, brothy, burnt, bitterness, low flavor intensity and milk fat flavor. In terms of texture, RFCC is perceived to be firmer, rubbery, waxier, more fracturable, less sticky and cohesive. The production of RFCC with up to 75% fat reduction has found some success, and is commercially available in the market (Schepers, 2005). However, we did not find any low-fat Cheddar cheese (LFCC), which is > 82% fat reduction, in the market place. This is because the larger the fat

reduction, the more severe the flavor and texture defects in the cheese. It was clearly shown by Childs and Drake (2009) from choice-based conjoint analysis and consumer acceptance test that flavor followed by texture of cheese are important attributes that determine consumption, and the consumer acceptance of a commercial RFCC (75% reduced fat) dropped dramatically due to profound differences in flavor and texture when compared to regular FFCC.

The effect of fat reduction on flavor development in Cheddar cheese was studied by Drake et al. (2010). It was found that flavor differences between FFCC and LFCC was not apparent at 2 wk of ripening, but by 9 mo of ripening pronounced flavor difference was observed. The FFCC had higher brothy, sulfur, milkfat flavor than LFCC at 9 mo of ripening. In addition, LFCC had higher bitterness than FFCC, and developed burnt rosy flavor that was not detected in FFCC. Likewise, instrumental analysis showed similar key odorants in LFCC and FFCC at 2 wk of ripening, however the key odorants in FFCC and LFCC showed more differences at 9 mo of ripening. It was also reported by Drake et al. (2010) that FFCC and LFCC were composed of identical volatile compounds, but in different concentrations. These differences might be related to differences in microbiology and proteolysis during aging that were caused by the difference in fat level and balance of compounds in the aqueous phase of the cheese. Fenelon et al. (2000a) showed that the rate of growth of nonstarter lactic acid bacteria decreased with lower fat content in cheese, but found a small effect on the starter population throughout 225-d ripening among cheese with various fat contents. They found lower primary proteolysis, as reflected in pH 4.6 water-soluble nitrogen as a percentage of total nitrogen, in lower-fat cheeses, but no differences in secondary proteolysis, as reflected in amino acid nitrogen as a percentage of total nitrogen, in cheeses with different fat contents. Another challenge in the flavor of RFCC and LFCC is the fact that volatile compounds have different

threshold levels depending on the environment they are in. Hydrophobic compounds have higher threshold level in FFCC (less polar) than RFCC or LFCC (more polar) because they are more soluble in the former environment, and preventing their release in the headspace (Leksrisompong et al., 2010; Kim et al., 2011).

The effect of fat reduction on the texture of Cheddar cheese can be explained in the context of filled-gel model, introduced by Visser (1991). Cheese consists of gel matrix and filler. Casein and bound mineral in a cheese serves as the gel matrix, whereas the rest of the constituents are filler. The casein gel matrix determines the solid nature of cheese. The higher the matrix volume, the firmer is the cheese. The reduction in fat in cheese concomitantly increases the protein content in cheese (Bryant et al. 1995, Drake et al. 2010; Fenelon et al. 2000a,b; Guinee et al., 2000), causing an increase in matrix and reduction in filler. This explains the high firmness in reduced-fat cheese. The microstructure difference between FFCC and LFCC is also evident from the scanning electron micrograph, showing a more compact protein matrix per given volume and less open space occupied by the milk fat globules in LFCC than FFCC (Bryant et al. 1995; Emmons et al., 1980).

Research studies have been extensively done to overcome defects in RFCC and LFCC. One of them is the use of adjunct culture to improve the flavor in RFCC and LFCC. Fenelon et al. (2002) demonstrated the use of *Lactobacillus helviticus* as adjunct culture and in combination with *Leuconostoc cremoris, Lactococcus lactis* var *diacetyl lactis, Streptococcus thermophilus* to produce RFCC (50% fat reduction) that had higher preference score than the RFCC without adjunct culture. The RFCC with these adjunct cultures showed higher degree of peptide hydrolysis and greater free amino acid concentration. However, even the most acceptable RFCC in the study by Fenelon et al. (2002) was still described as having different flavor profile than

typical FFCC, and had burnt off-flavor. To improve the texture of RFCC and LFCC, cheesemakers try to maximize moisture retention (i.e., increase filler volume) in the curd. This can be done by modifying make-procedure, such as increasing milk pasteurization temperature (Guinee et al., 1998), lowering scald temperature (Banks et al., 1989) , washing curd with cold water (22°C) (Johnson and Chen, 1995), milling curd at higher pH (Guinee et al., 1998), or by incorporating denatured whey protein (Lo and Bastian, 1998), hydrocolloids (Konuklar et al., 2004), exopolysaccharide-producing cheese starter cultures (Dabour et al., 2006). Some of these techniques improved the quality RFCC, however none of them has been successfully applied to produce LFCC with commercially acceptable quality.

Nelson and Barbano (2004) introduced a nonconventional method for producing RFCC by removing fat from an aged FFCC in which the typical Cheddar-characteristic flavors had already developed. This method was able to remove as much as 53% of the fat, and avoid flavor and texture problems that were common in RFCC made with conventional process. The RFCC made by the fat removal process was softer and creamier than the original FFCC (Nelson and Barbano, 2004), and also maintained the same flavor intensity as in the FFCC, which was evident from the consumer test (Carunchia Whetstine et al., 2006). The similarity of flavor profile in FFCC and RFCC produced by this method confirmed previous finding that the taste active compounds in Cheddar cheese reside in the water soluble extract (McGugan et al., 1979; Aston and Creamer, 1986; Andersen et al., 2010). While the filler volume was increased in the cheese produced by fat removal process, the major improvement in texture was caused by the change in the matrix structure caused by the manufacturing process (Nelson and Barbano, 2004).

A different approach for making LFCC would be to build it from its components and avoid the cheese aging process. The main components of the fat-free portion of the Cheddar

cheese are protein (mainly casein), water, and minerals. Micellar casein concentrate (MCC), which is a new dairy ingredient made by microfiltration of skim milk, consists of primarily casein micelles, water (before being spray dried), and minerals. In other words, hydrated MCC has a similar composition to that of the fat-free portion of cheese. A RFCC produced by the fat removal process can be made into LFCC by combining it with MCC to achieve the target fat of 3 g per 50 gram reference amount or 1.7 g per 28 g serving. The objective of our study was to develop an alternative process to produce LFCC by combining RFCC made by a fat removal process with MCC to try to achieve a texture and flavor characteristics of FFCC.

MATERIALS AND METHODS

Experimental Design and Statistical Analysis

An aged full-fat Cheddar cheese (FFCC), approximately 2.7 kg, was obtained from a commercial manufacturer. Half of the FFCC was used to produce reduced-fat Cheddar cheese (RFCC) using a fat removal process (Nelson and Barbano, 2004), while the remainder of FFCC was stored for chemical and sensory analyses. In making low-fat Cheddar cheese (LFCC), RFCC was combined with hydrated micellar casein concentrate (MCC) powder to achieve a fat content of 6% or 3 g of fat per 50 g of the product to comply with the FDA standard for a low-fat label claim (CFR 21 [101.62b]; FDA-DHHS, 2002). The production of LFCC was replicated 3 times starting with different lots of FFCC to make RFCC. The sensory and chemical analyses were conducted on LFCC, as well as on the corresponding FFCC, commercial 50% reduced-fat Cheddar cheese (50%RFCC) and commercial 75% reduced-fat Cheddar Cheese (75%RFCC). The 50%RFCC and 75%RFCC were obtained from the same manufacturer as the FFCC, and from 3 different lot numbers which were randomly assigned as replicate 1, 2 or 3

Chemical and sensory data were analyzed by ANOVA using the Proc GLM procedures of SAS (SAS version 8.02; SAS Institute Inc., Cary, NC) to determine if there were significant differences (i.e., $P < 0.05$) in chemical composition or sensory properties among treatments (FFCC, 50%RFCC, 75%RFCC, LFCC). The GLM model for chemical analyses was dependent variable = treatment + replicate + error. The GLM model for analysis of descriptive sensory data was dependent variable = treatment + replicate + panelist + treatment x replicate + treatments x panelist + replicate x panelist + error. Any main effects and interactions that were not significant (i.e., $P > 0.05$) were removed in a stepwise order starting with the term with the lowest type III sum of squares. To visualize any differences among cheese of different treatments with respect to sensory attributes, the scores from descriptive sensory analyses was used to construct a principal component analysis (PCA) biplot using XLStat (Addinsoft, New York, NY).

