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Manufacture of Ingredients for Use in Clean Label Process Cheese Products

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MANUFACTURE OF INGREDIENTS FOR USE IN CLEAN LABEL PROCESS CHEESE PRODUCTS

BY

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DISSERTATION ACCEPTANCE PAGE

Ahmed Hammam

This dissertation is approved as a creditable and independent investigation by a candidate for the Doctor of Philosophy degree and is acceptable for meeting the dissertation requirements for this degree. Acceptance of this does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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This dissertation is dedicated to all of my family. You have made me stronger, better, and more fulfilled than I could have ever imagined.

Thank you for your love and sacrifice

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CONTENTS

ABBREVIATIONS

CCP	Calcium phosphate complexes
CF	Concentration factor
CN(s)	Casein(s)
DF	Diafiltration
DSR	Dynamic stress rheometer
ES	Emulsifying salts
GMP	Glycomacropeptide
GP	Graded permeability
IMC	Imitation Mozzarella cheese
MT	Metric tons
MCC	Micellar casein concentrate
MF	Microfiltration
MPC	Milk protein concentrate
NCN	Noncasein nitrogen
NF	Nanofiltration
NFDM	Nonfat Dry Milk

NPN	Nonprotein nitrogen
PC	Process cheese
PCF	Pasteurized process cheese food
PCP	Process cheese products
PCS	Process cheese spread
Ppo	Permeate pressure outlet
PVDF	Polyvinyldeneflouride
RO	Reverse osmosis
Rpi	Retentate pressure inlet
Rpo	Retentate pressure outlet
RVA	Rapid visco analyzer
SP	Serum protein
SW	Spiral-wound
TMP	Transmembrane pressure
TPr	Total protein
TP	True protein
TS	Total solids

TPA	Texture profile analysis
UF	Ultrafiltration
UTP	Uniform transmembrane pressure
WPC	Whey protein concentrate
WPI	Whey protein isolate
β-CN	β-casein
αS_1-CN	αS_1 casein
αS_2-CN	αS_2 casein
κ-CN	κ-casein
γ-CN	γ-casein
α-LA	α-lactalbumin
β-LG	β-lactoglobulin
tan δ	Tan delta

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ABSTRACT

MANUFACTURE OF INGREDIENTS FOR USE IN CLEAN LABEL PROCESS CHEESE PRODUCTS

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Micellar casein concentrate (MCC) is a high protein ingredient that can be used in several applications, such as manufacture of acid curd and process cheese products (PCP). Acid curd is one of the casein (CN) products, which can be obtained by precipitating the CN at a pH of 4.6 (isoelectric point) using starter cultures or direct acids. Acid curd has low mineral and calcium content due to the solubility of colloidal calcium phosphate at the isoelectric point in the whey. Acid curd and MCC can be utilized in manufacture of clean label PCP formulations. PCP is a dairy food prepared by blending dairy ingredients (such as natural cheese, protein concentrates, butter, non-fat dry milk: NFDM, whey powder, and permeate) with nondairy ingredients (such as sodium chloride, water, emulsifying salts: ES, color, and flavors) and then heating the mixture with continuous agitation to produce a homogeneous product with an extended shelf-life. If acid curd is mixed with MCC, it may be possible to create a partially deaggregated casein network without the use of ES. The ratio of acid curd to MCC will have an impact on the level of deaggregation and the pH of the final PCP. We hypothesize that a ratio of 2 parts of protein from acid curd and 1 part of protein from MCC will create a partially deaggregated casein network similar to a typical process cheese that utilizes ES.

The objectives of the first study were to determine the optimum protein content (3, 6, and 9% protein) in MCC to produce acid curd and to manufacture PCP using a combination of acid curd cheese and MCC that would provide the desired improvement in the emulsification capacity of caseins without the use of ES. To produce acid curd, MCC was acidified using lactic acid to get a pH of 4.6. In the experimental formulation, the acid curd was blended with MCC to have a 2:1 ratio of protein from acid curd relative to MCC. The PCP was manufactured by blending all ingredients in a kitchenaid to produce a homogeneous paste. A 25 g sample of the paste was cooked in a rapid visco analyzer (RVA) for 3 min at 95°C at 1000 rpm stirring speed during the first 2 min and 160 rpm for the last min. The cooked PCP was then transferred into molds and refrigerated until further analysis. This trial was repeated three times using different batches of acid curd. MCC with 9% protein resulted in acid curd with more adjusted yield. The end apparent viscosity (402.0-483.0 cP), hardness (354.0-384.0 g), melting temperature (48.0-51.0°C), and melting diameter (30.0-31.4 mm) of PCP made from different batches of acid curd showed were slightly different from the characteristics to typical process cheese produced with conventional ingredients and ES (576.6 cP end apparent viscosity, 119.0 g hardness, 59.8°C melting temperature, and 41.2 mm melting diameter) due to the differences in pH of final PCP (5.8 in ES PCP compared to 5.4 in no ES PCP). We concluded that acid curd can be produced from MCC with different protein content. Also, we found that PCP can be made with no ES when the formulation utilizes a 2:1 ratio of acid curd relative to MCC (on a protein basis).

The objectives of the second study were to develop a process to produce acid curd from MCC using starter cultures and to manufacture imitation Mozzarella cheese (IMC)

using a combination of acid curd and MCC that would provide the required emulsification ability to the caseins without the use of ES. The formulations were targeted to produce IMC with 18.0% protein, 49.0% moisture, 20.0% fat, and 1.5% salt. In the IMC formulation (FR-2:1), the acid curd was blended with MCC so that the formula contained a 2:1 ratio of protein from acid curd relative to MCC. Additional dairy and nondairy ingredients (milk permeate, vegetable oil, and salt) were also utilized in the formulations. Another IMC formulation was made using conventional ingredients and ES as a control. The IMC was prepared by mixing all ingredients in a kitchen aid to produce a homogeneous paste. A 20 g of the mixture was cooked in the RVA for 3 min at 95°C with a 1000 rpm stirring speed during the first 2 min and 160 rpm during the last min. The cooked IMC was then transferred into molds and refrigerated until further analysis. This trial was repeated 3 times using 3 different batches of acid curd. The end apparent viscosity of IMC was approximately 5711.0 cP for control and 7500.0 cP for FR-2:1, while the hardness was 301.0 g for control and 95.0 g for FR-2:1. The melt temperature was 55.5 and 50.0°C, melt diameter was 29.4 and 31.6 mm), melt area was 679.6 and 783.1 mm2, and stretchability was 12.5 and 12.3 cm of control and FR-2:1 IMC, respectively. The melt and stretch characteristics of IMC made from FR-2:1 were similar compared to control IMC. We conclude that IMC can be made with no ES when the formulation utilizes a 2:1 ratio of protein from acid curd relative to MCC.

The objectives of the third study were to produce MCC using MF membranes and develop a process to produce a novel culture-based acid curd powder ingredient. Skim milk was pasteurized at 76°C for 16 sec and then microfiltered (MF) in 3 MF stages using graded permeability (GP) ceramic membranes. The skim milk was MF in a 3 stages

process at 50°C with a 3× concentration factor (CF) and diafiltration (DF) to get MCC with >9% true protein (TP) and >13% total solids (TS). Part of the MCC was dried to produce MCC powder. The rest of the MCC was used to produce acid curd. The MCC was fortified with milk permeate as a source of lactose and inoculated with 0.5% starter cultures at 43°C to get the pH of 4.6 in 10-14 h. The curd was subsequently cut, drained, washed, and pressed. The curd was then milled and dried at 70-75°C outlet temperature for 3-4 h. The dried curd was then milled to produce acid curd powder. The skim milk, MF permeate, liquid MCC, modified MCC, acid curd, acid whey, MCC powder, and acid curd powder were compositionally analyzed. This trial was repeated 3 times using 3 different batches of skim milk. The skim milk had approximately 0.7, 3.4, 0.3, 0.9, 0.6, 9.0, and 4.4% ash, total protein (TPr), nonprotein nitrogen (NPN), noncasein nitrogen (NCN), serum protein (SP), TS, and lactose, respectively. The fortified MCC had 1.4% ash, 10.9% TPr, 0.2% NPN, 1.4% NCN, 1.2% SP, 17.4% TS, and 4.2% lactose. The curd prior drying showed approximately 1.0, 36.4, 0.7, 1.3, 0.6, 40.4, and 0.80% for ash, TPr, NPN, NCN, SP, TS, and lactose, respectively. The acid curd powder had approximately 2.0% ash, 86.9% TPr, 2.2% NPN, 2.3% NCN, 0.08% SP, 96.4% TS, and 1.4% lactose. The acid curd prior drying and acid curd powder were successfully produced from MCC. Future studies will be performed to utilize the acid curd and MCC powders at different ratios in process cheese products formulations and examine the functional properties of the cheese.

The objective of the fourth study was to produce PCP without ES using different ratios of protein from novel cultured micellar casein concentrate ingredient (cMCC) and MCC powders. Three PCP treatments were formulated with 3 different ratios of cMCC:

MCC including 2.0:1.0, 1.9:1.1, and 1.8:1.2 on a protein basis. The composition of PCP was targeted to 19.0% protein, 45.0% moisture, 30.0% fat, and 2.4% salt. This trial was repeated 3 times using different batches of cMCC and MCC powders. All PCP were evaluated for their final functional properties. No significant differences ($P>0.05$) were detected in the composition of PCP made with different ratios of cMCC and MCC except for the pH. It was expected to increase slightly with elevating the MCC amount in the PCP formulations. The end apparent viscosity was significantly higher ($P<0.05$) in 2.0:1.0 formulation (4305 cP) compared to 1.9:1.1 (2408 cP) and 1.8:1.2 (2499 cP). The hardness ranged from 407 to 512 g with no significant differences ($P>0.05$) within the formulations. However, the melting temperature showed significant differences ($P<0.05$) with 2.0:1.0 having the highest melting temperature (54.0°C), while 1.9:1.1 and 1.8:1.2 showed 43.0 and 42.0°C melting temperature, respectively. The melting diameter (38.8 to 43.9 mm) and melt area (1183.9 to 1538.6 mm2) did not have any differences in different PCP formulations. The PCP made with a 2.0:1.0 ratio of protein from cMCC and MCC showed better functional properties compared to other formulations.

Keywords: Micellar casein concentrate; Microfiltration membranes; Process cheese products; Imitation Mozzarella cheese; Acid curd; Functional characteristics

CHAPTER I: REVIEW OF LITERATURE

1. Abstract

Micellar casein concentrate (MCC) is a novel ingredient with high casein content. Over the past decade, MCC has emerged as one of the most promising dairy ingredients having applications in beverages, yogurt, cheese, and process cheese products. Industrially, MCC is manufactured by microfiltration (MF) of skim milk and is commercially available as a liquid, concentrated, or dried containing ≥9, ≥22, and ≥80% total protein, respectively. As an ingredient, MCC not only imparts a bland flavor but also offers unique functionalities such as foaming, emulsifying, wetting, dispersibility, heat stability, and water-binding ability. The high protein content of MCC represents a valuable source of fortification in a number of food formulations. For the last 20 years, MCC is utilized in many applications due to the unique physiochemical and functional characteristics. It also has promising applications to eliminate the cost of drying by producing concentrated MCC. This work aims at providing a succinct overview of the historical progress of the MCC, a review on the manufacturing methods, a discussion of MCC properties, varieties, and applications.

Keywords: Applications of micellar casein concentrate; Casein products; Fractionation; Functional properties; Micellar casein concentrate; Microfiltration membranes

2. Introduction

Milk protein is one of the best choices of protein sources in the human diet due to a number of health benefits associated with their consumption. Muscle synthesis, satiety control, glucose control, and weight management are examples of health benefits of milk

proteins (McGregor and Poppitt, 2013; Nongonierma and FitzGerald, 2015). As a result, food products formulated with milk proteins are the fastest growing segment in the food industry (Bombe, 2020). Also, the world market of milk proteins has significantly increased over the last decade and is expected to elevate during the coming years with increasing population (Lagrange et al., 2015; Bombe, 2020). The population is increasing by 1.1% annually to reach 9.9 billion in 2050, while the food demand will increase at a range of 59.0 to 98.0% by 2050. Consequently, milk protein will increase proportionally since milk and its products are one of the essential foods (Bombe, 2020). The unique physicochemical and functional characteristics of milk protein and its products are also helping to commercialize this product globally (Figure 1).

Currently, various technologies are commonly utilized to separate milk proteins from the serum phase, such as fractionation, concentration, coagulation, and drying. This has led to the manufacture of different protein products that are available in the markets, including casein (CN) products and whey/serum protein (SP) products. The production of CN in the world was estimated to be 274,000 metric tons (MT) in 2004. In 2013, the global export of CN reached 110,675 MT with the United States, Europe, and Mexico being the largest markets (Lagrange et al., 2015). As a result, the number of new products containing CN increased in the United States by approximately 22.0% per year from 2000 to 2008 (Affertsholt, 2009).

As shown in Table 1, the characteristics of CN (e.g., amphiphilic, open, and flexible structures) have been utilized in food applications to provide foaming, emulsifying, and water binding properties (Rollema and Muir, 2009). In addition to those functional properties, it provides necessary amino acids to the human body, such as

valine, leucine, isoleucine, phenylalanine, tyrosine, and proline (Pritchard and Kailasapathy, 2011). Also, CN micelles provide the body with calcium, which is essential for strong bones (Walstra et al., 2006). There are different types of commercial CN products that are available in the market; including rennet casein, acid casein, caseinates, co-precipitates, and milk protein concentrate (MPC). The production and characteristics of CN products have been reviewed (Modler, 1985; Fox, 2001; Rollema and Muir, 2009; Augustin et al., 2011; Zhang et al., 2011). The functional and sensory characteristics (e. g. solubility, foaming, viscosity, heat stability, and flavor) of these CN products vary based on the manufacturing conditions used in each product. Besides those CN products, microfiltration (MF) has been recently utilized to produce a novel CN ingredient called micellar casein concentrate (MCC) or microfiltered milk protein. One review has been published on MCC since it is a novel ingredient in research and the dairy industry (Carter et al., 2021). Therefore, the objective of this review is to highlight and review the manufacturing conditions, properties, varieties, and applications of MCC.

3. Historical overview

Table 2 lists important scientific and technological milestones considered as the forerunner in the development of CN ingredients. Caseins (CNs) were first identified more than two hundred years ago by Schubler in 1818, who suggested the term “merely suspended” portion in milk (Schubler, 1818). Decades later (1880), Sheldon pointed out an insoluble portion in the serum that did not pass through a dialysator (Sheldon, 1880). Soon after, such a portion was designated as colloidal proteins (Duclaux, 1887). Years later, Kastle and Roberts in 1909 separated the CN from the serum with Pasteur-Chamberland porcelain filters. Subsequently, the colloidal particles were found to contain

calcium phosphate (Kastle and Roberts, 1909). In 1910, it was hypothesized that CN exists in a protective colloidal model, where the CN (irreversible unstable colloid) was covered by lactalbumin (reversible stable colloid). This hypothesis was then examined by coagulating bovine, human, and donkey's milk with rennet (Alexander, 1910; Alexander and Bullowa, 1910). The produced gel from human and donkey's milk was very weak as compared to bovine milk because of the lower CN and higher lactalbumin. In 1914, Wiegner described the physical state of CN as large particles associated together and disassembled them by displacing the calcium with sodium (Wiegner, 1914). Subsequently, Duclaux described macro clusters of CN as micelle (Duclaux, 1920).

Palmer and Richardson (1925) rejected the protective colloidal model and postulated the precipitation of CN due to the cations. Palmer and Richardson coined a new terminology for the casein micelle, including colloidal calcium caseinate, calcium caseinate, or calcium caseinate-calcium phosphate (Palmer and Richardson, 1925). Concomitantly, Sorensen employed the term "component system" to describe the CN macro clusters instead of the "micelle" term (Sorensen, 1930).

During that period, the isolation of the CN structure was a significant challenge. In 1936, Pederson ultracentrifuged milk to obtain CN and studied its polydispersity (Pedersen, 1936). Pederson's development represented a significant milestone to make it possible to study different characteristics of CN in milk. Years later, Langmuir and Waugh reported the hydrophilic and hydrophobic properties of CN (Langmuir and Waugh, 1940). During the 1950s, the term "casein micelle" was widely used to describe the state of milk CN (Pyne, 1953), where non-covalent forces maintained the protein association and aggregation (Waugh, 1954).

The aggregation of the CN network was an active area of research throughout 1960s. The role of pH, temperature, and protein content were extensively evaluated (von Hippel and Waugh, 1955). Waugh and von Hippel in 1956 invented a methodology to isolate the κ-casein (κ-CN) that allowed an in-depth study of the CN structure (Waugh and von Hippel, 1956). In 1957, the κ-CN was found to be about 12.0-15.0% of the CN content when the calcium was soluble, and 10 times more when the micelle was aggregated, reaching a size up to 2000 Å with an average of 1000 Å (Fox and Foster, 1957). After that, many studies were performed on the structure of the CN micelle. In 1958, the first microscopy study of the CN was reported (Barbaro and Calapuj, 1958), which make it possible to propose the first CN micelle model (Waugh, 1958). Before the 1960s, CN was manufactured for industrial applications (e.g., plastic, paints, and glues). After the 1960s, Australia and New Zealand initiated to utilize CN as a food ingredient. In 1972, researchers studied some functional characteristics of CN, such as viscosity and surface tension in CN solutions of buffalo and cow milk (Puri et al., 1972). Badertscher and Chaveron have invented a process to produce CN and caseinates in 1978 (Badertscher and Chaveron, 1978). Ten years later, the emulsifying characteristics of CN micelle were studied in bovine milk (Haque et al., 1988). Years later, in 1992, alternative models to sub-micelles associated together by calcium phosphate complexes (CCP) were proposed (Visser, 1992). At the beginning of 1990, MF has been used in the dairy industry to reduce the bacterial count, as well as, fractionation of CN and SP without heat treatment or chemicals, which keep the CN in its native status (Haeusl et al., 1990; Olesen et al., 1990). In 1994, MF has applied to examine the phage distribution in permeate and retentate produced from skim milk (Gautier et al., 1994). The protein

composition of MCC produced from MF was studied in 1999 (Jost et al., 1999). Since then, MF has been applied to milk to produce many dairy products, especially cheese (Lidberg and Bredahl, 1990; Garem et al., 2000). CN has different characteristics and a wide range of applications; so many researchers are still studying and will continue to examine these properties. The CN is widely used nowadays as a functional food ingredient (Huppertz et al., 2004). As a result, Guinee and others have patented MCC powders with different ratios of calcium to CN for making cheese (Guinee et al., 2009). Between 2000 and 2008, the number of new products containing CN grew by 22.0% in the United State annually (Affertsholt, 2009) and expected to have a continual increase in the future.

4. Structure of casein micelle

The CN structure in bovine milk is composed of several protein fractions (αS_1, αS_2, β, and κ-casein which presents approximately 38.0, 10.0, 36.0, and 12.0% of CN, respectively), forming a multi-molecular granular structure (Figure 2) (Holt, 1992). These fractions are linked by CCP, serving as a packaging system for calcium and phosphate. The high proline content in CN leads to a lack of organized secondary and tertiary structures of CN (Huppertz et al., 2004).

The CN is presented in a micellar form, and several models of CN micelles have been proposed (Waugh, 1958; Rose, 1969; Schmidt, 1982; Walstra, 1990, 1999; Holt, 1992; Horne, 2003, 2006). Most of the CN micelle models suggest that the micelles are formed from sub-micelles and cross-linked by CCP. The most accepted model was proposed by Holt (Holt, 1992), which described the CN micelle as a matrix of CN in

which the CCP macro clusters are dispersed (Figure 2). This model suggested that the CN fractions are not distributed evenly throughout the micelle and proposed that κ-CN is located on the surface of the micelle, which has a significant effect on the micelle stability.

The κ-CN fraction has glycomacropeptide (GMP), which provides negative charges and this makes the CN micelle stable through electrostatic repulsion of adjacent micelles (Holt, 1992). All models agree that CN micelle is covered by κ-CN, but not completely. Thus β-CN is mostly interior, while αS_1-CN presents within the structure (Dalgleish and Corredig, 2012). It is also postulated that κ-CN limits the self-binding process, which results in the CN micelle stability (Dalgleish and Corredig, 2012; de Kruif et al., 2012). The κ-CN makes the micelles stable and protects them from aggregation in the presence of calcium. The other highly phosphorylated CN would aggregate together if κ-CN is not presented in the micelle.

CN provides different functional properties, as shown in Table 1. The surface properties of the CN micelle are mainly responsible for the functional characteristics of the micelles more than the interior structure (Dalgleish and Corredig, 2012; de Kruif et al., 2012). Processing and drying techniques do not affect or modify the CN micelles, however, more studies need to be done to validate this (Dalgleish and Corredig, 2012). The internal structure of CN micelles is affected by heating at normal pH (Dalgleish and Corredig, 2012). The instability of CN micelles at high temperatures is related to the denaturation of SP and their interaction with CN micelles, and this, in turn, leads to changes in the calcium equilibrium (Singh and Creamer, 1992; Dalgleish and Corredig, 2012).

4.1.Colloidal calcium phosphate

In bovine milk, approximately two-thirds of the calcium content and half of the inorganic phosphate are present in a colloidal form, while the rest of these minerals are present in a soluble form. The main inorganic constituent in the CN micelle is CCP. The nature of CCP is complex, and these salts could be present in several forms, including tricalcium phosphate, calcium brushite, or exist in amorphous or different crystalline structures (Lucey and Horne, 2009). The phosphate groups of the CN phosphoserine residues are the primary binding sites of calcium phosphate in the CN micelle. Based on the phosphoserine content of the CNs, the capacity for CCP binding decreases as followed in that order; $\alpha S_2 > \alpha S_1 > \beta >$ κ-CN (Gaucheron, 2005).

CCP is an important constituent in maintaining the stability of CN micelles. It has been reported that CCP participates when CN micelles change during dairy processing, such as heating, cooling, and rennet coagulation (Aoki, 1991). The solubility of calcium phosphates present in the serum decreases at high temperatures. The elevated temperature can lead to changes in the structure and composition of the original micellar calcium phosphate (Visser et al., 1986; Aoki et al., 1990). Acidification of milk leads to solubilization of CCP, and this depends on the pH and temperature of acidification (Dalgleish and Law, 1989; Singh et al., 1996). The exact nature of CCP, its interactions with CN molecules, and the effects on the heat stability of CN micelles are still unresolved.

5. Production of MCC

5.1.Overview of filtration technology

There are different types of membrane filtration technologies used in the dairy industry, such as MF, ultrafiltration (UF), nanofiltration (NF), and reverse osmosis (RO). As shown in Figure 3, each type of filtration has a specific pore size that is utilized based on the milk components that need to be fractionated. The MF has the largest pore sizes (0.1-10 µm) while Ro has the smallest pore sizes (<0.001 µm). When MF is applied to skim milk, CN is separated in the retentate side and SP is produced as a byproduct in the permeate side, which can be utilized in other applications such as manufacture of whey protein isolate (WPI). The MF permeate (a byproduct of MF) can be further concentrated using UF to concentrate the SP and produce lactose, minerals, and water as a byproduct. Again, the UF byproduct can be fractionated into lactose and minerals using NF membranes while RO can be used to separate the water and minerals (Figure 3). Since CN is the largest component in skim milk, MF is utilized to fractionate the CN to produce MCC. MF is a membrane process that is utilized to fractionate the CN (0.1-0.4 µm) and SP (0.003-0.01 µm) using a 0.1 µm semi-permeable membrane. The CN in the MF retentate is present in a micellar form which is called MCC.

5.1.1. MF filtration

The MCC is manufactured using different MF membranes, which are divided into two main categories, namely ceramic membranes and polymeric membranes. Each category of MF membranes has different characteristics and, thereby, different performances. The efficiency of MF membranes is limited by concentration polarization and membrane fouling that accumulated on the surface of the membrane and blocks the membrane pores eventually. As a result, the membrane flux (L/m^2 per hour) and the efficiency of SP removal (%) are decreased.

5.2.Types of MF membranes

5.2.1. Polymeric membranes

The polymeric membranes can be found in different geometries, such as flat sheets, tubular, and spiral-wound (SW) (Cheryan, 1998). The most popular type of polymeric membranes is SW membranes because they have a higher surface area per length of the membrane as compared to other geometries.

5.2.1.1.Polymeric spiral-wound (SW)

The common material of polymeric SW MF membranes is polyvinyldeneflouride (PVDF) and polyethersulfone (Belfort et al., 1994). The PVDF material is widely utilized in all polymeric MF membranes. The SW membrane system is equipped with feed and retentate recirculation pumps. SW membranes have improved over the last 50 years and they are utilized in many applications at low temperatures < 7°C (Govindasamy-Lucey et al., 2004, 2005) to reduce the probability of SP denaturation and microbial growth during processing. The SW membranes are cheaper and have lower operating costs but have a limited viscosity range, low chemical stability, and shorter life as compared to ceramic membranes (Zulewska et al., 2009). Many experiments have been conducted on SW membranes to study the efficiency of SP removal (Zulewska et al., 2009; Beckman et al., 2010; Beckman and Barbano, 2013). It has been reported that the percentage of SP removal from skim milk using SW membranes during the first MF stage (3× concentration factor; CF) is 38.6% (Beckman et al., 2010; Zulewska et al., 2009) as compared to 68.0% theoretical SP removal (Figure 4). The mean cumulative SP removal is 38.6, 59.3, and 70.3% in the first, second, and third stages, respectively, in SW MF

with diafiltration (DF) (Beckman et al., 2010). The theoretical SP removal is 68, 90, and 97.0% in the first, second, and third stages, respectively (Figure 4). The efficiency of SP removal from SW MF membranes is low as compared to the theoretical values, and this could be due to the heat denaturation of SP on CN or due to the fouling of membrane which leads to rejection of SP passage through the membrane (Hurt and Barbano, 2010). Additionally, the membrane itself or a combination of the membrane plus membrane foulants (concentration polarization and membrane fouling) could lead to increasing the SP rejection and thereby decreasing the SP removal. As a result, increasing the efficiency of SP removal in SW membranes to approximately 95.0% is required 7 MF stages with DF and 17.98 m^2 membrane surface area (Figure 5) (Zulewska et al., 2009; Adams and Barbano, 2013), which is adding more processing cost.

5.2.2. Ceramic membranes

Due to the challenges in SW MF membranes, ceramic membranes, such as uniform transmembrane pressure (UTP), graded permeability (GP), and Isoflux membranes were designed to improve the efficiency of MF although their high costs relative to SW membranes. The typical process of MF is carried out using ceramic membranes at 50 to 55°C (Maubois, 2002). It has been found that the optimum temperature for MF of skim milk is 50°C (Hurt and Barbano, 2010). Thus, elevating the temperature during MF can lead to denaturation of SP on CN and thereby decrease the SP removal. Also, applying MF at lower temperatures can lead to increasing the viscosity of the product and thereby increasing the accumulated fouling on the membrane which is eventually decreasing the SP removal.

5.2.2.1.Uniform transmembrane pressure (UTP)

The UTP membrane is designed to decrease fouling. The UTP MF membranes are equipped with feed, retentate, and permeate recirculation pumps. Before the incorporation of the permeate recirculation pump, the permeate pressure outlet side (Ppo) was constant while there was a difference between the feed or retentate pressure inlet (Rpi) and retentate pressure outlet (Rpo), which means the flux at the inlet of the membrane was higher than at the outlet (Figure 6). As a result, fouling at the inlet end of the membrane was accelerated, and thereby efficient flux over the entire length of the membrane was limited. Equipping the UTP system with a permeate recirculation pump led to producing co-current permeate flow, and thereby, decreasing the fouling and increasing the SP removal (Zulewska et al., 2009). As a result, UTP membranes require a higher investment and operating costs relative to GP membranes due to the need for a permeate recirculation pump. This makes the transmembrane pressure (TMP) between the permeate and retentate sides of the membrane relatively constant along the length of the membrane. Consequently, the constant flux will be stable along the length of the membrane. It has been reported that the flux of UTP MF membranes is much higher as compared to SW MF membranes (54 vs. 16 L/m^2 per hour). The UTP system is equipped with polymeric beads in the permeate channel. It has been reported that SP passage to permeate was increased by beads packed on the permeate side of the membrane (Dai et al., 1999). The cumulative SP removal using UTP MF with DF is around 63.66, 85.59, and 95.23% during the first, second, and third stages, respectively (Hurt et al., 2010). The SP removal from UTP system using a 3× CF in a feed and bleed mode is higher relative to SW system and much closer to the theoretical SP removal values (Figure 4) which

requires only 3 MF stages and 2.8 m^2 membrane surface area (Figure 5) (Zulewska et al., 2009; Adams and Barbano, 2013).

5.2.2.2.Graded permeability (GP)

Different methods have been applied to ceramic membranes to allow a constant flux along the length of the membrane to be similar to the flux profile of UTP system without the need for the permeate recirculation pump to decrease the operation cost. Researchers have accomplished two methods: the first method was innovated to develop a membrane with a decreasing hydraulic resistance in the support layer from the inlet (higher resistance) to the outlet (lower resistance) (Garcera and Toujas, 2002). The second method was developed to make the ceramic membrane by decreasing the thickness of the selective membrane layer instead of the ceramic support material (Grangeon et al., 2002). GP membranes eliminate the need for the permeate recirculation pump and the associated electrical costs due to their ability to maintain a constant and uniform flux. It has been reported that the flux of GP MF membranes is higher as compared to UTP and SW using a 3× CF in a feed and bleed mode (Zulewska et al., 2009). The mean cumulative of SP removal was 55.97, 82.64, and 96.45% during the first, second, and third stages, respectively, of GP MF with DF (Zulewska and Barbano, 2014). The SP removal by the GP and UTP system is higher relative to SW system and is much closer to the theoretical SP removal rate using a 3× CF in a feed and bleed mode (Figure 4). The GP membranes require less surface area of 1.9 m^2 as compared to 2.8 m^2 in UTP to remove approximately 95.0% SP removal (Figure 5) (Zulewska et al., 2009; Adams and Barbano, 2013), which results in a low processing cost.

5.2.2.3.Isoflux membranes

The Isoflux membrane contains a selective layer on the interior surface of the flow channels that tapers in thickness from the inlet end of the membrane to the outlet (Grangeon et al., 2002). It is designed to provide a constant ratio of TMP to selective layer thickness that is purported to deliver equal permeate flux across the entire length of the membrane (Grangeon et al., 2002). TAMI Inc (ceramic membranes filtration company) creates this membrane to involve sequentially adding selective layers to the internal surfaces of the flow channels within the support structure (Grangeon et al., 2002). The manufacturer should select four selective layers; the first and second layers will apply to the entire length and three-quarters of the membrane, respectively. The third layer will apply to the first half of the membrane, while the fourth layer will apply to the first quarter of the membrane. This process results in a uniformly stepped selective layer gradient. The mean cumulative SP removal in Isoflux membranes was 39.5, 58.4, and 70.2% during the first, second, and third stages, respectively. The cumulative SP removal from Isoflux membranes was low as compared to other ceramic membranes using 3 MF stages with 3× CF (Figure 4). This could be due to the higher rejection of SP by the Isoflux ceramic membranes as compared to GP and UTP ceramic membranes. Isofulx membranes require 7 MF stages and 6.9 m^2 to increase the efficiency of SP removal (Figure 5) (Zulewska et al., 2009; Adams and Barbano, 2013).

5.3.Challenges of using MF membranes

Membrane fouling and concentration polarization in the MF membranes during milk processing are significant challenges that affect the efficiency of these membranes.

Membrane fouling occurs during filtration due to the low molecular weight components that pass through the membrane and become absorbed inside the membrane pores, or the colloidal components rejected on the membrane surface, which results in formation of the cake layer. Also, concentration polarization occurs during filtration when the dissolved components are convectively driven to the surface of membrane where they build up a boundary layer near the membrane surface. As a result of concentration polarization, the removal of SP and low molecular weight components are decreased and thereby decreasing the efficiency of membranes. Different approaches have been applied to MF membranes to decrease membrane fouling and concentration polarization, such as modifying the surface of the membrane, increasing the back-transport of particles away from the membrane by increasing the shear rate or changing the water recovery rate or DF (Bian et al., 2000; Saboyainsta and Maubois, 2000).

5.4.Manufacturing of MCC

MCC is a high-protein ingredient produced using 3 MF stages with 3× CF and DF to separate CN and SP from milk without adding chemicals. In the first stage of MF, skim milk is heated to 50°C and microfiltered in a feed and bleed mode through MF membranes (0.1 μm) so CNs and CN-bound minerals are retained by the membrane while SP, lactose, and unbound minerals pass through the membrane to the permeate (Figure 7). The retentate of the first stage is approximately 33.0% of the total feed weight when 3× CF is applied and around 66.0% is SP. The retentate of the first stage is diluted 2× with RO water to get the original volume of feed (2 kg of RO water: 1 kg of retentate) to increase the removal of SP and lactose in the subsequent stages (Nelson and Barbano, 2005). The diluted retentate is heated to 50°C and microfiltered using 3× CF again in the

second stage. Subsequently, the retentate of the second stage is added to 2× DF RO water, heated to 50°C, and microfiltered. The retentate of the third stage (MCC) has approximately 9.0% true protein (TP) and 13.0% total solids (TS) and these values can slightly change based on the MF membranes (Figure 7). As mentioned previously, MCC has been manufactured using different types of membranes including SW membranes (Beckman et al., 2010), UTP (Hurt et al., 2010), GP (Zulewska et al., 2009; Hammam and Metzger, 2018), and Isoflux ceramic membranes (Yin et al., 2004). The type of membrane used to produce MCC affects the SP removal, the protein content in the retentate, the amount of CN in the permeate, and the process cost (Zulewska et al., 2009). The SP removal from skim milk is higher in GP and UTP membrane systems as compared to SW and Isoflux membranes when MF is applied at 50°C using a 3× CF with DF in a feed and bleed mode (Yin et al., 2004; Zulewska et al., 2009). However, the GP and UTP membranes systems have more transmission of SP to the permeate side relative to SW and Isoflux membranes (Zulewska et al., 2009). The SW and Isoflux membranes need more MF and DF stages as well as a bigger surface area of the membranes (Figure 5) to increase the SP removal.

Theoretically, the SP removal is approximately 97.0% using 3 stages of MF with a 3× CF and 3× DF (Figure 4), assuming no rejection of SP and complete rejection of CN. The SP removal could be affected by many factors during the MF process (Hurt and Barbano, 2010). Hurt and Barbano reported that increasing the thermal processing of milk to 85°C increases the denaturation of SP on CN, and thereby decreases the amount of SP available for removal during MF. The SP removal factors are different depending on the membrane types, which reflect the resistance of the membrane to pass SP.

Increasing the rejection of SP leads to increasing the true protein content in the retentate of each stage, while the cumulative SP removal decreases (Hurt and Barbano, 2010). In addition to the membrane type, the initial composition of milk, CF, and DF are other factors that could affect the CN and SP fractionations (Hurt and Barbano, 2010).

5.5.Types of MCC

MCC can be manufactured in different forms, including liquid (9.0% total protein: TPr and 13.0% TS), concentrated (22.0% TPr and 25.0% TS), and dried (80.0-84.0% TPr and 95.0-96.0% TS).

5.5.1. Liquid MCC

Liquid MCC is a fresh product of retentate and could be obtained by MF of skim milk. One stage 3× CF produces a retentate with >8.0% TPr and >14.0% TS content (Zulewska et al., 2009). The final retentate (Figure 7) of 3 MF stages with 3× CF contains >9.0% TP and >13.0% TS using different MF membranes, such as SW, UTP, GP, and Isoflux membranes (Yin et al., 2004; Hurt et al., 2010). The liquid MCC is a high moisture product and should be refrigerated; thus, these conditions add more cost when the MCC is transported for long-distance. The high cost of transporting MCC can limit the growth of this product in many applications. The liquid MCC would be suitable and more cost effective when it is utilized at the same place where being manufactured.

5.5.2. Concentrated MCC

The liquid MCC can be further concentrated to increase the TPr and TS to more than 22.0% and 25.0%, respectively. The concentrated MCC has lower moisture as

compared to the liquid MCC (Amelia, 2012). The MF (Hammam and Metzger, 2018), UF, or both (Amelia and Barbano, 2013) membranes can be utilized to remove more moisture from the liquid MCC to concentrate the MCC. Increasing the viscosity of MCC during MF leads to accumulation of the fouling on the pores of the membrane, and thereby, the flux is decreased (Eykamp, 1995). This challenge could be more when UF is utilized to concentrate the liquid MCC which limits the production of concentrated MCC with higher solids. To avoid that, Hammam and Metzger developed a process to produce concentrated MCC (~22.0% TP and 25.0% TS) with higher SP removal using GP ceramic membranes (Hammam and Metzger, 2018). After manufacturing the typical liquid MCC, the product was then processed at approximately 62°C in a continuous MF using recirculation mode to remove as much as possible of SP till the TS was reached 21-22.0%. Subsequently, the temperature of MF was increased to 73°C to decrease the viscosity and continue removing more SP. Concentrating MCC reduces the volume of the product during transportation, thereby decreasing the transportation cost. Manufacture of concentrated MCC using MF is a good way of storing a valuable source of intact CN and producing co-products, such as producing WPI. The dairy industry counteracts a problem with milk production during the season. During the season of milk production, there is a peak at which excess milk is used to manufacture storable products, such as butter and nonfat dry milk (NFDM). The excess skim milk is transported a long distance to a drying facility to produce NFDM, which is adding more cost besides the drying cost (Amelia, 2012). However, the production of concentrated MCC would eliminate the drying and transportation cost when MF system is set up in a milk processing plant in a high milk production area. The cost of installing MF system is low and takes less space compared

to building an evaporator and a tower dryer. Different shelf-life studies have examined the stability of concentrated MCC. The major low molecular weight compounds, such as lactose and nonprotein nitrogen (nutrients for microbial growth) can be removed in permeate. It has been reported that increasing the removal of low molecular weight compounds during manufacturing of concentrated MCC could minimize the microbial growth and then increasing its shelf-life up to 2 to 4 months at 4°C (Amelia, 2012; Hammam and Metzger, 2018).

5.5.3. Dried MCC

Liquid or concentrated MCC could be dried using attrition dryers to produce dried MCC with a long shelf-life. It has been reported that the MCC powder can contain up to 84.0% TPr and 96.0%TS (Nasser et al., 2018). Dried MCC can be handled, transported, and stored easily; however, it needs to be reconstituted to be used in some applications (Amelia, 2012). The solubility of powder is decreasing during the shelf-life, which is another challenge besides the production cost. It has been reported that the solubility or rehydration of high protein powder ingredients such as MPC and MCC is decreased during storage (Anema et al., 2006; Fyfe et al., 2011; Gazi and Huppertz, 2015; Carter et al., 2018). Consequently, the dairy industry is also focusing on production of concentrated MCC as an alternative method for drying, which has more solubility and can maintain the original flavor plus eliminating the cost of drying (Carter et al., 2018; Hammam, 2019; Hammam et al., 2019).

6. MCC and other CN products

The average size of CN is 0.1 µm, which is around 100 times the size of SP (Walstra et al., 2006). The CN and SP could be separated using different methods based on their sizes (0.1-0.4 µm for CN and 0.003-0.01 µm for SP) or their characteristics (Table 3), such as pH of 4.6, rennet coagulation, and heat stability. As a result, there are many casein products available in the markets, such as rennet casein, acid casein, sodium caseinate, calcium caseinate, calcium casein caseinates, and co-precipitates with different compositions (Figure 8). There are variations in the composition of CN products due to the differences in methodology of manufacture, and thereby, substantial effect on the physicochemical and functional properties.

6.1.Rennet caseins

Rennet caseins can be manufactured by using proteolytic enzymes (chymosin or rennet) which hydrolyze the polypeptide chain of κ-CN between Phe105-Met106. The surface charge and steric repulsion (which maintain CN micelles in a colloidal status) are removed from the surface of the micelle when κ-CN is hydrolyzed, thereby, CN micelles aggregate and form the gel or curd. The curd is cooked at high temperatures (60°C) to increase the syneresis of curd, firmness, and to inactivate the coagulant enzymes. This is the principle of making rennet set cheeses like Cheddar and Mozzarella. The curd can be washed and dried by using roller dryers. Based on the drying process, a grinding step might be required to produce the desired particle size. Coagulation of milk using rennet at neutral pH retains the minerals associated with the CN. Mulvihill and Ennis reported that the solubility of rennet casein in water is low; however, it could be solubilized in water at a pH of ≥ 9 with the addition of calcium sequestering salts (e.g., sodium phosphates, sodium citrates) (Mulvihill and Ennis, 2003). Rennet caseins are widely utilized to

produce cheese analogues, which include the addition of polyphosphates (Fox et al., 2015).

6.2.Acid caseins

Acid casein is one of the casein products which can be obtained by precipitating the CN at a pH of 4.6 (isoelectric point) by using starter cultures (produce lactic acid) or direct acids (such as HCl, HNO_3, and H_2SO_4). The CCP in the micelles is dissolved in the whey and produces acid curd with low calcium or low mineral content opposite to rennet casein (Figure 8). This is the principle of making yogurt and cottage cheese curd. Similar to rennet casein, acid casein is not soluble in water (Figure 1).

6.3.Caseinates

Caseinates are manufactured by adding alkali, such as NaOH, NH_4OH, KOH, and $Ca(OH)_2$ to the acid casein to increase the pH to 7. The composition of different caseinates varieties (e. g. sodium caseinate, calcium caseinate, and calcium casein caseinates) is shown in Figure 8. Caseinates can be utilized in many applications (Table 4). The pH of 6.8 makes the caseinates soluble in water. The caseinates produce a high viscous solution (Figure 1), which limits the further concentration of solids content in caseinates solutions to be handled easily during production (Ann Augustin et al., 2011). As a result, the efficiency of drying caseinates is poor. Rollema and Muir reported that calcium caseinates behave differently from other caseinates, which resulted in the interaction of calcium with the phosphoserine residues in the CN (Rollema and Muir, 2009). The appearance of calcium caseinates is milky because of forming highly

aggregated colloidal dispersions, while the appearance of other caseinates could be clear or slightly opalescence (Rollema and Muir, 2009).

6.4.Co-precipitates

The composition of co-precipitates is shown in Figure 8. Co-precipitates can be obtained by heating the skim milk at temperature ranges from 90 to 95°C for 30 min which results in denaturation of the majority of SP on CN by forming disulfide interactions between β-LG and κ-CN (Modler, 1985; Singh, 1995; Rollema and Muir, 2009). To precipitate SP with CN, acidification is applied by using mineral acid to reach a pH of 4.6. The $CaCl_2$ is also added during this process to recover the majority of milk proteins (Rollema and Muir, 2009), then the co-precipitates are washed and dried. Co-precipitates are relatively soluble in water and form viscous solutions. Co-precipitates could be utilized in many applications (Table 4), such as infant formulation due to their high nutritional value as compared to CN.

6.5.Milk protein concentrate (MPC)

The composition of different milk protein concentrate (MPC) products is presented in Figure 9. MPC is obtained using separation technology which maintains the milk protein structure without using any chemicals for acidifications as in co-precipitates. MPC is produced using UF to concentrate the CN and SP from skim milk and remove the lactose and minerals. MPC could be further concentrated to get around 85.0-90.0% TPr as a percentage of TS using UF and DF steps. Phillips and Williams reported that MPC could have a range of protein content from 42.0 to 85.0% (Figure 9) (Phillips and Williams, 2011). The MPC can be utilized in a concentrated form or dried form. The

solubility of MPC powder decreases during storage period (Havea, 2006) but higher than acid casein and calcium caseinate (Figure 1).

6.6.Micellar casein concentrate (MCC)

The composition and properties of MCC are different from other casein products. The CN in other casein products (e.g., rennet caseins, acid caseins, caseinates, and co-precipitates) are not in a micellar form. However, MCC is maintained the casein micelles form which makes it unique and can be used in different applications as compared to other CN ingredients. MCC also contains the bound minerals associated with the micelles, while these minerals in acid caseinates are dissolved in the whey. The MCC is a good source of intact CN whereas in rennet casein GMP of κ-CN is dissolved in the whey. The existence of oligosaccharides in GMP improves the hydrophilicity of the CN (Huppertz et al., 2004). The CN structure is similar to MCC and MPC, but the difference is that MPC has a higher content of SP which could decrease its heat stability. Several MCC products are commercially available and have a range of protein content from 42 to 90.0% (Figure 10). Each product is identified by a number that represents the protein content of that product.

7. The properties of MCC

7.1.Functional properties of MCC

The great interest in MCC and its ability to be utilized in many applications is due to the unique functional properties of this product (Table 1 and Figure 1). The functional properties of MCC have been examined and shown in Table 5. The functional properties of MCC are similar to MPC (Figure 1) but the difference in SP content, which is low in

MCC that makes it more heat stable (Carter et al., 2021; US-Dairy-Export-Council, 2020). The CN micelle characteristics were examined in different studies (Gaiani et al., 2005; Karlsson et al., 2005). It has been found that the addition of Sodium chloride resulted in increasing the voluminosity of CN micelles while decreasing the pH from 6.5 to 5.5 led to a decrease in the voluminosity (Karlsson et al., 2005). Also, it was reported that the rehydration of CN improved when ultrafiltrate powder was added before drying (Gaiani et al., 2005). Another reconstitution study was conducted by Schokker who found that the rehydration of MCC is decreased during storage at 30°C due to cross-linking changes in micelles. However, This study exemplified that reconstitution improved when sodium casein was added to liquid MCC before drying (Schokker et al., 2011). As mentioned previously, the type of membrane affects the composition of MCC and SP content (Figure 4). Thus, it has been reported that increasing the SP content and temperature in MCC decreased the viscosity; however, increasing the CN content elevated the viscosity of MCC (Sauer et al., 2012).

The MCC is a heat stable product due to the high CN content with low SP content. It has been found that MCC aggregates at a range of temperature from 110 to 150°C oil bath and pH less than 6.7. However, it is more stable with no aggregation when the pH is elevated to >6.9 (Sauer and Moraru, 2012). It was also found that the suspendability and solubility of concentrated MCC were increasing with elevating the temperature to 50°C. To improve the suspendability and solubility of MCC at low temperatures (≤20°C), samples can store overnight. Additionally, trisodium citrate enhanced the suspendability and solubility of MCC at low temperatures (Lu et al., 2015). Storage of MCC at different temperatures (20, 40, and 60°C) led to decreasing the

solubility as expected but temperature increased the browning of MCC powder (Nasser et al., 2017). Another study reported that the lactose content did not affect the solubility of MCC powder during storage but it increased the browning of powder (Nasser et al., 2018). It has been found that the MCC forms a gel at room temperature and this functionality improves after adding sodium chloride (Lu et al., 2016). A study exemplified that the functionality of MCC, such as overrun and foam stability were higher than other dairy products. It also found the overrun, air face fraction, foam stability, and yield stress of MCC are decreased when the product is turned into a dried form especially the foam stability, which could be due to the denaturation of SP. However, the heat stability of liquid and dried MCC did not change (Carter et al., 2018).

Novel technologies have been used recently to study the characteristics of MCC. The impact of transglutaminase on various functional properties of MCC was studied. Transglutaminase enzyme has elevated alcohol and heat stability of MCC (Salunke, 2013). However, the solubility of MCC decreased when it was hydrated in the water at room temperature and hot water. High-pressure and ultrasound processing have been applied to MCC (Cadesky et al., 2017; Zhang et al., 2018). It has been found that applying the high pressure to MCC resulted in weak gels and increase the rennet coagulation time (Cadesky et al., 2017). On the other hand, using ultrasound in MCC increased conductivity, solubility, emulsifying, gelling, and surface hydrophobicity as the ultrasonic time prolonged (Villamiel and de Jong, 2000).

7.2.Sensory characteristics of MCC

The sensory characteristics of MCC and other casein products are shown in Table 5 and Figure 11. The sensory profile of MCC is characterized by a mild or bland flavor. Rennet and acid casein typically have higher aroma intensity, cardboard, dirty, and tortilla flavors as compared to other casein products. Dirty off-flavor that originates during manufacture was elevated in acid casein relative to rennet casein. MCC and MPC products exhibited similar aroma intensity. There was a significant difference in the cardboard flavor in rennet casein and MPC 80; however, it was similar to other casein products. The sour flavor was detected in acid casein products (Figure 11). The sweet aromatic was varied within MPC products.

Additionally, the flavor profile of MCC and other CN products was examined in a couple of studies (Smith et al., 2016; Carter et al., 2018). It has been reported that MCC powder was high in aroma intensity and cooked flavors relative to the liquid form. Furthermore, the MCC powder had more cardboard flavor as compared to the liquid MCC which was correlated with volatile aldehydes (Carter et al., 2018). As a result, concentrated MCC would be favorable due to a reduction in flavor development that occurred in MCC powder during drying. The same trend in MPC was reported in the same study. Another study found that MCC showed aroma intensity as well as sweet aromatic, cooked/milky, cardboard, and tortilla flavors (Smith et al., 2016).

