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Production and Storage Stability of High Concentrated Micellar Casein and its Effect on the Functional Properties of Process Cheese Products

Ahmed Hammam
South Dakota State University

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PRODUCTION AND STORAGE STABILITY OF HIGH CONCENTRATED MICELLAR CASEIN AND ITS EFFECT ON THE FUNCTIONAL PROPERTIES OF PROCESS CHEESE PRODUCTS

BY

AHMED HAMMAM

A thesis submitted in partial fulfillment of the requirements for the

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South Dakota State University

2019
PRODUCTION AND STORAGE STABILITY OF HIGH CONCENTRATED
MICELLAR CASEIN AND ITS EFFECT ON THE FUNCTIONAL PROPERTIES OF
PROCESS CHEESE PRODUCTS

Ahmed Hammam

This thesis is approved as a creditable and independent investigation by a
candidate for the Master of Science degree and is acceptable for meeting the thesis
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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Major and Thesis Advisor  Date

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Dairy Science Department  Date

Dean, Graduate School  Date
This thesis is dedicated to my loving parents, my wife, Preksam and my daughter, Alma, my loving brothers and sisters. 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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## Abbreviations

<table>
<thead>
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<th>Description</th>
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<tbody>
<tr>
<td>CGE</td>
<td>Capillary gel electrophoresis</td>
</tr>
<tr>
<td>CF</td>
<td>Concentration factor</td>
</tr>
<tr>
<td>cfu</td>
<td>Colony-forming unit</td>
</tr>
<tr>
<td>CN</td>
<td>Casein</td>
</tr>
<tr>
<td>DSR</td>
<td>Dynamic stress rheometer</td>
</tr>
<tr>
<td>GP</td>
<td>Graded permeability</td>
</tr>
<tr>
<td>MCC</td>
<td>Micellar casein concentrate</td>
</tr>
<tr>
<td>HC-MC</td>
<td>High concentrated micellar casein</td>
</tr>
<tr>
<td>MF</td>
<td>Microfiltration</td>
</tr>
<tr>
<td>MPC</td>
<td>Milk protein concentrate</td>
</tr>
<tr>
<td>NCN</td>
<td>Non-casein nitrogen</td>
</tr>
<tr>
<td>NFDM</td>
<td>Non-fat Dry Milk</td>
</tr>
<tr>
<td>NPN</td>
<td>Non-protein nitrogen</td>
</tr>
<tr>
<td>PC</td>
<td>Process cheese</td>
</tr>
<tr>
<td>PCP</td>
<td>Process cheese products</td>
</tr>
<tr>
<td>RVA</td>
<td>Rapid viso analyzer</td>
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</tbody>
</table>
SP  Serum protein
SW  Spiral wound
TN  Total nitrogen
TP  True protein
TPA Texture profile analysis
UF  Ultrafiltration
UTP Uniform transmembrane pressure
WPI Whey protein isolate
β-CN  β-casein
αS1-CN  αS1 casein
αS2-CN  αS2 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

PRODUCTION AND STORAGE STABILITY OF HIGH CONCENTRATED MICELLAR CASEIN AND ITS EFFECT ON THE FUNCTIONAL PROPERTIES OF PROCESS CHEESE PRODUCTS

AHMED HAMMAM

2019

Micellar casein is a high-protein ingredient that can be used in process cheese products (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, color, and flavors) and then heating the mixture with a continuous agitation to produce a homogeneous product with an extended shelf-life.

The first objective of this study was to produce a highly concentrated micellar casein (HC-MC) and evaluate its storage stability. Skim milk was pasteurized at 76°C for 16 sec and kept at ≤4°C until the following day. The skim milk was heated to 50°C using a plate heat exchanger and microfiltered (MF) with graded permeability (GP) ceramic MF membrane system (0.1μm) in a continuous feed-and-bleed mode (flux of 71.43 L/m² per hour) using a 3× concentration factor (CF). Subsequently, the retentate of the first stage was diluted 2× with soft-water (2 kg of water: 1kg of retentate) and again MF at 50°C using a 3× CF. The retentate of the second stage was then cooled to 4°C and stored overnight. The following day, the retentate was heated to 63°C and MF in recirculation-mode (retentate recirculated to system balance tank) until total solid (TS) was
approximately 22% (wt/wt). Consequently, the MF system temperature was increased to 74°C and MF continued until permeate flux reached less than 3 L/m² per hour. The HC-MC was then divided into three aliquots of approximately 10 kg each. The first portion was a control, while 1% sodium chloride added to the second portion (T1) and 1% sodium chloride + 1% sodium citrate was added to the third portion (T2). Treated HC-MC retentates were transferred at 74°C to sterilized vials and stored at 4°C to study the storage stability every 30 d. This trial was repeated three times using separate lots of skim milk. The HC-MC at d = 0 (immediately after manufacturing) contained average 25.41% TS, 21.65% true protein (TP), 0.09% nonprotein nitrogen (NPN), and 0.55% noncasein nitrogen (NCN). No difference (P > 0.05) was detected in the composition of control, T1, and T2 HC-MC during the 60 d of storage at 4°C. However, the NCN content increased significantly (P < 0.05) from 0.55 to 0.76%, 0.55 to 0.82% and 0.55 to 0.94% in control, T1, and T2, respectively, during the 60 d of storage at 4°C. Mean aerobic bacterial count in control, T1, and T2 at 0 d was 2.6, 2.5 and 2.8 log cfu/mL, respectively, and increased significantly (P < 0.05) to 4.3, 4.06 and 5.3 log cfu/mL, respectively, after 60 d storage at 4°C. Coliform, yeast, and mold were not detected during the 60 d of storage. This study determined that HC-MC with > 25% TS and > 95% casein as % of TP can be manufactured using ceramic MF membranes and could be stored up to 60 d at 4°C with no significant changes in the composition.

The second objective of this study was to utilize the high concentrated micellar casein (HC-MC) as an ingredient in making PCP and examine the effect of its storage on the functionality of PCP. The functionality of PCP was measured by determining the cooked apparent viscosity by using the rapid visco analyzer (RVA), hardness by using
texture profile analysis (TPA), and melting temperature by using dynamic stress rheometer (DSR). Three treatments of HC-MC (Control= HC-MC; T1= HC-MC + 1% sodium chloride; T2= HC-MC + 1% sodium chloride and 1% sodium citrate) were examined for the shelf-life at 0, 30, and 60 d. A 300 gm batch of each formula was prepared by mixing all ingredients (aged Cheddar cheese, HC-MC, water, unsalted Butter, deproteinized whey, sodium phosphate dibasic, salt, and sodium citrate) in a kitchenaid at room temperature for 30 min to get a homogenous paste. A 25gm sample of the paste was then weighed in a canister and tempered at 38°C for 15min. The PCP canisters were cooked in the RVA for 4 min at 90°C. The stirring speed was 1000 rpm for the first 2 min and 160 rpm for the last 2 min. Once the PCP was cooked, it was filled in molds and kept to the next day for further analysis. This trial was repeated three times using three separate batches of HC-MC at 0, 30, and 60 d of storage. Significant differences (P<0.05) were detected between treatments in the pH and moisture content of PCP. Also, the functionality of PCP was affected (P<0.05) by each treatment of HC-MC. However, no significant difference (P>0.05) was found in the functionality of PCP during the shelf-life of HC-MC at 0, 30, and 60 d. Overall, the addition of sodium chloride and sodium citrate to HC-MC during the shelf-life improved the melt characteristics of PCP.
CHAPTER I: REVIEW OF LITERATURE

1. INTRODUCTION

Microfiltration (MF) is a membrane process (pore size of 0.1 µm) that can be applied to milk to separate CN (approximately 80% of milk protein) and serum protein (SP) (approximately 20% of milk protein) (Maubois, 2002). When whole or skim milk is microfiltered using MF membranes, CN is retained in the retentate portion (micellar CN) and is called micellar casein concentrate (MCC), while low molecular weight components (such as lactose, soluble calcium, and serum proteins) pass into the permeate (Saboyainsta and Maubois, 2000; Maubois, 2002). Micellar CN (0.1 to 0.4 µm size) and SP (0.003 to 0.010 µm size) are separated from milk using a 0.1 µm MF semi-permeable membrane based on the differences in their sizes (Table 1).

Membrane fouling and concentration polarization of the MF membranes during milk processing are significant challenges that affect the efficiency of MF. 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 cake layer. Also, concentration polarization occurs during filtration when the dissolved components are convectively driven to the membrane surface 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 decrease. Over the last twenty years, different approaches have been applied to MF membranes (such as modifying the surface chemistry of membrane, increasing the back-transport of particles away from the
membrane by increasing the shear rate, and changing the water recovery rate or diafiltration) to decrease membrane fouling and concentration polarization (Bian et al., 2000; Saboyainsta and Maubois, 2000).

Polymeric spiral-wound (SW) membranes have improved over the last 50 years. SW membranes are used 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 than ceramic membranes. However, SW have limited viscosity range, low chemical stability, and shorter life compared to ceramic membranes. As a result, different membranes were designed to improve the efficiency of MF, such as uniform transmembrane pressure (UTP) and graded permeability (GP) membranes. The typical process of MF is carried out using ceramic membranes at 50 to 55°C (Maubois, 2002). Uniform transmembrane pressure (UTP) membranes have high investment and high operating costs due to the recirculation pumping of permeate to produce co-current permeate flow that decreases the fouling and increases SP removal. However, The permeate recirculation pump is not required for the GP membranes because they already have uniform thickness and porosity of the selective membrane layer and a difference in permeability built into the ceramic support structure, which maintains a uniform and constant flux. It has been reported that the efficiency of GP membranes is similar to UTP membranes (Zulewska et al., 2009) with less cost. As a result, the GP MF membranes are widely utilized in the dairy industry to produce MCC.

Micellar casein concentrate (MCC) is a high protein ingredient and could be employed in many applications (Table 2) due to its high nutritional value, bland flavor,
and its physicochemical and functional properties, as shown in Table 3 (Southward, 1985). Also, MCC has an extraordinary water-binding capacity (Walstra, 1990), emulsifying, and foaming characteristics (Halling, 1981; Phillips et al., 1994). Some researchers showed that MCC might have advantages in retorted shelf-stable high-protein nutritive beverages (Beliciu et al., 2012; Sauer and Moraru, 2012). MCC is heat stable under a range of pH and temperature conditions (Table 1). Therefore, it could be utilized as an ingredient in beverages that require sterilization. CN micelles in the MCC resist the harsh conditions applied during commercial processing (Schmidt, 1982). As a result, the number of new products containing casein increased in the U.S. by approximately 22% per year from 2000 to 2008 (Affertsholt, 2009). In addition to the applications of MCC, the SP removed by the membrane can be utilized in making whey protein isolate (WPI) or in beverages due to its clarity and clean flavor profile (Evans et al., 2010; Jervis et al., 2012; Campbell et al., 2013). The objective of this literature is to review and highlight the manufacturing of MCC, properties, varieties, and applications.

2. Properties of caseins

Milk is a valuable source of protein as a food ingredient because of its nutritional value and functional characteristics. Bovine milk has ~3.5% total protein (~80% of the total protein is casein) that can be precipitated at pH 4.6 at temperatures > 8°C (Zhang et al., 2011). The remainder of the total protein (~20%) is called serum protein (SP), which contains β-lactoglobulin (β-LG), α-lactalbumin (α-LA), bovine serum albumin (BSA), and immunoglobulins (IG). The high proline content in CN leads to a lack of organized secondary and tertiary structure of CN. As a result, CN is heat stable (Huppertz et al., 2004). Due to the high content of CN in MCC, MCC is heat stable at 110°C and pH >6.9
(Sauer and Moraru, 2012). The average size of CN micelles is 0.1 µm, which is around 100 times larger than the SP size (Walstra et al., 2006). The CN and SP could be separated by different methods (Table 1) based on their sizes.

To prior 1960s, CN was manufactured for use in industrial applications (e.g., plastic, paints, and glues). In the 1960s, Australia and New Zealand initiated production of CN for use as a food ingredient; however, CN is widely used nowadays as a functional food ingredient (Huppertz et al., 2004). The characteristics of CN (e.g., amphiphilic, open, and flexible structures) have been used in food systems to provide foaming, emulsifying, and water binding properties (Rollema and Muir, 2009). In addition to these functional properties, it also 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 the bone developed (Walstra et al., 2006). Rennet caseins, acid caseins, caseinates, co-precipitates, and milk protein concentrate are some of the commercial casein products that are available today. The production and characteristics of CN products have been reviewed (Modler, 1985; Fox, 2001; Rollema and Muir, 2009; Ann Augustin et al., 2011; Zhang et al., 2011). In addition to these casein products, MF has been recently utilized to produce a novel casein ingredient called MCC.

2.1. Casein micelle structure

Casein is present in a micellar form, however, several models of CN micelles have been proposed (Waugh, 1958; Rose, 1969; Schmidt, 1982; Walstra, 1990, 1999; Holt, 1992; Horne, 2003, 2006). All models of CN micelles have been reviewed recently and are shown in Figure 1 (de Kruif et al., 2012). Most CN micelle models propose that
the micelles are formed from sub-micelles and cross-linked by colloidal calcium phosphate (CCP). The most accepted model is proposed by Holt (1992), which described the CN micelle as a matrix of CN in which the colloidal calcium phosphate nanoclusters are dispersed (Figure 2). This model suggests that the CN fractions are not distributed evenly throughout the micelle, and also propose that κ-CN has a significant role in the micelle stability, which is located on the surface of the micelle.

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 αS1-CN present throughout the structure (Dalgleish and Corredig, 2012). The surface zeta potential of -20 mV at pH 6.7 and steric stabilization (protruding κ-CN hairs) are responsible for the CN micelle stability (Fox et al., 2015). It is also thought 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 the 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 3. The surface properties of the CN micelle is 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 and have similar structures and properties to native micelles. However, no proper studies have been carried out (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 because of heating is related to the denaturation of whey protein (WP) 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).

2. 2. Minerals

The mineral content in milk is relatively small (Gaucheron, 2005) and include cations (such as calcium, magnesium, sodium, and potassium) and anions (such as inorganic phosphate, citrate, and chloride). The mineral content in milk could be soluble as in the serum (one-third of the mineral content) or colloidal associated with CN (two-thirds of the mineral content). Milk salts or minerals have a significant role in the properties of dairy foods because they maintain the equilibrium of mineral fractions in milk, which affects the structure, stability, and functionality of CN micelles (Swaisgood, 1992; Gaucheron, 2005). Sodium in milk is significant because of its nutritional effect (high blood pressure) and calcium for its nutritional value (natural source of calcium) and functional properties (texture building, the effect on heat stability, etc.).

2. 3. Colloidal calcium phosphate

In the bovine milk, approximately two-thirds of the calcium content and half of the inorganic phosphate are present in a colloidal form while the remainder of these minerals presents in a soluble form. The main inorganic constituent in the casein micelle is colloidal calcium phosphate (CCP). The nature of CCP is complex, and these salts could have many compositions, including tricalcium phosphate, calcium brushite, or exist in amorphous or different crystalline structures (Lucey and Horne, 2009). The phosphate groups of the casein phosphoserine residues are the primary binding sites of calcium
phosphate in the casein micelle. Based on the phosphoserine content of the caseins, the capacity for CCP binding is decreasing in the order; \( \alpha S_2 > \alpha S_1 > \beta > \kappa \)-casein (Gaucheron, 2005).