Production of MCC Powder

The MCC powder was produced by spray drying of liquid 95% serum protein reduced MCC (approximately 10% protein). Liquid MCC was produced by UF of skim milk, followed by 3 stages of microfiltration (MF). On d 1, the incoming raw milk was pasteurized at 72°C for 16 s with a plate heat exchanger (model 080-S, AGC Engineering, Manassas, VA), and then separated at 49°C using a cream separator (model 619, DeLaval Co., Chicago, IL). The pasteurized skim milk was ultrafiltered (50°C) using a polyethersulfone spiral-wound UF membrane with a nominal pore size of 10,000 Da (model 3838, GEA Niro Inc., Hudson,WI) in a batch recirculation mode (2.2X concentration factor or CF). The UF retentate inlet pressure was 276 kPa, and the retentate outlet pressure was 103 kPa. The UF retentate was diluted with cold reverse osmosis (RO) water to reach protein content in the original skim milk and then stored

overnight at 4°C. On d 2, the RO water diluted UF retentate was MF at 50°C using 0.1µm nominal pore diameter ceramic Membralox graded permeability (GP) membrane (model EP1940GL0.1µAGP1020, alumina, Pall Corp., Cortland, NY) with 1.7 m^2 surface area. This MF process was a continuous bleed and feed, and the CF was set to 3X with retentate and permeate removal rates of 60 L/h and 120 L/h, respectively, which produced a permeate flux of 71 L/m^2h. On d 3, 2 additional MF stages were performed as diafiltration, where the retentate from the previous stage was diluted back to its original weight with RO water and used as the feed intake. Diafiltration was done to achieve a more complete removal NPN and serum protein (SP). MF stages 2 and 3 were a continuous bleed and feed with a CF of 3X. The MF stage 3 retentate was further concentrated to approximately 10% protein and stored at 4°C before being spray dried the following day. A Niro spray dryer model 1 with an FU11 atomizer (Niro Atomizer Inc., Columbia, MD) was used with a rotating speed of 23,000 rpm and a feed rate of 14 kg/h. The inlet and outlet temperature of the spray dryer was 200°C and 95°C, respectively. It took approximately 3 h to dry 37 kg feed material. The final temperature of the powder was about 30 to 35°C. The powder from the first 10 min of spray drying was discarded. The remainder of the powder was mixed and packaged in a mylar ziplok bags (Sorbent Systems, Los Angeles CA).

Production of Reduced-fat Cheddar Cheese

The RFCC was made using the fat removal process of Nelson and Barbano (2004). The shredded FFCC cheese was portioned in 250-mL polypropylene oakridge centrifuge tubes (approximately 60 g per tube) with screw top caps (Kendro Laboratory Products, Newtown, CT) and held at 4°C. The tubes were tempered in a shaking water bath (model 236 Versa-Bath S, Fisher Scientific, Pittsburgh, PA) set at 35°C and 40 rpm for approximately 1 h to achieve a

cheese temperature of 35°C. The tubes were immediately placed into a 35°C Sorvall RC2-B Superspeed centrifuge with a GSA rotor (Sorval Inc., Newtown, CT) operated at 23,500 x g for 5 min. The liquid fat was decanted from each tube. The collective cheese residues were mixed in a food processor with a Sabatier blade attachment (Kitchen Aid, St. Joseph, MI), followed by pressing them by hand in a cheese mold and vacuum packed (Multivac AGW, Koch, Inc., Kansas City, MO). The RFCC was stored at 4°C until needed for the production of LFCC and composition analysis.

Production of Low-fat Cheddar Cheese

The RFCC and MCC powder were analyzed for their composition, the formula to make LFCC was calculated to achieve 6% fat, 28% protein, 1.2% salt by combination of RFCC, MCC powder, salt, and RO water (Table 1). The MCC powder was hydrated gradually by adding it to 65°C RO water and mixed continuously using an over head stirrer (Model NQ-47, Fisher Scientific, Pittsburgh, PA). Salt was added to the mixture, followed by continuous mixing for 40 min to ensure proper hydration. Throughout the mixing process, the temperature of the system was maintained at 60°C in a water bath to aid in the hydration of MCC powder. The amount of moisture lost due to evaporation during the process, as calculated using mass balance, was added back to the mixture. The mixture was put in a food processor (Kitchen Aid, St. Joseph, MI) with a Sabatier blade attachment and mixed to achieve homogenous mixture with no lumps (approximately 10 sec of continuous mixing). The pre-shredded RFCC was added gradually into hydrated MCC mixture and mixed intermittently in the food processor. After all RFCC was added, the mixing was standardized across the three replicates by subjecting 30 times of 1-sec pulse mixing followed by 1 min of continuous mixing. The pH of the mixture was measured at

37°C using a Xerolyt combination electrode (model HA405, Metler Toledeo, Colombus, OH) and Accumet pH meter (model 925, Fisher Scientific, Springfield, NJ). DL-lactic acid 85% w/w USP/FCC (Fisher scientific, Pittsburgh, PA) was added drop by drop followed by mixing intermittently in the food processor, until the pH of the LFCC mix was 5.3. The LFCC mix was weighed to calculate the amount of rennet (Chy-max extra, double strength, activity: 600 International Milk Clotting Unit/mL, Chr. Hansen Inc., Milwaukee, WI) needed. The undiluted rennet addition rate was 0.00031 mL/g of protein derived from the MCC. The rennet was diluted to 1:20 ratio with water before being added to the LFCC mix (at 34 to 36°C) and blended in uniformly using a spatula. Approximately 1 mL of diluted rennet was needed for 1 kg of the LFCC mix. Following the incorporation of rennet, the LFCC mix was packed by hand into a cheese mold. The LFCC mix plus rennet was held at ambient temperature (21°C) for 30 min to allow reaction of the rennet and MCC. At the end of 30 min, there was a small amount of whey drainage, approximately 1.82% of the total cheese weight. The LFCC was removed from the mold and vacuum packed (Multivac AGW, Koch, Inc., Kansas City, MO) when the temperature of the cheese was approximately 22 to 25°C. The temperature of the cheese before vacuum packing was important for the cheese texture. If the temperature was too high, the cheese deformed when vacuum packed, whereas at lower temperature the cheese did not fuse together and achieve uniform texture. After vacuum packing, the LFCC was held at ambient temperature (21°C) for 30 min to allow the structure uniform and closed, and then it was refrigerated at 4°C.

MCC Powder and Cheese Composition, pH, and Titratable Acidity

The MCC powder was reconstituted to 10% solids in RO water for analyses. The moisture content and total nitrogen was measured in triplicate by forced-air oven drying at 100°C

for 4 h (AOAC, 2000; 33.2.44, method 990.20), and by the Kjeldahl method (AOAC, 2000; 991.20, method 33.2.11). Total fat was determined in duplicate using ether extraction (AOAC, 2000; 989.05 method 33.2.26). The pH was measured by a Xerolyt combination electrode (model HA405; Metler Toledo, Colombus, OH) and Accumet pH meter (model 925, Fisher Scientific, Springfield, NJ).

