ABSTRACT

WHITT, DANIEL MCKAY. The Effect of Micellar Casein Concentrate (MCC) Purity and Fat Content on Sulfur/Eggy Flavor in Ultrapasteurized Milk Protein-Based Beverages. (Under the direction of Dr. MaryAnne Drake).

Micellar casein concentrate (MCC) is made by the removal of milk derived whey protein (MDWP) from skim milk by microfiltration, using either a ceramic or polymeric membrane. The purity of MCC can be defined in one of two ways: percent MDWP removed from skim milk or the casein as a percentage of total protein (CN%TP) in MCC. The purity of MCC impacts its composition and flavor profile following thermal processing. Residual MDWP in MCC denatures in the presence of high heat and the volatile sulfur compounds hydrogen sulfide and carbon disulfide are formed. These volatile sulfur compounds are the source of undesirable sulfur/eggy flavors. The objectives of this study were to establish a MCC purity range above which sulfur/eggy flavor would not be detected in thermally processed MCC protein beverages, to determine the role that fat plays on sensory perception of sulfur/eggy flavors in MCC protein beverages, and to confirm an analysis technique to measure MCC purity.

Two studies were conducted. In study one, four model 6.3% protein beverages (15 g protein per 240 mL serving) were manufactured to represent a range of MCC purity levels. The beverages were manufactured by combining 95% MDWP reduced MCC, serum protein isolate (SPI), deionized (DI) water, carrageenan, cellulose gel, and dipotassium phosphate. The beverages were thermally processed by direct steam injection (DSI) ultrapasteurization (140°C for 3 sec) and analyzed at d 1 post-processing for sulfur/eggy flavor by both sensory and instrumental analysis. Additionally, Kjeldahl analysis was used to measure MCC purity before and after thermal processing. In study two, nine model beverages were made as described previously but with the addition of anhydrous milk fat (AMF) at 3 levels (0, 1%, 2%) to evaluate

the role that fat played on sulfur/eggy flavor detection. The nine beverages were then processed and analyzed using the same methods as study one but with the addition of SDS-PAGE to analyze MCC purity before and after thermal processing. Measurement of MCC purity of each beverage by Kjeldahl analysis increased ($p<0.05$) following UP heat treatment demonstrating that Kjeldahl cannot be used to determine MCC purity following high heat treatment. Sensory perception of sulfur/eggy flavor intensity and relative abundance of hydrogen sulfide and carbon disulfide increased with increasing level of residual MDWP ($p<0.05$). Beverages produced with 91% MDWP reduced MCC or that of higher purity were below sensory detection threshold for sulfur/eggy off flavors. MCC purity measurements by SDS-PAGE before and after UP had no statistical increase ($p>0.05$). The presence of fat decreased sensory intensity scores for sulfur/eggy flavor and relative abundance of hydrogen sulfide and carbon disulfide compared to beverages with similar % MDWP removal but no fat. MDWP removal from MCC must be greater than 91% to achieve a product with no sulfur off-flavors following thermal processing in a fat free protein beverage, but fat can be added to reduce sensory perception of undesirable sulfur/eggy flavors and utilize an MCC with lower purity.

The Effect of Micellar Casein Concentrate Purity and Fat Level on Sulfur-Eggy Flavors in Ultra-Pasteurized High Protein Beverages

by
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DEDICATION

This thesis is dedicated to my family. Without their constant support and encouragement, I would not have been able to make it to this point in my educational career. Thank you all for your loving support and supportive dedication, I love you.

BIOGRAPHY

Daniel McKay Whitt was born March 1st, 1993 to Samuel Wayne Whitt and Tracy Willis Whitt in Raleigh, North Carolina—where he grew up through high school. Daniel attended Brigham Young University as an undergraduate student, starting in 2012, where he later received his Bachelors of Science in Food Science in 2017. During his undergraduate studies, Daniel served a two-year mission trip for the Church of Jesus Christ of Latter-Day Saints in Belém, Brazil.

Daniel furthered his education at North Carolina State University under Dr. MaryAnne Drake in 2018—starting his graduate studies. After completion of his Master degree in Food Science, Daniel hopes to use his garnered skills and experience to help innovate the food/dairy industry.

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CHAPTER 1: LITERATURE REVIEW: THE PRODUCTION OF HIGH PROTEIN, ULTRA-PASTEURIZED MCC-BASED BEVERAGES

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Introduction

Current Statistics

Milk is as versatile as it is nutritious, making it a staple product in the food industry. The production of fluid milk has steadily increased every year. Production reached 496.84 million metric tons of fluid milk globally in 2015 and has since increased to 522.68 million metric tons in 2019 (Shahbandeh, 2020). However, as fluid milk production has increased, the consumption of fluid milk has been on a steady decline. Data from the USDA show that per capita fluid milk consumption in the U.S has declined 35% over the past 40 years (USDA, 2015); nevertheless, globally, fluid milk consumption and production has been on the rise. The global fluid milk market grew at a compound annual growth rate of 1.9% between 2011-2018— settling at a total volume of 222 billion liters in 2018 (IMARC, 2019). Global growth has been attributed to developing countries, such as China and India, while developed countries have begun to plateau or decline in their fluid milk consumption while simultaneously increasing production (IMARC, 2019). Consumption in the USA is projected to decline from 194.9 pounds per capita in 2010 to 155.3 pounds per capita by 2028 (Shahbandeh, 2019a). This decline of fluid milk consumption in developed countries, coupled along with the surge of dairy consumption in developing countries presents a unique opportunity to the US and global dairy industry, how to best utilize the excess fluid milk in nonconventional value-added applications and dairy products.

Current Dairy Products

An increasing growing demand for milk stems from the increased desire for milk protein: dairy proteins are generally superior to most plant-based proteins in nutritional quality and functionality, which has led to an increase in their demand (Hoffman and Falvo, 2004; Rutherford et al., 2015; Queiros et al., 2020). Examples of such innovative products that utilize

the high appeal of dairy proteins include Greek style yogurt, infant formula, canned milk, baked goods, and protein concentrates. These products appeal to consumers not only due to their nutritive content but also, in part, to their functional capabilities and clean labels. Consumer trends show that such products have become more popular and profitable because of the value added by dairy (Tunick, 2008; Turkmen et al., 2019). Greek style yogurt opened the market up to a wide variety of consumers; when it first hit shelves years ago and quickly became popular, taking over a larger market share of yogurt sales each year (Bong, 2013). In the USA, there were $391 million of Greek style yogurt sales, which increased to $3.7 billion in 2015 while non-Greek style yogurt sales over that same time period decreased from $4.4 billion to $4.0 billion (Guo, 2019). The appeal of Greek style yogurt has been widely been attributed to the high-protein content, perceived health benefits, and clean label. These key points contribute to its increasing popularity when juxtaposed with current consumer trends: healthy alternative foods, specifically convenient sources of protein (Tunick, 2008; Guo, 2019; Turkmen et al., 2019).

A second example of a dairy product riding the healthy/protein trend is whey protein; specifically, whey protein concentrate (WPC) and whey protein isolate (WPI). Whey protein products are among the most popular milk protein products currently on market and are traditionally isolated from cheese whey (Agarwal et al., 2015). With the advancement in membrane filtration technology, there has arisen two separate subcategories of whey proteins: those obtained directly from milk by filtration (milk derived whey proteins) and those obtained from cheese manufacture (whey proteins) (ADPI, 2018b). Some key differences and similarities between milk derived whey proteins (MDWP) and cheese whey proteins will be discussed further on in this review; however, a WPC or WPI product can be produced from either whey protein subcategory, differentiated by the subcategory of whey protein used: WPC if made from

cheese whey or milk WPC (mWPC) if made from MDWP (Nunes and Tavares, 2019; Carter et al., 2020). WPC is made by concentrating whey proteins from fluid whey using an ultrafiltration (UF) membrane to a percent protein as a percentage of total solids between 34% - 89% (U.S. Dairy Export Council, 2004). In contrast, mWPC is made by concentrating via UF the MDWP from a MF process to the aforementioned percent protein as a percentage of total solids range (Nunes and Tavares, 2019). UF is a filtration process that uses a 0.01 micron pore sized membrane to separate macromolecules such as proteins and suspended particles from the other components in milk based on their molecular weight. The UF membrane pore size allows for the separation of milk proteins (whey and casein) from lactose, water, and minerals and the separated liquids are termed retentate and permeate, respectively.

On the other hand, WPI contains >90% protein as a percentage of all solids and is produced through microfiltration (MF) or ion exchange of fluid whey (Huffman, 1996; USDA, 2010). As with concentrates, isolates are equally distinguished by the type of whey protein used in the manufacturing process: WPI if made from whey proteins derived from the cheese manufacturing process or milk whey protein isolate (mWPI) if the native whey proteins derived from a MF process are utilized (Nunes and Tavares, 2019). The MF process is required to remove the additional fat to achieve 90% protein. MF is a filtration process that uses a 0.1 micron pore sized membrane to separate casein proteins, fat, and bacteria to form a retentate stream from other the fractions in milk or whey which form the permeate stream— lactose, vitamins, minerals. WPC/WPI products are commonly used by athletes and strength training consumers in various forms: powders, protein bars, pre-mixed beverages, and even gummies. These products are desirable to consumers and their production/sales have steadily increased over the past decade to over 200 million tons of product produced every year, translating to an

increase from $9.97 billion in 2018 to a projected $15.4 billion in total sales worldwide in 2024 (Smithers, 2015, Shabandeh, 2019b).

A third category of alternative milk products is milk protein concentrates: milk protein concentrate (MPC), milk protein isolate (MPI) and micellar casein (MC). Milk protein concentrates maintain the same ratio of casein to whey protein as naturally found in milk: 80% casein to 20% whey (Agarwal et al., 2015). WPI/WPC are a concentrate of whey proteins to the exclusion of casein proteins, MPC and MPI concentrate all of the available protein in milk—casein and whey. MPC and MPI are typically made through the UF of skim milk to concentrate all available protein in their natural ratio as mentioned above while removing lactose, water, and minerals. The pore size of a UF membrane allows for the concentration of both casein and whey proteins while simultaneously removing lactose, minerals, and water (ADPI, 2018b). MPI, as with WPI or mWPI, is made by MF the milk beforehand to remove any fat. As with whey protein products, MPC and MPI are characterized by their overall protein concentration: MPC is classified as a product containing anywhere between 40-89% protein content and labeled according to the following guidelines: the abbreviation of the product name, followed by the percent of protein as a percentage of all solids in the product. An example of a product labeled according to these guidelines would be MPC80, meaning that this product contains 80% protein as a percentage of all solids in the same ratio as found naturally in fluid milk. While labeled in a similar fashion, MPI must contain a minimum of 90% protein (Burrington, 2017). MPC/MPI are commonly used in the powdered form as a food additive in beverage formulations of high protein products.

Micellar casein (MC) is defined by the American Dairy Product Institute (ADPI) as a microfiltered milk product that has an adjusted ratio of casein to whey protein (ADPI, 2018a). As

with MPC/MPI, the determination of the liquid or powder as a concentrate or isolate is done through the protein content as a percentage of total solids. To date, micellar casein products that have been produced or researched are primarily concentrates (ca 85% protein) and hence MCC is the most commonly referenced name (Carter et al., 2020). The main differentiating factor between MC and other milk protein products is the different ratio of casein to whey protein, with MC having a higher proportion of casein.

Composition

Milk is an important source of macronutrients such as fat, sugar (in the form of lactose), and proteins; additionally, milk contains nearly 10 other important micronutrients (Haug et al., 2007). Milk is composed of water (87% of the total weight) and solids: fat, lactose, protein, minerals, and enzymes (Walstra et al., 1999). Fats comprise ~36% of all the solid matter in milk and exists mainly as triglycerides, which comprise ~98% of all fat fractions found in milk. Fats can be easily removed from milk through centrifugation or filtration (Walstra et al., 1999). The composition of the fat varies greatly based upon region, the breed of the cow and even between cow-to-cow of the same breed; despite this, the physicochemical properties of the fat globules remain consistent. It has been hypothesized that the fat globule membrane could play a minor role in sulfur flavor development in heated milk products, which will be discussed further on in this review (Calvo and De la Hoz, 1992; Dill et al, 1962; Hutton and Patton, 1952).

Lactose is the primary carbohydrate in milk and can be removed through filtration. When left unremoved, lactose plays an important role in flavor and off-flavor development through Maillard browning. Lactose (a reducing sugar) reacts with sulfur-containing amino acids (cysteine/cystine, methionine) under high heat conditions to create brown colors and an assortment of caramel and burnt flavors (Hurt and Barbano, 2010; McGorrin, 2011; Jo et al

2018). Beyond lactose and fats, there are salts, minerals, enzymes, and other solids found in milk. However, the area of focus for this literature review is the proteins. Milk proteins make up only ~ 3% of the total weight of milk, are characterized as complete proteins (i.e. containing all the essential amino acids) and can be separated into two main groups: serum (whey) proteins and casein proteins. Dairy protein ingredients are gaining significant attention from both food and beverage manufactures due to their unique profile and functionality. Advancements in membrane technology and filtration processes has allowed for the isolation of both MDWP and casein micelles in their native structures that permit these proteins to be utilized in novel food and beverage applications that were previously incapable.

Milk Proteins

Whey Proteins

Whey proteins (or serum proteins) account for 20% of the protein in milk. Measuring 3-30 nm in diameter, they can be physically separated from their casein counterparts through microfiltration or chemically separated through the cheese making process. After they are separated from the other milk components, whey proteins are typically concentrated, dried, and used in a variety of food products as a functional or nutritional ingredient (Foegeding et al., 2002; Tunick, 2008). Several studies have documented the physical and functional properties of whey proteins that make them such viable food ingredient products: high emulsification, foaming, gelation, use as a thickening agent, high flavor binding potential, and their generally recognized as safe (GRAS) status (Nunes and Tavares, 2019).

Additionally, whey proteins are acid stable but not heat stable, make up 20% of the available protein in milk as fluid whey, and have varying isoelectric points depending on the type of whey protein: beta-lactoglobulin of pH 5.2, alpha-lactalbumin of pH 4.8, and lactoferrin of pH

8.8 (Nunes and Tavares, 2019; Carter et al., 2020). The acid stability of whey proteins is the main mechanism through which they are separated from caseins during cheese making: casein proteins will aggregate and fall out of solution at their isoelectric point of pH 4.6 while the acid stable whey proteins will not; allowing for their separation. Whey proteins are typically present as globular proteins with high hydrophobicity in compactly folded peptide chains (Walstra, 1999). Upon exposure to high temperatures, whey proteins will denature (unfold from their tertiary structure) and release reactive sulfhydryl groups, which will either bind with the casein micelles through disulfide bonds (leading to aggregation) or volatilize (leading to sulfur off-flavors) (Jang and Swaisgood, 1989; Dalgleish, 1990; Sharma et al, 1993; Walstra et al, 1999). Different types of whey proteins include β-lactogobulin, α-lactalbumin, serum albumin, immunoglobulins, and protease peptones. However, the two main groups of whey proteins are α-lactalbumin and β-lactoglobulin, which account for nearly 80% of whey protein (Schmidt et al.,1984; Jayaprakasha and Brueckner, 1999; Walstra, 1999). α-Lactalbumin and β-lactoglobulin are uniquely important because of the sulfur-containing amino acid that they carry as compared to other whey proteins and casein micelles: cysteine/cystine. The role that cysteine/cystine plays in the development of sulfur off-flavors is discussed further on in this review; however, studies have attributed this amino acid to sulfur flavor intensity as contributed by whey proteins because the only other sulfur-containing amino acid found in milk proteins (methionine) has been documented to be present at equal concentrations in both casein and whey proteins and therefore not responsible for the sulfur or cooked flavors as contributed by whey protein (Jo et al., 2019a,b).

Casein Proteins

Casein proteins account for the remaining 80% of the protein in milk and include a number of variations including: α1-caseins, α2-caseins, β-caseins, and κ-caseins (Mulvihill and Ennius, 2003). The majority of total available caseins in milk are α1 and α2, comprising nearly 50% of all available casein protein in milk (Fox, 2001). Casein proteins can be found primarily in a micelle form; this micellar structure is created through the aggregation of casein submicelles. Submicelles (12-15 nm in diameter) contain ~20-25 casein molecules and are formed through a combination of salt bridges and hydrophobic bonds between the individual casein proteins (Walstra, 1999). Submicelles vary in their composition but have been classified into two main groups: those with and without (or very little) κ-casein. The submicelles with very little κ-casein aggregate towards the center of the micelle, whereas the submicelles with κ-casein are forced to the outside, creating an outer layer with the κ-casein molecules projecting outwards from the micelle in finger-like structures. These κ-casein projections have been theorized to have a size-determining role on the micelle and to form a hydrophilic/negatively charged outer layer, which causes the casein micelles to repel one another (Holt, 1992). Submicelles are joined together in the presence of calcium and phosphate. These minerals lower the electric charge of the submicelles, allowing the submicelles to form bonds between themselves. K-casein does not bind with other κ-casein molecules, which eventually leads the submicelles to create a roughly spherical shape that is continuously covered in κ-casein "hairs", thus preventing further bonding (Walstra, 1999). The κ-casein "hairs" protect the casein micelle from Ca^{2+} precipitation and contain cysteine residues that are capable of forming disulfide bonds with denatured whey proteins, as mentioned above.

