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Manufacture, Concentration, and Functionality of Micellar Casein Concentrate

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MANUFACTURE, CONCENTRATION, AND FUNCTIONALITY OF MICELLAR CASEIN CONCENTRATE

BY

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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 candidates are necessarily the conclusions of the major department.

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This thesis is dedicated to my wife, who has always supported and encouraged me.

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ABSTRACT

MANUFACTURE, CONCENTRATION, AND FUNCTIONALITY OF MICELLAR CASEIN CONCENTRATE

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This research has been structured into 3 major parts. The first part evaluates a centrifugal evaporator to concentrate micellar casein concentrate (MCC). The second part evaluates a wiped film evaporator (WFE) to concentrate MCC. The third part evaluates heat stability of the high concentration MCC produced by WFE.

In part 1, the primary objective was to achieve >25% total solids (TS) without a loss of water removal rate associated with high solids materials. This is of importance, industrially, as this could reduce processing costs or eliminate drying all together. The major finding was that it did not offer any benefits over a standard falling film evaporator (FFE)

In part 2, WFE was used to achieve the same primary objective of >25% TS. WFE was found to be a viable for the production of MCC at ~29% solids without modification to the existing equipment. The resultant high concentration MCC (HCMCC) was found to have >99% dispersion at 2.5% solids in 50 °C water at a high sheer rate.

Part 3 focused on the heat stability of the HCMCC produced in part 2. The major findings were that: (i) there was a small decline in heat stability of HCMCC after reconstitution to 5 and 10% protein (ii) the lost heat stability could be recovered through

the use of trisodium citrate (TSC) (iii) when the pH was standardized to 6.8, the 10% protein treatments without TSC had greater heat stability than 5% protein treatments.

CHAPTER 1

REVIEW OF LITERATURE

Introduction

Micellar Casein Concentrate (MCC) has become a popular ingredient due to its unique functionality and demand from the nutritional supplement industry. MCC is produced by concentrating the colloidal fraction of milk. Traditional methods of concentration such as acidification, renneting, or coprecipitation result in the disruption of the casein micelle which negatively alter the physiochemical properties of MCC.(Beliciu et al., 2012) More recently, processors have used Microfiltration (MF) to produce MCC. While MCC produced by MF exhibits unique functionality, production challenges exist preventing wide spread usage. Improving the production process for MCC using a combination of new and existing technologies will increase its' viability as an ingredient.

Historically, the main serum proteins (SP), Alphalactalbumin, Betalactoglobulin and Bovine Serum Albumin, were a waste fraction (whey) generated during cheese manufacture. Today, the serum proteins are popular in the nutrition industry which has made them a value added stream demanding a premium. With increased demand and stronger pricing, new methods have been developed to simultaneously manufacture MCC and SP fractions. The use of Microfiltration to concentrate milk circumvents the need for a whey supply and provides unique functionality of the SP fractions.

Leveraging the physiochemical properties of MCC, developers have begun to investigate its functionality and use in foodstuffs.(Sauer and Moraru, 2012, Bong and Moraru, 2014) Bong and Moraru reported its use in a Greek style yogurt. The objective of this study was to avoid the straining step which is expensive and produces an acid whey waste stream.

They found some physiochemical differences compared to the commercial strained yogurts but felt that it was a reasonable alternative.

Loss of functionality occurs during UHT treatment and drying. (Sauer and Moraru, 2012) It has been associated with loss of heat stability, changes to the calcium phosphate partition, and dissociation of the caseins from the micelle. The wetting times have been found to be substantially higher than skim milk powder (SMP) or whey protein isolate (WPI) (Gaiani et al., 2005) A potential solution is through the elimination of the drying step. To achieve this, the MCC would have to be sufficiently concentrated and stored refrigerated or frozen. Functionality losses may be compensated for by the addition of chelators. (Lu et al., 2015a)Alternative methods should be assessed, however they must address microbial stability and maintenance of functionality.

Milk Composition

Bovine milk is a complex heterogeneous colloidal suspension which has a composition that varies as a function of duration of lactation, cow diet, seasonality, and milk age.(Fox and McSweeney, 1998) Quantification and characterization can be complicated by genetic variants and post translational modifications. Bovine milk contains ~3.4% protein (w/w) with about 80% existing in the colloidal state. (See Table 1.1) (Whitney, 1988, Swaisgood, 2013) Within the colloidal complex ~65% of the 30mM calcium resides. (Holt, 1985)

Table 1.1 Bovine Milk Composition

Total Solids	Fat	Protein	Lactose	Ash
12.7	3.7	3.4	4.8	.7

Adapted from (Fox and McSweeney, 1998)

Casein Proteins

Casein proteins were originally defined as the protein fraction that precipitated at pH 4.6 and temperature 20ºC (Jenness et al., 1956) This includes α-S1, α-S2, β Casein, and κ Casein (Table 1.2). Each protein has distinct physiochemical properties and structure but share some common features. All casein proteins have a high number of nonpolar residues that would suggest a low aqueous solubility, however, presence of carbohydrates in κ-casein, high phosphoryl groups, and low sulfur containing amino acids counterbalance the non-polar amino acid residues. (Fox and McSweeney, 1998) The tertiary structures are deficient of α-helix or β-sheet structures which make them readily available for proteolysis. Their secondary structure may be thought of as intrinsically unstructured.(Farrell et al., 2006) Limited secondary and tertiary structure and low sulphydryl content renders casein proteins resistant to thermal denaturation. (McSweeney and Fox, 2013)

Table 1.2 Casein protein composition in bovine milk

Protein	g/kg Milk	g/100g protein
Casein	26	78.3
α-S_1	10.7	32
α-S_2	2.8	8.4
β-Casein	8.6	26
κ-Casein	3.1	9.3

Adapted from (Walstra, 2006)

α-S_1

The α-S1 protein fraction is the most prevalent protein in raw milk at an average of ~10.7g/kg. (Walstra, 2006) It has been classified as a phosphoprotein due to the presence of 8 or 9 phosphoserine residues per mole(McSweeney and Fox, 2013). Calcium precipitation is possible but it is stabilized by κ-casein within the micelle. Walstra suggested that due to a reduced tertiary structure owing itself to the presence of a high proline content, κ-casein is not considered heat denaturable. It will, however, undergo chemical changes at temperatures above 120°C, rendering it insoluble.

α-S_2

The α-S_2 fraction is also considered a phosphoprotein having the highest amount of phosphoserine residues of all the milk proteins. It may vary from 10 to 13 residues per mole. The average concentration in bovine milk is ~2.8 g/kg. It has strong calcium

binding properties that make Ca++ precipitation possible. The α-S_2 has been found to be the most sensitive to Ca++ induced precipitation.(Toma and Nakai, 1973)

β-Casein

The β-Casein fraction is the second most prevalent milk protein at ~8.6 g/kg of milk. β-Casein is the most hydrophobic of the caseins and has several unique properties. At temperatures below 5°C, partial translocation outside of the micelle occurs. Higher temperatures have been shown to induce polymerization of β-Casein in thread-like chains up to 20 units at 8.5°C and cause aggregate formation at greater temperatures.(Fox and McSweeney, 1998) Due to its uneven charge distribution, β-Casein exhibits soap-like properties.(Walstra, 2006)

κ-Casein

κ-Casein represents ~3.1g/kg of milk and is classified as a glycoprotein. Glycosylated threonyl residues and the absence of phosphoseryl clusters is reported to reduce calcium binding capacity.(Swaisgood, 2013) This allows κ-Casein to shield the more sensitive α-S1, α-S2, and β-Casein from calcium binding. The distribution of κ-casein on the micelle surface is said to be critical in the prevention of hydrophobic associations between micelles.(Creamer et al., 1998)

Casein Micelle Assembly

The structure and mechanisms of association of the casein micelle is debated in the scientific literature. It is generally accepted that the casein micelle is somewhat spherical with a diameter ranging from 50 to 500 nm.(Fox, 2013)

The casein micelle is reported to have 3.5 kg of water per 1 kg of casein (Jeurnink and De Kruif, 1993) but make up 10% of the volume. (Dalgleish and Corredig, 2012) The micelles are somewhat resistant to heating or cooling, but are easily destabilized by proteases or acidification. This basic concept is the cornerstone of yogurt and cheese.

An explanation of how the casein micelle assembles may provide clues to the structure of the casein micelle as a whole. Most of κ-casein resides on the surface of the micelle, serving to stabilize the other caseins. (Dalgleish and Corredig, 2012) It is believed that κ-casein acts to limit the growth of the micelle, by preventing further interaction of the other caseins and calcium phosphate.

The surface chemistry of the casein micelle is the primary contributor of the functional properties of casein is often thought of as a "hard shell." The interior contributes more to the functional properties upon micellar disruption such as curd formation.

Theories of Casein Micelle Structure

A limitation of many of the studies on micellar structure is that they do not use native milk, rather they are rehydrated MPC, MCC, and SMP. Dalgleish suggests that they will have similar structure and function as the native micelle.

Fox states that valid micelle models must meet certain criteria including: the location of κ-casein such that it be able to stabilize α-S1, α-S2, and β-casein proteins, bulky proteases, such as chymosin, be able to readily hydrolyze κ-casein, and under heat, in the presence of serum proteins be able to form complexes with β-lactoglobulin.(Fox, 2013) He further suggests that the surface of the micelle be surrounded by a layer of κ-

casein. These are common features of the three popular categorical models: core-coat, internal structure (Holt, 1992, Horne, 1998, 2006), and sub-micelle model. (Walstra, 1999) Here is a brief overview of these models.

Core-Coat Model

The basis of the core-coat model is that β- casein forms a core or matrix which incorporates α-S1 and α-S2, with a κ-casein coat. This model is no longer considered to be valid and hence will not be reviewed further.

Submicelle Model

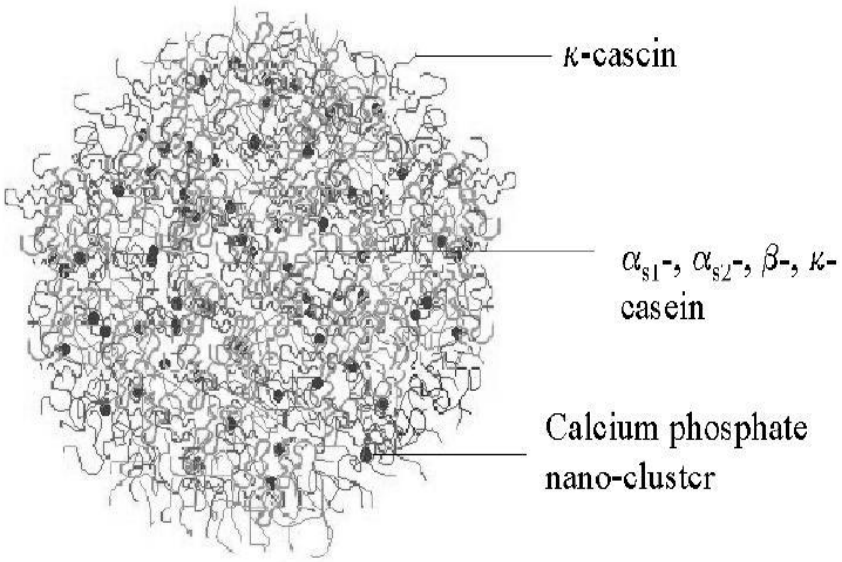
The submicelle model is a derivative of the core-coat model in that subunits of casein are again coated with κ-casein and bound to other casein subunits through CCP cement.(Walstra, 1999) It has been descriptively deemed the "raspberry model" due to its resemblance to the fruit. The submicelle model, while explaining many of the properties of casein, has begun to fall out of favor, likely due to the work put forth by McMahon and MacManus (McMahon and McManus, 1998) They were not able to find evidence of submicelles using novel cryopreparation electron microscope (EM) stereo-imaging. The study concluded that artifacts may occur during fixation of traditional EM, which may show electron density variations.