Cheeses were cut into 1 inch cubes and ground in a Waring blender (Model 31BL92, Waring Products, Torrington, CT) to a uniform particle size of 2 to 3 mm. After thoroughly being mixed, the ground cheese was packed into 2 oz universal vials (model CPP02, Capitol Vial, Inc., Auburn, AL) with no head space and stored at 4°C until the day of analysis. Cheese samples were analyzed within a week after the grinding. Moisture was determined (quadruplicate) by drying a 2-g portion in a forced-air oven at 100°C for 24 h (AOAC, 2000; 33.2.44, 990.20). Fat in cheese was determined (triplicate) using a Babcock method [(Frank and Wehr, 2004); method number 15.083]. Salt content was measured (duplicate) using the Volhard method [(Wehr and Frank, 2004); method number 15.052]. Cheese pH was measured using a Xerolyt combination electrode and Accumet pH meter. Cheese titratable acidity (TA) was measured as described by Lau et al. (1991).

Sensory analysis

Cheddar cheese samples were cut into cubes and placed into lidded 58 mL soufflé cups with 3-digit codes. Cheese samples were tempered to room temperature (25°C ± 4°C) before serving. Deionized water and crackers were used for palate cleansing in between samples. The descriptive sensory analysis adopted flavor lexicons established by Drake et al. (2001) with 15-point numerical universal spectrum intensity scale (Meilgaard et al, 1999). A group of trained

panelists (n = 8) at North Carolina State University evaluated the cheeses. Cheese samples from the same replicate were evaluated in the same session, therefore there were 3 sensory evaluation sessions corresponding to 3 replicates. Each cheese sample was evaluated in duplicate by each panelist.

Microstructure

Cheese samples (0.8 × 3 × 4 mm) were mounted to a specimen holder (ALT 118, Gatan Inc., Pleasanton, CA) and frozen by plunging into liquid nitrogen slush. Mounted samples were transferred under vacuum to the cryo-preparation chamber (ALTO 2500, Gatan Inc., Pleasanton, CA) and fractured using a scalpel. After sublimation, at -80°C for 15 min to reveal the fat and protein structure, the temperature was decreased to -155°C and coated with Pt. Sputter coating (20 mA) was performed twice at 1 min intervals to prevent sample heating. Coated samples were then transferred under vacuum to the field emission scanning electron microscope (Supra 40, Carl Zeiss Microscopy, Thornwood, NY). Imaging was performed at 2 kV (about 6 mm working distance and 30 μm aperture) with signals blended 50:50 from the in-lens and Everhart-Thornley detectors. The cheese samples were maintained at -155°C while imaging.

RESULTS AND DISCUSSION

Production of MCC Powder and Reduced-fat Cheddar Cheese

A MCC and 50% RFCC were the main ingredients used to produce LFCC in our study. The MCC powder used in this study contained 2.26% moisture, 2.39% fat, and 83.43% protein on a wet basis. The pH of reconstituted MCC powder was 7.13 at 21°C. The composition of hydrated MCC is similar to the fat-free portion of Cheddar cheese, which is primarily casein

micelles, water, and minerals. Thus, MCC is a good candidate for a low-fat ingredient derived from skim milk that can be used to build the structure of a low-fat cheese.

The fat removal process produced Cheddar cheese with 52.90%, 52.31%, and 54.30% reduced fat compared to the original FFCC, for replicate 1, 2, 3, respectively. The mean fat reduction for the 3 replicates was 53.17% for the RFCC ingredient.

Production of Low-fat Cheddar Cheese

Our target of 6% fat when formulating the LFCC cheese was established to comply with the FDA regulation for low-fat food labeling (CFR 21 [101.62b]; FDA-DHHS, 2002). The 28% protein and 1.2% salt targets were established based on empirical findings (from our preliminary testing, data not reported) that at this composition the product had desirable texture and saltiness perception. With a protein lower than 28% protein target, the texture of the LFCC cheese was too soft, while achieving a protein higher than the target posed a challenge because the MCC solution (MCC and RO water) was too viscous to mix properly. The formulation of LFCC for the 3 replicates is shown in Table 3.1. The small differences in ingredients usage among the 3 replicates was due to the slight variation in the RFCC fat content among replicates. Furthermore, the variation in the RFCC composition was caused by compositional difference among the 3 original FFCC and the amount of fat removed during the fat removal process. The pH of LFCC mix was greatly influenced by the amount of MCC and RFCC used in the formula. The buffering capacity of the MCC resisted pH change when RFCC was added. The pH of LFCC mix (before the addition of lactic acid) were 5.86, 5.70, and 5.77 for replicate 1, 2, and 3 respectively (Table 3.1). The replicate 1 formula had the lowest amount of RFCC and highest MCC, which explained its high pH of the mix before pH adjustment relative to replicate 2 and 3.

Meanwhile, replicate 2 had higher usage of RFCC, lower usage of MCC, and the RFCC in replicate 2 was more acidic compared to RFCC in replicate 1, as a result, the pH of the LFCC mix before pH adjustment in replicate 2 was lower. Lactic acid was added to the LFCC mixes until a pH of 5.3 was achieved. The LFCC in replicate 1 required more added lactic acid because it had the highest pH before the pH adjustment, while LFCC replicate 2 needed the least added lactic acid because it had the lowest initial pH before the pH adjustment. The pH of LFCC after the pH adjustment were 5.25, 5.28, and 5.28 for replicate 1, 2, and 3, respectively (Table 3.1). When all the cheese samples were analyzed for pH and the rest of compositional analyses after 2 wk in storage, the pH of LFCC had increased for all 3 replicates to a mean pH of 5.49 (Table 3.1). The increase in pH during 2 wk of refrigerated storage was presumably caused by the buffering action of the minerals from the MCC that gradually became soluble in the water phase of the low- fat cheese during 2 wk of storage at 4°C. Upreti and Metzger (2007) observed an increase in pH between d 1 and 14 of full-fat Cheddar cheese, made with different levels of calcium and phosphate, residual lactose, and S/M. They attributed the increase in pH to the gradual solubilization of calcium and phosphate entrapped by the cheese paracasein network. There seemed to be a delay in the solubilization of entrapped calcium and phosphate due to restricted mobility and its slow equilibrium (Upreti and Metzger, 2007). Because of this gradual shift in pH in our LFCC, the production of LFCC in the future (using the approach described in the present study) needs to target a pH lower than 5.3 at the time of processing, so that the pH of the final product would be approximately at 5.3 during refrigerated storage. Lowering the pH of the LFCC mix during processing to 5.3 served different purposes. First, from a sensory perspective, it improved the perception of acid taste. Second, it helped soften the LFCC protein matrix by releasing the bound calcium from the MCC and this may have caused the shift in

bound mineral from the MCC into the water phase producing the previously mentioned increase in pH during the first 2 wk. Hydrated MCC (approximately 27% protein) was a colloidal suspension of casein micelles in water. When pre-shredded RFCC was added to the hydrated MCC mixture, the particles of RFCC were dispersed in a continuous phase of hydrated MCC by the mixing. The addition of rennet acted on the κ-casein in the continuous phase of the hydrated MCC to create a continuous gel matrix with particulate RFCC imbedded in the matrix. When LFCC produced by this method is viewed in the context of a filled gel structure model, the matrix of the LFCC consisted of a protein network of RFCC embedded in a casein network of the renneted MCC. Based on observations in the present study (data not reported), the LFCC without the addition of rennet had short and crumbly texture, owing to minimal interaction of among casein micelles.

Table 3.1. Formula to produce low-fat Cheddar cheese (LFCC) for replicate 1, 2, and 3 and pH adjustment in LFCC by lactic acid to a target pH of 5.3.