Traditionally, casein for milk casein ingredients has been precipitated from milk by lowering the pH to their isoelectric point of 4.6 (acid casein); however, this leads to undesirable side effects by exposing the casein to additive materials (Mulvihill and Ennius, 2003). Due to evolving filtration and membrane technologies, casein proteins and MDWPs can be separated and removed from milk in their natural or native state, providing them with unique and more efficient functional and nutritional benefits (Sauer and Moraru, 2012a, 2012b). Amelia and Barbano (2013) noted how micellar casein obtained from microfiltration is unique because the native micellar form is not contaminated with additives (acids, alkalis or enzymes) that are common in other separation techniques that affect the overall flavor and functional profile.

Due in large part to their unique structure, caseins have a variety of novel properties: hydrophobicity, a fairly high negative charge, many prolines, and few cystine residues (Walstra et al, 1999). Additionally, due to both the micelle stability and the primary structure of individual casein proteins (meaning they have little secondary or tertiary structure), casein proteins have high thermal stability and are hardly ever denatured (Walstra et al, 1999). However, casein micelles are susceptible to precipitation from either low pH or in the presence of Ca^{2+} after chymosin splits the κ-casein layer from the outside of the micelle during the cheese making process (Walstra et al, 1999). The ability to utilize the high heat stability of natural casein proteins (derived by filtration) would be beneficial to the dairy industry in opening new areas of product development; such as, ready-to-drink (RTD) high protein beverages, high protein ice creams, or other thermally processed products with a higher protein content. Food and beverage products that were marketed with “high/source of protein” or “added protein” saw a compound annual growth rate of 20.7% in a four-year period covering 2014-2018 and the novelty of casein

micelles opens many doors for the dairy industry to take advantage of that market area. (Innova Market Insights, 2019).

In regard to the functional characteristics of caseins and whey proteins in nutritional and ingredient applications, caseins and whey proteins have a number of competitive advantages over other protein sources as well as differences between themselves. For instance, both casein and whey proteins contain all the essential amino acids making them complete proteins as opposed to plant proteins; furthermore, casein and whey proteins have high emulsification, foaming, gelling, color improvement, and flavor improvement properties (Modler, 2013). As opposed to caseins, MDWP have been characterized as having good clarity (at high or low pH ranges), high solubility, high foaming properties, a higher rate of satiation than caseins and other proteins, and heat gelation (Heino et al., 2007; Luck et al., 2013; Nunes and Tavares, 2019). Conversely, caseins have several functional attributes that make them uniquely desirable in ingredient applications: heat-stability, capability of binding large amounts of calcium, bland flavor, mouth-coating effect similar to that of fat, white color, pH sensitive, and slow absorbing in the body (Guo, 2019; Carter, 2020).

Micellar Casein Concentrate

Composition

Micellar casein concentrate (MCC) is the liquid concentrate of micellar casein proteins produced through the microfiltration (MF) of skim milk. While there currently is no standard of identity for MCC or MCC purity in the US or Europe, the American Dairy Product Institute (ADPI) defined a microfiltered milk product (microfiltered milk protein [MMP] or micellar casein [MC]) as a product that has an adjusted ratio of casein to MDWP (ADPI, 2018a). As opposed to other milk or whey protein products, MCC is the concentration of solely the casein

proteins. Other milk protein products such as MPI or MPC retain the same ratio of casein to whey protein as found naturally in milk (80:20) (ADPI, 2018a). MF allows for the passage of MDWP, lactose, and minerals through the membrane, which collect to form a liquid "permeate" phase. Everything else that does not pass through the membrane is concentrated into a liquid "retentate" phase: casein micelles, any residual fat, and microbes.

The purity of MCC can be characterized by one of two ways: casein as a percentage of true protein or percent of total milk derived whey protein (MDWP) reduction. The exact hurdles regarding the nomenclature and standardization of MCC purity will be addressed further on in this paper. Analytically, to measure the purity of spray dried MCC requires either high performance liquid chromatography or SDS-PAGE of the MCC to accurately ascertain the amount of MDWP in the sample.

Production

Current MF technology has advanced far beyond the humble beginnings of dairy filtration technology of the 1960's and is but a small part of possible membrane driven separation techniques. Based on pore size and molecule weight cut off, the separation processes are classified into 4 main groups: MF, UF (ultrafiltration), NF (nanofiltration), and RO (reverse osmosis) (Figure 1; Marella et al., 2013). MF is distinguished as the process with the largest pore size (0.1-10 µm), the lowest processing pressure (0.01-0.2 MPa), and is traditionally used as a pretreatment of milk to reduce the microbial load, but due to its wide pore size range, it can be utilized to separate different macromolecules – most recently, casein micelles (Pouliot, 2008). For example, using a 1.4 µm MF membrane, both bacteria (10-100 µm) and milk fat globules (10 µm) can be separated from other milk components, including casein micelles (Pouliot, 2008;

Smith, 2013). Conversely, using a 0.1 µm MF will separate bacteria, milk fat globules, and casein micelles; thusly, creating MCC.

There are currently two distinct MF processes by which MCC can be produced, and they differ on the type of membrane used for the MF process: spiral wound (polymeric) membranes and ceramic membranes. Many producers select spiral-wound membranes because of the lower overhead cost (nearly $1/10^{th}$ of the cost of a ceramic membrane); however, ceramic membranes have a longer life (at least 10 years compared to 0.5 years for the typical polymeric membrane), higher chemical/thermal stability, and less restrictive pH ranges (Beckman et al., 2010; Karasu et al., 2010; Park et al., 2014; Gitis and Rothenburg, 2016). Additionally, studies have shown that ceramic membranes are more efficient at removing MDWP—able to remove up to 95% of the total MDWP in skim milk while their polymeric counterparts can only reach up to ~70% MDWP removal through 3 stages of filtration with 2 stages of diafiltration at 50°C. (Beckman et al., 2010; Hurt et al., 2010). Beckman et al. (2010) showed that a spiral wound membrane was only capable of achieving a similar removal rate of 95% MDWP removal from skim milk after an 8 stage MF process with 7 stages of diafiltration at 50°C; however, this also will require additional time, resource, and costs.

The difference in separation capability is due to a variety of factors: ceramic MF membranes are designed to sustain a high cross flow velocity (CFV) while simultaneously maintaining a low transmembrane pressure (TMP). This design keeps the passage of permeate consistent down the entirety of the membrane; thus, preventing major fouling while allowing for a high rate of permeate passage (Karasu et al., 2010). Additionally, ceramic membranes have uniform pore sizes and distribution along the length of the membrane which allows for the separation of molecules by size. On the other hand, polymeric membranes form a foulant layer

(otherwise known as a concentration polarization layer) which is the actual method of separation as opposed to the declared molecular weight cutoff used in a ceramic membrane. Due to this design, polymeric membranes have lower performance and separation efficiency than their ceramic counterparts because of the high TMP and low CFV (Karasu et al., 2010). Separation of casein and MDWP is based on the size of the protein: micellar casein (50 -500 nm) is rejected by the MF membrane and dubbed retentate while MDWP (3-10 nm) are able to pass through the membrane as permeate (Saboya and Maubois, 2000; Fox and McSweeney, 2003). Due to the large pore size of MF membranes (compared to other filtration systems), they generally foul faster; however, fouling is prevented by applying a high CFV. A high CFV reduces fouling because it creates lift of the particles, keeping the pores of the membrane clear as a high wall shear stress washes away the foulant on the membrane service. Polymeric membranes are incapable of handling high CFV because the membranes will telescope, or in other words, suffer a mechanical deformation of the center of the membrane, leading to a pulling out of the outer wraps of the membrane. Even though the foulant layer is the responsible separation mechanism in spiral wound membranes, the low MDWP removal rate has been attributed to the lower CFV leading to impassable fouling caused by the higher hydrophobicity of polymeric membranes (Beckman et al., 2010).

Within ceramic membrane processing, there are sub-processes that differ based on a variety of factors: the pressure exerted on the membrane (uniform transmembrane pressure [UTP], Isoflux and graded permeability [GP]), the pore size of the membrane, and the pore shape of the membrane (Hurt and Barbano, 2010; Hurt and Barbano, 2015). UTP processing requires the use of both a retentate and a permeate pump to recirculate the liquids through the MF in order to maintain equal pressure throughout the entire membrane. By contrast, GP only utilizes the

permeate pump to recirculate all the liquid in the MF. Studies have shown that there is not a significant difference in the purity of MCC produced between these two methods (or the amount of MDWP removed), so GP has become more commonplace as it is the cheaper of the two options, requiring less energy to run a single pump versus two (Hurt and Barbano, 2010; Hurt and Barbano, 2015). Along with a GP process, a round 0.1 µm pore size/shape was selected for the purposes of the current thesis research as studies have also shown that particular pore size and shape to be the most effective or have little to no difference when compared to other pore sizes and shapes in GP processing (Zulewska et al., 2009; Hurt et al., 2010).

Studies have shown that a 3-stage filtration process utilizing a ceramic membrane is capable of producing a 95% MDWP reduced (or 98.35% casein as a percentage of true protein) MCC product, which is generally recognized as the standard for pure MCC (Hurt et al., 2010; Cheng et al., 2018). Outlined by Zulewska and Barbano (2009), the process for manufacturing MCC follows a continuous 3-stage MF process at 50°C, following a 3X concentration factor and diluting the MF retentate at the end of stages one and two with deionized (DI) water in a 2:1 (wt/wt) DI water to MF retentate ratio. Following this method, Hurt and Barbano (2009) established that at the end of stage 1, 70% MDWP reduction was achieved, 88% MDWP reduction at the end of stage 2, and 95% MDWP reduction at the end of stage 3 (Figure 2).

Current/Potential Uses of MCC

MCC offers a bevy of unique properties that make it potentially beneficial as a food ingredient and a focal point of research in recent years: high thermal stability, bland taste, white color, low lactose levels, ability to bind high amounts of calcium, high nutritive value, native casein proteins (clean label), mouth-coating effect, and emulsification properties (Barbano, 2009; Amelia, 2012; Sauer and Moraru, 2012b; Carter et al., 2016). Current beneficial uses that have

been documented for MCC include its inclusion in cheesemaking, nutritional meal replacement, whipped topping, coffee whitener (Papadaatos et al., 2003; Govindasamy-Lucey et al., 2007; Beckman, et al., 2010; Hurt et al., 2010; Marella et al., 2013; Lu etl., 2015), shelf-stabilizer, high protein beverages (Beliciu et al., 2012), Greek style yogurt (Bongh, 2013; Bong and Moraru, 2014), ice cream (Sipple et al., 2020), pizza cheese, cream liqueurs, fabricated meats, cereal products, (Fox, 2001), glues, plastics, gels, delivery systems (Huppertz et al., 2018), and as a protein fortifier in beverage, bakery, and meat products (Sauer et al., 2012).

In cheese manufacture, MCC has been used to increase cheese yield without compromising quality or functional attributes, reduce renneting time, and to standardize cheese milk before the cheesemaking process (Nelson and Barbano, 2005; Govindasamy-Lucey et al., 2007; Beckman et al., 2010; Hurt et al., 2010; Karasu et al., 2010; Huppertz et al., 2012; Lu et al., 2015; Lu et al., 2017). In the EU, MCC is commonly used for the standardization of cheese milk, thus improving cheese yields; however, the FDA has not permitted the use of microfiltered milk (MCC) in the manufacture of standard of identity cheeses in the USA (Culhane, 2016; Neocleous et al., 2002). Additional benefits of adding MCC to cheese manufacture include increasing the cheese yield by increasing the percentage of casein per unit volume— where typically non-fortified milk generates 1-part cheese and 9 parts whey, the fortified milk increases the cheese yield per hundred weight of milk used (Neocleous et al., 2002). Govindasamy-Lucey et al. (2007) made pizza cheese with MCC-fortified milk and found a statistically significant increase of the cheese yield (~3% increase from the control) without sacrificing the functional attributes or the quality of the cheese. Secondly, using MCC allowed for a more accurate casein standardization of the cheese milk; because current fat and protein standardization takes the whey protein into account (which are lost during the manufacture of most cheese varieties) and

use of MCC leads to a more accurate adjustment (Brandsma and Rizvi, 2001). Thirdly, MCC can be used to increase the solids and casein content of the cheese milk (with proper adjustments to overcome any gelatin kinetics) (Lu et al., 2016; Brandsma et al., 2001; Amelia and Barbano 2013a). Dalgleish (1990) showed that decreased micellar interactions of κ-casein and β-lactoglobulin minimized the impact of heat treatment on cheese making, allowing for MCC enriched cheese milks to be pasteurized at a higher time temperature combination. Papadatos et al. (2003) evaluated the economic feasibility of using MCC fortified cheese milk including the cost and production of coproducts. They found that the net revenue for the fortified cheese milks was higher in 30 out of the 36 months analyzed: net revenue compared to conventional cheesemaking was increased by $0.41 for Cheddar and $0.45 for mozzarella per 100 lb. of standardized milk. Their study strengthens the economic argument that MCC or MF milk should be allowed in the cheese make process.

In Greek style yogurt, MCC has been shown to decrease environmental impacts of dairy companies (seeing as it does not produce acid whey), increase the protein content, and act as a feasible food ingredient/protein fortifier (Bong, 2013; Bong and Moraru, 2014). Greek style yogurts made with MCC as opposed to traditional methods displayed similar chemical and physical properties to their control counterparts but with a shorter fermentation process and potential for higher protein (Bong and Moraru, 2014). Bong (2013) found that using MCC-fortified milk as the starter material for the production of Greek style yogurt resulted in a final product that manifested similar physical and chemical properties as traditionally made Greek style yogurt with the additional benefits of a shorter fermentation time and the creation of less waste by product (acid whey). This study concluded that MCC fortified Greek style yogurt was a feasible and potentially environmentally friendlier alternative to the traditional process.

MCC is well suited to be used in the beverage industry because of the bland taste, high capability of binding large amounts of calcium, lactose reduced, high nutritive value, mouth-coating effect, high thermal stability, and emulsification properties (Barbano, 2009; Amelia, 2012; Carter et al., 2016). Sauer and Moraru (2012b) concluded that MCC would be a viable and beneficial ingredient in shelf-stable, high-protein nutritional beverages due to the unique functionality of native casein micelles. Vogel (2019) manufactured and ultra-pasteurized vanilla-flavored milk protein beverages (15 and 25 g protein per 240 mL serving) using liquid MCC (95% MDWP reduced), liquid MPC, and liquid blends of MPC and SPI. Evaluation of these beverages over the course of 8 weeks documented sulfur/eggy flavors in MPC and MPC/SPI beverages but not in the samples made with MCC, highlighting a significant advantage of MCC over other dairy protein ingredients. Additionally, the same study showed that beverages produced with MCC had a higher vanilla flavor. The reduction (or removal) of sulfur/eggy flavors coupled with the higher vanilla flavor present a powerful argument for the expansion of the beverage market using MCC. Currently, the specific concentration range of MDWP that causes undesirable sulfur/eggy flavor is unknown. Lastly, studies have shown that milk-based protein beverages made with liquid MCC (or milk protein sources that are higher in casein content) will exhibit a lighter (i.e. whiter) color, which is more appealing to consumers without the use of additional additives (Cheng et al., 2019 a,b).

However, studies have also highlighted some of the drawbacks of using MCC: Sauer and Moraru (2012) showed that powdered MCC used in UHT and retort processed beverages resulted in significant aggregation and coagulation. When exposed to high processing temperatures as with UHT or retort sterilization, MCC was difficult to disperse and readily coagulated. The aforementioned study went on to show that decreasing the processing

temperature or increasing the pH of the sample helped prevent the observed aggregation/coagulation effects. Similar results were concluded by a study undertaken by Beliciu et al. (2012) with the notable difference: retort processing had a less significant effect on the casein micelles, leading to a more homogenous product using either liquid or powdered MCC.

While MCC has a wide range of possibilities in the food, beverage, and even pharmaceutical industry, there have been noted limitations in its application: heat stability and solubility. Heat stability is addressed further on in this literature review and could potentially be remedied with stabilizers commonly used in protein beverages, but solubility of dried MCC has been a constant limiting factor (Zhang et al., 2018; Burgain et al., 2016). Complete rehydration of the protein is necessary to ensure peak functionality and heat stability (Crowley et al., 2014). The principal theory to explain the poor rehydration of dried MCC is that the casein micelles release slowly from the powder particles into the solution (Schokker et al., 2011). Studies have put forth a variety of remedies to help with powdered MCC rehydration: increased time (30-40 mins), increased temperature (35-60C), high shear, increasing the ionic strength of the water, adding a calcium chelator (60 mM trisodium citrate), and lower storage temperature and time (Lu et al., 2015; Fang et al., 2010; Mimouni et al., 2010; Gaiani et al., 2007; Schuck et al., 1994b).

Liquid versus Dried MCC

As with other dairy protein ingredient, MCC can be processed, bought, and used in either a liquid or dried powder form. Carter et al. (2016) documented that dried MCC had poor rehydration properties and higher off-flavor attributes compared to liquid MCC, liquid/powdered MPC, and liquid/powdered MPI. It was concluded in the aforementioned study that liquid MCC had markedly better sensory perceptions and a blander flavor as a potential protein ingredient.

However, it was also shown that when resolubilized, powdered MCC did not have significant differences in its functional properties compared to liquid MCC. Additionally, liquid MCC has been shown to be more heat stable than its powdered/rehydrated counterpart (Beliciu et al., 2012; Lu et al., 2015). Experiments performed by Schuck et al. (2007) congruently concluded that powdered MCC was significantly more difficult to rehydrate compared to other dried milk protein powders, both MPC and MPI.

Studies have documented that rehydrated MCC powder was characterized by sweet aromatic, cooked/milky, and cardboard/doughy flavors; additionally, powdered MCC had a distinct corn chip flavor as opposed to the liquid product (Smith et al., 2016; Carter et al., 2016). These flavor drawbacks place powdered MCC at a disadvantageous position compared to the liquid form. Liquid MCC and MPC have been documented as similar in flavor, making liquid MCC more readily adaptable into beverage or other food products because of the established bland taste as compared to powdered MCC (Carter et al., 2016).