CCP is an important constituent in maintaining the stability of casein micelles. It has been reported that CCP participates when casein 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. Elevated heat treatments could 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 depending on both the pH and the temperature of acidification (Dalgleish and Law, 1989; Singh et al., 1996). The exact nature of CCP, its interactions with casein molecules, and the effects on the heat stability of casein micelles are still unresolved.

3. Casein products other than MCC

There are principal differences used to separate CN and WP (Table 1). As a result, there are many casein products in the market, such as rennet casein, acid casein, sodium caseinate, calcium caseinate, calcium casein caseinates, and co-precipitates with different composition (Table 4). Casein products can be manufactured using various approaches including acid, heat, charges, or by using enzymes (such as chymosin). There are variations in the composition of casein products due to the differences in the manufacturing process utilized, and thereby, substantial effect on the physicochemical and functional properties.
3. 1. Rennet caseins

Rennet caseins can be manufactured by using enzymes, such as chymosin (Figure 3), which cleaves the polypeptide chain of κ-casein between Phe105-Met106 (Hammam et al., 2017). The surface charge and steric repulsion (which maintain casein micelles in a colloidal status) decrease when the hydrolysis of κ-casein occurs, thereby, casein micelles coagulate in the presence of a sufficient amount of calcium. The curd is cooked at high temperatures (60°C) to increase the syneresis of curd, firmness, and inactivate the coagulant enzymes. Then the curd is washed and can be dried by using roller dryers or attrition dryers. Based on the drying process, a grinding step might be required to produce the desired particle size. The casein is coagulated using rennet at neutral pH, which retains the minerals associated with the casein. Mulvihill and Ennis (2003) reported that the solubility of rennet casein in water is low; however, it could be solubilized in water at high pH (pH >9) with the addition of calcium sequestering salts (e.g., sodium phosphates, sodium citrates). Rennet caseins are widely utilized to produce cheese analogues, which includes the addition of polyphosphates (Fox et al., 2015).

3. 2. Acid caseins

Acid casein is one of the casein products which can be obtained by precipitating casein at pH 4.6. Starter cultures which produce the lactic acid or acids (such as HCl, HNO₃, and H₂SO₄) are utilized to reduce the pH of casein to 4.6. The colloidal calcium phosphate in the micelles is dissolved during the acidification; thereby; the mineral content in the acid casein is lower than the rennet casein. Acid casein is not soluble in water, which is similar to the rennet casein.
3. 3. Caseinates

Caseinates are manufactured by adding alkali, such as NaOH, NH₄OH, 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 caseinates) is shown in Table 4. Caseinates can be utilized in many applications (Table 5). The high pH (~6.8) makes the acid casein soluble in water. The caseinates produce a viscous solution, which limits the solids content of caseinates solutions (~20%) to be handled easily during production (Ann Augustin et al., 2011). As a result, the efficiency of drying for caseinates is low. Rollema and Muir (2009) reported that calcium caseinates behave differently from other caseinates, which resulted in the interaction of calcium with the phosphoserine residues in the casein. 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).

3. 4. Co-precipitates

The composition of co-precipitates is shown in Table 4. CN and SP exist in a denatured form in co-precipitates. Co-precipitates can be obtained by heating the skim milk at temperature ranges from 90 to 95°C for 30 min which resulting in denaturation 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 co-precipitate SP with CN, acidification is applied by using mineral acid to reach pH 4.6. CaCl₂ is 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 5), such as infant formulations due to its high nutritional value compared to casein.

3.5. Milk protein concentrate (MPC)

The composition of milk protein concentrate (MPC) products presented in Table 6. 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 ultrafiltration (UF) to concentrate the CN and SP from skim milk, by removing the lactose and minerals. MPC could be further concentrated to get ~85-90% protein of % total solids (TS) using UF and DF steps. Phillips and Williams (2011) reported that MPC could have a range of protein content from 42 to 85%. The MPC can be utilized in a concentrated liquid form or dried form. The solubility of MPC powder decreases with storage period (Havea, 2006).

4. Micellar casein concentrate (MCC)

4.1. Manufacturing of MCC

MCC is a high protein product and produced using microfiltration (MF) to separate SP and CN from milk without adding chemicals. When skim milk is microfiltered through MF membrane (0.1 to 0.2 μm), caseins and casein-bound minerals are retained by the membrane; while SP, lactose, and unbound minerals pass through into the permeate. The retentate is diluted with reverse osmosis (RO) water to its original volume (diafiltration steps) to increase the SP removal and lactose in the subsequent stages (Nelson and Barbano, 2005). MCC has been manufactured using different types of membranes including: polymeric spiral-wound (SW) membrane (Beckman et al., 2010),
uniform transmembrane pressure (UTP) (Hurt et al., 2010), graded permeability (GP) (Zulewska et al., 2009; Hammam and Metzger, 2018), and Isoflux ceramic membrane (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 was higher in GP and UTP membrane systems compared to SW and Isoflux membrane when MF applied at 50°C using a 3× concentration factor (CF) with diafiltration (DF) in a feed and bleed mode (Yin et al., 2004; Zulewska et al., 2009). The GP and UTP membranes systems have more transmission of SP to the permeate side compared to SW and Isoflux membranes (Zulewska et al., 2009). The SW and Isoflux membranes would need more DF stages or enlarge the surface area of the membranes to increase the SP removal as in GP and UTP membranes.

Theoretically, the SP removal is ~97% using 3 stages of MF with a 3× CF and 3× DF (Figure 2), 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 (2010) 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 for 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 separations (Hurt and Barbano, 2010).
The composition and properties of MCC are different from other casein products. The casein in other casein products (rennet caseins, acid caseins, caseinates, and co-precipitates) are not in a micellar form, but MCC is maintained the casein micelles form. MCC also contains the bound minerals associated with the micelles, while these minerals in acid caseinates are solubilized and lost into the whey. The MCC is a good source of intact casein (intact structure of the proteins), whereas in rennet casein glycomacropeptides (GMP) of κ-casein is hydrolyzed in the whey. The existence of oligosaccharides in GMP improves the hydrophilicity of the casein (Huppertz et al., 2004). The casein structure is similar in MCC and MPC, but the difference between them is the presence of SP in MPC. MCC is a high and valuable protein ingredient that is different from other protein ingredients, which can be utilized for specific functions more than any other ingredients.

5. Forms of MCC

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 resulted in retentate contains >8% true protein (TP) and >14% total solids (TS) content (Zulewska et al., 2009). The final retentate (Figure 1) of a 3-stage MF with 3× CF contains >9% true protein (TP), and >13% total solids (TS) using different MF membranes, such as polymeric spiral-wound, ceramic UTP, GP membranes, and Isoflux (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 refrigerated MCC would
limit utilizing this product in many applications. The liquid MCC would be suitable and less cost when it is utilized at the same place where being manufactured.

5.2. Concentrated MCC

The concentrated MCC is lower moisture than liquid MCC (Amelia, 2012). The MF and UF membranes can be utilized to remove more moisture from the liquid MCC to produce concentrated MCC. Increasing the viscosity of MCC when more water is removed during MF leads to accumulation the fouling on the pores of the membrane, and thereby, the flux decreases (Eykamp, 1995). Concentrating MCC decreases the volume of the product during transportation, thereby decreasing the transportation cost. Production concentrated MCC using MF is a good way of storing a valuable source of intact casein and producing co-products, such as SP concentrate. The dairy industry counteracts a problem with milk production during the season. During the peak season of milk production, excess milk is used to manufacture storable products (e.g., butter and nonfat dry milk NFDM). The excess skim milk is transported long distance to a drying facility to produce NFDM (Amelia, 2012). This adds more cost besides the drying cost. Amelia (2012) reported that 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 costs of installing MF system is low and takes less space compared to building an evaporator and a tower dryer. The shelf-life study of concentrated MCC becomes an important factor to be used in many applications. The 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 concentrated MCC could minimize
the microbial growth and then increasing its shelf-life up to 16 weeks at 4°C (Amelia, 2012).

5. 3. Dried MCC

Liquid or concentrated MCC could be further dried to produce powder MCC with a long shelf-life (Amelia, 2012). Powder or dried MCC can be handled, transported, and storing easily; however, it needs to be reconstituted to use it again in some applications. It has been reported that the MCC powder can contain up to 84 % total protein and 96 % total solids (Nasser et al., 2018).

6. Possible applications of MCC

6. 1. Beverages

MCC could be employed in making high protein and low carbohydrate beverages (e.g., sports drinks, meal replacement drink) due to its high content of protein and low lactose content. MCC is also stable for high temperatures without precipitating, so it can be utilized in beverages that need sterilization. Also, MCC has a bland flavor and can provide a good mouthfeel in the absence of fat.

6. 2. Greek-style Yogurt

It has been reported that the production and sales of Greek-style yogurt increased remarkably in recent years (Bong and Moraru, 2014). The MCC is a good source for protein fortification of the yogurt milk base (Bong and Moraru, 2014) due to the nutritive value and the functional properties of MCC (Nelson and Barbano, 2005; Affertsholt, 2009; Zhang et al., 2011; Sauer and Moraru, 2012). The protein fortification in yogurt led to change in the chemical composition of yogurt milk base, which affects the rheological
and physical properties of yogurt (Prentice, 1992; Skriver et al., 1999; Lucey, 2002; Peng et al., 2009). MCC at different concentrations of total protein (MCC-58 and MCC-88) have been utilized to fortify yogurt milk to 9.80% protein. The acidification rate was faster in the MCC-fortified Greek-style yogurt than the normal milk, regardless of the inoculation level, which was attributed to the higher nonprotein nitrogen content in the MCC-fortified milk (Bong and Moraru, 2014).

6.3. Cheesemaking

MCC is utilized to fortify milk or as an alternative for milk for cheese making. The cheese yield is increasing when MF is applied to the skim that used in cheese making (Papadatos et al., 2003) due to removing the whey. Papadatos et al., (2003) reported that there are economic benefits of using MF prior cheese making, which resulted in the production of valuable co-products from the MF permeates, such as WPI. In addition, the MF permeate is 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). The gel firmness and coagulation time of milk fortified with 4-5% protein solution from MCC powder increased due to the higher calcium content that is complexed with casein and retardation of rennet diffusion in higher protein cheese milk, respectively (Caron et al., 1997).

6.4. Low-fat cheese

The main components of low-fat cheese are protein, water, and minerals. The liquid MCC contains casein micelles, water, and minerals, which is similar to the composition of the fat-free portion or low-fat cheese. A study has been reported that 45%
reduced-fat Cheddar cheese was made by using different protein concentrate powders (such as diafiltered MF retentate, UF retentate powder, and calcium caseinate powder) to fortify milk with 3%, 4%, 5% and 6% casein with 1.61 ratio of CN to fat (St-Gelais et al., 1998). The fortified milk with diafiltered MF retentate was higher in the yield of cheese than UF retentate and calcium caseinate, especially at 5% and 6% casein. It has been reported that curd made from fortified milk with calcium caseinate did not retain the fat well, which resulted in increasing the fat content in the whey (St-Gelais et al., 1998). The MCC is a valuable ingredient to produce low-fat cheddar cheese with a good structure (Amelia, 2012).

6.5 Process cheese

Process cheese (PC) is made in the late 19\textsuperscript{th} century to increase the shelf-life of natural cheeses. It has several applications and consumed with other food items as an ingredient. PC has many forms available in marketing (Figure 3), 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 non dairy ingredients (salt, water, mold inhibitor, preservatives, emulsifying salts, 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. The principles of making PC are calcium sequestration, water binding, and emulsification (Henning et al., 2006) using emulsifying salts followed by blending, heating, and cooling the product. The quality of PC is affected by the level and type of emulsifying salt, conditions of manufacturing, and
characteristics of natural cheeses (Zehren and Nusbaum, 2000; Kapoor and Metzger, 2008). During PC manufacturing, calcium phosphate para-caseinate (rennet cheese) or calcium-casein-phosphate (isoelectric precipitation at pH 4.6) is converted from insoluble to soluble form using emulsifying salts 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).

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 (FDA, 2014). Pasteurized process cheese food, pasteurized process cheese spread, 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 not meets 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 could be used as non-cheese dairy ingredients to fulfill specific functionality requirements (Lu et al., 2007; Kammerlehner, 2009). The quality and functionality of the PC 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 PC.
6. 5. 1. Intact casein

The PC characteristic is significantly affected by the type and amount of protein (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 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). 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). 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% to 90% intact casein) is used to make block PC with good
d Sliceability and elasticity (Fox et al., 1996), while PC spread is manufactured by using
aged or mature cheese (60% to 75% intact casein). Similar ratios of cheddar cheese
blend to manufacture PC was reported by Tamime (2011) as shown in Table 7.

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 αS₁-CN in natural stages could be another reason of 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 change the proportion of protein interaction
(Marchesseau and Cuq, 1995; Guinee, 2011).

Protein-based interactions which occur during PC manufacturing resulted in
produce strong protein network with low flow characteristics (Purna et al., 2006). CN is
used to form a gel network in many applications (Ann 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).

6.5.2. Functional properties of process cheese

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). 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 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).

Texture profile analysis (TPA) is widely used for measuring unmelted textural properties, such as hardness, adhesiveness, springiness, cohesiveness, and gumminess. It has been reported that TPA hardness is a measurement of the unmelted cheese texture, which describes the firmness of this cheese (Breene, 1975).

Dynamic rheology is a test which measures the viscoelastic properties of cheese (Sutheerawattananonda and Bastian, 1998) to understand the viscoelastic nature of cheese (Rüegg et al., 1991). The $G'$ (storage modulus) and $G''$ (loss modulus) 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 at a constant frequency and constant strain (or stress) (Lannes,
2004). There is a strong relationship between $G'$, $G''$ and TPA hardness (Drake et al., 1999).

7. CONCLUSIONS

An overview of the MCC production, its functional properties, and potential applications has been provided in this review. There are different types of casein products, which considered as a good source of casein, such as rennet caseins, acid caseins, caseinates, MPC, and MCC. MCC is widely used as an ingredient to enhance and improve the protein content of many products and could be produced using MF. MCC has functional properties, such as foaming, emulsifying, and water binding ability. MCC is a good source of casein and could be used in a liquid, concentrated, or dried form. Recently, MCC has been used as an ingredient in making beverages, yogurt, low-fat cheese, and PC. The future work is required to understand the physicochemical, functional, and microbiological changes in the highly concentrated micellar casein (HC-MC) during the shelf-life, and study the effects of using liquid and dried MCC as ingredients on the functional properties of PC.
REFERENCES


Science, London, UK.