Internal Structure Model

The internal structure model as proposed by Holt (Holt, 1992, 1994) presents the micelle as a "tangled web" of casein molecules. A gel-like structure is produced, which is stabilized by nanoclusters of colloidal calcium phosphate (CCP) as well as κ-casein in high concentration at the micelle surface. Further clarified, this model consists of a lattice

structure that is “sponge-like”, forming channels that allow for some degree of water motility. (McMahon and Oommen, 2008, Trejo et al., 2011)

Fig 1.1 Holt model for the structure of the casein micelle



(Adapted from Holt, 1992)

A limitation of many of the studies on micellar structure is that they do not use native milk, rather they are rehydrated MPC, MCC, and SMP. Dalgleish suggests, however, that they will have similar structure and function as the native micelle.(Dalgleish and Corredig, 2012)

MCC Composition and Physiochemical Properties

MCC does not have a standardized definition. It is typically expressed as a function of serum protein removal, which account for approximately 20% of total protein. (Beckman et al., 2010) As a consequence of membrane separation, ~70 to 90% of serum

protein as well as an amount of lactose and minerals are removed (Hurt et al., 2010) Rejection profile will vary based on membrane composition, diafiltration protocol and operational parameters. This removal may further contribute to changes in physiochemical properties.

Holt et al has elegantly referred to the casein micelle as a "functional aggregate." (Holt et al., 2013b) Casein micelles have a propensity to form fibril, planar, and polygonal aggregates.(Glantz et al., 2010, Holt and Carver, 2012) This may be a contributing factor for high film forming capacity of MCC. Additionally, apparent viscosity is inversely correlated with serum protein content even at equivalent casein concentration.(Sauer et al., 2012) Sauer suggests that the soluble components (SP, lactose, NPN, and minerals) collectively interfere with casein-casein interactions. This interference causes the inverse correlation, as casein has been indicated as the main contributor to viscosity. Furthermore, MCC has been purported to reversibly gel when protein concentrations are ≥ 16%.(Lu et al., 2015b) The temperature of cold-gelation has been inversely correlated to protein concentration. Gelation of MCC occurs at 16%, 17%, 20% and 23% protein at 5°, 7°, 28° and 38 °C, respectively.

When compared to other dairy ingredients, (i.e. WPC, sodium caseinate) MCC has a higher ratio of bound water. (Schuck et al., 1998) The desorption curve of the bound water, (β) as defined by the slope of the sigmoidal part at inflection point, is also greater.(Schuck et al., 1998) This indicates that water is slower to be released from the casein micelle than from globular proteins or when the micelle has been solvated. This is likely due to a film formation caused by a rapid release of water and subsequent

tightening of the protein network. Rate of rehydration of MCC powder is also reported to be extended. (Gaiani et al., 2006)

Serum proteins, particularly betalactaglobulin, have been implicated in reduced heat stability of milk (Singh and Fox, 1987, Oldfield et al., 1998). The initial step of denaturation is the dimerization of the betalactaglobulin through disulfide bridges.(Oldfield et al., 1998) The dimer subsequently associates with alphalactalbumin and kappa casein ultimately resulting in increased viscosity and aggregate formation. The removal of the majority of serum proteins has been suggested to increase the heat stability of the resultant MCC. However, modern high thermal treatments and drying still result in aggregation.(Sauer and Moraru, 2012)

The whiteness associated with milk can be attributed primarily to light diffraction by the casein micelle.(Kaliappan and Lucey, 2011) It is postulated that an increase in casein concentration would correspond with an increased whiteness. A visible increase in whiteness has been observed by the author.

Mineral Composition of MCC

The mineral composition of milk based systems can have a dramatic effect on viscosity and heat stability. In turn, the casein micelle structure can affect mineral solubility(Bienvenue et al., 2003) Mineral salts in milk are most often phosphates, citrates, sulfates, carbonates, and bicarbonates with the primary elements associated with them being: sodium, potassium, calcium, and magnesium.(Fox and McSweeney, 1998) Other trace elements do exist.

Calcium and inorganic phosphate are critical to the stability of the casein micelle.(Bienvenue et al., 2003) A quasi-equilibrium exists between colloidal calcium

phosphate (CCP) nanoclusters and soluble states. This partition is purported to be influenced by changes in pH and temperature. Lower pH and temperatures will shift the equilibrium to a higher soluble phase concentration.(Holt et al., 2013a) The dissolution of the CCP, as it acts as an anchor point for the micelle structure. There is some resistance to dissolution due to hydrophobic interactions of the casein proteins. (Dalgleish and Corredig, 2012)

Gelation of Foodstuffs

Gelation of food products is thought to occur due to covalent chemical bonds or physical crosslinking due to noncovalent forces.(Zhong and Daubert, 2004) The formation of physical gels is often found in biopolymers and may be induced by heating a solution or cooling a solution. These gels may be thermoreversible, and have a tendency to exhibit a creep response.(Osswald and Osswald, 2010) Rheology of these thermogels are a function of time and temperature.(Zhong and Daubert, 2004)

Biopolymer gels may exist as either ordered or disordered. Dairy based gels would traditionally be ordered due to specific noncovalent interactions that occur in response to thermal or chemical treatment. Gels are viscoelastic by nature which makes dynamic rheology suitable for analysis. The storage modulus (G') and the loss modulus (G'') are measurements that describe the response of the sample to a given sheer. The stored and subsequently released storage modulus, G', is the elastic component. The G'' loss modulus is a measurement of the dissipation of applied energy and is the viscous component. Both G' and G'' are measured on per cycle and per unit volume bases. They are frequency dependent.(Gunasekaran and Ak, 2000) The stress response in the linear region of a viscoelastic material can be given by:

$$\sigma(t) = \gamma_o G'(\omega)\sin(\omega t) + \gamma_o G''(\omega)\cos(\omega t)$$

The loss tangent (tanδ (ω)) = G’’/G’ is a relative comparison of the viscous and elastic components. A large tanδ indicates that the viscous component predominates. If the tanδ is low, the elastic component predominates. A high relative G’ is indicative of the sample behaving like a solid and a high relative G’’ suggesting behavior consistent with a liquid.

In practice, a developed gel is typically subjected to three measurements: strain sweep, frequency sweep, and temperature sweep. The strain sweep identifies the region in which a linear viscoelastic region exists, the frequency sweep determines the elastic nature of the gel, and the temperature sweep is used to evaluate the thermal stability of the gel. (Gunasekaran and Ak, 2000)

Rheology of Foodstuffs

Rheology is defined as the study of deformation and flow characteristics in the transitory state between solids and liquids.(Tabilo-Munizaga and Barbosa-Cánovas, 2005) Data is generated by the deformation and change in flow characteristics due to an applied stress. These relationships determinations are a function of time.

Fundamental concepts of rheology are stress and strain (Tabilo-Munizaga and Barbosa-Cánovas, 2005) Stress (σ) is expressed as Pascals (Pa) and is defined as force per area. This is a vector quantity with normal stress being perpendicular and shear stress being tangential. In contrast, strain (γ) is a dimensionless value relative to the deformation of the sample.(Daubert and Foegeding, 2010) Hooke’s law states that for an

ideal elastic material, stress and strain are directly proportional with the proportionality constant, modulus (G), following the equation:

$\sigma = G\gamma$

In the case of an ideal viscous material, Newton's law states that there is direct proportionality between sheer stress and sheer rate (γ).

$\sigma = \eta\dot{\gamma}$

The proportionality constant in this case is shear viscosity (η). Thermodynamically, ideal viscous materials completely dissipate applied energy as heat. Conversely, an ideal elastic material will return all applied energy in deformation.(Gunasekaran and Ak, 2000)

The rheometer is a quantitative tool to assess this relationship. The analytical geometry will vary based on sample attributes and type of analysis performed. Variations may include: concentric cylinder, rotating cylinder, plate and plate, and capillary tubes. (Miri, 2011) For the purpose of this review, we will focus on rotational rheometry. It may be characterized as either steady or oscillatory shear rates, both having applications based on interest in viscosity or structure, respectively.(Miri, 2011)

Rheology of Micellar Casein Concentrate

The rheological properties of milk have been well documented in various applications.(Vélez-Ruiz and Barbosa-Cánovas, 1998, 2000, Karlsson et al., 2005)As MCC is an emerging ingredient, the body of research is still being developed (Gaiani et al., 2006, Sauer et al., 2012, Lu et al., 2015b) MCC exhibits Newtonian and non-Newtonian behavior during steady shear, as a function of concentration. This is very similar to milk. (Sauer et al., 2012) Sauer found that in 65% and 95% SP reduced MCC,

clear non-Newtonian shear thinning occurred when casein concentrations were ≥7.5% As previously mentioned, cold gelation may occur in MCC as a function of high concentration and low temperatures. (Lu et al., 2015a)

Production of MCC

MCC production involves unit operations common to US dairy plants. Starting with raw milk, processing involves five basic steps: cream separation, pasteurization, microfiltration, concentration, and drying. Cream separation and pasteurization procedures are not specific for MCC and will not be addressed in this review.

Microfiltration

Microfiltration is a pressure-driven membrane separation process for the purpose of differential concentration.(Saboyainsta and Maubois, 2000) Commercial viability was realized through advances in multichannel geometry and a high permeability of a structural support.(Gillot and Garcera, 1986) Filtration design is almost exclusively cross-flow type where liquid flow is tangential to the membrane surface. Nominal particle passage size is typically .1 to 10 µm, although this is a general guideline as there are many factors that contribute to the filtration dynamics.

Two primary categories of microfiltration membranes are ceramic and polymeric. Within these categories are a myriad of variations. Spiral wound design is the most prevalent polymeric membrane in the dairy industry (Schwinge et al., 2004) Polymeric membranes are more sensitive to chemical and thermal damage.(Cheryan, 1998) Ceramic membranes are resistant to chemicals and high temperatures, however they are susceptible to cracking due to physical stress or extreme temperature changes. Beckman

et al notes that polymeric membranes have lower capital investments with a tradeoff of lower efficiency.(Beckman et al., 2010) Hurt et al. suggests a low rejection rate using ceramic membranes nearing the theoretical maximum. The capital cost of ceramic units can be prohibitively expensive with the initial investment being up to ten times greater than polymeric units.

Microfiltration of Skim Milk

MCC is often produced with membrane pore sizes of .1 to .5µm.(Pierre et al., 1992, Saboyainsta and Maubois, 2000, Lawrence et al., 2008, Beckman et al., 2010) Polyvinylidene fluoride (PVDF) is the principle material for MF polymeric membranes.

A guideline for the production of an enriched micellar casein fraction was proposed by numerous authors. (Pierre et al., 1992, Schuck et al., 1994) It can be separated into three steps: Removal of permeate stream until a concentration factor (CF) of 3-4x is achieved, diafiltration with Reverse Osmosis (RO) water to give the desired serum protein removal, concentration of the diafiltered retentate until desired TS is achieved. (Saboyainsta and Maubois, 2000) Due to the susceptibility of milk to spoilage, MF is often performed at low temperatures ~10°C. At temperatures less than 10°C casein molecules have a propensity to dissociate from the micelle. (Seibel et al., 2015) This phenomenon may be exploited in the production of a modified composition MCC exhibiting different physiochemical properties such as reduced micellar size and weaker rennet gels.