Nomenclature of MCC

One of the first hurdles the dairy industry needs to overcome in regard to MCC is having a common language used to describe all milk protein ingredients. Other dairy protein concentrates and isolates (WPI, WPC, MPC, and MPI) follow the pattern of using a 3-letter code followed by a number that distinguishes the percent protein of the solids. For example, WPC80 is a whey protein concentrate where 80% of the solids are protein. MCC is different, as it either needs to communicate the purity of the product as the percentage of casein, or it needs to show the purity as a percentage of MDWP that has been removed by microfiltration processing from the original skim milk. In this thesis, both forms of purity descriptors will be used, as there has been no consensus reached by the dairy industry.

The American Dairy Product Institute (ADPI) has recently moved to help with the standardization of the nomenclature of non-casein protein products made from milk versus whey because of the general difficulty to understand the technical terms of milk serum proteins. Moving forward in the dairy industry, it has been proposed that serum proteins derived from milk be termed “milk derived whey proteins”, and the same proteins isolated from cheese whey be called “whey protein” (ADPI, 2018). However, this distinction does not provide any clarity or information on casein purity in MCC. MCC purity (or casein as a percentage of true protein) is one of the key factors that affects both the functionality and flavor profile of MCC and should be an integral part of the nomenclature. Without this distinction, there is no economic or functional differentiation between MCC from MPC or MPI.

Thermal Processing

Direct Steam Injection-Ultra Pasteurization (DSI-UP)

DSI-UP is an ultra-pasteurization (UP) or ultra-high-temperature (UHT) process that destroys or inactivates all pathogenic and spoilage bacteria by heating products to or above 138°C (280°F) for at least 2s (21 CFR 131.3, FDA, 2017). The distinguishing difference between UP and UHT processes is not the heat load to which the product is exposed but rather the packaging process. UHT milks are aseptically packaged, which is why such products are considered shelf stable (FDA, 2015). The DSI-UP process applies superheated culinary steam to the milk in order to pasteurize the product and then utilizes a vacuum to remove the water added by the steam from the product (Datta et al., 2002; Bylund, 2003). The use of a vacuum also instantaneously cools the product (Datta et al., 2002).

Comparison to Other Types of Thermal Processing Procedures

The DSI-UP process has a number of advantages and disadvantages compared to other common pasteurization techniques: high temperature, short time (HTST) and indirect heat-ultra pasteurization (IND-UP). HTST is the most common dairy pasteurization process in the United States and requires that the product be heated to 73°C for 15 s (Boor, 2001; Lee et al., 2017). This technique is designed to kill all pathogenic bacteria (Boor, 2001; Lee et al., 2017). Dairy products made with HTST pasteurization have a shelf-life of approximately 3 weeks and must be refrigerated to prevent spoilage. However, although the technique kills all pathogenic bacteria, the heat load delivered to the product is much lower than that of a UP process; consequently, these products have fewer off-flavors as perceived by consumers (Perkins and Deeth 2001).

Compared to HTST milks, DSI-UP products have the disadvantage of being distinguished by flavors such as cooked, sulfur/cabbage, sulfur/eggy, and sulfur/burnt (Lee et al., 2017; Jo et al., 2018). These off-flavors are generated from the more intense thermal processing of DSI-UP and the reactions that take place at those higher temperatures. Maillard browning, Strecker degradation, and the denaturation of proteins are common reactions that take place at DSI-UP temperatures and create the volatile compounds responsible for the off-flavors (Vazquez-Landaverde et al., 2005; Vazquez-Landaverde et al., 2006; Jo et al., 2018; Jo et al., 2019). These off-flavors have been shown to be drivers of dislike for American consumers (Perkins and Deeth, 2001; Lee et al., 2017). Despite this disadvantage, DSI-UP has a distinct advantage over products made using HTST methods: shelf life. Milk products that are treated with DSI-UP parameters have a shelf life up to 75 to 80 days; whereas, as previously noted, HTST products only have a shelf life of 3 weeks. DSI-UP products also have the potential to be shelf-stable if they are packaged under aseptic conditions. The capability of creating dairy-based

products that are shelf-stable and safe to consume for such a long period of time makes the DSI-UP process desirable for future products.

The second type of pasteurization process to compare DSI-UP against is IND-UP. IND-UP utilizes ultra-high temperatures to kill all pathogenic and spoilage bacteria but without directly injecting the steam into the product. The steam is used to heat a water medium that flows parallel and in the opposite direction of the product line to heat the milk. The contact between the product line and the superheated water line is the mechanism which heats the milk to the desired temperature (Boor, 2001; Lee et al., 2017). The major difference between these processes is the length of time to which the product is exposed to the high temperatures. DSI-UP uses a more efficient heat transfer method where the products have smaller areas under the curve in the time-temperature profile of the treated product, limiting product quality loss as a result from excess heat exposure (Datta et al., 2002; Bylund, 2003).

DSI-UP products are heated and cooled much more quickly than the IND-UP products, meaning that the creation of high-heat induced byproducts is lower for DSI-UP products than IND-UP products. Studies have highlighted this advantage of DSI-UP over IND-UP (Datta, 2002; Lee et al., 2017; Jo et al., 2019). Jo et al. (2019) tested products created under DSI-UP versus IND-UP conditions and evaluated them using both descriptive sensory analysis (DA) and sulfur volatile analysis. Results from this study and Jo et al. (2018) showed how DSI-UP products had lower intensities of sensory attributes that were previously mentioned to be associated with drivers of dislike for consumers: overall aroma and sulfur/eggy flavors. Results from those studies also highlighted a lower concentration of sulfur volatiles in DSI-UP milks than IND-UP milks. Congruent with the previously mentioned study, Patrick and Swaisgood

(1976) concluded that products processed under DSI-UP conditions had less whey protein denaturation (and consequently less sulfur flavor intensity) than its IND-UP counterparts. Creating a product with an extended shelf-life and bacteria inactivation with a more desirable flavor would make DSI-UP a unique process with a high upside for market use.

Products Produced with DSI-UP

Many products have already hit market using the DSI-UP process, and while these products are more common globally, there is still a large, untapped market for DSI-UP-processed products in the United States. Fluid milk is the most common DSI-UP product. Across the globe, shelf-stable milk is staple of home diets, and studies have shown that consumers that grow up with shelf stable products have no aversion to the higher cooked or sulfur flavors (Vazquez-Landaverde et al., 2005; Liem et al., 2016); however, these higher cooked and sulfur flavors represent a challenge to the U.S. industry. Another common product is shelf-stable ready-to-drink (RTD) beverages. As the beverage industry has grown exponentially in recent years, new products utilizing this trend have taken off, and shelf stable RTD beverages provide a level of convenience unavailable to consumers in years past. Following the trend of convenience and higher sales in the beverage sector, RTD protein beverages have become more popular as consumers look for easier routes to better nutrition.

Effect of Thermal Processing on Products

Products produced utilizing the DSI-UP process benefit with a longer shelf life and are shelf-stable when aseptically packaged. As mentioned before, while these benefits are valuable, a number of drawbacks have limited the general acceptance of DSI-UP milk products, especially in the USA. Such dislike stems from the perception of offflavors: higher sulfur flavors, cooked flavors, and an overall aroma intensity (Gould and Sommer, 1939; Horner et al, 1980; Perkin and

Deeth, 2001; Attabi et al, 2009; Lee et al, 2017; Ansari and Sahoo, 2018). A study conducted by Chapman and Boor (2001) showed that children (6-11 yr old) preferred HTST milk at d1 postprocessing over UHT milk at 24 to 30 d postprocessing. Lee et al. (2017) similarly demonstrated that child and adult consumers preferred the flavor of HTST milk over UP milk processed by DSI or IND.

Cooked flavors commonly experienced by consumers in high heat-treated milk products have been attributed to the formation of sulfides during the thermal processing (Gould and Sommer, 1939). Patrick and Swaisgood (1976) went on to show that the formation of sulfur volatiles was due to milk whey protein denaturation during thermal processing and that cooked flavor was attributed to the sulfhydryl groups formed during this denaturation process. They went on to explain that the more severe heat treatment processes denature the whey proteins, which leads to 1) inactivating enzymes in raw milk that catalyze the oxidation of sulfhydryl groups – essentially leading to reactive sulfhydryls and 2) exposing all available sulfhydryl groups; thusly, leading them to become reactive. The reactive sulfhydryl groups then participate in a variety of reactions: 1) volatilization 2) oxidization to form disulfides or 3) being reburied in protein structures/binding with casein micelles. Past research has measured the sulfhydryl groups formed in thermally processed milk products and have concluded that the conversion of -SH and -SS groups on the milk proteins to volatile compounds leads to the perceived cooked flavors. Additionally, it was shown that there are no measurable free sulfhydryl groups on casein micelles while the whey protein counterparts contained significant portions of such groups (Zweig and Block; 1953; Pocahontas and Vakaleris, 1968; Watanabe and Klostermeyer, 1976).

Studies have further documented the sulfur flavors associated with UP milk as sulfur/cabbage, sulfur/eggy, and sulfur/burnt (Jo et al., 2019). These different variations of sulfur

flavor derive from the different sulfur-containing amino acids (methionine, cysteine/cystine) that breakdown during protein denaturation at high temperatures and the subsequent creation of varying sulfur-compounds: hydrogen sulfide, carbon disulfide, dimethyl sulfide, dimethyl disulfide, and dimethyl trisulfide. Hydrogen sulfide has been documented as the source of sulfur/eggy flavor (Thomas et al., 1976; Jo et al., 2018), carbon disulfide as the source of sulfur/burnt (Jo et al., 2018), and dimethyl sulfides as the sources of sulfur/cabbage (Al-Attabi et al., 2009; Jo et al., 2018). Additionally, Badings et al. (1981) found an increased concentration of hydrogen sulfide in milk samples that were exposed to an increasingly severe heat treatment process: 0.1 µg/L in HTST, 0.7 µg/L in HHST, and 47.2 µg/L in indirect UHT. As will be discussed further on, hydrogen sulfide plays a significant role in sulfur/eggy flavors in thermally processed milk beverages.

Compared to most other dairy protein ingredients, MCC has a thermal stability because of the higher concentration of casein micelles, but, as previously stated, MCC (especially powdered MCC) suffers from coagulation and aggregation during high heat processes. Probable causes that have been attributed to the poor heat stability of MCC-based products during UP temperature ranges include: the heat induced dissociation of κ-casein or the precipitation of calcium phosphate at high temperatures (Carter, 2019). However, studies have shown that liquid MCC had improved thermal stability when compared to its powdered/ rehydrated counterpart under UHT conditions (Beliciu et al., 2012; Lu et al., 2015). Work was also undertaken in this thesis to address the issues of heat stability using different stabilizers during the DSI-UP process and their effectiveness at preventing coagulation/aggregation during this high temperature ranges to create stable and homogenous beverages using MCC.

Sulfur Flavors in Thermally Processed Milk

Role of Serum Protein

An increase in the amount of whey protein in a product is directly correlated with an increase of perceived sulfur/eggy flavor—primarily β-lactogobulin and α-lactalbumin (Jenner, 1954; Vogel et al., 2018; Jo et al., 2019). Whey proteins are the main contributor to the sulfur-containing amino acid (AA) cysteine/cystine, which leads to the creation of hydrogen sulfide and carbon disulfide via Strecker degradation (Hutton and Patton, 1952; Zhang and Ho, 1991; Elliot et al., 2005; Al-Attabi et al., 2009; Jo et al., 2018). The presence of hydrogen sulfide and carbon disulfide in a product are the main contributors to sulfur/eggy flavor (Jo et al., 2019) and are also the compounds of interest for this study. Methionine is another sulfur-containing AA in milk; however, research has suggested that it does not contribute to the development of sulfur/eggy flavors, but rather, methionine plays an important role in the creation of cooked flavor and overall aroma (Colahan-Sederstrom and Peterson, 2005; Belitz et al., 2009; Jo et al., 2018; Jo et al., 2019). Methionine is present in both casein and whey protein at equal levels (Belitz et al., 2009; Jo et al., 2019). Because whey protein is the main contributor to sulfur/eggy flavor in thermally processed milk products and cysteine/cystine is the only sulfur-containing AA that is present in significantly higher concentrations in whey proteins, it is reasonable to conclude that cystine/cysteine is the AA responsible for the release of sulfur volatiles that create the perception of sulfur/eggy flavor.

The amount of cysteine/cystine in milk varies depending on the milk protein fraction between casein and whey protein. However, the degradation of this AA has been documented to be a precursor to hydrogen sulfide and carbon disulfide during heat treatment of milk (Belitz, 2009; Jo et al., 2018). The heat-induced degradation of whey protein invokes changes in its

properties through the unfolding and subsequent aggregation of unfolded protein molecules and flavor formations (Kühn et al., 2006). Seeing as whey proteins are more heat sensitive than caseins, the effect of pasteurization on whey protein can be more significant than casein as far as sulfur/eggy flavor intensity is concerned (Brodkorb et al., 2016). When milk products are heated to UP processing temperatures, β-lactoglobulin and other whey proteins begin to denature as the protein uncoils, and the -SH and -SS groups that were originally buried inside the coiled structure become exposed and are free to react with the surrounding environment. As the sulfhydryl groups are released from the whey protein, they convert to volatile sulfur compounds, such as hydrogen sulfide or carbon disulfide. After conversion, they are free in the sample and react with the fat globules, caseins, or remain in the headspace of the product.

Volatile Sulfur Compounds

While many sulfur compounds have been identified in thermally processed milk, hydrogen sulfide and carbon disulfide have been documented as the main contributors of sulfur/eggy flavor (Jo et al., 2018). Other volatile sulfur compounds that have been identified in thermally processed milks include methanethiol, dimethyl sulfide, dimethyl disulfide, and dimethyl trisulfide (Jo et al., 2018; Jo et al., 2019; Vazquez-Landaverde et al., 2005; Vazquez-Landaverde et al., 2006;). Many of these compounds are responsible for cooked, sulfur/cabbage or sulfur/burnt flavors.

Hydrogen sulfide is composed of two hydrogen molecules bonded to a single sulfur molecule, giving the overall molecule a neutral charge. This sulfur compound is formed in milk as a result of the Strecker degradation of cysteine/cystine with a diketone (Figure 3). Examples of diketones available in milk include diacetyl and 2,3-pentadione (Al-Attabi et al., 2009).

The most abundant source of cysteine/cystine AA in milk comes from β-lactoglobulin, which represents nearly 60% of all the available whey protein in milk (Hutton and Patton, 1952; Al-Attabi et al., 2009). β-lactoglobulin is nearly completely denatured during UP due to the high pasteurization temperature, leading to higher concentrations of volatile sulfur compounds and off-flavors in milk. Studies have documented hydrogen sulfide as having a sulfur/eggy flavor rather than sulfur/cabbage or sulfur/burnt unlike other sulfur volatiles (Al-Attabi et al., 2009; Jo et al., 2018; Jo et al., 2019).

Carbon disulfide is made up of two sulfur molecules double bonded to a single carbon molecule. Carbon disulfide is formed in milk during thermal processing in a similar process to hydrogen sulfide: cysteine/cystine is broken down as the whey protein uncoils and reacts with the milk medium. Studies have documented carbon disulfide as having a sulfur/burnt flavor rather than sulfur/eggy or sulfur/cabbage as other volatile sulfur compounds are characterized (Jo et al., 2019).

While the presence of sulfur volatiles can be easily detected by consumers as the perception of sulfur off-flavors, it has been more troublesome to quantify the available concentration of sulfur compounds that are responsible for individual off-flavors in a particular product quantitatively. Previously used methods include static headspace, dynamic headspace (purge and trap), solid phase extraction, and solid phase microextraction (SPME) (Al-Attabi et al., 2009). SPME has become more common due to its high sensitivity, faster analysis time, and high affinity for sulfur compounds. In SPME, a polar fiber is introduced into a vial containing the milk sample. Using the headspace technique, the fiber is exposed to the headspace above the sample and left for a certain amount of time/temperature to equilibrize and adsorb the volatiles

onto the fiber (Attabi et al., 2009). After the fiber has adsorbed the volatiles, it is injected into a gas chromatograph (GC) for analysis.

The GC injector uses inert gas (helium) to sweep the volatiles from the SPME fiber onto a column where the separation of the volatile compounds occurs, and then specific separated volatiles elute and pass through a detector where detection and quantification occurs. Quantification happens inside the quadrupole of the gas chromatograph-mass spectrometer (GC/MS). A GC/MS can have a single or triple quadrupole. A triple quadrupole breaks down the volatile samples two times to elucidate, separate and detect the volatile compounds from the sample. Because the sample is fragmented twice and analyzed, triple-quadrupole GC/MS have much higher sensitivity and accuracy compared to single quadrupole GC/MS.