**Table 1.** The characteristics of Micellar CN and serum protein (SP). Adapted from Mulvihill (1992)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Micellar CN</th>
<th>Serum protein (SP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solubility at pH 4.6</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Rennet coagulation</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Heat stability</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Particle size</td>
<td>Large (micelles; molecular weight $10^8$)</td>
<td>Small (molecules; molecular weight $1.5-7.0 \times 10^4$)</td>
</tr>
</tbody>
</table>
Table 2. Applications of micellar casein in foods. Adapted from Salunke (2013)

<table>
<thead>
<tr>
<th>Product</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Confectionary</td>
<td>- Chewy texture, water binding, emulsifier, firmness</td>
</tr>
<tr>
<td></td>
<td>- Foaming, high-temperature stability, improves flavor</td>
</tr>
<tr>
<td></td>
<td>- Whipping properties</td>
</tr>
<tr>
<td>Special dietary preparations</td>
<td>- Dieting patients, bodybuilders, athletics, astronauts, nutritional fortification, low</td>
</tr>
<tr>
<td></td>
<td>lactose foods, bioactive peptides, casein hydrolysates</td>
</tr>
<tr>
<td>Textured products: puffed snack foods,</td>
<td>- Structuring, texturing, nutritional</td>
</tr>
<tr>
<td>protein-enriched snack, meat extenders</td>
<td></td>
</tr>
</tbody>
</table>
**Table 3.** The functional properties of Micellar CN. Adapted from Lorient et al. (1991)

<table>
<thead>
<tr>
<th>Functional properties</th>
<th>Micellar CN</th>
</tr>
</thead>
</table>
| Hydration             | - High water binding capacity  
                        | - At higher concentration gels  
                        | - Minimum at the isoelectric point |
| Solubility            | - Insoluble at the isoelectric point |
| Viscosity             | - Lowest viscosity at the isoelectric point  
                        | - 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 |
Table 4. The typical composition of different casein products. Adapted from Carić (1994) and Huffman and Harper (1999). Adapted from Mulvihill (1992)

<table>
<thead>
<tr>
<th>Parameters (%)</th>
<th>Rennet Casein</th>
<th>Acid Casein</th>
<th>Sodium Caseinate</th>
<th>Calcium Caseinate</th>
<th>Calcium Casein Caseinates</th>
<th>Co-precipitate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>80.6</td>
<td>87.3</td>
<td>90.4</td>
<td>90.5</td>
<td>89.3</td>
<td>89-94</td>
</tr>
<tr>
<td>Moisture</td>
<td>11.0</td>
<td>9.6</td>
<td>4.6</td>
<td>4.6</td>
<td>4.6</td>
<td>5</td>
</tr>
<tr>
<td>Ash</td>
<td>7.8</td>
<td>1.8</td>
<td>3.8</td>
<td>3.7</td>
<td>4.9</td>
<td>4.5</td>
</tr>
<tr>
<td>Fat</td>
<td>0.5</td>
<td>1.2</td>
<td>1.1</td>
<td>1.1</td>
<td>0.1</td>
<td>1.5</td>
</tr>
<tr>
<td>Lactose</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>1.5</td>
</tr>
<tr>
<td>Sodium</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>1.2</td>
<td>0.01</td>
<td>0.5</td>
<td>-</td>
</tr>
<tr>
<td>Calcium</td>
<td>2.8</td>
<td>0.02</td>
<td>0.03</td>
<td>1.3</td>
<td>1.3</td>
<td>-</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>1.6</td>
<td>0.7</td>
<td>0.8</td>
<td>0.8</td>
<td>0.9</td>
<td>-</td>
</tr>
<tr>
<td>pH</td>
<td>7.1</td>
<td>4.6</td>
<td>6.8</td>
<td>6.8</td>
<td>6.9</td>
<td>6.8</td>
</tr>
<tr>
<td>Product</td>
<td>Effect</td>
<td></td>
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<tr>
<td>---------------------------------------------</td>
<td>---------------------------------------------</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Imitation cheese</td>
<td>- Fat and water binding</td>
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<td>- Texture enhancing</td>
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<td>- Stringiness</td>
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<td>- Stringiness</td>
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<td>- Shredding properties</td>
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<td>Coffee creamers</td>
<td>- Emulsifier</td>
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<td>- Whitener</td>
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<td>- Gives body and texture</td>
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<td>- Resistance to feathering</td>
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<td></td>
<td>- Sensory properties</td>
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<td>Yogurt / cultured milk products</td>
<td>- Increase gel firmness</td>
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<td></td>
<td>- Reduce syneresis</td>
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<td>Milk beverages</td>
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<td>- Foaming properties</td>
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<td>High-fat powders, high-fat products</td>
<td>- Emulsifier</td>
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<td>(shortening, whipped toppings, butter-like</td>
<td>- Texture enhancing</td>
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<td>spread)</td>
<td>- Sensory properties</td>
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<td>Drinking chocolate, fizzy drinks and fruit</td>
<td>- Stabilizer</td>
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<td>beverages</td>
<td>- Whipping and foaming properties</td>
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<td>Cream liqueurs, wine apertifs</td>
<td>- Emulsifier</td>
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<td>Wine and beer industry</td>
<td>- Fines removal, clarification, reduce color</td>
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<td>and astringency</td>
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<td>Ice cream, frozen desserts</td>
<td>- Whipping properties</td>
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<td>- Body and texture</td>
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<td>Mousses, instant pudding, whipped topping</td>
<td>- Whipping properties</td>
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<td>- Emulsifier</td>
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<td>- Imparts body and flavor</td>
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Table 6. Composition of MPC varieties. Adopted from Salunke (2013)

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<th>Parameters (%)</th>
<th>MPC 42</th>
<th>MPC 56</th>
<th>MPC 70</th>
<th>MPC 75</th>
<th>MPC 80</th>
<th>MPC 85</th>
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<td>Moisture</td>
<td>3.5</td>
<td>3.8</td>
<td>4.2</td>
<td>5.0</td>
<td>3.9</td>
<td>4.9</td>
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<tr>
<td>Fat</td>
<td>1.0</td>
<td>1.2</td>
<td>1.4</td>
<td>1.5</td>
<td>1.8</td>
<td>1.6</td>
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<td>Protein</td>
<td>42.0</td>
<td>56.0</td>
<td>70.0</td>
<td>75.0</td>
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<td>Carbohydrate</td>
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<td>31.0</td>
<td>16.2</td>
<td>10.9</td>
<td>4.1</td>
<td>1.0</td>
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<tr>
<td>Ash</td>
<td>7.5</td>
<td>8.0</td>
<td>8.2</td>
<td>7.6</td>
<td>7.4</td>
<td>7.1</td>
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Table 7. Typical ratios of blends of cheddar cheese for the manufacture of PC. Adopted from Tamime (2011)

<table>
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<th>PC type</th>
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<th>Medium cheese</th>
<th>Aged cheese</th>
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<td>Block</td>
<td>70-75</td>
<td>25-30</td>
<td>25-30</td>
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<tr>
<td>Slices</td>
<td>30-40</td>
<td>50-60</td>
<td>10</td>
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<tr>
<td>Slices</td>
<td>55</td>
<td>35</td>
<td>10</td>
</tr>
<tr>
<td>Spread</td>
<td>30</td>
<td>50</td>
<td>20</td>
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</tbody>
</table>
Figure 1. Collection of (artists) impressions of the casein micelle particle. From (de Kruif et al., 2012)
Figure 2. Casein micelle model by Holt (1992)
Figure 3. The action and effect of rennet on casein. Adopted from Hammam et al. (2017)
Figure 4. 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× = the amount of retentate was mixed with 2 times of water. Adapted from Hammam and Metzger (2018)
Figure 5. Process cheese supermarket sales in the U.S.A. in 2005 based on form
(Source: IDFA, 2011) reported by Kapoor and Metzger (2008)
CHAPTER II: PRODUCTION AND STORAGE STABILITY OF HIGH
CONCENTRATED MICELLAR CASEIN

INTRODUCTION

Microfiltration (MF) is a membrane process that can separate casein (CN) micelles (0.1-0.40 µm) and serum whey proteins (SP) (0.003-0.010 µm) using a semi-permeable membrane with a pore size of 0.1 µm. When MF is applied to skim milk, the CN is concentrated in the retentate (micellar casein concentrate) whereas the SP, lactose, soluble minerals, and water pass through the membrane. The CN in the retentate is present in a micellar form, which is a relatively stable colloidal dispersion (Rollema and Muir, 2009).

Micellar casein concentrate (MCC) has been manufactured from skim milk using different MF membranes, such as polymeric spiral-wound (SW) membranes (Govindasamy-Lucey et al., 2007; Lawrence et al., 2008; Zulewska et al., 2009; Beckman et al., 2010; Beckman and Barbano, 2013) and ceramic membranes, including uniform transmembrane pressure (UTP) and graded permeability (GP) membranes (Zulewska et al., 2009; Hurt and Barbano, 2010; Hurt et al., 2010b; Adams and Barbano, 2013, 2016; Zulewska and Barbano, 2014). The SW membranes are cheaper and have lower operating costs but have limited viscosity range, low chemical stability, and a shorter life as compared to ceramic membranes (Zulewska et al., 2009). The MF is typically conducted at 50°C (Maubois, 2002). The flux was higher in UTP and GP ceramic membranes (54, and 72 kg/m² per hour, respectively) compared to SW membranes (16 kg/m² per hour) when MF of skim milk at 50°C in a continuous bleed-and-feed using a 3× CF (Zulewska
et al., 2009). Zulewska et al. (2009) reported that the efficiency of SP removal was 39% for SW, 64% for UTP, and 61% for GP MF membranes. Increasing the surface area of SW membranes, multiple MF and diafiltration stages would be required in SW membranes to increase the SP removal to be similar to UTP and GP membranes (Zulewska et al., 2009). As a result, UTP and GP ceramic membranes are used widely for MF of skim milk. UTP membranes have high investment and operating costs relative to GP membranes due to the need for a recirculation pumping of permeate to produce co-current permeate flow, and thereby, decreasing the fouling and increasing the SP removal (Zulewska et al., 2009). GP membranes eliminate the need for permeate recirculation pump and the associated electrical costs due to its ability to maintain a constant and uniform flux.

Micellar casein concentrate (MCC) is a high protein ingredient that can be utilized in many applications due to its unique physicochemical and functional properties (such as water-binding, emulsifying, whipping, and foaming properties) and can be utilized in a range of commercial applications, including protein fortification of dairy foods, ingredients for beverages, bakery, or meat products (Mulvihill and Ennis, 2003). The typical composition of liquid MCC using a GP MF (3-stage, and 3× CF with DF) is >9% true protein (TP) and >13% total solids (TS) (Zulewska et al., 2009). This MCC can be further concentrated to increase the TP and TS to 18% and 22%, respectively, by using 2.2× CF UF followed by 3-stage 3× CF with DF, and finally UF for more concentration (Amelia and Barbano, 2013). The MCC could also be concentrated by using vacuum evaporation (Lu et al., 2015). Lu et al. (2015) reported that high concentrated micellar casein (HC-MC) contained > 25% TS and >20 protein by using MF and vacuum
evaporation. Additionally, there is another possibility to continue using MF to produce HC-MC. The MCC can be dried to produce MCC powder with a long shelf-life (Amelia, 2012). The dried MCC can contain up to 84% total protein and 96% TS (Nasser et al., 2018). If the MCC is left in a liquid form it eliminates the drying cost and has more solubility compared to dried MCC; however, the liquid MCC has more moisture and shorter shelf-life as well as increased transportation costs compared to dried MCC (Amelia, 2012). As a result, the shelf-life of liquid MCC has become an important factor for the dairy industry.

Due to the high moisture content of liquid or concentrated MCC, it is a suitable environment for microorganisms’. Consequently, it has a limited shelf-life. Muir (1996) reported that the shelf-life of a dairy product is the time in which the product remains safe (no pathogenic bacteria) and shows no organoleptic defects (e.g. bitterness, acidic). It has been reported that the shelf-life of dairy products is limited by the growth of spoilage bacteria (Muir, 1996), which produce enzymes that can degrade milk constituents and cause unacceptable quality. The end of the shelf-life of HC-MC (18% and 22%) has been previously determined for over when the total bacterial count is >4.3 log cfu/mL (Amelia and Barbano, 2013) because it is the legal limit for the shelf-life of pasteurized milk based on the Pasteurized Milk Ordinance (FDA, 2009).

Some additives may have an impact on the functionality as well as the shelf-life. Addition of sodium chloride to HC-MC could increase the shelf-life of HC-MC and decrease the proteolysis (Guo et al., 2012). Also, the addition of emulsifying salts, such as sodium citrate increase the dispersibility and solubility of HC-MC (Lu et al., 2015), which could improve the functionality of process cheese when HC-MC used as an
ingredient. To date, no studies have reported the chemical (proteolysis) changes during storage of HC-MC.

Our objective was to develop a process to manufacture HC-MC (>25% TS and about 95% CN%TP) from pre-concentrated micellar casein (9.65% TS and approximately 90% CN%TP) using a GP ceramic MF system and evaluate its storage stability of HC-MC during 60 days of storage at 4°C.

MATERIALS AND METHODS

Experimental Design

Manufacturing of HC-MC was completed over 48 hours at the South Dakota State University dairy plant (Brooking, SD). The experiment was repeated three times, starting with different lots of skim milk each time. The chemical and microbiological analyses were done on HC-MC at 0, 30, and 60 days of storage period to examine the shelf-life stability of HC-MC at 4°C.

1. Preparation of Skim milk

Approximately 685 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 dairy plant. Subsequently, pasteurization (76°C/16 sec) was applied to the skim milk in a plate heat exchanger (model PR02-SH, AGC Engineering, Bristow, VA). The pasteurized skim milk was then kept at ≤ 4°C until the following day.
2. Microfiltration Operation and MCC Manufacturing

A pilot-scale ceramic GP MF system (TIA, Rond-point des, Portes de Provence, Rue Robert Schumann, 84500 BOLLENE, FRANCE) equipped with 7 ceramic tubes (19 channels with diameter of 3.3-mm each) ceramic Membralox GP membranes (0.1-μm pore size; 1.68 m² surface area; 1.02 m membrane length) mounted in the system vertically was utilized for MF. The GP MF system was equipped with a feed pump and a retentate recirculation pump, both from TIA (BOLLENE, FRANCE).

First Stage: Day 1. The GP MF system was started with soft-water at 50°C; subsequently, the system was transitioned from water to milk. Approximately 670 kg of skim milk was microfiltered with the GP MF system at a constant flux (71.42 L/m² per hour) using a 3× CF (1 kg retentate: 2 kg of permeate) in a feed and bleed mode at 50°C (Figure 1). Skim milk was heated before processing with a heat plate exchanger to 50°C (SABCO Plate-pro Sanitary Chiller (NP925-41). The water at the beginning of the process was flushed out with skim milk by collecting about 37 kg of permeate and 18 kg of retentate in cans that were discarded. The permeate flow rate was 120 L/h (flux of 71.42 L/m² per hour) and the retentate flow rate was 60 L/H. After this startup, retentate and permeate were collected and weighed continuously. The following conditions were applied during MF of skim milk: the gauge pressures Rpi (retentate pressure inlet), Rpo (retentate pressure outlet), and Ppo (permeate pressure outlet) were 400, 198.5, and 186.5 kPa, respectively. The CF was measured 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). During the MF process, the
collected retentate was kept in tanks at 4°C. At the end of the run, the retentate and permeate were sampled for compositional analysis. The processing time of the first stage of MF was approximately 4 hours.