Recent work has focused on optimization of membrane filtration, (Lawrence et al., 2008, Beckman et al., 2010, Hurt et al., 2010). Beckman states that the theoretical max of SP removal in a single stage 3x CF is 68%. Combining this with two diafiltration

stages, maximum removal of SP is 97%. Using this layout, only 70.3% of the serum protein was removed using a 0.3um PDVF membrane. Using .1 µm ceramic membranes in a uniform transmembrane pressure (UTP) MF, ~98.3% of the serum proteins were removed, subject to the calculations for serum protein removal.(Hurt et al., 2010). More recently, Hurt found that pre-processing skim milk through a UF system reduced the overall surface area and stages required to achieve a 95% reduced MCC.(Hurt and Barbano) Clearly, opportunities still exist to increase the efficiency of serum protein removal.

Concentration

A concentration step is typically used prior to spray drying to reduce the energy cost associated with drying low solids material. Two methods are typically employed. Vacuum evaporation uses approximately 10% of the energy per unit of water removal at low solids content vs. spray drying.(Schuck et al., 2015) Another method is to concentrate low solids material with reverse osmosis membrane filtration (RO). RO is not considered to be economically viable for concentrating high solids. If a high concentration factor is desired, a combination of both may be utilized.

Drying

Drying ultimately serves to preserve the organoleptic properties and inhibit microbial growth.(Schuck, 2002) Drying also decreases water weight prior to shipment, reducing the cost of transport. Spray drying is the most common method used in the dairy industry today, but other options include roll drying and freeze drying. Spray drying serves as a “sweet” spot, between being cost effective and maintaining product

functionality. Thermal processing, such as spray drying, does induce physiochemical changes that will vary based on operational conditions. (Schuck, 2008)

Drying and High Heat Treatment on Physiochemical properties of MCC

The removal of a substantial fraction of the heat sensitive serum proteins would suggest a high heat stability, however that loss of functionality still occurs during UHT treatment and drying.(Sauer and Moraru, 2012) The wetting times are substantially higher than skim milk powder (SMP) or whey protein isolate (WPI) (Gaiani et al., 2005) This may limit the product's commercial usefulness or result in the addition of unit operations. Research into the elimination of such detrimental heat treatments may make this ingredient more attractive to the food industry. Alternative methods should be assessed, however they must address microbial stability and maintenance of functionality. The use of vacuum evaporation technologies to achieve high solids may meet these requirements. Preliminary work indicates that at 18% protein and 4°C , microbial stability can be achieved with liquid MCC.(Amelia and Barbano, 2013) The Aerobic Plate Count stayed <20,000 cfu/g for 16 weeks. It should be noted that no organoleptic or functional assessments were performed.

Evaporation Methods

History of Evaporation

Water removal in the form of evaporation has existed for centuries. It has been documented since the 1200s, where Marco Polo mentions the production of a milk "paste"(Westergaard, 2004).

The most rudimentary form of evaporation technology was a simple open pan design. The solution was heated to the boiling point, where the vapor pressure was equal to

ambient atmospheric pressure. The rate of water evaporation is limited by the area exposed to the air. Additionally, high temperatures must be used, which can ultimately cause changes to the solution such as coagulation and Maillard effects.

Further advances in evaporation led to forced circulation evaporators in which the product enters the tubes from the bottom and then circulate into the vacuum chamber where the vapor is expelled. Steam is applied to the external surface of the heating tubes, heating the liquid through conduction. A feed-and-bleed method may be utilized where the concentrate returns to the heat exchange section, also known as the calandria. (Westergaard, 2004) It has been reported that evaporation is ~10-12 times more efficient per unit of water removal than spray drying.(Smith, 2011)

Factors Affecting Water Removal

Water removal is based on a combination of drying kinetics and physiochemical properties of the ingredient. These are not mutually exclusive, rather variations or modifications in one can have a dramatic impact on the other. Theoretical models may not accurately take into account some of the more subtle interactions, so experimental data is critical for operational design.

A comprehensive presentation of evaporation kinetics is beyond the scope of this review. In general, they are related to: evaporation surface of the equipment, partial pressure of the water vapor in proximity to the ingredient, and water migration within the ingredient. (Schuck, 2008) These can be broken down even further, but the underlying cause is based on these three ideas.

Physiochemical properties affecting water removal in dairy ingredients are: viscosity, bound-unbound water ratio, type and concentration of ions, protein structure

and concentration, fat content, and moisture content. (Schuck, 2002, Westergaard, 2004, Schuck, 2008) As previously mentioned, micellar proteins have been reported to have greater β slope values of desorption.(Schuck et al., 1998) Schuck additionally suggests that the addition of sodium chloride has a greater impact than the addition of calcium chloride, phosphate or citrate. He attributes this to the increased hygoscopicity of sodium chloride. It should be noted that globular proteins (i.e. whey protein concentrate (WPC)) present lower β with the addition of all studied ion additions, whereas only sodium chloride resulted in a lower β. Schuck proposes that this may be due to the development of an osmotic gradient, causing less of the water to be bound to the casein micelle.

Current Technology

Modern evaporation in the dairy industry is most often of a falling film type. As in the forced circulation evaporators, it is performed under vacuum. Initially vacuum is generated by a vacuum pump but is thereafter maintained through the condensation of the vapor generated through evaporation. The feed liquid enters the calandria, where it is evenly distributed to the heating tubes. Like the forced circulation method, steam is applied to the external surface of the heating tubes. Gravity and displacement due to flow refresh the internal tube contact surface, thereby increasing heat transfer. Both the liquid and steam exit the bottom of the heating tubes. A tangentially oriented vapor separator removes entrained liquid before being evacuated and subsequently condensed.

In an effort to increase water removal efficiency, multiple effect evaporators have been constructed. The principle behind multiple effect evaporators is to use the steam generated from one effect to heat the liquid in another effect. Greater vacuum is applied in the next effect to allow for evaporation at a lower temperature. (Miranda and Simpson,

2005). Successive effects are operated at higher vacuums then the preceding one as there will be a diminished heat capacity of vapors from the initial effects. Negative attributes of multiple effect evaporators include increased capital costs in addition to increased heat exposure and duration. This will have to be taken into account when designing an evaporation system.

Evaporator Adjuncts

Vapor recompression may be optionally used in vacuum evaporators to increase efficiency and can be classified as either thermal vapor recompression (TVR) or mechanical vapor recompression MVR. TVR comingles high pressure steam and the vapor stream thereby increasing steam pressure feeding subsequent effects. MVR relies on a high speed fan to increase recompressesion with the net result being an increased vacuum and ultimately a lower boiling point. (Westergaard, 2004) TVR and MVR may be used solely or in combination with each other based on the evaporator design and physiochemical properties of the feed stream.

While falling film evaporators have many benefits, there are limitations to their use. Products that have high heat lability, crystalize, or high viscosity may foul out the heating tubes, thereby reducing heat transfer rates. There is a positive feedback loop in that more steam is applied causing greater fouling and necessitating more steam.

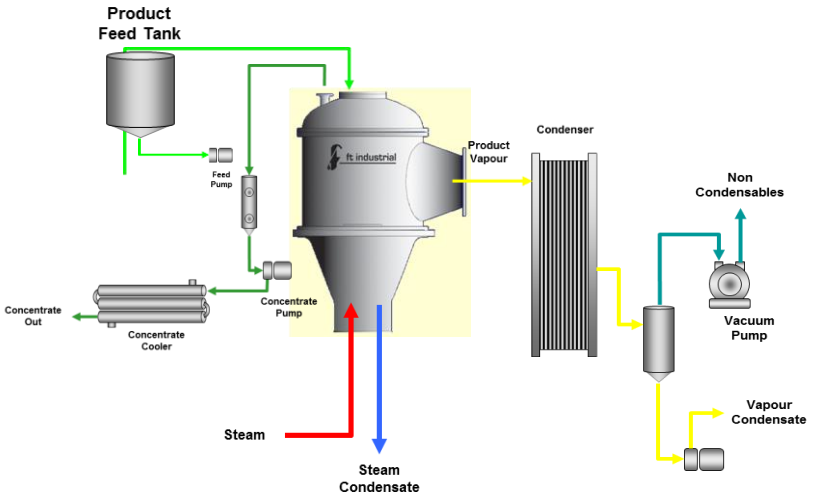
Centrifugal Film Evaporators (CTE)

Centrifugal Film Evaporators incorporate vacuum evaporation with the incorporation of a heated, cone shaped rotor. The rotor spins inducing a centrifugal force, which pushes the concentrate to the bottom of the cone. They have been indicated for use

in water removal of heat labile dairy ingredient solutions. (Jebson et al., 2009, Tanguy et al., 2015) The feasibility of CTE for the evaporation of MCC has not been researched.

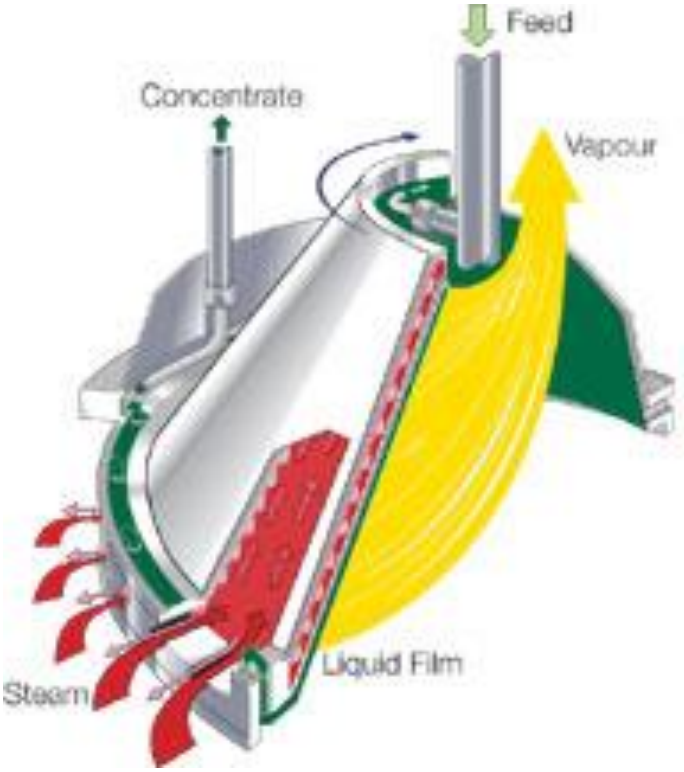
A CTE is a thin film vacuum evaporator utilizing a cone shaped rotor. Steam is applied to the outside of the cone with the condensate exiting through a port on the opposite side. The feed material is pumped through a feed tube at the narrow end of the cone. Through centrifugal force, the feed forms a turbulent thin film and travels radially towards the base of the cone into a concentrate port. (Tanguy et al., 2015) The design of a CTE allows for extremely short residence time within the evaporator, thus lending itself to use in heat labile applications. (Chen et al., 1997) The efficiency of CTE is due to the high overall heat transfer coefficient (HTC). Chen further proposes that primary variables affecting film thickness and HTC are: feed rate, cone rotational speed, cone length, cone angle and feed viscosity.

Fig 1.3 Layout of a centrifugal evaporator



(Centritherm operation guide, 2016)

Fig 1.4 Inlet and rotor design of a centrifugal evaporator



(Centritherm operation guide, 2016)

CTE has been proposed for use in high solids viscosity applications. Preliminary work has used CTE as a finishing evaporator for WPC80 and pre-concentrated skim milk to achieve ~42.5% and ~57.0% TS, respectively. (Tanguy et al., 2015) It has been found that for a recycled cheese whey at 70°C, the thermal resistance over a 4 hour period was lower than an FFE. (Jebson et al., 2009)

The centrifugal force generated by the CTE is substantially greater than simple gravitational force used in heat transfer in FFE. As MCC has a tendency to form a low moisture film, this mechanism could be pivotal in achieving high TS.