8. Possible applications of MCC

MCC could be utilized in many dairy and nondairy applications (Table 6) due to its high nutritional value, bland flavor, physicochemical, and functional properties. MCC is heat stable under a range of pH and temperature conditions due to the high CN content;

therefore, it could be utilized as an ingredient in making beverages that require sterilization (Beliciu et al., 2012; Sauer and Moraru, 2012). Due to the novelty of MCC, it has been utilized in a few applications (Table 7).

8.1.Beverages

MCC could be utilized in making high-protein and low carbohydrate beverages (e.g., sports drinks, meal replacement drinks) because it has a high content of protein and low level of lactose. Moreover, due to the unique micellar structure of caseins in MCC, it acts as a stable delivery system for calcium and phosphorous that can remain stable at high temperatures. As a result, it can be utilized in the manufacture of protein-fortified beverages that need sterilization which increases the shelf-life of these products. Also, MCC has a bland flavor and can provide a similar mouthfeel to 1.5 to 2.0% fat milk, making it suitable for low-fat versions of these beverages (Amelia, 2012; US-Dairy-Export-Council, 2020).

8.2.Greek-style Yogurt

Yogurt is produced by precipitating the CN in milk using starter culture to get a pH of 4.6. MCC is a good source for protein fortification of the yogurt milk base (Bong and Moraru, 2014) due to its nutritive value and functional properties (Nelson and Barbano, 2005; Affertsholt, 2009; Zhang et al., 2011; Sauer and Moraru, 2012). It has been found that the protein fortification in yogurt improved rheological and physical properties (Prentice, 1992; Skriver et al., 1999; Lucey, 2002; Peng et al., 2009). Bong and Moraru have recently utilized MCC in making Greek-style yogurt. They noticed that the addition of MCC increased the acidification rate during making Greek-style yogurt as

compared to normal milk (Bong and Moraru, 2014). This increase is due to the higher nonprotein nitrogen content in the MCC-fortified milk.

8.3.Cheese

Cheese is made by precipitating the CN by using rennet (Cheddar and Mozzarella) or acid (Cottage cheese or acid cheese curd). MCC is utilized to fortify milk or as an alternative for milk for cheese making. Guinee and others developed a process to produce MCC solutions with different calcium to CN ratios that can be utilized in manufacture of different varieties of cheese (Guinee et al., 2009). Additionally, different concentrations of recombined concentrated milk with different CN content and protein to fat ratios were produced by mixing concentrated MCC and cream (Lu et al., 2016). These different recombined solutions can be utilized in making cheese. The yield efficiency of cheese is typically increased when MF is applied to the milk before making the cheese (Papadatos et al., 2003). MCC has been utilized in making different cheese types, such as acid curd, rennet coagulated MCC, Cheddar cheese, and low-fat Cheddar.

Acid curd cheese was produced from MCC using direct acid and starter cultures (Hammam and Metzger, 2019, 2020a). Making acid curd from MCC has advantages as compared to skim milk, since manufacturing of MCC using MF results in milk-derived whey protein as a byproduct which can be utilized in many applications, particularly making WPI. On the other hand, using milk is producing tremendous amounts of acid whey (especially during making Cottage cheese curd), which is difficult to be utilized and this could limit the growth of these products due to the economic and environmental challenges. MCC has been used to produce acid curd (Cottage cheese curd) at a pH of 4.6

(Hammam and Metzger, 2019, 2020a). This study found that the yield efficiency of acid curd produced from MCC is high as compared to skim milk. Papadatos and others also reported that there are economic benefits of using MF prior to cheese making, which resulted in the production of valuable byproducts from the MF permeates, such as WPI (Mulvihill and Ennis, 2003). In addition, the MF permeate can be ultrafiltered to utilize the UF permeate as a diafiltrant to increase the removal of SP from skim milk by maintaining the same concentrations of skim milk from soluble minerals, nonprotein nitrogen, and lactose (Nelson and Barbano, 2005). Also, MCC has been utilized to produce rennet coagulated milk (Lu et al., 2016) and this found that the high content of cross-linked protein in MCC resulted in curd with a good matrix. Using MCC in making cheese also improves the characteristics and yield efficiency of cheese (Caron et al., 1997; Papadatos et al., 2003).

MCC has also been successfully used in making Cheddar cheese (Li et al., 2020) which is similar to the typical Cheddar produced from milk. This study reported that the proteolysis of Cheddar made from MCC was higher relative to control and this could be due to the high plasmin and chymosin activities. Another study has utilized SP reduced MCC in making Cheddar cheese (Xia et al., 2021). That study examined the effect of different heat treatments (pasteurization, 72°C/15s; and high heat treatment, 90°C/15s) applied to MCC on the characteristics of Cheddar cheese. Those temperatures did not affect the characteristics, composition, and yield of Cheddar cheese. MCC has also been utilized in manufacture of low-fat cheeses, such as low-fat Cheddar cheese (Amelia et al., 2013). The main components of low-fat cheese are protein, water, and minerals, which are similar to the components of MCC. A study has reported that 45.0% of reduced-fat

Cheddar cheese was made by using different protein concentrate powders, including diafiltered MF retentate, UF retentate powder, and calcium caseinate powder (St-Gelais et al., 1998). It has been found that the production of low-fat cheese made from MCC powder resulted in a soft texture cheese with bitter and grape-tortilla off-flavors (Amelia et al., 2013). The fortified milk with diafiltered MF retentate was higher in the yield as compared to UF retentate and calcium caseinate. The MCC is a valuable ingredient to produce Cheddar, low-fat Cheddar, or any type of cheese with a good structure (Amelia, 2012).

8.4.Process cheese

Process cheese (PC) was made in the late 19th century to increase the shelf-life of natural cheeses. It has several applications and is consumed with other food items as an ingredient. PC has many forms available in marketing (Figure 12), including slices, blocks, shreds, and sauces (Biswas et al., 2008); each requires some unique functional properties. PC is manufactured by mixing some dairy ingredients (protein, fat, carbohydrates sources, etc.) with nondairy ingredients (salt, water, mold inhibitor, preservatives, emulsifying salts (ES), color, flavor, additives, etc.) and heating the mixture to produce a pasteurized product with a long shelf-life (Mcsweeney, 2007; Kapoor and Metzger, 2008; Kammerlehner, 2009). Bowland and Foegeding (2001) described PC as a complex gel with emulsified fat dispersed within a protein network.

According to the Code of Federal Regulations (21CFR133.169 to 133.180), the PC is divided into four main categories depending on fat content, moisture content, final pH, and the number of ingredients that can be used in the formulation (FDA, 2014).

Pasteurized process cheese food (PCF), pasteurized process cheese spread (PCS), pasteurized blended cheese, and processed cheese analogues are the four categories of PC (Henning et al., 2006; Lu et al., 2007; Mcsweeney, 2007; Kapoor and Metzger, 2008; Chandan and Kapoor, 2011). In addition to these four categories, pasteurized process cheese products (PCP) are another undefined category. PCP is similar to the four categories, but it contains ingredients not permitted or do not meet the composition targets of the standard cheese categories (Lu et al., 2007; Kapoor and Metzger, 2008). PCP can be identified as a substitute or imitation cheese (Chandan and Kapoor, 2011). PCP cost less and non cheese dairy ingredients (such as MCC and WPI) could be used to fulfill specific functionality requirements (Lu et al., 2007; Kammerlehner, 2009). The quality and functionality of the PCP are affected by the amount of intact casein. The MCC is a valuable source of intact casein, which is used as an ingredient to enhance the quality of PCP.

8.4.1. Principles of making process cheese

The principles of making PC are calcium sequestration, water binding, and emulsification (Henning et al., 2006) using ES followed by blending, heating, and cooling the product. The quality of PC is affected by the level and type of ES, conditions of manufacturing, and characteristics of natural cheeses (Zehren and Nusbaum, 2000; Kapoor and Metzger, 2008). During PC manufacturing, the calcium in calcium phosphate para-caseinate (rennet cheese) or casein containing ingredients (calcium-casein-phosphate prepared at isoelectric point of pH 4.6) is converted from insoluble to soluble by using ES in the presence of heating and shear action while blending the ingredients of

PC. As a result, the PC becomes physicochemically stable by binding water and emulsifying fat (Guinee, 2011).

Figure 2 (A) is shown the structure of casein network in natural cheese that embedded fat and water. When we are zooming in, we can find the positive calcium ions, negative phosphate ions, and organic phosphate. The casein matrix is cross linked by calcium and phosphate. Calcium and phosphate microgranules play a critical role in the aggregation of the para-casein network. It is hard to make PC or PCP without ES that is responsible for sequestering part of the calcium from the para-casein network. A critical reaction that occurs during PC and PCP manufacture is calcium sequestration using ES (sodium citrate, disodium phosphate, etc). ES are critical for the functional characteristics of PC and PCP due to their role in improving the emulsification characteristics of casein by sequestrating a portion of the calcium from the calcium-casein-phosphate network in natural cheese or other casein containing ingredients (Figure 13). As shown in Figure 13, ES such as disodium phosphate are donating the sodium and sequestering the calcium from the calcium-casein-phosphate network. As a result, the major molecular forces that cross-link the various monomers of casein are partially disrupted by the sequestered calcium ES complexes. This disruption leads to hydration and dispersion of the protein. The partially dispersed monomers of casein have hydrophilic and hydrophobic portions that have emulsification properties. This, in turn, links the hydrophilic aqueous phase with the hydrophobic fat phase (Guinee et al., 2004), which prevents oil separation in PC and PCP. In the existence of heating and mixing, a homogeneous product with an extended shelf-life will be produced.

8.4.2. Functional properties

It has been reported that the functionality of the PC as an ingredient may be defined as its behavior during the preparation stages and consumption of the cheese in which it is incorporated (Guinee, 2002). The functionality of PC can be defined using two categories, namely unmelted textural properties and melted textural properties (Kapoor and Metzger, 2008). PC spread is utilized in several applications, such as dips, sauces, and spreads. The melting properties of PC are important in some applications where it is used. Also, consumers' acceptance is based on melting properties (Lefevere et al., 2000). The functionality of PC can be divided into three major types; rheology-related properties of the raw cheese (fracture characteristics), cooking properties (flowability), and flavor/aroma-related properties (Guinee, 2002).

8.4.2.1.Unmelted textural properties

Texture profile analysis (TPA) is widely used for measuring unmelted textural properties of PC (Figure 14), such as hardness, fracturability, cohesiveness, adhesiveness, gumminess, chewiness, slicing ability, and elastic and viscous properties at low temperatures (Kapoor and Metzger, 2008). It has been reported that TPA hardness is a measurement of the unmelted cheese texture, which describes the firmness of this cheese (Breene, 1975) and it is measured by compressing PC samples to a specific percentage or height and evaluate the exerted force in grams. The compression is sent down vertically with a specific crosshead speed using a flat probe to deform the cheese placed on a lower plate. The results are collected through software that generates curves with force–time and force-distance axis (Breene, 1975) (Figure 14). The hardness of PC can also be measured using penetration techniques, as described in several studies (Kalab et al.,

1987; Tamime et al., 1990). However, TPA is the most common technique utilized to measure the firmness or hardness of PC.

8.4.2.2.Melted textural properties

The rapid visco analyzer (RVA) is one of the equipment that can be utilized to cook the cheese on a small scale. Also, it is used to measure the apparent viscosity during cooking and stirring the PC at high temperatures (Kapoor et al., 2004; Kapoor and Metzger, 2005; Prow and Metzger, 2005; Hammam, 2019; Hammam and Metzger, 2019, 2020b; Hammam et al., 2019; Metzger and Hammam, 2020) (Figure 15). The apparent cooked viscosity is determined at the end of cooking time. It was reported that the RVA is produced PC with similar characteristics to those manufactured on the pilot scale (Kapoor and Metzger, 2005). There is a correlation between the end apparent viscosity and functional properties of PC so the RVA can be utilized to have an idea about the functionality of PC formulations at different parameters. Additionally, the apparent cooked viscosity was measured in the RVA during heating, holding, and cooling (Prow and Metzger, 2005). It was found that young cheese resulted in more extensive protein-protein and protein–fat interactions in the final PC (Purna et al., 2006). This resulted in increasing the end viscosity in the RVA. Thus, the PC made with young natural cheese is thicked faster during cooling compared to the PC that is made from old cheeses.

Dynamic stress rheology (DSR) is one of the common tests that are used to measure the melting properties of cheese (Figure 16). The meltability is the ability of cheese to melt and flow after heating (Gunasekaran and Ak, 2002). The DSR measures the viscoelastic properties of cheese (Sutheerawattananonda and Bastian, 1998) to

understand the viscoelastic status of cheese using the rheometer (Rüegg et al., 1991). The G' (storage modulus; measures the elastic), G" (loss modulus; measures the viscous), and tan δ (ratio of G"/ G') are recorded during this test. Based on the parameter that is kept constant, the test is called frequency sweep (strain or stress constant) at fixed temperature or temperature sweep if it is tested over a range of temperature (20-90°C) at a constant frequency and constant strain (or stress) (Lannes, 2004). When tan δ is <1 means the cheese is more elastic, while it becomes more viscous when tan δ is >1. The cheese melting at which tan δ=1, which refers to the solid and liquid characteristics are equal in the cheese (Gunasekaran and Ak, 2002). The G' is directly affected by the protein network matrix structure while G" is associated with the fat and water in cheese (Subramanian et al., 2006).

Schreiber melt test is also one of those tests used to measure the melting properties of PC. It was developed by Schreiber Foods and it was modified by some researchers (Bogenrief and Olson, 1995; Muthukumarappan et al., 1999). The principles of this test are to heat a PC cylinder (specific dimension) in the oven for a specific time at a specific temperature, then cooling and measuring the area or diameter of the melted cheese (Harvey et al., 1982; Park and Rosenau, 1984; Muthukumarappan et al., 1999).

It was found that proteolysis of αS_1-CN and β-CN in cheese resulted in increasing the meltability due to weakening the number and the strength of the protein-protein interactions between CN molecules (Lucey et al., 2003). The protein-protein/CN-CN and protein-fat interactions and a fibrous CN network are increased when the presence of more intact casein, while those interactions become weaker when CN is hydrolyzed (Taneya et al., 1980; Purna et al., 2006; Brickley et al., 2007). Part of the melting

characteristics is related to the fat at ≤40°C; however, the main effect is related to the number and strength of CN-CN interactions (Park and Rosenau, 1984). The storage modulus or elasticity increased with increasing the pH (Lee and Klostermeyer, 2001; Lee et al., 2004) and protein content (denser network), which is more difficult to deform (Fox et al., 2017). There is a correlation between G', G" and TPA hardness (Drake et al., 1999; Kapoor and Metzger, 2008).

Stretchability is one of the main properties besides melting that are required in some products, such as imitation Mozzarella cheese (IMC) and Mozzarella cheese due to their application in Pizza. The cheese is melted by itself or on Pizza at a high temperature and then the melted cheese is left using a fork until being broken. The strains of cheese are measured and reported in centimeters or millimeters. This test is the least to study because it has less standardization. Several studies measured the meltability using different methods (Cavella et al., 1992; Apostolopoulos, 1994; Guinee and O'Callaghan, 1997; Fife et al., 2002; Guinee et al., 2002; Hicsasmaz et al., 2004; Richoux et al., 2009; Ma et al., 2012; Berta et al., 2016; Bi et al., 2016; Samuel et al., 2018; Hammam and Metzger, 2020b). It was found that stretchability decreased with addition of inulin and resistant starch (Bi et al., 2016). The stretchability of goat milk Mozzarella cheese was improved using 3.0% Arabic gum (Samuel et al., 2018). Another study found that the stretchability of Mozzarella cheese elevated during the ripening time (Guinee et al., 2002). The same study reported that the Mozzarella cheese with higher pH and lower Ca resulted in more stretchability and flowability during ripening, while melt time was low.

8.4.3. Factors controlling the characteristics of process cheese

8.4.3.1.Intact casein

The PC characteristic is significantly affected by the type and amount of protein (Kapoor and Metzger, 2008; Salunke, 2013). The important structural and emulsifying proteins of cheese are casein or CN fractions (Shimp, 1985). The addition of casein or caseinates in PC formulations ameliorates the consistency of PC. It has been reported that intact casein is the most important ingredient in PC formulations and it is selected depending on type, flavor, maturity, consistency, texture, and pH of natural cheese (Zehren and Nusbaum, 2000). The PC properties are also affected by the amount of intact casein in natural cheese (Templeton and Sommer, 1930; Vakaleris et al., 1962; Berger et al., 1998; Zehren and Nusbaum, 2000; Piska and Štětina, 2004; Purna et al., 2006; Brickley et al., 2007; Kapoor et al., 2007; Kapoor and Metzger, 2008; Kammerlehner, 2009). Intact casein is referred to the non hydrolyzed CN, which is high in fresh cheese and decreases during the ripening of cheese because of the proteolysis (Purna et al., 2006). The enzymes, residual starters, and non starter lactic acid bacteria are breaking the protein down into peptides during cheese ripening and this results in reducing the casein that presents in intact form (unhydrolyzed). Natural cheese and rennet casein are good sources of intact CN for PC. Processors balance the ratio of young and aged cheese to have the optimum amount of intact casein in the final PC. Using aged natural cheese (less intact casein) in making PC results in decreasing the firmness and increasing the meltability of PC (Templeton and Sommer, 1930; Purna et al., 2006; Brickley et al., 2007; Kapoor and Metzger, 2008; Kammerlehner, 2009). It has been reported that the melting characteristic of cheese is affected by the interactions between CN molecules (Lucey et al., 2003). Another study examined the effect of Cheddar cheese ripening (2, 4, 6, 12, and 18 wk) on the characteristics of PCF produced with different trisodium citrate

as ES (2.0, 2.5, and 3.0%) (Purna et al., 2006). As the cheese was aged, the intact casein decreased in final PCF which resulted in low viscosity and firmness, and high melting. However, the flowability of the PCF elevated up to 12 wk and then decreased. This change in flowability was related to the over creaming phenomena which increases when natural cheese is extensively aged (Meyer, 1973; Kapoor and Metzger, 2008). When the natural cheese is ripened, protein is hydrolyzed into small peptides. The hydration and dispersion of those peptides are high which results in protein-based interactions (strong protein network) under PC manufacture conditions that decrease the flowability of final PC. The amount of intact casein in cheese, pH, and calcium to CN ratio affect the extent of casein hydration during PC manufacturing which influences the emulsification degree, CN aggregation degree, and elasticity of PC (Berger et al., 1998; Guinee, 2004). The age of natural cheese is determined based on the characteristics of the final PC. Young cheese (75.0% to 90.0% intact casein) is used to make block PC with good sliceability and elasticity (Fox et al., 1996), while PC spread is manufactured by using aged or mature cheese (60.0% to 75.0% intact casein). Similar ratios of cheddar cheese blend to manufacture PC was reported by Tamime (2011), as shown in Table 8.

The addition of natural cheese in PC formulations leads to increasing the softness of the final PC product. Mild cheese is contributed with a high amount in block PC, while medium and aged cheeses are used by a high ratio in manufacturing spread PC (Tamime, 2011). The hydrolysis of αS_1-CN in natural cheeses could be another reason for reducing the PC firmness (Vakaleris et al., 1962; Acharya and Mistry, 2005; Purna et al., 2006; Brickley et al., 2007; Tamime, 2011). Sliceable PC is thicker strands than spreadable PC (Guinee, 2011) due to the difference in pH and temperature. The stand thickness and

elasticity increase with decreasing the pH (Marchesseau et al., 1997) and increasing the holding time at high temperature before cooling (Kalab et al., 1987). This results in changes in the microstructure of PC due to the change in the proportion of protein interaction (Marchesseau and Cuq, 1995a; Guinee, 2011).

The firmness of PC is vary based on the emulsified gel network or microstructure of PC (Marchesseau et al., 1997), which is affected by some casein-based interactions including hydrophobic interactions, hydrogen bonds, and calcium mediated cross-links (Marchesseau and Cuq, 1995b; Marchesseau et al., 1997). So low intact casein in PC formulations leads to a weaker gel network due to the low long unhydrolyzed polymers that could cross-link through those interactions to produce a closely knit network (rigid structural network). The opposite effect occurs when a high level of intact casein is presented in PC formulations so a strong closely knit gel network of caseins is formed, which in turn increases the firmness and decreases the flowability of the final PC.

Protein-based interactions which occur during PC manufacturing result in producing a strong protein network with low flow characteristics (Purna et al., 2006). CN is used to form a gel network in many applications (Augustin et al., 2011), and CN provides unmelted firm texture and a stringy melted texture (Purna et al., 2006; Brickley et al., 2007; Metzger, 2007; Kapoor and Metzger, 2008; Kammerlehner, 2009; Chandan and Kapoor, 2011). As a result, CN is more valuable in PC manufacturing (Metzger, 2007). The fully ripened or too old natural cheese has a minimum amount of intact CN which results in the loss of emulsifying characteristics due to the high amount of hydrolyzed protein (Chambre and Daurelles, 2000; Brickley et al., 2007).

8.4.3.2.Calcium content

The total calcium content has a significant role in the functional properties of PC. As the total calcium content elevates in PC formulations, it becomes more difficult to produce PC with required characteristics due to the high level of calcium that needs to be sequestered from the casein of natural cheese using ES (Sood et al., 1979; Caric et al., 1985; Cavalier-Salou and Cheftel, 1991; Zehren and Nusbaum, 2000; Kapoor and Metzger, 2008). It was found that elevating the calcium content coming from sodium caseinate in cheese analogs led to more firmness cheese with low meltability (Cavalier-Salou and Cheftel, 1991). This was similar to those results reported in other studies (Olson et al., 1958; Zehren and Nusbaum, 2000). The major source of calcium in PC formulation is natural cheese; as a result, the amount of natural cheese should be observed before making PC. There is a correlation effect between intact casein and calcium content on the viscosity PC. It was found that 18% intact casein when calcium increased from 0.45 to 0.65% led to increasing the end apparent viscosity of PCF (Kapoor, 2007). As the calcium content in PC formulations is elevated, the cross-linked casein network increased, which elevated the size of casein aggregates in PC (Sood et al., 1979; Van Hekken and Strange, 1997). This in turn could be responsible for elevating the end apparent viscosity with the high intact casein (Kapoor, 2007). Although the high calcium content could also induce and increase the cross-linked casein network at low intact casein (14%), Kapoor did not notice increase the viscosity of PC when calcium elevated at 14% intact casein used in the formulations (Kapoor, 2007). When the level of intact casein is low, the casein molecules are hydrolyzed to small peptides, so it is difficult to increase the size of casein aggregates in PC by only elevating the calcium

content to increase the viscosity. Additionally, the peptide regions with phosphoserine residues are hydrolyzed and separated from the casein molecules. As a result, cross linking of peptides rich in phosphoserine residues at high calcium level are not affecting the viscosity since they are not associated with the structure of casein network in PC.

8.4.3.3.pH

The pH also has a significant effect on the quality, microstructure, and type of protein interaction in PC (Palmer and Sly, 1943; Meyer, 1973; Marchesseau et al., 1997). The pH is affected by type and age of natural cheese as well as the type and amounts of ES (Kapoor, 2007; Kapoor and Metzger, 2008). To produce a good quality PC, the pH should range from 5.4 to 5.8 (Palmer and Sly, 1943; Marchesseau et al., 1997; Kapoor and Metzger, 2008) because the stability of PC emulsion reduces as the pH is <5.4 or >5.8 (Palmer and Sly, 1943). Another study found that the PC with a pH of 5.2 had more protein-protein interactions (microstructure is knit) since the pH was close to the isoelectric point and this increased the aggregation of protein and thereby poor emulsification of the fat in PC (Marchesseau et al., 1997). However, they found that PC with a pH of 6.1 resulted in an open structure PC (poor emulsification), while PC with 5.7 pH produced a uniform fat emulsion with a closely knit protein network. This referred to the significant impact of pH on the structure and functional properties of PC. Another study found that as the pH of PC increased from 5.0 to 5.8, the firmness elevated; however, the PC firmness decreased when the pH exceeded 5.8 at the range of 5.8 to 6.2 (Templeton and Sommer, 1932). For melting and pH, no correlation was found between melting characteristics of PC and pH (Arnott et al., 1957).

The viscosity of PC after manufacture increases as the pH is elevated. It is known that the iso electric point is 4.6, which referred to removing the net negative charges on the casein molecules. Elevating the pH is increasing the net negative charges on casein micelles, which is increasing the water holding capacity of casein micelles (Fox and McSweeney, 1998). The increase in water holding capacity of casein molecules leads to elevating the viscosity of PC. This was confirmed by Zoller, who noticed an increase in the viscosity of casein pure solutions when the pH of this casein solution was elevated from 5 to 7 (Zoller, 1921).

A correlation between pH and intact casein on the firmness of PCF was found (Kapoor, 2007). It was found that 18% intact casein produced firmer PCF as the pH elevated from approximately 5.5 to 6.1; however, 14 and 16% intact casein did not affect the firmness in the same pH range. Increasing the firmness of PC in the existence of high level of intact casein and elevating the pH might be due to increasing the net negative charges on casein micelles, which resulted in stronger hydrogen bonds and more calcium mediated cross-links casein molecules in PC. This, in turn, increases the strength of PC gel network that increases the firmness of final PC with a high intact casein level (18%).

Another correlation was found by Kapoor between calcium content and pH on the hot apparent viscosity (flowability) of PC (Kapoor, 2007). It was found that 0.45 and 0.55% calcium did not affect the hot apparent viscosity of PCF as the pH increased from 5.5 to 6.1; however, 0.65% calcium increased the hot apparent viscosity of PCF as the pH elevated in the same range. As the pH elevated, the net negative charges on casein micelles increased, which promoted the calcium mediated cross-links casein molecules and this, in turn, strengthen the PC gel network. Increasing the strength of PC gel

network led to restricting the movement of casein chains in PC during reheating which increased the hot apparent viscosity and decreased the flowability. The low calcium content with increasing the pH should have the same effect but the calcium is not enough to cross links the casein network to elevate the hot apparent viscosity.

8.4.3.4.Emulsifying salts

The ES have a significant role in making PC due to their effects on the emulsification characteristics of PC. ES are ionic compounds composed of monovalent cations and polyvalent anions. ES are utilized in PC for two main effects, which are pH adjustment and calcium sequestration (donating the sodium and removing the calcium from the casein network in natural cheese). As mentioned earlier, this helps to hydrate and disperse of casein to link with the fat and water phase to produce a homogeneous product. Approximately 13 ES are approved by the CFR and allowed to use individually or combined in PC formulations (21CFR133.169 to 133.180) (Kapoor and Metzger, 2008). Each ES has a different ability of calcium sequestration and a different mechanism for removing the calcium from the casein network. However, the calcium sequestration mechanism is still not fully understood. Trisodium citrate and disodium phosphate are commonly used in PC formulations in the United States. Sodium hexametaphosphate and sodium aluminum phosphate are also utilized in PC formulations. The amount and type of ES are used based on the application. Trisodium citrate is used in slice on slice PC, while disodium phosphate is commonly used in making loaf and spreads PC, and sometimes sodium hexametaphosphate is added with it in a few applications. Sodium aluminum phosphate has been utilized in IMC to provide some desirable functional characteristics to be used in frozen pizza instead of natural Mozzarella (Kapoor and

Metzger, 2008). Different studies examined several ES to study the effect of those salts on the functional characteristics of PC. It was noticed that trisodium citrate and sodium aluminum phosphate resulted in more meltability PC compared to disodium phosphate, while the hardness of PC produced from those salts was similar (Gupta et al., 1984). Another study found that ES, such as trisodium citrate, disodium phosphate, and sodium hexametaphosphate did not find differences in the emulsion strength of PC (45% moisture; with no pH adjustment); however, the lowest meltability and firmness were noticed using sodium hexametaphosphate and trisodium citrate, respectively (Thomas et al., 1980). The abovementioned three ES were used in PC formulations (38-39% moisture and 33% fat) in another study at 0.25, 1.5, and 2.75% with adjusting pH to 5.6 (Shirashoji et al., 2005). This study found that elevating the concentration of all ES led to more firmness with low meltability PC. The firmest and less meltable PC at 2.75% ES was noticed in sodium hexametaphosphate, followed by disodium phosphate and trisodium citrate. Another study used trisodium citrate, disodium phosphate, sodium hexametaphosphate, and tetrasodium pyrophosphate in PC formulation (46% moisture and 19% protein) at a 2.5% level without standardizing the pH (Shirashoji et al., 2006). Sodium hexametaphosphate produced PC with the lowest pH (5.3) compared to other ES (5.9-6.0). Tetrasodium pyrophosphate resulted in PC with low meltability and flowability then sodium hexametaphosphate, while those properties in PC produced using trisodium citrate and disodium phosphate were not different. PC with a mushy and crumbly texture was noticed using disodium phosphate and sodium hexametaphosphate, while a tough and rubbery texture was produced using tetrasodium pyrophosphate. Another study found that as the concentration of trisodium elevated from 2.0 to 3.0% in PCF (44% moisture

and 25% fat) using Cheddar cheese during ripening for 2, 4, 6, 12, and 18 wk, the flow properties and meltability reduced while firmness was not affected (Purna et al., 2006).

8.4.3.5.Lactose content

The lactose content in PC formulation is another critical component. There are two main ingredients that are used as sources of lactose in PC, including nonfat dried milk and dried whey. Lactose could lead to some defects in PC such as crystallization and Maillard browning (lactose-protein interaction). Lactose crystallization is formed in PC when the lactose content in water is higher than 17% at 20°C (Templeton and Sommer, 1932, 1934; Thomas, 1973; Berger et al., 1998) so the lactose content in water should not be higher than 17% in PC formulations. The other defect that could be noticed as a result of high lactose content is browning and flavor development that results from the Maillard reaction (Thomas, 1969). As reported by Thomas, storage temperature, time, and pH have impacts on the browning of PC. This study found that browning of PC was noticed in PC when it was stored at 35°C for more than 4 to 6 wk.

8.4.3.6.Whey protein

The SP is presented with approximately 20.0% of milk protein. The main SP fractions are β-lactoglobulin (β-LG) and α-lactalbumin (α-LA) with approximately 10.0 and 5.0% of the SP, respectively. The β-LG is susceptible to high temperatures due to its high content of reactive-free sulfhydryl group in its primary structure. β-LG starts denaturation at >70°C on κ-CN through disulfide bonding (Singh, 1995; Wong et al., 1996). The heat-induced disulfide interactions involving free sulfhydryl groups in β-LG have the ability to cross within itself and with κ-CN (irreversible action). NFDM or whey

protein concentrate (WPC) can be used as a source of SP or WP in PC or PCP formulations. PC is manufactured at high temperatures so SP is denatured (WP could cross links among themselves and with casein via disulfide bonds) when it is found at high levels. This also can affect the sensory and functional characteristics of the final PC. SP in PC formulations can make the cheese firmer but with less meltability.

WPC (26.1% TS, 20.0% protein, and 6.0% lactose) was utilized to replace 20.0% of the TS in formulations of PCF. It was found that up to 8.0% of WP can be added in PCF formulations with no effect on the acceptability of PC (Gupta and Reuter, 1992). Another study reported that the body and texture of PCS became better when WPC (38.0% protein) was utilized to replace the TS at 3.0 and 4.5%; however, the meltability reduced with elevating the level of WPC in the formulations (Pinto et al., 2007). Although another study stated that increasing the WPC (28.0% TS, 15.0% WP) in PCS formulations (57.0% moisture, 3.0% ES) up to 40.0% of the final blend enhanced the meltability and sensory characteristics. Additionally, this resulted in higher moisture (might be responsible for the better melting and sensory properties), lactose, and pH by 0.8%, 2.5%, and 0.3%, respectively, in the final PCS (Abd El-Salam et al., 1996). Another study found that up to 2.0% CN can be replaced by WP in PC formulations (17% CN, 24% fat, and 2% ES). The addition of WP resulted in firmer cheese with less meltability (Mleko and Foegeding, 2000). The high firmness can be related to the denaturation of SP on casein. Another study found that the level of denaturation of SP on CN as well as the pH affects the functionality of PCS (Lee and Anema, 2009). This study found that the rheological properties and texture of PCS were affected by the addition of WP compared to PCS with no WP.

8.4.3.7.Rework

Rework is referred to the PC that is remade including PC that could not be sold, lost in the production line, edge trimmings removed during slicing, residuals from the cooker, rejected cheese due to defects in weight or packaging, etc (Kichline and Scharpf, 1969; Zehren and Nusbaum, 2000). Since rework PC has emulsifying salts, it could affect the functional characteristics when it is mixed with a new blend to remanufacture. The amount of rework PC ranges from 2 to 15% of total PC produced (Lauck, 1972). It was reported that the addition of rework to a fresh blend could affect the functional characteristics of final PC (Kichline and Scharpf, 1969; Lauck, 1972; Kalab et al., 1987). Kichline and Scharpf reported that the maximum rework PC should not exceed 4% to avoid any undesirable defects in the characteristics of final PC (Kichline and Scharpf, 1969). The addition of rework produces more hardness PC with less meltability (Kalab et al., 1987). The addition of different rework was studied on the functionality of PC formulations (43% moisture, 24% fat, and 2.7% trisodium citrate ES with 5.5 to 5.7 pH). This study found that the hardness and viscosity of PC was increased and meltability was decreased when fresh rework vs old rework, more amounts (20% vs 10%), and overcooked PC were added to the new blend. Fresh rework is a weak dispersed protein structure (more long structure) so it provides less denser matrix and less overcreaming that could obtain from overcooking. The rework could be utilized at the recommended amount to provide specific functionality in the final PC.

8.4.3.8.Ingredients

Natural cheese. As we mentioned, natural cheese is one of the main ingredients in PC formulations due to its effect on the intact casein, calcium content, and pH of the final PC. Natural cheeses have different properties based on pH, calcium and phosphorus, age, and intact casein (Templeton and Sommer, 1930; Barker, 1947; Olson et al., 1958; Vakaleris et al., 1962; Meyer, 1973; Thomas, 1973; Harvey et al., 1982; Zehren and Nusbaum, 2000; Kapoor, 2007; Kapoor et al., 2007; Kapoor and Metzger, 2008). As a result, natural cheeses should be selected based on those parameters to have the desired functional characteristics in the final PC. Different natural cheeses could be used in PC formulations, such as Cheddar cheese, Swiss cheese, Gouda cheese, etc (Meyer, 1973). Natural cheeses could be used at a range of 51 to 80% in PC formulations (FDA, 2006). Differences in the amount of natural cheese used in the blend lead to differences in intact casein, pH, and calcium content and thereby affecting the functional properties of the final PC.

NFDM/ dried whey/ milk permeate/ WPC. NFDM, dried whey or milk permeate, and WPC are the main sources of lactose and WP in PC formulations. Those ingredients are used in the PC blend due to their low cost. However, the high lactose and WP levels those ingredients provide in PC could result in changes and defects in the properties of the final cheese as mentioned. Consequently, the amount of those ingredients should be considered in PC formulations based on the lactose and WP they could provide in the final PC.

Food gums/ hydrocolloids. The gums or hydrocolloids could be used in PCS (up to 60% moisture) at a level of ≤0.8%. Those additives, such as carob bean gum, gum karaya, gum tragacanth, guar gum, gelatin, sodium carboxymethylcellulose (cellulose

gum), carrageenan, oat gum, algin (sodium alginate), propylene glycol alginate, or xanthan gum singly or in combination. could be used single or combined (Kapoor and Metzger, 2008). The main purpose of gums or hydrocolloids is water binding, increase viscosity, thickening, and enhancing mouthfeel since those materials are used in PCS but such additives could reduce the meltability of PCS. It was reported that gums and hydrocolloids are selected based on dispersibility, solubility, hydration, moisture holding capacity, cook viscosity, compatibility with milk proteins and other ingredients in PC formulations, and pH range (Zehren and Nusbaum, 2000).

8.4.4. MCC in PC formulations

MCC has been utilized as an ingredient in manufacture of PCP (Salunke, 2013; Hammam, 2019; Hammam and Metzger, 2019; Hammam et al., 2019; Metzger and Hammam, 2020) and IMC (Salunke, 2013; Hammam and Metzger, 2020b) due to the high intact CN content. MCC is utilized to give the desired compositional specification, texture, structure, meltability, and stability in the final PCP (Hammam and Metzger, 2019; Hammam et al., 2019, 2021; Metzger and Hammam, 2020). The actual amount of intact CN content in PC formulations can be balanced by the use of CN rich concentrate or powders such as MCC. The proteolysis of MCC was determined during 60 d of storage at 4°C (Hammam and Metzger, 2018; Hammam, 2019). A slight increase in the noncasein nitrogen (NCN) and nonprotein nitrogen (NPN) was noticed in MCC during storage. During storage, β-CN was degraded by the proteolytic enzymes and produce γ-casein (γ-CN) and small peptides. Then, the effect of proteolysis on PCP characteristics was studied (Hammam and Metzger, 2018; Hammam, 2019) by using the MCC as ingredients in the PCP formulations using ES. However, not many differences were

noticed in the functionality of PCP made from MCC during storage. MCC has also been utilized to produce novel clean label PCP with no ES using a 2:1 ratio of protein from acid curd to protein from MCC (Hammam and Metzger, 2019; Metzger and Hammam, 2020). Using the same methodology, it was able to produce IMC without ES using 2 portions of protein from acid curd and one portion of protein from MCC (Hammam and Metzger, 2020b). It has been found that the composition and functional properties of PCP and IMC made from MCC were similar to the conventional PCP and IMC in the same category (Hammam and Metzger, 2019, 2020b; Hammam et al., 2019).

9. Outlook

MCC is one of the most valuable CN products available nowadays and has been used to manufacture of few products. The global market value of micellar CN will increase the revenues significantly and gain more than $700 million by the end of 2026. It is expected that MCC will attribute to approximately 30.0% of the global revenues of North America by 2026, with more than $ 200 million contributed to MCC sales in the US (Cottage, 2018). This product is expected to have a promising trend and be essential in manufacture of many dairy products.

10. Conclusions

An overview of CN micelle history, manufacture, functional properties, and potential applications of MCC has been provided in this review. MCC can be manufactured using different MF membranes by fractionating the CN and SP using 0.1 μm semi-permeable membranes. There are different types of casein products other than MCC, which are considered a good source of CN, such as rennet caseins, acid caseins,

caseinates, and MPC. Compared to other CN products, MCC has the CN in a native state that can widely be used as an ingredient to enhance and improve the protein content of many products. MCC has unique functional properties, such as foaming, emulsifying, and water-binding ability. Also, it is a good source of intact casein and could be produced in a liquid, concentrated, or dried form. MCC has been successfully used as an ingredient in making some dairy products, such as beverages, yogurt, cheese, and PC. Future work is needed to utilize MCC in making more dairy products and study the functionality of these products.

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Tables

Table 1. The functional properties of milk casein (CN)

Functional properties	Casein (CN)
Hydration	High water binding capacity At higher concentration gels
Solubility	Minimum at the isoelectric point (pH= 4.6) Insoluble at the isoelectric point (pH= 4.6)
Viscosity	Low viscosity at the isoelectric point (pH= 4.6) A viscous solution at neutral and alkaline pH
Gelation	Micelle gelation by rennet enzyme No thermal gelation except in the presence of calcium
Emulsifying properties	Excellent emulsifying properties at neutral and alkaline pH
Foaming properties	Good foaming properties and overrun but low foam stability
Flavor binding	Good flavor binding

Adapted from (Lorient et al., 1991)

Table 2. Selected scientific and commercial milestones in casein (CN) micelle

Year	Milestone	Reference
1818	First work identified CN in milk	(Schubler, 1818)
1880	First described CN as insoluble in milk	(Sheldon, 1880)
1887	First referred to CN as a colloid	(Duclaux, 1887)
1909	First recognized CN as colloidal particles contain calcium phosphate and are retained by Pasteur-Chamberland porcelain filters	(Kastle and Roberts, 1909)
1910	First recognized CN particles as irreversible, unstable colloid and stabilized by reversible stable colloid (lactalbumin) when the milk is coagulated by rennet (protective colloid model)	(Alexander, 1910; Alexander and Bullowa, 1910)
1914	First described physical state of CN as large CN particles	(Wiegner, 1914)
1920	First used the term "micelle"	(Duclaux, 1920)
1925	First dismissed the protective colloid idea and suggested rennet coagulation due to cations and first used the term "colloidal calcium caseinate'', ''calcium caseinate'' or ''calcium caseinate–calcium phosphate''	(Palmer and Richardson, 1925)
1930	The term ''component system'' was proposed rather than ''micelle''	(Sorensen, 1930)
1936	Analytical ultracentrifuge experiment was performed to study the polydispersity of the CN particles in milk	(Pedersen, 1936)
1940	Association of protein that has hydrophilic and hydrophobic portions was studied	(Langmuir and Waugh, 1940)
1953	The term ''casein micelle'' became widely used	(Pyne, 1953)
1954	Non-covalent forces involved in protein association	(Waugh, 1954)
1955	Temperature, pH, and protein concentration on the equilibrium between CN monomers and polymers were examined	(von Hippel and Waugh, 1955)
1956	Isolation of κ-CN and novel ideas on the structure of the CN micelle	(Waugh and von Hippel, 1956)
1957	The size of CN micelle was proposed (average of 1000Å)	(Fox and Foster, 1957)
1958	Electron microscopy was utilized to study the CN micelle	(Barbaro and Calapuj, 1958)
1958	The first model of the CN micelle	(Waugh, 1958)
1972	Study the viscosity and surface tension of CN solutions in buffalo and cow milk	(Puri et al., 1972)
1978	Casein and caseinates production	(Badertscher and Chaveron, 1978)
1988	The emulsifying characteristics of casein micelle in bovine milk were examined	(Haque et al., 1988)
1992	Micelle is made up of CN molecules linked together by calcium phosphate complexes (CCP) microcrystals and hydrophobic bonds	(Visser, 1992)
1994	Effect of microfiltration (MF; 0.1 μm) on the distribution of phage particles in the retentate and permeate produced from raw milk	(Gautier et al., 1994)
1999	The protein composition of micellar casein produced from MF of skim milk was evaluated	(Jost et al., 1999)

2000	Cheese was manufactured from a combination of MF and ultrafiltered (UF) retentate powder to stimulate the characteristics of Mozzarella cheese made from raw milk	(Garem et al., 2000)
2009	MCC powder was produced with different levels of calcium to casein ratios for making cheese	(Guinee et al., 2009)

Table 3. The characteristics of casein (CN) and serum protein (SP)

Characteristics	**Casein (CN)**	**Serum protein (SP)**
Solubility at pH 4.6	No	Yes
Rennet coagulation	Yes	No
Heat stability	High	Low
Particle size	Large (micelles; molecular weight 10^8)	Small (molecules; molecular weight 1.5-7.0 x 10^4)

Adapted from (Mulvihill, 1992)

Table 4. Application of casein (CN), caseinates, and co-precipitates in dairy-based foods

Product	**Effect**
Imitation cheese	Fat and water binding Texture enhancing Melting properties Stringiness Shredding properties Emulsifier
Coffee creamers	Whitener Gives body and texture Resistance to feathering Sensory properties
Yogurt / cultured milk products	Increase gel firmness Reduce syneresis Nutritional
Milk beverages	Emulsifier Foaming properties
High-fat powders, high-fat products (shortening, whipped toppings, butter-like spread)	Emulsifier Texture enhancing Sensory properties
Drinking chocolate, fizzy drinks and fruit beverages	Stabilizer Whipping and foaming properties
Cream liqueurs, wine apertifs	Emulsifier
Wine and beer industry	Fines removal, clarification, reduce color and astringency
Ice cream, frozen desserts	Whipping properties Body and texture
Mousses, instant pudding, whipped topping	Whipping properties Film former Emulsifier Imparts body and flavor

Adapted from (Salunke, 2013)

Table 5. Summary of some studies on the functional and sensory properties of micellar casein concentrate (MCC)

Year	Properties	Reference
2005	The voluminosity of the CN micelles increased with addition of NaCl and decreased when pH was decreased from 6.5 to 5.5.	(Karlsson et al., 2005)
2005	Rehydration of CN was improved when ultrafiltrate powder was added before spray drying.	(Gaiani et al., 2005)
2011	The reconstitution of MCC decreased during storage at 30°C due to cross-linking changes in micelles. However, addition of sodium casein to MCC before drying improved reconstitution.	(Schokker et al., 2011)
2012	SP in MCC, as well as high temperatures, resulted in decreasing viscosity but while CN led to increasing the viscosity	(Sauer et al., 2012)
2012	MCC aggregates at 110 to 150°C and pH <6.7 but no aggregation occurred at pH >6.9	(Sauer and Moraru, 2012)
2013	Transglutaminase increased MCC stability against alcohol and heat but solubility decreased at room temperature water and in hot water.	(Salunke, 2013)
2015	Functionality and flavor of MCC were reported	(US-Dairy-Export-Council, 2020)
2015	Suspendability and solubility were enhanced at 50°C and at ≤20°C overnight storage and improved with trisodium citrate at ≤20°C.	(Lu et al., 2015)
2016	The cold gelling properties of recombined concentrated milk from MCC were improved by calcium chloride	(Lu et al., 2016)
2016	The sensory properties of MCC (aroma intensity, Cooked/milky, Sweet aromatic, Sour aromatic, Cardboard, Potato/ brothy, Animal, Tortilla/corn chip, soapy, fatty, sour, astringency) was studied	(Smith et al., 2016)
2016	The high pressure processing on MCC resulted in weak gel and increase rennet coagulation time	(Cadesky et al., 2017)
2017	Storage temperature is increasing the browning of MCC powder during storage at 40 and 60°C while the solubility decreased at all storage temperatures (20, 40, and 60°C)	(Nasser et al., 2017)
2018	Conductivity, solubility, emulsifying, and gelling in MCC increased significantly as the ultrasonic time prolonged, also increasing the surface hydrophobicity	(Zhang et al., 2018)
2018	The overrun, air phase fraction, foam stability, and yield stress of liquid MCC were higher compared to dried MCC. However, flavor properties (Aroma intensity, Sweet aromatic, Cardboard, Cooked milky, Corn chip/ tortilla, Astringency) were higher in dried MCC than liquid MCC	(Carter et al., 2018)
2018	Lactose does not affect the solubility of MCC but led to increasing the browning during storage	(Nasser et al., 2018)

Table 6. Applications of milk protein/micellar casein (MCC)

Product	**Effect**
Pasta products: macaroni, pasta, imitation pasta	Nutritional, texture, freeze-thaw stability, microwavable
	Chewy texture, water binding, emulsifier, firmness
Confectionery	Foaming, high-temperature stability, improves flavor
	Whipping properties
Meat products	Emulsifier, water binding improves consistency, releases meat proteins for gel formation and water binding
	Pre-emulsion, gelation, yield
Special dietary preparations	Dieting patients, bodybuilders, athletics, astronauts, nutritional fortification, low lactose foods, bioactive peptides, casein hydrolysates
Textured products: puffed snack foods, protein-enriched snacks, meat extenders	Structuring, texturing, nutritional
Convenience foods	Whitening agents, dairy flavor, flavor enhancer, emulsifier, stabilizer, viscosity controller, freeze-thaw stability, egg yolk replacement, lipid replacement
Others	Toothpaste, cosmetics, wound treating preparations

Adapted from (Salunke, 2013)

Table 7. Summary of some studies on the applications of micellar casein concentrate (MCC)

Year	Applications	Reference
2012	MCC was utilized in making low-fat Cheddar cheese	(Amelia et al., 2013)
2013	Using MCC in making Greek-style yogurt	(Bong and Moraru, 2014)
2013	MCC has been used to make imitation Mozzarella cheese	(Salunke, 2013)
2015	MCC can be added to Low-fat and shelf-stable beverage	(US-Dairy-Export-Council, 2020)
2016	Preparation of recombined MCC concentrated milk	(Lu et al., 2016)
2017	MCC made recombined concentrated milk	(Lu et al., 2017)
2019	Using MCC as an ingredient in making process cheese products (PCP) with no emulsifying salts	(Hammam and Metzger, 2019; Hammam et al., 2019; Metzger and Hammam, 2020)
2020	MCC has been utilized in making Cheddar cheese	(Li et al., 2020)
2020	Using MCC in making Acid curd	(Hammam and Metzger, 2019, 2020a)
2020	Using MCC in making imitation Mozzarella cheese with no emulsifying salts	(Hammam and Metzger, 2020b)
2020	MCC was used to make Cheddar cheese	(Xia et al., 2021)

Table 8. Typical ratios of blends of cheddar cheese for the manufacture of process cheese (PC)

PC type	Mild cheese	Medium cheese	Aged cheese
Block	70-75	25-30	25-30
Slices	30-40	50-60	10
Slices	55	35	10
Spread	30	50	20

Adapted from Tamime (2011)