GC-MS methods have been used and improved to more accurately quantitate the amount of sulfur compounds in a particular product. Vazques-Landaverde et al. (2006) used GC-MS with pulsed flame photometric detection to quantify the presence of sulfur compounds in milk. This included the detection of hydrogen sulfide and carbon disulfide along with methanethiol, dimethyl sulfide, dimethyl disulfide, and dimethyl trisulfide. Their work found that milk products processed under UP conditions had substantially higher concentrations of sulfur compounds than the same products processed under HTST conditions. Jo et al. (2018, 2019) also quantified volatile sulfur compounds using a GC/MS triple quadrupole equipped with a sulfur selective flame photometric detector. Using this method of detection and quantification, the mentioned studies were able to accurately quantify a number of sulfur compounds that distinguished UP milks from HTST pasteurized milks: hydrogen sulfide, carbon disulfide, methional, dimethyl sulfide, dimethyl disulfide, dimethyl trisulfide, and dimethyl sulfoxide. Work done by Colahan-Sederstrom and Peterson (2005) highlighted the production of methional

via the Strecker degradation of methionine in both whey and casein proteins after UP processing of bovine milk. Through a combination of both GC and trained sensory analysis, they documented the high aroma impact that methionine has on the cooked flavors of high-heat processed milk products. Christensen and Reineccius, (1992) were able to establish a correlation between cooked flavor and the relative concentration of hydrogen sulfide in heated milk products.

Thresholds of Sulfur Volatiles in Milk

The threshold of hydrogen sulfide and carbon disulfide have been well-documented in a variety of mediums. The thresholds of particular interest are the values that have been reported in skim milk and water. For hydrogen sulfide, the best estimate threshold (BET) in skim milk has been reported at 22.5 µg/L (Jo et al., 2019) and in water at 10 µg/L (Pippen and Mecchi, 1969). For carbon disulfide, the BET in skim milk has been reported at 35.2 µg/L (Jo et al., 2019) and in water at 5 µg/L (Vazquez-Landaverde et al., 2006). The BET of each compound found in skim milk was established by Jo et al. (2019) following the methodology prescribed by the American Society for Testing and Materials procedure E676—9 (ASTM, 2004) where panelists established individual BET values and the group BET was calculated as the geometric mean of those individual BET values. Individual BET values were determined using a 7-series-3-ascending-forced choice test in which panelists evaluated 3 sets of samples in ascending order at 7 different compound concentrations orthonasally in the selected medium.

While useful, threshold testing is affected by several different variables that must be controlled for. The temperature of the product affects the threshold of a compound and should be served at room temperature. The threshold will be higher if the medium is at refrigerated temperatures and lower if the medium is evaluated at a higher temperature. The amount of time

between preparation and evaluation also affects the BET score. If the sample is not given sufficient time to come to equilibrium within the sample cup, the threshold value will be inconsistent between panelists and yield inaccurate results. Conversely, if too much time is given between preparation of samples and evaluation of those samples, volatiles will dissipate, and the threshold will be recorded as higher than what it actually is. Studies have also shown that time of day, overhead lighting, pH, medium composition, fat level, salt/sugar levels, and many other variables all play into effecting the panelist's ability to accurately and consistently measure the BET of the same compound in the same medium (Leksrisompong et al., 2010). The sample medium play a significant role on the range of sulfur thresholds, which are generally low. For example, comparing the thresholds of carbon disulfide and hydrogen sulfide in water versus skim milk clearly highlights the effect that medium plays on the detectable threshold limit of sulfur compounds: Jo et al. (2019) established the threshold of carbon disulfide and hydrogen sulfide in skim milk at 35.2 and 22.5 µg/L, respectively, while in the water those same compounds have detectable threshold limits of 5 µg/L (Vazquez-Landaverde et al., 2006) and 10 µg/L (Pippen and Mecchi, 1969)—significantly lower.

Sulfur volatile threshold results were also identified by Vazquez-Ladaverde et al. (2006) in water for methanethiol at 0.2µg/kg, dimethyl disulfide at 0.16 µg/kg, and dimethyl trisulfide at 8 ng/kg. These same compounds (among others) were analyzed by Jo et al. (2018) in skim milk: methanethiol at 0.51 µg/kg, dimethyl disulfide at 1.10 µg/kg, and dimethyl trisulfide at 1.41 µg/kg. Clearly, sulfur volatile thresholds are 1) present at low concentrations and 2) variable dependent upon the medium that they are measured in. The threshold for sulfur compounds in milk is naturally going to be higher because of the varying interfering compounds found in milk, such as proteins. Similar conclusions were drawn by Hoffmann et al. (2001) who concluded that

a threshold of a compound mainly depends on two variables, 1) its volatility and 2) its reactivity with matrix compounds. They go on to show that pH, protein concentration, and fat content in the matrix all play a significant role in the detectable threshold limit of volatile compounds. Sulfur compounds are highly volatile, leading to their generally low threshold scores, and, as has already been shown, their overall difficulty to detect and quantify.

Effects on Consumer Preference

Studies have shown that consumers dislike UP milk products versus HTST (Horner et al, 1980; Attabi et al, 2009; Lee et al, 2017; Ansari and Sahoo, 2018); citing the intense overall aroma, sulfur/eggy flavor, and cardboard flavors as drivers of disliking. As outlined above, the high heat load associated with UP breaks down proteins, releases volatiles, and starts reactions that create these undesirable flavors at higher concentrations than HTST or other lower heat pasteurization techniques. The intense heat treatment also effects the color of milk products, which has been shown to effect consumer preference. Phillips et al. (1995) concluded that a whiter appearance of milk was preferred by consumers and UP leads to a change in color from white to brown that is undesirable to consumers (Al-Attabi et al, 2009). At UP temperatures, Maillard reactions take place which leads to a brown-like color in UP milk products as the lactose reacts with lysine or sulfur-containing amino acids to produce a yellow/brown color (Ansari and Sahoo, 2018).

Effect of Fat in Dairy Products

Role of Fat in Masking Flavors

Fat plays an important role on the sensory and physicochemical properties of food products. A majority of aroma compounds are fat-soluble, which means that the presence of fat in food products can produce a masking effect on the flavor perception for different foods to

consumers (Hatchwell, 1996). Fat globules present in milk and dairy products are composed of 98% triglycerides with a membrane covering the fat globule that acts as an emulsifying agent to prevent aggregation/coalescence of milk fat (Lopez, 2005). These globules consist of the majority of solids present in milk (~36%) and are largely responsible for emulsifying properties and binding sites for a variety of volatiles and proteins. The total available membrane space is not sufficient to cover the now greater fat surface area, which makes the fat more readily available for reaction with proteins and fat-soluble, volatile compounds. Homogenization breaks apart the fat globules, making them more accessible for reaction. Thermal processing then releases the volatile compounds from the cystine AA from the whey proteins, making them available in the headspace for reaction with the smaller fat globules. As mentioned above, H_2S and CS_2 are both fat soluble compounds and readily dissolve into the fat globules post-homogenization and thermal processing. As such, the presence of fat may modulate sensory thresholds for the sulfur compounds and impact consumer liking. A study done by Christensen and Reineccius (1992) found that fat had a masking effect on sulfur compounds in milk products: working with dibutyl sulfide (a fat soluble compound), they found as little as 1% fat in a milk sample decreased the vapor pressure of some compounds significantly, thus, lowering their concentration and sensitivity in the headspace.

Role of Fat in Consumer Preference

Fat has long been documented to improve sensory attributes of dairy products and consumer preference. Studies have also documented fat as increasing the creaminess, mouthfeel, and tactile attributes (Phillips et al., 1995; Guinard and Mazzuchelli, 1999; McCarthy et al., 2017). Additionally, the presence of fat affects the color of samples: increasing the whiteness and decreasing the blue/green color (Phillips et al., 1995), and this increase has been shown to

improve consumer preference scores and perceived mouthfeel. Attributes such as creaminess or mouthfeel have been shown to be more desirable by consumers and are therefore highly sought-after sensory attributes for product developers (Richardson-Harman et al., 2000; McCarthy et al., 2017; Harwood et al., 2018).

The presence of fat has been clearly documented to have a masking effect for fat-soluble flavor volatiles. Threshold testing completed has shown that the threshold for one compound is significantly higher for that same compound with the addition of fat. While fat has recently been perceived as a negative attribute, the addition of fat to food products has a variety of positive effects on consumer preference. McCarthy et al. (2017) discovered that milk does have a positive response from consumers up to a certain point (the background norm of fat percentage played a heavy role in consumer perception). A study done by Richardson-Harman et al. (2000) found similar results: consumers prefer products with a higher fat content and perceived creaminess.

Recently, Cheng et al. (2018a,b) documented that the perceived color (or whiteness) of a skim milk or fat free beverage was greatly influenced by the temperature, but that the presence of fat in milk-based beverages counteracted this effect as there was no documented impact on color due to processing temperature in a sample containing fat. While fat plays an important role in consumer preference, Lee et al. (2017) found that preference was more strongly directed by heating method rather than fat percentage. The aforementioned study found that consumers preferred milks HTST pasteurized regardless of fat percentage. The mouthfeel and textural properties that fat adds to a product does not overshadow overall flavors perceived by consumers in UP milks.

Release of Sulfur Volatiles from Fat Globules

While fat may play a role in binding and masking the sulfur volatiles created during thermal processing and raising the threshold of sulfur compounds in shelf-stable milk products, it has also been hypothesized that the milk fat globule could play a role in increasing the concentration of sulfur compounds. Hutton and Patton (1952) and Dill et al. (1962) suggested that the proteinaceous material associated with the milk fat globule membrane could serve as a potential, albeit, minor source of sulfur flavor. These studies were further supported by Gaafar (1987) who concluded that more reactive -SH groups and more intense sulfur flavors were present in UP processed whole milk than skim milk, and that increase was attributed to the milk fat globule membrane. Mehta (1980) and Calvo and de la Hoz (1992) have both highlighted the proteins in the milk fat globule as contributors to sulfur flavor due to thermal treatment. The role of fat as a modulator of cooked flavors has been documented for many years. A study done by Gould and Sommer (1939) found that milks with a higher fat content (comparing whole versus skimmed milk) had a lower cooked flavor temperature and theorized that cooked flavor was attributed to the formation of sulfide when milk products were heated to high temperatures and that possible release of sulfhydryls and volatile compounds from the fat globular membrane.

Conclusions

MCC is an under-explored ingredient of the dairy industry that has high potential to become a major contributor to both sales and product innovation. The potential for MCC in food products can be attributed to the native casein protein structure, high nutritional value, sensory attributes, and functional properties. The lack of a clear definition on the purity level for milk products to be classified as MCC and the processing parameters needed to make such a product creates an important hurdle that needs to be overcome in order to use this ingredient to its highest

capacity. The effect of purity on the detection of sulfur off-flavors due to thermal processing needs to be carefully studied and outlined in order to help establish purity level cutoffs. The objectives of this thesis were to determine first, how MCC purity changes as a function of the percent MDWP removal, second, the purity of MCC that is required to avoid the development of volatile sulfur off-flavors from thermally processed milk-based beverages at 6.3% protein, third, if heat treatment impacts measurement of MCC purity by Kjeldahl expressed as casein as a percentage of true protein, and fourth, if the presence of fat has a masking effect on the perceptibility of sulfur off-flavors in thermally processed milk-based beverages at 6.3% protein.

References

ADPI, 2018a. Concentrated Milk Proteins Standard. Accessed Apr 08, 2020 https://www.adpi.org/Portals/0/Standards/ConcentratedMilkPowder_book.pdf

ADPI, 2018b. Milk whey protein standard. Accessed Apr 08, 2020 https://www.adpi.org/Portals/0/Stanadrds/Milk%20Whey%20Protein.pdf.

Al-Attabi, Z., B.R. D'Arcy, and H.C. Deeth. 2009. Volatile sulphur compounds in UHT milk. Crit. Rev. Food Sci. Nutr., 49:28-47.

Agarwal, S., R.L.W. Beausire, S. Patel, and H. Patel. 2015. Innovative uses of milk protein concentrates in product development. J. Food Sci, 80:A23-A29.

Ansari, I.A., and P.K. Sahoo. 2018. Color variation of ultra high temperature (UHT) sterilized milk during storage. Int. J. Chem. Studies. 2:754-757.

Amelia, I., and D.M. Barbano. 2013. Production of an 18% protein liquid micellar casein concentrate with a long-refrigerated shelf life. J. of Dairy Sci. 96:3340-3349.

Badings, H.T., J.J.G. Van De pol, and R. Neeter. 1981. Aroma compounds which contribute to the difference in flavor between pasteurized milk and UHT milk flavor. 3rd Weurman Symposium: Proceedings of the International Conference. Pp. 683-692. Munich, Germany: Walter de Gruyter.

Beckman, S.L., J. Zulewska, M. Newbold, D.M. Barbano. 2010. Production efficiency of micellar casein concentrate using polymeric spiral-wound microfiltration membranes. J. of Dairy Sci. 93:4506-4517.

Beliciu, C.M., A. Sauer, and C.I. Moraru. 2012. The effect of commercial sterilization regimens on micellar casein concentrates. J. Dairy Sci. 95:5510-5526.

Belitz, H.D., W. Grosch, and P. Schieberle. 2009. Milk and dairy products. Pages 498-544 in Food Chemistry. 4th ed. Springer, Heidelberg, Germany.

Bong, D. Evaluation of an alternate processing method for Greek style yogurt using micellar casein concentrate. 2013. Technical and Business Aspects. Masters thesis. Cornell University, Ithaca, NY.

Bong, D.D., and C.I. Moraru. 2014. Use of micellar casein concentrate for Greek-style yogurt manufacturing: Effects on processing and product properties. J Dairy Sci. 97:1259-1269.

Boor, K.J. 2001. Fluid dairy product quality and safety: looking to the future. J. Dairy Sci. 84:1-11.

Brandsma, R.L., and S.S.H. Risvi. 2001. Manufacture of mozarella cheese from highly-concentrated skim milk microfiltration retentate depleted of whey protein. Int. J. Food Sci. Technology. 36:611-624.

Brodkorb, A., T. Croguennec, S. Bouhallab, and J.J. Kehoe. 2016. Heat-induced denaturation, aggregation and gelation of whey proteins. In McSweeny P., O'Mahony J. (Eds) Advanced Dairy Chemistry. Springer, New York, NY.

Burgain, J., J. Schedule, J. Petit, G. Francius, and C. Gaiani. 2016. Link between particle surface hardening and rehydration impairment during micellar casein powder storage. Food Hydrocoll 61: 277-285.

Burrington, K.J. 2017. Differences between milk protein and whey protein ingredients. ADPI Intelligence. 5:8.

Bylund, G. 2003. Long life milk, Pages 215-232 in Dairy Processing Handbook, Lund Sweden. TetraPak Processing Systems AB.

Calvo, M.M., and L. De la Hoz. 1992. Flavor of heated milks - a review. Int. Dairy J. 2:69-81

Carter, B. 2020. The efficiency of whey protein production and improvements on whey product applications. Doctorate dissertation. North Carolina State University, Raleigh, NC.

Carter, B., H. Patel, D. M. Barbano, and M. A. Drake. 2018. The effect of spray drying on the difference in flavor and functional properties of liquid and dried whey proteins, milk proteins, and micellar casein concentrates. J. Dairy Sci. 101:3900-3909.

Chapman, K.W., and K.J. Boor. 2001. Acceptance of 2% ultra-pasteurized milk by consumers, 6-11 years old. J. Dairy Sci., 84:951-954.

Cheng, N., D.M. Barbano, and M.A. Drake. 2018. Hunter versus CIE color measurement systems for analysis of milk-based beverages. J. Food Sci. 75:S513-521.

Cheng, N., D.M. Barbano, and M.A. Drake. 2019a. Effect of pasteurization and fat, protein, casein to serum protein ration, and milk temperature on milk beverage color and viscosity. J. Dairy Sci. 102: 2022-2043.

Cheng, N., D.M. Barbano, and M.A. Drake. 2019b. Effects of milk fat, casein, and serum protein concentrations on sensory properties of milk based beverages. J. Dairy Sci. 102: 8670-8690.

Christensen, K.R., and G.A Reineccius. 1992. Gas chromatographic analysis of volatile sulfur compounds from heated milk using static headspace sampling. J. Dairy Sci. 75:2098-2104.

Colahan-Sederstrom, P.M., and D.G. Peterson. 2005. Inhibition of key aroma compound generated during ultrahigh-temperature processing of bovine milk via epicatechin addition. J. Agric. Food Chem. 53:398-402.

Crowley, S.V., B. Desautel, I. Gazi, A.L. Kelly, T. Huppertz, and J.A. O'Mahony. 2015. Rehydration characteristics of milk protein concentrate powders. J. Food. Eng. 149:105-113.

Culhane, M. 2016. Filtered milk for cheese and ingredient production. US Dairy Export Council.

Dalgleish, D. D. 1990. Denaturation and aggregation of serum proteins and caseins in heated milk. J. Agric. Food Chem. 38:11.

Datta, N., A.J. Elliott, M.L. Perkins, and H.C. Deeth. 2002. Ultra-high-temperature (UHT) treatment of milk: comparison of direct and indirect modes of heating. Aust. J. Dairy Technol., 57:211-227.

Dill, C.W., W.M. Roberts, and L.W. Aurand. 1962. Production of sulfur compounds in skim milk heated by direct steam injection. J. Dairy Sci. 11:1332-1335.

Elliott, A.J., N. Datta, B. Amenu, and H.C. Deeth. 2005. Heat-induced and other chemical changes in commercial UHT milks. J. Dairy Res. 72:442-446.

Fang Y., C. Selomulya, and X.D. Chen. 2010. Characterization of milk protein concentrate solubility using focused beam reflectance measurement. Dairy Sci. Technol. 90:253-270.

FDA (Food and Drug Administration). 2015. Grade "A" Pasteurized Milk Ordinance. Accessed https://www.fda.gov/downloads/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/Milk/UCM513508.pdf.

FDA (Food and Drug Administration). 2017. 21 CFR 131.3 Accessed April 8, 2020. https://accessdata.fda.gov/scripts/cdrh/dfcocs/cfcfr/CFRSearch.cfm?fr=131.3.