**Second Stage: Day 1.** The retentate from the first stage was diluted with soft-water (Approximately 204 kg of retentate was mixed with 408 kg of water) to obtain a diafiltration factor (DF) of 2x. After mixing, the diluted retentate was heated to 50°C and processed with the GP MF system using a 3x CF as described previously. The water at the beginning of the process was flushed out of the system with the diluted retentate as described in the first stage. The typical gauge pressures Rpi, Rpo, and Ppo were 400, 197, and 205 kPa, respectively. Permeate and retentate were weighed and sampled, as described in the first stage. The retentate was collected in cans, cooled to 4°C, and stored overnight at ≤ 4°C. The processing time of the second stage was approximately 3.5 hours.

**Third Stage: Day 2.** The following day, about 154 kg of the retentate (9.65% TS and approximately 92% CN%TP) was microfiltered in a recirculation-mode to produce highly-concentrated micellar casein (HC-MC). As soon as MF was started, the retentate was further heated to 63°C to reduce the viscosity while minimizing denaturation of whey protein. The following conditions were applied: the gauge pressures (Rpi 398, Rpo 199, and Ppo 200 kPa), and flow rates (120 L/h permeate and 60 L/h retentate). When the TS reached approximately 22% using a CEM (CEM Smart System5 SL7199), the temperature was increased to 74°C to maximize the final TS content that could be obtained. Pp was decreased until reached to 0 kPa due to the accumulation of the fouling layers on the membrane during recirculation of the retentate. The process was stopped when the permeate flux reached approximately 3 L/m² per hour and Ppo reached 0 kPa.
The retentate from the third stage (approximately 30 kg) was collected and sampled. A composite sample of the permeate was taken for compositional analysis. The processing time for the third stage was about 2 hours. Tanks and milk cans were covered during processing to minimize the airborne contamination from the plant environment. The retentate of the third stage was divided into three lots; approximately 10 kg each. The first portion was used as a control, while 1% of sodium chloride was added into the second portion (T1) and 1% sodium chloride + 1% sodium citrate was added to the third portion (T2). HC-MC from all treatments were sampled in small sterilize vials and stored at 4°C to study chemical and microbiological composition during storage. This trial was repeated three times using three separate lots of raw milk.

**Cleaning before and after processing.** 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 feed pump running. Subsequently, 30 kg of soft-water was added in 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 11. This solution was recirculated for 30 min at a 350 L/h permeate flow rate (flux of 208 L/m² per hour). 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 was 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 pH 2. The recirculation of acid solution was applied for 20 min at a 350 L/h permeate flow rate (flux of 208 L/m² per hour). Subsequently, the system was stopped. Before processing, the acid solution was flushed with soft-water until the pH reached ~ 8.3 or 8.5. The flux was measured again after flushing the acid solution out of the system.

**Statistical Analyses**

Data were analyzed by R package (R x64-3.3.3). All data of manufacturing was analyzed by ANOVA using a GLM for each processing variable constructed for each stage. Mean separation was done using the least significant difference (LSD) comparison test at P < 0.05 if significant differences were detected. During the storage period, statistical analysis was performed to study the effect of treatments, time, or the interaction on the proteolysis of HC-MC. An ANOVA was done to obtain the mean squares (MS) and P-values using the GLM procedure available in R software. LSD comparison test was also used to detect the significant difference between treatments, time, or their interaction at P < 0.05.

**Chemical Analyses**

Skim milk, permeate, and retentate samples collected during the process were analyzed using an infrared spectrophotometer to check if the system was running normally. Ash (AOAC, 2000; method 945.46; 33.2.10), TS (AOAC, 2000; method 990.20; 33.2.44), total nitrogen TN (AOAC, 2000; method 991.20; 33.2.11), nonprotein nitrogen NPN (AOAC, 2000; method 991.21; 33.2.12), and noncasein nitrogen NCN (AOAC, 2000; method 998.05; 33.2.64) were determined in skim milk, retentate, and
permeate samples for each stage and during the shelf-life of HC-MC treatments. NCN was subtracted from TN and multiplied by 6.38 to calculate the CN, NPN was subtracted from TN and multiplied by 6.38 to calculate true protein (TP), and NPN was subtracted from NCN and multiplied by 6.38 to calculate the SP content.

**SP Removal**

A mass-balance was conducted to determine the efficiency of SP removal from skim milk. The mass of SP in permeate was divided by the mass of SP in the skim milk and multiplying by 100 to calculate the percentage of SP removal of a given stage in the MF process. The mass of SP in permeate was calculated by multiplying the weight of removed permeate by the SP concentration in this permeate, while the total mass of SP present in skim milk was calculated by multiplying the weight of skim milk by the % SP in the skim milk.

**Capillary Gel Electrophoresis (CGE)**

The protein fractions in the skim milk, permeate, and HC-MC were determined using capillary gel electrophoresis (CGE). Samples with high protein content were diluted to approximately 1% protein with distilled water. Subsequently, 10 µL of each diluted sample, 85 µL of sample buffer (ProteomeLab™ SDS-MW Analyses Kit, Beckman-Coulter), and 5 µL of β-mercaptoethanol were pipette into a PCR-vial (Fisher Scientific CO LLC, Florence, KY) and heated in a water bath at 90°C for 15 min. The samples were analyzed with the CGE (Beckman P/ACE MIDQ, Beckman-Coulter, Fullerton, CA, USA) equipped with a UV detector set at 214 nm. The test was performed using a 50 µm bare fused silica capillary (20.2 cm effective length from the inlet to the detection window). Solution and reagents were obtained as a part of the ProteomeLab™
SDS-MW Analyses Kit (Beckman-Coulter) that is designed for the separation of protein SDS complexes using a replaceable gel matrix. The gel is formulated to provide an effective sieving range of approximately 10 to 225 kDa. An SDS-MW size standard (recombinant proteins 10-225 kDa supplied with the ProteomeLabTM SDS-MW Analyses Kit) was used to estimate the molecular weight of the proteins in each sample. A capillary preconditioning method (basic rinse-0.1N NaOH-5 min-50 psi, acidic rinse-0.1N HCl-2 min-50 psi, distilled water rinse-2 min-50 psi, and SDS gel rinse-10 min-40 psi) was run every three samples, and then the sample was electrokinetically introduced at 5 kV for 20 sec. The separation was done using the following conditions: a constant voltage of 15 kV, temperature 25°C, and 20 bar pressure with reverse polarity in the SDS molecular weight gel buffer. Molecular weight standards (ProteomeLab and Beckman-Coulter) and available pure milk protein fractions (Sigma, USA) were also separated using the method as described above to calculate migration times.

The migration time of the peaks resulting from the capillary electropherogram was compared to molecular weight standards and pure standard samples to identify the peaks. Also, the peaks were compared to those results reported by other researchers (Creamer and Richardson, 1984; Miralles et al., 2000, 2003; Anema, 2009; Salunke, 2013). The area of each identified peak was calculated from the electropherogram using a valley-to-valley approach, as described by Miralles et al. (2003). The area of each identified individual CN fraction (such as, αS₁, αS₂, β, κ, and γ-CN), SP fractions (such as α-LA, β-LG), and peptides (peaks between 10-20 kDa) was calculated as a percentage of total area (positive peaks) to compare with the Kjeldahl analyses.
**Microbiological Analyses**

All HC-MC treatments were analyzed for total aerobic bacterial, Coliform, yeast, and mold at 0, 30, and 60 days of the storage period. The samples were stored at 4°C during the shelf-life testing. The samples were packed in sterile 45-ml plastic hinged lid vials (model 3040-00; Thermo Scientific-Capitol Vial Inc.). Method 6.040; was used to determine the total aerobic bacteria, method 7.074; was used to determine the coliform count, and method 8.115; was used to determine yeast and mold count (Wehr et al., 2004). Petrifilms (3M, Marshfield, WI) were utilized for the total aerobic bacterial count, Coliform count, yeast, and mold count. A sterile phosphate buffer was used for dilutions (Weber Scientific, Hamilton, NJ). Coliform petrifilm plates were incubated at 32°C ± 1°C for 24 h ± 2 h, total count petrifilms were incubated at 32 ± 1°C for 48 h ± 3 h, yeast and mold petrifilm plates were incubated at 25°C ± 1°C for 5 d. All petrifilms were counted and rounded, as described by Wehr and Frank (2004).

**Shelf-life Study**

Samples were analyzed chemically (Ash, TN, NCN, NPN, TS, and CGE) and microbiologically (aerobic count, coliform, yeast, and mold count using 3M Petrifilm) every 30 days for 60 days of storage period. The end of shelf-life was defined as total bacterial count > 20,000 cfu/ml (> 4.3 log cfu/mL) as described by Amelia and Barbano (2013).
RESULTS AND DISCUSSION

Composition of Skim Milk

The composition of pasteurized skim milk used to produce HC-MC is shown in Table 1. There was a small amount of variation (0.12, 0.19, 0.02, 0.01, 0.17, 0.03, and 0.04% SD for the TS, TN, NCN, NPN, CN, SP, and ash content, respectively), among the three replicates. The CN as a percentage of TN (CN%TN) and the CN%TP were typical (Beckman et al., 2010; Hurt and Barbano, 2010; Adams and Barbano, 2013) and ranged from 79.23 to 80.45% for CN%TN and from 84.15 to 85.06% for CN%TP. The skim milk was pasteurized at 76°C for 16 s. Temperatures over 70°C can lead to interactions between β-LG and κ-CN through disulfide bonding (Singh, 1995). Consequently, an elevated pasteurization temperature can decrease the SP available for removal during MF (Hurt and Barbano, 2010).

Composition of Permeate

The composition of permeate from each stage of MF is shown in Table 2. The TS, TN, NPN, and SP content of the permeate significantly decreased (P < 0.05) with each subsequent stage of MF. The SP concentrations in the permeate were 0.33, 0.26, and 0.15% for the first, second, and third stages, respectively. The SP content in the first stage permeate (0.33%) was lower than SP in the permeate portion of the pasteurized skim milk (0.47%). This was primarily due to some rejection of SP by the membrane. It has been reported by Tremblay-Marchand et al. (2016) that the permeate of the first stage contained 0.35% SP resulted from MF skim milk (0.49% SP) using GP ceramic membranes. As calculated using the method described by Hurt and Barbano (2010),
permeate of the first, second, and third stages should contain 0.49, 0.26, and 0.16% of SP, respectively (Zulewska and Barbano, 2014). In our study, the permeate SP in the first, second, and third stages were 0.33, 0.26, and 0.15%, respectively. The differences in the permeate of MF first stage could be due to the skim milk composition (3.2% TP in Hurt study vs. 3.1% TP in our study) as well as the type of membrane (UTP membrane in Hurt study vs. GP membranes in our study). However, no difference was found (P > 0.05) between SP content in the permeate of the second and third stage as compared to Hurt and Barbano calculations. Also, Zulewska and Barbano (2014) reported that the SP in the permeate of the first, second, and third stages was 0.51, 0.25, and 0.13%, respectively, using 0.1-μm ceramic GP membranes. The processing conditions (such as the flux; 72.49 kg/m² per hour) used by Zulewska and Barbano (2014) were different compared to our study (71.42 kg/m² per hour), and this could affect the SP removal, especially in the first stage. However, the SP removal in the second and third stage was similar to our study. Another study has been reported that the SP in the permeate of the first, second, and third stages was 0.35, 0.13, and 0.06%, respectively (Tremblay-Marchand et al., 2016) using GP membranes 3-stage, 3× CF with DF (flux was 90 kg/ m² per hour, 115.4 kg/ m² per hour, and 139.3 kg/ m² per hour in the first, second, and third stages, respectively). The ash content decreased significantly (P < 0.05) between the first and second stage, however, the ash content decreased in the third stage but not significantly (P > 0.05) different from the second stage. This could be due to the majority of the remaining ash content after the second stage being bound within the CN micelle, which is rejected by the membrane.
**Composition of Retentate**

The retentate composition from 3-stages MF is shown in Table 3. The TS and TN content decreased between stage 1 and stage 2 due to the passage of soluble minerals, lactose, and SP through the membrane (Zulewska and Barbano, 2014). The TS, TN, TP, CN, and ash values of the retentate were significantly high (P < 0.05) in the third stage compared to the first and second stages due to the rejection of CN by the membrane, which resulted in an increase the TS, TN, and TP in the third stage. Since 2/3 of the ash is bound to the CN (Hurt and Barbano, 2010), the ash content in the third stage increased with increasing the CN content. The SP content in the retentate decreased (P < 0.05) with each successive stage of MF due to their passage through the membrane into the permeate. As expected, the CN%TP and TN%TS increased significantly (P < 0.05) with each subsequent stage of MF. The CN%TP increased from 84.66% in the skim milk to approximately 98% in the final retentate (HC-MC).

**SP removal**

Theoretical SP removal from skim milk during the 3-stages of MF, the cumulative percentage of SP removal during each stage, and the stage CF are shown in Table 4. Theoretically, the cumulative SP removal from skim milk is 67.8, 89.8, and 97.8% in the first, second, and third stages, respectively (Hurt and Barbano, 2010). The actual percentage of SP removal in our study was 46.20, 77.20, and 83.10% in the first, second, and third stages, respectively. It was expected that the theoretical SP removal would be higher than the actual SP removal from skim milk because theoretically, SP is not rejected by the membrane and completely pass through the membrane. Hurt and Barbano (2010) have reported that the percentage of cumulative SP removal from MF skim milk
(CN%TP=85.00%) is 56, 74, and 80% in the first, second, and third stage, respectively, using UTP membranes. We hypothesize that the differences in the composition of skim milk and type of membrane could affect the SP in the permeate. Tremblay-Marchand et al. (2016) reported that the cumulative SP removal in the first, second, and third stages was 47.0, 73.8, and 82.3%, respectively, using GP MF system which is similar to our results.

The concentration factor (CF) is the ratio of feed mass for each stage to the retentate mass of the same stage. In practice, there was variation or limitation in the ability of the system, but the CF was close to the targeted values of 3× (for the first and second stage) and 5x (for the third).

**Composition of HC-MC**

The composition of the HC-MC after manufacturing (d=0) and during 60 days of storage is shown in Table 5 and 6, respectively. Also, ANOVA with MS and P-values for the composition of HC-MC during shelf-life are shown in Table 7. As expected, no significant differences (P > 0.05) were found in the TS, TN, NCN, NPN, TP, CN, CN%TP, and TN%TS between treatments after processing (Table 5). However, there was a significant difference (P < 0.05) in the ash content between control and T2, which was expected due to the addition of sodium chloride and sodium citrate to T2. Addition of 1% sodium chloride in T1 and 1% sodium chloride and 1% sodium citrate in T2 should result in an increase in the TS content by 1% and 2% in T1 and T2, respectively, compared to control HC-MC. The ash content should also be higher in T1 and T2 by 1% and 2%, respectively, compared to control. The TS and ash contents were not as high as expected in T1 and T2 compared to the control due to the salts were mixed quickly in the HC-MC
at high temperature and before cooling, which did not disperse the salts evenly with the HC-MC after manufacturing. Also, there were substantial variations between replicates; however, we did not find significant differences between treatments in the TS content.