Figures

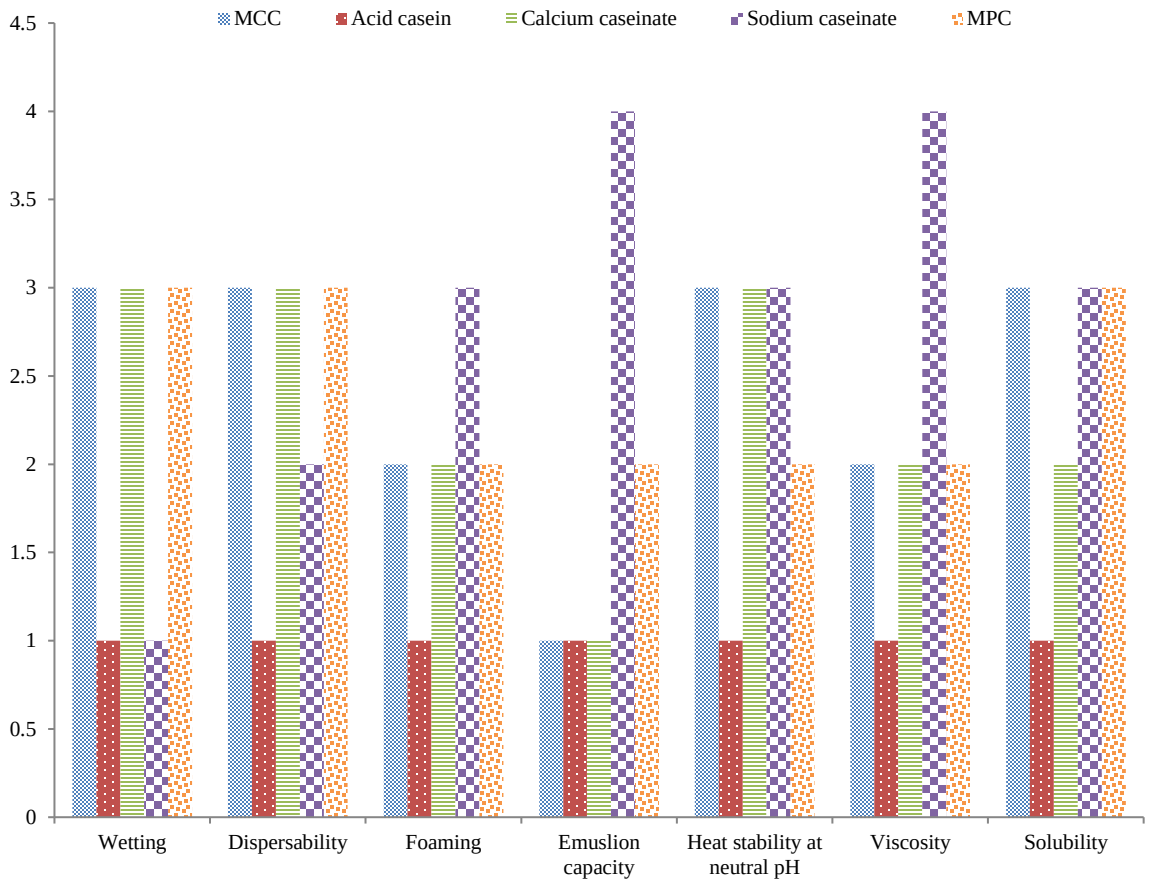

Figure 1. The functional properties of micellar casein concentrate (MCC), milk protein concentrate (MPC), and other casein (CN) products

Scores: 1= low; 2= medium; 3= high; 4= very high. Adapted from (US-Dairy-Export-Council, 2020)

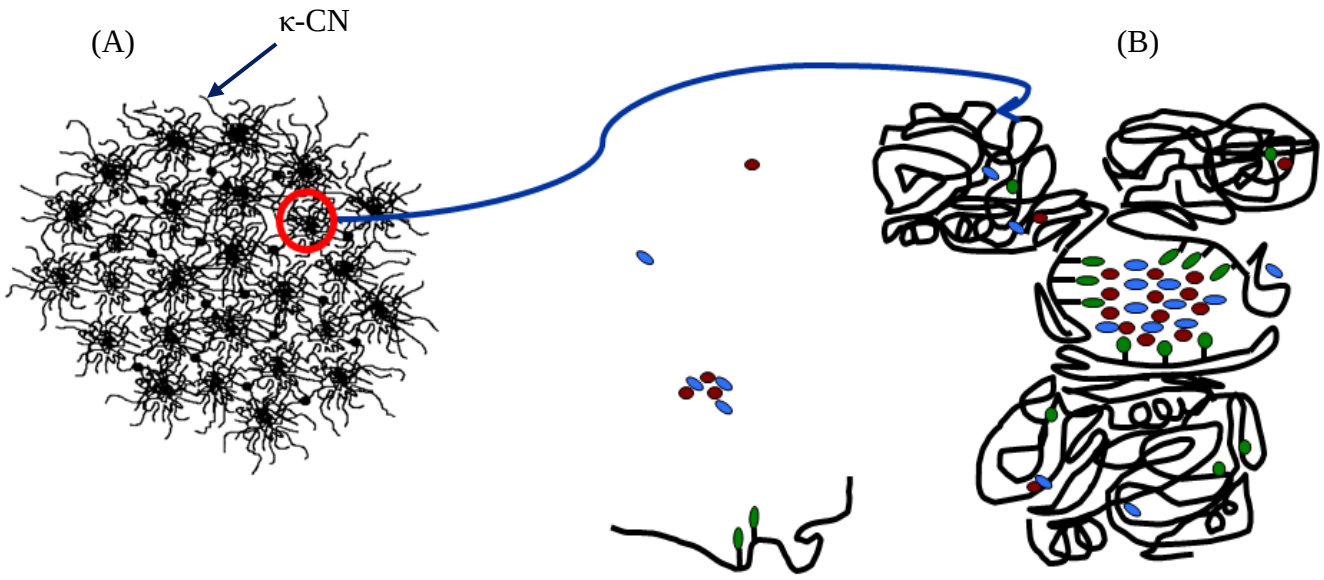

Figure 2. Casein micelle model

(A) Model adapted from (Holt, 1992); (B) Zoomed in image. κ-CN= κ-casein; Ca=calcium; PO= phosphate

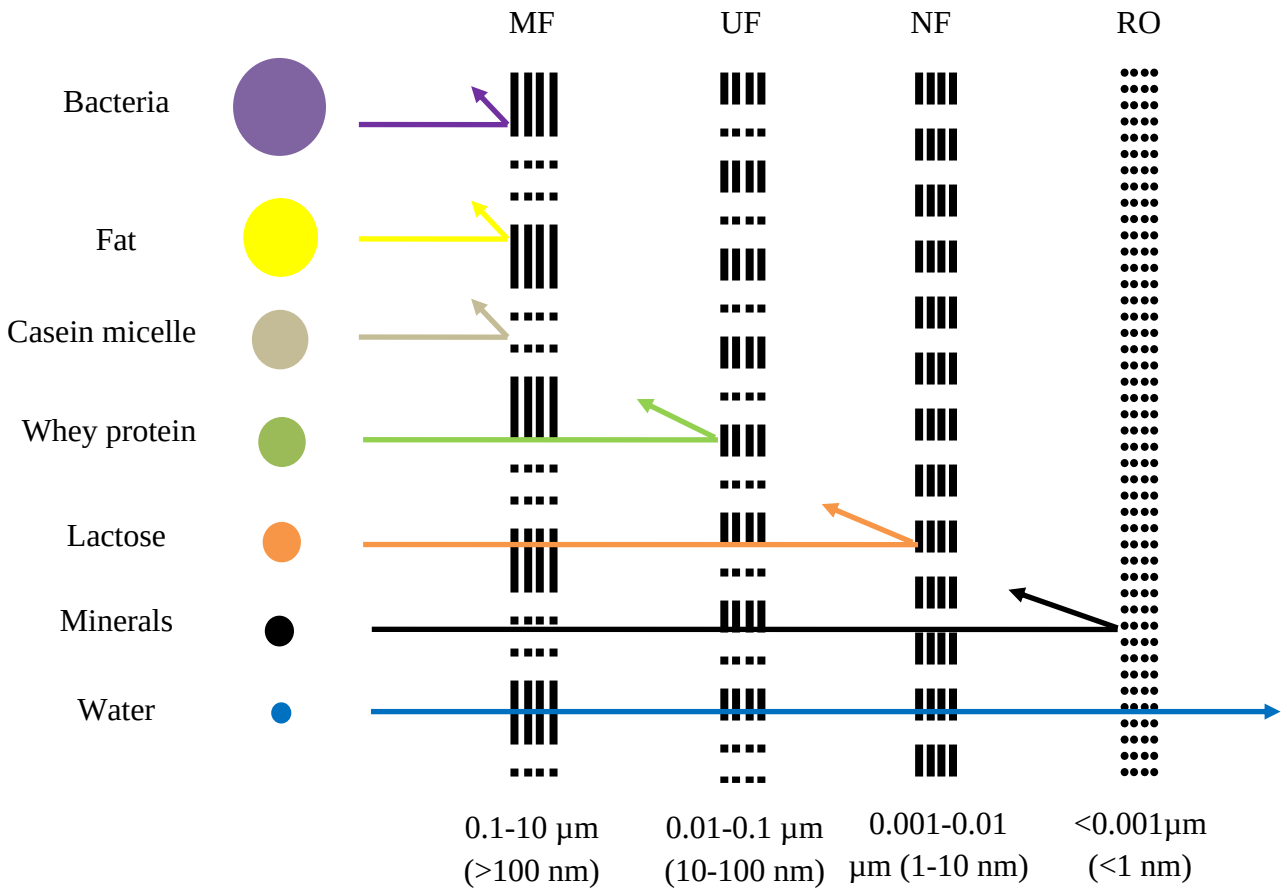

Figure 3. Membrane technologies in the fractionation of dairy components. MF=microfiltration; UF=ultrafiltration; NF=nanofiltration; RO=reverse osmosis

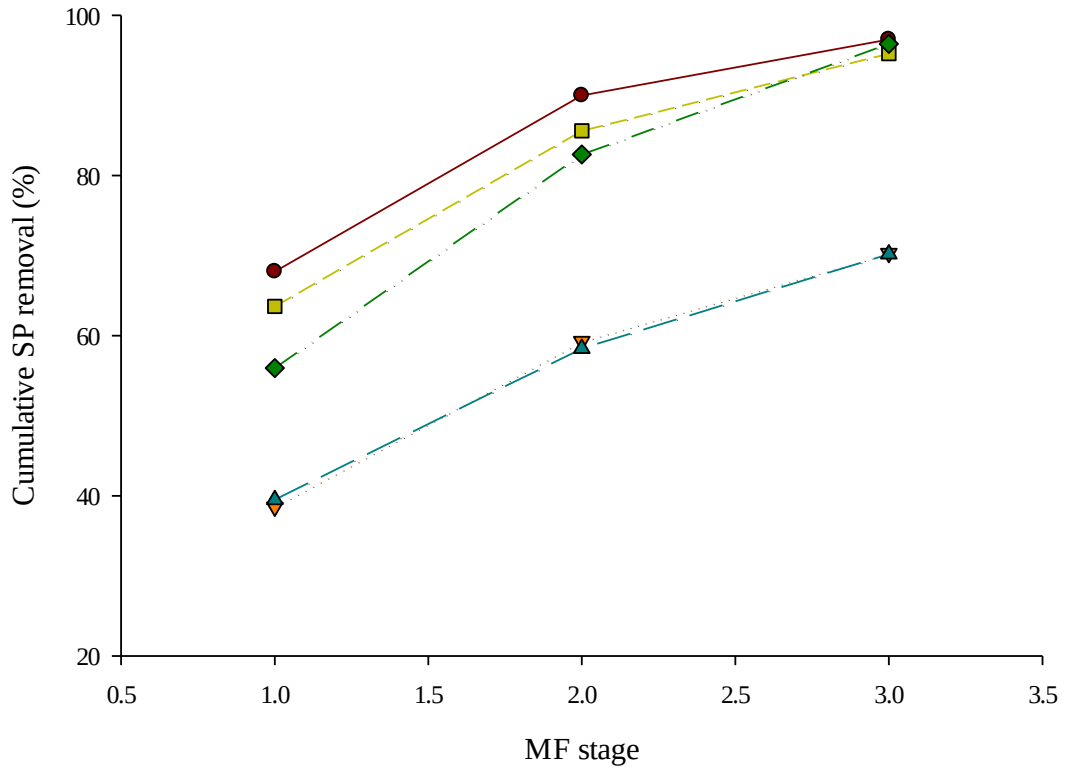

Figure 4. Cumulative serum protein (SP) removal in different microfiltration (MF) membranes

Theoretical values (●): assuming no rejection of serum protein (SP) and complete rejection of casein (CN)

SP removal from skim milk using polymeric spiral-wound (SW) membranes (▼). Adapted from (Beckman et al., 2010)

SP removal from skim milk using uniform transmembrane pressure (UTP) ceramic membranes (■). Adapted from (Hurt et al., 2010)

SP removal from skim milk using graded permeability (GP) ceramic membranes (♦). Adapted from (Zulewska and Barbano, 2014)

SP removal from skim milk using Isoflux ceramic membranes (▲). Adapted from (Adams and Barbano, 2013)

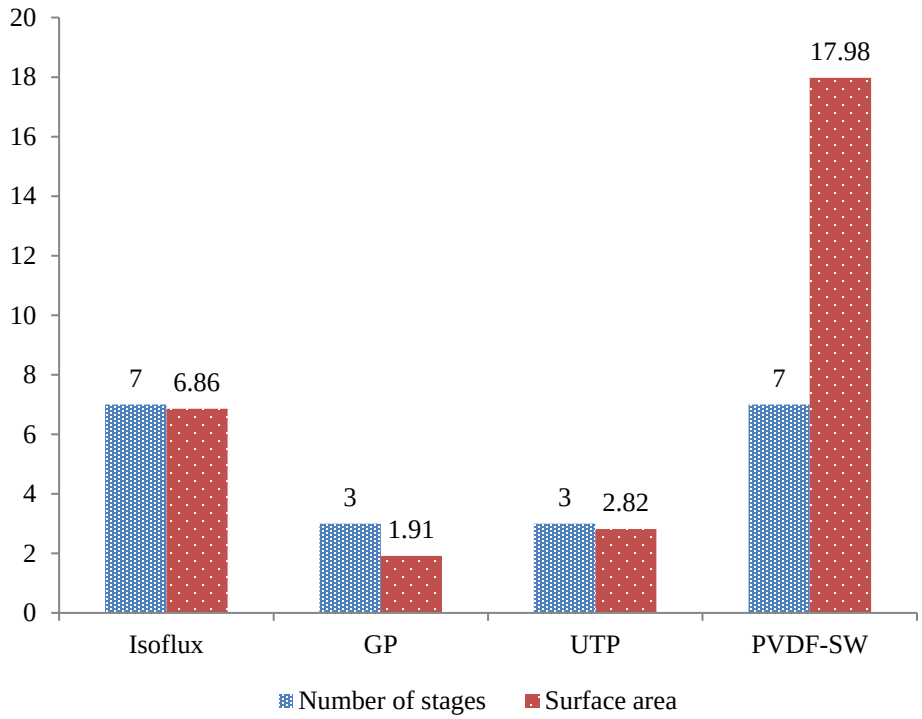

Figure 5. The number of stages and membrane surface area (m2) required to remove 95.0% serum protein (SP) in different microfiltration (MF) membranes

Using 0.14-μm Isoflux ceramic membrane; 0.10-μm graded permeability (GP) ceramic membrane; 0.10-μm uniform transmembrane pressure (UTP) ceramic membrane; 0.30-μm polymeric spiral-wound (SW) membranes. Adapted from (Zulewska et al., 2009; Adams and Barbano, 2013)

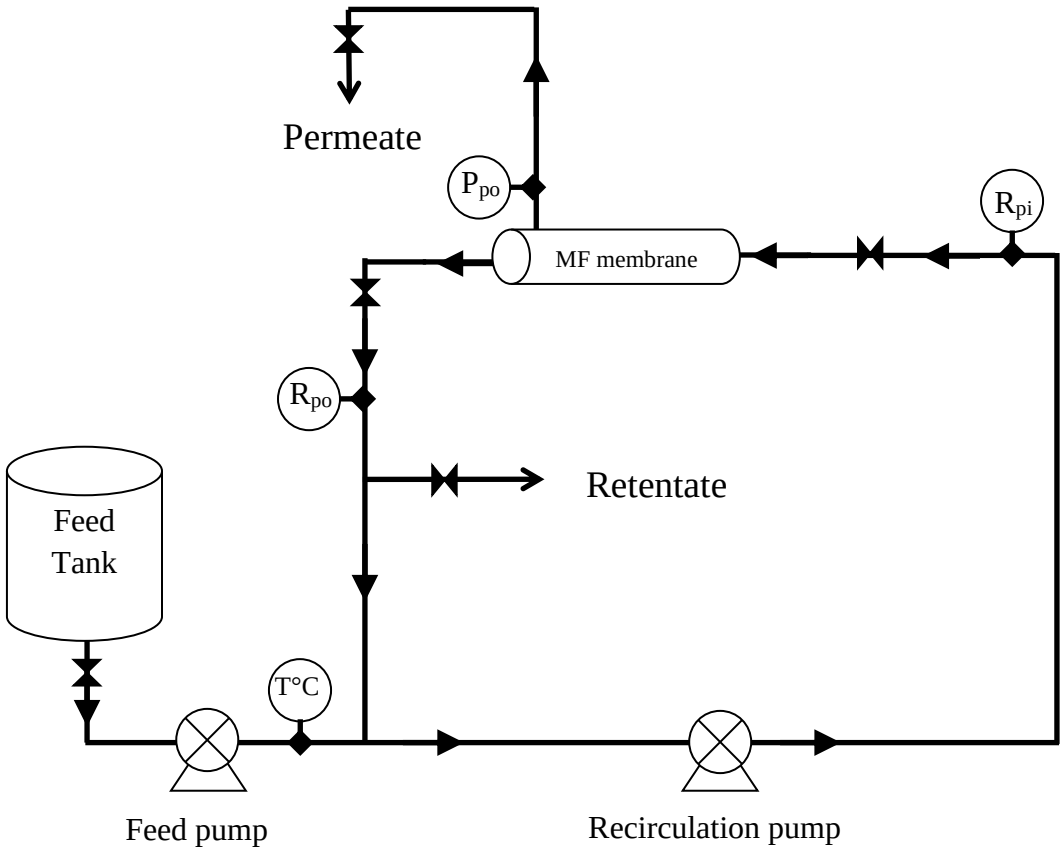

Figure 6. Microfiltration (MF) system used to fractionate skim milk into permeate and retentate

T°C= temperature; Rpi= retentate pressure inlet; Rpo= retentate pressure outlet; Ppo= permeate pressure outlet

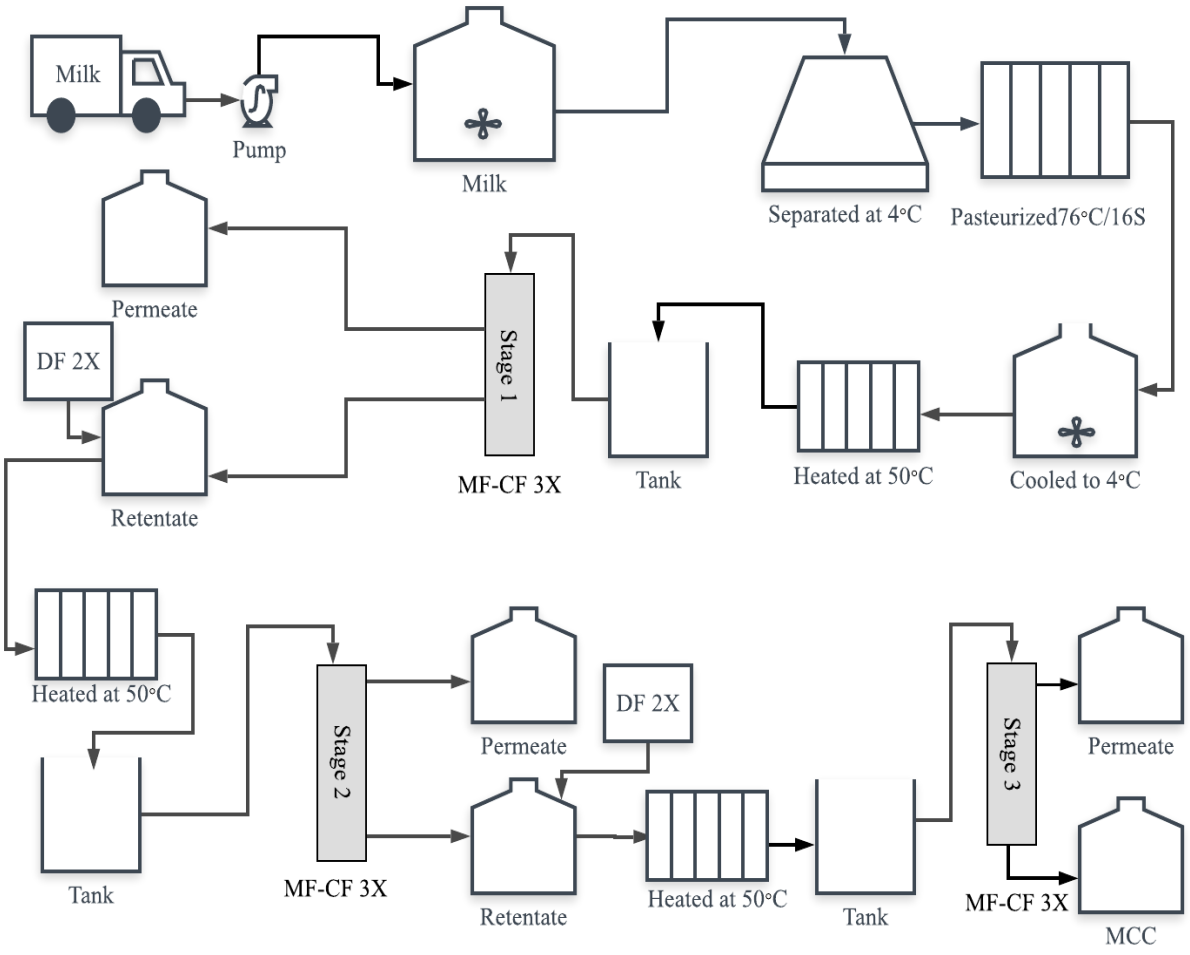

Figure 7. Diagram of manufacturing micellar casein concentrate (MCC) using 3-stages, 3× CF

MF= microfiltration; CF = concentration factor= 3×=2 kg of permeate: 1 kg of retentate;

DF = diafiltration= 2×= the amount of retentate mixed with 2 times of water

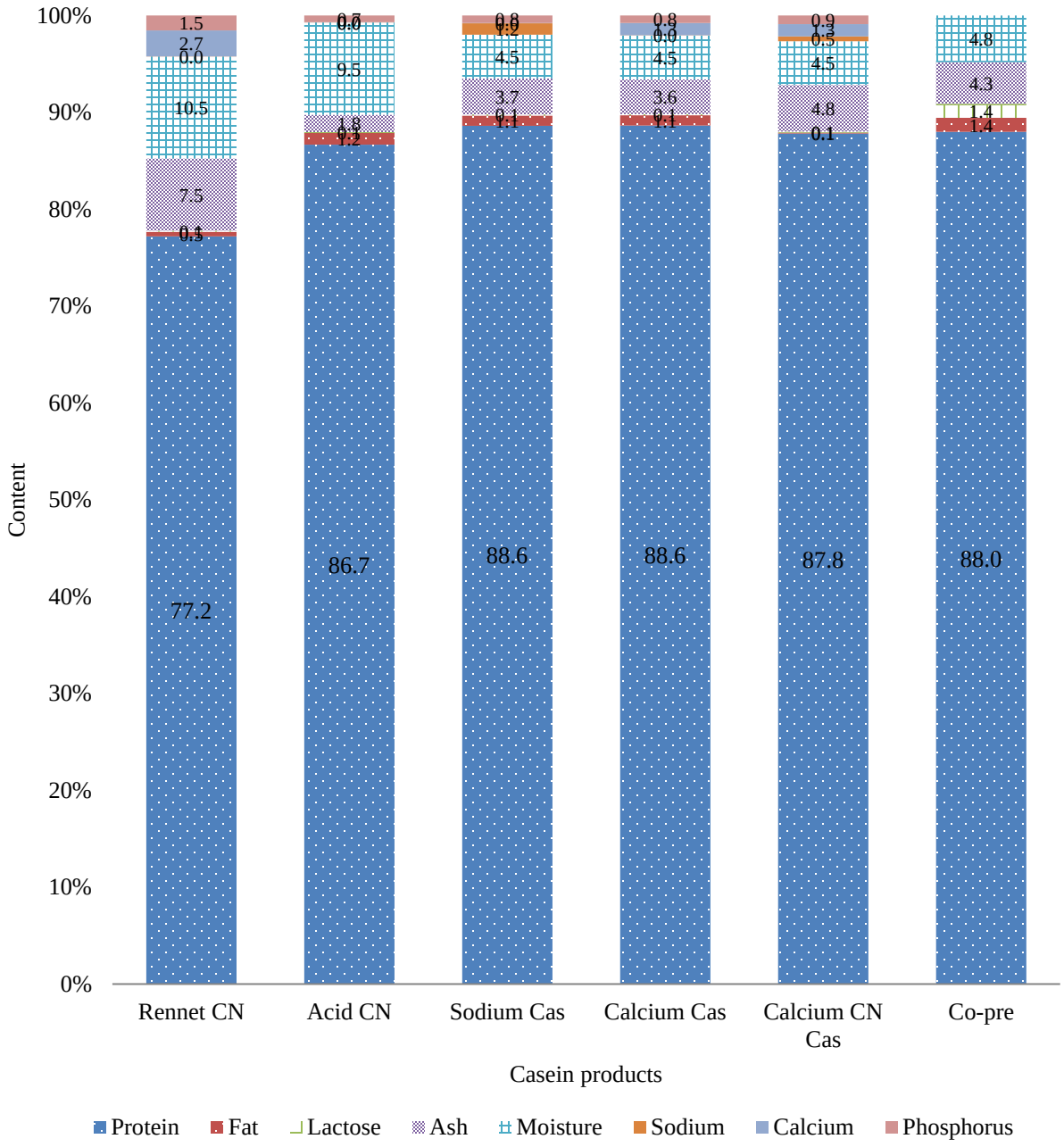

Figure 8. The typical composition of different casein products

Adapted from (Huffman and James Harper, 1999; US-Dairy-Export-Council, 2020). CN= casein; Ca= calcium; Cas= caseinates; Co-pre= Co-precipitates

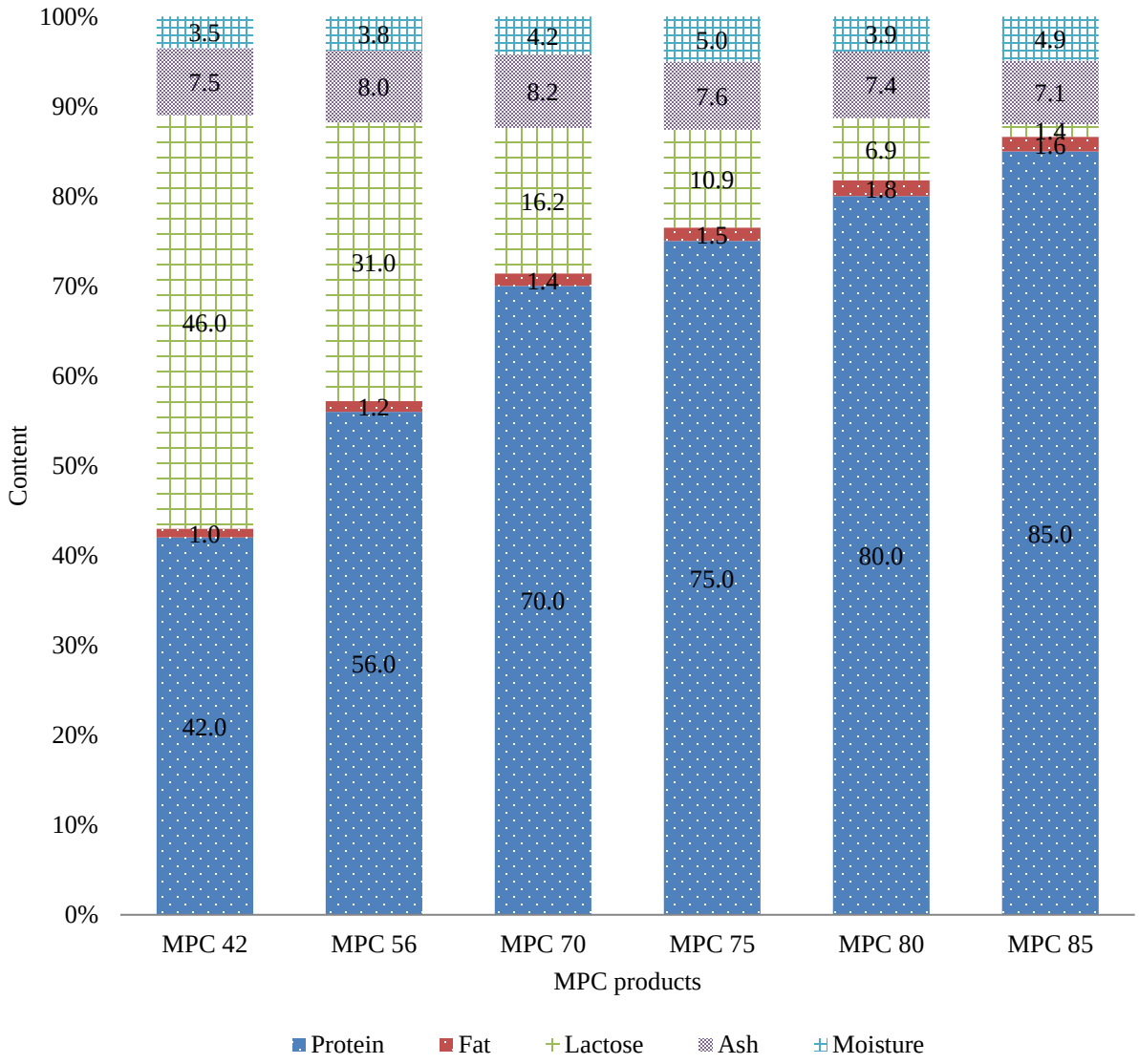

Figure 9. Composition of different milk protein concentrate (MPC) products

Adapted from (Salunke, 2013)

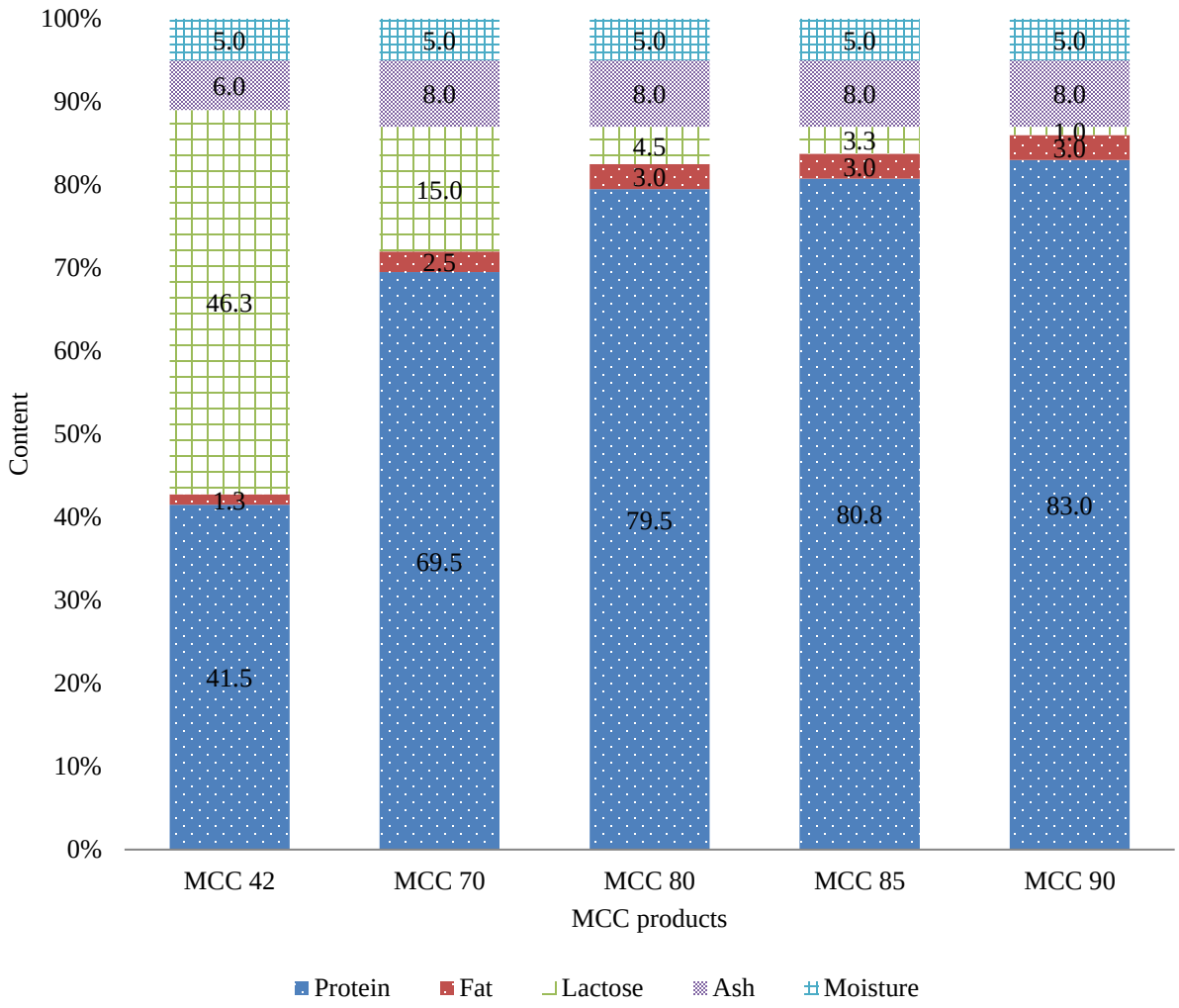

Figure 10. Composition of different micellar casein concentrate (MCC) products

Adapted from (US-Dairy-Export-Council, 2020)

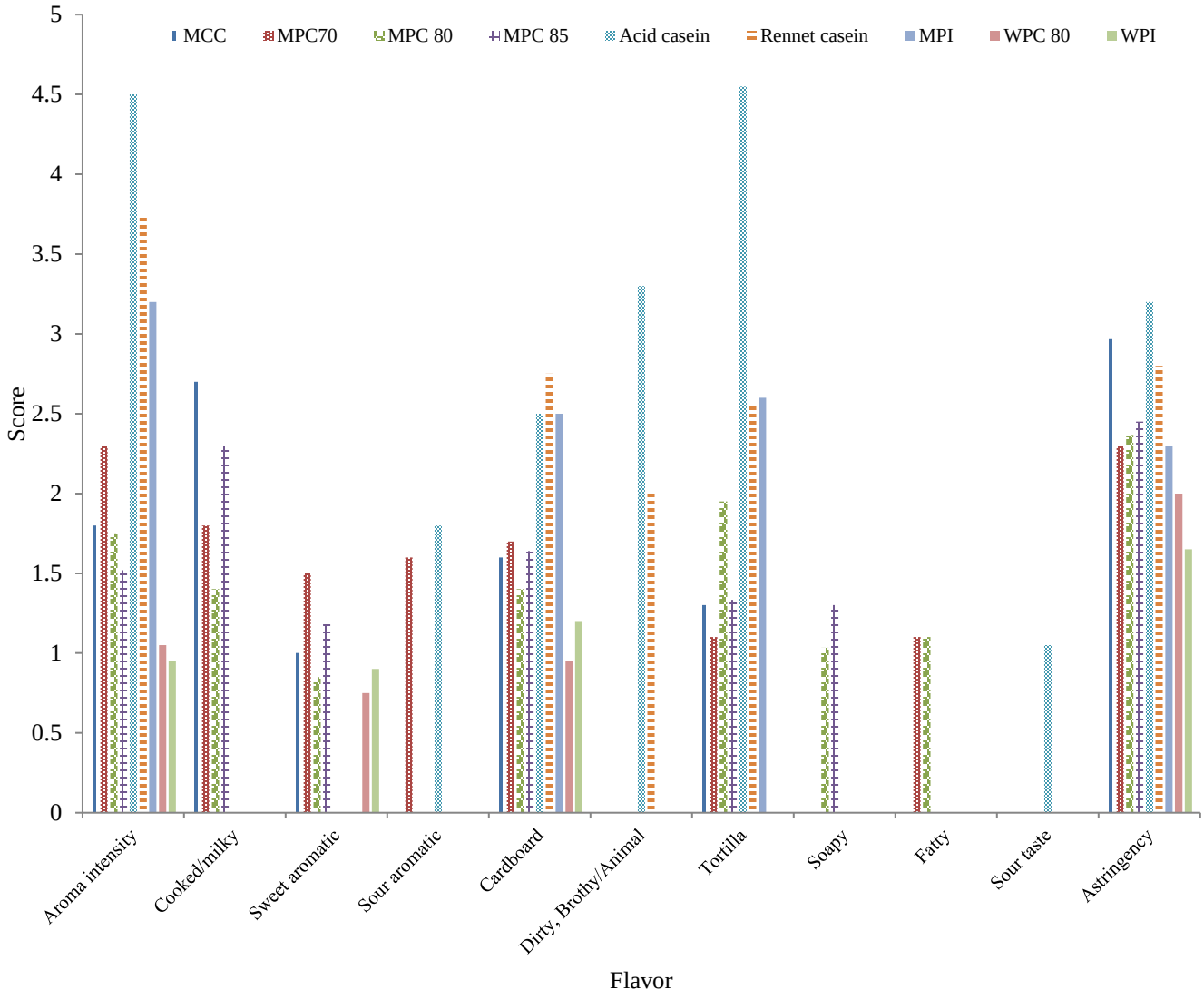

Figure 11. Sensory profiles of micellar casein concentrate (MCC) and other protein products

Adapted from (Smith et al., 2016; Carter et al., 2018; US-Dairy-Export-Council, 2020). MPC= milk protein concentrate; MPI= milk protein isolate; WPC= whey protein concentrate; WPI= whey protein isolate. Attribute intensities were scored on a 0 to 15 point universal intensity scale where 0 = absence of the attribute and 15 = very high intensity of the attribute

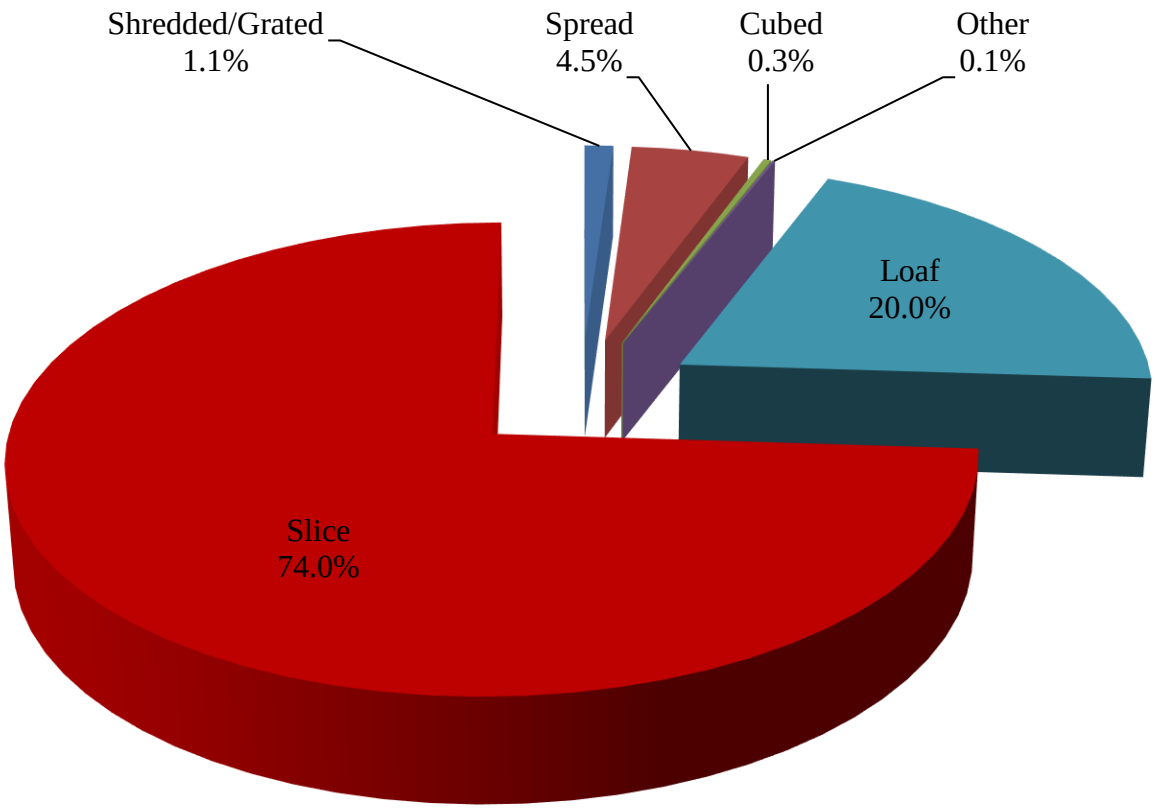

Figure 12. Process cheese supermarket sales in the U.S.A. in 2005

Source: IDFA (2011) reported by Kapoor and Metzger (2008)

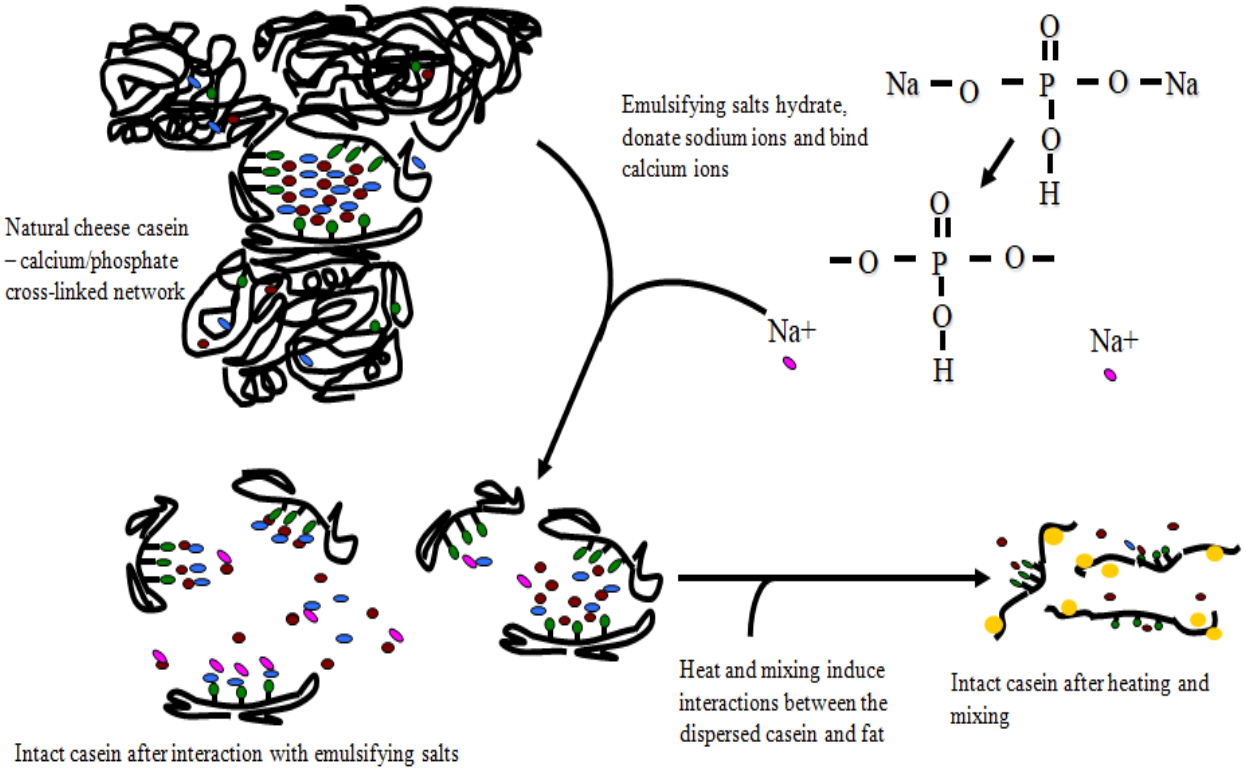

Figure 13. Emulsifying salts (ES) interaction during making process cheese (PC) or process cheese products (PCP)

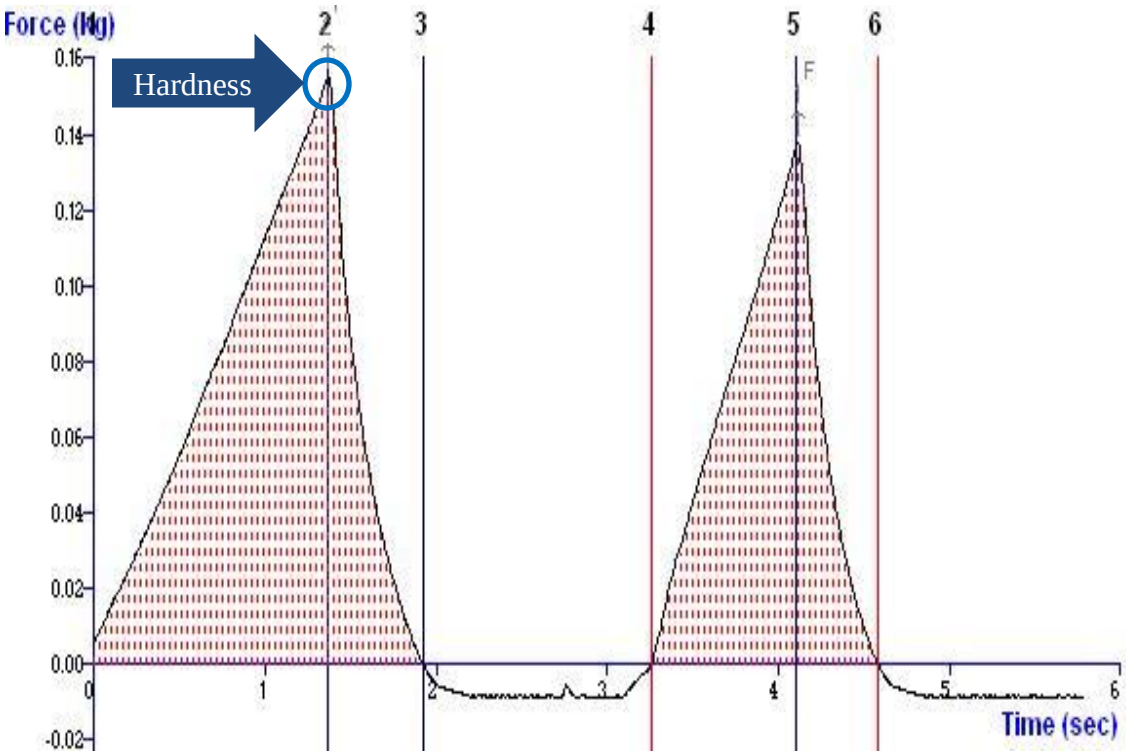

Figure 14. Measuring the hardness of process cheese (PC) using the texture profile analyzer (TPA)

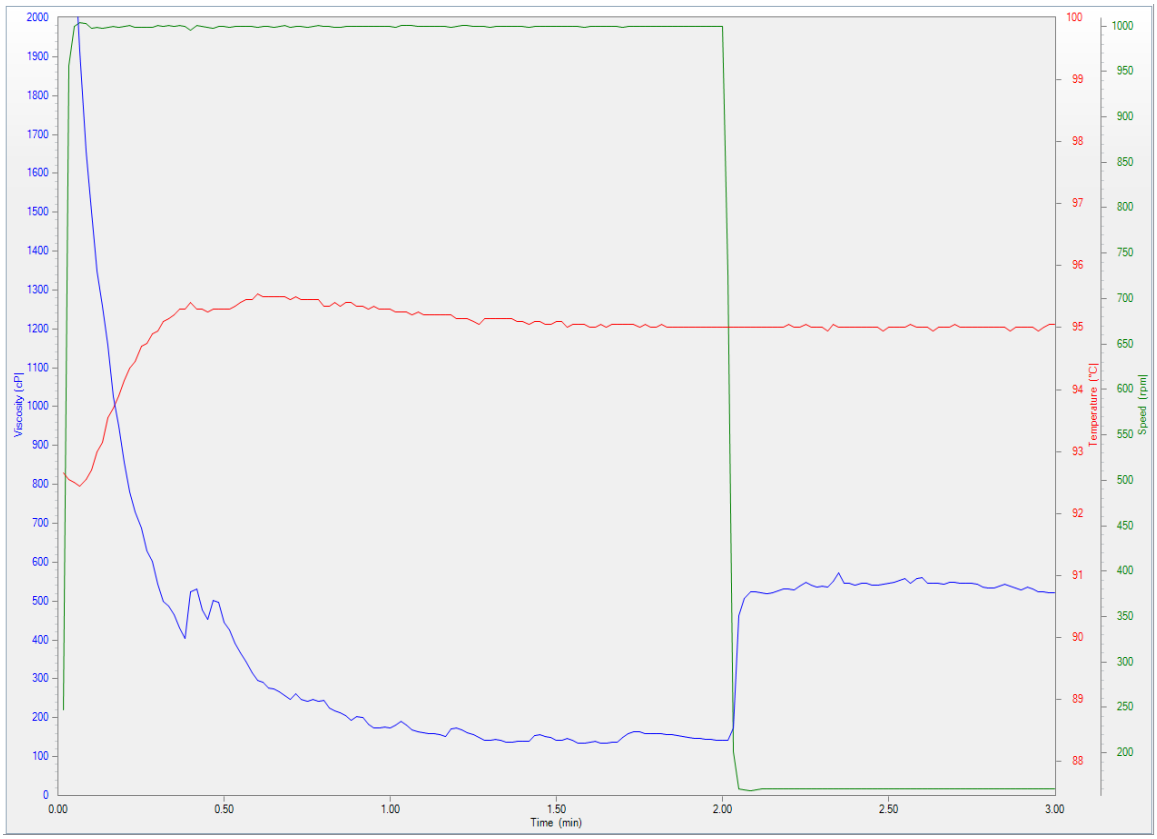

Figure 15. The rapid visco analyzer (RVA) graph that is generated during cooking the process cheese (PC)

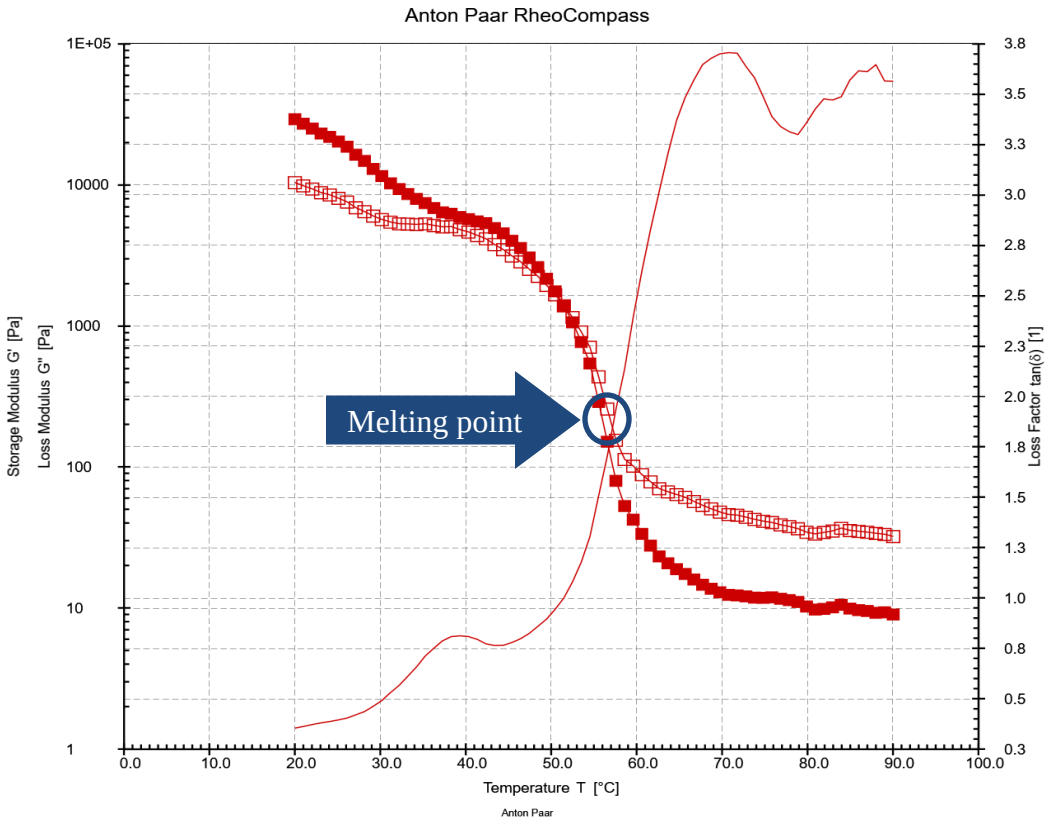

Figure 16. Measuring the melting point of process cheese (PC) using the dynamic stress rheometer (DSR)

CHAPTER II: MANUFACTURE OF PROCESS CHEESE PRODUCTS WITHOUT EMULSIFYING SALTS USING ACID CURD AND MICELLAR CASEIN CONCENTRATE

Abstract

Process cheese product (PCP) is a dairy food prepared by blending dairy ingredients (such as natural cheese, protein concentrates, butter, nonfat dry milk, whey powder, and permeate) with nondairy ingredients (such as sodium chloride, water, emulsifying salts; ES, color, and flavors) and then heating the mixture to get a homogeneous product with an extended shelf-life. Emulsifying salts (ES), such as sodium citrate and disodium phosphate, are critical for the unique microstructure and functional properties of PCP because they improve the emulsification characteristics of casein by displacing the calcium phosphate complexes that are present in the insoluble calcium-paracaseinate-phosphate network in natural cheese. The objectives of this study were to determine the optimum protein content (3, 6, and 9% protein) in micellar casein concentrate (MCC) to produce acid curd and to manufacture PCP using a combination of acid curd cheese and MCC that would provide the desired improvement in the emulsification capacity of caseins without the use of ES. To produce acid curd, MCC was acidified using lactic acid to get a pH of 4.6. In the experimental formulation, the acid curd was blended with MCC to have a 2:1 ratio of protein from acid curd relative to MCC. The PCP was manufactured by blending all ingredients in a kitchenaid to produce a homogeneous paste. A 25 g sample of the paste was cooked in a rapid visco analyzer (RVA) for 3 min at 95°C at 1000 rpm stirring speed during the first 2 min and 160 rpm for the last min. The cooked PCP was then transferred into molds and refrigerated until

further analysis. This trial was repeated three times using different batches of acid curd. MCC with 9% protein resulted in acid curd with more adjusted yield. The end apparent viscosity (402.0-483.0 cP), hardness (354.0-384.0 g), melting temperature (48.0-51.0°C), and melting diameter (30.0-31.4 mm) of PCP made from different batches of acid curd showed were slightly different from the characteristics to typical process cheese produced with conventional ingredients and ES (576.6 cP end apparent viscosity, 119.0 g hardness, 59.8°C melting temperature, and 41.2 mm melting diameter) due to the differences in pH of final PCP (5.8 in ES PCP compared to 5.4 in no ES PCP). We concluded that acid curd can be produced from MCC with different protein content. Also, we found that PCP can be made with no ES when the formulation utilizes a 2:1 ratio of acid curd relative to MCC (on a protein basis).