Foegeding, E.A. J.P. Davis, D. Doucet, and M.K. McGuffey. 2002. Advances in modifying and understanding whey protein functionality. Trends in Food Sci. & Techol. 13:151-159

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

Fox, P.F., and P.L.H McSweeney. 2003. Advanced Dairy Chemistry. Dairy Chemistry and Biochemistry. 2nd rev. ed. Kluwer Academic/Plenum Publishers, New York, NY.

Gaafar, A.M.M. 1987. Investigation into the cooked flavor in heat-treated milk. PhD thesis University of Reading.

Gaiani, C., P. Schuck, J. Scher, S. Desobry, and S. Banon. 2007. Dairy powder rehydration: influence of protein state, incorporation mode, and agglomeration. J. Dairy Sci. 90:570-581.

Gitis, V., and G. Rothenburg. 2016. Ceramic membranes: new opportunities and practical applications. Wiley-VCH, Weinheim, Germany.

Gould, I.A., and H.H. Sommer. 1939. Effect of beat on milk with especial reference to the cooked flavor. Michigan Agr. Expt. Sta., Tech. Bull. 164.

Govindasamy-Lucey, S., J.J. Jaeggi, M.E. Johnson, T. Wang, and J.A. Lucey. 2007. Use of cold microfiltration retentates produced with polymeric membranes for standardization of milks for manufacture of pizza cheese. J. Dairy Sci. 90:4552-4568.

Guinard, J.X., and R. Mazzuchelli. 1999. Effect of sugar and fat on the sensory properties of milk chocolate: descriptive analysis and instrumental measurements. J. Sci. Food Agric. 79:1331-1339.

Guo, M., 2019. Whey protein production, chemistry, functionality, and applications. John Wiley & Sons. 1st ed. Hoboken, NJ

Hatchwell, L.C. 1996. Pages 14-23 in Flavor-food interactions. Mt. Prospect, IL.

Haug, A., A.T. Hostmark, and O.M. Harstad. 2007. Bovine milk in human nutrition — a review. Lipids Health Dis. 6:25.

Heino, A. T., Uusi-rauva, J. O., Rantamäke, P.R., and O. Tassavainen. 2007. Functional properties of native and cheese whey protein concentrate powders. Int. J. Dairy Technol. 60: 277-285.

Hoffmann, J.R., and M.J. Falvo. 2004. Protein- which is best? J. Sports Sci. Med. 3:118-130.

Hoffmann, T., M. Czerny, S. Calligaris, and P. Schieberle. 2001. Model studies on the influence of coffee melanoidins on flavor volatiles of coffee beverages. J. Agric. Food Chem. 49:2382-2386.

Holt, C. 1992. Structure and Stability of Bovine Casein Micelles. Adv Protein Chem. 43:63-151.

Horner, S. A., E. Stanley, and F.C. Wallen. 1980. Sensory aspects of UHT milk combined with whole pasteurized milk. J. Food Protection. 1:54-57.

Huffman, L.M. 1996. Processing whey protein for use as a food ingredient. Food Technol. 2:49-52.

Huppertz, T., P.F. Fox, and A.L. Kelly. 2018. Pages 49-92 Proteins in Food Processing. 2nd Ed. Woodhead Publishing. Oxford, UK.

Hutton, J.T., and S. Patton. 1952. The origin of sulfhydryl groups in milk proteins and their contributions to “cooked” flavor. J. Dairy Sci. 35:699-705.

Hurt, E., and D.M. Barbano. 2010. Processing factors that influence casein and serum protein separation by microfiltration. J. Dairy Sci. 93:4928-4941.

Hurt, E., J. Zulewska, M. Newbold, and D.M. Barbano. 2010. Micellar casein concentrate production with a 3X, 3-stage, uniform transmembrane pressure process at 50C. J. Dairy Sci. 93:5588-5600.

IMARC Group, 2019. Fluid Milk Market: Global industry trends, share, size, growth, opportunity and forest 2019-2024. Accessed July 27, 2020. https://www.researchandmarkets.com/reports/4763048/fluid-milk-market-global-industry-trends-share?utm_source=GN&utm_medium=PressRelease&utm_code=2p79bk&utm_campaign=1236666+-+Fluid+Milk+Market+-+Global+Industry+Trends%2c+Share%2c+Size%2c+Growth%2c+Opportunity+and+Forecasts+2019-2024&utm_exec=chdo54prd

Jang, H. D., and H. E. Swaisgood. 1989. Disulfide bond formation between thermally denatured b-lactoglobulin and k-casein in casein micelles. J. Dairy Sci. 73:900-904.

Jayaprakasha, H.M., and H. Brueckner. 1999. Whey protein concentrate: a potential functional ingredient for food industry. J. Food Sci. Tech. 36:189-204.

Jenness, R. 1954. Effects of heat treatment on serum proteins. Agr. Food Chem. 2:75-81.

Jo, Y., B.G. Carter, D.M. Barbano, and M.A. Drake. 2019. Identification of the source volatile sulfur compounds produced in milk during thermal processing. J. Dairy Sci. 102:8658-8669.

Jo, Y., D.M. Benoist, D.M. Barbano, and M.A. Drake. 2018. Flavor and flavor chemistry differences among milks processed by high-temperature, short-time pasteurization or ultra-pasteurization. J. Dairy Sci. 101:3812-3828.

Josephson, D.V., and F.J. Doan. 1939. Observations on cooked flavor in milk, its source and significance. Milk Dealer. 29:35-54.

Karasu, Kensuke, N. Glennon, N. D. Lawrence, G. W. Stevens, A. J. O’Connor, A. R. Barber, S. Yoshikawa, and S. E. Kentish. 2010. A comparison between ceramic and polymeric membrane systems for casein concentrate manufacture. Int. J. Dairy Technol. 63:284-289.

Kühn, J.T., Considine, and H. Singh. 2006. Interactions of milk proteins and volatile flavor compounds: implications in the development of protein foods. J. Food Sci. 71:72-82.

Lee, A.P., D.M. Barbano, and M.A. Drake. 2017. The influence of ultra-pasteurization by indirect heating versus direct steam injection on skim and 2% fat milks. J. Dairy Sci. 100:1688-1701.

Leksrisompong, P., D.M. Barbano, A. E. Foegeding, P. Gerald, and M.A. Drake. 2010. The roles of fat and pH on the detection thresholds and partition coefficients of three compounds: Diacetylmorphine, gamma-dacalactone and furaneol. J. Sens. Stud. 25:347-370.

Liem, D.G., D.P. Bolhuis, X. Hu, and R.S.J. Keast. 2016. Short communication: influence of labeling on Australian and Chinese consumers' liking of milk with short (pasteurized) and long (UHT) shelf life. J. Dairy Sci. 99: 1747-1754.

Lopez, C. 2005. Focus on the supramolecular structure of milk fat in dairy products. Reprod. Nutr. Dev. 45:497-511.

Lu, Y., D.J. McMahon, L.E. Metzger, A. Kommineni, and A.H. Vollmer. 2015. Solubilization of rehydrated frozen highly concentrated micellar casein for use in liquid food applications. J. Dairy Sci. 98:5917-5930.

Lu, Y., D.J. McMahon, and A.H. Vollmer. 2017. Investigating rennet coagulation properties of recombined highly concentrated micellar casein concentrate and cream for use in cheese making. J. Dairy Sci. 100:892-900.

Luck, P.J., B. Vardhanabhuti, Y.H. Yong, T. Laundon, D.M. Barbano, and E.A. Foegeding. 2013. Comparison of functional properties of 34% and 80% whey protein and milk serum protein concentrates. J. Dairy Sci. 96: 5522-5531.

Marella, C., K. Muthukumarappan, and L.E. Metzger. 2013. Application of membrane separation technology for developing novel dairy food ingredients. J. Food Process Technol. 4:9

McCarthy, K.S., K. Lopetcharat, and M.A. Drake. 2017. Milk fat threshold determination and the effect of milk fat content on consumer preference for fluid milk. J. Dairy Sci. 100:1702-1711.

McGorrin, R.J. 2011. The significance of volatile sulfur compounds in food flavors. Pages 3-31 in Volatile Sulfur Compounds in Food. M.C. Qian, E. Fan, and K. Mahattanatawee, ed. ACS, Washington, DC.

Mehta, R.S. 1980. Milk processed at ultra-high-temperatures— A review. J. Foo Prot. 43:212-225

Mimouni, A., H.C. Deeth, A.K. Whittaker, M.J. Girdles, and B.R. Bhandari. 2010. Investigation of the microstructure of milk concentrate powders during rehydration: alternations during storage. J. Dairy Sci. 93:463-472.

Modler, H.W. 2013. Functional properties of nonfat dairy ingredients – a review. modification of lactose and products containing whey proteins. J. Dairy Sci. 68:2206-2214.

Mulvihill, D.M., and M.P. Metzger. 2003. Functional milk proteins: production and utilization. In: Fox P.F., McSweeney P.L.H (eds) Advanced Dairy Chemistry— 1 Proteins. Springer, Boston, MA.

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

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

Nunes, L., and G. M. Tavares. 2019. Thermal treatments and emerging technologies: impacts on the structure and techno-functional properties of milk proteins. Trends Food Sci Technol. 90:88-99.

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

Park, S.H., Y.G. Park, J. Lim, and S. Kim. 2014. Evaluation of ceramic membrane applications for water treatment plants with a life cycle cost analysis. Desalin. Water Treat. 54: 545-559.

Patrick, P.S., and H.E. Swaisgood. 1976. Sulfhydryl and disulfide groups in skim milk as affected by direct ultra-high-temperature heating and subsequent storage. J. Dairy Sci. 59:594-600.

Perkins, M.L., and C.H. Deeth. 2001. A survey of Australian consumers' attitudes towards UHT milk. Aust. J. Dairy Technol. 56:28-34.

Phillips, L. G., M. L. McGiff, D. M. Barbano, and H. T. Lawless. 1995. The influence of fat on the sensory properties, viscosity, and color of low fat milk. J. Dairy Sci. 78:1258-1266.

Pippen, E.L., and E.P. Mecchi. 1969. Hydrogen sulfide, a direct and potential indirect contributor to cooked chicken aroma. J. Food Sci. 34:443-446

Pofahl, T.R., and D.G. Vakaleris. 1968. Effects of heat on sulfhydryl and disulfide groups of milk proteins as measured by the spectrofluorometric method. J. Dairy Sci. 9:1345-1348.

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

Queiros, M.S., R. L. S. Viriato, D.A. Vega, A.P.B. Ribeiro, and M.L. Gigante. 2020. Milkfat nanoemulsions stabilized by dairy proteins. J. Food Sci. Techn. 57:3295-3304.

Richardson-Harman, N. J., R. Stevens, S. Walker, J. Gamble, M. Miller, M. Wong, and A. McPherson. 2000. Mapping consumer perceptions of creaminess and liking for liquid dairy products. Food Qual Prefer. 11:239-246.

Rutherford, S.M., A.C. Fanning, B.J. Miller, and P.J. Moughan. 2015. Protein digestibility-corrected amino acid scores and digestible indispensable amino acid scores differentially describe protein quality in growing male rates. J. Nutr. 145:372-379.

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

Sauer, A., I. Doehner, and C.I. Moraru. 2012a. Steady shear rheological properties of micellar casein concentrates obtained by membrane filtration as a function of shear rate, concentration, and temperature. J. Dairy Sci. 95:5569-5579.

Sauer, A., and C.I. Moraru. 2012b. Heat stability of micellar casein concentrates as affected by temperature and pH. J. Dairy Sci. 95:6339-6350.

Schmidt, R.H., V.S. Packard, and H.A. Morris. 1984. Effect of processing on whey protein functionality. J. Dairy Sci. 67:2723-2733.

Schokker, E., J. Church, J. Mata, E. Gilbert, A. Puvanenthiran, and P. Udabage. 2011. Reconstitution properties of micellar casein powder: effects of composition and storage. Int. Dairy J. 21:877-886.

Schuck P., M. Piot, S. Méjean, J. Fauquant, C. Brulé, and J.L. Maubois. 1994. Dehydration of an ultra-clean milk and micellar casein enriched milks. Le Lait. 74: 47-63.

Schuck, P., V. Briard, S. Mejean, M. Pilot, M.H. Famelart, and J.L. Maubois. 2007. Dehydration by desorption and by spray drying of dairy products: influence of the mineral environment. Dry Technol: Int. J. 17:1347-13587

Shahbandeh, M. 2020. Cow milk production worldwide from 2015 to 2019. Available: https://www.statista.com/statistics/263952/production-of-milk-worldwide/. Accessed April 08, 2020.

Shahbandeh, M. 2019a. U.S. Milk Market – Statistics & Facts. Available: https://www.statista.com/topics/1284/milk-market/. Accessed April 08, 2020

Shahbandeh, M. 2019b. Size of the whey protein market worldwide 2018 & 2024. Available: https://www.statista.com/statistics/728005/global-whey-protein-market-size/

Sharma-Shri, K., and D. G. Dalglesih. 1993. Interactions between milk serum proteins and synthetic fat globule membrane during heating of homogenized whole milk. J. Agric. Food Chem. 41:1407-1412.

Sipple, L., D.M. Barbano, and M.A. Drake. 2020. Impact of milk protein type and concentration on the composition, physical, and sensory properties of low-fat, high-protein ice cream. ADSA presentation 2020.

Smith, K. 2013. Development of membrane processes. Pages 1-9 in A. Y. Tamime (Ed.) Membrane processing: dairy and beverage application. Oxford, UK: Blackwell Publishing Ltd.

Smith, T.J., R.E. Campbell, Y. Jo, and M.A. Drake. 2016. Flavor and stability of milk proteins. J. Dairy Sci. 99:4325-4346.

Smithers, G.W. 2015. Whey-ing up the options – Yesterday, today, and tomorrow. Int. Dairy J. 48:2-14

Thomas, E.L., G.A. Reinceccius, G.J. DeWaard, and M.S. Slinkard. 1976. Determiniation of hydrogen sulfide in heated milks by gas-liquid chromatographic headspace analysis. J. Dairy Sci. 59:1865-1869

Tunick, M.H. 2008. Whey protein production and utilization: a brief history. Pages 1-15 in Whey Processing, Functionality, and Health Benefits. Onwulata, C.I., Huth, P.J., eds. Wiley-Blackwell, Ames, Iowa. Pp. 480.

Turkmen, N., C. Akal, and B. Özer. 2019. Probiotic dairy-based beverages – a review. J. Funct. Foods. 53:62-75.

USDA, Economic Research Service. 2015. Food Availability Per Capita Data System. Accessed April 8, 2020. http://www.ers.usda.gov/data-products/dairy-data.aspx.

USDA. 2010. Dairy Products 2010 Summary: USDA. Available: http://usda.mannlib.cornell.edu/usda/current/DairProdSu/DairProdSu-04-27-2011.pdf. Accessed April 08, 2020.

U.S. Dairy Export Council. 2004. Reference Manual for US Whey and Lactose Products. Available: http://usdec.org/files/pdfs/us08D_04.pdf. Accessed April 08, 2020.

Vazquez-Landaverde, P.A., J.A. Torres, and M.C. Qian. 2006. Quantification of trace volatile sulfur compounds in milk by solid-phase microextraction and gas chromatography-pulsed Flame photometric detection. J. Dairy Sci. 89:2919-2927.

Vazquez-Landaverde, P.A., J.A. Torres, and M.C. Qian. 2005. Quantitative determination of thermally derived off-flavor compounds in milk using solid-phase microextraction and gas chromatography. J. Dairy Sci. 88:3764-3772.

Vogel, K.G. III. 2019. Effect of protein amount and milk protein ingredients on sensory properties and physicochemical properties of ready-to-drink protein beverages. Master thesis, North Carolina State University, Raleigh, NC.

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

Watanabe, K., and H. Klostermeyer. 1976. Heat-induced changes in sulphydryl and disulphide levels of b lactoglobulin A and the formation of polymers. J. Dairy Res. 43:411-418.

Zhang, R., X. Pang, J. Lu, L. Liu, S. Zhang, and J. Lv. 2018. Effect of high intensity ultrasound pretreatment on functional and structural properties of micellar casein components. Ultrason. Sonochem. 47:10-16.

Zhang, Y. and C.-T. Ho. 1991. Comparison of the volatile compounds formed from the thermal reaction of glucose with cysteine and glutathione. J. Agric. Food Chem. 39:760-763.

Zweig, G., and R. Block. 1953. The effect of heat treatment on the sulfhydryl group in skim milk and nonfat dry milk. J. Dairy Sci. 4:427-436.