	Usage (%)			
Ingredients	Replicate 1	Replicate 2	Replicate 3	Mean
Reduced-fat Cheddar cheese (RFCC)	32.05	33.50	35.32	33.62
Micellar casein concentrate powder	21.61	20.61	19.86	20.69
Water	44.83	44.82	43.68	44.44
Salt	0.51	0.45	0.30	0.42
Lactic acid racemic (85% w/w)	1.00	0.62	0.85	0.82
Total	100.00	100.00	100.00	100.00
pH of RFCC	5.28	5.07	5.28	5.21
pH of LFCC mix before pH adjustment	5.86	5.70	5.77	5.78
pH of LFCC mix after pH adjustment	5.25	5.28	5.28	5.27
pH measured after 2 wk storage	5.37	5.57	5.54	5.49

Cheese composition

Comparison among commercial FFCC, 50%RFCC, 75%RFCC. The mean cheese composition across 3 replicates is reported in Table 3.2. As the Cheddar cheese fat content was

reduced using a conventional Cheddar cheesemaking approach, the moisture and protein content of the cheese (Table 3.2) increased ($P < 0.05$). An increase in moisture and protein percentage was expected, because of the absence of fat. With increased moisture and lower amount of fat, both 50%RFCC and 75%RFCC had lower fat in the dry matter (FDM) than FFCC. The protein in the dry matter (PDM) in 50%RFCC and 75%RFCC was higher than FFCC. No difference was detected ($P > 0.05$) in pH, titratable acidity (TA) and salt among FFCC, 50%RFCC, and 75%RFCC. Given the higher moisture content of the 50%RFCC and 75%RFCC compared to FFCC and no salt difference, the percentage salt in the moisture (S/M) in 50%RFCC (3.69%) and 75% RFCC (3.35%) was lower ($P < 0.05$) than in FFCC (4.97%). If the S/M was to be kept the same, then extra salt addition would have been needed, and that might not be desirable from sensory and nutritional perspective. Mistry and Kasperson (1998) studied the effect of S/M (2.7, 3.7, 4.5% S/M) on the quality of RFCC (about 50% reduced fat) made with conventional cheesemaking process and ripened for 24 wk. Low-S/M RFCC scored higher on flavor intensity, and had better body and texture than high-S/M RFCC. However, the bitter flavor increased in low-S/M RFCC with the concomitant increase in proteolysis. Thus, production of low S/M RFCC by conventional cheese making technology used by Mistry and Kasperson (1998) needs to be coupled with the addition of cultures containing peptidase activity to hydrolyze hydrophobic peptides that are associated with bitterness (El Abboudi et al., 1992; Sridhar et al., 2005).

The moisture in the nonfat substance (MNFS) in the 50%RFCC and 75%RFCC were higher ($P < 0.05$) than the FFCC in our study (Table 3.2). A decrease in MNFS has been reported in several studies as fat was reduced in cheese (Bryant et al., 1995; Fenelon et al., 2000a; Guinee et al., 2000). This means that the commercial RFCC used in our study retained more moisture in the curd, probably due to a modification in the traditional cheesemaking, than the RFCC made in

the previously mentioned research studies. The MNFS has been used by cheesemakers as an important indicator of the likely flavor and body development in mature full-fat cheeses (Pearce, 1978). Lawrence and Gilles (1980) reported that full-fat Cheddar cheese with MNFS higher than 56% has a marked tendency develop defects during maturation. The 50%RFCC and 75%RFCC had MNFS higher than 56%, however no previous studies have been reported on how higher MNFS affects the quality of lower fat Cheddar cheese during aging. Emmons et al. (1980) suggested that MNFS in low-fat Cheddar cheese needed to be higher than in the full-fat counterpart to achieve a texture closer to the full-fat counterpart, however the effect of higher MNFS on flavor of low-fat Cheddar cheese was not discussed.

Table 3.2. Mean (n=3) chemical composition of full-fat Cheddar cheese (FFCC), commercial 50% reduced-fat Cheddar cheese (RFCC), commercial 75% RFCC, experimental RFCC, and experimental low-fat Cheddar cheese (LFCC).

Composition	Commercial FFCC	Commercial 50% RFCC	Commercial 75% RFCC	Experimental RFCC	Experimental LFCC	SE
Fat %	34.69[a]	14.50[b]	7.31[c]	16.25[b]	5.97[c]	0.359
Gram of fat/serving[1]	9.71[a]	4.06[b]	2.05[c]	4.55[b]	1.67[c]	0.100
Moisture %	35.65[e]	49.23[c]	52.52[b]	45.04[d]	58.07[a]	0.260
Protein[2] %	24.21[d]	29.45[c]	32.62[a]	31.22[a,b]	29.69[b,c]	0.347
FDM[3] %	53.91[a]	28.56[b]	15.38[c]	29.57[b]	14.24[c]	0.599
PDM[4] %	37.63[c]	58.01[b]	68.70[a]	56.81[b]	70.81[a]	0.609
pH	5.22[b]	5.15[b]	5.10[b]	5.21[b]	5.49[a]	0.056
TA[5]	1.09[a,b]	0.77[b]	0.77[b]	1.38[a]	0.64[b]	0.123
Salt %	1.77[b]	1.82[b]	1.76[b]	2.29[a]	1.20[c]	0.072
S/M[6] %	4.97[a]	3.69[b]	3.35[b]	5.09[a]	2.07[c]	0.172
MNFS[7] %	54.59[c]	57.58[b]	56.65[b]	53.79[c]	61.76[a]	0.358

[a-d]Means in the same row not sharing a common superscript are different ($P < 0.05$).
[1]One serving of cheese = 28 g
[2]Total nitrogen x 6.38
[3] FDM = Fat in dry matter
[4] PDM = Protein in dry matter
[5] TA = Titratable acidity
[6] S/M = (salt/moisture) x 100
[7] MNFS = Moisture in nonfat substance

Comparison among reduced-fat Cheddar cheeses (50%RFCC, 75%RFCC, experimental RFCC). The term "reduced-fat" is used for a product with at least 25% fat reduction compared to the full-fat counterpart (CFR 21 [101.62b]; FDA-DHHS, 2002). In other words, reduced-fat Cheddar cheese must not exceed 7.2g fat per serving, and can be as low as 1.68 g per serving, below which the term "low-fat" can be used. The 50%RFCC, 75%RFCC and experimental RFCC contained 4.06, 2.05, and 4.55 g of fat per serving, respectively, which qualified them to be labeled "reduced-fat". Experimental RFCC had lower ($P < 0.05$) moisture than both commercial RFCC. The protein content of the experimental RFCC was higher ($P < 0.05$) than the commercial 50% RFCC but no difference in protein content ($P > 0.05$) from the 75% RFCC was detected (Table 3.2). If the moisture content of the experimental RFCC was increased to match that of the commercial 50% RFCC, the percentage fat, protein, and grams of fat per serving would have been comparable. There was no difference ($P > 0.05$) detected in FDM and PDM of 50% RFCC and experimental RFCC. No difference ($P > 0.05$) in pH among three cheeses was detected. The TA and salt were higher ($P < 0.05$) in experimental RFCC than the two commercial RFCC because these compounds were concentrated in the nonfat phase of the cheese during the production of experimental RFCC. Due to higher salt and lower moisture content, S/M of experimental RFCC was higher ($P < 0.05$) than 50%RFCC and 75%RFCC. The fat removal process should not affect MNFS of the resulting RFCC. Thus, MNFS of experimental RFCC was not different ($P > 0.05$) from FFCC, and they had lower ($P < 0.05$) MNFS than 50%RFCC and 75%RFCC. As reported previously (Nelson and Barbano, 2004; Carunchia Whetstine et al., 2006), the experimental RFCC produced by the fat removal process has a Cheddar flavor intensity comparable to that of full-fat Cheddar

Comparison between commercial FFCC and experimental LFCC. The amount of fat per serving was reduced from 9.71 g in FFCC to 1.67 g in LFCC to comply with the FDA regulation for low-fat labeling (Table 3.2). This translated to an 83% reduction of fat from FFCC. Moisture and protein were higher ($P < 0.05$) in LFCC than FFCC, as a result PDM was much higher ($P < 0.05$) in LFCC (70.81%) than FFCC (37.63%). With lower fat and higher moisture, LFCC had lower ($P < 0.05$) FDM than FFCC. The mean pH of LFCC (5.49) was higher ($P < 0.05$) than FFCC (5.22). The mean TA of FFCC and LFCC were 1.09 and 0.64, respectively. No difference ($P > 0.05$) in TA was detected among the two cheese types because there was a large variation in TA (up to 0.4% difference) among replicates within each sample (individual TA value for each replicate was not reported). The TA of LFCC was lower than expected, given that an average of 0.82% of racemic lactic acid (85% w/w) was added to the LFCC mixture (Table 3.1). The added lactic acid was a racemic mixture, and it is known the D(-)-lactic acid is less soluble than the L(+)-lactic acid (Dybing et al., 1980; Johnson et al., 1990) in cheese and forms insoluble calcium lactate crystals. This may have created a problem in the recovery of lactic acid in the measurement method for TA of cheese. The method for TA determination in cheese has an initial water extraction step where the cheese combined with warm RO water were blended and filtered. An aliquot sample of the filtrate was then titrated with 0.1 N NaOH, and the TA was calculated. We suspected that part of the lactic acid that was added was not soluble in warm water, didn't pass through the filter, and hence was not quantified.