This does suggest that this technology may be suitable for the concentration of MCC, therefore the objective of this experiment is to evaluate CTE for the concentration of MCC to a TS in excess of 25%.

Wiped Film Evaporators

Wiped film evaporators (WFE) are well suited for use in heat sensitive, viscous or "hard-to-handle" applications, as the design provides a good heat transfer coefficient. (Chawankul et al., 2003, Solutions, 2015) WFE similarly operates under vacuum, with indirect steam used as the primary heat source. However, it has one larger cylindrical surface rather than the multiple heating tubed calandria in FFE. For the sake of congruity, I will refer to the heated chamber of the WFE as a calandria. Additionally, rather than the use of gravity and fluid dynamics to refresh the contact layer, WFE uses blades that periodically scrape the heating wall, leading to the relatively high heat transfer coefficient. (Chuaprasert et al., 1999) The hydrodynamic modifications of the system may be made based on feed material characteristics and extent of water removal.

Wiped Film Evaporation (WFE) technology utilizes a typical thin film technique for efficient heat transfer. (Zeboudj et al., 2005) In contrast to CTE or FFE, WFE incorporates a rotor that serves to refresh the heat transfer surface with new product periodically. WFE has been indicated for use in heat labile and high viscosity feed solutions. (Cvengroš, 1995, Zeboudj et al., 2005)

Heat Exchanger Surface Fouling

Fouling dynamics of heat exchanger surfaces in the evaporation of bovine milk has been frequently studied, however a consensus of the mechanisms has not been reached. It can be said that the cleaning of process equipment due to fouling is a substantial portion of the processing costing in the dairy industry. (Bansal and Chen, 2006) For the purposes of this section, an evaporator may be thought of as a heat exchanger under vacuum.

Fouling of milk on heat exchanger surfaces has often been classified into two categories: type A occurring at 75-110°C and type B occurring above 110°C. (Burton, 1968, Lund and Bixby, 1975, Changani et al., 1997) Bansal proposes that type A deposits are 70% protein, ~30-40% minerals and 4-8% fat. (Bansal and Chen, 2006) Type B deposits are 70-80% minerals, 15-20% proteins and 4-8% fat. The morphology of the deposits differ with the former being white and spongy and the latter compact, hard, and grey in color.

Initiation of Fouling

Due to high heat lability β-Lg, is often proposed to be implicated in the process of fouling. (Lyster, 1970) Jebson et al suggest that at 70°C bovine serum albumin (BSA) is causally implicated in fouling of WPC on a CTE.(Jebson et al., 2009) Foster et al present a model where at 100°C, a mineral rich sublayer forms, followed by the proteinaceous layer. (Foster et al., 1989)

Many challenges exist in the efficient production of MCC and the potential use of a HCMCC gel in the food industry. Currently, technologies have not been identified to efficiently produce HCMCC in excess of ~25%. We have identified two technologies that

may overcome the challenges associated with evaporation to high solids. In addition to high TS, it is important to consider commercial feasibility. For the purpose of this discussion, we can consider feasibility to be the absence or minimal presence of a foulant layer on the heat exchanger surface or the decrease of water removal capacity during a small scale trial. The duration of lab/pilot scale trial are typically orders of magnitude shorter in duration than commercial production. If these conditions are seen on a short time scale, this will likely be compounded upon scale up. Once a suitable technology is found, a functional evaluation of the resultant HCMCC should be performed. As some of the initial applications of MCC are in beverages and soups, an assessment of the heat stability is a critical first step. We therefore propose 7 objectives.

Objectives

1. Determine the feasibility to produce a high solids MCC containing >25% total solids (w/v) using a Centritherm centrifugal thin film evaporator
2. Determine the feasibility to produce a high solids MCC containing >25% total solids (w/v) using a wiped film evaporator
3. Evaluate the role of increased solids and processing conditions on dynamic rheological properties.
4. Evaluate the Dispersibility of high solids MCC gel under high shear conditions.
5. Evaluate aggregation of residual serum proteins due to wiped film evaporation.
6. Compare heat stability of WFE evaporated MCC with starting MCC.
7. Identify strategies to maintain heat stability of the HCMCC.

CHAPTER 2

EVALUATION OF A CENTRITHERM EVAPORATOR FOR CONCENTRATING MICELLAR CASEIN

Introduction

Microfiltration of skim milk to produce casein levels from 7%-18% has been previously produced with 7%-10% being typical (Nelson and Barbano, 2005, Beckman et al., 2010) Amelia (Amelia and Barbano, 2013) produced an 18% protein MCC with 95% serum protein removal using exclusively membrane filtration. A highly concentrated MCC (HCMCC) has been further produced using evaporation to achieve 24.9 and 30.14 TS with concomitant protein of 18.9% and 22.7%, respectively, using a 2 stage multi-pass falling film evaporator. (Lu et al., 2015a) It was found that with the ~30% solids protocol, excessive fouling occurred.

Our internal research supports the finding that at TS excess of 25% fouling prevents production without frequent CIP cycles. (unpublished data, L.E. Metzger) This implies that feasibility to scale up this process is not realistic as cleaning duration could surpass production time. There exists, therefore, the opportunity for alternate water removal technologies for the efficient water removal in MCC, prior to or in lieu of spray drying.

Objectives

1. Evaporate MCC to maximum attainable solids.
2. Determine the feasibility to produce a high solids MCC containing >25% total solids (w/v) using a Centritherm centrifugal thin film evaporator

Materials and Methods

MCC Production

MCC was produced from pasteurized skim milk (72°C for 20s) in a parallel 2 vessel continuous flow Abcor MF (Koch Membrane Systems, Wilmington, Massachusetts). The membranes were Parker FH3838-S01 (Parker Process Advanced Filtration Division, Oxnard, CA) fitted with 43 mil spacers. Cleaning cycles were performed at the termination of each run as well in addition to a short cleaning immediately prior to processing. The pre-production protocol was a water rinse to neutral pH and a 30 minute alkaline cleaner recirculation with Ultrasil 110 (.6% vol/vol) and Ultrasil (.02% vol/vol) (Ecolab Inc., St Paul, MN) A water rinse to neutral pH followed a 10 minute recirculation sanitation step using Oxonia Active (.2% vol/vol)(Ecolab Inc., St Paul, MN). A final water rinse to neutral pH was performed prior to MCC production. All chemical cleaning steps were performed at 47°C to 50°C.

After each processing batch a long cleaning was performed. A water rinse cycle until the effluent was clear, followed by a 30 minute chlorinated alkaline cleaning recirculation with Ultrasil 110 (.6% vol/vol), Ultrasil 01(.02% vol/vol), and XY 12 (.088% vol/vol) (Ecolab Inc., St Paul, MN). A water rinse was used until neutral pH was achieved followed by a 45 minute alkaline enzyme cleaning recirculation step with Ultrasil 110 (.13% vol/vol) and Ultrasil 63 (.04% vol/vol) (Ecolab Inc., St Paul, MN) A water rinse was then used until neutral pH was achieved. This was followed by a 30 minute acid wash recirculation step using Ultrasil 65 (.2% vol/vol) and subsequent water rinse to neutral pH. A final 30 minute chlorinated alkaline cleaning recirculation with Ultrasil 110 (.6% vol/vol), Ultrasil 01(.02% vol/vol), and XY 12 (.088% vol/vol) (Ecolab

Inc., St Paul, MN) was used with a subsequent water rinse to neutral pH. All cleaning steps were performed at 47°C to 50°C. The final step is a soak rinse with Ultrasil MP (.26% vol/vol) (Ecolab Inc., St Paul, MN) at 32 °C.

To verify cleaning efficacy, water flux measurements from pre-production and post production were compared. Pre and post production flux rates were within 10%.

The batch size was 317 kg. The MF process used a temperature of 14-16 °C with a baseline pressure of 4 PSI, a maximum differential pressure of 28 PSI and a diafiltration percentage of ~62.8. The overall concentration factor was 3.7 with the diafiltration water split into five increments.

CTE Evaporation

25 gallons of ~10% protein MCC was concentrated using a Centritherm model CT1-09RM (Flavourtech, Griffith, Australia). The trials were performed in triplicate with three passes in each trial. Initial trials were used to determine optimal parameters. The starting TS were 12.21%, 12.24%, and 11.72% for replicates R-1, R-2, and R-3, respectively. Feed rate was maintained at 50L/hr. Heating temperature was set to 80°C and a pressure of -20 in Hg.

Results

The results are presented in table 2.1. The percent increase in TS was similar for all passes. The condensate flowrate was 11 to 13 l/hr for passes one and two, however declined to 7-8 l/hr on pass three and was significantly different. The corresponding loss

of evaporative efficiency was ~27% to 46%. The passes were terminated at that point as this was determined to not be commercially feasible.

A substantial foulant layer formed at the contact surface of the cone in all replicates. It was solid with a slight yellow color. The third pass for each replicate had a substantially larger fouling area than the first two passes.

Figure 2.1 Incursion of fouling on CTE rotor surface

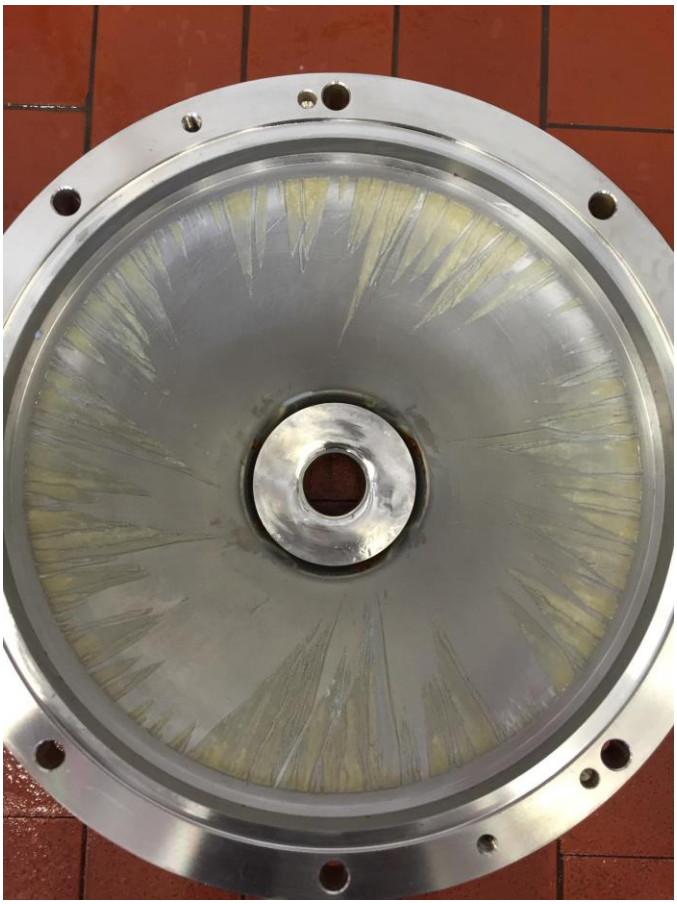


Table 2.1 Process parameters during the CTE evaporation of MCC

	Rep. Number	*Feed Solids (%TS)*	*Concentrate Solids (%TS)*	*Condensate flowrate (l/hr)*	*Percent Evaporation (condensate flow/ feed flowrate) (%)*
Pass 1	R-1	12.21	15.63	12	6
	R-2	12.24	15.19	12	6
	R-3	11.72	15.25	13	6.5
Pass 2	R-1	15.63	18.09	12	6
	R-2	15.19	18.91	11	5.5
	R-3	15.25	19.12	12.5	6.25
Pass 3	R-1	18.09	22.49	8	4
	R-2	18.91	21.92	7	3.5
	R-3	19.12	22.93	8	4

Discussion

The present study found that evaporation efficiency of a CTE was substantially reduced when TS was greater than 19%. This decline suggests interactions specific to or at least more prevalent in MCC than other concentrated dairy proteins.