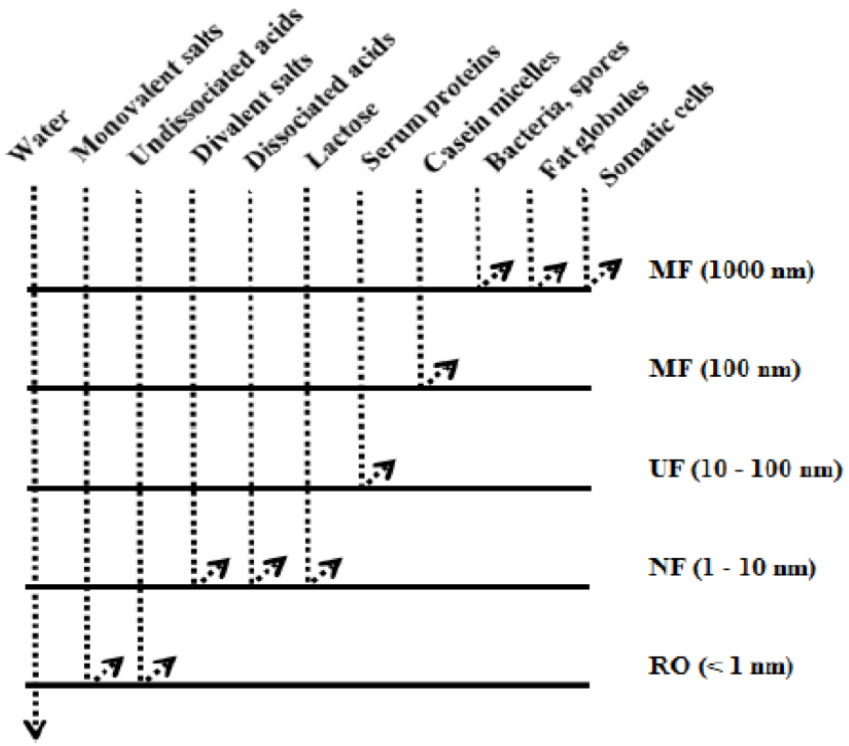

Figure 1.1 Passed and rejected dairy components based on membrane pore size (modified from Adams, 2012)

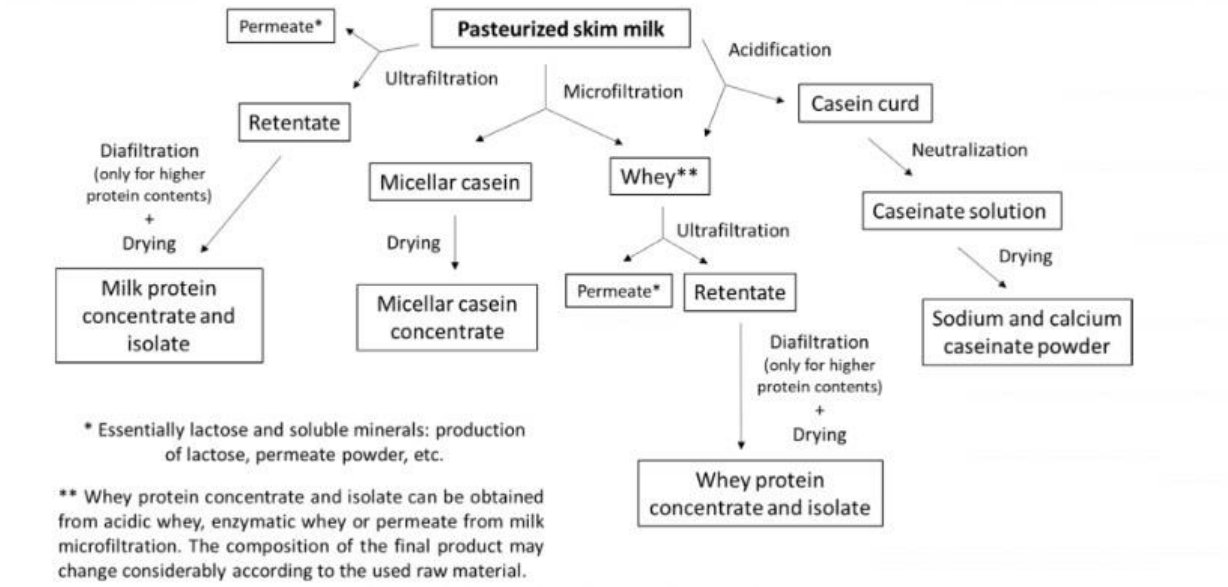

Figure 1.2 Schematic of MCC Production and MDWP Manufacture (Nunes and Tavares, 2019)

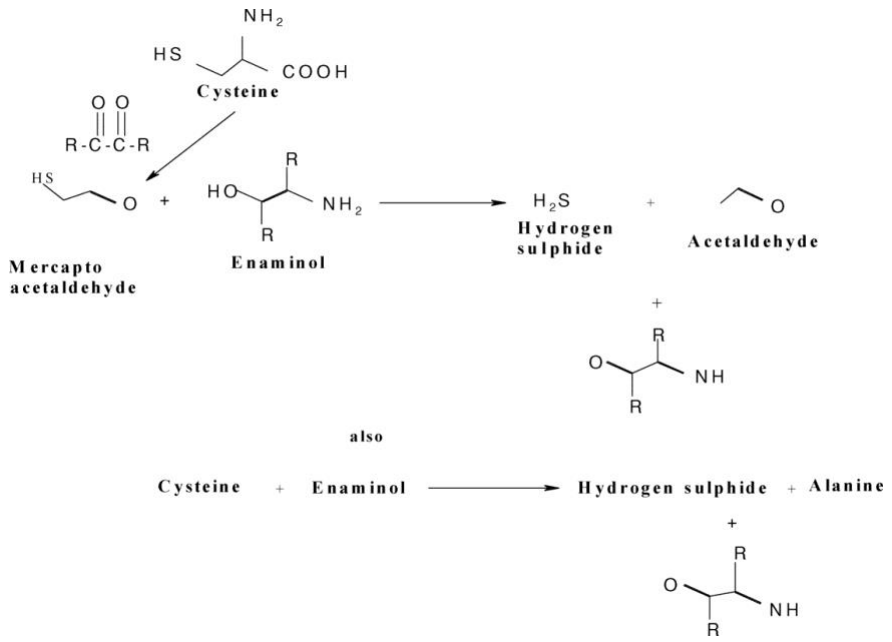

Figure 1.3 Strecker Degradation of cysteine to hydrogen sulfide (taken from Al-Attabi et al., 2009

CHAPTER 2: THE EFFECT OF MICELLAR CASEIN CONCENTRATE PURITY ON SULFUR/EGGY FLAVOR IN ULTRA-PASTUERIZED MILK PROTEIN-BASED BEVERAGES

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Abstract

Micellar casein concentrate (MCC) is made through the microfiltration of skim milk using either a ceramic or polymeric membrane. Micellar casein concentrates differ from milk protein concentrates (MPC) in that the ratio of casein:milk derived whey protein (MDWP) has been altered where it is not in MPC. MCC purity is defined by the percent milk derived whey protein (% MDWP) removed from the starting skim milk or by the casein as a percentage of true protein (CN%TP) as measured in the final MCC. Following high heat treatment such as ultrapasteurization (UP), MDWP denature and form volatile sulfur off flavors. Identification of the necessary range limit of MCC purity necessary to eliminate sulfur flavors from thermally processed MCC-based products would help in the establishment of a standard of identity and provide an additional functional point of difference from MPC/MPI. The objectives of this study were to determine the level of MDWP removal necessary to achieve no detectable sulfur-eggy flavor in direct steam injection (DSI) UP MCC based beverages at 0.15% (w/w) fat with 15 g protein per 240 mL serving and to determine if heat treatment impacted the measurement of MCC purity by Kjeldahl expressed as CN%TP. The orthonasal threshold limit for hydrogen sulfide and carbon disulfide were established in 95% MDWP reduced MCC following a 7-series AFC threshold test. Four model beverages were prepared with freshly produced 95% MDWP reduced MCC, serum protein isolate, carrageenan, cellulose gel, and dipotassium phosphate to mimic a geometric progression of MDWP removal of 95.2%, 91.0%, 83.2%, and 69.2% which correspond to MCC purities (i.e., casein % of true protein) of 93.8, 93.2, 92.2 90.2%, respectively. The beverages were subjected to direct steam injection UP (140C for 3 sec), bottled, and cooled to 4°C. Residual MDWP and MCC purity were measured by Kjeldahl prior to and following UP. At d 1 post-processing, the beverages were evaluated by a trained panel

and triple quadrupole GC-MS/MS for sulfur volatiles. Measurement of MCC purity of each beverage by Kjeldahl analysis increased ($p<0.05$) following UP heat treatment demonstrating that Kjeldahl cannot be used to determine MCC purity following high heat treatment. Sensory perception of sulfur/eggy flavor intensity and relative abundance of hydrogen sulfide and carbon disulfide increased with increasing level of residual MDWP ($p<0.05$). Beverages produced with 91% MDWP reduced MCC or that of higher purity were below sensory detection threshold for sulfur/eggy off flavors. Serum protein removal from MCC must be greater than 91% to achieve a product with no sulfur off-flavors following thermal processing. To eliminate sulfur eggy flavor in a milk protein beverage at 15 g protein and 0.35 g fat per 240 mL serving, the residual MDWP concentration needs to be $\leq$ 0.96 g/240 mL beverage.

Key Words: sulfur-eggy flavor, milk protein-based beverage, micellar casein, milk derived whey protein

Introduction

Micellar casein concentrate (MCC) is made through the removal of milk derived whey protein (MDWP) from skim milk by means of microfiltration, using either a ceramic or polymeric (spiral wound) membrane (Zulewska et al., 2009). Zulewska and Barbano (2014) found that ceramic membranes were more efficient at removing MDWP from skim milk than polymeric membranes. Additionally, despite their higher overhead cost, ceramic membranes had a higher thermal and chemical stability, operated under a higher limiting flux, and have a longer usability life. Adams et al. (2015) evaluated other membrane variations to improve processing efficiency and found that diamond shaped pores in ceramic membranes can be more energy efficient, but round pores are better at separating casein from MDWP. Zulewska and Barbano (2014) found that under similar conditions, at 50°C, and with 3 stages of filtration (2 of which included diafiltration), a microfiltration apparatus equipped with a ceramic membrane removed 95% MDWP while the same microfiltration unit equipped with a polymeric membrane was only capable of achieving 70% MDWP removal. 95% MDWP removal has been shown to be possible using a polymeric membrane but only after 8 stages of diafiltration (Beckman et al., 2010).

Currently, there is no standard of identity for MCC or its purity level. Recent studies have demonstrated that MDWP is the milk protein fraction responsible for undesirable sulfur/eggy flavor in UP milk (Lee et al., 2017; Jo et al., 2018, 2019). Vogel (2019) also demonstrated that sulfur/eggy flavors were documented in 6.3 and 10.5% protein beverages made from MPC, but this flavor was not detected in the same beverages made with 95% MDWP removed MCC. Purity for MCC is defined as casein as percentage of true protein (CN%TP) or as the percent MDWP removed from starting skim milk. While the two definitions both relate to the purity of the MCC, % MDWP removed refers to the percentage of MDWP removed from the starting skim milk. For

example, MCC processed to 95% MDWP reduced means that 95% of the MDWP in the starting skim milk was removed from the final MCC product. MCC purity as expressed by CN%TP is a measurement of the total amount of casein present in the final MCC as a percentage of the measured protein by Kjeldahl analysis. As way of example, a MCC product produced to a purity level of 95% MDWP reduction would have the equivalent CN%TP value of 93.83%, meaning that 93.83% of the protein in the final MCC product is casein. For the purposes of this paper, both values will be cited; however, higher emphasis will be placed on % MDWP because it offers a greater distinction between purity levels (skim milk already starts out at 80% CN%TP) and provides a more accurate representation of the purity of the MCC as achieved through microfiltration. MCC purity (CN%TP) can be measured using the Kjeldahl method as documented by Lynch et al. (1998) by indirectly measuring the amount of casein by determining the total nitrogen available in the sample and then subtracting the noncasein nitrogen (NCN) in the filtrate after precipitating the casein at pH 4.6. Lynch et al. (1998) went on to theorize that such an analysis should be done prior to thermal processing to obtain in accurate measurement of the NCN in MCC.

The flavor and compositional profile of MCC is dependent on its purity because the residual MDWP is the main contributor to sulfur/eggy flavor in UP processed milk and protein beverages (Lee et al., 2017; Jo et al., 2019). When subjected to high temperatures, MDWPs denature and expose the sulfur containing amino acids cysteine/cysteine which degrade to hydrogen sulfide and carbon disulfide through Strecker degradation (Zhang and Ho, 1991). Hydrogen sulfide and carbon disulfide have been documented as the volatile sulfur compounds responsible for sulfur/eggy flavor in UP milk products (Jo et al., 2019). Establishing a range limit of the level of MCC purity necessary to eliminate sulfur flavors from thermally processed MCC-based products would provide an additional functional point of difference from MPC/MPI and aid

in the establishment of a standard of identity. Our objectives were to determine the level of MDWP removal necessary to achieve no detectable sulfur-eggy flavor in the direct steam injection (**DSI**) UP micellar casein concentrate based beverage at 0.15% (w/w) fat with 15 g protein per 240 mL serving and to determine if heat treatment impacted the measurement of MCC purity by Kjeldahl expressed as CN%TP.

Materials and Methods

Experimental Design Overview

MCC with 95% milk derived whey protein (MDWP) removal was produced from skim milk (50ºC) using a 3X, 3-stage ceramic MF process using 0.1 µm pore size graded permeability membranes (n=3). Milk protein-based beverages at 15 g protein per 240 mL serving (i.e. about 6.1% (w/w) true protein) were formulated at a fat content of 0.15% (w/w) fat at 4 different levels of MDWP removal percentages (95.2%, 91.0%, 83.2%, and 69.2%). These MDWP removal percentages corresponded to MCC purities (casein % of true protein) of 93.8, 93.2, 92.2, and 90.2%, respectively. The MDWP removal percentages also corresponded to g of SP/100g of protein (0.39g, 0.42g, 0.50g, and 0.60g, respectively). The experiment used a model beverage formulation with constant fat content of 0.15% fat for all four MCC purity levels and was designed to determine the level of MDWP removal necessary to achieve no detectable sulfur-eggy flavor in DSI UHT milk protein-based beverages. The experiment was replicated three times with different lots of milk. Each experimental replication was comprised of two production days. Micellar casein concentrate was manufactured from skim milk on day 1 and beverages were formulated and processed on day 2.

Milk Based Ingredients

Raw Skim Milk. Raw bovine skim milk (0.087% ± 0.03 fat, SCC <300,000) was obtained from the North Carolina State University Dairy Enterprise System (Raleigh, NC) on the morning of each processing run. Raw skim milk was collected and held at 4°C in a glycol-chilled, jacketed storage tank until use (ca 2 h).

Micellar Casein Concentrate (MCC). Liquid MCC was made the day before beverage formulation as described by Cheng et al. (2018), with minor modifications. Raw skim milk (570 kg) was prefiltered before pasteurization at 4°C using a Nexis T-filter (NXT 10-30U-M7S, Pall Corp., Port Washington, NY.), pasteurized (720 kg/h) with a plate heat exchanger (model T4 RGS-16/2, SPX Flow Technology, Greensboro, NC) at 72°C for 16 s, and then ~30 kg of pre-filtered, HTST pasteurized skim milk was used to flush out the de-ionized (DI) water from the membrane system before collection began. A 3 stage, 3x microfiltration (MF) process described by Zulewska and Barbano (2014) was used to produce a 95% MDWP reduced MCC with true protein concentration between 7.3 to 7.5% using a pilot scale MF system (Tetra Alcross MFS-7, TetraPak Filtration Systems) equipped with 0.1-µm nominal pore diameter graded permeability ceramic Membralox (model EP1940GL0.1u, AGP1020, alumina, Pall Corp.) membranes. The 95% MDWP reduced ~7.3% TP (w/w) MCC was cooled in milk cans to 10°C using a glycol-chilled water bath immediately post-process and then stored at 4°C in a walk-in cooler until use (ca 12 h). The MCC was used as a fresh liquid ingredient in beverage formulation the following day for each experimental replication to limit storage effects.

Serum Protein Isolate (SPI). To produce enough SPI for the study, the SPI was produced in a large batch at South Dakota State University (Brookings, SD), frozen, and shipped overnight to NC State University and held frozen (-20) until used in the beverage study. Briefly, MF

permeate from the production of MCC was concentrated using ultra-filtration (UF) to a high protein (about 25% w/w) SPI by UF, and the concentrate was packaged in half gallon plastic jugs (Upstate Niagara Cooperative, Buffalo, NY), and placed in a -20^{o}C freezer for storage. Three days before formulation beverage, the frozen SPI containers were placed in a 4^{o}C cooler to thaw slowly prior to formulation of beverages.

Orthonasal Threshold Measurement

H_2S and CS_2 were selected for orthonasal threshold testing based from work done by Jo et al. (2019), who showed that these two compounds are the main contributors to sulfur/eggy flavor in thermally processed milk-based products. Orthonasal thresholds for each of these compounds were determined in 95% MDWP reduced MCC to provide further clarity on concentrations of these two compounds that were below sensory detection in this matrix. A modification of the American Society for Testing and Materials procedure E679-9 (ASTM, 2004), an ascending forced-choice method of limits, was used to determine the best estimate orthonasal threshold (BET) value for each compound. This method had panelists evaluate the samples in a 7-series 3-ascending forced-choice procedure.

Stock solutions of each compound were prepared in 95% ethanol (Sigma Aldrich, St Louis, MO). Portions of each stock solution were added to the selected medium (95% MDWP reduced, ~7.3% TP [w/w] MCC, HTST pasteurized). MCC was prepared the day before threshold tests and HTST pasteurized (74C, 15 sec) using a Microthermics EHVH pasteurization unit (Microthermics, Raleigh, NC). The concentration of H_2S and CS_2 in HTST pasteurized MCC are below the instrumental limit of detection and HTST pasteurized MCC has no discernible sulfur/eggy aroma or flavor as determined by (Jo et al., 2019). Blank solutions were prepared using MCC with no

added compounds. The orthonasal detection threshold of each compound was determined individually.