Keywords: Process cheese products; Micellar casein concentrate; Acid curd; Functional characteristics

Introduction

Microfiltration (**MF**) is a pressure-driven membrane process that is utilized to fractionate casein (**CN**) and serum protein (**SP**) from skim milk using a 0.1 µm semipermeable membrane. The retentate stream in this process which holds the CN is called micellar casein concentrate (MCC), which is mostly native casein. Micellar casein concentrate is a high protein ingredient that is typically manufactured in 3 MF stages using a 3× concentration factor (**CF**) with diafiltration (**DF**). Several MF membranes have been utilized to produce MCC (Hammam et al., 2021), such as spiral-wound membranes (Govindasamy-Lucey et al., 2007; Lawrence et al., 2008; Zulewska et al.,

2009; Beckman et al., 2010; Beckman and Barbano, 2013; Marella et al., 2021), isoflux ceramic membranes (Adams and Barbano, 2013; McCarthy et al., 2017), uniform transmembrane pressure (**UTP**) ceramic membranes (Vadi and Rizvi, 2001; Hurt et al., 2010), and graded permeability (**GP**) ceramic membranes (Zulewska and Barbano, 2014; Tremblay-Marchand et al., 2016; Hammam and Metzger, 2018; Hammam, 2019; Yang et al., 2020).

MCC has promising applications in some dairy and nondairy products due to its unique physicochemical and functional characteristics (e.g., foaming, emulsifying, wetting, dispersibility, heat stability, bland flavor, and water-binding ability) (Carter et al., 2021; Hammam et al., 2021). The high casein content in MCC makes it heat stable and thereby it can be used in beverages that require sterilization (Beliciu et al., 2012; Sauer and Moraru, 2012). Nondairy applications of MCC are pasta, confectionery, meat products, special dietary preparations, textured products, convenience foods, toothpaste, cosmetics, and wound treating preparations (Salunke, 2013; Hammam et al., 2021). Dairy applications for MCC include Cheddar cheese (Amelia et al., 2013; Li et al., 2020; Xia et al., 2021), Greek-style yogurt (Bong and Moraru, 2014), imitation Mozzarella cheese (**IMC**) (Salunke, 2013; Hammam and Metzger, 2020a), recombined MCC (Lu et al., 2016, 2017), process cheese (**PC**) and process cheese products (**PCP**) (Hammam et al., 2019; Hammam and Metzger, 2019; Metzger and Hammam, 2020; Hammam et al., 2022), and acid curd (Hammam and Metzger, 2019, 2020b; Metzger and Hammam, 2020).

Process cheese and PCP are dairy foods prepared by blending dairy ingredients (such as natural cheese, protein concentrates, butter, nonfat dry milk, whey powder, and

permeate) with nondairy ingredients (such as sodium chloride, water, emulsifying salts (**ES)**, color, and flavors) and then heating the mixture with continuous agitation to produce a homogeneous product with an extended shelf-life (Meyer, 1973; Thomas, 1973; Caric et al., 1985; Guinee et al., 2004; Kapoor and Metzger, 2008). PC and PCP are similar products but the differences in the ingredients that are allowed to utilize in each one as listed in the Code of Federal Regulations (**CFR**).

A critical reaction that occurs during PC and PCP manufacture is calcium sequestration using ES, such as sodium citrate and disodium phosphate. Emulsifying salts are critical for the functional characteristics of PC and PCP due to their role in improving the emulsification characteristics of casein by sequestrating a portion of the calcium from the calcium-casein-phosphate network in natural cheese or other casein containing ingredients (Figure 1). As shown in Figure 1, ES such as disodium phosphate sequester the calcium from the calcium-casein-phosphate network by donating sodium ions. As a result, the major molecular forces (calcium phosphate based cross-links) that cross-link the various monomers of casein are partially disrupted. This disruption leads to hydration and dispersion of the protein. The partially dispersed monomers of casein, like any other protein, have hydrophilic and hydrophobic portions which are now free to interact with the water phase and with the fat phase respectively leading to their improved emulsification properties (Kapoor and Metzger, 2008). The above process in the presence of heat and agitation produces an emulsified, homogeneous product with an extended shelf-life.

Acid curd is a protein concentrate, which is obtained by precipitating casein at the pH of 4.6 (isoelectric point of caseins) using starter cultures or acids without the use of

rennet (Hammam et al., 2021). At the isoelectric point of caseins, the colloidal calcium phosphate present in the casein micelles solubilizes in the whey, which consequently results in the acid curd with low mineral or calcium content. In contrast to the low mineral content of acid curd, MCC has a high level of casein bound calcium due to its pH (6.5-6.7). If acid curd is mixed with MCC (Figure 2), it may be possible to create a partially deaggregated casein network without the use of ES. The ratio of acid curd to MCC will have an impact on the level of deaggregation and the pH of the final PCP. We hypothesize that a ratio of 2 parts of protein from acid curd to 1 part of protein from MCC will create a partially deaggregated casein network similar to a typical PC that utilizes ES (Figure 2).

Acid curd could be produced from skim milk in a process similar to cottage cheese manufacture. There is a possibility of using MCC instead of skim milk to produce acid curd. Making acid curd from MCC has advantages as compared to skim milk, since manufacturing MCC using MF results in milk-derived whey protein as a by-product which can be utilized in many applications, particularly making whey protein isolate (**WPI**). In contrast, acid curd produced from skim milk results in acid whey as a by-product, which is more difficult to utilize. The typical composition of MCC (3-stages using 3× CF with DF) is >9% true protein (**TP**) and >13% total solids (**TS**) (Zulewska et al., 2009). This MCC can be used as is to make acid curd or diluted to lower protein levels before making acid curd if required.

Many consumers are perceiving ES in PC as chemicals, which is reducing the consumption of those products. Also, ES increase the level of sodium in PC which could elevate the blood pressure. As a result, few attempts were performed to produce PC with

no ES using whey proteins (Yee et al., 1998), calcium reduced ingredients (Smith and Rivera, 2017), and blends of sheared and non-sheared amounts of fat (Kimmel et al., 2014). To date, no study related to manufacture PCP with no ES is available in the literature. Therefore, the objectives of this study were to develop a process to produce acid curd from MCC and to determine if PCP could be produced without ES using a novel method by combining acid curd and MCC at a 2:1 ratio on a protein basis in the formulation.

Materials and Methods

Experimental Design

Manufacture of MCC (as described below) was completed in approximately 10 h in one day at Davis Dairy Plant at South Dakota State University (Brookings, SD). The experiment was repeated 3 times with different lots of skim milk. Part of the final MCC was dried using a spray dryer to produce MCC powder, while the rest of the MCC was divided into 3 aliquot solutions and diluted with water to standardize the protein content to 3, 6, and 9% before making acid curd.

Preparation of Skim Milk

The MCC was manufactured as described in our previous studies with some modifications (Hammam and Metzger, 2018, 2019; Hammam, 2019; Metzger and Hammam, 2020). Approximately 800.0 kg of whole bovine milk was separated (Model MSE 140-48-177 AirTight centrifuge; GEA Co., Oelde, Germany) at 4°C at the South Dakota State University Davis Dairy Plant and then pasteurized in a plate heat exchanger (model PR02-SH, AGC Engineering, Bristow, VA) at 76°C for 16 sec. The pasteurized

skim milk was then kept at ≤ 4°C until MF was conducted. Tanks and cans were sanitized and covered during processing to minimize airborne contamination.

Microfiltration Operation

To fractionate skim milk into CN and SP to produce MCC, a pilot-scale ceramic GP MF system (TIA, Rond-point des, Portes de Provence, Rue Robert Schumann 84500, Bollène, France) was utilized. The GP MF system was equipped with 7 ceramic tubes (19 channels with a diameter of 3.3-mm) mounted in the system vertically. The ceramic GP MF membranes had a 0.1-μm pore size, 1.68 m^2 surface area, and a 1.02 m membrane length. The GP MF system was also equipped with a feed pump and a retentate recirculation pump (TIA, Bollène, France). The MF of skim milk (approximately 730.0 kg) was performed in 3 stages to produce MCC.

Manufacture of MCC

First Stage: The GP MF system was started with soft water at 50°C using 3× CF (1 kg retentate: 2 kg of permeate) in a feed and bleed mode (one way pass) with 400 kPa retentate pressure inlet (**Rpi**), 200 kPa retentate pressure outlet (**Rpo**), and 200 kPa permeate pressure outlet (**Ppo**). The skim milk with ~10.6% Brix (Misco, Palm Abbe Digital Refractometer #PA201, USA) was heated to 50°C with a plate heat exchanger (SABCO Plate-pro Sanitary Chiller; NP925-41) before processing. When the processing conditions were stable while running with water, the system was transitioned to skim milk. The skim milk was microfiltered with the GP MF system at a constant flux (71.42 L/m^2 per h) using a 3× CF in a feed and bleed mode at 50°C (Figure 3). The water at the beginning of the process was flushed out with skim milk by collecting about 37.5 kg of

permeate and 19.5 kg of retentate. The permeate flow rate was set at 120 L/h (flux of 71.42 L/m^2 per h) and the retentate flow rate was 60 L/h to produce a 3× retentate. After this startup, retentate and permeate were collected and weighed continuously. During MF of skim milk, Rpi, Rpo, and Ppo were targeted to maintain 400, 200, and 200 kPa, respectively. The CF was calculated every 15 min by collecting permeate and retentate samples. The composition of retentate and permeate during MF was monitored using an infrared spectrophotometer (MilkoScan FT1-Lactoscope FTIR, FOSS Instruments-FOSS Analytical A/S- Hillerod, Denmark). The collected retentate was kept in tanks during the MF process. The processing time of the first stage was approximately 4 h.

Second Stage: The retentate (~17.7% Brix) from the first stage was diluted with soft water (approximately 219.0 kg of retentate mixed with 438.0 kg of water) to obtain a DF of 3× to get back the original volume of skim milk. After mixing, the diluted retentate (~5.9% Brix) was heated to 50°C and processed with the GP MF system using a 3× CF, as described in the first stage. The water at the beginning of the process was flushed out of the system with the diluted retentate by collecting about 37.0 kg of permeate and 18.0 kg of retentate. The Rpi, Rpo, and Ppo were set at 400, 200, and 200 kPa, respectively. The permeate flow rate was 120 L/h (flux of 71.42 L/m^2 per h) and the retentate flow rate was 60 L/h. Permeate and retentate were weighed continuously, as described in the first stage. The retentate was collected in sanitized cans. The processing time of the second stage was approximately 4 h.

Third Stage: Approximately 200.0 kg of the retentate (~13.0% Brix) was microfiltered in a recirculation mode. The retentate of the second stage was placed in the tank of MF unit and then proceeded to the third stage using a 3× CF at 50°C. The following conditions

were applied: Rpi, Rpo, and Ppo were 400, 200, and 200 kPa, respectively, while the permeate flow rate was 120 L/h (flux of 71.42 L/m^2 per h) and the retentate flow rate was 60 L/h. The retentate was recirculated while permeate was collected until the TS reached 13.0-14.0% (CEM Smart System5 SL7199) or ~16.0% Brix. Increasing the solids content of MCC during MF led to a reduction in Ppo. This reduction is related to the concentration polarization and membrane fouling that accumulated on the membrane during recirculation. The final MCC resulting from the third stage was collected. The processing time for the third stage was around 1 h. The MCC was then pasteurized at 63°C/30 min. Part of the liquid MCC was dried using a spray dryer to produce MCC powder, while the rest of the liquid MCC was utilized in making acid curd. This trial was replicated 3 times using three separate lots of raw milk.

Cleaning the Membrane

After processing, the GP MF system was flushed with soft water to remove all retentate residues from the system. The initial flux was measured with approximately 60 kg of soft water at 27°C. During the flux measurement, the retentate valves were closed and the permeate valves were completely opened with the feed pump running. Subsequently, 30.0 kg of soft water was added to the system and heated to 74°C, then 900 ml of Ultrasil 110 Alkaline cleaner (Ecolab Inc. 370 Wabasha Street N., St Paul, MN) and 200 ml of XY 12 (Ecolab Inc. 370 Wabasha Street N., St Paul, MN) was added to get pH of 11 (Accumet, Fisher Scientific, USA). This solution was recirculated for 30 min at a 350 L/h permeate flow rate (flux of 208 L/m^2 per h). After cleaning the MF system with the alkaline solution, the membrane was cooled to 50°C (less than 10°C per min). The alkaline solution was flushed out of the MF system with soft water until the pH

of outlet water ranged from 8.3 to 8.5. The flux was measured again, as described previously. The system was cleaned with an acid solution (Ultrasil 78 acid cleaner) by adding 30 kg of soft water and heated to 52°C; subsequently, 400 ml of Ultrasil 78 (Ecolab Inc. 370 Wabasha Street N., St Paul, MN) was added to get a pH of 2. The recirculation of the acid solution was applied for 20 min at a flux of 208 L/m^2 per h. Subsequently, the machine was stopped, and the acid was retained inside the system. Before using the system again, the acid solution was flushed out with soft water until the pH reached 8.3-8.5. The flux was measured again after flushing the acid solution. Within each MF stage, membrane was flushed using water, and flux was measured. The membrane was cleaned within stages using the abovementioned procedures when the flux did not show the original values.

MCC Drying

A pilot scale spray dryer at Davis Dairy Plant at South Dakota State University was utilized to dry the MCC. The nozzle used to dry the MCC had a core size of 21 and an orifice size of 66. The inlet pressure was set at 2250.0 psi using hi-pressure pump speed and it was adjusted manually through the fan (30.0%). The supply fan and exhaust fan were set at 80.0 and 90.0%, respectively. The inlet temperature was 175°C, while the outlet temperature was 82°C. The dryer was connected to a fluid bed (Dahmes Stainless INC: DSI, Model no 10011-11, New London, MN) that was equipped with sieves. The fluid bed was attached to three fans (hot=71°C and 40.0% speed; warm=50°C and 50.0% speed; cool=21°C and 40.0% speed). The liquid MCC was heated to 50°C in a water bath before feeding into the dryer. The powder was collected in an airtight container and stored at room temperature until further analysis.

Manufacture of Acid Curd

The liquid MCC was diluted with water to standardize the protein content to 3 (acid curd-3), 6 (acid curd-6), and 9% (acid curd-9). Lactic acid (88% Lactic Acid FCC, product code: 175820, lot number: 1501277028, Faries Parkway, Decatur, IL 62526) was added to reduce the pH to 4.6 (isoelectric point) at 4°C. Approximately 0.6, 1.2, and 2.0% of lactic acid were added to MCC 3, 6, and 9% protein, respectively to achieve the pH of 4.6. The acidified MCC was then warmed to 25°C, left to set, then cut, and mixed gently during heating to 45°C. The whey was subsequently drained from the curd, and the curd was then pressed for 1 h at 80 psi. After pressing, the curd was kept in the freezer at -20°C until further analyses. The adjusted moisture yield of the acid curd was calculated considering 60% moisture as the desired water content (adjusted moisture). This experiment was repeated three times.

Manufacture of Process Cheese Products

Techwizard (Excel-based-formulation software program provided by Owl Software) was used to develop the PCP formulations (Metzger, 2010). The percentage of ingredients utilized in PCP formulations is shown in Table 1 to produce PCP with 49.0 % moisture, 20.0 % fat, 18.0 % protein, and 2.0 % salt. In each formulation (**FR-3**; PCP made from acid curd 3, **FR-6**; PCP made from acid curd 6, and **FR-9**; PCP made from acid curd 9), the amount of protein from acid curd and MCC was adjusted to have a ratio of 2:1, respectively. The ingredients were aged natural Cheddar cheese (Kraft Heinz Aged Cheddar), unsalted butter (Land O'Lakes, INC., Arden Hills, MN), acid curd, MCC powder, deproteinized whey (Bongards' Creameries, Perham, MN), and salt (Cargill,

Minneapolis, MN). Approximately 10% of aged cheddar was added into PCP formulations (FR-3, FR-6, and FR-9) to get a mild Cheddar flavor in the final PCP. The deproteinized whey was used to standardize the solids content in the PCP formulations. Control PCP was also manufactured using disodium phosphate (Lot 085651, Fisher Scientific, Fair Lawn, New Jersey 07410) as the ES, commercial MCC powder (CasPro 8500, Lot # NF8109A1, Milk Specialties Global, Eden Prairie, MN 55344), young and aged Cheddar cheeses, deproteinized whey, and salt. Young and aged Cheddar cheeses, as well as commercial MCC, were utilized in control formulation to have the typical intact casein (unhydrolyzed casein) to produce PCP with typical functionality. PCP formulations were prepared by mixing all ingredients in a kitchenaid at room temperature for 30 min to produce a homogeneous paste. A 25 g sample of the paste was weighed in a canister and then a paddle was inserted. The canisters were warmed in a water bath at 40°C for 10 min and then cooked in the rapid visco analyzer (**RVA**) for 3 min at 95°C. The stirring speed was 1000 rpm for the first 2 min and 160 rpm for the last min. Each batch (300 g) was divided into 10 canisters. The cooked PCP was then poured into copper molds (diameter=20 mm; height=30 mm) to measure the hardness using texture profile analysis (**TPA**). Also, it was poured into plastic molds (diameter=28.3 mm; height=25 mm) to measure the melt temperature using dynamic stress rheometry (**DSR**) and melt diameter using the Schreiber melt test.

Chemical Analyses

TS (AOAC, 2000; method 990.20; 33.2.44), total protein (TPr= total nitrogen × 6.38) (AOAC, 2000; method 991.20; 33.2.11), ash (AOAC, 2000; method 945.46; 33.2.10), and pH (Thermo Scientific, Orion Star A321 pH Meter, Indonesia) of the MCC

and acid curd were determined before they were used in PCP formulations. Also, the TS and pH of the final PCP were determined.

Functional Analyses

End Apparent Viscosity. The end apparent viscosity of the PCP was measured at the end of the cooking time using the RVA at 95°C by calculating the mean value of the last five values (Figure 4), which is referred to the end apparent viscosity. The end apparent viscosity was measured in all canisters of each batch.

Texture Profile Analysis (TPA). The hardness of the PCP was measured using the TPA. The PCP was prepared by pulling the cheese out from the copper cylinders and then cutting the cheese into 20 mm height. The PCP was analyzed for hardness using a TA.XT-Plus Texture Analyzer (TA.XT-Plus, 6 Patton Drive, South Hamilton, MA) equipped with a 38 mm diameter cylindrical flat probe (TA-4) and using uniaxial double bite 10% compression with 1 mm/s crosshead speed. The maximum force of the first compression (Figure 5) was referred to the hardness of the cheese. This test was repeated 6 times for each batch.

Dynamic Stress Rheometry (DSR). The PCP was prepared by cutting the cheese into slices (2 mm thick and 28.3 mm diameter) using a wire cutter (Salunke, 2013). A stress sweep test of the PCP was performed at a frequency of 1.5 Hz, and stress ranged from 1 to 1000 Pa at 20°C using a rheometer with parallel plate geometry (MSR 92, Anton Paar, Graz, Austria). The stress sweep experiment determined that the maximum stress limit for the linear viscoelastic region was 50 Pa. The dynamic rheological properties of the PCP were then analyzed with a dynamic temperature ramp test that ranged from 20 to

90°C with a ramp rate of 1°C/min using a frequency of 1.5 Hz and constant stress of 50 Pa. The temperature at which tan δ=1 (G″/G′) was referred to the cheese melting temperature (Figure 6). A duplicate was performed on each batch.

Schreiber Melt Test. The PCP samples were cut into cylinders (diameter=28.5 mm and height=7 mm) and placed in glass Petri dishes (95.0 mm diameter) (Figure 7A). The dishes were transferred to a forced draft oven at 90°C for 7 min (Salunke, 2013). After cooling the dishes (Figure 7B), the diameter of the melted PCP samples was measured in four different places using a vernier caliper and reported in millimeters. This test was repeated four times for each batch.

Statistical Analysis

Statistical analysis was performed to study the effect of treatments on the functional properties of PCP. ANOVA was done to obtain the mean squares (MS) and P-values using the GLM procedure available in R software (R ×64-3.3.3, R Foundation for Statistical Computing). Differences were tested using the least significant difference test when a significant difference was detected at $P<0.05$.

Results and Discussion

PCP Formulations

PCP formulations made with and without ES are shown in Table 1. Acid curd was not used in control PCP formulation. Additionally, young and aged Cheddar were used at the ratio of 1:1 in control PCP formulations to have the desired functionality.

Composition of Ingredients

The composition of MCC before and after drying is shown in Table 2. The TS, TPr, and ash content were approximately 13.8, 9.5, and 1.0%, respectively, in liquid MCC. It has been reported that the retentate of 3× MF GP membranes had 89.6% CN%TP with 0.92% SP and 15.3% TS (Tremblay-Marchand et al., 2016). Similar results were also found in other studies; however, the TS and protein of MCC produced in other studies were high relative to our study due to the extra DF stages utilized in those studies (Hurt and Barbano, 2010; Hurt et al., 2010; Zulewska and Barbano, 2014). The composition of MCC can vary depending on many factors, such as membrane type, DF, as well as the composition of skim milk (Hurt and Barbano, 2010; Hammam et al., 2021). After spray drying of MCC, it resulted in MCC powder with 97.2% TS, 65.4% TPr, and 7.1% ash. The pH of MCC before and after drying was approximately 6.8. The composition of MCC powder was relatively similar to liquid MCC on a dry basis.

The TS, TPr, ash, pH, and moisture adjusted yield of acid curd made from 3, 6, and 9% protein MCC are shown in Table 3. The mean TS of acid curd made from 3, 6, and 9% protein MCC was around 37.5, 43.8, and 41.6%, respectively. The TPr content was 32.1% in acid curd made from 3% protein MCC, while it was 37.9 and 34.5% in acid curd made from 6 and 9% protein MCC, respectively. The ash content ranged from 0.7 to 1.3% in acid curd made from MCC with a protein content of 3 to 9%. The moisture adjusted yield significantly increased ($P<0.05$) with the increased protein content in MCC as expected, which was 6.6, 16.9, and 32.0% for acid curd produced from MCC with 3, 6, and 9% protein, respectively. There was no significant difference ($P>0.05$) in the composition of acid curd made from MCC with 3, 6, and 9% protein. Although there

were substantial variations between replicates, we did not find any significant differences in the composition of acid curd made from the three treatments. We think that the replicate effect may have resulted from differences in pressing time and amount of lactic acid used to acidify and set the curd at the pH of 4.6. Since no ash modification was done, we expected that the acid curd made from 3% protein MCC would be lower in ash as compared to 6 and 9% protein MCC. The target pH in acid curd was 4.6; however, the pH of acid curd made from 9% protein MCC (4.00) was lower than other treatments (~ 4.6). As the protein content increased in MCC, it was more challenging to adjust the pH with lactic acid possibly due to increased buffering leading to an increase in the amount of used lactic acid. Therefore, 0.3% of sodium hydroxide (40%) was used to standardize the pH of acid curd-9 treatment to elevate the pH to 4.6. The composition of acid curd made from MCC in our study was in the range of typical acid curd made from skim milk on a dry basis (Blanchette et al., 1996; Klei et al., 1998; Sarode et al., 2016; Hammam et al., 2021). The composition of acid curd depends on the composition of starting material, final pH, and process conditions (e.g., cooking temperature, washing curds, pressing) (Wong et al., 1976). The adjusted yield of acid curd made from 6 and 9% protein MCC was high as compared to Cottage cheese curd made from skim milk (Klei et al., 1998; Hallab et al., 2007). These results demonstrate the efficiency of making acid curd from 6 and 9% protein MCC, which contributed to more yield.

Composition of PCP

The moisture content and pH of PCP made with ES (control) and without ES (FR-3, FR-6, and FR-9) are shown in Table 4. Mean squares and P-values for the moisture and pH of the final PCP are shown in Table 5. The moisture content of PCP made from

acid curds (no ES) ranged from 48.1 % to 48.5%, while the moisture content of control PCP was approximately 48.5%. Because all formulations were standardized to have the same composition, no significant difference ($P>0.05$) was detected in the moisture content of all PCP formulations. The targeted moisture content of PCP was 49.0%; however, there was some moisture loss during cooking in the RVA, which led to a decrease in the final moisture content of the PCP. This loss could be compensated by the addition of 0.5 g of water in each canister before cooking in the RVA (Purna et al., 2006).

The pH of PCP made from acid curd was approximately 5.4 and did not show any differences ($P>0.05$) within treatments made with no ES; however, the pH of control was significantly higher ($P<0.05$), which was 5.8. The differences in the type and age of natural cheese, type and level of ES can result in differences in the final pH of PCP (Gupta et al., 1984; Shirashoji et al., 2006; Kapoor and Metzger, 2008; Bulut-Solak and Akin, 2019). The differences in the final pH between the control and experimental PCP may be attributed to the use of ES. In addition to calcium chelation, the other important function of ES is pH buffering, which leads to an increase in the final pH of process cheeses (Kapoor and Metzger, 2008). It has been reported that the pH of a good quality PCP using ES should range from 5.4 to 5.8 (Palmer and Sly, 1943; Marchesseau et al., 1997; Kapoor and Metzger, 2008; Bulut-Solak and Akin, 2019), which is similar to those results in Table 4. Palmer and Sly (1943) stated that the emulsion of PC is low when the pH is lower than 5.4 or higher than 5.8. The differences in pH of control PCP made with ES relative to PCP made with no ES could affect the structure and quality of final PCP and thereby its functional properties due to its effects on the protein interactions in the final PCP emulsion (Palmer and Sly, 1943; Meyer, 1973; Marchesseau et al., 1997). It

was found that as the pH of PC drops to 5.2, the protein-protein interaction increases (Marchesseau et al., 1997) because the pH is close to the isoelectric point of caseins (4.6). This induces the aggregation of protein, which in turn, results in poor emulsion of fat in PC. On the other hand, the PC had an open structure when the pH elevated to 6.1, which eventually led to weaker emulsification (Marchesseau et al., 1997). Marchesseau et al. (1997) also found that the pH of 5.7 resulted in PC with better uniform fat emulsion with a closely knit protein network.

Functional Characteristics of PCP

The mean values of end apparent viscosity and hardness of PCP are illustrated in Table 6. Mean squares and P-values for the end apparent viscosity and hardness of the final PCP are shown in Table 7. The end apparent viscosity of PCP in FR-3, FR-6, and FR-9 treatments were approximately 483.2, 402.1, and 474.9 cP, respectively. The end apparent viscosity of control PCP was around 576.6 cP. Although the end apparent viscosity of PCP made with ES (control) was slightly higher relative to PCP made with no ES (FR-3, FR-6, and FR-9), no significant differences ($P>0.05$) were detected.

For the pH effect on end apparent viscosity; it was stated that as the pH elevated in PC, the net negative charges on the casein increased (Kapoor, 2007; Mulsow et al., 2007; Lu et al., 2008). This induced the calcium mediate cross-linking of the casein molecules around each other in PC gel network. The calcium induced cross-linking of the casein molecules limits the movement of casein chains and thereby reduces the flowability of PC. When the PC is heated, the hydrophobic interactions increase as the temperature elevates which could decrease the flowability. When pH is low and close to

the isoelectric point, more protein-protein interactions elevate. Increasing the pH elevates the net negative charges on casein molecules and thereby elevates the water holding capacity of casein molecules (Fox et al., 2015). This results in swelling of casein molecules and increasing the viscosity of PC emulsion. This was also proved when the pH of casein solutions was elevated from 5.0 to 7.0, which resulted in higher viscosity (Zoller, 1921). However, the high pH in PCP made with ES (control) did not result in significant differences in the end apparent viscosity compared to PCP made with no ES.

The hardness of the PCP was 383.7, 363.3, and 354.6 g for FR-3, FR-6, and FR-9, respectively (Table 6). No significant difference was found ($P>0.05$) in the hardness of PCP among the three experimental treatments made with no ES. However, the PCP made using ES (control) had a lower hardness of 119.0 g ($P<0.05$) as compared to PCP made without ES using different acid curd at a ratio of 2:1 protein from acid curd to protein from MCC, respectively. In our previous study, we found that the hardness of PCP made using MCC and ES ranged from 100.0 to 212.0 g (Hammam, 2019). In another study, the hardness of PCP made from MCC using ES was 110.0-135.0 g (Salunke, 2013), which is similar to control PCP we made in this study using ES. The results indicate that PCP made without ES was firmer than the control PCP.

The hardness of PC is mainly affected by the emulsified-gel network (Marchesseau et al., 1997). Several casein-based interactions are responsible for forming the basis for the emulsified gel network and stabilizing casein network, such as hydrophobic interactions, hydrogen bonds, and calcium mediated cross-links (Marchesseau et al., 1997). The changes in pH could affect those interactions. Typically, the net negative charges on casein are increased when the pH is elevated, which induces

the robust hydrogen bonds and calcium mediated cross links within the casein molecules in PCP, and this, in turn, strengthens the gel network of PCP and makes it firmer. Some studies reported that increasing the pH in a specific range led to an increase in the hardness, while others proved the opposite suggestions. It was reported that the pH affects the hardness (Kapoor, 2007). Kapoor found that the firmness of PCF elevated as the pH increased from 5.5 to 6.1 with increasing the intact casein. Another study found that the hardness of PC made with sodium hexametaphosphate as an ES increased with elevating the pH from 5.6 to 5.9 (Lu et al., 2008). Another study found that increasing the pH led to increasing the hardness of fat free PC spreads (Swenson et al., 2000). On the other hand, other studies reported that increasing pH resulted in low hardness PC (Cavalier-Salou and Cheftel, 1991; Lee et al., 1996; Lee and Klostermeyer, 2001; Awad et al., 2002). In a different study, it was found that elevating the pH of PC from 5.7 to 6.7, and decreasing pH from 5.7 to 5.2, led to less firmed PC (Marchesseau et al., 1997). The increased firmness of PCP with no ES, when compared to control in this study, might be due to increases protein-protein interactions of caseins at pH 5.4 leading to a firmer product than control (pH 5.8).

The melting characteristics of the control and experimental PCP are illustrated in Table 8. Mean squares and P-values for the melting properties of the PCP are shown in Table 9. The melting temperature of PCP made from FR-3, FR-6, and FR-9 was 51.3, 48.4, and 50.5°C, respectively. The melting temperature of PCP made with ES (Control) was higher with an average of 59.8°C compared to FR-3, FR-6, and FR-9. No significant difference ($P>0.05$) was detected in the melting temperature of PCP made from FR-3,

FR-6, and FR-9. However, there was a significant difference ($P<0.05$) in the melting temperature of PCP made with no ES relative to control.

The differences in the onset of melting (melting temperature) can be explained by the differences in pH of those cheeses. As the pH drops to the isoelectric point, the net negative charges on caseins reduce which increases the protein-protein interactions, and this leads to aggregation of protein and thereby poor emulsification (Kapoor, 2007). The higher pH in control PCP resulted in a uniform fat emulsion with a closely knit protein network. This led to a higher melting temperature of PCP made with ES relative to other PCP made with no ES.

The melt diameter of FR-3, FR-6, and FR-9 was 29.9, 30.2, and 31.4 mm, respectively; with a melt area of 704.3, 717.9, and 775.1 mm^2, respectively. The melt diameter of those treatments was not significantly different ($P>0.05$) (Table 8). The melting diameter and melting area of PCP made from different acid curds were significantly different ($P<0.05$) as compared to PCP made using ES (control), with an average of 41.2 mm diameter or 1331.2 mm^2.

The PCP made with ES (control) resulted in increased meltability due to the high pH in this formulation compared to other PCP formulations made with no ES. This is due to the increased protein-protein interactions (high at lower pH) in PC made with no ES.

The storage modulus (G′) and loss modulus (G″) of PCP measured during heating from 20 to 90°C at 10°C increments are shown in Tables 10 and 11, respectively. The ANOVA Table with mean squares and P-values at 20, 70, and 90°C for both elastic (G′: Pa) and viscous (G″: Pa) moduli of the PCP made from acid curd and MCC are shown in

Table 12. The effect of using different acid curds in manufacture of PCP with no ES compared to control PCP on G′ and G″ during heating from 20 to 90°C is also presented in Figures 8 and 9, respectively.

The G′ (at a temperature range of 20 to 90°C) of PCP made with no ES using different acid curds (made from 3, 6, and 9% protein MCC) was higher than PCP made with ES (Figure 8) from 20 to 40°C. The G′ of control PCP was high from 50 to 70°C compared to FR-3, FR-6, and FR-9, while it was lower than those treatments from 80 to 90°C. These differences were not significant ($P>0.05$) at many points (Table 10); however, the G′ was significant ($P<0.05$) between control and other treatments at 20°C (room temperature), 50 and 60°C (near melting temperatures). More G′ indicates more firmness or hardness for the final PCP (Kapoor and Metzger, 2008), which was noticed with low pH in PCP made with no ES at 20°C and firmness was measured at the same temperature. The differences in pH play a significant role in the melting characteristics of PCP as mentioned as well as the G′ values. This reflected in the G′ values of final PCP. It was found that the G′ values of PCP with a pH of 5.77 were higher compared to PCP with 5.55 pH (Salunke and Metzger, 2022). Another study found that the G′ values increase as the pH decrease (Lee et al., 1996) which is similar to our study.

The G″ (at a temperature range of 20 to 90°C) of PCP made with no ES using different acid curds was higher than control PCP (Figure 9) from 20 to 40°C. Similar to the G′ trend, the G″ of control PCP was high from 50 to 70°C compared to FR-6, and FR-9, while it was lower than those treatments from 80 to 90°C. These differences were not significant ($P>0.05$) at several points during running the DSR test (Table 11). However, PCP made from FR-9 (acid curd made from MCC with 9% protein) resulted in the

highest G″ than FR-3, FR-6, and control, which followed the same trend as in G′. It was found that the G″ values of PCP with a pH of 5.77 were higher compared to PCP with 5.55 pH (Salunke and Metzger, 2022). Another study found that the G″ values increase as the pH decrease (Lee et al., 1996) which is similar to our study.

The G′ of PCP before melting was higher than G″. This indicates that the PCP has more elastic behavior (gel) than the viscous behavior (liquid). The G′ (elastic) and G″ (viscous) are decreased during measuring the melting point using the DSR. Both moduli are decreased with increasing the temperature until the cross point, which is the cheese melting point. This is due to the low protein-protein interactions in the casein network while heating which lead to fat separations (Salunke and Metzger, 2022). This trend was similar to those found in other studies (Hennelly et al., 2005; Subramanian et al., 2006; Zhong et al., 2007; Guinee and O'Kennedy, 2009; Kommineni et al., 2012; Hosseini-Parvar et al., 2015; Salunke and Metzger, 2022).

The functional characteristics and composition of PCP can be affected by formulations, ingredients, pH, intact casein, calcium content, cooking time, cooking temperature, stirring speed, type and amount of ES, and cooling speed. The functionality of the PCP made without ES was in the range of typical PCP made with ES. This indicates that PCP can be manufactured using a 2:1 ratio of acid curd protein to MCC protein and has similar functionality as compared to PCP made with ES.

Conclusions

In this research, we determined that acid curd could be made efficiently from MCC with different protein contents (3, 6, and 9%). The adjusted yield of acid curd

increased with increasing the protein content, therefore 9% protein MCC could be a good option to make acid curd commercially as it would generate less acid whey. Acid curds produced from MCC with different protein content were successful in the manufacture of PCP without ES using 2 parts of protein from acid curd to 1 part of protein from MCC. The 2:1 ratio creates a partially deaggregated casein network that results in PCP with functionality similar to PCP produced with ES. Although there were differences observed in the melted and un-melted texture of PCP made without ES when compared to control, these can be explained due to the possible microstructural interactions induced in the final PCP due to their pH differences. Future studies will focus on devising methodologies to produce PCP using acid curd with similar pH as conventional PCP. Additionally, no differences were detected in the functionality of PCP produced from acid curds with different protein levels.

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Tables

Table 1. Mean (n=3) ingredients used in process cheese products (PCP) formulations

Ingredients (%)	Treatment[1]			
	Control	FR-3	FR-6	FR-9
Acid curd	0.00	32.76	26.64	30.83
MCC powder (produced)	0.00	7.80	7.80	7.80
MCC powder (commercial)	3.40	0.00	0.00	0.00
Butter (unsalted)	0.00	20.55	20.55	20.55
Aged Cheddar cheese	30.05	10.00	10.00	10.00
Young Cheddar cheese	30.05	0.00	0.00	0.00
Deproteinized whey	7.20	6.44	6.65	5.80
Salt (sodium chloride)	1.00	2.00	2.00	2.00
Water	25.80	20.45	26.36	23.02
Disodium phosphate	2.50	0.00	0.00	0.00

[1]Treatments: Control= PCP formulations made with emulsifying salts (ES); FR-3= PCP formulations made from acid curd 3 (acid curd produced from MCC with 3% protein); FR-6= PCP formulations made from acid curd 6 (acid curd produced from MCC with 6% protein); FR-9= PCP formulations made from acid curd 9 (acid curd produced from MCC with 9% protein).

Table 2. The composition of liquid and dried micellar casein concentrate (MCC)

Treatment	Composition[1]			
	TS (%)	TPr (%)	Ash (%)	pH
Liquid MCC	13.79	9.54	1.03	6.80
MCC powder (produced)	97.23	65.38	7.13	6.80

[1]Composition: TS= total solids; TPr= total protein = total nitrogen × 6.38

Table 3. Mean values (n=3) of the composition of acid curd used in process cheese products (PCP) formulations

Treatment[1]	Composition[2]				
	TS (%)	TPr (%)	Ash (%)	pH	Moisture adjusted yield (%)
Acid curd-3	37.46	32.11	0.72	4.68	6.60[c]
Acid curd-6	43.77	37.86	1.07	4.54	16.90[b]
Acid curd-9	41.65	34.50	1.26	4.00	32.00[a]
SEM	1.79	1.70	0.10	0.15	4.40

[1]Treatments: Acid curd 3= acid curd produced from MCC with 3% protein; Acid curd 6= acid curd produced from MCC with 6% protein; Acid curd 9= acid curd produced from MCC with 9% protein.

[2]TS= total solids; TPr= total protein= total nitrogen × 6.38

$$\text{Adjusted yield} = \text{actual yield x } \frac{100 - \text{actual\%moisture}}{100 - \text{desired\%moisture}}$$

Table 4. Mean values (n=3) of the composition of the process cheese products (PCP) made from acid curd

Treatment[1]	Moisture (%)	pH
Control	48.53	5.76[a]
FR-3	48.54	5.41[b]
FR-6	48.09	5.44[b]
FR-9	48.54	5.37[b]
SEM	0.10	0.05

[1]Treatments: Control= PCP formulations made with emulsifying salts (ES); FR-3= PCP formulations made from acid curd 3 (acid curd produced from MCC with 3% protein); FR-6= PCP formulations made from acid curd 6 (acid curd produced from MCC with 6% protein); FR-9= PCP formulations made from acid curd 9 (acid curd produced from MCC with 9% protein).

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

Table 5. Mean squares and P-values (in parentheses) for the composition of the process cheese products (PCP) made from acid curd

Factor	df	Moisture	pH
Replicate	2	0.06 (0.70)	0.004 (0.19)
Treatment[1]	3	0.14 (0.47)	0.10 (<0.05)
Error	6	0.15	0.001

[1]Treatments: Control= PCP formulations made with emulsifying salts (ES); FR-3= PCP formulations made from acid curd 3 (acid curd produced from MCC with 3% protein); FR-6= PCP formulations made from acid curd 6 (acid curd produced from MCC with 6% protein); FR-9= PCP formulations made from acid curd 9 (acid curd produced from MCC with 9% protein).

Table 6. Mean values (n=3) of end apparent viscosity (cP) and hardness (g) of the process cheese products (PCP) made from acid curd

Treatment[1]	End apparent viscosity (cP)	Hardness (g)
Control	576.63	119.02[b]
FR-3	483.17	383.72[a]
FR-6	402.12	363.29[a]
FR-9	474.93	354.64[a]
SEM	63.90	49.80

[1]Treatments: Control= PCP formulations made with emulsifying salts (ES); FR-3= PCP formulations made from acid curd 3 (acid curd produced from MCC with 3% protein); FR-6= PCP formulations made from acid curd 6 (acid curd produced from MCC with 6% protein); FR-9= PCP formulations made from acid curd 9 (acid curd produced from MCC with 9% protein).

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

Table 7. Mean squares and P-values (in parentheses) for the end apparent viscosity (cP) and hardness (g) of the process cheese products (PCP) made from acid curd

Factor	df	End apparent viscosity	Hardness
Replicate	2	43660 (0.56)	52663 (0.08)
Treatment[1]	3	15369 (0.87)	46645 (0.09)
Error	6	67583	13614

[1]Treatments: Control= PCP formulations made with emulsifying salts (ES); FR-3= PCP formulations made from acid curd 3 (acid curd produced from MCC with 3% protein); FR-6= PCP formulations made from acid curd 6 (acid curd produced from MCC with 6% protein); FR-9= PCP formulations made from acid curd 9 (acid curd produced from MCC with 9% protein).

Table 8. Mean values (n=3) of melting properties of the process cheese products (PCP) made from acid curd

Treatment[1]	Melt temperature (°C)	Melt diameter (mm)	Melt area (mm^2)
Control	59.85[a]	41.16[a]	1331.23[a]
FR-3	51.31[b]	29.94[b]	704.30[b]
FR-6	48.44[b]	30.23[b]	717.90[b]
FR-9	50.47[b]	31.38[b]	775.10[b]
SEM	1.41	1.42	79.87

[1]Treatments: Control= PCP formulations made with emulsifying salts (ES); FR-3= PCP formulations made from acid curd 3 (acid curd produced from MCC with 3% protein); FR-6= PCP formulations made from acid curd 6 (acid curd produced from MCC with 6% protein); FR-9= PCP formulations made from acid curd 9 (acid curd produced from MCC with 9% protein).

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

Table 9. Mean squares and P-values (in parentheses) for the melting properties of the process cheese products (PCP) made from acid curd

Factor	df	Melt temperature	Melt diameter	Melt area
Replicate	2	4.26 (0.45)	1.55 (0.30)	4800 (0.26)
Treatment[1]	3	76.05 (<0.05)	86.08 (<0.05)	271730 (<0.05)
Error	6	4.72	1.07	2870

[1]Treatments: Control= PCP formulations made with emulsifying salts (ES); FR-3= PCP formulations made from acid curd 3 (acid curd produced from MCC with 3% protein); FR-6= PCP formulations made from acid curd 6 (acid curd produced from MCC with 6% protein); FR-9= PCP formulations made from acid curd 9 (acid curd produced from MCC with 9% protein).