The LFCC was formulated to have 1.20% salt in the final product. This low salt target was intentionally done because at this salt level, we felt that the perception of saltiness was comparable to a regular Cheddar cheese. In preliminary trials, a LFCC made at the salt level

similar to a regular Cheddar cheese (1.70% salt) was perceived to be too salty (data not reported). The mean sodium content of the cheese was calculated to be 195 mg in FFCC and 132 mg in LFCC per 28 g serving. The sodium content in LFCC was 32% lower than FFCC, which would be desirable from the nutritional perspective. The S/M in LFCC (2.07%) was lower than FFCC (4.97%). In replacing the fat, the moisture increase was larger than protein increase in LFCC, which resulted in higher MNFS ($P < 0.05$) in LFCC than FFCC. Both S/M and MNFS are important parameters to assess the potential quality of Cheddar cheese in a conventional cheesemaking. However, these parameters are less relevant in the cheese produced by our novel cheesemaking process because the LFCC produced by our process has no aging step required for flavor development. The flavor and texture characteristics are achieved at the day of processing. Any undesirable changes in flavor and texture would be the limiting factor of the LFCC during its shelf-life.

Sensory analysis

Texture. The ratio of matrix to filler volume of a cheese gives an insight on the expected cheese texture. For commercial Cheddar cheeses with a range of fat contents, the proportion of matrix increased and the filler volume decreased (Table 3.3), as fat was reduced (Table 3.2). In the opinion of three experienced cheese judges, 75%RFCC was the firmest (lowest filler volume: 67.38%, Table 3.3) and FFCC was the softest among the commercial cheeses (highest filler volume: 75.79%, Table 3.3). This observation is consistent with the filled gel model by Visser (1991) which indicates increasing filler volume makes cheese softer. To maintain a similar filler volume in 50%RFCC and 75% RFCC as in FFCC, the amount of moisture needs to be increased to make up for the lower amount of fat. This can be technically challenging in a conventional

Cheddar cheesemaking. As an illustration, the commercial 75%RFCC contained 7.31% fat (Table 3.2). In order to achieve the same filler volume as in commercial FFCC (75.79%), the moisture in commercial 75%RFCC needed to be approximately 68.48%, but it was only 52.52% (Table 3.2).

Table 3.3 Matrix and filler composition of commercial full-fat Cheddar cheese (FFCC), commercial 50% reduced-fat Cheddar cheese (RFCC), commercial 75% RFCC, experimental RFCC, and experimental low-fat Cheddar cheese (LFCC) based on a filled gel model by Visser (1991).

	Commercial FFCC	Commercial 50% RFCC	Commercial 75% RFCC	Experimental RFCC	Experimental LFCC
			%		
Matrix[1]	24.21^{d}	29.45^{c}	32.62^{a}	31.22^{a,b}	29.69^{b,c}
Filler[2]	75.79	70.55	67.38	68.78	70.31
Observed firmness	Medium	Harder	Hardest	Soft	Softest

$^{a-d}$Means in the same row not sharing a common superscript are different ($P < 0.05$).
[1] Estimated as protein %
[2] 100% - protein %

In comparing commercial FFCC to experimental RFCC and experimental LFCC, the FFCC was expected to be softer because it had higher filler volume than experimental RFCC and LFCC (Table 3.3). However, this was not the case. The experimental RFCC contained original cheese matrix (FFCC) that had been disrupted, hence the structural integrity of the matrix was weakened and the texture of the RFCC was softer than the FFCC. The disrupted matrix of the FFCC became the matrix of the experimental RFCC. When the experimental RFCC is dispersed in hydrated MCC to make LFCC, the experimental RFCC becomes an inclusion in the continuous gel matrix formed by the action of rennet on the casein in the MCC (i.e., primary matrix of LFCC). Does the RFCC contribute to matrix of the LFCC, or does it act as filler? For the experimental LFCC, the matrix reported in Table 3.3 is the total protein from two different sources: MCC and experimental RFCC. One can argue that the effective matrix in experimental

LFCC is solely from the protein in MCC, which is calculated to be 18.46%. In this case, the filler volume of the experimental LFCC would be 81.54%, not 70.31% as reported in Table 3.3, and the filler volume of the commercial FFCC is 75.79%. The observation by the three experience judges that experimental LFCC was softer than commercial FFCC (Table 3.3) is consistent with the idea that the protein in the RFCC ingredient is not acting as matrix in the LFCC.

A typical FFCC contain large fat inclusions due to the mechanical action during cheesemaking that ruptures milkfat globules, which causes fat to coalesce (Rogers et al., 2010). The fat removal process (Nelson and Barbano, 2004) to produce RFCC from FFCC tends to melt these large fat inclusions, which are then separated and decanted. What is left in the cheese residue (experimental RFCC) are the smaller original fat globules (ca. 1 μm) entrapped in the matrix. When the experimental RFCC is used as an ingredient for LFCC in this study, the concentration of these small fat globules is further diluted in a continuous phase of hydrated MCC. In comparison, a conventional process of making RFCC uses skim milk plus cream, which naturally contains normal size fat globules (ca. 1 to 4.5 μm). There are more original large milkfat globules from the cream apparent throughout the structure of commercial 75%RFCC (Figure 3.1a), while the experimental LFCC (Figure 3.1b) contains only smaller original milkfat globules, even though the total fat contents of the commercial 75%RFCC and experimental LFCC are almost the same. At higher magnification (100,000 x) scanning electron microscope images, the size of water pockets in experimental LFCC (Figure 3.1d) appears to be larger than in commercial 75%RFCC (Figure 3.1c). This is in agreement with the composition data which shows higher moisture in experimental LFCC (58.07%) than commercial 75%RFCC (52.52%). The difference in fat dispersion and size of water pockets in the cheese structure contributes to the difference in perceived texture between commercial 75%RFCC and experimental LFCC.