Sample solutions were made by geometrically serially diluting the stock solution with MCC using a step factor of 1.5. Thirty mL of each serial dilution or blank was poured into clean, 3-digit-coded, 59-mL lidded souffle cups (Dart Container Corp., Mason, MI). The samples for each compound were prepared 3 h before testing so that all solutions could come to equilibrium in the lidded cups (Lekrisompong et al., 2010). Panelists (n = 50) were instructed on the appropriate sniffing technique and testing procedure before testing as prescribed by Lekrisompong et al. (2010). Seven series of three sample sets were presented to each panelist in ascending order of compound concentration, with sample presentation randomized within each series. Each series had one signal cup containing the compound-spiked serial dilution and two blank cups containing MCC. Each panelist had to choose a sample cup within each individual series in which they believed to have detected the signal compound; whether or not such a signal could be detected. A 2-min rest was enforced between each set of three samples. Responses were collected using paper ballots. The individual best estimate threshold (BET) was taken as the geometric mean of the last concentration with an incorrect response and the first concentration with a correct response except for the following sequence: if the subject indicated a "not sure" response for the correct choice, that concentration was increased by a factor of 1.41 to adjust for the possibility of a chance correct response (Lawless et al., 2000). The estimate group BET was taken as the geometric mean of the individual BET values.

Beverage Formulation

Formulations (15,000 g per batch) were calculated using the Microsoft Excel linear optimization solver function (Redmond, WA) as described by Misawa et al. (2016) and Cheng et al. (2018). MCC, SPI, and DI water were used as the raw ingredients. Other non-dairy ingredients in the beverage formulations were: 2.5 g carrageenan (Ticaloid® 780 Stabilizer, Tic Gums, Belcamp, MD) 60 g of cellulose gel (Ticaloid Pro HC 988®, Tic Gums, Belcamp, MD), 22.5 g dipotassium phosphate (CAS No. 7758-11-4, ICL Food Specialties, St. Louis, MO) for each 15,000 g batch of beverage. These were unsweetened, unflavored model beverage base formulations.

There were four beverage formulations replicated three times, starting with freshly produced MCC for each replicate. MCC was diluted to 6.3% TP (w/w) using DI water and measured using MIR (Lactoscope FTIR, Delta Instruments, Drachten, the Netherlands). The beverages were formulated at a fat content of 0.15% fat at 4 different levels of MDWP removal percentages (95.2%, 91.0%, 83.2%, and 69.2%, respectively). These MDWP removal percentages correspond to MCC purities (i.e., casein % of true protein) of approximately 93.8, 93.2, 92.2 90.2%, respectively. After combining all ingredients, formulations were homogenized using a 2-stage homogenizer at 60°C (model NS2006H, GEA Niro Soave, Parma Italy) with 20.7 MPa total pressure and 3.4 MPa on the second stage to ensure homogeneity for all formulations. Beverages were cooled to 4°C after homogenization and stored at 4°C until UP processing.

Beverage Processing

UP and Homogenization. For each replicate, 15,000 g of each of beverage formulation was well mixed individually and continuously fed to a Microthermics EHVH pasteurization unit (Microthermics, Raleigh, NC) at a flow rate of 1.4/min and then DSI UP processed: preheated to

90°C, pasteurized at 140°C for 2.3 sec under 33 kPa pressure with direct culinary steam injection (model LG-30, Electro-Steam Generator Corp., Alexandria, VA), cooling by vacuum chamber under 1,040 mmHg and homogenized by a 2-stage in-line homogenizer (GEA Niro Soavi, Parma, Italy) and cooled to 10°C. Formulations were homogenized both before and after DSI-UP to activate hydrocolloids and ensure homogeneity of the samples. DSI UP beverages were packaged in 473 mL high-density polyethylene (HDPE) dairy bottles (VWR®, Radnor, PA) under a clean fill hood (model EL422TT-ST, HEPA filter, 0.3 micron size filter) and stored at 4°C. Beverages were sampled for testing at d1 post-processing since Jo et al. (2018) established that sulfur volatile compounds diminish over time in refrigerated storage and the goal of this study required the formulations be evaluated at their highest possible volatile compound concentration.

Analysis Methods

Chemical Composition of Beverages. Milk based beverages and ingredients were analyzed in triplicate using the following analytical methods: total solids was analyzed by direct forced-air method (AOACI 2016, method number 990.20), fat by ether extraction (AOACI 2016, method number 989.05), lactose by an enzymatic method (AOACI, 2016; method number 2006.06), total nitrogen (TN) (AOACI 2016, method number 990.20), nonprotein nitrogen (NPN) (AOACI, 2016; method number 990.21), noncasein nitrogen (NCN) AOACI, 2016; method number 998.05). True protein (TP) was calculated as TN minus NPN multiplied by 6.38, CN was calculated as TN minus NCN multiplied by 6.38, and SP content was calculated by subtracting NPN from NCN and multiplying by 6.38 (Carter et al, 2018).

For monitoring, skim milk, micellar casein concentrate (**MCC**) and microfiltration (**MF**) permeate, SPI and UF permeate composition, (i.e., fat, protein, and lactose concentration g/100 g milk) during the MF and UF processing runs, samples were analyzed using a mid-infrared

(**MIR**) spectrophotometer (Lactoscope FTA, Delta Instruments, Drachten, Netherlands). The MIR milk product group was calibrated using modified milk samples produced at Cornell University as described by Kaylegian et al. (2006). The reference chemistry for the calibration samples was all lab mean reference chemistry for the modified milk calibration samples as described by Wojciehowski et al. (2016). A milk product testing group was set up on the Delta FTA using traditional virtual filter models for fat A, fat B, true protein and anhydrous lactose measures using during the run to monitor the process and ensure the processing was running properly. The wavelengths, scale factors (i.e., primary slope) and intercorrection factors for each virtual filter model were as described by Kaylegian et al. (2009).

Measurement of CS_2 and H_2S H_2S and CS_2 in MCC and beverages were measured using an Agilent 7890B gas chromatograph applied to an Agilent 7000C triple quad mass spectroscopy (GC-MS/MS) and sulfur selective flame photometric detector (FPD; Agilent Technologies Inc., Santa Clara, CA) equipped with a ZB-5ms column (30 m length x 0.25 mm i.d. x 0.25 µm film thickness; Phenomenex, Torrance, CA) as prescribed by Jo et al. (2019). Samples were introduced using a CTC Analytics CombiPal Autosampler (CTC Analytics, Zwingen, Switzerland). Five g of each formulation was weighed into a 20-mL solid-phase microextraction autosampler vial with a steel screw top containing silicone septa faced in Teflon (Microliter Analytical, Suwannee, GA) along with 20 µL of internal standard (ethyl methyl sulfide in ethyl ether at 1.65 mg/kg; Sigma-Aldrich). The analytical conditions and the multiple reaction monitoring transition for selected compounds were followed as described in Jo et al. (2019). Formulations were run in triplicate for each experiment replication. MassHunter Qualitative and Quantitative Analysis software (Agilent Technologies Inc.) were used for data analysis. The relative concentration of each compound was calculated based on the response ratio of each quantified ion to that of the internal standard.

Descriptive Analysis Descriptive analysis was conducted in accordance with the North Carolina State University Institutional Review Board for the Protection of Human Subjects in Research regulations. The milk protein beverages were evaluated for overall aroma and sulfur/eggy and cooked flavors (Jo et al., 2018, 2019) by 7 panelists (3 males, 4 females, ages 24-50 y) at d1 post processing. Each panelist had a minimum of 80 h of prior descriptive analysis experience documenting flavors of milks and dairy protein beverages using the Spectrum™ method with a 0 to 15 point intensity scale (Meilgaard et al., 2007). Thirty mL of each beverage was poured into 59-mL souffle cups, capped (Dart Container Corp.), and labeled with a randomized 3-digit blinding code. Samples were prepared with overhead lights off to prevent light oxidation. Beverages were evaluated at 15C. Panelists evaluated each treatment in duplicate. Data was collected on paper ballots.

Statistical Analysis The general linear models (**GLM**) procedure of SAS (version 9.4, SAS Institute Inc., Cary, NC) was used. The ANOVA model included category variables for MDWP removal (n=4) and replicate (n=3) for chemical analysis data and for sensory data category variables were MDWP removal (n=4), panelist (n=7) and replicate (n=3). Partial least squares regression was used to relate analytical instrument with DA sensory data and determine the capability of volatile sulfur compounds to predict sensory characteristics.

Results and Discussion

Beverage composition

The composition of the formulated beverages and their MCC purity levels are shown in Table 1. Data from the compositional analysis of the four formulated MCC-based beverages with varying levels of MCC purity (i.e. % MDWP removal) show that the formulated beverages

achieved a consistent true protein concentration across all formulations of the study design. Additionally, the beverages achieved the desired statistical difference in CN%TP concentrations (i.e. MCC purity levels) between each sample. Statistical analyses between the samples show that while the beverages are not statistically significant in regard to their crude and true protein values, the beverages are statistically significant in their CN%TP and MCC purity values- affirming that the beverages were properly prepared with MCC purity being the only difference in their composition and the only variable of difference.

Measured MCC purity (CN%TP) decreased as the MDWP removal decreased in unheated beverages (Table 1). Consistent with previous research, the results of this experiment demonstrate that MCC purity acts as a function of the % MDWP removed from the starting skim milk material: as MDWP is removed from skim milk through the microfiltration process, the purity of the MCC increases (Hurt et al., 2010; Zulweska et al., 2009; Cheng et al., 2019a). The measured MDWP concentration in the unheated beverages increased as MDWP removal decreased.

Measurement of MCC beverage purity before and after UP treatment

The measurement of MCC purity (CN%TP) was consistently different among beverages as measured before and after UP processing (Table 2). There was a statistical increase in the apparent MCC purity as determined by Kjeldahl of the same beverage when measured after UP as opposed to prior. Kjeldahl measurements were done following the established method described in Lynch et al. (1998) using the indirect method, which has been historically more accurate, as opposed to the direct casein determination method. Briefly, the total nitrogen (TN) of the sample and the nitrogen in the filtrate are determined using Kjeldahl anaylsis and subtracted from each other to indirectly determine the total casein of the sample, where filtrate is defined as the

remaining material after casein is precipitated and removed at pH 4.6. Whey proteins denature when exposed to excessively high temperatures such as UP, and then bind with κ-caseins through the formation of disulfide bonds (Jang and Swaisgood, 1989; Dalgleish, 1990, Sharma and Dalgleish, 1993; Walstra et al., 1999). This binding process makes measurement of the MCC purity or CN%TP falsely elevated by Kjeldahl analysis following UP heat treatment because the method is incapable of distinguishing between the two types of proteins in their bonded state. This indistinction will lead to an overestimation of the purity of the MCC by overestimating the amount of casein by mistakenly attributing the presence of bonded whey protein as casein. In place of Kjeldahl, a different type of measurement that can differentiate between bonded whey and casein proteins, such as HPLC or SDS-PAGE with a reducing agent, is likely needed after MCC-based products have undergone thermal processing for an accurate assessment of purity.

Best Estimate Thresholds

The orthonasal BET values for hydrogen sulfide and carbon disulfide in 95% MDWP reduced MCC were 33.4 $\pm$ 5.2 µg/kg and 172.8 $\pm$ 51 µg/kg, respectively. The orthonasal BET for these compounds is higher than what was previously reported in skim milk by Jo et al. (2019) (22.5 µg/kg for hydrogen sulfide and 35.2 µg/mg for carbon disulfide) presumably due to the higher protein concentration in MCC that may bind sulfur compounds and reduce their headspace concentrations (Hoffmann et al., 2001). Threshold values for these compounds have not been reported in 95% MDWP reduced MCC. Sensory thresholds can vary in different mediums, matrices, or methodologies (Leksrisompong et al., 2010). The comparison of an established BET for the compounds of interest with GC-MS/MS relative abundance values and trained panel profiling of beverages is critical to firmly establish the MCC purity range necessary to eliminate

sulfur off-flavors. Additionally, the current study elaborates the flavor and sensory perspective of sulfur/eggy flavors in milk-based products because the sensory threshold has been theorized to, potentially, be more significant than the concentration of the volatile compounds (Teranishi et al., 1991).

Trained Panel Profiling and Sulfur Volatile Analysis of Beverages

Trained panel profiling of the high protein beverages with varying MCC purity levels demonstrated that overall aroma, cooked flavor, and sulfur/eggy flavor increased with increasing residual MDWP levels (Table 3, Figure 1). These results mirror what has been achieved in previous studies. Vogel (2019) evaluated 6.3 and 10.5% protein beverages made with various protein blends (serum protein isolate, MCC, and MPC). The beverages made with a higher concentration of MDWP as a percentage of true protein had higher concentrations of sulfur/eggy flavor by descriptive analysis. The aforementioned study, amongst others, also demonstrated that sulfur/eggy flavor intensity decreased with storage time (Jo et al., 2018; Lee et al., 2017; Mehta, 1980) which is why the analyses in the current study were conducted within 24 h post UP processing. Because the only difference among the formulated beverages was the amount of MDWP present (i.e. the purity of the MCC), it is reasonable to conclude that the amount of MDWP in the MCC is a major contributing factor to the overall aroma and cooked flavor as well as the main contributing factor to sulfur/eggy flavor as also demonstrated by Jo et al. (2019) in reformulated milks with 3.2% TP. The intensity of sulfur/eggy flavor increased with decreasing percentage of MDWP removal, but there were no detectable sulfur/eggy flavor in beverages with 95% MDWP removal and beverages with 91% MDWP removal had only a trace of sulfur/eggy

flavor (0.6 intensity on a 0 to 15 point scale). As such, a MDWP removal of less than 91% will result in consistently detected sulfur/eggy flavors following UP heat treatment.

Congruent with data obtained from trained descriptive sensory analysis, higher MDWP removal resulted in relative abundance of hydrogen sulfide and carbon disulfide (Table 3, Figure 2). These results are also consistent with sensory BET values since it is reasonable to expect that trained panelists would be as or more sensitive than consumer BET values. Jo et al. (2019) demonstrated that these 2 compounds, hydrogen sulfide and carbon disulfide, were the main contributors to sulfur/eggy flavor and were derived from MDWP denaturation. Jo et al. (2019) demonstrated that 95% MDWP removal from 3.2% TP milk-based beverages resulted in no discernable sulfur/eggy flavor, consistent with the results of this study. Jo et al. (2019) also demonstrated that hydrogen sulfide was the primary contributor to sulfur/eggy flavor which is consistent with the instrumental and BET values from this study. However, the role of matrix on compound volatility and thus SPME instrumental analysis cannot be discounted. Carbon disulfide relative abundance values may be distinct in this study compared to Jo et al. (2019) due to lower volatility in the headspace. Regardless, our results establish a range of percent MDWP removal above which sulfur/eggy flavors would not be detected by the average consumer in a thermally processed MCC-based beverage: 91% MDWP removal (93.22% CN%TP). Vogel (2019) also demonstrated, using 5 different liquid milk protein blends: 100% MPC, 100% MCC, 50:50 SPI:MCC, 50:50 SPI:MPC and 18:82 SPI:MCC, that high protein beverages made with a blend of SPI (serum protein isolate or MDWP) and MCC exhibited higher concentrations of hydrogen sulfide and carbon disulfide when compared to beverages made with 95% MDWP MCC. They likewise concluded that higher levels of MDWP as a percentage of true protein were the main

contributing factor to higher concentrations of hydrogen sulfide and carbon disulfide and sulfur/eggy flavors.

Conclusions

The purity of MCC (CN%TP or % MDWP removal) has significant effects on the flavor profile of a heat treated product made with this novel dairy ingredient. Purity measurement by Kjeldahl must be done prior to UP heat treatment to avoid overestimation of MCC purity. Sensory, instrumental, and orthonasal threshold analysis results suggest that a purity level of 91% MDWP or higher (93.22 CN%TP or higher) will not have noticeable sulfur/eggy flavors to the average consumer. Increased concentration of MDWP as a percent of total protein resulted in increased sulfur/eggy flavor and higher concentrations of hydrogen sulfide and carbon disulfide in UP treated MCC-based beverages. These findings provide practical insight into the process parameters and purity levels necessary to eliminate sulfur/eggy flavors from MCC-based products produced under UP conditions.

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References:

ASTM (American Society for Testing and Materials). 2004. Standard practice for determination of odor and taste thresholds by a forced-choice ascending concentration series method of limits. E 679-04. ASTM, Philadelphia, PA.

AOAC. 2016. Official Methods of Analysis. 20th ed. Association of Official Analytical Chemists, Gaithersburg, MD.

Adams, M. C., E. E. Hurt, and D. M. Barbano. 2015. Effect of ceramic membrane channel geometry and uniform transmembrane pressure on limiting flux and serum protein removal during skim milk microfiltration. J. Dairy Sci. 98:7527–7543. http://dx.doi.org/10.3168/jds.2015-9753.

Beckman, S. L., J. Zulewska, M. Newbold, and D. M. Barbano. 2010. Production efficiency of micellar casein concentrate using polymeric spiral-wound microfiltration membranes. J. Dairy Sci. 93: 4506-4517.

Cheng, N., D.M. Barbano, and M.A. Drake. 2018. Hunter versus CIE color measurement systems for analysis of milk-based beverages. J. Dairy Sci. 75: S513-521.

Cheng, N., D. M. Barbano, and M. A. Drake. 2019a. Effect of pasteurization and fat, protein, casein to serum protein ratio, and milk temperature on milk beverage color and viscosity. J. Dairy Sci. 102: 2022-2043.

Cheng, N. D. M. Barbano, and M. A. Drake. 2019b. Effects of milk fat, casein and serum protein concentrations on sensory properties of milk based beverages. J Dairy Sci. 102:8670–8690.

Dalgleish, D. D. 1990. Denaturation and aggregation of serum proteins and caseins in heated milk. J. Agric. Food Chem. 38:11.

Hoffman, T., M. Czerny, S. Calligaris, and P. Schieberle. 2001. Model studies on the influence of coffee melanoindins on flavor volatiles of coffee beverages. J. Argic. Food Chem. 49:2382-2386.