Table 10. Mean elastic modulus (G′: Pa) of process cheese products (PCP) made from acid curd and micellar casein concentrate (MCC) during heating from 20 to 90°C using dynamic rheological analysis (DSR)

Temperature (°C)	Treatment[1]			
	Control	FR-3	FR-6	FR-9
20	18022.1[b]	40534.2[ab]	34806.5[ab]	70025.2[a]
30	7581.4[a]	13018.4[a]	12171.8[a]	19924.9[a]
40	2925.6[a]	5275.0[a]	4995.4[a]	5272.6[a]
50	2046.8[a]	1407.8[ab]	1060.5[ab]	834.6[b]
60	1227.5[a]	285.0[b]	365.2[b]	260.2[b]
70	326.4[a]	67.4[a]	181.5[a]	156.5[a]
80	10.0[a]	30.4[a]	79.8[a]	134.9[a]
90	1.4[a]	11.5[a]	27.2[a]	111.6[a]

[1]Treatments: Control= PCP formulations made with emulsifying salts (ES); FR-3= PCP formulations made from acid curd 3 (acid curd produced from MCC with 3% protein); FR-6= PCP formulations made from acid curd 6 (acid curd produced from MCC with 6% protein); FR-9= PCP formulations made from acid curd 9 (acid curd produced from MCC with 9% protein).

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

Table 11. Mean viscous modulus (G″: Pa) of process cheese products (PCP) made from acid curd and micellar casein concentrate (MCC) during heating from 20 to 90°C using dynamic rheological analysis (DSR)

Temperature (°C)	Treatment[1]			
	Control	FR-3	FR-6	FR-9
20	7041.1[b]	13048.2[ab]	11395.2[ab]	21466.5[a]
30	4159.7[a]	5277.9[a]	4831.6[a]	7352.5[a]
40	2054.9[a]	3093.8[a]	3363.2[a]	2784.2[a]
50	1619.3[a]	1303.2[a]	1265.7[a]	746.7[a]
60	1228.2[a]	462.5[a]	707.4[a]	362.1[a]
70	464.6[a]	150.2[a]	354.4[a]	271.0[a]
80	79.5[a]	66.2[a]	99.9[a]	230.5[a]
90	33.3[a]	17.5[a]	42.8[a]	227.7[a]

[1]Treatments: Control= PCP formulations made with emulsifying salts (ES); FR-3= PCP formulations made from acid curd 3 (acid curd produced from MCC with 3% protein); FR-6= PCP formulations made from acid curd 6 (acid curd produced from MCC with 6% protein); FR-9= PCP formulations made from acid curd 9 (acid curd produced from MCC with 9% protein).

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

Table 12. Mean squares and P-values (in parentheses) for elastic modulus (G′: Pa) and viscous modulus (G″: Pa) of process cheese products (PCP) made from acid curd and micellar casein concentrate (MCC) during heating from 20 to 90°C using dynamic rheological analysis (DSR)

Factor	df	G′			G″		
		20°C	70°C	90°C	20°C	70°C	90°C
Replicate	2	15,132,092	66,454	3,226	935,479	192,537	29,403
		(0.98)	(0.38)	(0.61)	(0.98)	(0.33)	(0.49)
Treatment[1]	3	1,408,926,975	34,636	7,578	109,540,943	52,928	29,305
		(0.19)	(0.64)	(0.38)	(0.19)	(0.77)	(0.53)
Error	6	653,356,947	58,295	6,145	50,746,681	142,187	36,217

[1]Treatments: Control= PCP formulations made with emulsifying salts (ES); FR-3= PCP formulations made from acid curd 3 (acid curd produced from MCC with 3% protein); FR-6= PCP formulations made from acid curd 6 (acid curd produced from MCC with 6% protein); FR-9= PCP formulations made from acid curd 9 (acid curd produced from MCC with 9% protein).

Figures

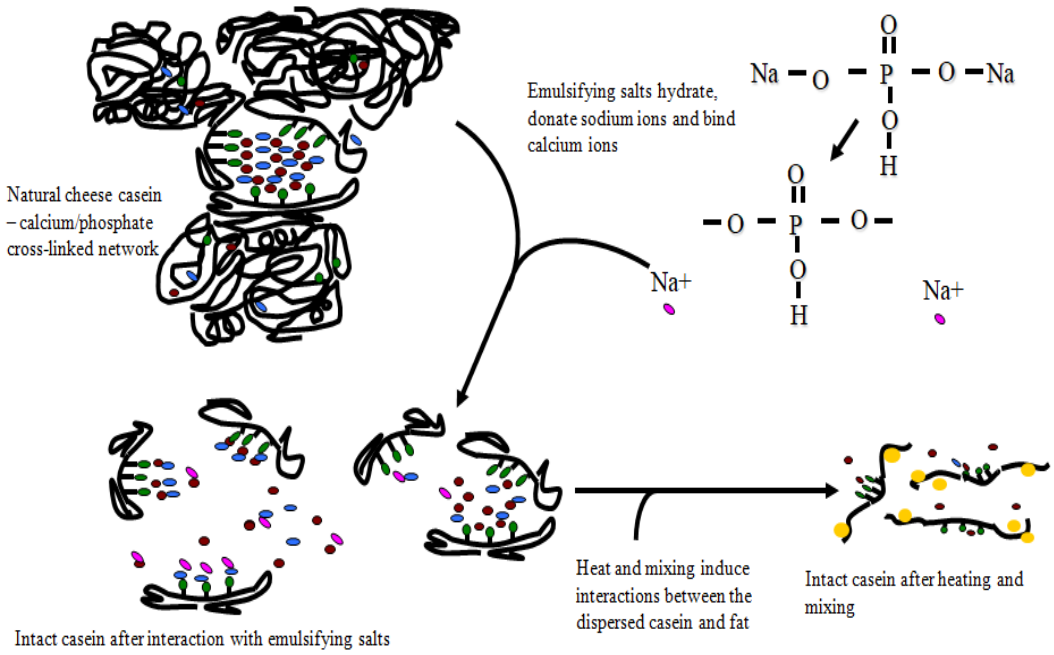

Figure 1. Emulsifying salts (ES) interaction during making process cheese (PC) or process cheese products (PCP)

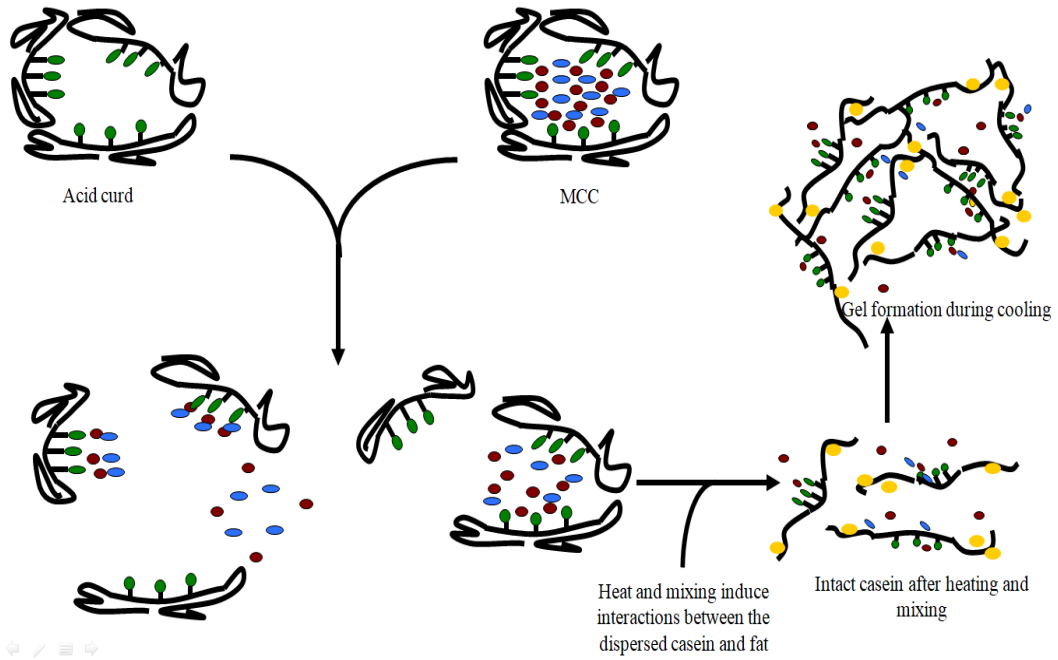

Figure 2. Acid curd and micellar casein concentrate (MCC) interaction in making process cheese products (PCP)

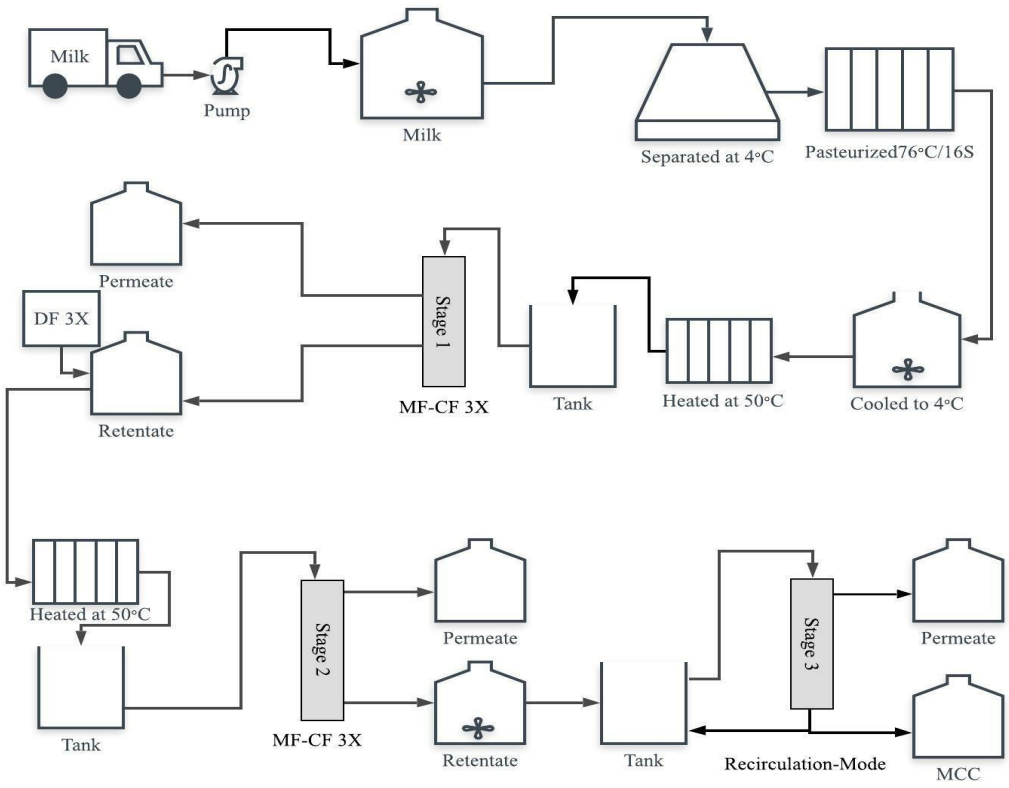

Figure 3. Diagram of manufacturing micellar casein concentrate (MCC) using 3-stages, 3× CF. MF= microfiltration; CF = concentration factor= 3×=2 kg of permeate: 1 kg of retentate; DF = diafiltration= 3×= 2 times of the amount of retentate water added

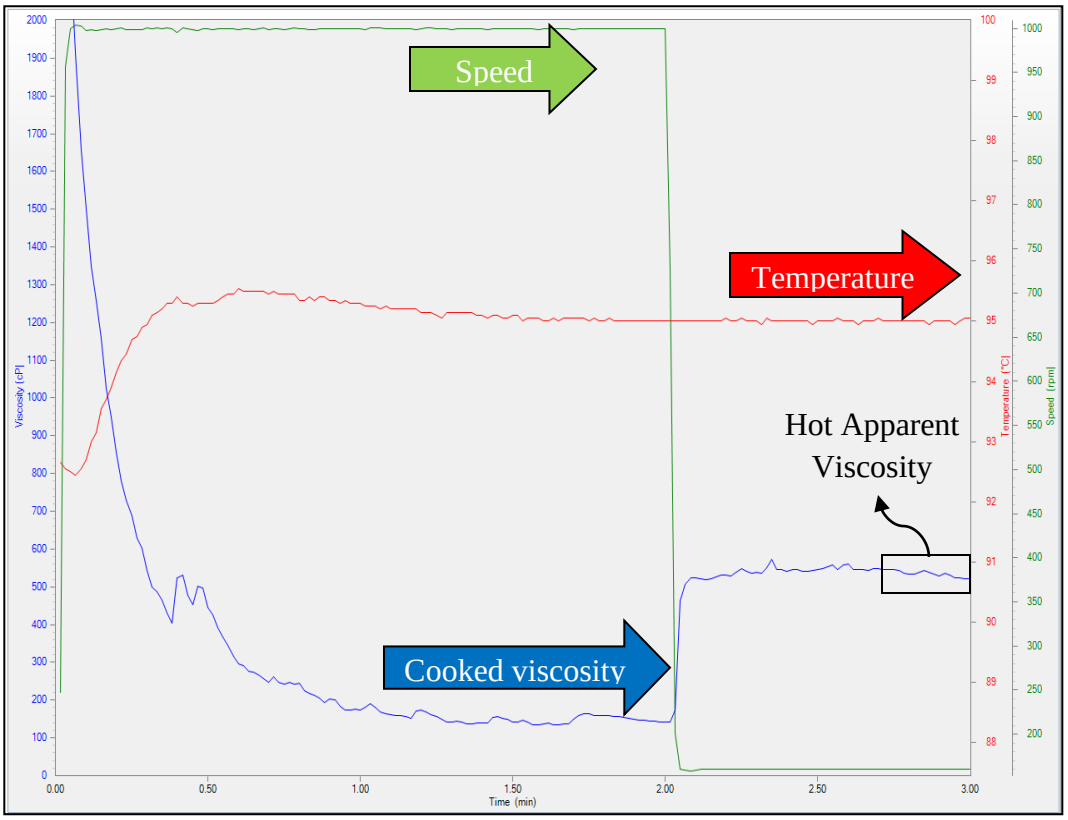

Figure 4. Measuring the end apparent viscosity (cP) of process cheese products (PCP) using the rapid visco analyzer (RVA)

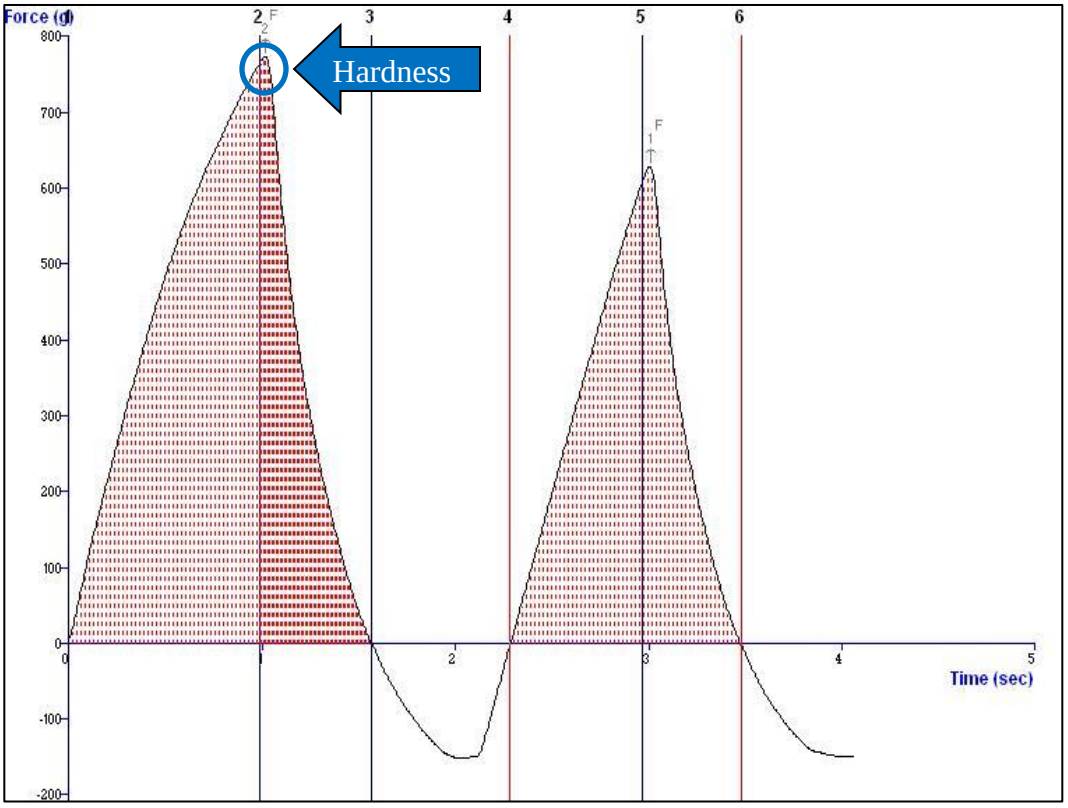

Figure 5. Measuring the hardness (g) of process cheese products (PCP) using the texture profile analysis (TPA)

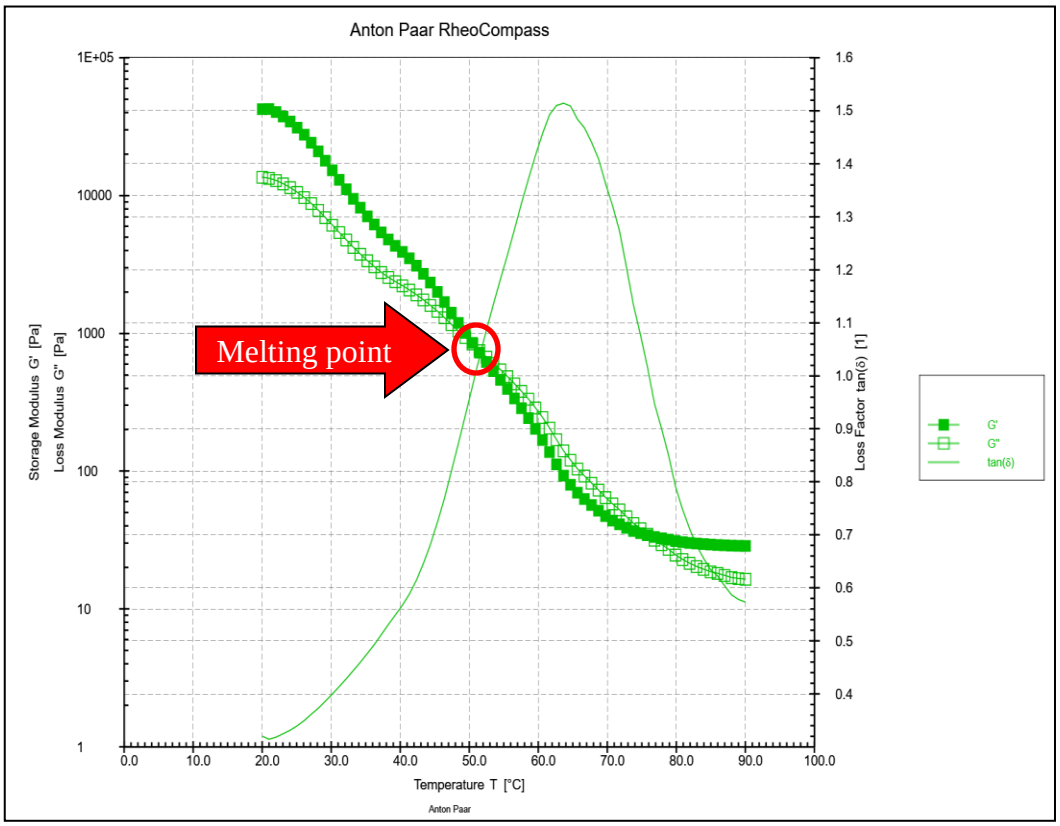

Figure 6. Measuring the melting temperature of process cheese products (PCP) using the dynamic stress rheometer (DSR)

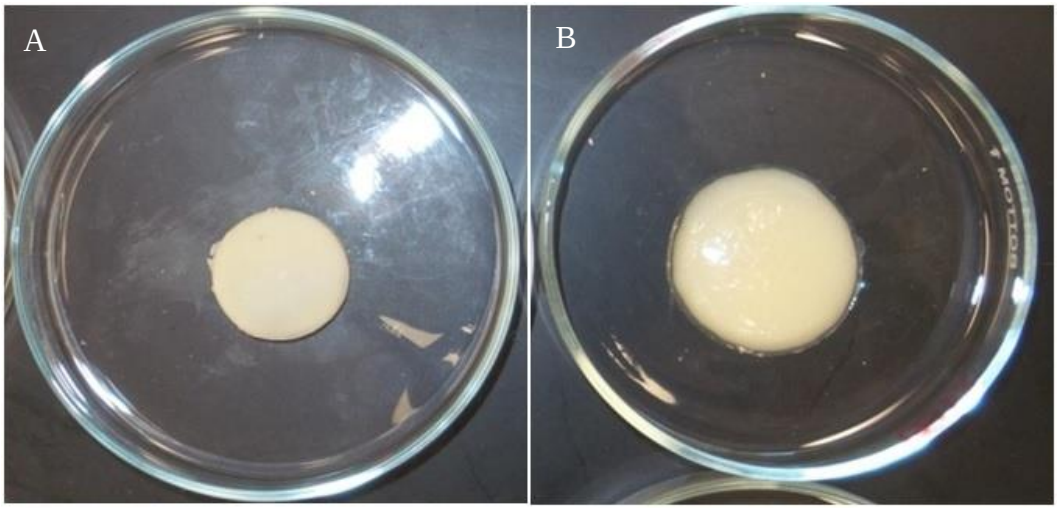

Figure 7. Schreiber melt test to measure the melting diameter of process cheese products (PCP): (A) Before melting; (B) After melting

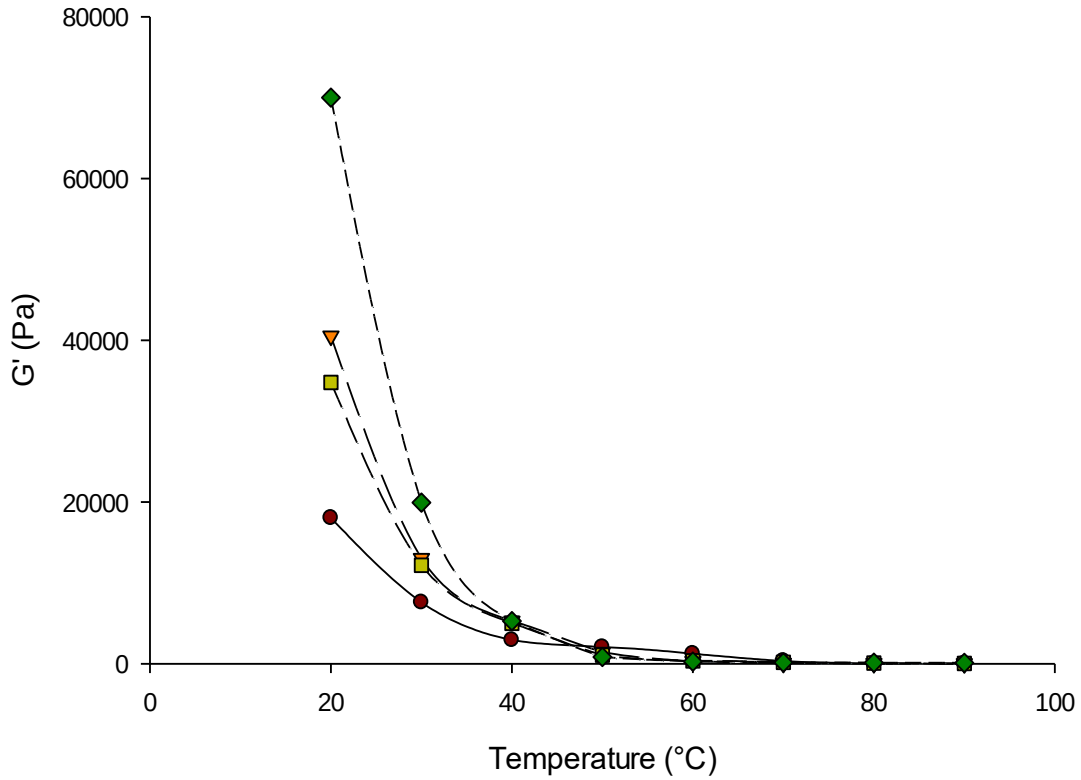

Figure 8. Elastic modulus (G′: Pa) of process cheese products (PCP) made from control (●)= PCP formulations made with emulsifying salts (ES); FR-3 (▼)= PCP formulations made from acid curd 3 (acid curd produced from MCC with 3% protein); FR-6 (■)= PCP formulations made from acid curd 6 (acid curd produced from MCC with 6% protein); FR-9 (♦)= PCP formulations made from acid curd 9 (acid curd produced from MCC with 9% protein) during heating from 20 to 90°C using dynamic rheological analysis (DSR)

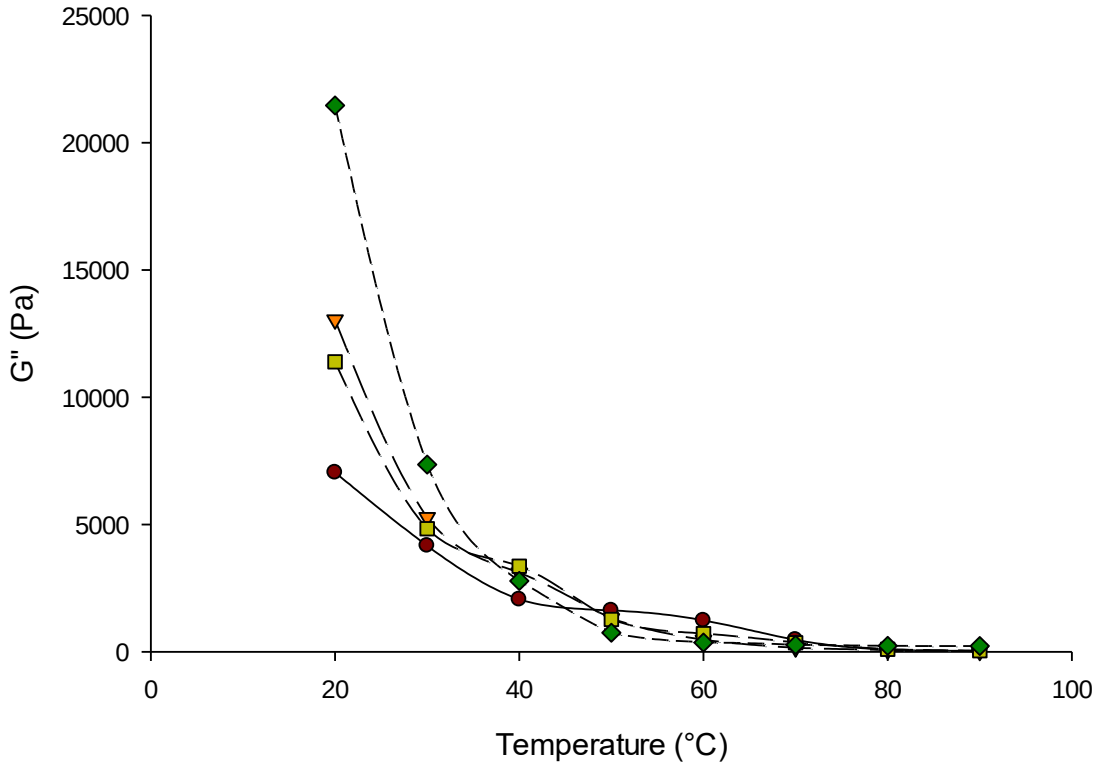

Figure 9. Viscous modulus (G″: Pa) of process cheese products (PCP) made from control (●)= PCP formulations made with emulsifying salts (ES); FR-3 (▼)= PCP formulations made from acid curd 3 (acid curd produced from MCC with 3% protein); FR-6 (■)= PCP formulations made from acid curd 6 (acid curd produced from MCC with 6% protein); FR-9 (♦)= PCP formulations made from acid curd 9 (acid curd produced from MCC with 9% protein) during heating from 20 to 90°C using dynamic rheological analysis (DSR)

CHAPTER III: CHARACTERISTICS OF IMITATION MOZZARELLA CHEESE MANUFACTURED WITH NO EMULSIFYING SALTS USING CULTURE BASED ACID CURD AND MICELLAR CASEIN CONCENTRATE

Abstract

Imitation Mozzarella cheese (IMC) is a dairy, partial or nondairy food based on the source of protein and fat used in the formulation. It has the same basic principles of manufacture as process cheese (PC), and it is prepared by blending dairy ingredients and nondairy ingredients with the aid of heat, shear, and emulsifying salts (ES) to produce a homogeneous product. Emulsifying salts are critical for the functional characteristics of IMC because they improve the emulsification characteristic of casein by displacing the calcium phosphate complexes that are present in the insoluble calcium-paracaseinate phosphate network in the casein containing ingredients. The objectives of this study were to develop a process to produce acid curd from micellar casein concentrate (MCC) using starter cultures and to manufacture IMC using a combination of acid curd and MCC that would provide the required emulsification ability to the caseins without the use of ES. The formulations were targeted to produce IMC with 18.0% protein, 49.0% moisture, 20.0% fat, and 1.5% salt. In the IMC formulation (FR-2:1), the acid curd was blended with MCC so that the formula contained a 2:1 ratio of protein from acid curd relative to MCC. Additional dairy and nondairy ingredients (milk permeate, vegetable oil, and salt) were also utilized in the formulations. Another IMC formulation was made using conventional ingredients and ES as a control. The IMC was prepared by mixing all ingredients in a kitchen aid to produce a homogeneous paste. A 20 g of the mixture was cooked in the rapid visco analyzer (RVA) for 3 min at 95°C with a 1000 rpm stirring

speed during the first 2 min and 160 rpm during the last min. The cooked IMC was then transferred into molds and refrigerated until further analysis. This trial was repeated 3 times using 3 different batches of acid curd. The end apparent viscosity of IMC was approximately 5711.0 cP for control and 7500.0 cP for FR-2:1, while the hardness was 301.0 g for control and 95.0 g for FR-2:1. The melt temperature was 55.5 and 50.0°C, melt diameter was 29.4 and 31.6 mm), melt area was 679.6 and 783.1 mm^2, and stretchability was 12.5 and 12.3 cm of control and FR-2:1 IMC, respectively. The melt and stretch characteristics of IMC made from FR-2:1 were similar compared to control IMC. We conclude that IMC can be made with no ES when the formulation utilizes a 2:1 ratio of protein from acid curd relative to MCC.

Keywords: Imitation Mozzarella cheese; Micellar casein concentrate; Acid curd; Functional characteristics; Melting properties

Introduction

Microfiltration (**MF**) is a membrane process that is utilized to fractionate casein (**CN**) and serum protein (**SP**) from skim milk using a 0.1 μm semipermeable membrane. The skim milk is driven force through the membrane to separate CN (retentate side) and SP (permeate side) based on their sizes (0.1-0.4 μm CN vs 0.003-0.01 μm SP). The retentate is called micellar casein concentrate (MCC), which is mostly native casein. Micellar casein concentrate is a high protein ingredient that is typically manufactured in 3 MF stages using a 3× concentration factor (**CF**) with diafiltration (**DF**). Several MF membranes have been utilized to produce MCC (Hammam et al., 2021), such as spiral-wound membranes (Govindasamy-Lucey et al., 2007; Lawrence et al., 2008; Zulewska et

al., 2009; Beckman et al., 2010; Beckman and Barbano, 2013; Marella et al., 2021), isoflux ceramic membranes (Adams and Barbano, 2013), uniform transmembrane pressure (**UTP**) ceramic membranes (Vadi and Rizvi, 2001; Hurt et al., 2010), and graded permeability (**GP**) ceramic membranes (Zulewska and Barbano, 2014; Tremblay-Marchand et al., 2016; Hammam and Metzger, 2018; Hammam, 2019a). The spiral-wound membranes are cheaper and have lower operating costs, but they have a shorter shelf-life, low chemical stability, limited viscosity range, and less efficiency to remove SP as compared to ceramic membranes. The UTP and GP membranes are commonly used to produce MCC due to their high SP removal. The GP membranes have low operating costs (do not require permeate recirculation pump) although they are more expensive compared to UTP membranes (Hammam et al., 2021).

MCC has promising applications in some dairy and nondairy products due to its unique physicochemical and functional characteristics (e.g., foaming, emulsifying, wetting, dispersibility, heat stability, bland flavor, and water-binding ability). The high casein content in MCC makes it heat stable and thereby it can be used in beverages that require sterilization (Beliciu et al., 2012; Sauer and Moraru, 2012). The nondairy applications of MCC are pasta, confectionery, meat products, special dietary preparations, textured products, convenience foods, toothpaste, cosmetics, and wound treating preparations (Salunke, 2013; Hammam et al., 2021). The dairy applications for MCC include Cheddar cheese (Amelia et al., 2013; Li et al., 2020; Xia et al., 2021), Greek-style yogurt (Bong and Moraru, 2014), imitation Mozzarella cheese (**IMC**) (Salunke, 2013; Hammam and Metzger, 2020a), recombined MCC (Lu et al., 2016, 2017), process cheese (**PC**) and process cheese products (**PCP**) (Hammam and Metzger,

2019; Hammam et al., 2019, 2022; Metzger and Hammam, 2020), and acid curd (Hammam and Metzger, 2019, 2020b; Metzger and Hammam, 2020). We recently developed a process to produce soft acid curd from MCC using direct acidification (lactic acid) (Hammam and Metzger, 2019; Metzger and Hammam, 2020).

Imitation Mozzarella cheese (IMC) or Mozzarella cheese substitute is a dairy, partial or nondairy food based on the source of protein and fat used in the formulation. The typical protein source for IMC is rennet casein and fat sources can range from milk fat to vegetable oils depending on the final nutritional, functionality, and cost targets desired. This type of cheese is one of the popular analogue cheeses in the United States due to its applications in pizza (Bachmann, 2001; O'Riordan et al., 2011). It also offers manufacturers the flexibility to produce a final product with targeted functional characteristics (shreddability, meltability, flowability, stretchability, chewiness, oiling off, and or browning on baking) that are more consistent over a longer shelf life when compared to natural cheese (Guinee et al., 1999; Bachmann, 2001; O'Riordan et al., 2011).

Imitation Mozzarella cheese has the same basic principles that are used in the manufacture of PC in terms of process and equipment. It is prepared by blending dairy and nondairy ingredients (edible oils/fat, protein, emulsifying salts: **ES**, and water) with the aid of heat and shear to produce a homogeneous product (Bachmann, 2001; O'Riordan et al., 2011; Metzger and Hammam, 2020). A critical reaction that occurs during IMC or PC manufacture is calcium sequestration using ES. Emulsifying salts such as sodium citrate and disodium phosphate, are critical for the functional characteristics of IMC due to their role in improving the emulsification characteristics of casein by

sequestrating a portion of the calcium from the calcium-casein-phosphate network in natural cheese or other casein containing ingredients (Figure 1). As shown in Figure 1, ES such as disodium phosphate donates the sodium and consequently sequesters the calcium from the calcium-casein-phosphate network. As a result, the major molecular forces that cross-link the various monomers of casein are partially disrupted by the sequestered calcium complexes. This disruption leads to hydration and dispersion of the protein. The partially dispersed monomers of casein have hydrophilic and hydrophobic portions that have emulsification properties. This, in turn, links the hydrophilic aqueous phase with the hydrophobic fat phase (Guinee et al., 2004), which prevents oil separation in IMC. In the presence of heating and mixing, a homogeneous product with an extended shelf-life is produced.

Acid curd is a protein concentrate, which can be obtained by precipitating casein at pH 4.6 (isoelectric point) using starter cultures or acids without the use of rennet. The colloidal calcium phosphate in the micelles is dissolved in the whey at that pH, which results in acid curd with low mineral or calcium content. In contrast to the low mineral content of acid curd, MCC has a high level of casein bound calcium due to its pH (6.5-6.7). If acid curd is mixed with MCC (Figure 2), it may be possible to create a partially deaggregated casein network without the use of ES. The ratio of acid curd to MCC will have an impact on the level of deaggregation and the pH of the final product. In our previous patent, we hypothesized that a ratio of 2 parts of protein from acid cur to 1 part of protein from MCC created a partially deaggregated casein network similar to a typical process cheese that utilizes ES (Figure 2) (Metzger and Hammam, 2020), and we hypothesize that this can also occur in IMC formulations.

Acid curd can be produced from skim milk in a process similar to Cottage cheese manufacture. There is a possibility of using MCC instead of skim milk to produce acid curd. Making acid curd from MCC has advantages as compared to skim milk, since manufacturing MCC using MF results in milk-derived whey protein as a by-product which can be utilized in many value-added applications, particularly making whey protein isolate (**WPI**). In contrast, acid curd produced from skim milk results in acid whey as a byproduct, which is more challenging to utilize. The typical composition of MCC (3-stages using 3× CF with DF) is >9% true protein (**TP**) and >13% total solids (**TS**) (Zulewska et al., 2009). This MCC could be used immediately in making acid curd or diluted to lower protein levels before making acid curd if required. In our previous studies, we tried to produce acid curd from MCC with different protein content. We found that MCC with 9% protein is the optimum product to produce acid curd using lactic acid (Hammam and Metzger, 2019, 2020b; Metzger and Hammam, 2020).

To date, no study related to manufacture IMC with no ES is available. Therefore, the objectives of this study were to develop a process to produce acid curd from MCC (~9% TP and ~13% TS) using starter cultures and to determine if IMC could be produced without ES using a combination of acid curd and MCC in the formulations.

Materials and Methods

Preparation of MCC Solution

The MCC solution (pH~ 6.6) was prepared and standardized by mixing MCC powder (CasPro 8500, Lot # NF8109A1, Milk Specialties Global, Eden Prairie, MN 55344), milk permeate (product lot: 19113D40, Idaho Milk Products, ID), and water to

produce recombined MCC with approximately 13.0% TS, 9.0% total protein (**TPr**), and 2.0% lactose (enough lactose for fermentation using the starter cultures to set the curd at pH of 4.6). Techwizard software (Excel-based-formulation software program provided by Owl Software) was used to develop the MCC formulations. Powder ingredients were mixed with water, left for hydration for 1 h at room temperature, and then pasteurized at 65°C for 30 min to be ready for fermentation.

Manufacture of Culture Based Acid Curd

Thermophilic cultures (i455, Batch no 3489654, Chr Hansen) were added at a rate of 0.005% to the recombined MCC and incubated at 43°C for approximately 15 h to decrease the pH to 4.6 (Hannah Edge Blu, Woonsocket, RI 02895). After reaching the pH of 4.6, the curd was cut and stirred gently during heating to 50°C in 1 h. The whey was subsequently drained, and the curd was washed with water at a 1:1 ratio, pressed, and kept in the freezer for further analyses. This trial was repeated three times.

Manufacture of Imitation Mozzarella cheese

Techwizard was also used to develop the IMC formulations (Metzger and Roland, 2017) to produce IMC with 49.0% moisture, 20.0% fat, 18.0% protein, and 1.5% salt. The percentage of ingredients utilized in IMC formulations is shown in Table 1. In the formulation of 2:1 (**FR-2:1**), the amount of protein from acid curd and MCC was adjusted to have a ratio of 2:1, respectively. The ingredients of FR-2:1 included salt (Morton salt, INC, Chicago, IL 60606), water, commercial vegetable oil (Wesson, Omaha, NE 68103), MCC powder, milk permeate, and acid curd. Control IMC formulation was made using rennet casein, which included salt, water, commercial

vegetable oil, rennet casein (Rennet Casein 90 Mesh, Fonterra INC, Rosemont, IL 60018), milk permeate, citric acid (KIC chemical Inc, New Peltz, NY 12561), and Kasal salt as ES (chelating salt blend, sodium aluminum phosphate basic powder, Innophos, Chicago Height, IL 60411). The milk permeate was used to standardize the solids content. The formulations were prepared by mixing all the ingredients in a kitchenhand at room temperature for 30 min to produce a homogeneous paste. A 20 g sample of the paste was weighed in a canister and then cooked in the rapid visco analyzer (**RVA**) for 3 min at 95°C. A 0.5 g of water was added into each canister to compensate for the water that evaporated during mixing and cooking in RVA. The stirring speed was 1000 rpm for the first 2 min and 160 rpm for the last min. Each batch was divided into 10 canisters. The cooked IMC was then poured into copper molds (diameter=20 mm; height=30 mm) to measure the hardness using texture profile analysis (**TPA**). Also, it was poured into plastic molds (diameter=28.3 mm; height=25 mm) to measure the melt temperature using dynamic stress rheometry (**DSR**) and melt diameter using the Schreiber melt test.

Chemical Analyses

The MCC and milk permeate before being utilized in preparing recombined MCC or IMC formulations were analyzed for TS (AOAC, 2000; method 990.20; 33.2.44), total protein (TPr= total nitrogen × 6.38) (AOAC, 2000; method 991.20; 33.2.11), and lactose (González de Llano et al., 1996; Amamcharla and Metzger, 2011). The recombined MCC was analyzed for TS, TPr, noncasein nitrogen (NCN) (Zhang and Metzger, 2011), and lactose. The acid curd was analyzed for TS, TPr, ash (AOAC, 2000; method 945.46; 33.2.10), lactose, lactic acid, and mineral profile (AOAC, 2000; method 985.01). The moisture adjusted yield efficiency (MAYE) was also calculated. The whey (a byproduct

of making curd) was analyzed for TS, TPr, ash, lactose, and lactic acid. Also, the TS and pH of the final IMC were determined.

Functional Analyses

The End Apparent Viscosity. The end apparent viscosity was measured following the same procedures we performed in our previous studies (Hammam and Metzger, 2019; Metzger and Hammam, 2020). The end apparent viscosity of the IMC was measured by the end of the cooking time using the RVA at 95°C by calculating the mean value of the last five values (Figure 3), which is referred to the end apparent viscosity. The end apparent viscosity was measured in all canisters of each batch.

Texture Profile Analysis (TPA). The hardness was measured following the methodologies we described in our previous studies (Hammam and Metzger, 2019; Metzger and Hammam, 2020). The hardness of the IMC was measured using the TPA. The IMC was prepared by pulling the cheese out from the copper cylinders and then cutting the cheese into 20 mm height. The IMC was analyzed for hardness using a TA.XT-Plus Texture Analyzer (TA.XT-Plus, 6 Patton Drive, South Hamilton, MA) equipped with a 38 mm diameter cylindrical flat probe (TA-4) and using uniaxial double bite 10% compression with 1 mm/s crosshead speed. The maximum force of the first compression (Figure 4) was referred to the hardness of the cheese. This test was repeated 6 times for each batch.

Dynamic Stress Rheometry (DSR). The DSR was performed following our previous studies (Hammam and Metzger, 2019; Metzger and Hammam, 2020). The IMC was prepared by cutting the cheese into slices (2 mm thick and 28.3 mm diameter) using a

wire cutter (Salunke, 2013). A stress sweep test of the IMC was performed at a frequency of 1.5 Hz, and stress ranged from 1 to 1000 Pa at 20°C using a rheometer with parallel plate geometry (MSR 92, Anton Paar, Graz, Austria). The stress sweep experiment determined that the maximum stress limit for the linear viscoelastic region was 500 Pa. The dynamic rheological properties of the IMC were then analyzed with a dynamic temperature ramp test that ranged from 20 to 90°C with a ramp rate of 1°C/min using a frequency of 1.5 Hz and constant stress of 500 Pa. The temperature at which tan δ=1 (G″/G′) was referred to the cheese melting temperature (Figure 5). A duplicate was performed on each batch.

Schreiber Melt Test. The IMC samples were cut into cylinders (diameter=28.5 mm and height=7 mm) and placed in Petri dishes (Figure 6A). The dishes were transferred to a forced draft oven at 90°C for 7 min (Salunke, 2013). After cooling the dishes (Figure 6B), the diameter of the melted IMC samples was measured in four different places using a vernier caliper and reported in millimeters. This test was repeated four times for each batch.

Stretchability Test. The stretchability of IMC samples was measured by placing a cylinder (diameter=28.5 mm and height=7 mm) in a glass Petri dish (95.0 mm diameter) and left in a forced draft oven at 232°C for 3 min and 30 sec. It was cooled for 30 sec and then a 4-pronged fork was inserted into the cheese. Subsequently, the fork was lifted vertically and the distance before breaking the cheese was measured in centimeters (Figure 7). This test was replicated four times.

Statistical Analysis

Statistical analysis was performed to study the effect of formulations on the functional properties of IMC. An ANOVA was done to obtain the mean squares (MS) and P-values using the GLM procedure available in R software (R ×64-3.3.3, R Foundation for Statistical Computing). Differences were tested using the least significant difference test when a significant difference was detected at $P<0.05$.

Results and Discussion

Composition of Ingredients

The composition of ingredients used in IMC formulations (MCC powder and milk permeate) as well as the composition of recombined MCC utilized in making acid curd is presented in Table 2. The MCC powder contained approximately 94.2% TS, 2.5% lactose, and 83.9% TPr. The composition of MCC powder used in this study is similar to the typical composition of MCC reported in previous studies (Bong and Moraru, 2014; Nasser et al., 2017, 2018; Hammam et al., 2021). The slight differences can be related to the composition of initial material, MF process conditions (e.g., temperatures, pressures, CF, DF), and types of membrane (e.g., ceramic membranes, spiral-wound membranes) (Hammam et al., 2021). The milk permeate used in the formulations had around 94.3, 3.3, and 62.8% TS, TPr, and lactose, respectively. The composition of milk permeate is in the range reported in previous studies (Kalab et al., 1991; Shrestha et al., 2008; Kuechel and Schoenfuss, 2018; Pandalaneni and Amamcharla, 2018; Tsermoula et al., 2021) based on the processing conditions. The MCC powder, milk permeate, and water were mixed to have the recombined MCC solution with approximately 13.0% TS, 9.2%

TPr, 1.0% NCN, and 2.0% lactose. The typical composition of MCC (3-stages using 3× CF with DF) is ~9% TPr and ~13% TS (Zulewska et al., 2009; Hammam et al., 2021), which is similar to the composition of recombined MCC solution used in this study.

Composition of Acid Curd and Acid Whey

The composition of acid curd produced from MCC is shown in Table 3. The mean composition of acid curd from three replicates were 25.8% TS, 23.7% protein, 0.9% ash, 0.2% lactose, 0.6% lactic acid, 0.2% Ca, and 0.1% P. The mean MAYE of acid curd was 97.5%. The average pH of acid curd was targeted to have 4.6. The composition of acid curd depends on the composition of initial materials, final pH, and process conditions (e.g., cooking temperature, washing curds, pressing) (Wong et al., 1976). The step of washing the curd has a significant role in decreasing the ash, Ca, P, lactose, and lactic acid content in the final acid curd. Since the lactose is converted into lactic acid using starter cultures as a result of fermentation, lactose decreased while lactic acid increased as the pH reached 4.6. It is also expected that the ash content is decreased when the MCC is converted into acid curd (McDowall and Dolby, 1935; Czulak et al., 1969; Wong et al., 1976; Hill et al., 1985; Lucey and Fox, 1993). The Ca is converted from insoluble (colloidal form) to soluble form and released in the whey as the pH decreases (Davies and White, 1960; Wong et al., 1976; Dalgleish and Law, 1989; Guinee et al., 1993). As a result, the Ca and P dropped as the pH decreased with having a low ratio of Ca to P (Kindstedt and Kosikowski, 1988; Lucey and Fox, 1993), which results in low ash content. The MAYE of acid curd in our study was close to the MAYE values of Cottage cheese curd reported in a previous study (Klei et al., 1998).

The composition of acid whey (a byproduct of making the curd from MCC) is illustrated in Table 4. The acid whey produced as a byproduct during making the curd from the three replicates showed an average of 5.0% TS, 1.4% protein, 0.9% ash, 0.6% lactose, and 1.4% lactic acid. The TPr%TS in acid whey produced on a lab scale was 28.0%. Approximately 1.4% lactose was required to reach a pH of 4.6 in the acid curd/whey. Using 2.0% lactose MCC left around 0.6% of lactose in the lab scale acid whey (Hammam and Metzger, 2020b). The loss of components in whey especially protein depends on the composition of initial material as well as handling the curd in the cheese vat and this loss gets higher with increasing the scale size. The composition of acid whey produced as a byproduct of making acid curd using MCC was similar to the acid whey produced from milk in previous studies (Peri and Dunkley, 1971; Durham and Hourigan, 2007; Saffari and Langrish, 2014; Chandrapala et al., 2015; Lievore et al., 2015; Chen et al., 2016; Hammam et al., 2017; Hammam, 2019b). Those studies found that the TS of acid whey can range from 5.0-7.0%, while protein and ash can range from 0.5 to 1.0 and 0.5 to 1.0%, respectively. The protein in acid whey can elevate to 1.4% as in our study with increasing the protein content in MCC (9.2% protein), which was expected. As a result, solids, ash, and protein contents in acid whey can be changed based on the composition of starting material. The lactose content in acid whey was different compared to other studies since we started with 2.0% lactose MCC not 4.5% lactose as in milk.