The NCN and NPN of HC-MC were monitored for 60 days to determine the proteolysis (Table 6). There was no significant difference (P > 0.05) between treatments in the NCN content; however, a significant difference (P < 0.05) was observed in the NCN content during 60 days of storage period at 4°C. This was expected due to degrading the β-CN by the proteolytic enzymes and produce γ-CN and small peptides which increase the NCN content. Also, the NPN increased slightly during the storage period but not significantly (P > 0.05). The NPN did not increase significantly (P > 0.05) during storage because NPN is considered a nutrient ingredient that can be easily metabolized by the bacteria (Amelia and Barbano, 2013). However, NPN showed significant differences (P < 0.05) between replicates (Table 7) due to the differences in the bacterial content in HC-MC and thereby different proteolysis level. It has been reported that the proteolysis increased in the milk during storage at 4°C (Bishop and White, 1985; Verdi et al., 1987; Paludetti et al., 2018) due to an increase in the proteolytic bacterial count. The activity of enzymes during storage could increase the fractions of NPN (i.e., amino acids and peptides) in milk and dairy products (Verdi et al., 1987; Paludetti et al., 2018).

Microbiological Analysis of HC-MC

The aerobic bacterial count (log cfu/mL) of HC-MC treatments during storage is shown in Table 8 and MS and P-values for the aerobic bacterial count shown in Table 9. The mean aerobic bacterial count of the HC-MC at d = 0 (i.e., immediately after
manufacturing) were 2.6, 2.5, and 2.8 log cfu/mL for control, T1 and T2, respectively (Table 6). Amelia and Barbano (2013) reported that the pasteurized MCC (18% protein and 22% TS) contained 2.1 log cfu/mL at day= 0. No significant difference (P > 0.05) was detected in the total aerobic bacterial count between treatments (Table 9) and this due to the differences in the source of milk, environment, and processing conditions. The mean aerobic bacterial count increased significantly (P < 0.05) in all treatments after 60 days of storage period at 4°C. Amelia and Barbano (2013) defined that the end of the shelf-life at which the aerobic bacterial count is over > 4.3 log cfu/mL, which is the legal limit for the shelf-life of pasteurized milk. This number is based on the Pasteurized Milk Ordinance (FDA, 2009); as a result, the end of the shelf-life of HC-MC in our study is 60 days when it stored at 4°C. Additionally, no coliform, yeast, or mold was detected (< 1 est) at any time point during the 60 days of storage.

**Protein Fractions**

**Skim milk and HC-MC**

A representative CGE electrophoreogram for skim milk is shown in Figure 2. Also, representative electrophoreograms of control, T1, and T2 HC-MC are shown in Figures 3, 4, and 5, respectively. Among the caseins fractions, the β-CN peak migrated first followed by αS₁-CN although the molecular weight of αS₁-CN is lower. Other researchers have reported similar results (Creamer and Richardson, 1984; Anema, 2009; Salunke, 2013). Creamer and Richardson (1984) reported that αS₁-CN has a reduced electrophoretic velocity due to its negatively charged regions, which extend its conformation in the presence of SDS thereby giving an increased apparent size and slower migration under SDS-PAGE conditions (Creamer and Richardson, 1984; Anema,
Although κ-CN has a low molecular weight as compared to other caseins, it eluted last after all other casein fractions. The late migration of κ-CN attributed to the glycosylation of κ-CN (Walstra and Jenness, 1984; Anema, 2009; Salunke et al., 2011). Any changes in the protein, such as hydrolysis, crosslinking, and heat-induced changes cause a change in molecular weight, and thereby, this will lead to change the peaks height and migration time.

The % of protein fractions in pasteurized skim milk and HC-MC (control) measured by capillary gel electrophoresis (CGE) are shown in Table 10. The percentage of peak areas in skim milk for CN fractions β-CN, αS₁-CN, αS₂-CN, κ-CN, and γ-CN determined by CGE were approximately 33.8, 35.2, 8.5, 4.3, 1.1%, respectively, whereas the percentage of major SP of β-LG and α-LA obtained were 9.9 and 5.2%, respectively, in skim milk. It has been reported that the CN fractions of the normal milk β-CN, αS₁-CN, αS₂-CN, κ-CN, and γ-CN were approximately 33.8, 34.4, 8.5, 8.5, and 3.0, respectively, and SP fractions of β-LG and α-LA percentage were 9.5 and 5.1%, respectively (Walstra and Jenness, 1984; Fox and McSweeney, 1998; Farrell et al., 2004), which is similar to our results. However, we noticed that the κ-CN determined by CGE is lower than we expected and this due to the carbohydrate moiety of κ-CN, which is hard to be detected by the UV detector (214 nm) in CGE. The percentage of peak areas in HC-MC for CN fractions β-CN, αS₁-CN, αS₂-CN, κ-CN, and γ-CN determined by CGE was approximately 37.0, 39.1, 8.6, 4.4, and 1.6%, respectively, while the percentage of major SP fractions β-LG and α-LA obtained were approximately 3.7 and 3.3, respectively, in HC-MC. The casein fractions of β-CN, αS₁-CN, and γ-CN were higher (P < 0.05) in HC-MC as compared to the skim milk due to the concentration of CN. It is noticeable from
Table 10 and the electrophoreograms of skim milk and HC-MC (Figure 2 and 3, respectively) that the β-LG and α-LA are significantly higher (P < 0.05) in skim milk relative to HC-MC. This is related to removing SP in the permeate, which resulted in decreasing β-LG and α-LA in the HC-MC. No differences in αS2-CN and κ-CN (P > 0.05) were detected between skim milk and HC-MC. Increasing the γ-CN in HC-MC compared to skim milk could result from the potential proteolysis by the bacteria during the two days of manufacturing. The percentage of CN and WP in skim milk are 80 and 20%, respectively, determined by gel electrophoresis (Walstra and Jenness, 1984), however variations have been reported in other literature and ranges from 74 to 86% for CN and from 14 to 26% for WP (Walstra and Jenness, 1984; Fox and McSweeney, 1998; Farrell et al., 2004). The percentage of CN%TP in skim milk using CGE was 84.61%, which is similar to the value of 84.66% (Kjeldahl analysis) reported earlier in Table 1.

Permeate

The protein fractions of the permeate created during MF processing while making HC-MC are shown in Table 11. The percentages of β-LG and α-LA content were calculated in the permeate of each stage. The percentage of β-LG and α-LA in the first stage were approximately 70.0 and 26.9%, respectively. Zulewska et al. (2009) reported that the percentage of β-LG and α-LA in MF permeate were 76.3 and 23.7 %, respectively, in the first stage with a 3× CF using GP ceramic membranes. The percentage of β-LG and α-LA in the second stage were 75.04 and 20.8%, respectively, while they were 72.1 and 22.3% in the third stage. It has been reported that the relative percentage of β-LG to β-LG plus α-LA in permeate were 71.5, 73.0, and 74.6% in the first, second, and third stages, respectively, with a 3× CF using Isoflux membranes.
(Adams and Barbano, 2013). The relative percentage of β-LG to β-LG plus α-LA in the permeate of our study were 72.9, 78.2, and 76.4% in the first, second, and third stages, respectively, which are close to Adams and Barbano values. These small differences can be related to the composition of skim milk, processing conditions, type of membranes, or experimental error, such as temperatures, flux, CF, and DF. Peptides increased but not significantly (P > 0.05) from 3.09% in the first stage to 5.6% in the third stage due to increasing the removal of small peptides in the permeate. Small particles of β-CN passed through the membrane in the second stage, which resulted in the presence of a small amount of CN in the permeate of the second stage. It has been reported that the permeate of the first stage MF had a 4.93% CN using GP ceramic membranes and 1.23% using UTP membranes (Zulewska et al., 2009). The retentate after each stage of MF was kept at 4°C. We hypothesize that β-CN (size < 0.1 µm) is separated from the CN micelles at 4°C with the addition of DF water. However, heating the retentate to 50°C before MF processing is not enough for the β-CN to be completely assembled with the CN micelles. As a result, some fractions of the unassembled β-CN are passed through the membrane during MF, which led to the present trace amount of CN in the permeate of the second stage. Also, this amount resulted in the permeate due to drops of skim milk or retentate unconsciously dropped into the permeate during sampling.

**The protein fractions of HC-MC**

The protein fractions of HC-MC treatments after processing (at d=0) are shown in Table 12. No significant difference (P > 0.05) was detected between control, T1 and T2 in the protein fractions content at d=0. Similar results of the protein fractions in MCC have been reported (Salunke, 2013). The protein fractions of HC-MC during the shelf-life
are shown in Figure 6. No significant differences were observed between control, T1, and T2 at 0, 30, and 60 days of storage. It was expected that γ-CN are produced from β-casein by proteolysis by plasmin; an indigenous proteinase in milk (Fox et al., 2015) or produced by psychrotrophic microorganism during storage at low temperatures (Nielsen, 2002). The proteolysis or increase in NCN did not detect by the CGE because it measured the individual protein fractions resulted from proteolysis, which is less sensitive to proteolysis compared to the Kjeldahl method. However, there was some proteolysis (Figure 6) as shown in T1 at 60 d of storage at 4°C. Thus, T1 at 60 d showed lower levels of β-CN and a higher level of peptides, which referred to the proteolysis. This is due to the bacteria, which produce enzymes that degraded β-casein.

**CONCLUSIONS**

The process to produce HC-MC with a long refrigerated shelf-life was developed. This study determined that HC-MC can be manufactured using ceramic GP MF system with over 25% TS and greater than 95% CN%TP. No significant differences (P > 0.05) were detected between the compositions of treatments after manufacturing (at d=0) and during the 60 days of storage period at 4°C. However, the NCN content increased significantly (p < 0.05) during the 60 days of storage, which referred to as increasing the proteolysis. The total aerobic bacteria count increased significantly (P < 0.05) during 60 days of storage at 4°C. The impact of the small increase in NCN and NPN in all treatments during 60 days of storage on process cheese characteristics will be evaluated in subsequent studies.
REFERENCES


### Table 1. Mean \((n = 3)\) composition of pasteurized skim milk \((\% \text{ by weight})\)

<table>
<thead>
<tr>
<th>Replicate</th>
<th>TS</th>
<th>TN</th>
<th>NCN</th>
<th>NPN</th>
<th>CN</th>
<th>SP</th>
<th>Ash</th>
<th>CN%TN</th>
<th>CN%TP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>8.79</td>
<td>3.10</td>
<td>0.64</td>
<td>0.20</td>
<td>2.46</td>
<td>0.44</td>
<td>0.74</td>
<td>79.23</td>
<td>84.78</td>
</tr>
<tr>
<td>1</td>
<td>8.94</td>
<td>3.48</td>
<td>0.68</td>
<td>0.18</td>
<td>2.80</td>
<td>0.49</td>
<td>0.66</td>
<td>80.45</td>
<td>85.06</td>
</tr>
<tr>
<td>2</td>
<td>9.04</td>
<td>3.26</td>
<td>0.66</td>
<td>0.17</td>
<td>2.60</td>
<td>0.49</td>
<td>0.67</td>
<td>79.57</td>
<td>84.15</td>
</tr>
<tr>
<td>3</td>
<td>Mean</td>
<td>8.92</td>
<td>3.28</td>
<td>0.66</td>
<td>0.19</td>
<td>2.62</td>
<td>0.47</td>
<td>80.75</td>
<td>84.66</td>
</tr>
<tr>
<td></td>
<td>0.12</td>
<td>0.19</td>
<td>0.02</td>
<td>0.01</td>
<td>0.17</td>
<td>0.03</td>
<td>0.04</td>
<td>0.62</td>
<td>0.028</td>
</tr>
</tbody>
</table>

TS= total solids; TN = total nitrogen \(\times 6.38\); NCN = noncasein nitrogen \(\times 6.38\); NPN = nonprotein nitrogen \(\times 6.38\); CN = Casein \((\text{TN} – \text{NCN})\); SP = Serum protein \((\text{NCN} – \text{NPN})\); CN%TN = CN as a percentage of TN; CN%TP = CN as a percentage of TP
Table 2. Mean (n = 3) composition of permeates (% by weight) from each stage of a 3-stage Graded Permeability (GP) ceramic membranes microfiltration (MF) System 0.1 µm from skim milk

<table>
<thead>
<tr>
<th>MF Stage</th>
<th>Composition (% by weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TS</td>
<td>TN</td>
</tr>
<tr>
<td>1</td>
<td>6.00&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>2.02&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>1.60&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>SEM</td>
<td>0.70</td>
</tr>
<tr>
<td>R&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0.99</td>
</tr>
</tbody>
</table>

<sup>a</sup><sup>c</sup>Means in the same column not sharing a common superscript are different (P< 0.05).

TS= total solids; TN = total nitrogen × 6.38; NPN = nonprotein nitrogen × 6.38; SP = Serum protein (TN-NPN)
Table 3. Mean (n = 3) composition of retentates (% by weight) from each stage of the 3-stage Graded Permeability (GP) ceramic microfiltration (MF) system 0.1 µm from skim milk

<table>
<thead>
<tr>
<th>MF stage</th>
<th>TS</th>
<th>TN</th>
<th>NCN</th>
<th>NPN</th>
<th>TP</th>
<th>CN</th>
<th>SP</th>
<th>Ash</th>
<th>CN%TP</th>
<th>TN%TS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>14.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.14&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.92&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.07&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.85&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>89.23&lt;sup&gt;c&lt;/sup&gt;</td>
<td>57.75&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>9.64&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.66&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.09&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.96&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.38&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.58&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.78&lt;sup&gt;c&lt;/sup&gt;</td>
<td>91.65&lt;sup&gt;b&lt;/sup&gt;</td>
<td>73.21&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>25.41&lt;sup&gt;a&lt;/sup&gt;</td>
<td>21.75&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.55&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.09&lt;sup&gt;b&lt;/sup&gt;</td>
<td>21.65&lt;sup&gt;a&lt;/sup&gt;</td>
<td>21.20&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.45&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>97.92&lt;sup&gt;a&lt;/sup&gt;</td>
<td>85.59&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>SEM</td>
<td>2.00</td>
<td>1.93</td>
<td>0.060</td>
<td>0.017</td>
<td>1.94</td>
<td>1.95</td>
<td>0.05</td>
<td>0.15</td>
<td>1.43</td>
<td>5.05</td>
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<tr>
<td>R&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0.98</td>
<td>0.99</td>
<td>0.96</td>
<td>0.93</td>
<td>0.99</td>
<td>0.99</td>
<td>0.93</td>
<td>0.98</td>
<td>0.98</td>
<td>0.98</td>
</tr>
</tbody>
</table>

<sup>a-d</sup>Means in the same column not sharing a common superscript are different (P< 0.05).