Casein micelles are known to be highly hydrated, however, only 15% of the 4g of water/ g of protein is bound. (Holt et al., 2003, Farrell Jr et al., 2013) The remaining is said to be occluded by the micelle supra molecular structure. Lu et al suggest a model where an overlap of casein protuberances occurs as casein concentration increases. (Lu et al., 2015a) In order to reach ~20% casein, removal of water from the casein surface must occur. This closely packs in the casein micelles, presumably hindering further water release.

Fouling of Heat Transfer Surface

Fouling of whole milk on heat exchanger surfaces has been categorized into either a type A deposit occurring at 70°C to 110°C or a type B occurring at greater than 110°C. The deposit morphology in the CTE did not correspond to a type A deposit, suggesting that it may be compositionally different. That is not surprising as MCC is substantially depleted of serum proteins, specifically β-lactoglobulin. This fraction is implicated in fouling development. (Dalgleish, 1990)

One potential mechanism for this layer formation is that it is not a standard fouling layer, rather a thin film of dehydrated MCC. Lu eta al presents cold gelling properties of MCC as a function of protein concentration. (Lu et al., 2015a) At 23% protein, cold gelation occurred at 38°C. Gelation decreased at a rate of 5°C for everyone percent decrease in protein. An extreme extrapolation of this formula yields that at 80°C the MCC would only have to be at ~31.4% protein to form a gel. It is plausible that localized concentrations at the heat exchanger surface could achieve those levels. The consequence of this phenomenon would be that the centrifugal force may not be sufficient to refresh the exchanger surface, further locally increasing the solids of the MCC. The end result would be a dehydrated MCC layer. Given sufficient time, a standard type A fouling would potentially occur.

Substantial work has been dedicated to reduction of surface fouling. (Changani et al., 1997, Bansal and Chen, 2006) Surface modification such as polyethylene glycol (PEG) grafting or super hydrophobic coatings may reduce the buildup during evaporation.

Conclusion

Key findings:

a. CTE may be used for water removal in MCC, however beyond ~19% TS a decrease in evaporative efficiency occurs.
b. When the feed TS exceeds 19% a decreased evaporative efficiency occurs. The morphology of the deposit differs from that of whole milk at similar temperatures, suggesting that it may be compositionally or mechanistically different.

It can be concluded that under the current processing conditions, CTE does not offer a benefit over FFE in regards to water removal at increased solids. Pretreatment steps may be used to optimize evaporative efficiency and reduced fouling. Further work should be performed to determine the fouling mechanisms of MCC and strategies to mitigate deposit formation.

CHAPTER 3

EVAPORATION OF MICELLAR CASEIN CONCENTRATE USING WIPED FILM EVAPORATION

Introduction

Wiped Film Evaporation (WFE) technology utilizes a typical thin film technique for efficient heat transfer. (Zeboudj et al., 2005) In contrast to CTE or FFE, WFE incorporates a rotor that serves to refresh the heat transfer surface rather than centrifugal or gravitational force as with CTE and FFE, respectively. WFE has been indicated for use in heat labile and high viscosity feed solutions.(Cvengroš, 1995, Zeboudj et al., 2005) Indeed, the fouling/dehydration deposit observed in the CTE trial may be overcome through the physical wiping action of the WFE. Removing a greater amount of water prior to drying is more cost efficient, therefore it is a goal of many optimization attempts in the food industry. Furthermore, an understanding of the physiochemical consequences of evaporation on the resultant HCMCC is necessary to assure no loss of functionality occurs.

Process Design of WFE

WFE is typically designed for use in single pass type evaporation. The evaporator vessel is under vacuum to reduce the boiling point and thereby increase evaporative efficiency. Steam is applied to the outer surface of the chamber wall and transferred to feed through conduction. The feed is pumped into the top of the evaporator, where a distributor plate applies a continuous stream into the calandria. The wiping action of the rotor combined with gravitational force causes a radial and downward movement of the solution inventory. The concentrate exits through the base of the evaporator. The water vapor with entrained concentrate may be separated internally as depicted in figure 3.1, or externally in a FFE style vapor separator.

The hydrodynamics of the agitated feed can be separated into 3 distinct zones: bow wave, air gap, and streaming film zone as shown in figure 3.2. (Taeymans, 1988) The contributions of these zones will ultimately determine the heat transfer rates given a static feed rate.

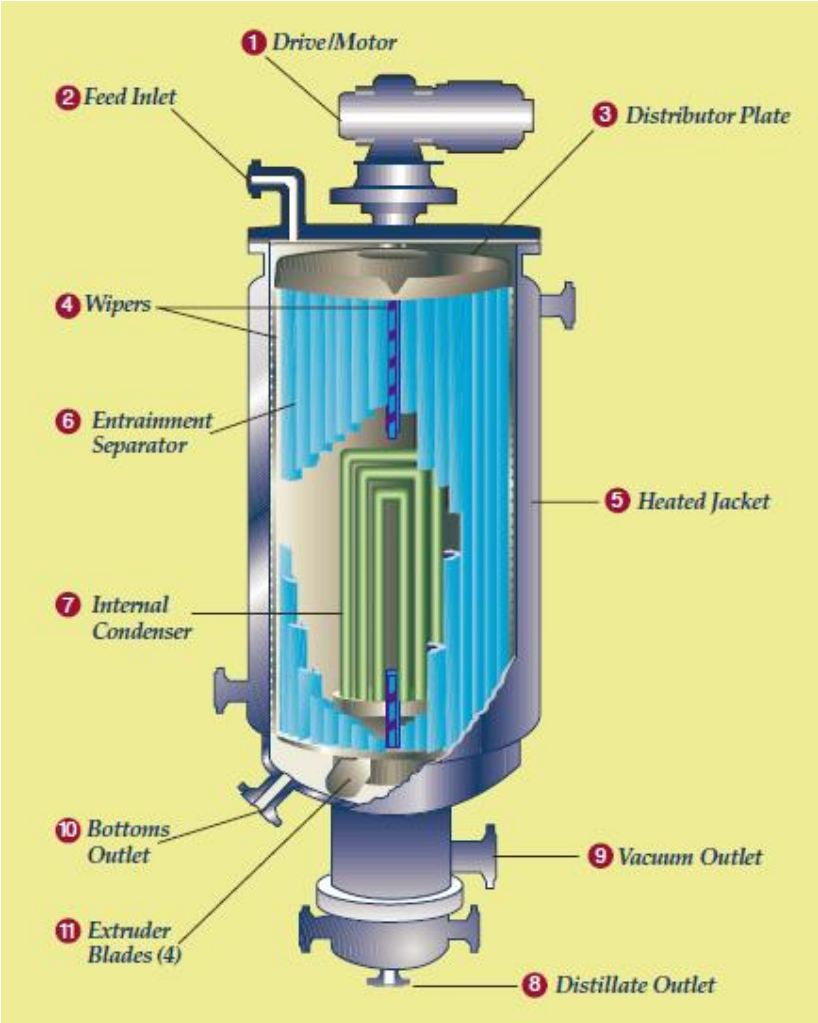


Fig 3.1 Wiped Film Evaporator Layout

http://www.tradeindia.com/fp760710/Wiped-Film-Evaporator.html

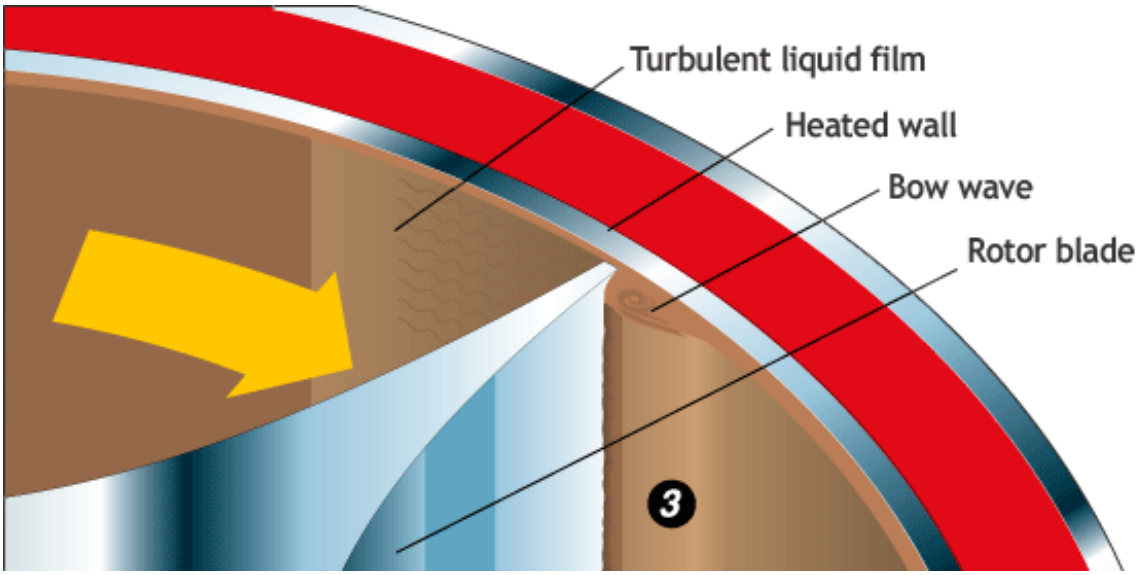


Fig. 3.2 Wiped Film Evaporator: Product path within calandria

http://www.lcicorp.com/thin_film_evaporators/category/operation

The results of our internal CTE trials and other work suggest that fouling at the heat exchanger surface may be due in part to the film forming properties of MCC. (Lu et al., 2015a) We predict that through the periodic renewal of the heat transfer surface through physical agitation, the tendency to foul will be reduced, thereby allowing for the production of high TS MCC. The achievement of greater than 25% TS will be an indicator of successful performance.

Objectives

1. Determine the feasibility of high solids MCC production containing >25% total solids (w/v) using a wiped film evaporator
2. Evaluate the role of increased solids and processing conditions on dynamic rheological profiles

3. Evaluate the Dispersibility of high solids MCC gel under high shear conditions

Materials and Methods

MCC Manufacture

MCC was manufactured as described in chapter 2. Total solids were 12.41, 11.48, and 12.76% for the replicates A, B, and C, respectively. The full results of the preliminary analysis can be found in table 3.1. The MCC replicates were frozen at -20 °C prior to evaporation.

WFE Evaporation

The frozen MCC was transferred to the Rtech Laboratories pilot facility (Land O' Lakes, Inc., Arden Hills, MN) where it was placed in a -15 °C blast freezer until the day before the trial. Prior to evaporation, samples were allowed to partially thaw overnight at 20°C. In the morning samples were tempered to 49.5 ± .7 °C in a 50 gallon hot water jacketed wiped surface tank and held for a minimum of 15 minutes prior to commencement of evaporation.

The evaporator used for the trials was a Pfaudler Wiped Film Evaporator model #8.8-12V-27 with 8.8 sq. ft. of evaporative surface area. It was equipped with a hot water jacket for conductive heat transfer to the calandria. Optimization was performed prior to the trial. The settings were based on maintaining a constant feed rate without causing excessive residence time. Feed rates below the designated optimum were associated with product bridging above discharge outlet and excessive adhesion in the vapor separator. When feed rates exceeded optimum, the TS was low and inconsistent solids material was produced.