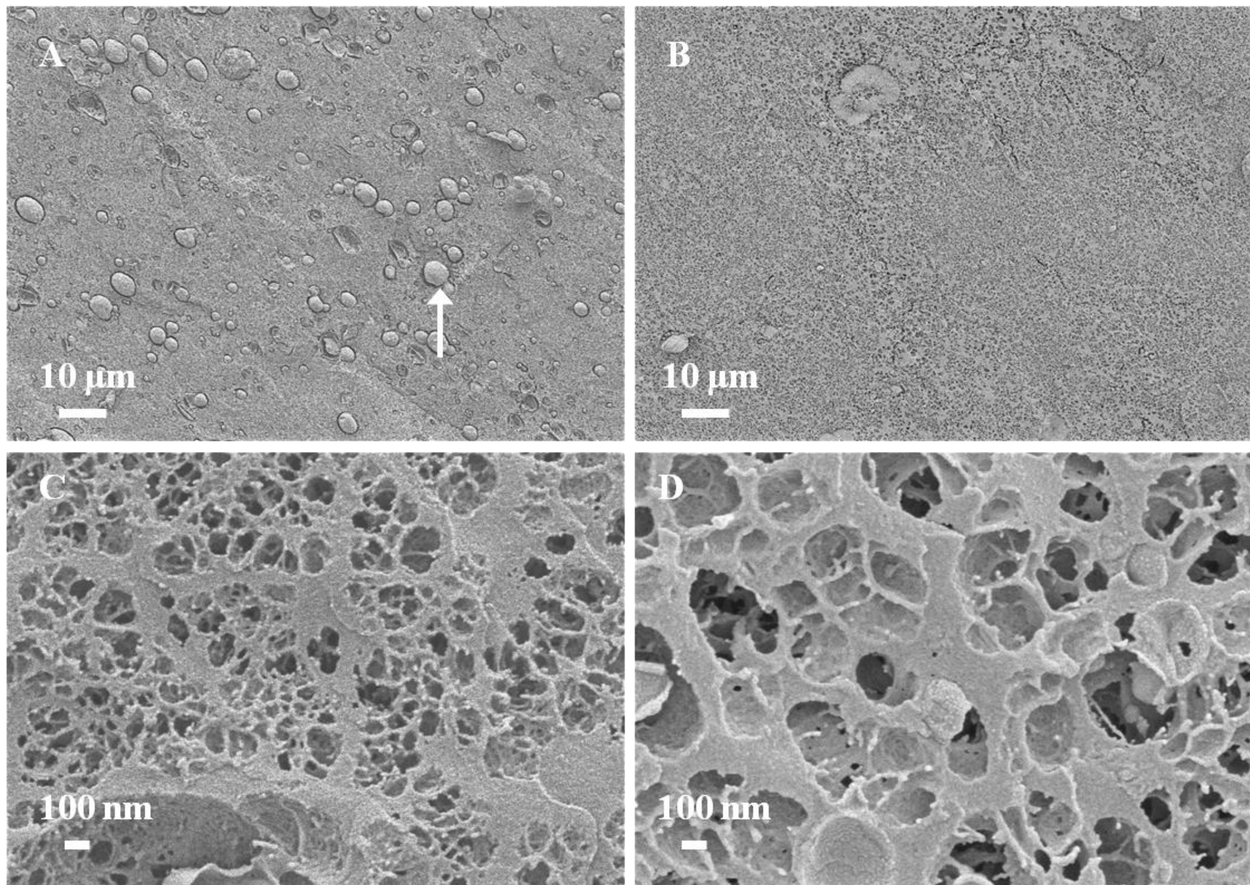

Figure 3.1. Scanning electron microscopy images of commercial 75% reduced-fat Cheddar cheese (A and C at 2,000 and 100,000 x magnification, respectively) and experimental low-fat Cheddar (B and D at 2,000 and 100,000 x magnification, respectively). White arrow in A indicates visible fat globules. Protein matrix appears as a lace-like structure at higher magnification (C, D).

To our knowledge, previous attempts on making LFCC have always resulted in much firmer texture than FFCC, which contributes to low consumer acceptability in LFCC (Childs and Drake, 2009). The technology demonstrated in the current study represents a different approach to produce LFCC and the demonstration that modification of the cheese matrix structure can produce LFCC with soft texture. A more formal and complete descriptive sensory and instrument analysis of the texture of LFCC produced by this new approach needs to be conducted in the future.

Flavor. From the principle component analysis (PCA) biplot (Figure 3.2), we were able to determine which flavor attributes strongly characterized each cheese type. The PCA plot also provided a view of how different or similar cheese types were based on the flavor attributes. About 61% variation among the cheese types can be explained by attributes closer to PC1 axis (horizontal), and the other 31% can be explained by attributes closer to PC2 axis (vertical). This means that the attributes closer to PC1 axis were the major flavor attributes that distinguished different cheese types. The FFCC had different flavor attributes than other cheeses. The FFCC was characterized by attributes (such as, nutty, fruity, milk fat, catty, brothy, sweet, and sulfur) close to the positive loading of PC1 axis (typical aged Cheddar character), whereas other cheeses were lacking of these attributes. Both 50%RFCC and 75%RFCC lay between the negative loading on PC1 and positive loading of PC2 axes, which was characterized by whey and cooked flavor. The LFCC had a distinct flavor character that was different from the rest of the cheeses that was between the negative loading of the PC1 and PC2 axes, which was characterized by bitter and grapey-tortilla.

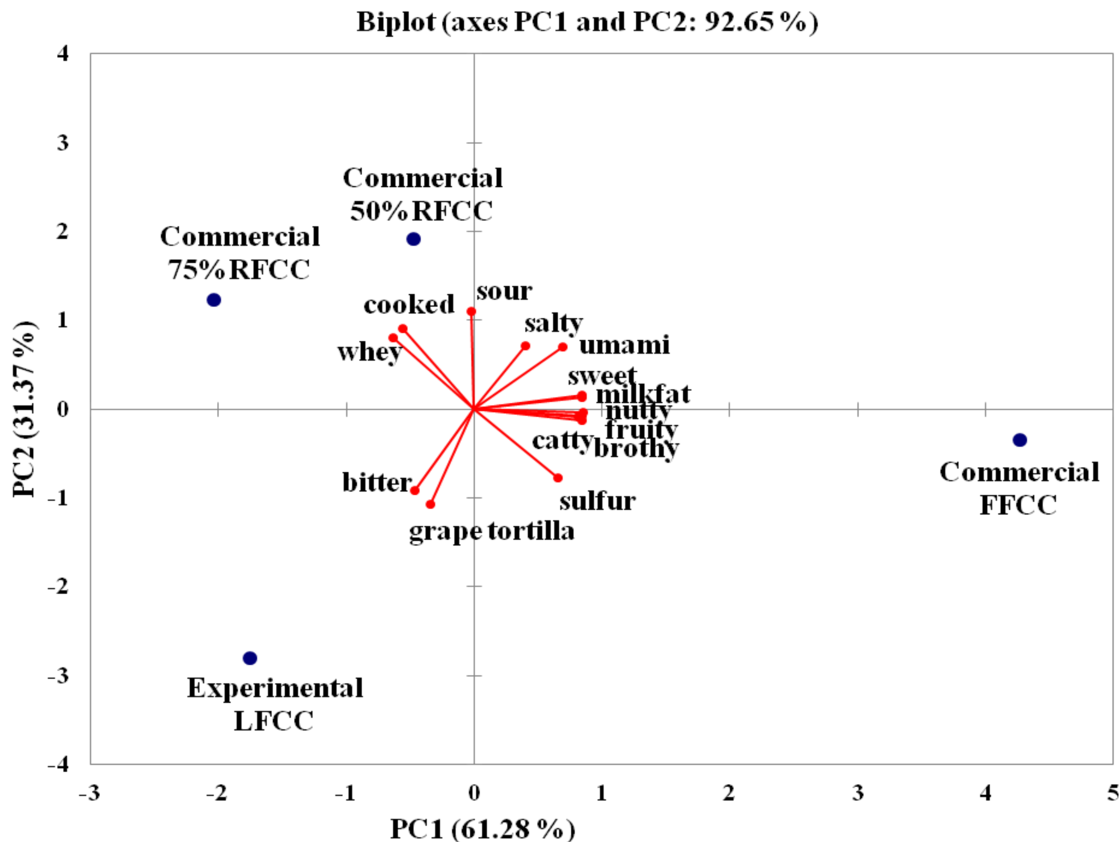

Figure 3.2. Principal compoment (1 and 2) biplot of descriptive analysis of Cheddar cheese sample with various fat content.

The mean descriptive flavor scores of flavor descriptive sensory are reported in Table 3.4. The FFCC had the highest brothy flavor. Brothy has been reported as a common flavor defect in lower fat Cheddar cheese (Milo and Reineccius, 1997). However, all of the lower fat Cheddar cheese in our study didn't exhibit this flavor. No difference ($P > 0.05$) in saltiness was detected among the different types of cheeses, despite the fact that LFCC was 32% lower in sodium than FFCC. The high moisture content of the LFCC might facilitate a faster release of this water soluble compound, hence the saltiness of LFCC was perceived to be similar to FFCC. The FFCC had the highest sulfur flavor among the cheese types. Low sulfur flavor in 50%RFCC and 75%RFCC was expected because previous studies (Dimos et al., 1996; Drake et al., 2010) had shown similar observation that the sulfur compounds in conventionally-produced lower fat

Cheddar cheese were reduced. Surprisingly, experimental LFCC had significantly higher sulfur flavor ($P < 0.05$) than commercial 50%RFCC and 75%RFCC. The sulfur flavor in LFCC was derived from experimental RFCC which retained the Cheddar flavor of the FFCC it was made from after the fat removal process (Carunchia Whetstine et al. 2006). Grapey-tortilla is not a common descriptor for Cheddar cheese, and it has not been reported in previous studies. This flavor was detected in LFCC, but none in other Cheddar cheeses. The grapey-tortilla came from the dried MCC. Fresh liquid MCC does not have this flavor.