TS= total solids; TN = total nitrogen × 6.38; NCN = noncasein nitrogen × 6.38; NPN = nonprotein nitrogen × 6.38; TP = true protein (TN – NPN); CN = Casein (TN – NCN); SP = TP – casein; CN%TP = CN as a percentage of TP; TN%TS = TN as a percentage of TS
Table 4. Mean (n = 3) serum protein (SP) removal as a percentage of SP in starting skim milk determined by Kjeldahl analysis of NPN and NCN during each stage and concentration factor (CF) of a 3-stage GP ceramic microfiltration (MF) system 0.1 µm from skim milk

<table>
<thead>
<tr>
<th>MF stage</th>
<th>Theoretical SP removal(^1)</th>
<th>Cumulative SP removal</th>
<th>CF</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>67.78</td>
<td>46.20(^\text{c})</td>
<td>3.01(^\text{b})</td>
</tr>
<tr>
<td>2</td>
<td>89.78</td>
<td>77.20(^\text{b})</td>
<td>3.01(^\text{b})</td>
</tr>
<tr>
<td>3</td>
<td>97.78</td>
<td>83.10(^\text{a})</td>
<td>5.12(^\text{a})</td>
</tr>
<tr>
<td>SEM</td>
<td>5.75</td>
<td>0.41</td>
<td></td>
</tr>
<tr>
<td>R(^2)</td>
<td>0.98</td>
<td>0.71</td>
<td></td>
</tr>
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</table>

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

\(^1\) Data in this column represent theoretical SP removal, assuming no rejection of serum proteins and complete rejection of casein.

\[%SP \text{ removal of each stage} = \left(\frac{\%SP \text{ in Permeate of each stage} \times \text{the amount of Permeate}}{\%SP \text{ in the feed} \times \text{the amount of feed}}\right) \times 100\]

CF= concentration factor
Table 5. Mean (n = 3) composition (% by weight) of the high concentrated micellar casein (HC-MC) of all treatments measured after manufacturing

<table>
<thead>
<tr>
<th>Treatments</th>
<th>TS</th>
<th>TN</th>
<th>NCN</th>
<th>NPN</th>
<th>TP</th>
<th>CN</th>
<th>Ash</th>
<th>CN%TP</th>
<th>TN%TS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>25.41</td>
<td>21.75</td>
<td>0.55</td>
<td>0.09</td>
<td>21.65</td>
<td>21.20</td>
<td>2.00b</td>
<td>97.89</td>
<td>85.60</td>
</tr>
<tr>
<td>T1</td>
<td>25.62</td>
<td>21.23</td>
<td>0.55</td>
<td>0.08</td>
<td>21.14</td>
<td>20.67</td>
<td>2.26ab</td>
<td>97.80</td>
<td>82.86</td>
</tr>
<tr>
<td>T2</td>
<td>26.13</td>
<td>21.15</td>
<td>0.55</td>
<td>0.13</td>
<td>21.02</td>
<td>20.60</td>
<td>2.52a</td>
<td>98.02</td>
<td>80.95</td>
</tr>
<tr>
<td>SEM</td>
<td>0.48</td>
<td>0.38</td>
<td>0.00</td>
<td>0.01</td>
<td>0.38</td>
<td>0.46</td>
<td>0.09</td>
<td>0.05</td>
<td>0.85</td>
</tr>
</tbody>
</table>

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

TS= total solids; TN = total nitrogen × 6.38; NCN = noncasein nitrogen × 6.38; NPN = nonprotein nitrogen × 6.38; TP = true protein (TN − NPN); CN = TN − NCN; CN%TP = CN as a percentage of TP; TN%TS = TN as a percentage of TS

Control= HC-MC; T1= HC-MC + 1% salt; T2= HC-MC+ 1% salt and 1% sodium citrate
Table 6. Mean (n = 3) composition (% by weight) of the high concentrated micellar casein (HC-MC) measured over 60 days of storage period at 4°C

<table>
<thead>
<tr>
<th>Composition (% by weight)</th>
<th>Time</th>
<th>Treatments</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>T1</td>
</tr>
<tr>
<td>NCN</td>
<td>0</td>
<td>0.55</td>
<td>0.55</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>0.76</td>
<td>0.82</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>0.66</td>
<td>0.68</td>
</tr>
<tr>
<td>NPN</td>
<td>0</td>
<td>0.09</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>0.12</td>
<td>0.13</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>0.10</td>
<td>0.11</td>
</tr>
</tbody>
</table>

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

NCN = noncasein nitrogen × 6.38; NPN = nonprotein nitrogen × 6.38

Control = HC-MC; T1 = HC-MC + 1% salt; T2 = HC-MC + 1% salt and 1% sodium citrate
Table 7. Mean squares and P-values (in parentheses) of the high concentrated micellar casein (HC-MC) measured over 60 days of storage period at 4°C

<table>
<thead>
<tr>
<th>Factor</th>
<th>df</th>
<th>NCN</th>
<th>NPN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replication</td>
<td>2</td>
<td>0.021 (0.26)</td>
<td>0.0099 (0.021)*</td>
</tr>
<tr>
<td>Treatment$^1$</td>
<td>2</td>
<td>0.012 (0.22)</td>
<td>0.0012 (0.51)</td>
</tr>
<tr>
<td>Time$^2$</td>
<td>1</td>
<td>0.38 (0.000023)**</td>
<td>0.0035 (0.18)</td>
</tr>
<tr>
<td>Treatment x Time</td>
<td>2</td>
<td>0.012 (0.23)</td>
<td>0.0009 (0.61)</td>
</tr>
<tr>
<td>Error</td>
<td>10</td>
<td>0.007</td>
<td>0.001</td>
</tr>
</tbody>
</table>

*Statistically significant at P < 0.05
$^1$Treatment= Control, T1, and T2
$^2$Time= 0, 30, and 60 days of storage
Table 8. Mean (n = 3) log of total aerobic bacterial count (cfu/mL) of the high concentrated micellar casein (HC-MC) measured every 30 days over 60 days of storage period at 4°C

<table>
<thead>
<tr>
<th>Total aerobic bacterial count</th>
<th>Time</th>
<th>Treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>log cfu/mL</td>
<td>0</td>
<td>2.63</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>3.53</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>4.33</td>
</tr>
<tr>
<td>SEM</td>
<td></td>
<td>0.45</td>
</tr>
</tbody>
</table>

Control= HC-MC; T1= HC-MC + 1% salt; T2= HC-MC+ 1% salt and 1% sodium citrate
Table 9. Mean squares and P-values (in parentheses) of total aerobic bacterial count (cfu/mL) of the high concentrated micellar casein (HC-MC) measured every 30 days over 60 days of storage period at 4°C

<table>
<thead>
<tr>
<th>Factor</th>
<th>df</th>
<th>Total aerobic bacterial count (log cfu/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replication</td>
<td>2</td>
<td>3.06 (0.08)</td>
</tr>
<tr>
<td>Treatment(^1)</td>
<td>2</td>
<td>1.17 (0.35)</td>
</tr>
<tr>
<td>Time(^2)</td>
<td>2</td>
<td>8.75 (0.003)**</td>
</tr>
<tr>
<td>Treatment x Time</td>
<td>4</td>
<td>0.32 (0.87)</td>
</tr>
<tr>
<td>Error</td>
<td>16</td>
<td>1.05</td>
</tr>
</tbody>
</table>

*Statistically significant at \( P < 0.05 \)

\(^1\)Treatment= Control, T1, and T2

\(^2\)Time= 0, 30, and 60 days of storage
Table 10. Mean (n = 3) relative protein fractions measured by using capillary gel electrophoresis of pasteurized skim milk and the high concentrated micellar casein (HC-MC)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>β-CN(^1)</th>
<th>α(_\text{S1})-CN(^1)</th>
<th>α(_\text{S2})-CN(^1)</th>
<th>κ-CN(^1)</th>
<th>γ-CN(^1)</th>
<th>α-LA(^2)</th>
<th>β-LG(^2)</th>
<th>Peptide(^3)</th>
<th>CN</th>
<th>SP</th>
<th>NPN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skim milk</td>
<td>33.76(^b)</td>
<td>35.24(^b)</td>
<td>8.5</td>
<td>4.31</td>
<td>1.13(^b)</td>
<td>5.17(^a)</td>
<td>9.91(^a)</td>
<td>1.96(^b)</td>
<td>82.95(^b)</td>
<td>15.08(^a)</td>
<td>1.96</td>
</tr>
<tr>
<td>HC-MC</td>
<td>36.97(^a)</td>
<td>39.15(^a)</td>
<td>8.59</td>
<td>4.40</td>
<td>1.63(^a)</td>
<td>3.30(^b)</td>
<td>3.75(^b)</td>
<td>2.19(^a)</td>
<td>90.74(^a)</td>
<td>7.06(^b)</td>
<td>2.19</td>
</tr>
<tr>
<td>SEM</td>
<td>0.85</td>
<td>0.91</td>
<td>0.19</td>
<td>0.10</td>
<td>0.11</td>
<td>0.48</td>
<td>1.38</td>
<td>0.27</td>
<td>1.78</td>
<td>1.82</td>
<td>0.27</td>
</tr>
<tr>
<td>SD</td>
<td>0.70</td>
<td>0.91</td>
<td>0.01</td>
<td>0.03</td>
<td>0.92</td>
<td>0.73</td>
<td>0.98</td>
<td>0.03</td>
<td>0.95</td>
<td>0.96</td>
<td>0.03</td>
</tr>
</tbody>
</table>

\(^{a,b}\) Means in the same column not sharing a common superscript are different (P< 0.05).

\(^1\) Each fraction calculated as a percentage of total casein (CN) area.

\(^2\) Each fraction calculated as a percentage of total serum protein (SP) area.

\(^3\) Peptides = peptide peaks (10-20 kD) other than α-LA and β-LG.
Table 11. Mean (n = 3) relative protein fractions measured by using capillary gel electrophoresis of permeate during 3-stages of MF processing from skim milk

<table>
<thead>
<tr>
<th>Stage</th>
<th>β-CN&lt;sup&gt;1&lt;/sup&gt;</th>
<th>α-LA&lt;sup&gt;2&lt;/sup&gt;</th>
<th>β-LG&lt;sup&gt;2&lt;/sup&gt;</th>
<th>Peptides&lt;sup&gt;3&lt;/sup&gt;</th>
<th>CN</th>
<th>SP</th>
<th>NPN</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>26.87&lt;sup&gt;a&lt;/sup&gt;</td>
<td>70.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.09</td>
<td>96.91</td>
<td>3.09</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.83</td>
<td>20.85&lt;sup&gt;b&lt;/sup&gt;</td>
<td>75.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.28</td>
<td>0.83</td>
<td>95.89</td>
<td>3.28</td>
</tr>
<tr>
<td>3</td>
<td>22.28&lt;sup&gt;b&lt;/sup&gt;</td>
<td>72.10&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>5.62</td>
<td>94.38</td>
<td>5.62</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SEM</td>
<td>1.00</td>
<td>0.95</td>
<td>0.55</td>
<td>0.52</td>
<td>0.55</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0.82</td>
<td>0.58</td>
<td>0.54</td>
<td>0.48</td>
<td>0.54</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a-b</sup>Means in the same column not sharing a common superscript are different (P< 0.05).

<sup>1</sup>Each fraction calculated as a percentage of total casein (CN) area

<sup>2</sup>Each fraction calculated as a percentage of total serum protein (SP) area

<sup>3</sup>Peptides= peptide peaks (10-20 kD) other than α-LA and β-LG
Table 12. Mean (n = 3) relative protein fractions measured by using capillary gel electrophoresis of the high concentrated micellar casein (HC-MC)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$\beta$-CN$^1$</th>
<th>$\alpha_S$1-CN$^1$</th>
<th>$\alpha_S$2-CN$^1$</th>
<th>$\kappa$-CN$^1$</th>
<th>$\gamma$-CN$^1$</th>
<th>$\alpha$-LA$^2$</th>
<th>$\beta$-LG$^2$</th>
<th>Peptides$^3$</th>
<th>CN</th>
<th>SP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>36.97</td>
<td>39.15</td>
<td>8.59</td>
<td>4.40</td>
<td>1.63</td>
<td>3.30</td>
<td>3.75</td>
<td>2.19</td>
<td>90.74</td>
<td>7.06</td>
</tr>
<tr>
<td>T1</td>
<td>37.80</td>
<td>39.49</td>
<td>7.78</td>
<td>3.65</td>
<td>1.98</td>
<td>4.07</td>
<td>3.33</td>
<td>1.98</td>
<td>90.72</td>
<td>7.40</td>
</tr>
<tr>
<td>T2</td>
<td>37.25</td>
<td>38.51</td>
<td>7.52</td>
<td>4.42</td>
<td>2.82</td>
<td>3.73</td>
<td>3.75</td>
<td>1.88</td>
<td>90.53</td>
<td>7.48</td>
</tr>
<tr>
<td>SEM</td>
<td>0.50</td>
<td>0.29</td>
<td>0.43</td>
<td>0.21</td>
<td>0.29</td>
<td>0.34</td>
<td>0.19</td>
<td>0.13</td>
<td>0.21</td>
<td>0.26</td>
</tr>
<tr>
<td>SD</td>
<td>1.49</td>
<td>0.89</td>
<td>1.31</td>
<td>0.63</td>
<td>0.87</td>
<td>1.03</td>
<td>0.57</td>
<td>0.38</td>
<td>0.64</td>
<td>0.78</td>
</tr>
</tbody>
</table>

No significant difference was detected at P< 0.05.

$^1$Each fraction calculated as a percentage of total casein (CN) area

$^2$Each fraction calculated as a percentage of total serum protein (SP) area

$^3$Peptides= peptide peaks (10-20 kD) other than $\alpha$-LA and $\beta$-LG
**Figure 1:** Schematic process diagram of manufacturing high solids micellar casein concentrate (HC-MC). CF = concentration factor; DF = diafiltration.
Figure 2. Capillary gel electrophoreogram (CGE) of skim milk
Figure 3. Capillary gel electrophoreogram (CGE) of HC-MC (Control)
Figure 4. Capillary gel electrophoreogram (CGE) of HC-MC (T1)
Figure 5. Capillary gel electrophoreogram (CGE) of HC-MC (T2)
Figure 6. Mean (n = 3) relative protein fractions observed in capillary gel electrophoresis of the high concentrated micellar casein (HC-MC) during 60 days of storage at 4°C.
CHAPTER III: EFFECT OF STORAGE OF HIGH CONCENTRATED MICELLAR CASEIN ON THE FUNCTIONAL PROPERTIES OF PROCESS CHEESE PRODUCTS

INTRODUCTION

Process cheese (PC) and process cheese products (PCP) are dairy foods manufactured by mixing dairy ingredients (such as natural cheese, protein concentrates, butter, NFDM, whey powder, and permeate) with nondairy ingredients (such as sodium chloride, water, emulsifying salts, color, mold inhibitors, and flavors) and then heating the mixture with continuous agitation to produce a homogeneous product with a long shelf-life (Meyer, 1973; Thomas, 1973; Guinee, 2007; Kapoor and Metzger, 2008; Kammerlehner, 2009). The PC and PCP are categorized based on the composition and permitted ingredients that utilized in making these types of cheese (Code of Federal Regulations, 2004), thus PCP contains not permitted ingredients or do not meet the typical composition of the standard cheese listed in the Code of Federal Regulations (CFR) (Lu et al., 2007). 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. PC is one of the leading varieties of cheese in the world and has several applications as an ingredient (Sorensen, 2001; Kapoor and Metzger, 2008).