The jacket water temperature was set at 69 ± 1°C throughout the experiments. Wiper rotor speed was set at 202-203 RPM. Chamber vacuum was -23 to -24 PSI. WFE rotor speed was held at a constant 202 ± 1 RPM. Chamber vacuum was held at -23 to -24 PSI throughout the trial. MCC was feed rate to the WFE at 2.0 ±.2 lbs. /min.

Compositional and Statistical Analysis

All compositional tests were performed in triplicate unless otherwise noted. Experimental results were analyzed by R (ver. 3.2.0) to determine statistical difference between samples with a p of less than .05 considered significantly different.

Total solids (TS) as determined by the reference method for the feed and evaporated were 29.85, 29.19, and 29.06% respectively, total nitrogen (TN), casein nitrogen (CN), ash, non-protein nitrogen (NPN) of the feed and evaporated MCC solutions were performed according to reference method (Hooi, 2004a, c, b)

Dispersibility

Sample Preparation

To assess dispersibility or rather the disruption of the MCC gel matrix by agitation, a method adapted from Lu et al. in high sheer conditions was utilized. (Lu et al., 2015b) One hundred gram samples were thawed at room temperature. To 200 mL of 50°C deionized water, MCC was added to achieve a 2.5% wt/wt solution. To each aliquot, .5 mL of TRANS-10A antifoam Trans-Chemco Inc., Bristol, WI) was added. High shear mixing was achieved by using a Polytron PT2100 (Kinematica Co., Lucerne, Switzerland) at 11000 rpm for 1 min.

Dispersibility procedure

Prepared samples were poured through a 250 µm standard sieve using the filtrate to rinse the beaker 3 times. Retained particulates were transferred to a pre-weighed, pre-dried filter paper with ~800 mL deionized water, and placed in a vacuum oven at 107°C overnight. Dispersibility was calculated by percentage of dry weight retained as compared to total solids in the MCC.

Dynamic Rheological Analysis

Sample preparation

Analysis of dynamic rheology was performed using a Stresstech HR high resolution controlled stress rheometer (ATS Rheosystems, Rheological Instruments Inc., Borden-town, NJ) The method was adapted from Kommineni et al. (Kommineni et al., 2012) A temperature sweep was used to determine the melting point of the gel. MCC of ~100g were placed into Whirlpak bags (Nasco Inc., Fort Atkinson, WI) and immersed in a 60°C water bath, keeping the opening closed but above the water level. Aliquots were allowed to equilibrate for minimum 30 minutes. Forms for the samples were made from polypropylene flip top vials (28 mm diameter) with the tops cut off. A small amount of non-stick cooking spray (Pam Original, ConAgra Foods Inc., Omaha, NE) was applied to the inside surface with the excess removed with paper towels. Aliquots of 2 grams were rapidly weighed into each vial and tapped on the counter to disperse and remove air bubbles. Vials were placed into refrigeration (4°C), inverted and covered with plastic wrap for minimum 60 minutes to set the gel. Sample were transferred directly onto the temperature controlled (30°C) rheometer stage from the refrigerator to maintain sample integrity. The plate was lowered to a 2mm gap and mineral oil was applied to the exposed

surfaces. Samples were allowed to equilibrate for 5 minutes prior to test commencement. Stress and frequency sweeps were performed to establish the linear range for each replicate. Based on these preliminary tests, 100 Pa and frequency 1.5 Hz were selected. Temperature ramp for Rep 2 was 30-60°C at a rate of 2°C sec^{-1} and was extended to 30-75°C for Reps 1 and 3 at the same rate. The increased temperature ramp rate was necessary due to the tendency of the exposed sample surface to dry out, even with the application of the mineral oil.

Results

Composition

The compositions of the concentrated MCC are shown in table 3.2. The mean for the total solids was 29.37%. Replicate A was significantly higher than B and C, however B and C were not different. The ash was significantly higher in A than B and C, however the increased TS of replicate A contributed to this difference. The total calcium was significantly higher in B than A and C. There was a numerically lower NCN in replicate C, however it was not considered significant. Composition of the feed material can be found in Table 3.2. All replicates had complete dispersibility (Table 3.3) at 50°C after 1 minute of high sheer at 99.94, 99.94, and 99.92 for replicates A, B, and C, respectively.

Evaporation

The CN: TN ratio was not significantly different among samples. No differences were seen from feed material and evaporated MCC. This indicates that the evaporator did not cause aggregation of the residual serum proteins.

Fig 3.1 Pfaudler WFE used in the evaporation of MCC

Fig 3.2 Positive displacement discharge pump during experiment.

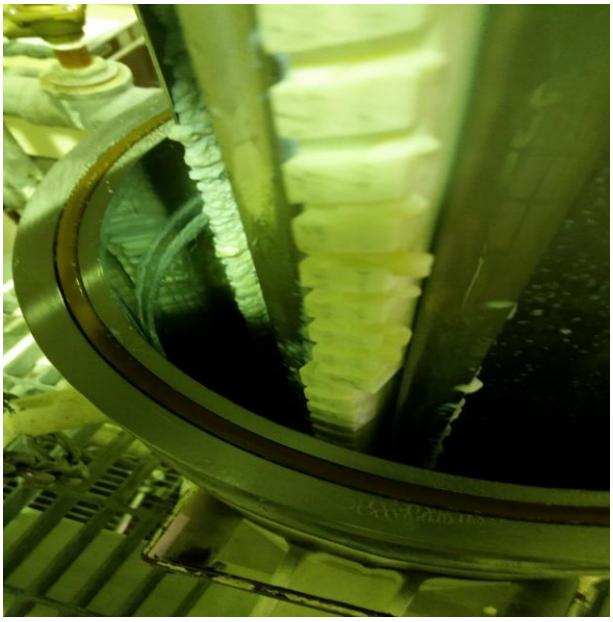


Fig 3.3 Rotor assembly post evaporation prior to water rinse.

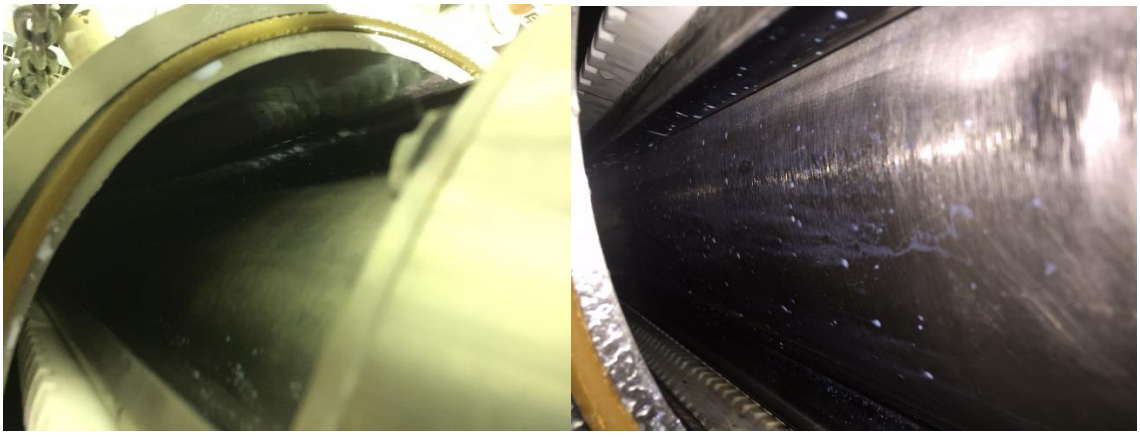


Figure 3.4 Rotor assembly post evaporation prior to full cleaning, but after water rinse.

Table 3.1 Composition of Feed MCC after MF filtration

Attribute	*Rep-A*	*Rep-B*	*Rep-C*
TS	12.41	11.48	12.76
Protein	10.40	9.61	10.61
NCN	1.48	1.44	1.64
CN	8.92	8.17	8.96
CN:TN	.86	.85	.85
Ash	0.99	0.92	1.04
*Calcium**	259	272	273
Fat	0.27	0.23	0.36

**calculated based on evaporated results*

Table 3.2 Compositional data of WFE evaporated MCC

Attribute	*Rep-A*	*Rep-B*	*Rep-C*	*Average of all replicates*
TS	29.85[b]	29.19[a]	29.06[a]	29.37
Protein	23.55[a]	23.33[a]	23.17[a]	
NCN	3.49[a]	3.53[a]	3.17[a]	
CN	20.06[a]	19.79[a]	19.99[a]	
CN:TN	0.85[a]	0.85[a]	0.86[a]	
Ash	2.41[b]	2.37[a]	2.36[a]	
Calcium (mg/100g)	623[a]	694[b]	626[a]	

Results in the same row with the same letter are not significantly different.

Table 3.3 Dispersibility of HCMCC in water at 50°C after high shear mixing

	Rep-A	*Rep-B*	*Rep-C*
Percent Dispersibility	99.94	99.94	99.92

Dynamic Rheological Analysis

Replicate C had a mean tan-δ of 1 at ~65°C while Replicates A and B had tan-δ=1 at ~51 °C. This defines the transition from elastic to viscous dominance. Protein, NCN, CN, and ash content were not significantly different among the samples. While not

statistically significant, the NCN of Replicate C was numerically lower than Replicates A and B. This may have contributed to the increased temperature of tan-δ

The method for the temperature sweep was modified from that used by other investigators. A ramp of 1°C per minute is often used, however we used a 2°C per minute ramp. It was found that even when a mineral oil vapor barrier was used, sample dehydration occurred after extended analysis times, because sample pucks absorbed the oil over time, exposing the outside layer to air.