Table 3.4 . The mean sensory attribute flavor scores from descriptive sensory analysis[1] of commercial full-fat Cheddar cheese (FFCC), commercial 50% reduced-fat Cheddar cheese (RFCC), commercial 75% RFCC, and experimental low-fat Cheddar cheese (LFCC).

Attribute	Commercial FFCC	Commercial 50%RFCC	Commercial 75%RFCC	Experimental LFCC	SE
Bitter	0.36[c]	0.23[c]	0.61[b]	0.85[a]	0.064
Brothy	2.98[a]	2.15[b]	2.26[b]	2.16[b]	0.056
Catty	1.18[a]	ND[2]	ND	ND	0.045
Cooked	2.62[b]	3.32[a]	3.40[a]	2.79[b]	0.057
Diacetyl	ND	ND	ND	ND	NA[3]
Fruity	0.51[a]	ND	ND	ND	0.038
Free fatty acid	ND	ND	ND	ND	NA
Grapey-tortilla	ND	ND	ND	2.95[a]	0.025
Milkfat	3.50[a]	2.20[b]	1.15[d]	1.30[c]	0.049
Nutty	1.11[a]	0.16[b]	ND	ND	0.053
Salty	3.55[a]	3.95[a]	3.01[a]	2.97[a]	0.306
Sour	3.10[b]	3.18[a,b]	3.29[a]	2.87[c]	0.047
Sulfur	3.17[a]	1.88[c]	1.56[d]	2.63[b]	0.053
Sweet	2.60[a]	2.10[b]	1.78[c]	1.78[c]	0.052
Umami	3.09[a]	2.80[b]	2.67[b]	2.24[c]	0.048
Whey	1.51[c]	2.53[a]	2.70[a]	1.90[b]	0.063

[a-d]Means in the same row not sharing a common superscript are different ($P < 0.05$).
[1]Scores were based on the 15-point universal intensity Spectrum scale where 0 = not detected to 15 = very high (Meilgaard et al., 1999; Drake et al., 2001).
[2]ND = Not detected.
[3]NA = Not applicable.

The FFCC had higher ($P < 0.05$) milkfat flavor than other cheeses. Lactones contribute to milkfat flavor (Drake et al., 2001). Milk with lower fat content had lower lactone concentration, and there were less lactone precursors in the cheese (Drake et al., 2010) which explained lower milkfat flavor in lower fat cheeses. Although milkfat is required for the production of lactones (Wijesundera and Watkins, 2000), the removal of fat from an aged FFCC does not alter milkfat flavor intensity because lactones reside in the aqueous phase of the cheese (Carunchia Whetstine et al. 2006). Furthermore, lactones have lower sensory threshold level in water than in oil (Leksrisompong et al., 2010). Lower milkfat flavor in experimental LFCC might be attributed to the fact that two thirds of the ingredients used to make LFCC (e.g. MCC, water, salt, lactic acid) did not contain lactones, and these ingredients reduced the flavor contribution of lactones from RFCC, which only accounted for one third of the ingredients to make LFCC (Table 3.1). It was also possible that lactones were bound to protein from MCC. Flavorants bound to protein will not contribute to taste and aroma, if they are not released during mastication (Plug and Haring, 1993; Damodaran, 2008). Catty and slight fruity flavor were detected in FFCC but not in other cheeses. Nutty flavor was detected only in FFCC and in 50%RFCC at a lesser level. The catty, fruity and nutty flavors have been reported previously as typical attributes of aged full-fat Cheddar cheese (Urbach, 1997; Drake et al., 2001). From a previous study on RFCC made by the fat removal process (Carunchia Whetstine et al. 2006), the flavor compounds for catty, fruity, and nutty flavors were soluble in the fat fraction of the cheese, hence the resulting RFCC had less intensity of these flavors. In this study, RFCC only contributed one third of the total ingredients to make the LFCC. This explains why these flavors were no longer detectable in LFCC. The LFCC in the current study was perceived to have more bitterness than other cheeses, but even so, LFCC had a bitter score < 1 on a 15 point scale. A comprehensive sensory analysis

of Cheddar cheeses from around the world had a broad range of bitterness in Cheddar cheese from a score 0 (not detectable) to 3.0 (Drake et al., 2001). Thus, it is reasonable to state that all the cheese samples in this study were relatively low in bitterness. Cooked and whey flavor was higher in 50%RFCC and 75%RFCC than in FFCC and LFCC. In the production of 50%RFCC and 75%RFCC, more moisture was retained in the curd (less whey was expelled) than in FFCC. This may account for higher whey flavor in the commercial RFCC than FFCC. In comparison, MCC used as a major ingredient to produce LFCC contained very little whey protein and lactose. The LFCC had a lower sourness intensity score ($P < 0.05$) than other cheeses. The umami and sweet flavor was higher in FFCC than other cheeses. Diacetyl and FFA flavors were not detected in any of the cheeses.

In comparison to commercial RFCC, LFCC contained some flavor and particularly texture attributes that were similar to FFCC. Nonetheless, the flavor of LFCC still needs improvement to make it comparable to FFCC. This includes increasing sourness, umami, sweet, milkfat, brothy, nutty, fruity and catty flavors, and reducing bitter and grapey-tortilla flavor. A possible improvement strategy is to incorporate a flavoring ingredient which can enhance the missing flavors and at the same time mask undesirable flavors. Enzyme modified cheese (EMC) is an example of flavor ingredients that can be added to the formula to serve these roles. A preliminary experiment (data not reported) with EMC addition did show masking of the grapey-tortilla flavor.

Possible new industrial process

A 50% RFCC can be made from FFCC that is subjected to a fat removal process (Nelson and Barbano, 2004). The FFCC can be from trim from a cut and wrap Cheddar cheese packaging

plant. For a continuous industrial process, we envision that FFCC is shredded and placed on a running belt into a heat tunnel. The warm shredded cheese will be fed into a continuous horizontal decanter centrifuge (Figure 3.3) to separate the liquid milk fat from RFCC. Meanwhile in a separate unit process, MCC powder is hydrated in a cooker (60°C) with continuous mixing. The RFCC, salt and lactic acid are added to the hydrated MCC mixture, while maintaining the heating and mixing until uniform. The LFCC mix is cooled to 35 to 38°C. Rennet is added, followed by immediate mixing. The final LFCC mix is injected into a mold to achieve a rectangular shape and allow for a small volume of whey drainage over a period of 30 min. The LFCC is removed from the mold and packaged. The packaged LFCC is maintained at ambient temperature (21°C) for 30 min to allow the structure become uniform and closed. The LFCC is then cooled and stored at 4°C.