The principle of making PC and PCP is the calcium sequestration by using emulsifying salts (sodium citrate, disodium phosphate, etc.). Emulsifying salts are critical
for the functional characteristics of PC due to their role in improving the emulsification characteristics of casein by sequestrating the calcium phosphate complexes from the insoluble calcium-paracaseinate-phosphate network in natural cheese or aggregated casein network in casein containing ingredients. As a result, the major molecular forces that cross-link the various monomers of casein in the network are disrupted by the calcium sequestrating ingredients. 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 presence of mixing and heating.

Intact casein is referred to as the non hydrolyzed CN, and it is the most critical property in PC formulations. The source of intact casein (such as natural cheese and rennet casein) is selected depending on type, flavor, maturity, consistency, texture, and pH (Zehren and Nusbaum, 2000) due to its effects on the final PC and PCP characteristics. All hydrophilic and hydrophobic casein fractions are bound together to form the stable casein micelles. When proteolysis occurs in cheese or any source of casein, small peptides and free amino acids (soluble or hydrophilic portion) are separated from the casein micelles, which results in separation the hydrophilic and hydrophobic portions and thereby affecting the functional properties of PC and PCP. The intact CN is high in fresh cheese and decreases during the ripening of cheese because of the proteolysis (Purna et al., 2006). Aged natural cheese (less intact casein) in making PC and PCP formulations 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). The amount of intact casein in cheese, its pH, and calcium to CN ratio affect the extent of casein hydration during PC and PCP manufacturing, which influence the emulsification degree, CN aggregation degree, and elasticity of PC (Berger et al., 1998; Guinee, 2007; Guinee, 2004). Consequently, processors usually balance the ratio of young and aged cheese to have the optimum functionality in the final PC.

Micellar casein concentrate (MCC) is a high protein ingredient that is used in many applications. When skim milk is microfiltered through MF membrane (0.1 μm), caseins and casein-bound minerals are retained by the membrane while SP, lactose, and unbound minerals pass through into the permeate. The typical composition of liquid MCC using a GP MF (3-stage, and 3× CF with DF) is >9% true protein (TP) and >13% total solids (TS) (Zulewska et al., 2009). This MCC can be further concentrated to increase the TP and TS to 18% and 22%, respectively, by using 2.2× CF UF followed by 3-stage 3× CF with DF, and finally UF for more concentration (Amelia and Barbano, 2013). The MCC can be dried to produce MCC powder with a long shelf-life (Amelia, 2012). The dried MCC can contain up to 84 % total protein and 96 % TS (Nasser et al., 2018).

High concentrated micellar casein (HC-MC) is a good source of intact casein and has unique characteristics, such as water-binding, emulsifying, whipping, foaming, and texture properties. Due to these properties, HC-MC is utilized in a range of commercial applications, including protein fortification of dairy foods, ingredients in PC making, ingredients for beverages, bakery, or meat products (Mulvihill and Ennis, 2003). The advantages of HC-MC are lower moisture content and thereby longer shelf-life compared
to liquid MCC; also, HC-MC saves the cost of drying because it does not need to be dried. Due to the importance of HC-MC and its applications, there is interest in studying the changes that happen to HC-MC during the storage. HC-MC is a suitable environment for microorganisms’ due to their moisture content and the presence of low molecular weight compounds (such as lactose and NPN) that consider as nutrients for microbial growth. These organisms cause proteolysis (protein degradation), which result in organoleptic defects (e. g. bitterness, acidic) and cause unacceptable quality. The proteolysis is a chemical process which degrades the protein and resulted in small peptides, and this, in turn, leads to decreasing the intact casein in HC-MC. As a result, this could affect the characteristics of products that used HC-MC as an ingredient, such as PC and PCP. Increasing the proteolysis in HC-MC that is used in making PC and PCP formulations lead to increasing the meltability and decreasing the firmness of the PC and PCP. To date, no studies have reported the effect proteolysis of HC-MC on the properties of PCP. The objective of this study was to evaluate the effects of storage of HC-MC on the functional characteristics of PCP.

MATERIALS AND METHODS

Experimental Design

HC-MC was manufactured using MF GP ceramic membrane. The HC-MC was divided into three parts; approximately 10 kg each. The first portion was the control, while 1% of sodium chloride and 1% sodium chloride + 1% sodium citrate were added to the second (T1) and third portions (T2), respectively. The HC-MC was kept at 4°C to study the shelf-life of the treatments. The composition of different HC-MC treatments is
shown in Table 1. Three replicates of HC-MC were produced and stored for 60 days at 4°C. At 0, 30, 60 days, a sample of each treatment was frozen. Subsequently, the samples were thawed and utilized in making PCP. PCP was made from each treatment at time 0, 30, and 60 days of storage. A formula was used for each replicate of each treatment.

**Process Cheese Formulations**

The ingredients used in each formulation are shown in Table 2. The ingredients used in making PCP were aged Cheddar (Great Value, Extra Sharp Cheddar Cheese, Bentonville, AR), HC-MC, water, unsalted butter (Land O Lakes Half Stick Unsalted Butter, INC., Arden Hills, MN), deproteinized whey (Bondgrads’ Creameries, Perham, MN), dibasic sodium phosphate (Fisher Scientific, Fair Lawn, New Jersey), sodium chloride salt (Cargill, Minneapolis, MN), and trisodium citrate (KIC Chemical Inc., New Paltz, NY). Techwizard, which is an Excel-based-formulation software program, is used to develop the PC formulations (Metzger, 2010) provided by Owl Software (2301 Wood Street, Lancaster, PA). Each formulation was balanced for moisture, fat, protein, and salt at 49, 21, 16.5, and 1.5%, respectively. The protein content was balanced between cheddar cheese and HC-MC to get a ratio of 2:1, respectively. Trisodium citrate and sodium chloride were standardized in each formula depending on each HC-MC treatment to have the same composition in all formulations.
Process Cheese Manufacture

Preblend Preparation

All ingredients (Table 2) were weighed and blended in a kitchenaid at room temperature for approximately 30-40 min to get a homogenous paste. A 300 g of each formula was prepared to make the PCP.

Cooking in the Rapid Visco Analyzer (RVA)

A 25g sample of the paste was weighed in a canister when the blend was completely mixed. The canisters were tempered at 38°C/15-20 min in a water bath before being manufactured in the RVA (Perten RVA 4500, Macquarie Park NSW 2113, Australia). The canisters were then cooked in the RVA for 4 min at 90°C. The stirring speed was 1000 rpm for the first 2 min and 160 rpm for the last 2 min. The cooked PCP was poured in copper cylinders (20 mm diameter × 30 mm height) for texture profile analysis (TPA) and plastic molds (28.3 mm diameter × 25 mm height) for dynamic rheological analysis (DSR). Then the cylinders and molds were sealed with aluminum foil and kept at 4°C for the next day for further analysis. Three replicates of PCP from each HC-MC treatment were manufactured.

Chemical Analyses

HC-MC were analyzed for Ash (AOAC, 2000; method 945.46; 33.2.10), TS (AOAC, 2000; method 990.20; 33.2.44), total nitrogen TN (AOAC, 2000; method 991.20; 33.2.11) and fat (Mojonnier method: Atherton and Newlander, 1977) before being utilized in PCP formulations. Also, the TS content and pH of the final PCP were determined.
**Analyses of the Functional Properties**

**Cooked apparent viscosity**

The cooked apparent viscosity of the PCP was measured at 90°C at the end of cooking time in the RVA by calculating the mean of the last 5 values of viscosity (Figure 1). This test was repeated 6 times for each replicate.

**Texture Profile Analysis (TPA)**

Texture profile analysis (TPA) was used to determine the hardness of the PCP samples. The PCP samples were removed out from the copper molds and cut into cylinders (20 mm high) using a wire cutter. The TPA was performed using a TA.XT-Plus Texture Analyzer (TA.XT-Plus, 6 Patton Drive, South Hamilton, MA). The following conditions were applied as follow: Uniaxial 10% double bite compression, 50-mm diameter cylindrical flat probe (TA-25), and 1 mm/s crosshead speed. The maximum force during the first compression was referred to as the hardness of PCP (Figure 2). TPA was performed on 6 samples of each replicate.

**Dynamic Stress Rheometer (DSR)**

A dynamic rheological analysis was performed using a rheometer (MSR 92, Anton Paar, Graz, Austria) to analyze PCP meltability using 25-mm parallel plate geometry. DSR test was done using the modified method as described by Sutheerawattananonda and Bastian (1998). The PCP was prepared by removed the PCP partially from the plastic molds and then cutting it into slices (2 mm thick) using a wire cutter. All cheese samples were tempered at room temperature for 10 min before performing the test. Initially, a stress sweep test for PCP was performed at a frequency of
1.5 Hz and a range of 1 to 1000 Pa stress at 20°C using the rheometer (MSR 92, Anton Paar, Graz, Austria) with parallel plate geometry. The stress sweep experiment determined that the maximum stress limit for the linear viscoelastic region was 50 Pa.

The DSR properties of the PCP were then analyzed using a dynamic temperature ramp test. The ramp test was performed using the same rheometer at temperature 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 (linear viscoelastic region). Elastic modulus (G’), viscous modulus (G’’), tangent angle (tan δ), and melt temperature were determined. The temperature at which tan δ=1 (G’’/ G’) was referred to as the cheese melt temperature (Figure 3). DSR test was performed in duplicates.

Statistical Analysis

Statistical analysis was performed to study the effect of treatments and shelf-life of HC-MC 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). When a significant difference was detected between treatments, time, or their interaction, differences were tested using the least significant difference (LSD) comparison test at P < 0.05.

RESULTS AND DISCUSSION

Composition

The mean composition of control, T1, and T2 HC-MC is shown in Table 1. No significant difference (P > 0.05) was detected in the TS, TN, and fat contents of HC-MC treatments. A significant difference (P < 0.05) was found between the ash content of the
control and T2 treatment of HC-MC due to the addition of 1% sodium chloride and 1% sodium citrate in T2 which led to an increase in the ash content. However, ash contents did not significantly differ (P > 0.05) between control and T1 treatments or between T1 and T2 treatments. Addition of 1% and 2% salts in T1 and T2, respectively, should result in an increase in the TS content by 1% and 2% in T1 and T2, compared to control HC-MC. The ash content should also be higher in T1 and T2 by 1% and 2%, respectively, compared to control. The lack of a significant difference could be due to the salts did not mix evenly with the HC-MC after manufacturing, and this results in low TS and ash contents in T1 and T2 HC-MC compared to the expected values. The implementation of this could result in variations in the composition of T1 and T2 HC-MC during storage due to the differences within the vials that used to store the HC-MC. As a result, this could lead to some differences in the composition and functional properties of PC and PCP made from T1 and T2 HC-MC.

Mean compositional analysis for PCP made from control, T1, and T2 HC-MC is shown in Table 3. The ANOVA with MS and P-values for moisture and pH of the PCP is shown in Table 4. The moisture content of PCP made with control, T1, and T2 was 47.70, 48.20, and 46.83 %, respectively (Table 3). There was a significant difference (P < 0.05) in the moisture content of PCP made with different treatments of HC-MC. Also, there was a significant (P < 0.05) replicate effects in the moisture content of the final PCP. However, the moisture content of PCP made with control and T1 was non-significant (P > 0.05). The low moisture content for PCP made from T2 (HC-MC with 1% sodium chloride and 1% sodium citrate) could be due to the distribution of salt in T2 HC-MC. Additionally, there is some water loss during the cooking in the RVA; also, the
addition of sodium chloride and sodium citrate in HC-MC could have an impact on the hydration of water in the preblend before cooking. The storage period of HC-MC at 0, 30, 60 days had no significant effect (P > 0.05) on the moisture content of PCP. Also, the interaction between the storage time of HC-MC and treatments was non-significant (P > 0.05).

Table 3 presented the pH of PCP made with different HC-MC treatments at 0, 30, and 60 days of storage. The pH of the final PCP was 5.7, 5.7, and 5.8 made from control, T1, and T2 HC-MC, respectively. The pH of PCP made from T2 treatment was significantly (P < 0.05) higher compared to control and T1 PCP. The slight difference in the composition of ingredients used in PCP formulations could lead to small variations in the pH. However, the storage period of HC-MC at 0, 30, and 60 days, and the interaction between the storage time of HC-MC and treatments were non-significant (P > 0.05). It has been reported that the pH of a good-quality process cheese should be ranged from 5.4 to 5.8 (Palmer and Sly, 1943; Marchesseau et al., 1997) which is similar to our results.

**Functional Properties**

**Cooked apparent viscosity**

The mean values of cooked viscosity (cP) of PCP determined by the RVA are shown in Table 5. The ANOVA with MS and P-values for cooked viscosity of the PCP is shown in Table 6. The viscosity of PCP made from control, T1, and T2 HC-MC was 760.73, 506.53, and 569.61 cP, respectively. The PCP made from control HC-MC had significantly (P < 0.05) higher viscosity compared to T1 and T2 PCP. The addition of sodium chloride in T1, and sodium chloride + sodium citrate in T2 HC-MC increased the
solubility of T1 and T2 HC-MC during the shelf-life (Piska et al., 1999; Guinee, 2004; Hladká et al., 2014; Toro et al., 2016) and this might have an impact on the behavior of HC-MC during the storage period, and thereby, affecting the functional properties of PCP made from T1 and T2 treatments (Kapoor and Metzger, 2008; Salunke, 2013). Also, the variations in the composition of T1 and T2 HC-MC have an impact on the apparent cooked viscosity of PCP. However, the storage period of HC-MC at time 0, 30, 60 days had no significant effect (P > 0.05) on the cooked viscosity of PCP. Thus the viscosity of PCP made from HC-MC was 587.52, 616.74, and 632.61 cP at 0, 30, and 60 days of the storage period, respectively (Table 5). The interaction between the storage time of HC-MC and treatments had no significant effect (P > 0.05) on the apparent viscosity of PCP.

**Hardness**

Mean values of TPA hardness (g) of the PCP made from HC-MC during the storage period are shown in Table 7. The ANOVA with MS and P-values for TPA hardness of the PCP is shown in Table 8. The hardness of PCP made from control, T1, and T2 HC-MC was 119.56, 113.84, and 195.80 g, respectively. The T2 PCP had significantly (P < 0.05) higher hardness than control and T1 PCP (Table 5). However, the TPA hardness of PCP made from control and T1 HC-MC was non-significant (P > 0.05). The variation in the composition (low moisture content) of T2 HC-MC compared to control and T1 HC-MC led to an increase in the hardness of PCP made from T2 HC-MC (Kapoor and Metzger, 2008; Salunke, 2013). Also, the addition of sodium citrate in HC-MC during storage may be contributed to the high hardness in PCP made from T2 HC-MC, which improved the functional properties of PCP. The hardness of PCP made from HC-MC was 142.68, 145.88, and 140.64 g at 0, 30, and 60 days of the storage period,
respectively (Table 5). The storage time of HC-MC did not affect (P > 0.05) on the TPA hardness of PCP made from HC-MC. The interaction of treatment and time had no significant effect (P > 0.05) on the TPA hardness of PC made from HC-MC.