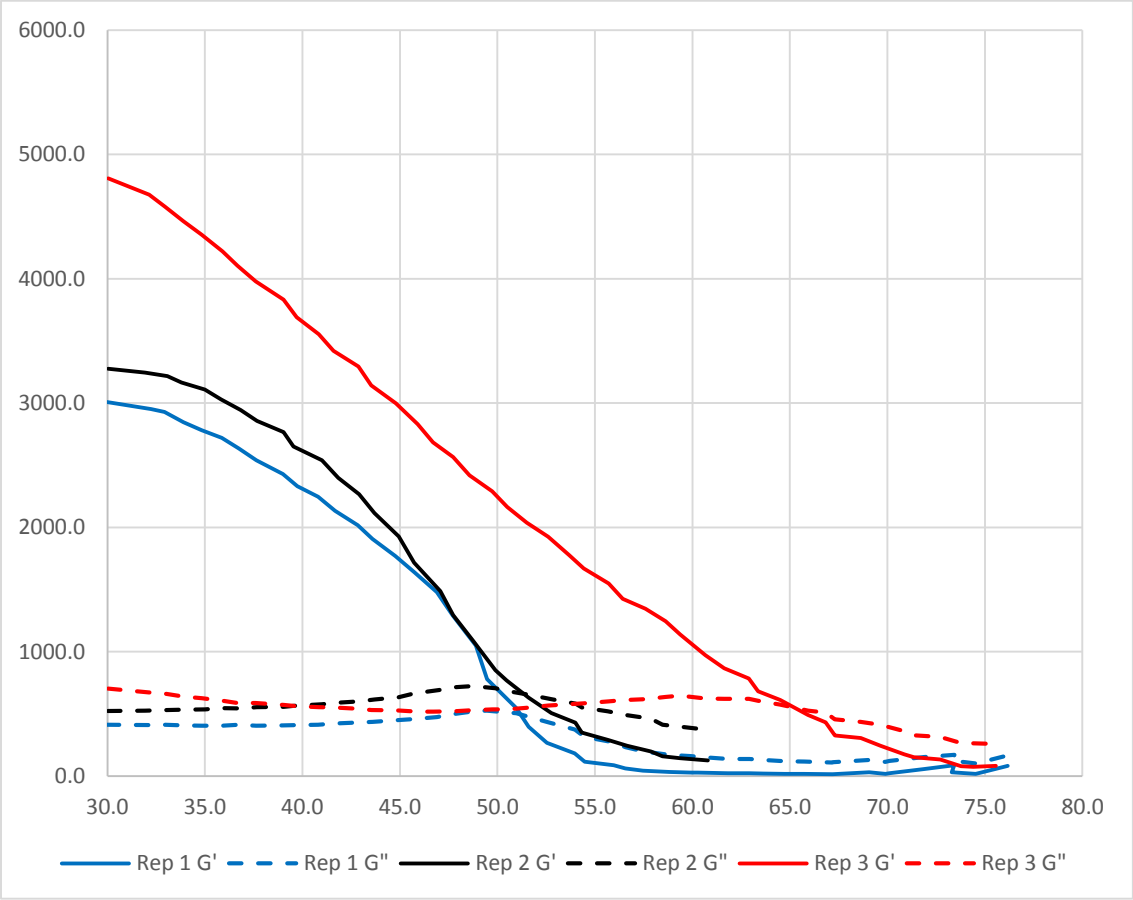


Figure 3.5 Mean results of Dynamic rheological analysis of evaporated HCMCC

Discussion

Evaporation Process

Previous attempts to evaporate MCC to greater than ~23% solids have been marginally effective as there is a high propensity for fouling even during short trials.(Lu et al., 2015a) Metzger L.E., unpublished). The current work presents a viable alternative to falling film evaporation. Further optimization should be performed to increase energy recovery and throughput. During preliminary experiments, a preconcentration step using FFE was used to get to 23% solids. Throughput was approximately double that of the current trial. A cost analysis should be factored in when considering this option.

As seen in figure 3.3 and 3.4, no substantial surface fouling occurred .The buildup that was formed was considered "normal." Indeed, the buildup became dislodged easily during the product flush cycle with ~50°C water. This is an important aspect in extended production runs, since full cleanouts due to fouling are costly and time consuming.

In contrast, the cleaning during CTE was substantial. A standard CIP cycle was not sufficient to clean the rotor. Between each pass of the CTE, it had to be disassembled and manually scrubbed, combined with a ~30 minute caustic/chlorine soak to remove the foulant layer.

Rheology

At a cursory glance, the high tan-δ=1 of replicate C may seem to be an anomaly, however subtle differences in composition may have a dramatic effect on the rheology. The casein to solids ratio was higher thereby creating more overlap of the casein hydration spheres. The casein protuberances, presumably κ-casein, would be allowed to interact to a larger extent. (Dalgleish et al., 2004, McMahon and Oommen, 2008)

The lower NCN in replicate C means there is less residual serum protein. Serum proteins may act the inhibit casein-casein interactions. It should be noted that neither of these value comparisons were significantly different.

Conclusion

Key Findings

1. The WFE was found to produced HCMCC with TS >29%.
2. No fouling was observed and throughput remained constant throughout the trials.
3. High temperatures (~50°C) combined with high sheer rate causes near complete dispersion of the HCMCC.
4. No changes in NCN ratio occurred indicating no heat or concentration based denaturation of the residual serum fraction.
5. HCMCC forms a reversible cold gel at room temperature, but will liquefy upon heating.
6. MCC composition may be a contributing factor in the shift from elastic to viscous temperatures.

Initial findings suggest minimal effects of evaporation on physiochemical properties of the HCMCC while still achieving a high TS. Shelf and heat stability are important steps for evaluating viability of this process. Efforts to scale up the WFE process should evaluate pre-concentration using FFE or WFE in a multi-effect format to increase throughput and cost effectiveness.

CHAPTER 4

EFFECT OF PREVIOUS HIGH CONCENTRATION ON MCC HEAT STABILITY

Introduction

MCC is an attractive ingredient to the food industry due to the partial removal of the serum phase (serum proteins, soluble minerals, lactose) components. (de Kort et al., 2012). High protein beverage systems are a target application. These beverages are typically subjected to high heat treatments to extend shelf life. It is critical that the ingredient system does not aggregate, coagulate, precipitate, or form gels during high heat treatment. Heat Coagulation Time (HCT) is a measure of these manifestations and is often used synonymously and can be accomplished through numerous methods.(Singh, 2004) One method proposed by Davies and White uses glass vials filled with a predetermined quantity of sample.(Davies and White, 1966) The vials are attached to a platform and immersed in an oil bath, typically at 120-140ºC. The platform is rocked at a given frequency until the onset of coagulation or precipitation occurs. While this is considered a subjective method, it is widely used for research purposes, likely due to simplicity and repeatability.(Singh, 2004)

Milk can be classified based on the HCT profile over a pH range.(O'Connell and Fox, 2000, Singh, 2004) Type A milks exhibit a maximum HCT at pH 6.7 and minimum at 6.9, subsequently increasing as pH is increased. Type B milks have been sufficiently altered, such that the HCT increases as pH increases. (O'Connell and Fox, 2000) Singh summarizes strategies that may be utilized for the conversion of type A to type B milks. (See table 4.1). Based on these strategies, MCC fulfills two of them: depletion of serum proteins and reduced soluble minerals. Indeed, it has been found to follow a type B HCT profile.(de Kort et al., 2012)

Heat stability may be affected by the inclusion of calcium chelators as this may impact the micellar structure due to the integration of CCP.(Augustin and Clarke, 1990, Singh et al., 1995) These chelators are used to increase the heat stability of milk. Indeed, they have been shown to increase HCT, however, at sufficiently high concentrations they can cause the disruption of the micelle structure. The consequence is a negative impact on HCT. It has been suggested by De Kort et al that a loss of turbidity is an indicator of such disruption. (de Kort et al., 2012) Citrate is an often used chelator, typically at concentrations up to 40mM, in beverage applications. The implied mechanism is binding of the serum calcium, whereas phosphate has been proposed to associate with calcium on the micelle surface. (de Kort et al., 2012)

Heat stability may be negatively impacted by previously applied water removal steps. While modern evaporation technology utilizes low heat/ high vacuum systems to accomplish water removal, a decline in subsequent heat stability still occurs. The increase in destabilizing components such as an increased protein, calcium, and lactose, is implied in reduced subsequent heat stability.

The previous chapter presented a method for the production of a HCMCC containing greater than 29% TS, without surface fouling reported using other methods. It is unknown whether this material maintains the functionality of the unconcentrated material.

Objectives:

1. Evaluate the heat coagulation time of MCC concentrated by wiped film evaporation.
2. Compare heat coagulation time of the pre and post evaporation MCC.
3. Determine the effects of trisodium citrate on the heat coagulation time of the evaporated MCC

Materials and Methods

Sample Preparation

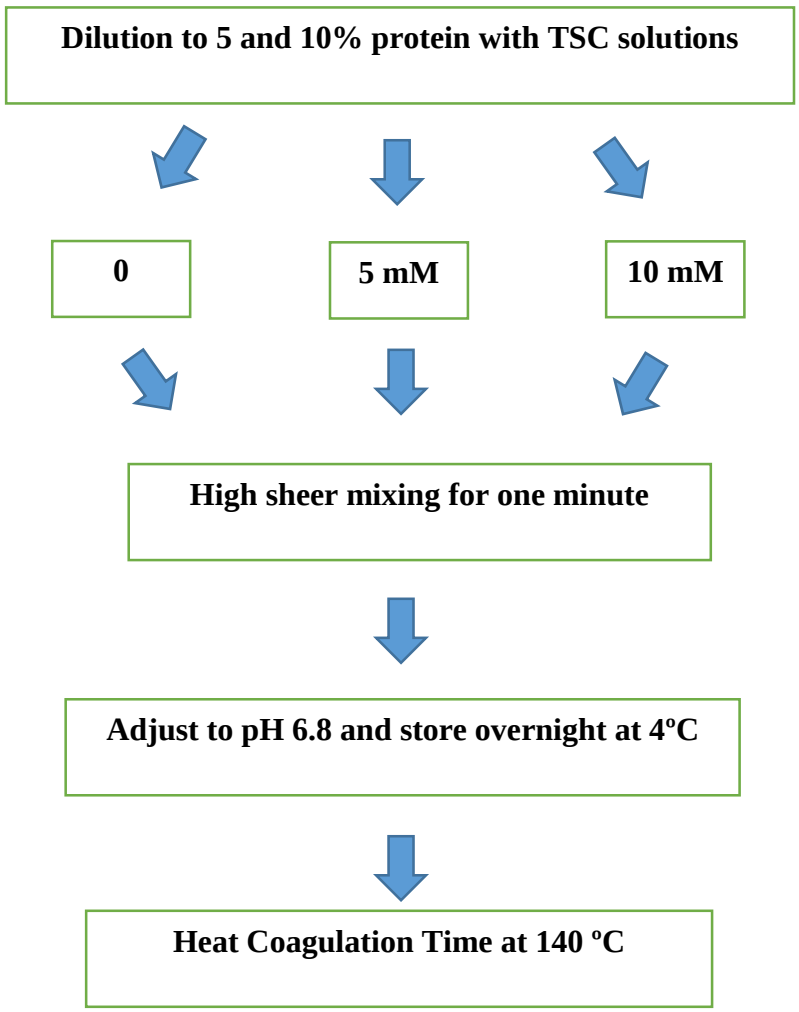
The three HCMCC replicates produced in the previous chapter were frozen until the day prior to use. A summary of the experimental protocol can be found in figure 4.1. The sample containers were partially immersed in 23 ºC water and allowed to thaw overnight. Samples were mixed using scoopulas for a minimum of one minute to ensure even compositional distribution. Trisodium citrate (TSC) was added to double distilled water to achieve 0 (NC), 5 (LC), and 10mM (HC) solutions. The protocol utilized in the dispersion method was used to dilute the samples to 5 and 10% protein. The amount of antifoam (Trans 10A, Transchemco, Bristol, WI) was reduced from .5mL to ~.1 mL in order to limit potential impact on the HCT. Sodium azide was added to at .02% to inhibit microbial growth. After HS mixing, samples were allowed to rest at 23 ºC for 1 hour for the sample to cool and foam to break. Samples were measured for pH (Fieldscout pH 110, Spectrum Technologies Inc., Aurora, IL) and adjusted to pH 6.80±.05 with either

1M hydrochloric acid (Thermo Fisher Scientific Inc., Waltham, MA) or 1M sodium hydroxide (Thermo Fisher Scientific Inc., Waltham, MA) to raise or lower pH, respectively. Samples were refrigerated at 4 ºC overnight. In the morning, samples were immersed in a 30 ºC water bath for a minimum of 2 hours. A final pH measurement was performed and minor adjustments were made. Control samples were made using the unevaporated MCC (feed) used in the WFE trials and were prepared by diluting to 5 and 10% protein with double distilled water. Replicate 2 of the unevaporated MCC had an as is protein of 9.61, therefore, no dilution was performed. The control samples were adjusted to pH 6.8±.05. Sodium azide at .02% was added, and they were refrigerated with the trial samples overnight. In the morning, samples were immersed in a 30 ºC water bath for a minimum of 2 hours. A final pH measurement was performed and minor adjustments were made. As a follow-up, feed and 0mM samples of the 3 replicates were prepared in the above manner, except no pH adjustment was made.