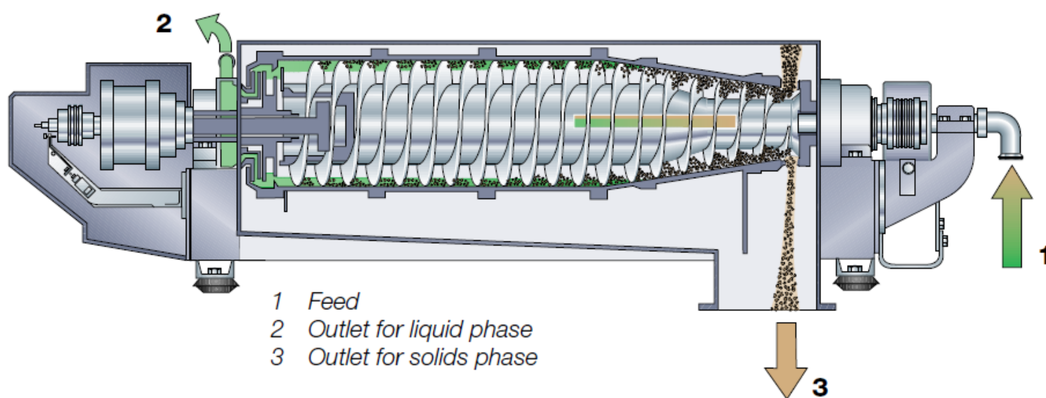

Figure 3.3. An illustration of a continuous horizontal decanter centrifuge (source: Anonymous, 2003)

In our study, we did not investigate whether the small amount of whey produced (1.82% of the total cheese) after the addition of rennet would be reabsorbed into the cheese, if the cheese was packaged immediately and cooled from 38°C to 4°C. Olabi and Barbano (2002) demonstrated temperature-induced moisture migration within 290 kg cheese blocks using a laboratory-scale apparatus that stimulated the temperature gradient that developed during cooling

of a cheese block (Reinbold and Ernstrom, 1988; Reinbold et al., 1992; Barbano, 2001). As the cheese began to cool from the surface of the cheese, hydrophobic interactions between proteins on the surface of the cheese are weakened, and protein-water interaction becomes favored. As a result, moisture migrates from higher temperature region (in the center of the cheese) to lower temperature region (on the surface of the cheese). If the whey produced in our LFCC making can be reabsorbed back into the cheese by protein-water interaction induced by cooling, the whey draining step (after depositing the cheese into a mold) can be eliminated, the LFCC can be packaged right away after being shaped in a mold and removed from the mold. The LFCC made using this novel process is expected to be marketed directly without aging. Flavor can be enhanced by addition of a flavor ingredient such as enzyme modified cheese. The absence of aging process for the LFCC translates to a significant cost saving related to storage space and inventory because LFCC can be produced to meet fluctuating market demand without aging.

CONCLUSIONS

A new approach to produce LFCC was developed by combining 50% RFCC made using the fat removal process of Nelson and Barbano (2004) with hydrated MCC, lactic acid and salt. The LFCC made by this process complies with the FDA low-fat label requirements. The LFCC had 83% less fat, 32% less sodium, higher protein and moisture than FFCC. When the cheese texture was evaluated in the context of a filled-gel model consisting matrix and filler (100% minus % matrix), the LFCC had lower filler volume than FFCC, yet the LFCC had a softer texture than FFCC. The LFCC contained some of the original FFCC cheese matrix that had been disrupted by the fat removal process, and this original FFCC matrix was embedded in a LFCC matrix formed by the action of rennet on casein from the continuous phase of hydrated MCC.

Thus, the texture of the LFCC was desirable and softer than the FFCC it was made from, whereas commercial 50%RFCC and 75%RFCC were firmer than the FFCC. The sulfur flavor in LFCC was closer to FFCC than the commercial 50%RFCC and 75%RFCC. The LFCC had bitter and grape-tortilla off-flavors which came from the dried MCC ingredient. The commercial RFCC and LFCC made in this study were missing the typical aged Cheddar character (catty, nutty, fruity, brothy, milkfat) found in FFCC. Future work to improve the flavor of LFCC made by the process described in this study should include the addition of a flavoring ingredient, e.g. enzyme modified cheese, to enhance the aged Cheddar flavors and mask undesirable flavors.

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Chapter 4

Conclusions and Future Work

The caseins in milk can be physically separated from other milk components by microfiltration. The retantate of MF skim milk is called micellar casein concentrate (MCC) which is rich in caseins in their micellar form and low in lactose and serum protein. The MCC has distinct properties that are different from any available commercial protein products (e.g. acid casein, rennet casein, caseinates, milk protein concentrate, co-precipitates), thus MCC might serve particular functions that other ingredients can't deliver. The production of MCC from skim milk can be used as an alternative to NDM production to balance milk production seasonality. The production of MCC avoids the cost of transporting excess skim milk to a drying facility and eliminates the cost of evaporating and drying. The fresh liquid MCC can be concentrated by ultrafiltration to remove excess water which translates to a saving in transportation cost

To leverage its competitiveness as a protein ingredient, shelf-life stability of MCC becomes an important factor. The 1st study in this thesis demonstrated the production of high concentration liquid MCC (18% protein or MCC18) with a long refrigerated shelf life. In order to have a long refrigerated shelf-life, the processing of MCC18 was designed to maximize the removal of low molecular weight (MW) compounds (e.g. lactose, nonprotein nitrogen) which can be easily metabolized by microbes and used as nutrient sources. The manufacturing process involved UF and multiple stages of MF in which low MW compounds were removed with the permeate. Another strategy to prolong refrigerated shelf-life was to minimize the microbial count in the final product which was achieved through gravity separation and pasteurization. The MCC18 produced in this study maintained a bacterial count of < 20,000 cfu/mL for 16 wk when stored at refrigeration temperature of 4°C. The MCC18 formed a solid gel at temperatures

$< 22^{o}$C, but it reverted back to a liquid when warmed from 4°C temperature to $> 22^{o}$C. This provides a unique opportunity in ingredient handling and packaging and eliminates the challenges encountered in reconstitution of dried milk protein ingredients.

Further study is needed to determine if there are changes in the organoleptic and functional properties of MCC18 during its refrigerated storage. We propose that a descriptive sensory evaluation is conducted on fresh MCC18 (at the day of processing), and MCC18 at different stages of storage time (e.g. interval of 4 wk) to detect any changes in flavor. Similarly, evaluation on functional properties of MCC18 should be conducted on fresh MCC18 and at different stages of storage time. Evaluation on functional properties of MCC18 may include solubility, viscosity, emulsification, and heat stability. The MCC18 in this study was made in a pilot scale that was limited in equipment capacity and staffing, therefore the manufacturing process took 5 days. This processing condition was less ideal from a microbial contamination and microbial growth perspective. Future work may include producing MCC18 in a more continuous process that could run multiple stages of filtration within the same day. By doing so, microbial contamination and growth can be minimized, and the resulting MCC18 might have a longer shelf-life.

The 2nd study developed a new approach to produce low fat Cheddar cheese (LFCC) by combining 50% reduced-fat Cheddar cheese made using a fat removal process with hydrated MCC, lactic acid and salt. The LFCC had 83% less fat, 32% less sodium, higher protein and moisture than full-fat Cheddar cheese (FFCC). When the cheese texture was evaluated in the context of a filled-gel model consisting matrix and filler (100% minus % matrix), the LFCC had lower filler volume than FFCC, yet the LFCC had a softer texture than FFCC. The LFCC contained some of the original FFCC cheese matrix that had been disrupted by the fat removal

process, and this original FFCC matrix was embedded in a LFCC matrix formed by the action of rennet on casein from the continuous phase of hydrated MCC. Thus, the texture of the LFCC was desirable and softer than the FFCC it was made from, whereas commercial 50% reduced- and 75% reduced-fat Cheddar cheese (50%RFCC and 75%RFCC, respectively) were firmer than the FFCC. The sulfur flavor in LFCC was closer to FFCC than the commercial 50%RFCC and 75%RFCC. The LFCC had bitter and grape-tortilla off-flavors which came from the dried MCC. The commercial RFCC and experimental LFCC made in this study were missing the typical aged Cheddar character (catty, nutty, fruity, brothy, milkfat) found in FFCC.

Future work should include flavor improvement of LFCC made by this process. The addition of a flavoring ingredient, e.g. enzyme modified cheese, might be used to enhance the aged Cheddar flavors and mask undesirable flavors. If flavoring ingredients can deliver the desired Cheddar cheese flavor, it might be possible to eliminate the use of reduced fat Cheddar cheese made by the fat removal process, and use FFCC and MCC as the building block of LFCC. By doing so, the LFCC-making can be simplified and cost associated with its production can be reduced. The melting properties of LFCC could also be evaluated, especially when LFCC is intended to be used as an ingredient in the food service.