**Melting temperature**

The DSR melt test measures the initial melt characteristics of PCP and indicates molecular interactions. The DSR melt temperature has been used to quantify the melting characteristic of PCP. DSR was used to measure the melt temperature where tan \( \delta \) \((G''/G') = 1\) is a convenient measure of the melting point of PCP because this is the lowest temperature where a material change from primarily elastic to primarily viscous (Sutheerawattananonda and Bastian, 1998; Prow and Metzger, 2005). The mean values of melt temperature (°C) of PCP are shown in Table 9. The ANOVA with MS and P-values for melt temperature (°C) of the PCP is shown in Table 10. The melt temperature of PCP was 57.62, 65.34, and 65.96 °C made from control, T1, and T2 HC-MC, respectively. The melt temperature of PCP made from T1 and T2 HC-MC was significantly (P < 0.05) higher than the PCP made from the control. The low melt temperature of PC made from control HC-MC may be related to the high insoluble calcium in HC-MC. This leads to the formation of heat-induced irreversible gel and produces a PCP that has higher restricted melt characteristics (Purna et al., 2006; Chemistry, 2007; Kapoor and Metzger, 2008; Kammerlehner, 2009). The addition of sodium chloride in T1 and sodium chloride + sodium citrate in T2 HC-MC might have an impact on the behavior of HC-MC during the storage period, and thereby, affecting the melt temperature of PCP made from T1 and T2 treatments. The melt temperature of PCP made from HC-MC at 0, 30, and 60 days of storage period was 64.44, 62.75, and 61.74 °C, respectively (Table 6). The storage time
of HC-MC during the 60 days decreased the PCP melt temperature slightly but not significantly (P > 0.05). The protein proteolysis (increasing the NCN and NPN) that occurred during the shelf-life or storage period of HC-MC contributed to the small decrease of PCP melt temperature. The interaction of treatment and time had no significant effect (P > 0.05) on the melt temperature of PCP made from HC-MC.

CONCLUSIONS

The HC-MC could be stored for 60 days at 4°C and can be used in PCP formulations. No differences (P > 0.05) were detected in the functionality of PCP made from HC-MC at 0, 30, and 60 days of storage. However, the functionality of PCP was affected (P<0.05) by each treatment of HC-MC. Overall, the addition of sodium chloride and sodium citrate in HC-MC during the 60 days of storage improved the melt and textural characteristics of PCP.
REFERENCES


### Tables

**Table 1.** Mean (n = 3) composition (% by weight) of the high concentrated micellar casein (HC-MC) of all treatments measured after manufacturing.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Composition (% by weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TS</td>
</tr>
<tr>
<td>Control</td>
<td>25.41</td>
</tr>
<tr>
<td>T1</td>
<td>25.62</td>
</tr>
<tr>
<td>T2</td>
<td>26.13</td>
</tr>
<tr>
<td>SEM</td>
<td>0.48</td>
</tr>
</tbody>
</table>

<sup>a,b</sup>Means in the same column not sharing a common superscript are different (P < 0.05).

TS = total solids; TN = total nitrogen × 6.38

Control = HC-MC; T1 = HC-MC + 1% salt; T2 = HC-MC + 1% salt and 1% sodium citrate
Table 2. Mean (n=3) composition of process cheese products (PCP) formulations made with different HC-MC treatments at time 0, 30, and 60 days of storage.

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Control</th>
<th>T1</th>
<th>T2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aged Cheddar cheese</td>
<td>42.78</td>
<td>42.78</td>
<td>42.78</td>
</tr>
<tr>
<td>HC-MC</td>
<td>27.53</td>
<td>27.27</td>
<td>26.94</td>
</tr>
<tr>
<td>Water</td>
<td>10.54</td>
<td>10.77</td>
<td>11.16</td>
</tr>
<tr>
<td>Unsalted Butter</td>
<td>8.17</td>
<td>8.18</td>
<td>8.18</td>
</tr>
<tr>
<td>Deproteinized whey</td>
<td>6.73</td>
<td>7.02</td>
<td>7.19</td>
</tr>
<tr>
<td>Disodium Phosphate</td>
<td>2.50</td>
<td>2.50</td>
<td>2.50</td>
</tr>
<tr>
<td>Salt</td>
<td>1.50</td>
<td>1.22</td>
<td>1.23</td>
</tr>
<tr>
<td>Trisodium Citrate</td>
<td>0.25</td>
<td>0.25</td>
<td>0.00</td>
</tr>
</tbody>
</table>
Table 3. Mean (n=3) composition of process cheese products (PCP) made with control, T1 and T2 of HC-MC at 0, 30, and 60 days of storage.

<table>
<thead>
<tr>
<th>Composition</th>
<th>Time</th>
<th>Treatments</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>T1</td>
</tr>
<tr>
<td>Moisture (%)</td>
<td>0</td>
<td>47.14</td>
<td>48.04</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>48.08</td>
<td>48.33</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>47.88</td>
<td>48.25</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>47.70&lt;sup&gt;a&lt;/sup&gt;</td>
<td>48.20&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>pH</td>
<td>0</td>
<td>5.71</td>
<td>5.71</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>5.70</td>
<td>5.74</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>5.71&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.70</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>5.71&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.72&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a,b</sup>Means in the same row not sharing a common superscript are different (P < 0.05).

Control= HC-MC; T1= HC-MC + 1% salt; T2= HC-MC+ 1% salt and 1% sodium citrate
Table 4. Mean squares and P-values (in parentheses) of process cheese products (PCP) made with control, T1 and T2 of HC-MC at 0, 30, and 60 days of storage.

<table>
<thead>
<tr>
<th>Factor</th>
<th>df</th>
<th>Moisture</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replication</td>
<td>2</td>
<td>1.17(0.04)*</td>
<td>0.0003(0.58)</td>
</tr>
<tr>
<td>Treatment¹</td>
<td>2</td>
<td>4.35(0.0003)***</td>
<td>0.024(0.0000003)***</td>
</tr>
<tr>
<td>Time²</td>
<td>2</td>
<td>0.37(0.32)</td>
<td>0.0005(0.40)</td>
</tr>
<tr>
<td>Treatment x Time</td>
<td>4</td>
<td>0.23(0.57)</td>
<td>0.0006(0.34)</td>
</tr>
<tr>
<td>Error</td>
<td>16</td>
<td>0.31</td>
<td>0.0005</td>
</tr>
</tbody>
</table>

*Statistically significant at P < 0.05
Treatment= Control, T1, and T2
Time= 0, 30, and 60 days
**Table 5.** Mean (n=3) apparent cooked viscosity of process cheese products (PCP) determined by rapid visco analyzer (RVA) made with control, T1 and T2 of HC-MC at 0, 30, and 60 days of storage.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Time</th>
<th>Treatments</th>
<th></th>
<th></th>
<th></th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>T1</td>
<td>T2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apparent cooked viscosity (cP)</td>
<td>0</td>
<td>769.16</td>
<td>403.78</td>
<td>589.61</td>
<td>587.52</td>
<td></td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>757.87</td>
<td>481.11</td>
<td>611.24</td>
<td>616.74</td>
<td></td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>755.14</td>
<td>634.70</td>
<td>507.98</td>
<td>632.61</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>760.73&lt;sup&gt;a&lt;/sup&gt;</td>
<td>506.53&lt;sup&gt;b&lt;/sup&gt;</td>
<td>569.61&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a-c</sup>Means in the same row not sharing a common superscript are different (P < 0.05).

Control= HC-MC; T1= HC-MC + 1% salt; T2= HC-MC+ 1% salt and 1% sodium citrate
Table 6. Mean (n=3) squares and P-values (in parentheses) of apparent cooked viscosity of process cheese products (PCP) determined by rapid visco analyzer (RVA) made with control, T1 and T2 of HC-MC at 0, 30, and 60 days of storage.

<table>
<thead>
<tr>
<th>Factor</th>
<th>df</th>
<th>Apparent cooked viscosity (cP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replication</td>
<td>2</td>
<td>44001(0.09)</td>
</tr>
<tr>
<td>Treatment&lt;sup&gt;1&lt;/sup&gt;</td>
<td>2</td>
<td>157676(0.001)&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>Time&lt;sup&gt;2&lt;/sup&gt;</td>
<td>2</td>
<td>4709(0.74)</td>
</tr>
<tr>
<td>Treatment x Time</td>
<td>4</td>
<td>22902(0.26)</td>
</tr>
<tr>
<td>Error</td>
<td>16</td>
<td>15789</td>
</tr>
</tbody>
</table>

*Statistically significant at P < 0.05  
Treatment= Control, T1, and T2  
Time= 0, 30, and 60 days
Table 7. Mean (n=3) hardness of process cheese products (PCP) determined by texture profile analyses (TPA) made with control, T1 and T2 of HC-MC at 0, 30, and 60 days of storage.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Time</th>
<th>Treatments</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>T1</td>
</tr>
<tr>
<td>Hardness (g)</td>
<td>0</td>
<td>134.73</td>
<td>104.69</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>118.68</td>
<td>106.75</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>105.27</td>
<td>130.08</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>119.56&lt;sup&gt;b&lt;/sup&gt;</td>
<td>113.84&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>abc</sup>Means in the same row not sharing a common superscript are different (P < 0.05).

Control= HC-MC; T1= HC-MC + 1% salt; T2= HC-MC+ 1% salt and 1% sodium citrate
Table 8. Mean (n=3) squares and P-values (in parentheses) of the hardness of process cheese products (PCP) determined by texture profile analyzer (TPA) made with control, T1 and T2 of HC-MC at 0, 30, and 60 days of storage.

<table>
<thead>
<tr>
<th>Factor</th>
<th>df</th>
<th>Hardness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replication</td>
<td>2</td>
<td>2232.8(0.13)</td>
</tr>
<tr>
<td>Treatment</td>
<td>2</td>
<td>18839.5(0.0005)**</td>
</tr>
<tr>
<td>Time</td>
<td>2</td>
<td>62.8(0.94)</td>
</tr>
<tr>
<td>Treatment x Time</td>
<td>4</td>
<td>897.7(0.47)</td>
</tr>
<tr>
<td>Error</td>
<td>18</td>
<td>966.0</td>
</tr>
</tbody>
</table>

*Statistically significant at P < 0.05
Treatment= Control, T1, and T2
Time= 0, 30, and 60 days
Table 9. Mean (n=3) melt temperature of process cheese products (PCP) determined by dynamic stress rheometry (DSR) made with control, T1 and T2 of HC-MC at 0, 30, and 60 days of storage.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Time</th>
<th>Control</th>
<th>T1</th>
<th>T2</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melt temperature (°C)</td>
<td>0</td>
<td>58.41</td>
<td>66.36</td>
<td>68.56</td>
<td>64.44</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>58.07</td>
<td>65.51</td>
<td>64.67</td>
<td>62.75</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>56.38</td>
<td>64.16</td>
<td>64.67</td>
<td>61.74</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>57.62&lt;sup&gt;a&lt;/sup&gt;</td>
<td>65.34&lt;sup&gt;a&lt;/sup&gt;</td>
<td>65.96&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a,c</sup>Means in the same row not sharing a common superscript are different (P < 0.05).

Control= HC-MC; T1= HC-MC + 1% salt; T2= HC-MC+ 1% salt and 1% sodium citrate
Table 10. Mean (n=3) squares and P-values (in parentheses) of melt temperature of process cheese products (PCP) determined by dynamic stress rheometry (DSR) made with control, T1 and T2 of HC-MC at 0, 30, and 60 days of storage.

<table>
<thead>
<tr>
<th>Factor</th>
<th>df</th>
<th>Melt temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replication</td>
<td>2</td>
<td>10.07(0.43)</td>
</tr>
<tr>
<td>Treatment(^1)</td>
<td>2</td>
<td>194.5(0.0001)***</td>
</tr>
<tr>
<td>Time(^2)</td>
<td>2</td>
<td>16.81(0.26)</td>
</tr>
<tr>
<td>Treatment x Time</td>
<td>4</td>
<td>2.78(0.91)</td>
</tr>
<tr>
<td>Error</td>
<td>16</td>
<td>11.48</td>
</tr>
</tbody>
</table>

*Statistically significant at P < 0.05

Treatment= Control, T1, and T2
Time= 0, 30, and 60 days
Figure 1. Measuring the apparent cooked viscosity of process cheese products (PCP) by using the RVA
Figure 2. Measuring the hardness of process cheese products (PCP) by using the TPA
Figure 3. Measuring the melting point of process cheese products (PCP) by using the DSR
OVERALL CONCLUSIONS AND FUTURE WORK

Microfiltration (MF) is a membrane process to separate casein (CN) and serum whey protein (SP) using 0.1 µm to produce micellar casein concentrate (MCC). MCC is a high-protein ingredient that is widely used in many applications in the dairy industry due to its distinct properties compared to other commercial protein products (such as acid casein, rennet casein, caseinates, milk protein concentrate, co-precipitates). The MCC can be further concentrated using MF membranes to produce highly concentrated micellar casein (HC-MC). The production of HC-MC using MF would eliminate, or reduce, the costs of expensive thermal concentrating processes, such as evaporation, and drying. MCC has many functional properties, such as foaming, emulsifying, and water binding ability. MCC is a good source of casein and can be used as a liquid, concentrated, or dried. Recently, MCC has been used as an ingredient in making beverages, yogurt, low-fat cheese, and PCP formulations.

The process to produce HC-MC with a long refrigerated shelf-life was developed as our first objective. This study determined that HC-MC can be manufactured using ceramic GP MF system with over 25% TS and greater than 95% CN%TP. The HC-MC formed a solid gel at room temperatures and it needs high temperatures to revert to a liquid. No significant differences (P > 0.05) were detected between the compositions of treatments after manufacturing (at d=0) and during the 60 d of storage period. However, the NCN content increased significantly (p < 0.05) during 60 d of storage; also, the NPN slightly increased during the storage period of HC-MC, which indicates that some proteolysis occurred in the HC-MC during this storage period. The total aerobic bacteria count increased significantly (P < 0.05) during 60 d of storage at 4°C. The HC-MC
produced in this study maintained a bacterial count 3.5, 3.5, and 4.1 log cfu/mL in control, T1 and T2, respectively, for 60 d. As a result, we had to study the impact of the small increase in NCN and NPN in all treatments during 60 d of storage on process cheese characteristics.

The HC-MC could be stored for more than 60 d and can be used in many applications, such as PC and PCP manufacturing. No differences (P > 0.05) were detected in the functionality of PCP made from HC-MC at 0, 30, and 60 d of storage. However, the functionality of PCP was affected (P<0.05) by each treatment of HC-MC. Overall, the addition of sodium chloride and sodium citrate in HC-MC during the shelf-life improved the melt characteristics of PCP made from HC-MC. We concluded that HC-MC with over 60 d of storage can be used in the manufacture of PCP with no change in the functionality of this cheese.

Future studies are needed to examine the changes in the sensory and functional characteristics of HC-MC at 4°C. The descriptive sensory characteristics should be monitored for fresh HC-MC and at different times during the storage. The solubility, viscosity, emulsification, and heat stability of HC-MC should be examined with adding sodium chloride and sodium citrate. The HC-MC in this study was manufactured in 2 d in a pilot scale; therefore, these conditions were ideal for microbial growth perspective. As a result, the manufacturing of HC-MC should be done in a continuous process on the same day to reduce the potential of microbial growth and proteolysis, which increase the shelf-life of HC-MC.