Composition of IMC

The moisture content and pH of IMC are shown in Table 5. Also, the mean squares and P-values for the moisture content and pH of IMC are presented in Table 6.

The IMC formulations were targeted to have 49.0% moisture. The actual moisture content was approximately 48.3% in IMC made with ES (control) and 48.2% in IMC made with no ES (2:1 formulation). No significant differences ($P>0.05$) were detected in the moisture content of IMC within the treatments. It was expected to have some moisture loss during mixing and cooking the IMC. It was found in previous studies that there is evaporated (loss) water during cooking PC and IMC (Kommineni et al., 2012; Salunke, 2013; Hammam, 2019a; Hammam and Metzger, 2019, 2020a; Hammam et al., 2019, 2022; Metzger and Hammam, 2020). However, we added 0.5 g of water was added to compensate the evaporated water from the cooked cheese as described by Purna et al., (2006). The added water could be increased to 1.0 g to get the targeted moisture content in IMC.

The pH of IMC made from control was approximately 5.7, while the pH of IMC made with no ES was around 5.4. Significant differences ($P<0.05$) were detected in the pH of IMC made with ES and with no ES. We previously manufactured PCP with no ES using acid curd and MCC at the same ratio. The pH of the PCP was approximately 5.4 (Hammam and Metzger, 2019; Metzger and Hammam, 2020). The main roles of using ES in PC formulations are calcium sequestration and pH adjustment (Kapoor and Metzger, 2008; Shirashoji et al., 2010). ES are sequestering the Ca from the casein network to produce a deaggregated casein network. The buffering capacity of ES is different which could affect the amount of casein-bound calcium and thereby the pH of final PC (Brickley et al., 2008; Shirashoji et al., 2010). This might affect the characteristics of final PC, as a result, the type of ES and typical amounts should be considered to have the desired PC (Kapoor and Metzger, 2008). The pH of our PC made

with no ES had low pH compared to control since ES were not utilized. The pH of IMC can range from 5.4 to 5.8 when ES are utilized (Mounsey and O'Riordan, 1999; Noronha et al., 2008; Jana et al., 2010; Salunke, 2013; Bi et al., 2016), which is similar to the typical pH of PC or PCP (Palmer and Sly, 1943; Marchesseau et al., 1997; Kapoor and Metzger, 2008; Bulut-Solak and Akin, 2019). PC or PCP and IMC have the same basic principles and same interactions during cooking the cheese. Palmer and Sly (1943) stated that the emulsion of PC is low when the pH is lower than 5.4 or higher than 5.8. The differences in pH of control IMC made with ES relative to IMC made with no ES could affect the structure and quality of final IMC and thereby its functional properties due to its effects on the protein interactions in the final IMC emulsion (Palmer and Sly, 1943; Meyer, 1973; Marchesseau et al., 1997). It was found that as the pH of PC drops to 5.2, the protein-protein interaction increases (Marchesseau et al., 1997) because the pH is close to the isoelectric point of caseins (4.6). This induces the aggregation of protein, which in turn, results in poor emulsion of fat in IMC. On the other hand, the PC had open structure when the pH elevated to 6.1, which eventually led to a weaker emulsification (Marchesseau et al., 1997). Marchesseau et al. (1997) also found that the pH of 5.7 resulted in PC with better uniform fat emulsion with a closely knit protein network.

Functional Characteristics of IMC

The end apparent viscosity of IMC is presented in Table 7. The mean squares and P-values for the end apparent viscosity of IMC are shown in Table 8. The end apparent viscosity is referred to the cheese's flowability when completely melted, which is measured at the end of cooking time (Prow and Metzger, 2005). The end apparent viscosity of IMC was approximately 5711.0 and 7500.0 cP for IMC made with ES

(control) and without ES (FR-2:1), respectively. Although the 2:1 IMC showed higher end apparent viscosity relative to control IMC, no significant differences (P>0.05) were detected.

The hardness, mean squares, and P-values of IMC are also illustrated in Tables 7 and 8, respectively. The hardness of IMC made using ES was around 301.0 g compared to 95.0 g in 2:1 formulation IMC. No significant differences (P>0.05) were detected in the hardness of IMC made from control and FR-2:1 formulation. It was found that the hardness (a measure of unmelted cheese firmness) is not correlated with the functional melt properties (Prow and Metzger, 2005). Noronha and others reported that the hardness of IMC (48.0-48.3% moisture, 24.4-25.4% fat, and 22.4-23.2% protein) ranged from 392.0 to 533.0 g with a pH of 5.9 (Noronha et al., 2008). Another study found that IMC (55.5-57.0% moisture, 9.0-11.0% fat, and 22.0-22.5% protein) with a pH of 5.7 showed approximately 3600.0 g hardness (Jana et al., 2010). Other IMC formulations (48.0% moisture, 21.0% fat, 1.0% salt, and 20.0% protein) made using MCC and MPC presented a hardness of 2640.0-3660.0 g, while the hardness of IMC made from rennet casein as control was 3214.0 g with a pH of 5.8 (Salunke, 2013).

Although the hardness values between the control and treatment were not significantly different, the experimental IMC had a lower hardness value. This could be due to the pH effects. Kapoor found that the firmness of PCF elevated as the pH increased from approximately 5.5 to 6.1 (Kapoor, 2007). Another study found that the hardness of PC increased as the pH elevated from 5.6 to 5.9 using ES (Lu et al., 2008). Typically, the net negative charges on casein are increased as the pH elevates, which induces the robust hydrogen bonds and calcium mediated cross links within the casein

molecules in IMC leading to a stronger gel network of IMC and consequently making it firmer.

The melting characteristics of IMC are shown in Table 9. The mean squares and P-values for the melting properties are exemplified in Table 10. The melt temperature was approximately 55.5°C relative to 50.0°C in IMC made with no ES. The melt temperature of IMC made from control was not significantly different ($P>0.05$) as compared to the FR-2:1 formulation. The melting diameter and melt area were 29.4 mm and 679.6 mm^2, respectively, in IMC made with ES; while they were 31.6 mm and 783.1 mm^2 in IMC made with no ES (FR-2:1). The melting diameter and melt area were less ($P<0.05$) in control IMC compared to IMC made with no ES. However, control IMC was as stretchable ($P>0.05$) as IMC made with no ES. The stretchability was approximately 12.5 and 12.3 cm in control and FR-2:1 formulation, respectively.

Noronha's study found that the melting temperature of IMC ranged from 65.3 to 69.5°C (Noronha et al., 2008). Salunke reported that the melting temperature of IMC made from MCC and MPC ranged from 70.0 to 71.0°C compared to 73.7°C in rennet casein IMC (Salunke, 2013). Salunke's study also found that the melted diameter and stretching on pizza were 31.81-32.82 mm (area of 846.0-795.0 mm^2) and 6.0-14.0 mm, respectively in IMC made with MCC and MPC, while it was 37.56 mm (area of 1108.2 mm^2) and 25.6 mm (area of 514.7 mm^2), respectively, in IMC made with rennet casein (Salunke, 2013). The stretching of IMC (44.3% moisture and 21.8% fat) made in another study was 34.0-45.0 cm (Shah et al., 2010).

The slight differences in the onset of melting (melting temperature) can be explained by the differences in pH of IMC. As the pH drops to the isoelectric point, the net negative charges on caseins reduce which increases the protein-protein interactions and this led to aggregation of protein and thereby poor emulsification (Kapoor, 2007). The higher pH in control IMC resulted in a uniform fat emulsion with a closely knit protein network. This led to a higher melting temperature of IMC made with ES relative to IMC made with no ES. As the pH elevated, the net negative charges on casein micelles increased, which promoted the calcium mediated cross-links casein molecules and this, in turn, strengthen the IMC gel network. Increasing the strength of IMC gel network led to restricting the movement of casein chains in IMC during reheating which decreased the flowability, melt diameter, and melt area of IMC with higher pH made with ES (Kapoor, 2007). This phenomenon was not pronounced in the melting temperature and stretchability of IMC, but it was more differentiated in the melt diameter and melt area.

Conclusions

Culture based acid curd was produced from liquid MCC (>9% TPr and >13% TS). Acid curd and MCC can be mixed in a specific ratio and market to be ready for making different types of cheeses including PCP and IMC. The MCC and acid curd were utilized successfully in manufacture of IMC with no ES in the ratio of 2:1 protein from acid curd to protein from MCC. This ratio of acid curd and MCC creates a partially deaggregated casein network that results in IMC with similar functionality to IMC produced with ES. IMC with no ES could be produced with the similar functional characteristics of IMC made with ES.

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Tables

Table 1. Imitation Mozzarella cheese (IMC) formulations made with different acid curd and micellar casein concentrate (MCC)

Ingredients	Treatment[1]	
	Control	FR-2:1
Salt	1.50	1.50
Water	46.44	11.56
Vegetable oil, canola	19.98	19.89
MCC powder	0.00	7.19
Acid curd	0.00	49.77
Rennet casein	21.05	0.00
Milk permeate powder	8.73	10.09
Citric acid	0.90	0.00
Kasal salt (sodium aluminum phosphate)	1.40	0.00
Total	100.0	100.0

[1]Treatment: Control= IMC made with rennet casein and emulsifying salts (ES); FR-2:1= IMC made with no ES using a 2:1 ratio of protein from acid curd to MCC

Table 2. Composition (% by weight) of MCC powder, milk permeate (used to produce liquid MCC), and prepared MCC (used in acid curd manufacture)

Ingredient	Composition (%)[1]			
	TS	TPr	NCN	Lactose
MCC powder	94.24	83.88	-	2.48
Milk permeate powder	94.34	3.35	-	62.78
Recombined MCC	13.02	9.18	1.01	1.96

[1]Composition: TS= total solids; TPr= total protein= total nitrogen × 6.38; NCN=non casein nitrogen

Table 3. Composition (% by weight) of acid curd used in imitation Mozzarella cheese (IMC) formulations

Replicate	Composition (%)[1]							
	TS	TPr	Ash	Lactose	Lactic acid	Ca	P	MAYE
1	27.74	25.12	0.87	0.19	0.60	0.18	0.13	97.20
2	25.30	23.41	0.93	0.22	0.68	0.20	0.15	97.65
3	24.39	22.57	0.86	0.21	0.61	0.19	0.12	97.49
Mean	25.81	23.70	0.89	0.21	0.63	0.19	0.14	97.45
SD	1.73	1.30	0.04	0.02	0.05	0.01	0.02	0.23

[1]TS=total solids; TPr= total protein= total nitrogen × 6.38; Ca=calcium; P= phosphate; MAYE= % Moisture adjusted yield efficiency; Actual yield (AY)= (curd wt)/(MCC wt)*100; Theoretical yield (TY)= (MCC TPr – NCN)/0.9/0.19; Moisture adjusted yield (MAY)= (AY)*(curd TS)/100/0.19; Moisture adjusted yield efficiency= (MAY)/(TY)*100

Table 4. Composition (% by weight) of acid whey produced as a byproduct of making acid curd

Replicate	Composition (%)[1]				
	TS	TPr	Ash	Lactose	Lactic acid
1	5.06	1.41	0.91	0.61	1.47
2	4.92	1.39	0.88	0.55	1.41
3	5.04	1.44	0.86	0.60	1.44
Mean	5.01	1.42	0.88	0.58	1.44
SD	0.07	0.03	0.02	0.03	0.03

[1]Composition: TS=total solids; TPr= total protein= total nitrogen × 6.38

Table 5. Mean (n=3) composition (% by weight) of the imitation Mozzarella cheese (IMC) made from acid curd

Treatment[1]	Moisture (%)	pH
Control	48.29	5.72[a]
FR-2:1	48.21	5.37[b]
SEM	0.09	0.08

[1]Treatment: Control= IMC made with rennet casein and emulsifying salts (ES); FR-2:1= IMC made with no ES using a 2:1 ratio of protein from acid curd to MCC

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

Table 6. Mean squares and P-values (in parentheses) for the composition of imitation Mozzarella cheese (IMC) made from acid curd

Factor	df	Moisture	pH
Replicate	2	0.04 (0.69)	0.0003 (0.53)
Treatment[1]	1	0.01 (0.76)	0.19 (<0.05)
Error	2	0.099	0.0004

[1]Treatment: Control= IMC made with rennet casein and emulsifying salts (ES); FR-2:1= IMC made with no ES using a 2:1 ratio of protein from acid curd to MCC

Table 7. Mean (n=3) composition (% by weight) the end apparent viscosity (cP) and hardness (g) of the imitation Mozzarella cheese (IMC) made from acid curd

Treatment[1]	End apparent viscosity (cP)	Hardness (g)
Control	5711.33	300.98
FR-2:1	7499.59	94.92
SEM	451.34	50.68

[1]Treatment: Control= IMC made with rennet casein and emulsifying salts (ES); FR-2:1= IMC made with no ES using a 2:1 ratio of protein from acid curd to MCC

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

Table 8. Mean squares and P-values (in parentheses) for the end apparent viscosity (cP) and hardness (g) of the imitation Mozzarella cheese (IMC) made from acid curd

Factor	df	End apparent viscosity	Hardness
Replicate	2	351155 (0.46)	2746 (0.59)
Treatment[1]	1	4796775 (0.06)	63693 (0.06)
Error	2	306089	3938

[1]Treatment: Control= IMC made with rennet casein and emulsifying salts (ES); FR-2:1= IMC made with no ES using a 2:1 ratio of protein from acid curd to MCC

Table 9. Mean (n=3) composition (% by weight) the melting properties of the imitation Mozzarella cheese (IMC) made from acid curd

Treatment[1]	Melt temperature (°C)	Melt diameter (mm)	Melt area (mm^2)	Stretchability (cm)
Control	55.54	29.41[b]	679.60[b]	12.50
FR-2:1	49.96	31.57[a]	783.10[a]	12.30
SEM	2.16	0.51	24.68	0.26

[1]Treatment: Control= IMC made with rennet casein and emulsifying salts (ES); FR-2:1= IMC made with no ES using a 2:1 ratio of protein from acid curd to MCC

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

Table 10. Mean squares and P-values (in parentheses) for the melting properties of the imitation Mozzarella cheese (IMC) made from acid curd

Factor	df	Melt temperature	Melt diameter	Melt area	Stretchability
Replicate	2	35.04 (0.19)	0.25 (0.44)	619.5 (0.44)	0.60 (0.40)
Treatment[1]	1	46.70 (0.25)	6.98 (<0.05)	16064.9 (<0.05)	0.08 (0.69)
Error	2	11.96	0.20	487.1	0.41

[1]Treatment: Control= IMC made with rennet casein and emulsifying salts (ES); FR-2:1= IMC made with no ES using a 2:1 ratio of protein from acid curd to MCC

Figures

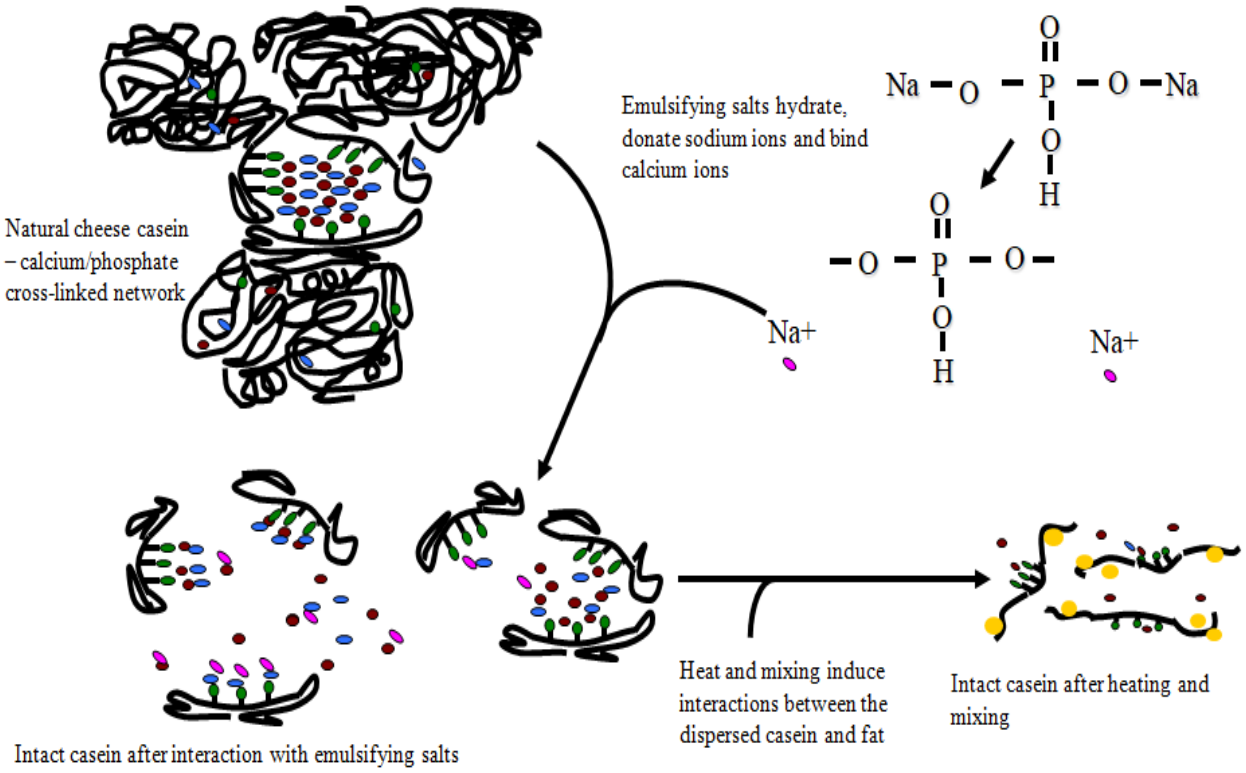

Figure 1. Emulsifying salts (ES) interaction during making process cheese (PC) or imitation Mozzarella cheese (IMC)

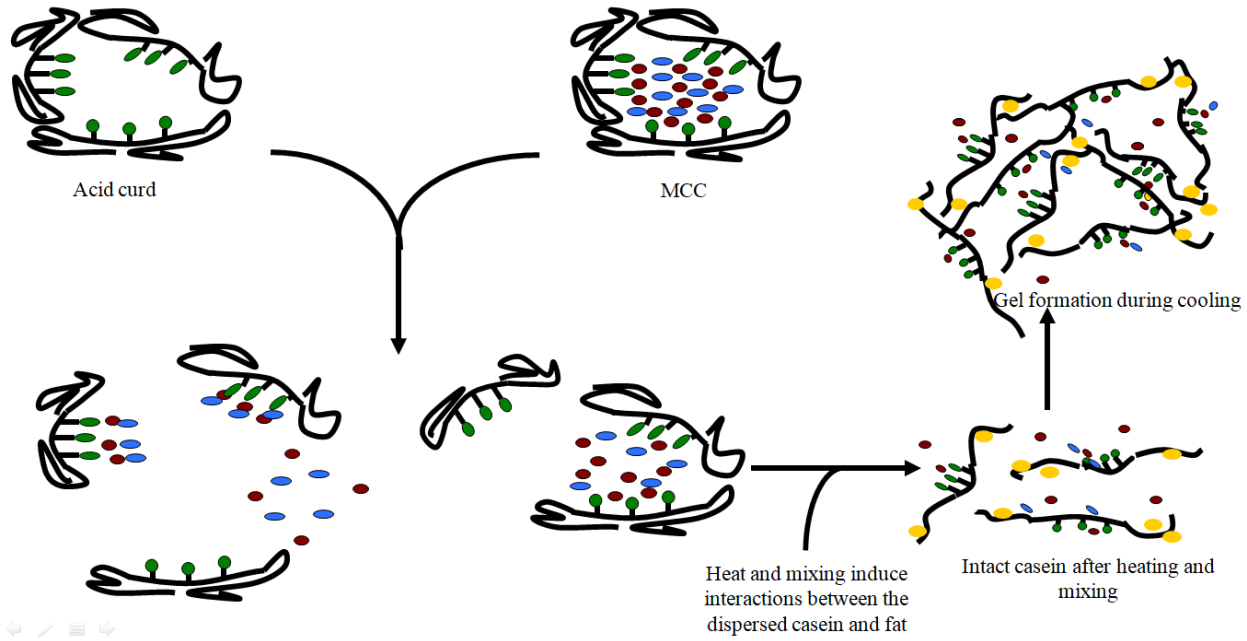

Figure 2. Acid curd and micellar casein concentrate (MCC) interaction in making imitation Mozzarella cheese (IMC)

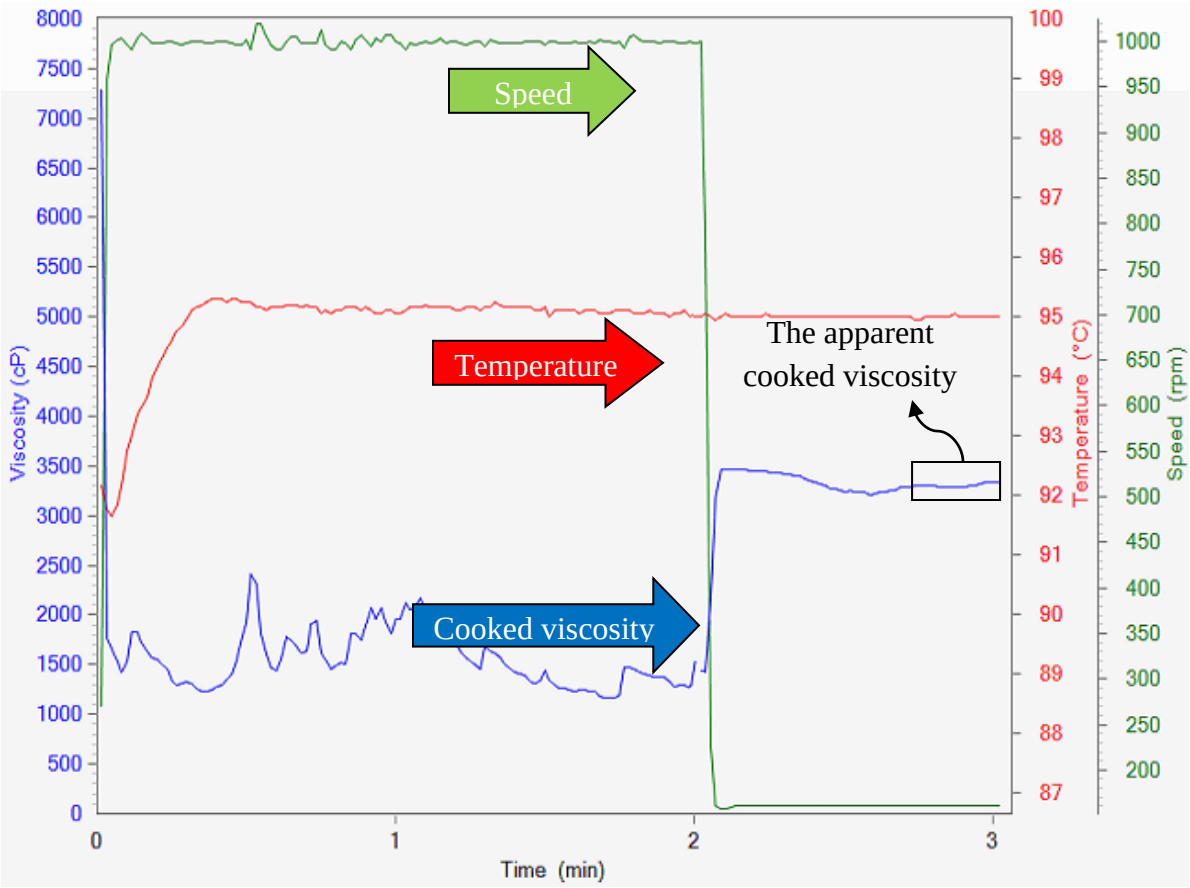

Figure 3. Measuring the apparent end apparent viscosity (cP) of imitation Mozzarella cheese (IMC) using the rapid visco analyzer (RVA)

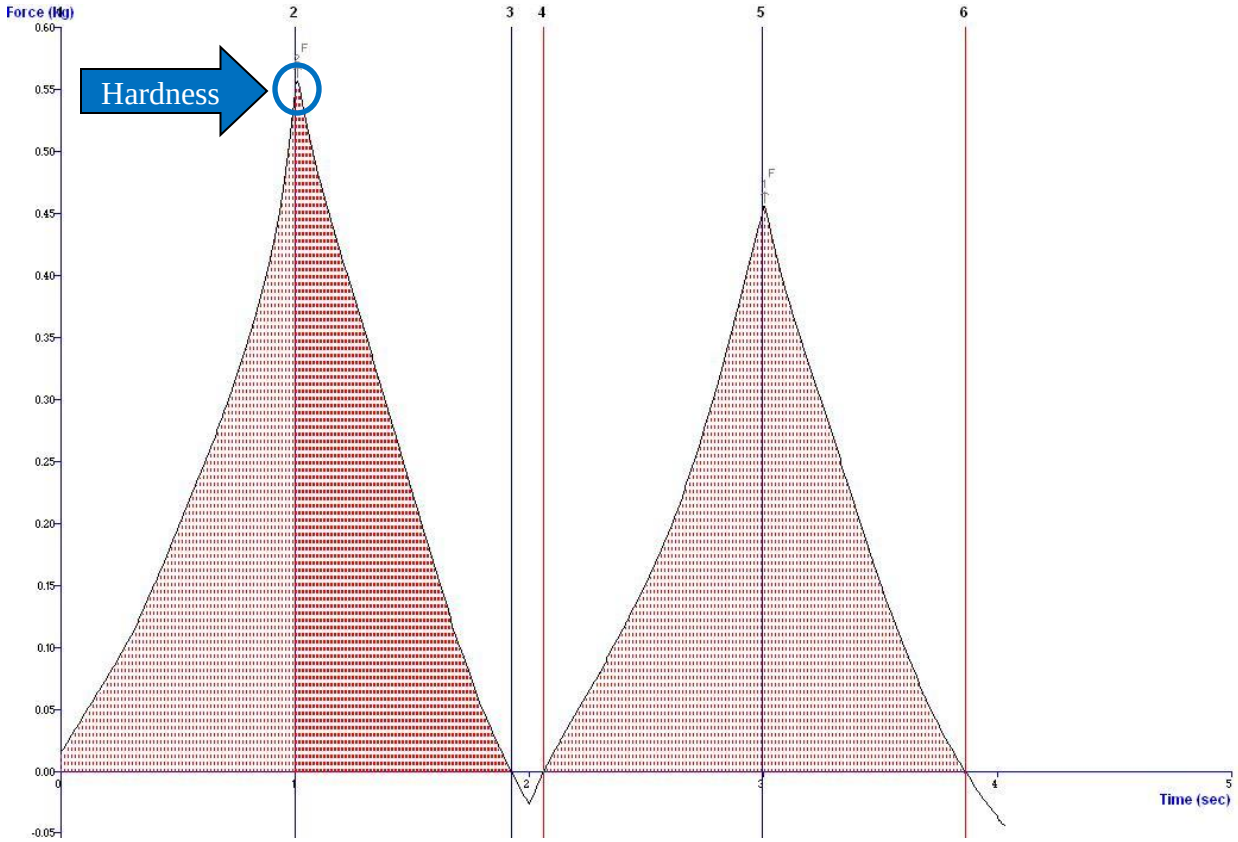

Figure 4. Measuring the hardness (g) of imitation Mozzarella cheese (IMC) using the texture profile analysis (TPA)

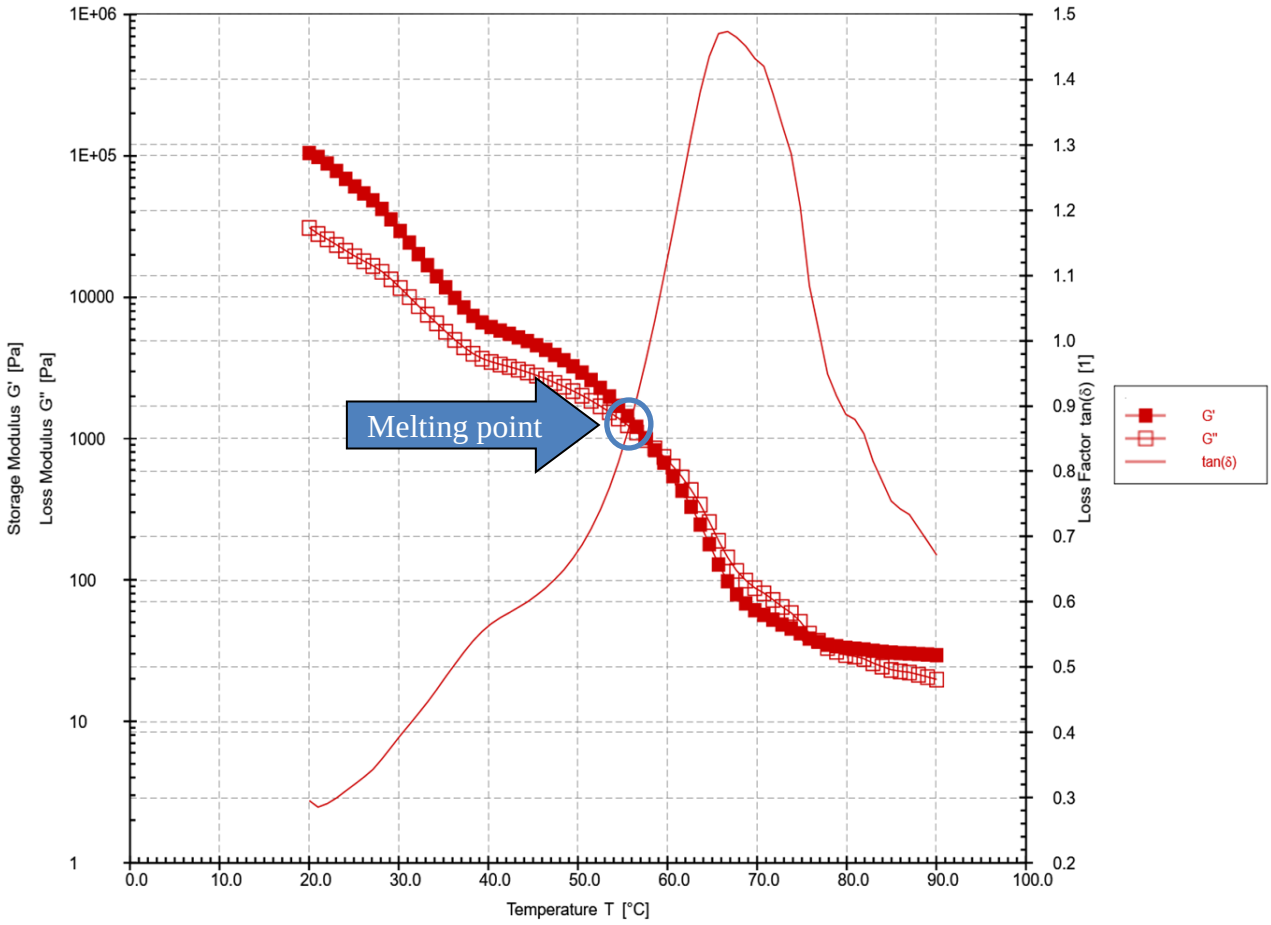

Figure 5. Measuring the melting point of imitation Mozzarella cheese (IMC) using the dynamic stress rheometer (DSR)

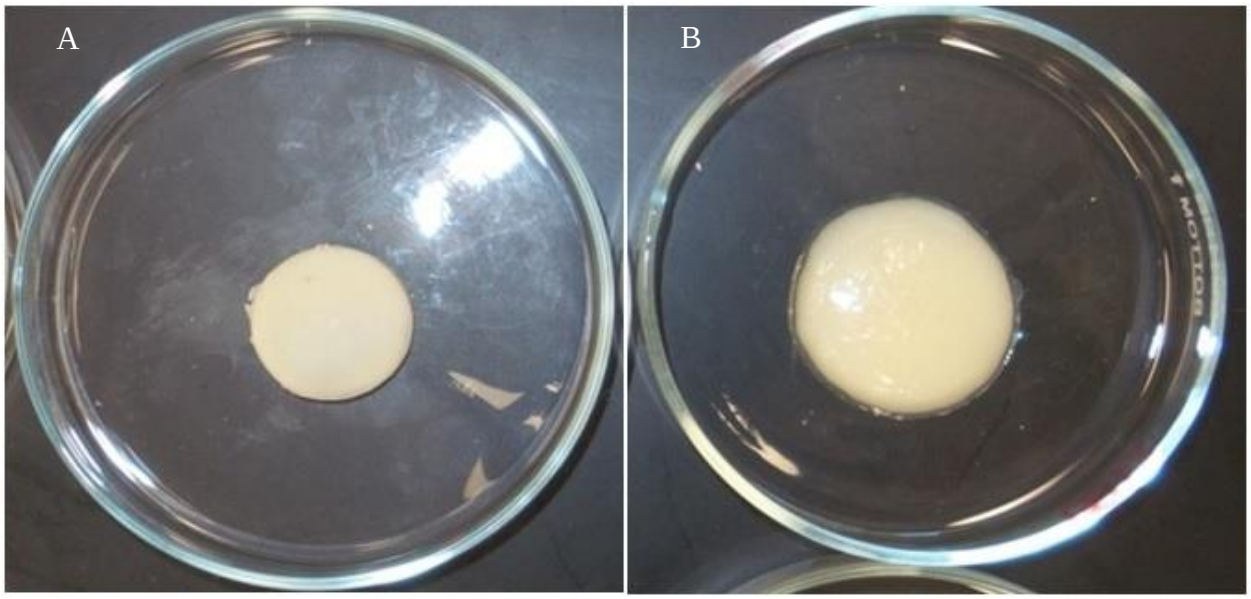

Figure 6. Schreiber melt test to measure the melting diameter of imitation Mozzarella cheese (IMC): (A) Before melting; (B) After melting

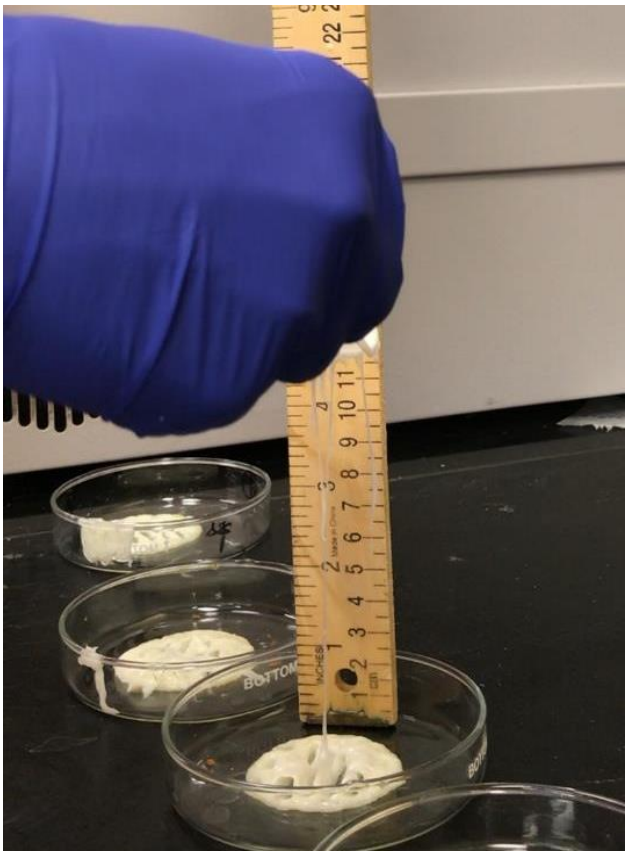

Figure 7. Stretchability test for measuring the stretchability of imitation Mozzarella cheese (IMC)

CHAPTER IV: MANUFACTURE OF A NOVEL CULTURED MICELLAR CASEIN CONCENTRATE INGREDIENT FOR DAIRY FOOD APPLICATIONS

Abstract

Micellar casein concentrate (MCC) is a high protein ingredient that is typically produced using 3 stages of microfiltration (MF) with a 3× concentration factor (CF) and diafiltration (DF). The objectives of this study were to produce MCC using MF membranes and develop a process to produce a novel culture-based acid curd powder ingredient. Skim milk was pasteurized at 76°C for 16 sec and then microfiltered (MF) in 3 MF stages using graded permeability (GP) ceramic membranes. The skim milk was MF in a 3 stages process at 50°C with a 3× CF and DF to get MCC with >9% true protein (TP) and >13% total solids (TS). Part of the MCC was dried to produce MCC powder. The rest of the MCC was used to produce acid curd. The MCC was fortified with milk permeate as a source of lactose and inoculated with 0.5% starter cultures at 43°C to get the pH of 4.6 in 10-14 h. The curd was subsequently cut, drained, washed, and pressed. The curd was then milled and dried at 70-75°C outlet temperature for 3-4 h. The dried curd was then milled to produce acid curd powder. The skim milk, MF permeate, liquid MCC, modified MCC, acid curd, acid whey, MCC powder, and acid curd powder were compositionally analyzed. This trial was repeated 3 times using 3 different batches of skim milk. The skim milk had approximately 0.7, 3.4, 0.3, 0.9, 0.6, 9.0, and 4.4% ash, total protein (TPr), nonprotein nitrogen (NPN), noncasein nitrogen (NCN), serum protein (SP), TS, and lactose, respectively. The fortified MCC had 1.4% ash, 10.9% TPr, 0.2% NPN, 1.4% NCN, 1.2% SP, 17.4% TS, and 4.2% lactose. The curd prior drying showed approximately 1.0, 36.4, 0.7, 1.3, 0.6, 40.4, and 0.80% for ash, TPr, NPN, NCN, SP, TS,

and lactose, respectively. The acid curd powder had approximately 2.0% ash, 86.9% TPr, 2.2% NPN, 2.3% NCN, 0.08% SP, 96.4% TS, and 1.4% lactose. The acid curd prior drying and acid curd powder were successfully produced from MCC. Future studies will be performed to utilize the acid curd and MCC powders at different ratios in process cheese products formulations and examine the functional properties of the cheese.

Keywords: Microfiltration, Ceramic membranes; Micellar casein concentrate; Culture based acid curd; Acid curd powder; Process cheese products; Compositional analyses

Introduction

Microfiltration (MF) is a membrane process that is utilized to fractionate casein (CN) and serum protein (SP) from skim milk using a 0.1 μm semipermeable membrane. The skim milk is force-driven through the membrane to separate CN (retentate side) and SP (permeate side) based on their sizes (0.1-0.4 μm vs 0.003-0.01 μm, respectively). The product on the retentate side is called micellar casein concentrate (MCC), which is mostly native casein. MCC is a high protein ingredient that is typically manufactured in 3 MF stages using a 3× concentration factor (CF) with diafiltration (DF). Details on various membranes used for the production of MCC and their advantages and disadvantages have been extensively covered in chapter 1 and in our previous publications (Hammam et al., 2021, 2022a).

The MCC has promising applications in various dairy and nondairy products due to its unique physicochemical and functional characteristics (e.g., foaming, emulsifying, wetting, dispersibility, heat stability, bland flavor, and water-binding ability). The high casein content in MCC makes it heat stable and thereby it can be used in beverages that

require sterilization (Beliciu et al., 2012; Sauer and Moraru, 2012). The nondairy applications for MCC are pasta, confectionery, meat products, special dietary preparations, textured products, convenience foods, toothpaste, cosmetics, and wound treating preparations (Salunke, 2013; Hammam et al., 2021). The dairy applications for MCC include Cheddar cheese (Amelia et al., 2013; Li et al., 2020; Xia et al., 2021), Greek-style yogurt (Bong and Moraru, 2014), imitation Mozzarella cheese (IMC) (Salunke, 2013; Hammam and Metzger, 2020a), recombined MCC (Lu et al., 2016, 2017), process cheese (PC) and process cheese products (PCP) (Hammam et al., 2019; Hammam and Metzger, 2019; Metzger and Hammam, 2020; Hammam et al., 2022b), and acid curd (Hammam and Metzger, 2019, 2020b; Metzger and Hammam, 2020). We recently developed a process to produce acid curd (basically Cottage cheese curd) from MCC using direct acidification (lactic acid) (Hammam and Metzger, 2019; Metzger and Hammam, 2020) and starter cultures (Hammam and Metzger, 2020b).

Acid curd is a protein concentrate, which can be obtained by precipitating the casein at pH 4.6 (isoelectric point) using starter cultures or direct acids without the use of rennet. The colloidal calcium phosphate in the micelles is dissolved at the isoelectric point, which leads to a low mineral (calcium) content in the acid curd. Acid curd can be produced from skim milk in a process similar to Cottage cheese manufacture (Klei et al., 1998). There is a possibility of using MCC instead of skim milk to produce acid curd. Making acid curd from MCC has advantages as compared to skim milk, since manufacturing MCC using microfiltration (MF) results in milk-derived whey protein as a byproduct which can be utilized in many applications, particularly making whey protein isolate (WPI). Furthermore, the yield of acid curd produced from MCC can be higher. In

contrast, acid curd produced from skim milk results in acid whey as a byproduct, which is more difficult to utilize.

The typical composition of MCC (3-stages using 3× CF with DF) is >9% true protein (TP) and >13% total solids (TS) (Zulewska et al., 2009; Hammam et al., 2021). This MCC could be used immediately in making acid curd or diluted to lower protein levels before making acid curd if required. In our previous studies, we tried to produce acid curd from MCC with different protein content (3, 6, and 9% protein). We found that MCC with 9% protein is the optimum product to produce acid curd (Hammam and Metzger, 2019, 2020b; Metzger and Hammam, 2020). No studies have utilized MCC in making acid curd as well as producing a novel culture-based acid curd powder ingredient. Therefore, the objectives of this study were to produce MCC using MF membranes and develop a process to produce a novel culture-based acid curd powder ingredient.

Materials and Methods

Experimental Design

Manufacture of MCC was completed in approximately 10 h in one day at Davis Dairy Plant at South Dakota State University (Brooking, SD). The experiment was repeated 3 times with different lots of skim milk. Part of the final MCC was dried using a spray dryer to produce MCC powder, while the rest of the MCC was used in making culture based acid curd.

Preparation of Skim milk

The MCC was manufactured as described in our previous studies with some modifications (Hammam and Metzger, 2018, 2019; Hammam, 2019a; Metzger and Hammam, 2020). Approximately 750 kg of whole bovine milk was separated (Model MSE 140-48-177 AirTight centrifuge; GEA Co., Oelde, Germany) at 4°C at the South Dakota State University Davis Dairy Plant and then pasteurized in a plate heat exchanger (model PR02-SH, AGC Engineering, Bristow, VA) at 76°C for 16 sec. The pasteurized skim milk was then kept at ≤ 4°C until MF was conducted.

Microfiltration Operation

To fractionate skim milk into casein (CN) and serum protein (SP) to produce MCC, a pilot-scale ceramic graded permeability (GP) MF system (TIA, Rond-point des, Portes de Provence, Rue Robert Schumann 84500, Bollène, France) was utilized. The GP MF system was equipped with 7 ceramic tubes (19 channels with a diameter of 3.3-mm) mounted in the system vertically. The ceramic GP MF membranes had a 0.1-µm pore size, 1.68 m^2 surface areas, and a 1.02 m membrane length. The GP MF system was also equipped with a feed pump and a retentate recirculation pump (TIA, Bollène, France). The MF of skim milk (approximately 670 kg) was performed in 3 stages to produce MCC.

Manufacture of MCC

First Stage: The GP MF system was started with soft water at 50°C using 3× concentration factor (CF) (1 kg retentate: 2 kg of permeate) in a feed and bleed mode (one way pass) with 400 kPa retentate pressure inlet (Rpi), 200 kPa retentate pressure

outlet (Rpo), and 200 kPa permeate pressure outlet (Ppo). The skim milk (~10.3% Brix) was heated to 50°C with a heat plate exchanger (SABCO Plate-pro Sanitary Chiller; NP925-41) before processing. When the processing conditions were stable while running with water, the system was transitioned to milk. The skim milk was MF with the GP MF system at a constant flux (71.42 L/m^2 per h) using a 3× CF in a feed and bleed mode at 50°C (Figure 1). The water at the beginning of the process was flushed out with skim milk by collecting about 36.0 kg of permeate and 17.0 kg of retentate. The permeate flow rate was set at 120 L/h (flux of 71.42 L/m^2 per h) and the retentate flow rate was 60 L/H to produce a 3× retentate. After this startup, retentate and permeate were collected and weighed continuously. During MF of skim milk, Rpi, Rpo, and Ppo were targeted to maintain 400, 200, and 200 kPa, respectively. The CF was calculated every 15 min by collecting permeate and retentate samples. The composition of retentate and permeate during MF was monitored using an infrared spectrophotometer (FT-IR spectroscopy, Model Dairyspec FT, Bentley Instrument INC, Chaska, MN 55318). A composite permeate sample was collected during the process. The collected retentate was kept in tanks during the MF process. The processing time of the first stage was approximately 4 h.

Second Stage: The retentate (~17.0% Brix) from the first stage was diluted with soft water (approximately 212.0 kg of retentate was mixed with 424.0 kg of water) to obtain a DF of 3× to get back the original volume of skim milk. After mixing, the diluted retentate (~5.5% Brix) was heated to 50°C and processed with the GP MF system using a 3× CF, as described in the first stage. The water at the beginning of the process was flushed out of the system with the diluted retentate by collecting about 36.0 kg of permeate and 18.0

kg of retentate. The Rpi, Rpo, and Ppo were set at 400, 200, and 200 kPa, respectively. The permeate flow rate was 120 L/h (flux of 71.42 L/m^2 per h) and the retentate flow rate was 60 L/h. Permeate and retentate were weighed continuously, as described in the first stage. A composite permeate sample was collected during the process. The retentate was collected in sanitized cans. The processing time of the second stage was approximately 3.5 h.

Third Stage: Approximately 175.0 kg of the retentate (~13.0% Brix) was MF in a recirculation mode. The retentate of the second stage was placed in the tank of MF unit and then proceed to the third stage using a 3× CF at 50°C. The following conditions were applied: Rpi, Rpo, and Ppo were 400, 200, and 200 kPa, respectively, while the permeate flow rate was 120 L/h (flux of 71.42 L/m^2 per h) and the retentate flow rate was 60 L/h. The retentate was recirculated while permeate was collected until the TS reached approximately 14-15% (CEM Smart System5 SL7199) or ~16.0-17.0% Brix. Increasing the solids content of MCC during MF led to decreasing the Ppo. The decrease of Ppo is related to the concentration polarization and membrane fouling that accumulated on the membrane during recirculation. The final MCC resulting from the third stage (approximately 125.0 kg) was collected. A composite sample of the permeate was sampled for compositional analysis. The processing time for the third stage was around 1 h. The MCC was then pasteurized at 63°C/30 min. Approximately 50.0 kg of the liquid MCC was dried using a spray dryer to produce MCC powder, while the rest of the liquid MCC (75.0 kg) was utilized in making acid curd. This trial was replicated 3 times using three separate lots of raw milk.

Cleaning the Membrane

After processing, the GP MF system was flushed with soft water to remove all retentate residues from the system. The initial flux was measured with approximately 60 kg of soft water at 27°C. During the flux measurement, the retentate valves were closed and the permeate valves were completely opened with the feed pump running. Subsequently, 30 kg of soft water was added to the system and heated to 74°C, then 900 ml of Ultrasil 110 Alkaline cleaner (Ecolab Inc. 370 Wabasha Street N., St Paul, MN) and 200 ml of XY 12 (Ecolab Inc. 370 Wabasha Street N., St Paul, MN) were added to get pH 11 (Accumet, Fisher Scientific, USA). This solution was recirculated for 30 min at a 350 L/h permeate flow rate (flux of 208 L/m^2 per h). After cleaning the MF system with the alkaline solution, the membrane was cooled to 50°C (less than 10°C per min). The alkaline solution was flushed out of the MF system with soft water until the pH of outlet water ranged from 8.3 to 8.5. The flux was measured again, as described previously. The system was cleaned with an acid solution (Ultrasil 78 acid cleaner) by adding 30 kg of soft water and heated to 52°C; subsequently, 400 ml of Ultrasil 78 (Ecolab Inc. 370 Wabasha Street N., St Paul, MN) was added to get a pH of 2. The recirculation of the acid solution was applied for 20 min at a flux of 208 L/m^2 per h. Subsequently, the machine was turned off, and the acid was retained inside the system. Before using the system again, the acid solution was flushed out with soft water until the pH reached 8.3-8.5. The flux was measured again after flushing the acid solution. Within each MF stage, membrane was flushed using water, and flux was measured. The membrane was cleaned within stages using the abovementioned procedures when the flux did not show the original flux.