Heat Coagulation Time

HCT was adapted from the method presented by Davies and White. (Davies and White, 1966) Aliquots (3 mL) of the diluted MCC were transferred to 8 mL Wheaton glass tubes (D- 17 mm × H- 61 mm). (Thermo Fisher Scientific Inc., Waltham, MA) The tubes were capped until air tight and clamped into the oil bath rocker assembly. The oil bath was maintained at 140 ºC. The time began when samples were placed into the bath. The rocker assembly was maintained at a 7-8 second cycle time. For the purpose of the experiment, HCT was defined as the onset of a visible aggregate or gelation. At minimum, duplicate samples were tested for HCT.

Figure 4.1 Experimental protocol for the evaluation of 5 and 10% reconstitutions of HC



Results

The results of the HCT are shown in table 4.1. Within each WFE replicate, samples made with 10 mM TSC had the longest HCT. The 5 mM TSC samples had the next longest followed by the feed samples and the 0 mM TSC samples.

In all replicates, the 10% protein samples had a longer HCT than the corresponding 5% protein samples at 0mM TSC. Conversely, the 5 and 10mM TSC samples had a longer HCT in the 5% than the corresponding 10% protein samples.

The HCT pH unadjusted results for the feed and concentrated treatments are shown in tables 4.2 and 4.3, respectively. The pH results of the feed and 0mM TSC are shown in tables 4.4 and 4.5. The samples without pH adjustment increased in HCT as pH increased and with lower protein concentration ($P < 0.05$). No significant difference was seen between the feed and NC samples when factoring for the difference in pH although the p-value was on the threshold of significance.

The HCT of the pH adjusted samples were significantly different based on citrate level with HC>LC>NC, respectively ($P < 0.05$). The feed samples were not significantly different than the NC samples, although within replicates, a slightly lower HCT was found for NC.

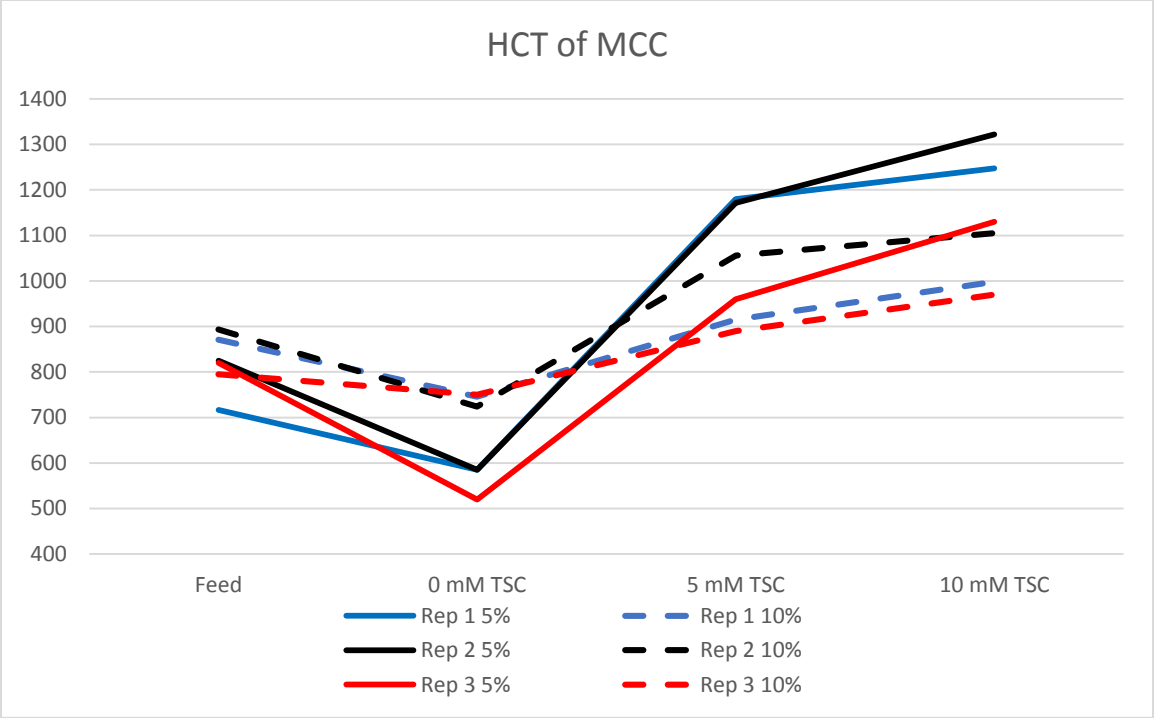


Fig 4.2 HCT of MCC at pH 6.8 and 5 and 10% protein solutions.

Table 4.1 Average HCT (s) of MCC with or without the addition of sodium citrate at pH 6.8.

Sample	*Feed*	*0 mM TSC*	*5 mM TSC*	*10 mM TSC*
Rep 1 5%	716.6	585.0	1180.0	1247.0
Rep 1 10%	871.0	746.0	915.0	998.3
Rep 2 5%	825.0	585.0	1171.6	1322.0

Rep 2 10%	893.3	724.0	1056.0	1105.0
Rep 3 5%	820.0	520.0	960.0	1130.0
Rep 3 10%	795.0	750.0	890.0	970.0

Table 4.2 Average HCT (s) for pH unadjusted feed samples

Protein level	*Rep 1*	*Rep 2*	*Rep 3*
5% Protein	1338	1296	1305
10% Protein	1005	1000	1005

Table 4.3 Average HCT (s) for pH unadjusted product samples

Protein level	*Rep 1*	*Rep 2*	*Rep 3*
5% Protein	1167	965	1238
10% Protein	1245	975	1065

Table 4.4 pH of unadjusted feed samples

Protein level	*Rep 1*	*Rep 2*	*Rep 3*
5% Protein	7.09	7.04	7.07
10% Protein	6.94	6.91	6.94

Table 4.5 pH of unadjusted 0mM TSC samples

Protein level	*Rep 1*	*Rep 2*	*Rep 3*
5% Protein	6.91	6.79	7.08
10% Protein	6.98	6.83	6.93

WFE evaporation effect on heat stability

A significant decline from feed to NC in the 5% protein samples compared to a still significant but modest decline in the 10% protein samples suggest a mechanism not specifically related to the overall protein level. It is generally thought that in milk, as protein increases, the heat stability decreases. (Singh, 2004) The addition of sodium chloride has been reported to been reported in type A milk to shift the HCT/pH curves towards alkaline side and an increase in maximum stability(Grufferty and Fox, 1985) Huppertz and Fox proposed that even though the addition of NaCl caused a decrease in ζ-potential, it may reduce the dissociation of κ-casein.(Huppertz and Fox, 2006) Grufferty did, however, find that at 300mM levels, NaCl reduces HCT.(Grufferty and Fox, 1985)

Discussion

While the HCMCC samples did have a modest but consistently lower HCT than the feed MCC, the cause has not been fully determined. The likely contributing factors are the increased protein concentration as a result of evaporation as well as exposure to high temperatures. We found that the addition of TSC can return the HCT to equal or greater values than the feed samples. This is important as a target application of MCC is

in high protein beverages that may be subjected to UHT or retort treatment. Initial work has suggested that increasing the pH or lower heat treatment temperatures may further increase the heat stability.(Sauer and Moraru, 2012)

As proposed by others, type B milks, with higher pH exhibit a higher HCT.(Singh, 2004, Sauer and Moraru, 2012) The results of the current study agree with this. There was no substantial difference seen between the 5 and 10% protein treatments. When standardized to pH 6.8, however, the HCT of the NC at 10% was greater than the 5% treatments. The increased buffering capacity with the higher protein treatments caused an increased use of HCl or NaOH to reach the target pH. We propose that the increased usage caused a deviation from the calcium concentration to ionic strength relationship. The result likely causing a reduced calcium ionic strength. Ultimately, this may increase the HCT. No calcium ion activities were performed, so this cannot be proven based on the data.

When TSC was added, this was not the case. Both 5mM and 10mM treatments had higher HCT in the 5% protein samples than the 10% samples. The HCT was directly proportional to TSC concentration for all samples. The 5% protein sample with 10mM TSC had the highest HCT.

TSC is reported to bind calcium serum calcium ions, however, it has finite binding capacity. Because binding is limited, the unbound calcium ion concentration is higher in the 10% protein samples. Higher unbound calcium ion concentration in these samples reduces the electrostatic propulsion forces of the casein micelles. Therefore, aggregation is faster in the 10% protein samples.

An alternative explanation is that the 10% protein samples had an elevated ion content such that the ζ-potential was sufficiently low, reducing impact of the TSC as suggested by Grufferty and Fox.(Grufferty and Fox, 1985) To support this explanation, the HCT increase from 5mM to 10mM was lower in the 10% than the 5%, potentially due to ζ-potential reduction.

Beyond what has been discussed above, other variables may have an effect on HCT. These are beyond the scope of this work but include:

1. Variations in ionic strength.
2. Calcium ion activity
3. Protein to solids ratio
4. NCN content
5. Differences in urea concentration.
6. Variations in residual SP

Urea content variation is not thought to alter HCT in concentrated milk. It is unknown if this applies to MCC as well(Singh, 2004).

Conclusion

Key Findings

1. A modest but not significant decline in HCT occurred to the HCMCC as a result of evaporation.
2. TSC solutions at 5 and 10mM may be used to dilute the HCMCC at 5 and 10% protein to restore and increase HCT beyond starting values.

3. NC preparations with 10% protein (pH 6.8) had a higher HCT than those at 5% which may be attributed to a reduced calcium ion activity.
4. In MCC preparations without pH adjustment, the HCT was positively correlated with pH regardless of protein concentration.

We have found that WFE does not have a significant effect on heat stability. The use of a chelator, such as TSC in 5 and 10mM solutions increase heat stability. Further work should focus on stability over time under various storage conditions.

CHAPTER 5

SUMMARY AND CONCLUSION

This research was structured into 3 major parts. The first part evaluated a centrifugal evaporator to concentrate micellar casein concentrate (MCC). The second part evaluated a wiped film evaporator (WFE) to concentrate MCC. The third part evaluated heat stability of the high concentration MCC produced by WFE.

In part 1, the primary objective was to achieve >25% total solids (TS) without a loss of water removal rate associated with high solids materials. The major finding was that it did not offer any benefits over a standard falling film evaporator (FFE). We propose that MCC adhesion to the rotor surface due to localized dehydration was greater than the generated centrifugal force generated by the CTE. The dehydration layer then would have sufficient residence time to generate a foulant layer.

In part 2, WFE was used to achieve the same primary objective of >25% TS. No visible foulant layer or reduced throughput was observed, thus fulfilling our criteria for feasibility. WFE was found to be able to achieve MCC at ~29% solids without modification to the existing equipment. The resultant high concentration MCC (HCMCC) was found to have >99% dispersion at 2.5% solids in 50 °C water at a high sheer rate.

Part 3 focused on the heat stability of the HCMCC produced in part 2. The major findings were that: (i) there was a small decline in heat stability of HCMCC after reconstitution to 5 and 10% protein levels (ii) the lost heat stability could be recovered through the use of trisodium citrate (TSC) (iii) when the pH was standardized to 6.8, the 10% protein treatments without TSC had greater heat stability than 5% protein treatments.

Recommendations for further research

1. Based on the finding that 29% TS MCC could be produced feasibly, post evaporation processing should be evaluated. Due to the high viscosity of the product stream, traditional spray drying techniques are unlikely to be successful. We would recommend researching packaging methods in the concentrate form.
2. In chapter 4, we found that the use of WFE to concentrate MCC did not have an immediate effect on heat stability, however no evaluation on age related effects were evaluated. We recommend assessing heat stability and microbial effects over time.

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