MCC Drying

A pilot scale spray dryer at Davis Dairy Plant at South Dakota State University was utilized to dry the MCC. The nozzle used to dry the MCC had a core size of 21 and an orifice size of 66. The inlet pressure was set at 2250.0 psi using hi-pressure pump speed and it was adjusted manually through the fan (30.0%). The supply fan and exhaust fan were set at 80.0 and 90.0%, respectively. The inlet temperature was 175°C, while the outlet temperature was 82°C. The dryer was connected to a fluid bed (Dahmes Stainless INC: DSI, Model no 10011-11, New London, MN) that was equipped with sieves. The fluid bed was attached to three fans (hot=71°C and 40.0% speed; warm=50°C and 50.0% speed; cool=21°C and 40.0% speed). Approximately 50.0 kg of the liquid MCC was heated to 50°C in a water bath before feeding into the dryer. The powder was collected in an airtight container and stored at room temperature until further analysis. The weight of the final powder was about 7.0 kg.

Manufacture of acid curd

The rest of the liquid MCC (75.0 kg) was fortified with lactose by adding 4.7% milk permeate powder (IdaPro milk permeate, Idaho milk products, Jerome, Idaho 83338) to add approximately extra 3% lactose to assure there was enough lactose for fermentation using starter cultures. The milk permeate was hydrated in the MCC and then the mix was pasteurized at 65°C for 30 min and cooled to 43°C to be ready for fermentation. The fortified MCC was then inoculated with 0.5% starter cultures (i455, Batch No 3489654, Chr. Hansen, Hørsholm, Denmark) at 43°C to get the pH of 4.6 in 12-14 h. A small amount (100 mL) of lactic acid (88% Lactic Acid FCC, product code:

175820, lot number:1501277028, Faries Parkway, Decatur, IL 62526) was added before cutting if the pH did not drop to the desired pH (4.6). Once the pH of 4.6 was reached, the curd was completely cut and left to heal for 30 min with gentle mixing, and then it was heated gradually to 50°C in 1 h. The whey was subsequently drained from the curd and sampled. The water at 50°C was used to wash the curd for 5 min at a ratio of 1:1 and then the water was drained. When the curd was washed with water, the pH of the curd was increased slightly due to the buffering capacity. As a result, approximately 50 mL of lactic acid was added to the water (~100 lb) before washing the curd to keep the pH at 4.6. The washing step was repeated 3 times. The water was drained, and the curd was pressed for 6 h at 80 psi. The pressed curd was kept at ≤4°C until the next day.

Acid Curd Drying

After pressing, the curd was milled into small pieces using a grinder (Humboldt Model 5DPJ3, split phase motor from Dayton, Lake Forest, IL 60045) equipped with a 4.5 mm diameter stainless plate.

A pilot scale spray dryer at Davis Dairy Plant at South Dakota State University was utilized to dry the acid curd. The dryer was not turned on only the exhaust fan and three fans connected to the fluid bed were running manually. The exhaust fan was set at 20%. The three fans attached to the fluid bed were set as follows: hot air fan=71.0°C and 40.0% speed; warm air fan=71.0°C and 50.0% speed; cool air fan=21.0°C and 15.0% speed.

The curd was then placed on the sieve of the fluid bed, which is part of the spray dryer. The curd was evenly distributed on the surface of the fluid bed. The temperature of

fans was elevated or decreased by controlling the speed of fans manually. The curd was moved and mixed every 30 min upside down. The curd was left in the fluid bed for around 4 h to assure the curd was completely dried. The dried curd was collected in an airtight container and stored at room temperature until grinding. The weight of the dried curd was approximately 14.0 kg. The dried curd was then turned into powder using a high speed multifunctional grinder (Item No HC-700Y). The powder was then separated using a metal sieve (Item No 8RTG5, Sieve #70, Grainger, Minooka, IL 60447) with a 212 µm pore size to have the typical particle size of powders. Powder particles with over 212 µm were collected and milled again in the Retsch Ultracentrifugal mill (Brinkmann Retsch ZM1) equipped with a 0.2 mm stainless steel ring sieve to have a fine powder. The acid curd powder was collected in an airtight container and stored at room temperature until further analysis.

Chemical Analyses

Skim milk, liquid MCC, MF permeate (composite from all three stages), MCC powder, fortified MCC (used in making curd), acid curd prior drying, acid whey, and acid curd powder were analyzed for total solids (TS) (AOAC, 2000; method 990.20; 33.2.44), total protein (TPr=total nitrogen× 6.38) (AOAC, 2000; method 991.20; 33.2.11), noncasein nitrogen (NCN) (AOAC, 2000; method 998.05; 33.2.64; Zhang and Metzger, 2011), nonprotein nitrogen (NPN) (AOAC, 2000; method 991.21; 33.2.12), lactose content and lactic acid (Amamcharla and Metzger, 2011), ash (AOAC, 2000; method 945.46; 33.2.10), and mineral profile (AOAC, 2000; method 985.01). The skim milk, MCC powder, and acid curd powder were also analyzed for fat using Mojonnier method (AOAC, 2000; method 989.05; 33.2.26).

Statistical Analysis

Means and standard deviations (SD) within the three replicates were calculated using formulations of Microsoft Office Excel (Microsoft Office Excel 2007, Microsoft Corporation).

Results and Discussion

MF Filtration to Produce MCC

Composition of skim milk. The mean composition of skim milk used in this study is shown in Table 1. The skim milk showed an average of 3.37, 0.29, 0.87, 0.58, 9.01, 4.41, 0.20, and 0.21% for TPr, NPN, NCN, SP, TS, lactose, lactic acid, and fat, respectively. The TP in skim milk was 3.08% with 2.50% CN. The CN as a percentage of TP was approximately 81.08%, while the ash content in skim milk was 0.72% including 0.12% calcium (Ca) and 0.09% phosphate (P). No noticeable differences were detected in the composition of skim milk within the three replicates. The mean composition of skim milk did not differ from the skim milk reported in other MF studies (Beckman et al., 2010; Hurt and Barbano, 2010; Adams and Barbano, 2013; Hammam, 2019a). The high pasteurization temperature can result in denaturation of β-LG on κ-CN through disulfide bonding (Singh, 1995), which can increase the CN%TP and thereby decrease the SP removal during MF (Hurt and Barbano, 2010). The CN%TP in this study was typically similar to other studies (Hurt and Barbano, 2010; Adams and Barbano, 2013; Beckman and Barbano, 2013) since the pasteurization temperature did not exceed the recommended value. The extensive pasteurization can be noticed in the NCN values, which is increasing with higher temperature since SP is denaturated on CN. It has been

found that the Ca and P in milk were around 0.11 and 0.09%, respectively (Wong et al., 1976), and those values are similar to the Ca and P values reported in Table 1.

Composition of permeate. The composite permeate composition collected from the three stages of MF is shown in Table 2. The composite permeate had 0.44, 0.16, 0.43, 0.26, 4.01, 3.11, and 0.17% for TPr, NPN, NCN, SP, TS, lactose, and lactic acid, respectively. There was 0.01% CN in the permeate while TP was 0.28%, therefore the CN%TP (percent of casein to true protein) was 4.28%. The ash content in the permeate was 0.32% with 0.01% Ca and 0.02% P. The composition of permeate was similar within replicates. We have produced MCC previously using the same procedures to have >25% TS and >20% protein (Hammam, 2019a). The composite permeate of the first and second stages in that study can be utilized for comparison reason to this study since we did not go further to produce highly concentrated MCC. No differences were detected in the composition of permeate of our previous study relative to the current study. It showed 4.01% TS, 0.43% TPr, 0.14% NPN, 0.29% SP, and 0.32% ash, which are similar to the current study. The composition of permeate in this study was also similar to the permeate reported in other studies (Hurt and Barbano, 2010; Hurt et al., 2010; Zulewska and Barbano, 2014; Tremblay-Marchand et al., 2016). The slight differences in the composition of permeate within studies can be due to the differences in membrane type, DF steps, as well as the composition of skim milk.

Composition of MCC. The composition of MCC made from MF of skim milk is illustrated in Table 3. The average composition of MCC made from the three replicates was 11.15% TPr, 0.16% NPN, 1.26% NCN, 1.10% SP, 14.06% TS, 1.32% lactose, and 0.18% lactic acid. The ash content was 1.18% with 0.34% Ca and 0.20% P. The CN%TP

was approximately 90% with an average of 9.89% CN and 10.99% TP. We previously produced MCC with similar composition using the GP MF ceramic membranes following the same procedures (Hammam and Metzger, 2019). We found that the TS, TPr, and ash were 13.79, 9.54, and 1.03%, respectively. Similar results were also found in Tremblay-Marchand's study (Tremblay-Marchand et al., 2016). That study reported that the retentate of 3× MF GP membranes had 89.59% CN%TP with 0.92% SP and 15.31% TS. However, the CN%TP of MCC produced in other studies was high relative to our study due to the extra DF stage that those studies utilized between the second and third stages (Hurt and Barbano, 2010; Hurt et al., 2010; Zulewska and Barbano, 2014). This led to increasing the CN%TP in MCC due to the high SP amount that was removed during MF.

Culture Based Acid Curd

Composition of fortified MCC. The composition of MCC used in making acid curd is exemplified in Table 4. The same MCC product produced from MF was used in making acid curd after fortification with milk permeate powder. The TPr, NPN, NCN, SP, TS, lactose, and lactic acid in fortified MCC were 10.95, 0.25, 1.45, 1.20, 17.37, 4.54, and 0.24%, respectively. The ash content was 1.39% including 0.35% Ca and 0.23% P. The CN%TP was 88.78% with 9.50 and 10.70% CN and TP, respectively. No differences were detected between the MCC solutions before and after fortification with milk permeate powder except for TS and lactose. It was expected that lactose will be elevated by >3% due to the addition of milk permeate powder as a source of lactose to assure there is enough lactose for fermentation using starter cultures. Additionally, the TS increased by around 3.3% for the same reason.

Composition of acid whey. The composition of acid whey produced as a byproduct of making acid curd from MCC is presented in Table 5. The whey showed approximately 3.82, 0.37, 1.52, 1.14, 10.61, 2.49, and 2.30% for TPr, NPN, NCN, SP, TS, lactose, and lactic acid, respectively. The whey showed around 1.37, 0.33, and 0.16% ash, Ca, and P. The TPr presented in whey includes 3.44% TP and 2.30% CN to show 65.55% CN%TP. We previously produced acid curd from MCC (13.02% TS, 1.96% lactose, 1.01% NCN, and 9.18% TPr) using starter cultures at a lab scale (Hammam and Metzger, 2020b). The composition of acid whey produced as a byproduct of making acid curd at a lab scale did not differ much from that produced on the plant scale. The TPr%TS in acid whey produced on a lab scale was 28.1%, which is close to the value in this study (36.0%). Approximately 1.4-1.8% lactose was required to reach a pH of 4.6 in the acid curd/whey. As a result, around 2.5% lactose from a total of 4.23% was left in the whey. Using 2.0% lactose MCC left around 0.6% of lactose in the lab scale acid whey (Hammam and Metzger, 2020b). The loss of components in whey especially protein depends on the composition of initial material as well as handling the curd in the cheese vat and this loss gets higher with increasing the scale. The composition of acid whey produced as a byproduct of making acid curd using MCC was different from the acid whey produced from milk in previous studies (Peri and Dunkley, 1971; Durham and Hourigan, 2007; Saffari and Langrish, 2014; Chandrapala et al., 2015; Lievore et al., 2015; Chen et al., 2016; Hammam et al., 2017; Hammam, 2019b) due to the differences in the composition of starting material (MCC), which was expected. As a result, TS, ash, TPr, lactose, and lactic acid contents in acid whey can be changed based on the composition of MCC. Those studies found that the TS of acid whey can range from 5.0-7.0%, while TPr and

ash can be 0.5-1.0 and 0.5-1.0%, respectively. The TS, TPr, and ash in acid whey produced from MCC were high since they were high in MCC compared to acid whey produced from making curd from milk.

Composition of soft acid curd. The composition of soft acid curd produced from MCC is shown in Table 6. The TPr, NPN, NCN, SP, TS, lactose, and lactic acid content in produced acid curd was 36.45, 0.74, 1.33, 0.58, 40.37, 0.80, and 1.34%, respectively. The ash content was 0.96% including 0.11% Ca and 0.18% P. The CN in acid curd was 35.13%, while TP was 35.71% to present 98.36% CN%TP. The average pH of acid curd was targeted to have 4.60. The composition of acid curd depends on the composition of initial materials, final pH, and process conditions (e.g., cooking temperature, washing curds, pressing) (Wong et al., 1976). The step of washing the curd has a significant role in increasing the ratio of CN to TP and decreasing the ash, Ca, P, lactose, and lactic acid content in the final acid curd. Since the lactose is converted to lactic acid using starter cultures as a result of fermentation, lactose decreases, and lactic acid increases as the pH reached 4.6. It is also expected that the ash content decreases when the MCC is turned into acid curd (McDowall and Dolby, 1935; Czulak et al., 1969; Wong et al., 1976; Hill et al., 1985; Lucey and Fox, 1993). The Ca is converted from insoluble (colloidal form) to soluble form and released in the whey as the pH decreases (Davies and White, 1960; Wong et al., 1976; Dalgleish and Law, 1989; Guinee et al., 1993). As a result, the Ca and P are dropped as the pH decreases with having a low ratio of Ca to P (Kindstedt and Kosikowski, 1988; Lucey and Fox, 1993). Wong and others have reported that the Ca in Cottage curd is reduced by 68.2% (washed 3 times) relative to the Ca content in skim milk (Wong et al., 1976), which is similar to our results (68.6%). Another review

reported that the Ca and P in Cottage cheese were 0.08 and 0.16%, respectively (Lucey and Fox, 1993).

MCC and Acid Curd Powders

Composition of MCC powder. The liquid MCC was spray dried to produce MCC powder. Table 7 presents the composition of MCC powder. The average composition of MCC powder showed 75.77% TPr, 2.07% NPN, 9.66% NCN, 7.60% SP, 97.84% TS, 8.69% lactose, 1.73% lactic acid, and 2.98% fat. The ash content was 8.03% with 2.42% Ca and 1.29% P. The CN%TP was approximately 89.70% with an average of 66.10% CN and 73.70% TP. The composition of MCC powder is relatively similar to the liquid MCC on a dry basis. The composition of MCC powder is similar and falls in the range of MCC reported in other studies (Nasser et al., 2017, 2018; Hammam et al., 2021). The slight differences can be related to the composition of initial material, MF process conditions (e.g., temperatures, pressures, CF, DF), and types of membrane (e.g., ceramic membranes, spiral-wound membranes) (Hammam et al., 2021).

Composition of acid curd powder. The composition of acid curd powder is exemplified in Table 8. The acid curd powder showed 86.88, 2.22, 2.29, 0.08, 96.42, 1.41, 2.55, and 2.55% for TPr, NPN, NCN, SP, TS, lactose, lactic acid, and fat. The ash content in acid curd was 2.05% with 0.17% Ca and 0.29% P. The majority of TP in acid curd was CN. The acid curd had 84.58% CN and 84.66% TP to present approximately 99.90% CN%TP. The composition of acid curd powder was relative to the composition of soft acid curd on a dry basis and similar to this range reported in previous studies (Huffman and James Harper, 1999; Hammam et al., 2021). The acid curd made from milk can have

less ash content as well as Ca and P due to the low content of those components in milk as compared to MCC, which has higher ash, Ca, and P contents.

Conclusions

The GP MF membranes can be effectively utilized to produce MCC with >9% TP and >13% TS using 3 stages MF with a 3× CF and DF. Additionally, culture based acid curd was produced from the liquid MCC. A unique method was developed to produce acid curd powder by drying and grinding the curd. The novel ingredients (acid curd and MCC powders) produced in this study can be mixed in a specific ratio and market to be ready for making different types of cheeses including PCP and IMC. The MCC and acid curd powders will be utilized in manufacture of clean label PCP with no emulsifying salts and the functional properties of that cheese will be examined.

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Tables

Table 1. Mean (n=3) composition (% by weight) of pasteurized skim milk utilized in manufacture of micellar casein concentrate (MCC)

Replicate	Composition (%)[1]													
	Ash	TPr	NPN	NCN	SP	TS	Lactose	Lactic acid	Fat	Ca	P	TP	CN	CN%TP
1	0.74	3.41	0.31	0.84	0.53	8.92	4.33	0.21	0.22	0.13	0.10	3.11	2.58	82.92
2	0.70	3.40	0.26	0.85	0.59	8.98	4.12	0.21	0.20	0.12	0.09	3.14	2.55	81.22
3	0.72	3.30	0.30	0.92	0.63	9.14	4.78	0.18	0.21	0.12	0.08	3.01	2.38	79.11
Mean	0.72	3.37	0.29	0.87	0.58	9.01	4.41	0.20	0.21	0.12	0.09	3.08	2.50	81.08
SD	0.02	0.06	0.03	0.05	0.05	0.11	0.34	0.02	0.01	0.01	0.01	0.07	0.11	1.91

[1]Composition: TPr= total protein= total nitrogen × 6.38; NPN = nonprotein nitrogen × 6.38; NCN = noncasein nitrogen × 6.38; SP = serum proteins = NCN - NPN; TS= total solids; Ca = calcium; P = phosphate; TP = true protein= TPr – NPN; CN = casein= TPr – NCN; CN%TP = CN as a percentage of TP= (CN/TP) × 100

Table 2. Mean (n=3) composition (% by weight) of permeate produced as a byproduct from microfiltration (MF) of skim milk during manufacture of micellar casein concentrate (MCC)

Replicate	Composition (%)[1]												
	Ash	TPr	NPN	NCN	SP	TS	Lactose	Lactic acid	Ca	P	TP	CN	CN%TP
1	0.33	0.41	0.12	0.40	0.28	3.87	2.82	0.17	0.00	0.00	0.29	0.01	2.71
2	0.30	0.46	0.17	0.45	0.27	4.07	3.01	0.17	0.02	0.03	0.29	0.01	4.87
3	0.32	0.44	0.19	0.43	0.24	4.08	3.51	0.18	0.02	0.03	0.25	0.01	5.27
Mean	0.32	0.44	0.16	0.43	0.26	4.01	3.11	0.17	0.01	0.02	0.28	0.01	4.28
SD	0.02	0.03	0.04	0.02	0.02	0.12	0.36	0.01	0.01	0.02	0.02	0.00	1.38

[1]Composition: TPr= total protein= total nitrogen × 6.38; NPN = nonprotein nitrogen × 6.38; NCN = noncasein nitrogen × 6.38; SP = serum proteins = NCN - NPN; TS= total solids; Ca = calcium; P = phosphate; TP = true protein= TPr – NPN; CN = casein= TPr – NCN; CN%TP = CN as a percentage of TP= (CN/TP) × 100

Table 3. Mean (n=3) composition (% by weight) of micellar casein concentrate (MCC) produced from microfiltration (MF) of skim milk

Replicate	Composition (%)[1]												
	Ash	TPr	NPN	NCN	SP	TS	Lactose	Lactic acid	Ca	P	TP	CN	CN%TP
1	1.18	11.36	0.14	0.98	0.84	14.15	1.18	0.17	0.37	0.25	11.22	10.38	92.51
2	1.12	10.29	0.17	1.32	1.15	13.18	1.32	0.18	0.31	0.19	10.12	8.97	88.62
3	1.25	11.80	0.18	1.49	1.31	14.85	1.47	0.19	0.35	0.17	11.62	10.31	88.73
Mean	1.18	11.15	0.16	1.26	1.10	14.06	1.32	0.18	0.34	0.20	10.99	9.89	89.95
SD	0.07	0.78	0.02	0.26	0.24	0.84	0.15	0.01	0.03	0.04	0.78	0.79	2.21

[1]Composition: TPr= total protein= total nitrogen × 6.38; NPN = nonprotein nitrogen × 6.38; NCN = noncasein nitrogen × 6.38; SP = serum proteins = NCN - NPN; TS= total solids; Ca = calcium; P = phosphate; TP = true protein= TPr – NPN; CN = casein= TPr – NCN); CN%TP = CN as a percentage of TP= (CN/TP) × 100

Table 4. Mean (n=3) composition (% by weight) of micellar casein concentrate (MCC) fortified with milk permeate powder to produce acid curd

Replicate	Composition (%)[1]												
	Ash	TPr	NPN	NCN	SP	TS	Lactose	Lactic acid	Ca	P	TP	CN	CN%TP
1	1.44	10.93	0.22	1.36	1.14	17.68	4.55	0.17	0.37	0.25	10.71	9.57	89.35
2	1.30	10.54	0.23	1.42	1.19	16.51	4.36	0.36	0.32	0.21	10.30	9.11	88.45
3	1.44	11.38	0.30	1.58	1.27	17.91	4.71	0.19	0.37	0.22	11.08	9.81	88.53
Mean	1.39	10.95	0.25	1.45	1.20	17.37	4.54	0.24	0.35	0.23	10.70	9.50	88.78
SD	0.08	0.42	0.05	0.11	0.07	0.75	0.18	0.10	0.03	0.02	0.39	0.35	0.50

[1]Composition: TPr= total protein= total nitrogen × 6.38; NPN = nonprotein nitrogen × 6.38; NCN = noncasein nitrogen × 6.38; SP = serum proteins = NCN – NPN; TS= total solids; Ca = calcium; P = phosphate; TP = true protein= TPr – NPN; CN = casein= TPr – NCN; CN%TP = CN as a percentage of TP= (CN/TP) × 100

Table 5. Mean (n=3) composition (% by weight) of acid whey produced as a byproduct produced from making acid curd

Replicate	Composition (%)[1]												
	Ash	TPr	NPN	NCN	SP	TS	Lactose	Lactic acid	Ca	P	TP	CN	CN%TP
1	1.40	3.00	0.36	1.41	1.04	10.19	2.44	2.10	0.34	0.18	2.63	1.59	60.39
2	1.29	4.68	0.38	1.43	1.05	11.08	2.43	2.07	0.31	0.16	4.30	3.25	75.58
3	1.42	3.78	0.38	1.71	1.34	10.55	2.59	2.72	0.34	0.14	3.40	2.06	60.69
Mean	1.37	3.82	0.37	1.52	1.14	10.61	2.49	2.30	0.33	0.16	3.44	2.30	65.55
SD	0.07	0.84	0.01	0.17	0.17	0.45	0.09	0.37	0.02	0.02	0.84	0.86	8.68

[1]Composition: TPr= total protein= total nitrogen × 6.38; NPN = nonprotein nitrogen × 6.38; NCN = noncasein nitrogen × 6.38; SP = serum proteins = NCN – NPN; TS= total solids; Ca = calcium; P = phosphate; TP = true protein= TPr – NPN; CN = casein= TPr – NCN; CN%TP = CN as a percentage of TP= (CN/TP) × 100

Table 6. Mean (n=3) composition (% by weight) of soft acid curd produced from micellar casein concentrate (MCC)

Replicate	Composition (%)[1]													
	Ash	TPr	NPN	NCN	SP	TS	Lactose	Lactic acid	Ca	P	TP	CN	CN%TP	pH
1	1.11	36.16	0.83	1.48	0.65	39.58	0.66	1.35	0.18	0.28	35.33	34.67	98.15	4.66
2	0.92	36.16	0.82	1.35	0.53	39.88	0.76	1.20	0.07	0.14	35.34	34.81	98.50	4.58
3	0.85	37.05	0.58	1.15	0.57	41.66	0.98	1.47	0.07	0.12	36.46	35.89	98.43	4.55
Mean	0.96	36.45	0.74	1.33	0.58	40.37	0.80	1.34	0.11	0.18	35.71	35.13	98.36	4.60
SD	0.14	0.51	0.14	0.17	0.06	1.13	0.16	0.14	0.06	0.09	0.65	0.67	0.19	0.06

[1]Composition: TPr= total protein= total nitrogen × 6.38; NPN = nonprotein nitrogen × 6.38; NCN = noncasein nitrogen × 6.38; SP = serum proteins = NCN – NPN; TS= total solids; Ca = calcium; P = phosphate; TP = true protein= TPr – NPN; CN = casein= TPr – NCN; CN%TP = CN as a percentage of TP= (CN/TP) × 100

Table 7. Mean (n=3) composition (% by weight) of micellar casein concentrate (MCC) powder produced using the spray dryer

Replicate	Composition (%)[1]													
	Ash	TPr	NPN	NCN	SP	TS	Lactose	Lactic acid	Fat	Ca	P	TP	CN	CN%TP
1	8.19	76.03	2.36	8.62	6.25	99.30	8.56	1.73	3.37	2.61	1.59	73.66	67.41	91.51
2	7.93	75.58	1.92	10.04	8.12	97.26	9.62	1.79	3.06	2.22	1.17	73.67	65.55	88.98
3	7.96	75.70	1.92	10.34	8.42	96.96	7.88	1.68	2.50	2.43	1.12	73.78	65.36	88.58
Mean	8.03	75.77	2.07	9.66	7.60	97.84	8.69	1.73	2.98	2.42	1.29	73.70	66.11	89.69
SD	0.14	0.23	0.26	0.92	1.17	1.27	0.88	0.06	0.44	0.20	0.26	0.07	1.13	1.59

[1]Composition: TPr= total protein= total nitrogen × 6.38; NPN = nonprotein nitrogen × 6.38; NCN = noncasein nitrogen × 6.38; SP = serum proteins = NCN – NPN; TS= total solids; Ca = calcium; P = phosphate; TP = true protein= TPr – NPN; CN = casein= TPr – NCN; CN%TP = CN as a percentage of TP= (CN/TP) × 100

Table 8. Mean (n=3) composition (% by weight) of acid curd powder produced from drying the soft acid curd using the fluid bed

Replicate	Composition (%)[1]													
	Ash	TPr	NPN	NCN	SP	TS	Lactose	Lactic acid	Fat	Ca	P	TP	CN	CN%TP
1	2.65	86.02	1.92	2.03	0.11	96.75	1.67	2.95	3.30	0.20	0.33	84.10	83.99	99.87
2	1.55	87.55	2.59	2.64	0.05	96.23	1.73	2.47	1.88	0.17	0.27	84.96	84.91	99.94
3	1.94	87.07	2.14	2.21	0.07	96.27	0.83	2.22	2.48	0.16	0.27	84.92	84.85	99.92
Mean	2.05	86.88	2.22	2.29	0.08	96.42	1.41	2.55	2.55	0.17	0.29	84.66	84.58	99.91
SD	0.56	0.78	0.34	0.31	0.03	0.29	0.50	0.37	0.71	0.02	0.03	0.48	0.52	0.04

[1]Composition: TPr= total protein= total nitrogen × 6.38; NPN = nonprotein nitrogen × 6.38; NCN = noncasein nitrogen × 6.38; SP = serum proteins = NCN – NPN; TS= total solids; Ca = calcium; P = phosphate; TP = true protein= TPr – NPN; CN = casein= TPr – NCN; CN%TP = CN as a percentage of TP= (CN/TP) × 100

Figures

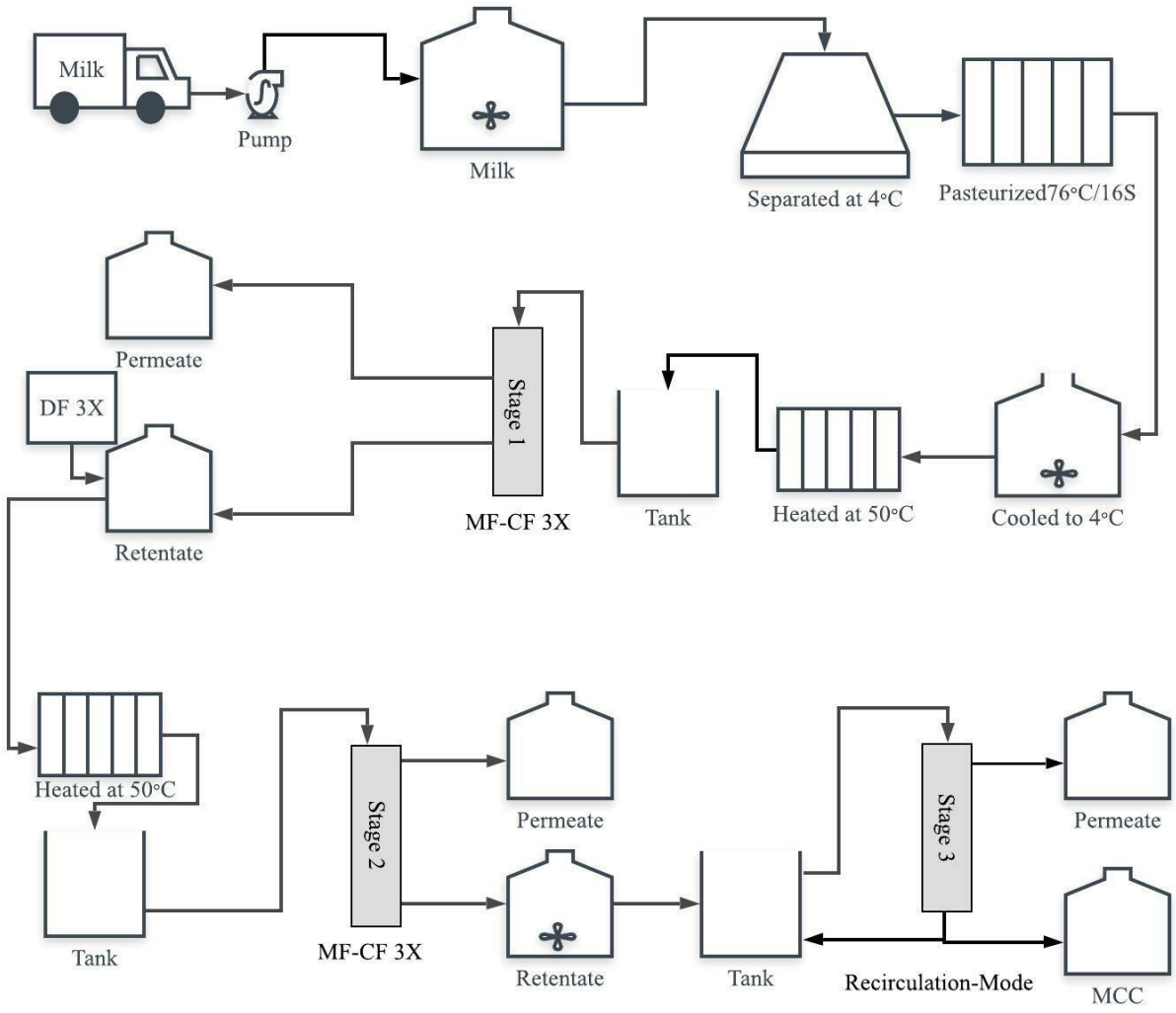

Figure 1. Manufacture of micellar casein concentrate (MCC) using microfiltration (MF) graded permeability (GP) ceramic membranes using 3 MF stages with 3× concentration factor (CF) and diafiltration (DF)

CHAPTER V: MANUFACTURE OF EMULSIFYING SALT FREE PROCESS CHEESE PRODUCTS USING A NOVEL CULTURED MICELLAR CASEIN CONCENTRATE INGREDIENT

Abstract

Process cheese product (PCP) is a dairy food prepared by blending dairy ingredients with nondairy ingredients and then heating the mixture to get a product with an extended shelf-life. Emulsifying salts (ES) are critical for the desired functional characteristics of PCP because of their role in calcium sequestration and pH adjustment. The objective of this study was to produce PCP without ES using different ratios of protein from novel cultured micellar casein concentrate ingredient (cMCC) and micellar casein concentrate (MCC) powders. Three PCP treatments were formulated with 3 different ratios of cMCC: MCC including 2.0:1.0, 1.9:1.1, and 1.8:1.2 on a protein basis. The composition of PCP was targeted to 19.0% protein, 45.0% moisture, 30.0% fat, and 2.4% salt. This trial was repeated 3 times using different batches of cMCC and MCC powders. All PCP were evaluated for their final functional properties. No significant differences ($P>0.05$) were detected in the composition of PCP made with different ratios of cMCC and MCC except for the pH. It was expected to increase slightly with elevating the MCC amount in the PCP formulations. The end apparent viscosity was significantly higher ($P<0.05$) in 2.0:1.0 formulation (4305 cP) compared to 1.9:1.1 (2408 cP) and 1.8:1.2 (2499 cP). The hardness ranged from 407 to 512 g with no significant differences ($P>0.05$) within the formulations. However, the melting temperature showed significant differences ($P<0.05$) with 2.0:1.0 having the highest melting temperature (54.0°C), while 1.9:1.1 and 1.8:1.2 showed 43.0 and 42.0°C melting temperature, respectively. The

melting diameter (38.8 to 43.9 mm) and melt area (1183.9 to 1538.6 mm^2) did not have any differences in different PCP formulations. The PCP made with a 2.0:1.0 ratio of protein from cMCC and MCC showed better functional properties compared to other formulations.

Keywords: Process cheese products; Micellar casein concentrate; Acid curd powder; Clean label; Functional characteristics; Hardness; End apparent viscosity; Melting properties

Introduction

Microfiltration (MF) is a membrane process that is utilized to fractionate casein (CN) and serum protein (SP) from skim milk using a 0.1 μm semipermeable membrane. The skim milk is driven force through the membrane to separate CN (retentate side) and SP (permeate side) based on their sizes (0.1-0.4 μm vs 0.003-0.01 μm, respectively). The retentate is called micellar casein concentrate (MCC), which is mostly native casein. MCC is a high protein ingredient that is typically manufactured in 3 MF stages using a 3× concentration factor (CF) with diafiltration (DF). Several MF membranes have been studied to produce MCC (Hammam et al., 2021), such as spiral-wound membranes (Govindasamy-Lucey et al., 2007; Lawrence et al., 2008; Zulewska et al., 2009; Beckman et al., 2010; Beckman and Barbano, 2013; Marella et al., 2021), isoflux ceramic membranes (Adams and Barbano, 2013), uniform transmembrane pressure (UTP) ceramic membranes (Vadi and Rizvi, 2001; Hurt et al., 2010), and graded permeability (GP) ceramic membranes (Zulewska and Barbano, 2014; Tremblay-Marchand et al., 2016; Hammam and Metzger, 2018; Hammam, 2019). The spiral-wound membranes are

cheaper and have lower operating costs, but they have a shorter shelf-life, low chemical stability, limited viscosity range, and less efficiency to remove SP as compared to ceramic membranes. The UTP and GP membranes are commonly used to produce MCC due to their high SP removal. The GP membranes have low operating costs (do not require permeate recirculation pump) although they are expensive compared to UTP membranes (Hammam et al., 2021).

The MCC has promising applications in some dairy and nondairy products due to its unique physicochemical and functional characteristics (e.g., foaming, emulsifying, wetting, dispersibility, heat stability, bland flavor, and water-binding ability). The high casein content in MCC makes it heat stable and thereby it can be used in beverages that require sterilization (Beliciu et al., 2012; Sauer and Moraru, 2012). The nondairy applications for MCC are pasta, confectionery, meat products, special dietary preparations, textured products, convenience foods, toothpaste, cosmetics, and wound treating preparations (Salunke, 2013; Hammam et al., 2021). The dairy applications for MCC include Cheddar cheese (Amelia et al., 2013; Li et al., 2020; Xia et al., 2021), Greek-style yogurt (Bong and Moraru, 2014), imitation Mozzarella cheese (IMC) (Salunke, 2013; Hammam and Metzger, 2020a), recombined MCC (Lu et al., 2016, 2017), process cheese (PC) and process cheese products (PCP) (Hammam et al., 2019; Hammam and Metzger, 2019; Metzger and Hammam, 2020; Hammam et al., 2022), and acid curd (Hammam and Metzger, 2019, 2020b; Metzger and Hammam, 2020). We recently developed a process to produce soft acid curd (basically Cottage cheese curd) from MCC using direct acidification (lactic acid) (Hammam and Metzger, 2019; Metzger and Hammam, 2020) and starter cultures (Hammam and Metzger, 2020b) to set the curd.

Acid curd is a protein concentrate, which can be obtained by precipitating casein at pH 4.6 (isoelectric point) using starter cultures or acids without the use of rennet. The colloidal calcium phosphate that exists in the micelles is dissolved at the isoelectric point, which leads to a low mineral (calcium) content in the acid curd. In contrast to the low mineral content of acid curd, MCC has a high level of casein bound calcium due to its pH of 6.5-6.7. Acid curd could be produced from skim milk in a process similar to cottage cheese manufacture (Klei et al., 1998). There is a possibility of using MCC instead of skim milk to produce acid curd. Making acid curd from MCC has advantages as compared to skim milk, since manufacturing MCC using microfiltration (MF) results in milk-derived whey protein as a by-product, which can be utilized in many applications, particularly making whey protein isolate (WPI). In contrast, acid curd produced from skim milk results in acid whey as a by-product, which is more difficult to utilize. The typical composition of MCC (3-stages using a 3× concentration factor with a diafiltration) is >9% true protein (TP) and >13% total solids (TS) (Zulewska et al., 2009). This MCC could be used immediately in making acid curd or diluted to lower protein levels before making acid curd if required. For the rest of this paper, we will refer to acid curd made from micellar casein using cultures as cultured micellar casein concentrate ingredient (cMCC).

Process cheese (PC) and process cheese products (PCP) are dairy foods prepared by blending dairy ingredients (e.g., natural cheese, protein concentrates, butter, nonfat dry milk, whey powder, permeate) with nondairy ingredients (e.g., sodium chloride, water, emulsifying salts: ES, color, and flavors) and then heating the mixture with continuous agitation to produce a homogeneous product with an extended shelf-life

(Meyer, 1973; Thomas, 1973; Caric et al., 1985; Schmid, 1992; Guinee et al., 2004; Mcsweeney, 2007; Kapoor and Metzger, 2008; Kammerlehner, 2009; Hammam, 2019; Hammam and Metzger, 2019, 2020a; Hammam et al., 2019, 2021, 2022; Metzger and Hammam, 2020). PC and PCP are remarkably similar products however PC is defined by the Code of Federal Regulations (CFR), whereas PCP does not have a CFR definition. The major ingredient in PC is natural cheese, while PCP may or may not contain cheese. In that case, they typically rely on other ingredients, such as MCC and whey protein concentrate (WPC) for structure building (Lu et al., 2007; Hammam, 2019; Hammam et al., 2021). PC has been made since the late nineteenth and early twentieth century to extend the shelf-life of natural cheeses. Approximately one-third of all natural cheese produced in the United States is used in making PC (Kommineni et al., 2012). PC is one of the leading varieties of cheese in the world and has several applications (Sorensen, 2001; Kapoor and Metzger, 2008).

A critical reaction that occurs during PC and PCP manufacture is calcium sequestration using ES (sodium citrate, disodium phosphate, etc.). ES are critical for the functional characteristics of PC and PCP due to their role in improving the emulsification characteristics of casein by sequestrating a portion of the calcium from the calcium-casein-phosphate network in natural cheese or casein containing ingredients (Figure 1). As shown in Figure 1, ES such as disodium phosphate sequester the calcium from the calcium-casein-phosphate network by donating their sodium ions. As a result, the major molecular forces that cross-link the various monomers of casein are partially disrupted. This disruption leads to hydration and dispersion of caseins. The partially dispersed monomers of casein, like any protein, have hydrophilic and hydrophobic regions. These

amphiphilic dispersed caseins, in the presence of heat and mixing, get hydrated via hydrophobic interactions and at the same time emulsify fat phase via hydrophobic interactions to produce an emulsified process cheese (Guinee et al., 2004).

If cMCC is mixed with MCC (Figure 2), it may be possible to create a partially deaggregated casein network without the use of ES. The ratio of cMCC to MCC will have an impact on the level of deaggregation and the pH of the final PC product. We recently patented a method of producing PCP with no ES using cMCC and MCC (Metzger and Hammam, 2020). We hypothesized that a ratio of 2 parts of protein from cMCC: 1 part of protein from MCC will create a partially deaggregated casein network similar to a typical process cheese that utilizes ES (Figure 2). The objectives of this study were to produce PCP without ES using different combinations of protein from cMCC and MCC in the formulations (2.0:1.0, 1.9:1.1, and 1.8:1.2) as well as to study the functional characteristics of the cheese and determine the optimum ratio of protein from cMCC to MCC.

Materials and Methods

Manufacture of Process Cheese Products

Techwizard (Excel-based-formulation software program provided by Owl Software) was used to develop PCP formulations (Metzger, 2010) with different ratios of cMCC and MCC (2.0:1.0= FR-2.0:1.0, 1.9:1.1= FR-1.9:1.1, and 1.8:1.2= FR-1.8:1.2) to produce PCP with 45% moisture, 30% fat, 19% protein, and 2.4% salt. The cMCC and MCC powders were manufactured as described in our previous chapter. The percentage of ingredients utilized in PCP formulations is shown in Table 1. In each formulation, the

amount of protein from cMCC and MCC was adjusted based on the formulation. As a result, the amount of cMCC was decreased while the amount of MCC was increased from 2.0:1.0 to 1.8:1.2 formulations. The ingredients included aged natural Cheddar cheese (Kraft Heinz Cheddar), salted butter (Land O'Lakes, INC., Arden Hills, MN), cMCC, MCC, milk permeate (product lot: 19113D40, Idaho Milk Products, ID, USA), and salt (Morton salt, INC, Chicago, IL 60606). Approximately 15% of aged cheddar was added into PCP formulations to get mild Cheddar flavor in the final PCP. Milk permeate powder was utilized to standardize the solids content in all formulations. PCP formulations were prepared by mixing cMCC powder, MCC powder, and water for 10 min in a kitchenaid at room temperature. Then other ingredients were added and mixed for 30 min to produce a homogeneous paste. A 20 g sample of the paste was weighed in a canister, warmed in a 40°C water bath for 30 min, and then cooked in the rapid visco analyzer (RVA) for 3 min at 95°C. A 0.5 g of water was added into each canister to compensate for the water that evaporated during mixing and cooking in RVA. The stirring speed was 1000 rpm for the first 2 min and 160 rpm for the last min. Each batch was divided into 10 canisters. The cooked PCP was then poured into copper molds (diameter=20 mm; height=30 mm) to measure the hardness by using texture profile analysis (TPA). PCP was poured into plastic molds (diameter=28.3 mm; height=25 mm), which was used to measure the melt temperature by using dynamic stress rheometry (DSR) and melt diameter by using the Schreiber melt test. This experiment was repeated 3 times using different batches of cMCC and MCC powders.

Chemical Analyses

MCC and cMCC were analyzed for total solids (TS) (AOAC, 2000; method 990.20; 33.2.44), total protein (TPr=total nitrogen× 6.38) (AOAC, 2000; method 991.20; 33.2.11), noncasein nitrogen (NCN) (AOAC, 2000; method 998.05; 33.2.64; Zhang and Metzger, 2011), nonprotein nitrogen (NPN) (AOAC, 2000; method 991.21; 33.2.12), lactose content and lactic acid (Amamcharla and Metzger, 2011), ash (AOAC, 2000; method 945.46; 33.2.10), mineral profile (AOAC, 2000; method 985.01), and fat using Mojonnier method (AOAC, 2000; method 989.05; 33.2.26). Also, TS, ash, and pH (Hannah Edge Blu, Woonsocket, RI 02895) of the final PCP were determined.

Functional Analyses

The End Apparent Viscosity. The end apparent viscosity of the PCP was set to be measured by the end of the cooking time in the RVA at 95°C after 3 min by calculating the mean of the last five apparent viscosity readings from the RVA curve (Figure 3). However, due to the high viscosity of the treatments in this study, the RVA automatically stopped after 1 min of cooking, so this cooking time was standardized in all treatments and the last point referred to the end apparent viscosity. The end apparent viscosity was measured in all canisters of the batch.

Texture Profile Analysis (TPA). The hardness of the PCP was measured by using the TPA. The PCP was prepared by pulling the cheese out from the copper cylinders and then cutting the cheese into 20 mm height. The PCP was analyzed for hardness using a TA.XT-Plus Texture Analyzer (TA.XT-Plus, 6 Patton Drive, South Hamilton, MA) equipped with a 38 mm diameter cylindrical flat probe (TA-4) and using uniaxial double

bite 10% compression with 1 mm/s crosshead speed. The maximum force of the first compression (Figure 4) was referred to as the hardness of the cheese. This test was repeated 6 times for each batch.

Dynamic Stress Rheometry (DSR). The PCP was prepared by cutting the cheese into slices (2 mm thick and 28.3 mm diameter) using a wire cutter (Salunke, 2013). A stress sweep test of the PCP was performed at a frequency of 1.5 Hz, and stress ranged from 1 to 1000 Pa at 20°C using a rheometer with parallel plate geometry (MSR 92, Anton Paar, Graz, Austria). The stress sweep experiment determined that the maximum stress limit for the linear viscoelastic region was 500 Pa. The dynamic rheological properties of the PCP were then analyzed with a dynamic temperature ramp test that ranged from 20 to 90°C with a ramp rate of 1°C/min using a frequency of 1.5 Hz and constant stress of 500 Pa. The temperature at which tan δ=1 (G''/G') was referred to as the cheese melting temperature (Figure 5). A duplicate was performed on each batch.

Schreiber Melt Test. The PCP samples were cut into cylinders (diameter=28.5 mm and height=7 mm) and placed on 0.75 mm thick aluminum plates (100 mm × 90 mm) (Figure 6A). The plates were transferred to a forced draft oven at 90°C for 7 min (Salunke, 2013). After cooling the plates (Figure 6B), the diameter of the melted PCP samples was measured in four different places using a vernier caliper and reported in millimeters. This test was repeated four times for each batch.

Statistical Analysis

Statistical analysis was performed to study the ratio of protein from MCC and cMCC on the functional properties of PCP. An ANOVA was done to obtain the mean

squares (MS) and P-values using the GLM procedure available in R software (R x64 3.3.3 using R studio). Differences were tested using the least significant difference (LSD) comparison test when a significant difference was detected at $P<0.05$.

Results and Discussion

Composition of Ingredients

Table 2 shows the composition of MCC and cMCC powders used in PCP formulations. The MCC powder had approximately 75.8% TPr, 2.1% NPN, 9.7% NCN, 97.8% TS, 8.7% lactose, 1.7% lactic acid, and 3.0% fat. The ash content of MCC was 8.0% with 2.4% calcium. The composition of MCC powder in this study is similar to the composition of commercial MCC 80 reported previously (Hammam et al., 2021). The MCC 80 has approximately 80.0% TPr, 95.0% TS, 4.5% lactose, 3.0% fat, and 8.0% ash. Similar results were reported in several studies (Nasser et al., 2017, 2018; Carter et al., 2018). Those studies found that TPr, TS, lactose, and ash ranged from 80.0 to 83.0, 94.0 to 95.0, 1.0 to 2.5, and 7.0-8.0%, respectively. The lactose content in the commercial MCC and other studies is low due to the multiple DF stages that are used to deplete more lactose and low molecular weight components in the permeate during MF.

The cMCC powder had around 86.9, 2.2, 2.3, 96.4, 1.4, 2.5, and 2.5% TPr, NPN, NCN, TS, lactose, lactic acid, and fat, respectively. The ash of cMCC was 2.0% with 0.17% calcium. The acid casein or acid curd made from skim milk can have up to 86.7% TPr, 95.0% TS, 0.5% lactose, 1.2% fat, and 1.8% ash (Hammam et al., 2021). The slight differences in the composition of cMCC are due to the differences in the composition of the initial material and process used to make the curd.

Composition of PCP

The composition of PCP made from cMCC and MCC is shown in Table 3. The mean squares and the P-values for the composition of PCP are indicated in Table 4. The moisture content was 44.19, 44.21, and 44.15% in PCP made from FR-2.0:1.0, FR-1.9:1.1, and FR-1.8:1.2, respectively. The ash content of PCP made from the 2.0:1.0 ratio of protein from cMCC to MCC was 3.28%, while it was 3.42 and 3.40% in the PCP made from 1.9:1.1 and 1.8:1.2, respectively. No differences ($P>0.05$) were detected in the moisture and ash contents of PCP because all formulations were standardized before cooking in the RVA to have the same composition. The PCP exhibited 0.31, 0.33, and 0.41% calcium in formulations of FR-2.0:1.0, FR-1.9:1.1, and FR-1.8:1.2, respectively. Although a slight increase in the calcium content was noticed with decreasing the cMCC and increasing the MCC, no significant difference ($P>0.05$) was detected in the calcium content. It was reported that the calcium of PC made using ES and natural cheeses ranged from 0.3 to 0.4% (Kapoor et al., 2007), which is similar to our values. However, increasing the amount of MCC led to differences ($P<0.05$) in the pH of final PCP, which was 5.25 in 2.0:1.0 formulation, 5.32 in 1.9:1.1 formulation, and 5.37 in 1.8:1.2 formulation. This was expected as the MCC had higher pH when compared to cMCC. The ratio of cMCC to MCC has an impact on the level of deaggregation and the pH of the final PCP. It was indicated that the Ca and P, residual lactose, and salt-to-moisture ratio in natural cheeses affect the pH of PC (Kapoor et al., 2007). It has been reported that the typical pH of PC made from natural cheeses and ES can fall in the range of 5.4 to 5.8 (Marchesseau et al., 1997; Kapoor and Metzger, 2008; Lu et al., 2008; Hammam, 2019; Hammam et al., 2019, 2022). The pH of PCP in this study was close to the typical values.

However, it has been found that pH of PC can get to 5.1-5.2 and still shows good functional properties (Kommineni et al., 2012). We previously have found that the pH of PCP or IMC produced with no ES using cMCC and MCC ranged from 5.3 to 5.5 (Hammam and Metzger, 2019, 2020a; Metzger and Hammam, 2020), which is similar to this study. The differences in pH could affect the structure and quality of final PCP and thereby its functional properties due to its effects on the protein interactions (Palmer and Sly, 1943; Meyer, 1973; Marchesseau et al., 1997). Palmer and Sly (1943) stated that the emulsion of PC is low when the pH is lower than 5.4 or higher than 5.8. It was found that as the pH of PC drops to 5.2, the protein-protein interaction increases (Marchesseau et al., 1997) because the pH is close to the isoelectric point of caseins (4.6). This induces the aggregation of the protein, which in turn, results in a poor emulsion of fat in PCP. On the other hand, the PC had an open structure when the pH elevated to 6.1, which eventually led to weaker emulsification (Marchesseau et al., 1997). Marchesseau et al. (1997) also found that as the pH increased up to 5.7 it resulted in PC with better uniform fat emulsion with a closely knit protein network.

Functional Characteristics of PCP

End apparent viscosity of PCP. The end apparent viscosity of PCP made from cMCC and MCC is illustrated in Table 5. The mean squares and the P-values for the end apparent viscosity and hardness of PCP are presented in Table 6. The end apparent viscosity of PCP made from FR-2.0:1.0, FR-1.9:1.1, and FR-1.8:1.2 was approximately 4305.0, 2408.8, and 2499.3 cP, respectively. The end apparent viscosity was different ($P<0.05$) within treatments showing the highest viscosity in the FR-2.0:1.0 formulation. The highest viscosity in FR-2.0:1.0 could be due to the differences in pH. The viscosity

of PC increased as the pH decreased. As the pH dropped to the isoelectric point (4.6), the protein-protein interactions increased which led to high viscosity (Kapoor, 2007).

Hardness of PCP. The hardness of PCP made from cMCC and MCC is shown in Table 5. The mean squares and the P-values for the hardness of PCP are exemplified in Table 6. The hardness was approximately 424.0 g for the 2.0:1.0 ratio, 512.0 g for the 1.9:1.1 ratio, and 407.0 g for the 1.8:1.2 ratio. The hardness of PCP did not show any significant differences ($P>0.05$) between different formulations made from different ratios of protein from cMCC and MCC. Although the hardness should be correlated with viscosity (Kapoor and Metzger, 2008) and pH (Kapoor, 2007), we did not find differences in the hardness of the PCP made from different ratios of protein from cMCC and MCC. The hardness of our PCP looks better compared to those PCP made using ES. In our previous study, we found that the hardness of PCP made using MCC and ES ranged from 100.0 to 212.0 g (Hammam et al., 2022). Another study reported that the hardness of PCP made from MCC using ES was 110.0-135.0 g (Salunke, 2013).

Melting characteristics of PCP. The melting characteristics of PCP made from cMCC and MCC are shown in Table 7. The mean squares and the P-values for the melting characteristics of PCP are illustrated in Table 8. There was a significant difference ($P<0.05$) in the melting temperature of PCP made from 2.0:1.0, 1.9:1.1, and 1.8:1.2 formulations. The highest melting temperature was 54.0°C in the 2.0:1.0 ratio, while it was 43.0 and 42.0°C in the 1.9:1.1 and 1.8:1.2 ratios, respectively. It was expected that 2.0:1.0 formulation will have the highest melting temperature since the PCP showed higher end apparent viscosity than other formulations. There is a correlation between viscosity and melt temperature. It was mentioned that the melt temperature is elevated as

the end apparent viscosity increases (Kapoor and Metzger, 2008), which our work pointed out in Table 5. Additionally, the melting of PC increased as the pH decreased. As the pH decreased to the isoelectric point (4.6), the protein-protein interactions increased which required more temperature to be melted (Kapoor, 2007).

It has been reported that the melt diameter or melt area should increase as the melt temperature decreases (Kapoor and Metzger, 2008). Although there was a significant difference in the melting temperature of PCP, no differences ($P>0.05$) were detected in the melt diameter or area. There was an increase in the melt diameter of PCPs, but this increase was not significant. The melt diameter and melt area increased with elevating the protein ratio from MCC compared to cMCC. The melt diameter was 38.8, 43.9, and 42.4 mm for PCPs made with 2.0:1.0, 1.9:1.1, and 1.8:1.2 of protein from cMCC to MCC, respectively. The melt area was approximately 1184.0, 1539.0, and 1415.0 mm^2 for PCPs made with 2.0:1.0, 1.9:1.1, and 1.8:1.2 of protein from cMCC to MCC, respectively. The melting temperature is an indicator of the melt diameter. As a result, a similar trend was found in the melt diameter compared to the melt temperature.

The differences in the onset of melting can be explained by the differences in pH of those cheeses. As the pH drops to the isoelectric point, the net negative charges on caseins reduce which increase the protein-protein interactions, and this leads to aggregation of protein and thereby low meltability. This led to a higher melting temperature with less melted diameter and area.

The rheological properties of PCP. The storage modulus (G′) and loss modulus (G″) of PCP measured during heating from 20 to 90°C at 10°C increments are shown in Tables 9

and 10, respectively. The ANOVA table with mean squares and P-values at 20, 70, and 90°C for both elastic (G′: Pa) and viscous (G″: Pa) moduli of the PCP made from cMCC and MCC are shown in Table 11. The effect of using different ratios of protein cMCC and MCC (2.0:1.0, 1.9:1.1, and 1.8:1.2) in manufacture of PCP with no ES on G′ and G″ during heating from 20 to 90°C is presented in Figure 7 and 8, respectively.

The G′ values of PCP made using different ratios of protein from cMCC and MCC (2.0:1.0, 1.9:1.1, and 1.8:1.2) were not different ($P>0.05$) at a temperature range of 20 to 30°C (Figure 7). However, there were differences ($P<0.05$) in G′ values of PCP made using different ratios of protein from cMCC and MCC from 40 to 80°C (Table 9). These differences in G′ values of PCP made using different ratios of protein from cMCC and MCC were not found (Table 11) at 90°C (Table 9). PCP made from 2.0:1.0 (protein from cMCC to MCC) gave the highest G′ values at temperatures of 40 to 80°C, followed by 1.9:1.1, and 1.8:1.2 formulations.

The G″ of PCP made using different ratios of protein from cMCC and MCC (2.0:1.0, 1.9:1.1, and 1.8:1.2) followed the same trend of G′ at a temperature range of 20 to 90°C (Figure 8). The G″ values of PCP made using different ratios of protein from cMCC and MCC (2.0:1.0, 1.9:1.1, and 1.8:1.2) were not different ($P>0.05$) at a temperature range of 20 to 30°C (Table 10). However, there were differences ($P<0.05$) in G″ values made using different ratios of protein from cMCC and MCC from 40 to 80°C (Table 10). These differences in G″ values of PCP made using different ratios of protein from cMCC and MCC were not found at 90°C (Table 11). PCP made from 2.0:1.0 (protein from cMCC to MCC) gave the highest G′ values at temperatures of 40 to 80°C, followed by 1.9:1.1, and 1.8:1.2 formulations.

The G′ of PCP made using different ratios of protein from cMCC and MCC (2.0:1.0, 1.9:1.1, and 1.8:1.2) before melting was higher than G″. This indicates that the PCP has elastic behavior (gel) more than the viscous behavior (liquid). The G′ (elastic) and G″ (viscous) are decreased during measuring the melting using the DSR. Both moduli are decreased until the cross point, which is the cheese melting point. The same trend was found in other studies (Hennelly et al., 2005; Subramanian et al., 2006; Zhong et al., 2007; Guinee and O'Kennedy, 2009; Kommineni et al., 2012; Hosseini-Parvar et al., 2015). The improvement in the functional characteristics of PCP with no ES made using different ratios of protein from cMCC and MCC (2.0:1.0, 1.9:1.1, and 1.8:1.2) may be a result of a better emulsion of cMCC and MCC during making the cheese.

We hypothesized that a ratio of 2 protein portions from cMCC: 1 protein portion from MCC is the optimum ratio to create a partially deaggregated casein network similar to a typical process cheese that utilizes ES. We think that the 2.0:1.0 ratio is typical because we hypothesized that ES in typical PC are sequestering 2 parts of the calcium, which we applied to our patent by using 2 portions of protein from cMCC with low/no calcium content (Metzger and Hammam, 2020).

Conclusions

Different ratios of protein from cMCC powder to MCC can be utilized in making PCP with no ES. The cMCC and MCC protein of different ratios create a partially deaggregated casein network that results in a process cheese with functionality similar to process cheese produced with ES. We also found that PCP made from the 2.0:1.0 formulation showed better functional properties as compared to other formulations.

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Tables

Table 1. Mean (n=3) composition of process cheese products (PCP) formulations made with different ratios of cultured micellar casein concentrate (cMCC) and micellar casein concentrate (MCC)

Ingredients (%)	Treatment[1]		
	FR-2.0:1.0	FR-1.9:1.1	FR-1.8:1.2
Cheddar cheese (aged)	15.00	15.00	15.00
Salt	1.62	1.62	1.62
Water	34.02	34.03	34.04
Milk permeate powder	0.40	0.31	0.22
Butter (salted)	30.86	30.86	30.86
MCC powder	6.60	7.25	7.90
cMCC powder	11.50	10.93	10.36
Total	100	100	100

[1]Treatment: FR-2.0:1.0=PCP made with 2.0:1.0 ratio of protein from cMCC to MCC; FR-1.9:1.1=PCP made with 1.9:1.1 ratio of protein from cMCC to MCC; FR-1.8:1.2=PCP made with 1.8:1.2 ratio of protein from cMCC to MCC

Table 2. Mean (n=3) composition (% by weight) of micellar casein concentrate (MCC) and cultured micellar casein concentrate (cMCC)

Treatment	Composition (%)[1]								
	Ash	TPr	NPN	NCN	TS	Lactose	Lactic acid	Fat	Ca
MCC	8.03	75.77	2.07	9.66	97.84	8.69	1.73	2.98	2.42
cMCC	2.05	86.88	2.22	2.29	96.42	1.41	2.55	2.55	0.17

[1]Composition: TPr= total protein= total nitrogen × 6.38; NPN = nonprotein nitrogen × 6.38; NCN = noncasein nitrogen × 6.38; TS= total solids; Ca = calcium; TP = true protein (TPr – NPN); CN = casein (TPr – NCN)

Table 3. Mean (n=3) composition of process cheese products (PCP) made with different ratios of cultured micellar casein concentrate (cMCC) and micellar casein concentrate (MCC)

Treatment[1]	Moisture (%)	pH	Ash (%)	Ca (%)
FR-2.0:1.0	44.19	5.25[c]	3.28	0.31
FR-1.9:1.1	44.21	5.32[b]	3.42	0.33
FR-1.8:1.2	44.15	5.37[a]	3.40	0.41
SEM	0.04	0.02	0.04	0.02

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

[1]Treatment: FR-2.0:1.0=PCP made with 2.0:1.0 ratio of protein from cMCC to MCC; FR-1.9:1.1=PCP made with 1.9:1.1 ratio of protein from cMCC to MCC; FR-1.8:1.2=PCP made with 1.8:1.2 ratio of protein from cMCC to MCC

Table 4. Mean squares and P-values (in parentheses) for the composition of the process cheese products (PCP) made with different ratios of cultured micellar casein concentrate (cMCC) and micellar casein concentrate (MCC)

Factor	df	Moisture (%)	pH	Ash (%)	Ca (%)
Treatment[1]	2	0.003 (0.90)	0.011 (<0.05)	0.016 (0.27)	0.009 (0.17)
Replication	2	0.008 (0.75)	0.001 (<0.05)	0.036 (0.10)	0.002 (0.61)
Error	4	0.026	0.0000444	0.008	0.003

[1]Treatment: FR-2.0:1.0=PCP made with 2.0:1.0 ratio of protein from cMCC to MCC; FR-1.9:1.1=PCP made with 1.9:1.1 ratio of protein from cMCC to MCC; FR-1.8:1.2=PCP made with 1.8:1.2 ratio of protein from cMCC to MCC

Table 5. Mean values (n=3) of end apparent viscosity (cP) and hardness (g) of the process cheese products (PCP) made with different ratios of cultured micellar casein concentrate (cMCC) and micellar casein concentrate (MCC)

Treatment[1]	End apparent viscosity (cP)	Hardness (g)
FR-2.0:1.0	4305.06[a]	424.09
FR-1.9:1.1	2408.78[b]	511.89
FR-1.8:1.2	2499.33[b]	407.10
SEM	339.98	28.98

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

[1]Treatment: FR-2.0:1.0=PCP made with 2.0:1.0 ratio of protein from cMCC to MCC; FR-1.9:1.1=PCP made with 1.9:1.1 ratio of protein from cMCC to MCC; FR-1.8:1.2=PCP made with 1.8:1.2 ratio of protein from cMCC to MCC

Table 6. Mean squares and P-values (in parentheses) for the end apparent viscosity (cP) and hardness (g) of the process cheese products (PCP) made with different ratios of cultured micellar casein concentrate (cMCC) and micellar casein concentrate (MCC)

Factor	df	End apparent viscosity (cP)	Hardness (g)
Treatment[1]	2	3432339 (<0.05)	9489 (0.43)
Replication	2	149899 (0.63)	2763.7 (0.75)
Error	4	289512	8992

[1]Treatment: FR-2.0:1.0=PCP made with 2.0:1.0 ratio of protein from cMCC to MCC; FR-1.9:1.1=PCP made with 1.9:1.1 ratio of protein from cMCC to MCC; FR-1.8:1.2=PCP made with 1.8:1.2 ratio of protein from cMCC to MCC

Table 7. Mean values (n=3) of melting properties of the process cheese products (PCP) made with different ratios of cultured micellar casein concentrate (cMCC) and micellar casein concentrate (MCC)

Treatment[1]	Melt temperature (°C)	Melt diameter (mm)	Melt area (mm^2)
FR-2.0:1.0	54.02[a]	38.80	1183.95
FR-1.9:1.1	43.02[b]	43.90	1538.62
FR-1.8:1.2	42.01[b]	42.36	1415.34
SEM	2.18	1.53	107.17

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

[1]Treatment: FR-2.0:1.0=PCP made with 2.0:1.0 ratio of protein from cMCC to MCC; FR-1.9:1.1=PCP made with 1.9:1.1 ratio of protein from cMCC to MCC; FR-1.8:1.2=PCP made with 1.8:1.2 ratio of protein from cMCC to MCC

Table 8. Mean squares and P-values (in parentheses) for the melting properties of the process cheese products (PCP) made with different ratios of cultured micellar casein concentrate (cMCC) and micellar casein concentrate (MCC)

Factor	df	Melt temperature (°C)	Melt diameter (mm)	Melt area (mm^2)
Treatment[1]	2	133.0 (<0.05)	20.54 (0.48)	97263 (0.49)
Replication	2	29.3 (0.056)	17.33 (0.53)	85511 (0.53)
Error	4	4.56	23.07	115371

[1]Treatment: FR-2.0:1.0=PCP made with 2.0:1.0 ratio of protein from cMCC to MCC; FR-1.9:1.1=PCP made with 1.9:1.1 ratio of protein from cMCC to MCC; FR-1.8:1.2=PCP made with 1.8:1.2 ratio of protein from cMCC to MCC

Table 9. Mean elastic modulus (G′: Pa) of process cheese products (PCP) made with different ratios of cultured micellar casein concentrate (cMCC) and micellar casein concentrate (MCC) during heating from 20 to 90°C using dynamic rheological analysis (DSR)

Temperature (°C)	Treatment[1]		
	FR-2.0:1.0	FR-1.9:1.1	FR-1.8:1.2
20	69179.3[a]	70611.0[a]	72193.2[a]
30	30019.5[a]	27004.3[a]	27120.2[a]
40	11594.9[a]	5982.3[b]	3556.3[c]
50	6055.7[a]	2182.2[b]	623.8[b]
60	926.6[a]	353.0[b]	164.9[b]
70	201.5[a]	68.6[b]	60.3[b]
80	103.5[a]	24.0[b]	36.1[b]
90	40.7[a]	7.3[a]	10.0[a]

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

[1]Treatment: FR-2.0:1.0=PCP made with 2.0:1.0 ratio of protein from cMCC to MCC; FR-1.9:1.1=PCP made with 1.9:1.1 ratio of protein from cMCC to MCC; FR-1.8:1.2=PCP made with 1.8:1.2 ratio of protein from cMCC to MCC

Table 10. Mean viscous modulus (G″: Pa) of process cheese products (PCP) made with different ratios of cultured micellar casein concentrate (cMCC) and micellar casein concentrate (MCC) during heating from 20 to 90°C using dynamic rheological analysis (DSR)

Temperature (°C)	Treatment[1]		
	FR-2.0:1.0	FR-1.9:1.1	FR-1.8:1.2
20	23228.8[a]	24522.5[a]	25684.8[a]
30	11385.6[a]	11052.7[a]	11450.5[a]
40	6881.0[a]	4258.1[b]	2926.8[c]
50	4661.1[a]	2491.5[b]	1029.9[c]
60	1345.6[a]	739.2[b]	421.6[c]
70	438.7[a]	229.8[b]	214.0[b]
80	299.1[a]	99.4[b]	150.0[b]
90	143.3[a]	53.2[a]	70.9[a]

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

[1]Treatment: FR-2.0:1.0=PCP made with 2.0:1.0 ratio of protein from cMCC to MCC; FR-1.9:1.1=PCP made with 1.9:1.1 ratio of protein from cMCC to MCC; FR-1.8:1.2=PCP made with 1.8:1.2 ratio of protein from cMCC to MCC

Table 11. Mean squares and P-values (in parentheses) for elastic modulus (G′: Pa) and viscous modulus (G″: Pa) of process cheese products (PCP) made with different ratios of cultured micellar casein concentrate (cMCC) and micellar casein concentrate (MCC) during heating from 20 to 90°C using dynamic rheological analysis (DSR)

Factor	df	G′			G″		
		20°C	70°C	90°C	20°C	70°C	90°C
Replicate	2	95627858	1057.6	642.0	6119707	7533	5282
		(0.06)	(0.54)	(0.25)	(0.14)	(0.40)	(0.20)
Treatment[1]	2	6818056	18829.8	1032.8	4528264	47191	6836.4
		(0.69)	(0.02)	(0.15)	(0.20)	(0.048)	(0.15)
Error	4	16655330	1470.1	327.0	1889423	6599	2203

[1]Treatment: FR-2.0:1.0=PCP made with 2.0:1.0 ratio of protein from cMCC to MCC; FR-1.9:1.1=PCP made with 1.9:1.1 ratio of protein from cMCC to MCC; FR-1.8:1.2=PCP made with 1.8:1.2 ratio of protein from cMCC to MCC

Figures

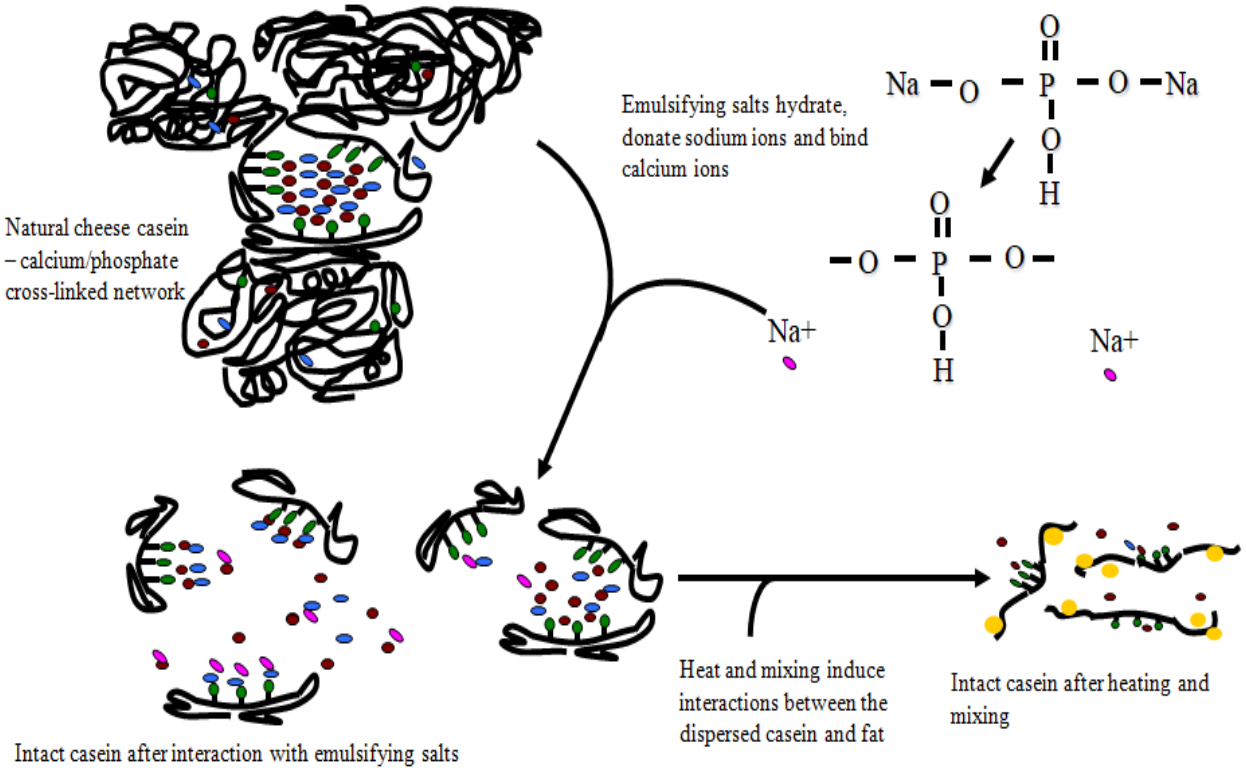

Figure 1. Emulsifying salts (ES) interaction during making process cheese (PC) or process cheese products (PCP)

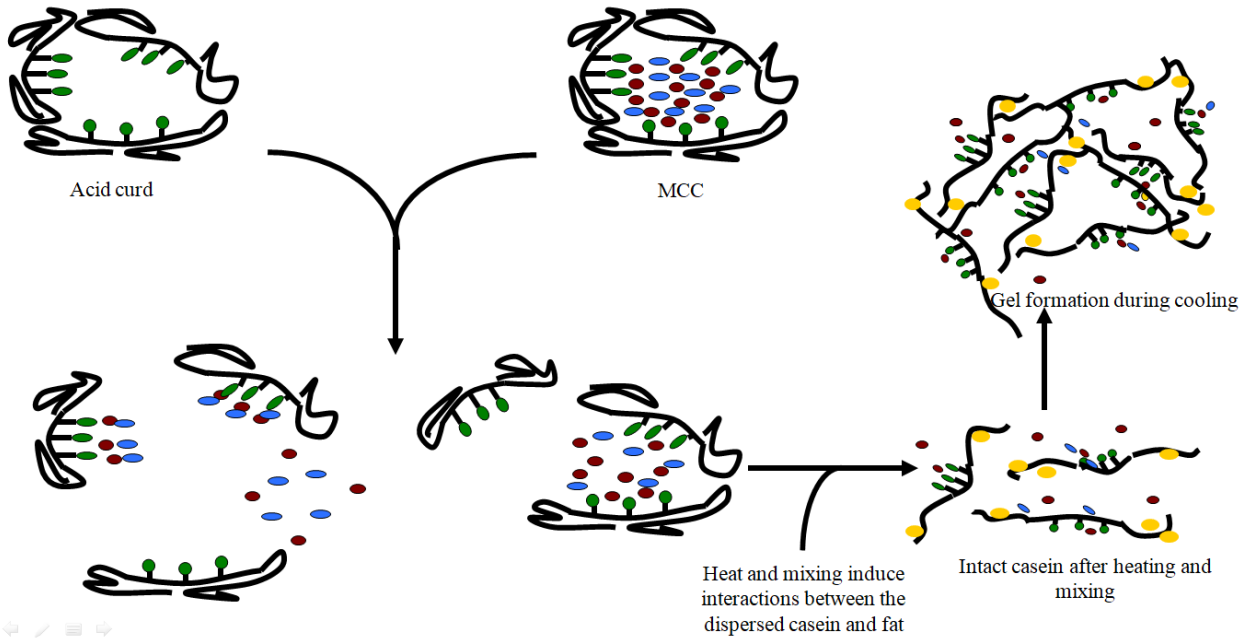

Figure 2. Cultured micellar casein concentrate (cMCC) and micellar casein concentrate (MCC) interaction in making clean label process cheese products (PCP)

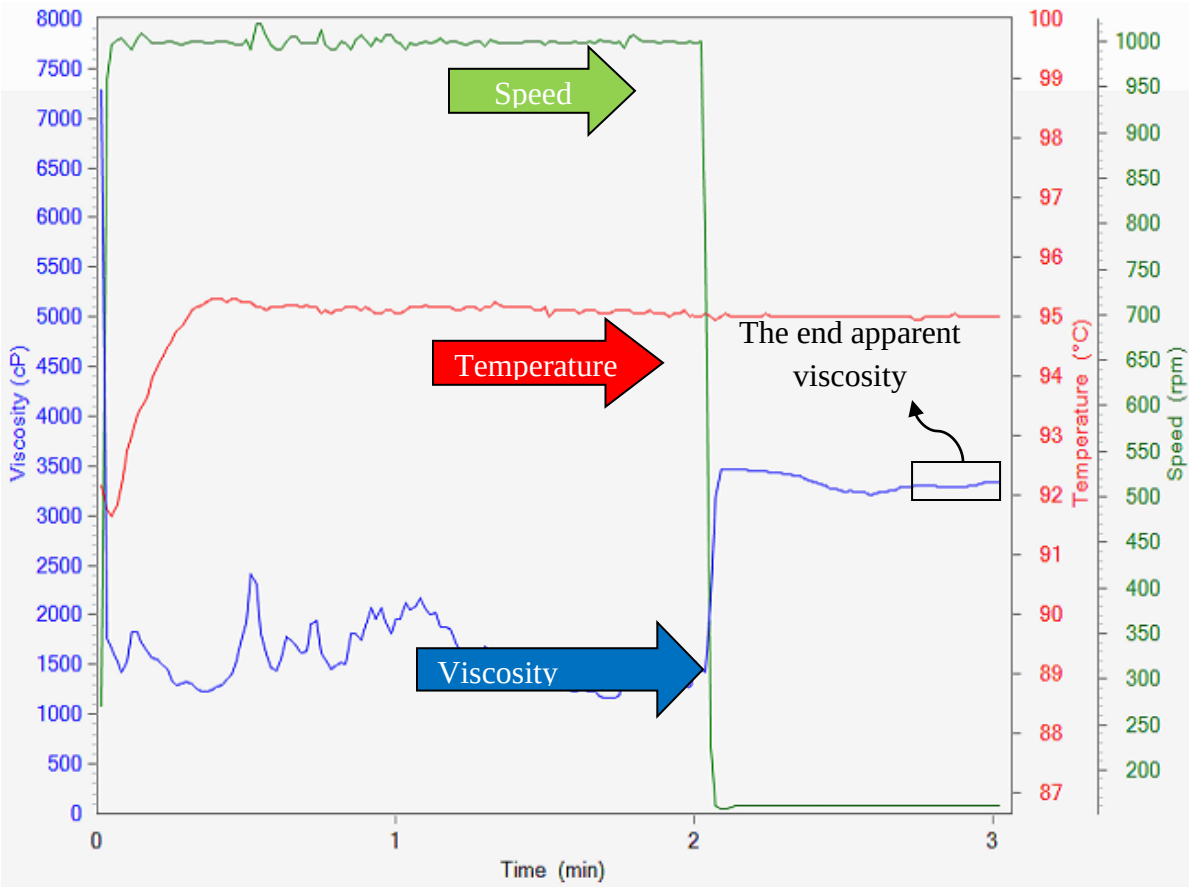

Figure 3. Measuring the end apparent viscosity (cP) of process cheese products (PCP) using the rapid visco analyzer (RVA)

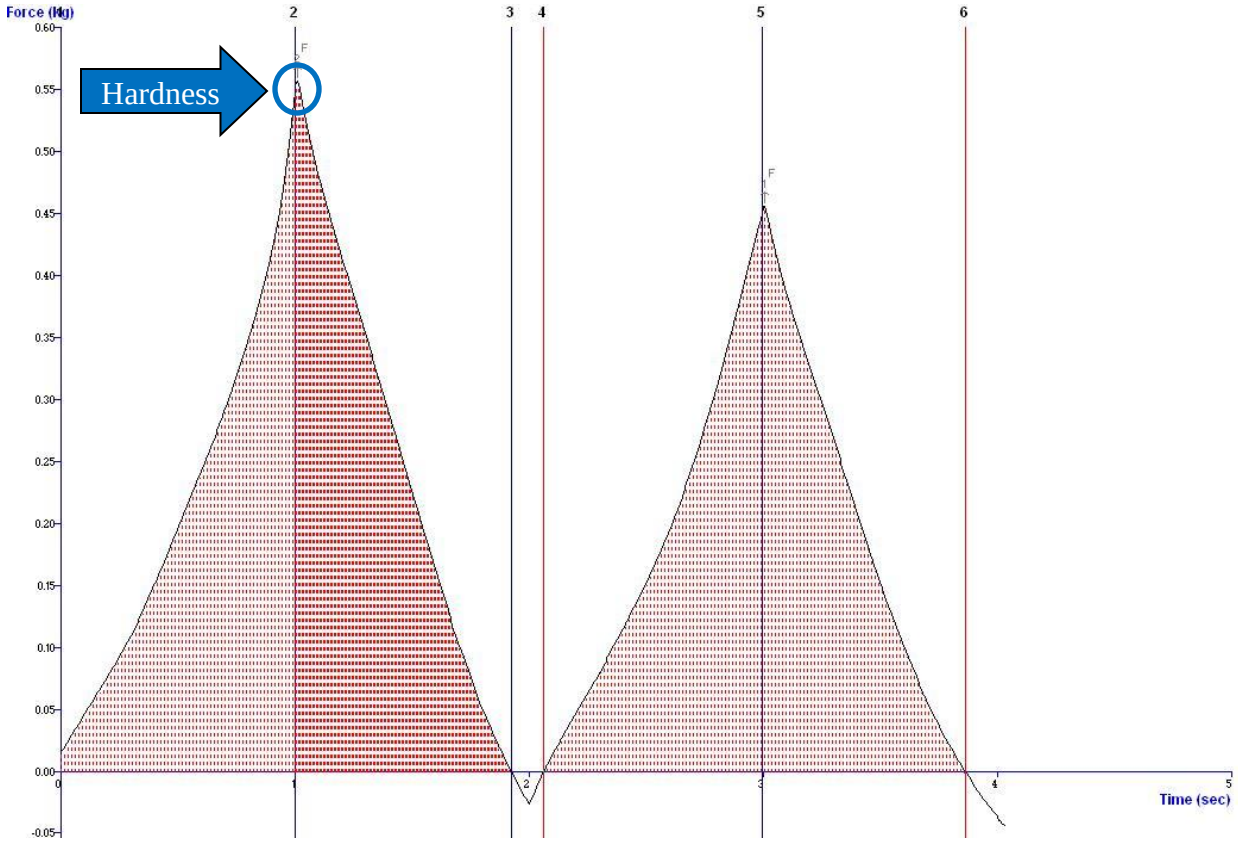

Figure 4. Measuring the hardness (g) of process cheese products (PCP) using the texture profile analysis (TPA)

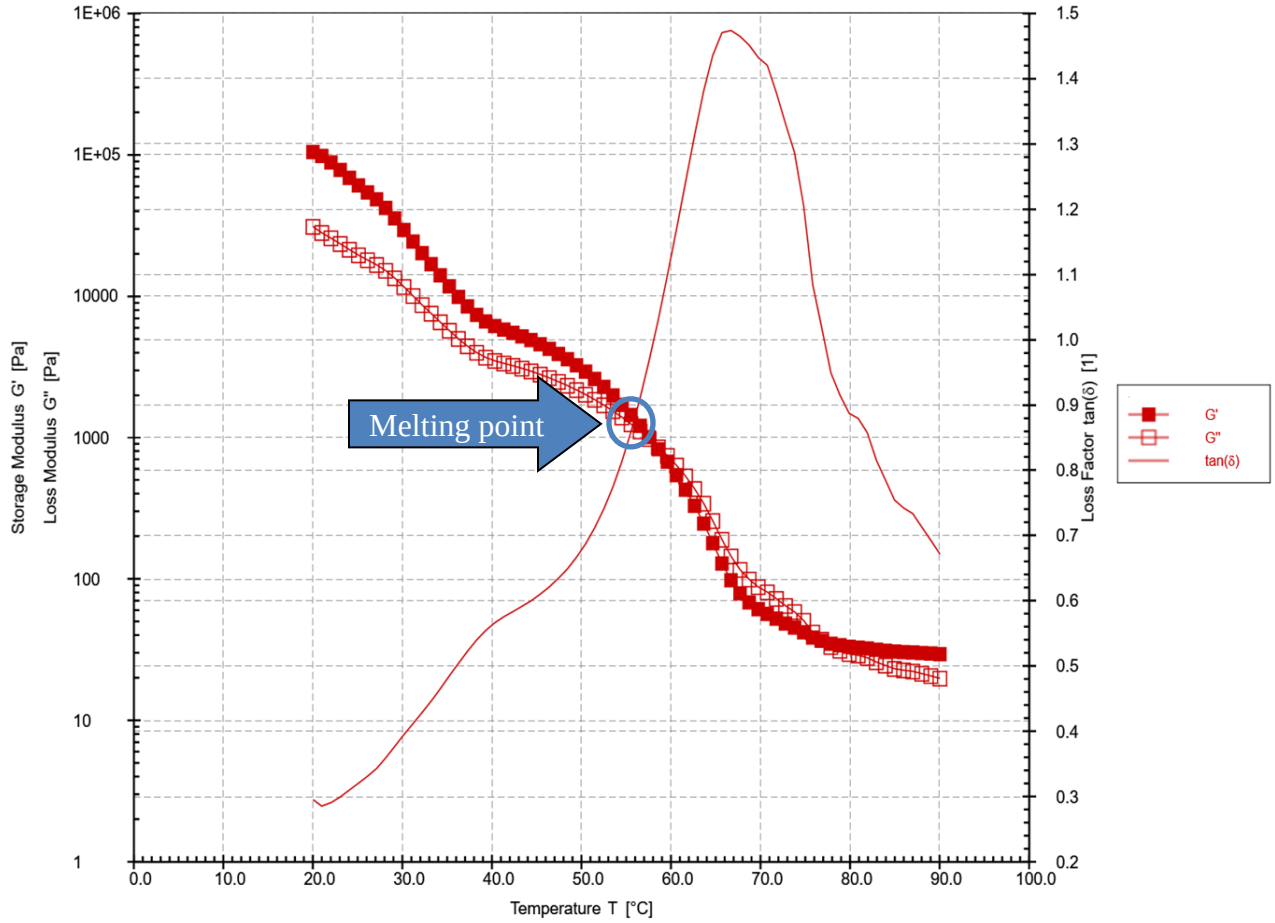

Figure 5. Measuring the melting point of process cheese products (PCP) using the dynamic stress rheometer (DSR)

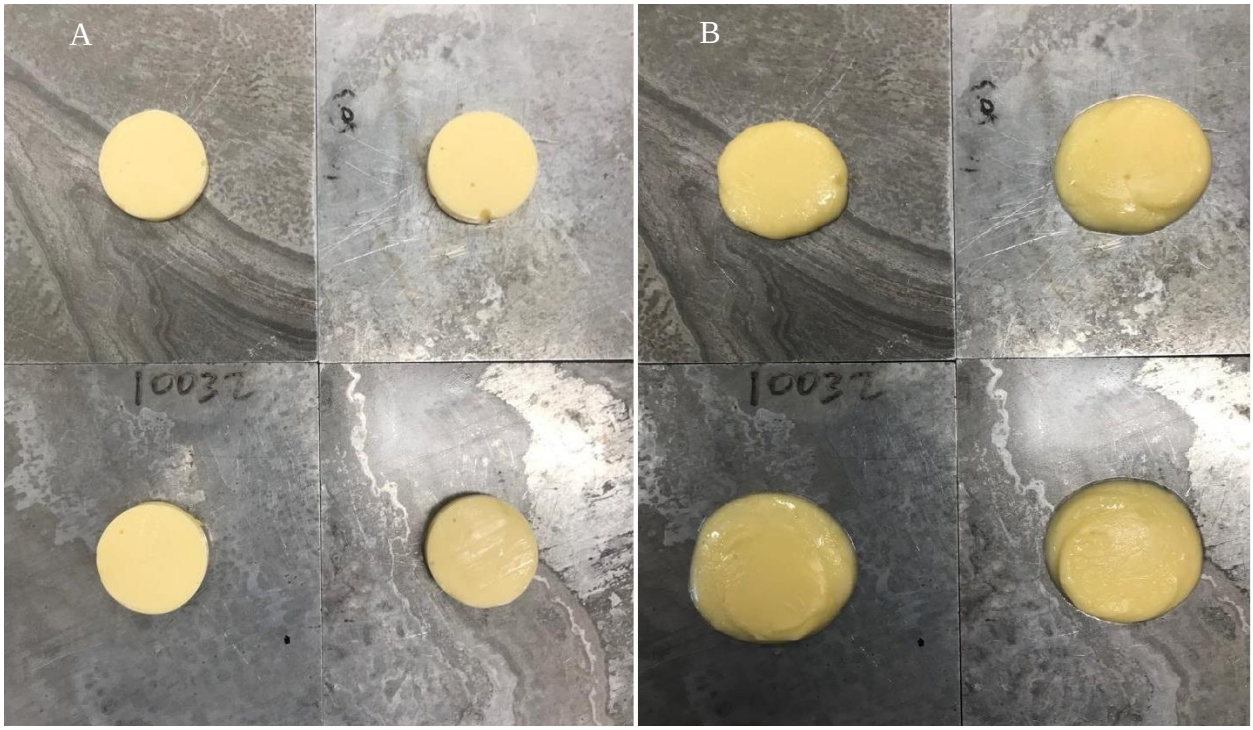

Figure 6. Schreiber melt test to measure the melting diameter of process cheese products (PCP): (A) Before melting; (B) After melting

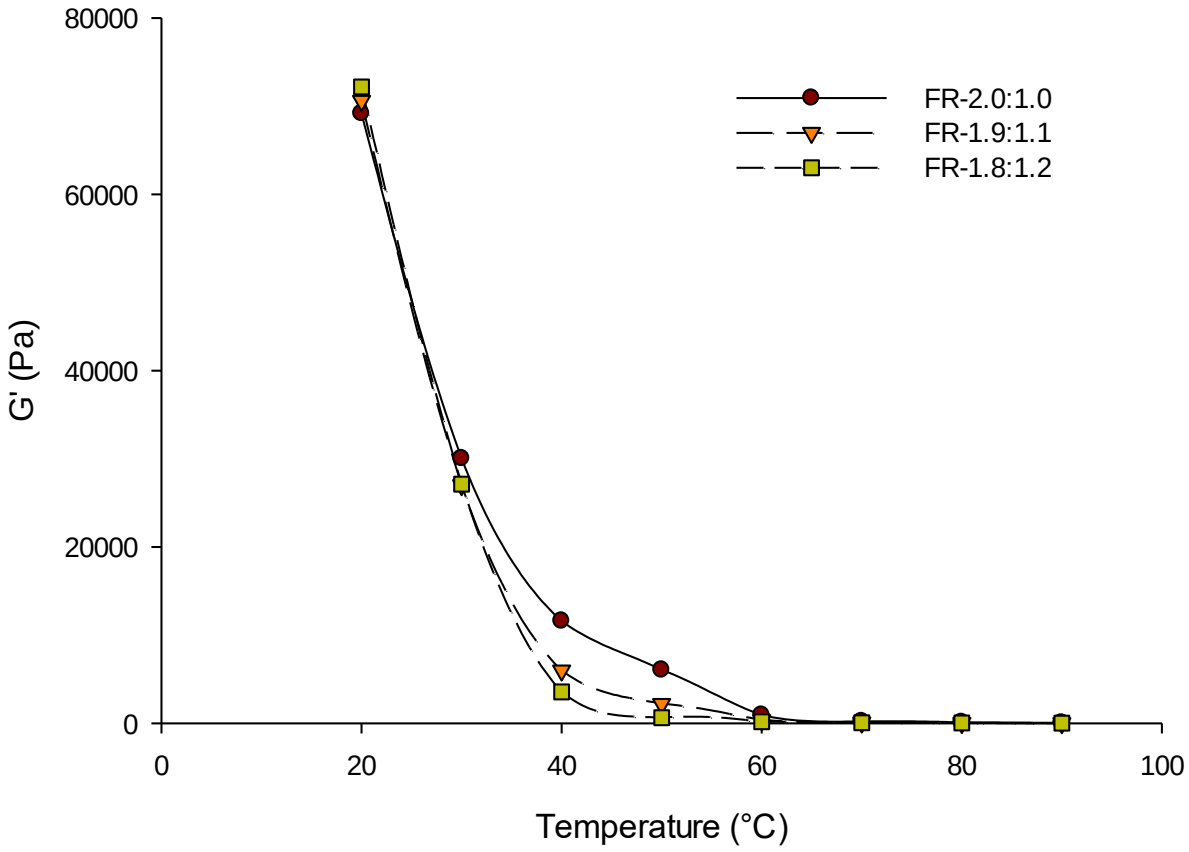

Figure 7. Elastic modulus (G′: Pa) of process cheese products (PCP) made from FR-2.0:1.0 (●)=PCP made with 2.0:1.0 ratio of protein from cultured micellar casein concentrate (cMCC) to micellar casein concentrate (MCC); FR-1.9:1.1 (▼)=PCP made with 1.9:1.1 ratio of protein from cMCC to MCC; FR-1.8:1.2 (■)=PCP made with 1.8:1.2 ratio of protein from cMCC to MCC during heating from 20 to 90°C using dynamic rheological analysis (DSR)

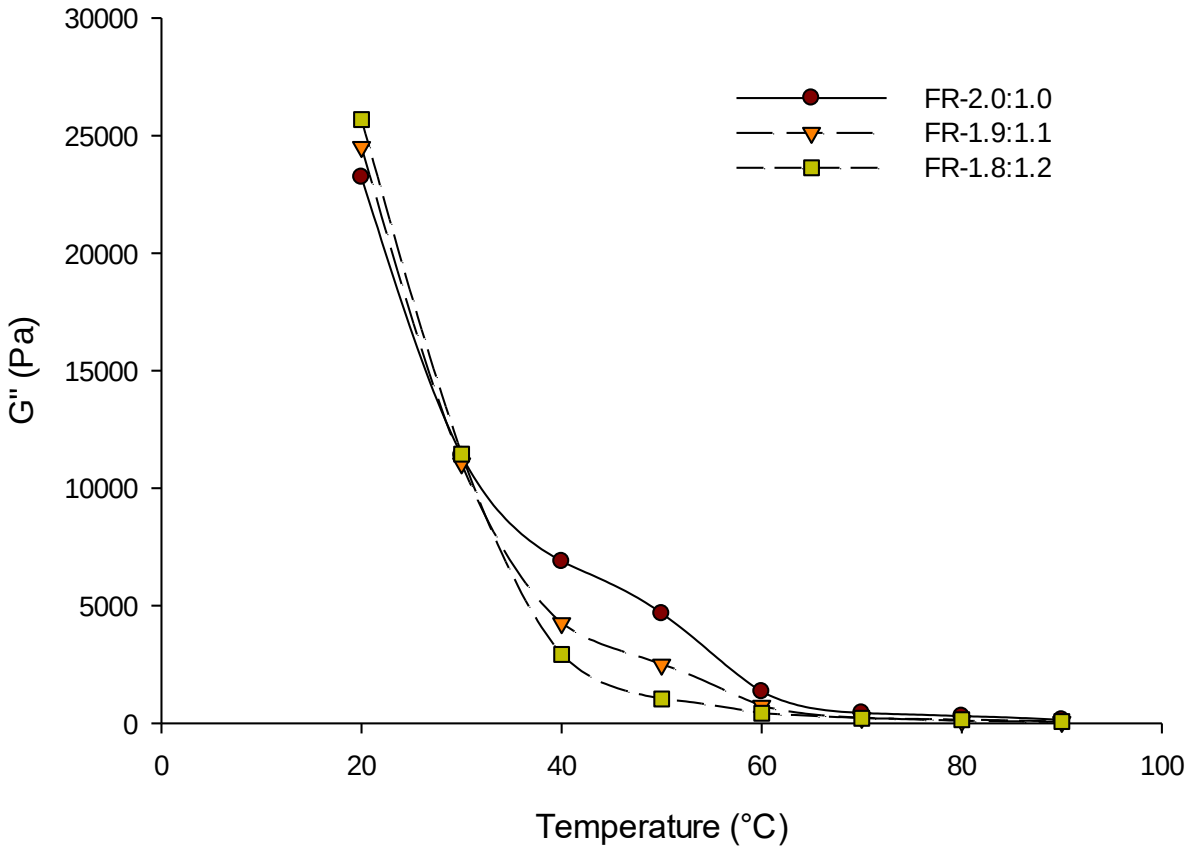

Figure 8. Viscous modulus (G″: Pa) of process cheese products (PCP) made from FR-2.0:1.0 (●)=PCP made with 2.0:1.0 ratio of protein from cultured micellar casein concentrate (cMCC) to micellar casein concentrate (MCC); FR-1.9:1.1 (▼)=PCP made with 1.9:1.1 ratio of protein from cMCC to MCC; FR-1.8:1.2 (■)=PCP made with 1.8:1.2 ratio of protein from cMCC to MCC during heating from 20 to 90°C using dynamic rheological analysis (DSR)

CHAPTER VI: OVERALL CONCLUSIONS

MCC can be manufactured using different MF membranes by fractionating the CN and SP using 0.1 µm semi-permeable membranes. There are different types of casein products other than MCC, which are considered a good source of CN, such as rennet caseins, acid caseins, caseinates, and MPC. Compared to other CN products, MCC has the CN in a native state that can widely be used as an ingredient to enhance and improve the protein content of many products. MCC has unique functional properties, such as foaming, emulsifying, and water-binding ability. Also, it is a good source of intact casein and could be produced in a liquid, concentrated, or dried form. MCC has been successfully used as an ingredient in making some dairy products, such as beverages, yogurt, cheese, and PC.

In the first study, we found that acid curd could be made efficiently from MCC with different protein contents (3, 6, and 9%). The adjusted yield of acid curd increased with increasing the protein content, therefore 9% protein MCC could be a good option to make acid curd commercially as it would generate less acid whey. Acid curds produced from MCC with different protein content were successful in the manufacture of PCP without ES using 2 parts of protein from acid curd to 1 part of protein from MCC. The 2:1 ratio creates a partially deaggregated casein network that results in PCP with functionality similar to PCP produced with ES. Although there were differences observed in the melted and un-melted texture of PCP made without ES when compared to control, these can be explained due to the possible microstructural interactions induced in the final PCP due to their pH differences. Future studies will focus on devising methodologies to produce PCP using acid curd with similar pH as conventional PCP.

Additionally, no differences were detected in the functionality of PCP produced from acid curds with different protein levels.

In the second study, we were able to produce culture based acid from liquid MCC (>9% TPr and >13% TS). Acid curd and MCC can be mixed in a specific ratio and market to be ready for making different types of cheeses including PCP and IMC. The MCC and acid curd were utilized successfully in manufacture of IMC with no ES in the ratio of 2:1 protein from acid curd to protein from MCC. This ratio of acid curd and MCC creates a partially deaggregated casein network that results in IMC with similar functionality to IMC produced with ES. IMC with no ES could be produced with the similar functional characteristics of IMC made with ES.

In the third study, we found that GP MF membranes can be effectively utilized to produce MCC with >9% TP and >13% TS using 3 stages of MF with a 3× CF and DF. Additionally, culture based acid curd was produced from the liquid MCC. A unique method was developed to produce acid curd powder by drying and grinding the curd. The novel ingredients (acid curd and MCC powders) produced in this study can be mixed in a specific ratio and market to be ready for making different types of cheeses including PCP and IMC. The MCC and acid curd powders will be utilized in manufacture of clean label PCP with no emulsifying salts and the functional properties of that cheese will be examined.

In the fourth study, we found that different ratios of protein from cMCC powder to MCC can be utilized in making PCP with no ES. The cMCC and MCC protein of different ratios create a partially deaggregated casein network that results in a process

cheese with functionality similar to process cheese produced with ES. We also found that PCP made from the 2.0:1.0 formulation showed better functional properties as compared to other formulations.