Hurt, E., J. Zulewska, M. Newbold, and D. M. Barbano. 2010. Micellar casein concentrate production with a 3X, 3-stage uniform transmembrane pressure process at 50°C. J. Dairy Sci. 93: 5588-5600.

Hutton, J.T., and S. Patton. 1952. The origin of sulfhydryl groups in milk proteins and their contributions to "cooked" flavor. J. Dairy Sci. 35:699-705.

Jang, H. D., and H. E. Swaisgood. 1989. Disulfide bond formation between thermally denatured b-lactoglobulin and k-casein in casein micelles. J. Dairy Sci. 73:900-904.

Jo, Y., D. M. Benoist, D. M. Barbano, and M. A. Drake. 2018. Flavor and flavor chemistry differences among milks processed by high-temperature, short-time pasteurization or ultra-pasteurization. J. Dairy Sci. 101:3812-3828.

Jo, Y., B.G. Carter, D.M. Barbano, and M.A. Drake. 2019. Identification of the source volatile sulfur compounds produced in milk during thermal processing. J. Dairy Sci. 102:8658-8669.

Kaylegian, K. E., G. E. Houghton, J. M. Lynch, J. R. Fleming, and D. M. Barbano. 2006. Calibration of infrared milk analyzers: Modified milk versus producer milk. J. Dairy Sci. 89:2817–2832.

Kaylegian, K. E., J. M. Lynch, J. R. Fleming, and D. M. Barbano. 2009. Influence of fatty acid chain length and unsaturation on mid-infrared milk analysis. J. Dairy Sci. 92:2485–2501. doi:10.3168/jds.2008-1910.

Lawless, H., C. Harono, and S. Hernandez. 2000. Thresholds and supra-threshold intensity functions for capsaicin in oil and aqueous based carriers. J. Sens. Stud. 15:437-447.

Lee, A. P., D. M. Barbano, and M. A. Drake. 2017. The influence of ultra-pasteurization by indirect heating versus direct steam injection on skim and 2% fat milks. J. Dairy Sci.100: 1688-1701.

Leksrisompong, P., D.M. Barbano, A.E. Foegeding, P. Gerald, and M.A. Drake. 2010. The roles of fat and pH on the detection thresholds and partition coefficients of three compounds: diacetyl, gamma-dacalactone, and furaneol. J. Sens. Stud. 25:347-370.

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

Meilgaard, M., G.V. Civille, B.T. Carr. 2007. Descriptive analysis techniques, Chapter 10. In: Sensory Evaluation Techniques, 4th ed. Boca Raton, Fla.: CRC Press.

Mehta, R.S. 1980. Milk processed at ultra-high-temperatures—a review. J. Food Prot. 43:212-225.

Misawa, N., D. M. Barbano, and M. A. Drake. 2016. Influence of casein as a percentage of true protein and protein level on color and texture of milks containing 1 and 2% fat. J. Dairy Sci. 99:5284–5304.

Sharma-Shri, K., and D. G. Dalgleish. 1993. Interactions between milk serum proteins and synthetic fat globule membrane during heating of homogenized whole milk. J. Agric. Food Chem. 41:1407-1412.

Teranishi, R., R.G. Buttery, D.J. Stern, and G. Takeoka. 1991. Use of odor thresholds in aroma research. Lebensm. Wiss. Technol. 24:1-5.

Vogel, K.G. III. 2019. Effect of protein amount and milk protein ingredients on sensory properties and physicochemical properties of ready-to-drink protein beverages. Master's thesis, North Carolina State University, Raleigh, NC.

Walstra, P., T.J. Geurts, A. Noomen, A. Jallema, and M.A.J.S. Van Boekel. 1999. 2.4.1.5 Denaturation. Page 77 in Dairy Technol: Principles of Milk Properties and Processes. Marcel Dekker Inc., New York, NY.

Wojciechowski, K. L., C. Melilli, and D. M. Barbano. 2016. A proficiency test system to improve performance of milk analysis methods and produce reference values for component calibration samples for infrared milk analysis. J. Dairy Sci. 99:6808–6827. http://dx.doi.org/10.3168/jds.2016-10936

Zhang, Y., and C.-T. Ho. 1991. Comparison of the volatile compounds formed from the thermal reaction of glucose with cysteine and glutathione. J. Agric. Food Chem. 39:760-763.

Zulewska, J., and D. M. Barbano. 2014. The impact of linear velocity and flux on performance of ceramic graded permeability membranes when processing skim milk at 50°C. J. Dairy Sci. 97:2619– 2632.

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

Table 2.1 Mean (n=3) beverage composition with differing MCC purity percentages at 6.3% true protein formulated to 15 g per 240 mL serving

% MDWP removal	CP[1] (TN x 6.38)	NPN[2] (NPN x6.38)	True Protein(TP) (TN-NPN)	CN (TN-NCN)[3]	MCC Purity (CN%TP)[3]	Residual MDWP%[3]
95.20	6.57[a]	0.04[a]	6.53[a]	6.15[a]	93.83[a]	0.40[d]
91.00	6.54[a]	0.05[a]	6.49[a]	6.08[b]	93.22[b]	0.44[c]
83.30	6.54[a]	0.04[a]	6.49[a]	6.01[c]	92.18[c]	0.51[b]
69.20	6.49[a]	0.05[a]	6.53[a]	5.84[d]	90.27[d]	0.63[a]
Standard Error	0.03	0	0.03	0.03	0.19	0.01
R^2	0.94	0.55	0.94	0.96	0.97	0.97

[a-d]Means in the same column followed by a different superscript differ ($P < 0.05$).
[1]CP = crude protein
[2]NPN = Non-protein nitrogen
[3]Corrected for pasteurization denaturation

Table 2.2 Mean (n=3) kjeldahl purity measurement before and after UHT processing of differing MCC purity percentages at 6.3% total protein formulated to 15g per 240 mL serving

%MDWP removal	MCC Purity (CN%TP) before UP[1]	MCC purity (CN%TP) after UP[1]	Apparent increase in MCC purity
95.20	93.83[a]	95.18[a]	1.35[d]
91.00	93.22[b]	94.76[a]	1.54[c]
83.30	92.18[c]	94.69[a]	2.51[b]
69.20	90.27[d]	94.36[a]	4.09[a]
Standard Error	0.194		
R^2	0.97		

[a-d]Means in the same column followed by a different superscript differ ($P < 0.05$).
[1]Corrected for pasteurization denaturation

Table 2.3 Mean (n = 3) sensory/volatile compound/MDWP concentrations for MCC-based beverages at 6.3% TP at varying MDWP removal percentages, formulated to 15 g per 240 mL serving at d 1 postprocessing

	Sensory Attribute[1]			Volatile Sulfur Compound[2] (µg/kg)		
MDWP %	Overall Aroma	Cooked	Sulfur/Eggy	Hydrogen Sulfide	Carbon Disulfide	Residual MDWP %[4]
95.20	1.8[d]	3.7[d]	ND[3]	9.27[d]	33.28[d]	0.40[d]
91.00	2.2[c]	4.0[c]	0.7[c]	21.88[c]	58.52[c]	0.44[c]
83.30	2.6[b]	4.2[b]	1.3[b]	35.37[b]	100.74[b]	0.51[b]
69.20	4.4[a]	4.4[a]	2.2[a]	58.65[a]	148.81[a]	0.63[a]
Standard Error	0.04	0.02	0.03	1.43	7.35	0.01
R^2	0.89	0.90	0.97	0.94	0.78	0.97

[a-d]Means in the same column followed by a different superscript differ ($P < 0.05$).
[1]Attribute intensities were scored on a 0- to 15-point universal intensity scale (Meilgaard et al., 2007).
[2]Relative mean concentration in triplicate (µg/kg).
[3]ND = Not detected.
[4]Corrected for pasteurization denaturation

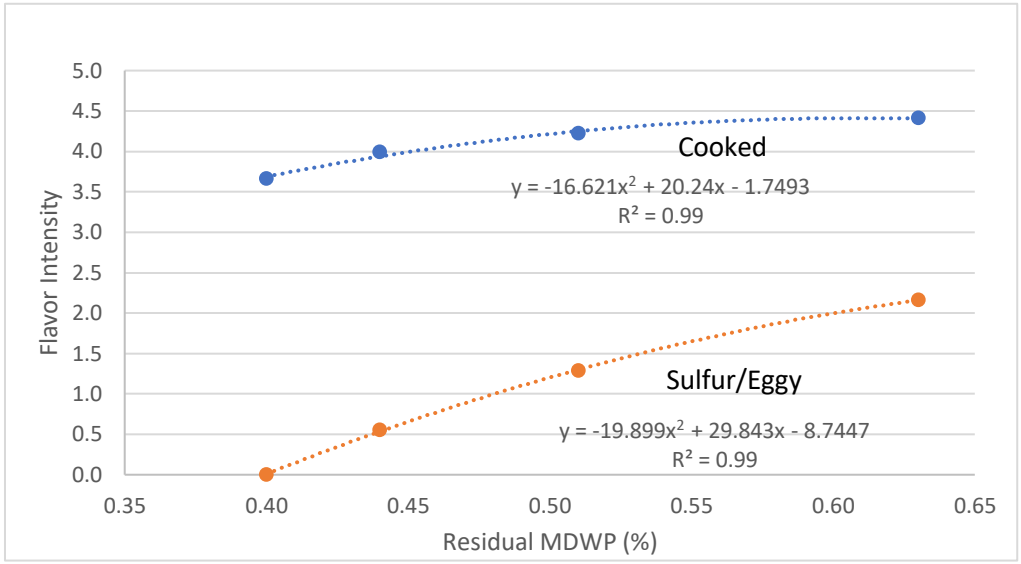

Figure 2.1 Mean (n=3) sensory descriptive analysis flavor intensity scores for cooked and sulfur/eggy flavors in MCC-based beverages based on residual MDWP % at 6.3% total protein, formulated to 15 g per 240 mL serving.

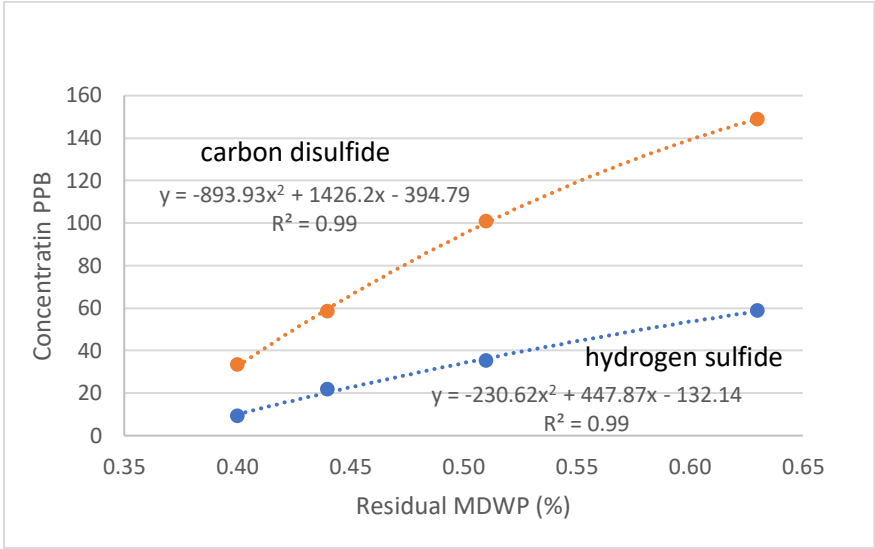

Figure 2.2 Mean (n=3) relative abundance (µg/kg) of hydrogen sulfide and carbon disulfide in MCC-based beverages based on residual MDWP % at 6.3% total protein, formulated to 15 g per 240 mL serving.

CHAPTER 3: THE EFFECT OF FAT CONTENT ON SULFUR/EGGY FLAVOR IN ULTRA-PASTEURIZED MILK PROTEIN-BASED BEVERAGES

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Abstract

The purity of micellar casein concentrate (MCC) is defined in one of two ways: the percent milk derived whey protein (% MDWP) removed from skim milk or the casein as a percentage of true protein (CN%TP) as measured in the final MCC. The purity of MCC can impact flavor in thermally processed products made with MCC because MDWP denature and form volatile sulfur off-flavors. The objectives of this study were to determine the role that fat content has on sulfur/eggy flavor in ultrapasteurized (UP) MCC beverages with varying levels of MCC purity and to determine if SDS-PAGE could be used to accurately determine MCC purity following UP heat treatment. Nine model beverages were prepared with freshly produced 95% MDWP reduced MCC, serum protein isolate, anhydrous milk fat, carrageenan, cellulose gel, and dipotassium phosphate following a factorial arrangement of treatments: 3 levels of MCC purity (% MDWP removal 95.2%, 83.3%, 69.2%) and 3 levels of fat content (0.1%, 1.0%, 2.0%). Formulated beverages were thermally processed under direct steam injection (DSI) UP (140°C for 3 sec), bottled, and cooled to 4°C. Residual MDWP and MCC purity were measured by both Kjeldahl and SDS-PAGE prior to and following UP. At d-1 post-processing, the model beverages were analyzed by a trained sensory panel and by triple quadrupole GC-MC/MS for sulfur volatiles.. Measurement of MCC purity for each beverage by Kjeldahl analysis increased ($p<0.05$) following UP treatment, measurements by SDS-PAGE before and after UP had no statistical increase ($p>0.05$). Percent MDWP removal had a greater impact on sulfur/eggy flavor and relative abundance of hydrogen sulfide and carbon disulfide following UP than fat content, but both parameters impacted beverage flavor by sensory and instrumental analysis ($p<0.05$). The presence of fat decreased sensory intensity scores for sulfur/eggy flavor and relative abundance of hydrogen sulfide and carbon disulfide compared to beverages with similar % MDWP removal

but no fat. Fat can be added to milk protein beverages to reduced sensory perception of undesirable sulfur/eggy flavors.

Key Words: sulfur/eggy flavor, milk protein-based beverage, micellar casein, milk derived whey protein, milkfat

Introduction

The nutritive value, high functionality, and clean label potential of milk protein/dairy protein has led to a spur of innovation for dairy protein ingredients. Whey protein isolate (WPI) and whey protein concentrate (WPC) are proteins concentrated from fluid cheese whey or directly from skim milk (Carter et al., 2020). When these proteins are removed directly from milk in place of traditional cheese whey, they are called milk derived whey protein (MDWP). Milk protein concentrate (MPC) and milk protein isolate (MPI) are derived from fluid milk, and these products are concentrated milk protein and casein and whey protein in the natural ratio found in fluid milk (20:80 whey:casein protein) (Hoffmann and Falvo, 2004; Rutherford et al., 2015; Queiros et al., 2020; Carter et al., 2020). Micellar casein concentrate (most commonly produced in a concentrate form and hereafter termed MCC) is another dairy protein ingredient underutilized potential within the dairy industry. MCC is produced by microfiltration of skim milk such that the casein portion of the milk protein is concentrated. As such, MCC distinguished from MPC/MPI in that MCC has a higher proportion of casein to whey protein (Carter et al., 2020).

MCC is manufactured through the microfiltration of skim milk using either a ceramic or polymeric membrane (Zulewska et al., 2009). Ceramic membranes are more efficient at removing MDWP from skim milk than their polymeric counterparts under similar conditions, and, despite their overhead cost, ceramic membranes have a higher chemical and thermal stability, operate at a higher limiting flux, and have a long usability life. (Zulewska and Barbano, 2014). The previously cited study concluded that at 50°C and with 3 stages of filtration (2 of which included diafiltration), it was possible for a ceramic membrane to reach a purity level of 95% MDWP reduced while a polymeric membrane was only capable of achieving 70% MDWP

reduced. Beckman et al. (2010) demonstrated that 95% MDWP removal was possible using a polymeric membrane if the process was carried out to 8 stage of filtration (7 of which include diafiltration).

The compositional and flavor profile of MCC (or MCC-based products) that are thermally processed is dependent on MCC purity because the residual MDWP is the main contributor to sulfur/eggy flavor in UP processed milk-based protein products (Lee et al., 2017; Jo et al., 2019; Whitt et al., 2020). The purity of MCC is defined in one of two ways: percentage of the MDWP removed from skim milk through the microfiltration process or the casein as a percentage of true protein (CN%TP) in MCC. When exposed to high temperatures (such as during UP processing or spray drying), MDWP denature and the sulfur containing amino acids cysteine/cystine are released, which degrade to hydrogen sulfide and carbon disulfide through Strecker degradation (Zhang and Ho, 1991).

Whitt et al. (2020) established a workable MCC purity range, above which sulfur/eggy flavors would not be detected by the average consumer, 91% MDWP reduced (93.22% CN%TP), in 6.5% protein model beverages. However, research has shown that fat in dairy products can mask volatile compound flavors and/or modulate sensory perception (Christensen and Reineccius, 1992; Hatchwell, 1996; McCarthy et al., 2017; Cheng et al., 2019a, 2019b). Zheng et al. (1997) reported that measured vanillin concentrations decreased as the fat content increased in vanilla ice creams and that there were concurrent differences by sensory analysis as well, demonstrating that fat modulated perception of vanilla flavor. Whitt et al. (2020) recently demonstrated that following thermal processing of MCC beverages, denatured MDWP were bound to κ-caseins and were unable to be accurately measured by Kjeldahl analysis because the MDWP were mistakenly accounted for as casein, falsely elevating the purity of the MCC. Other

methods of protein analysis may provide more accurate purity measurements post-thermal processing, such as SDS-PAGE. The objectives of this study were twofold: to determine the role of fat content on sulfur/eggy flavor in protein beverages and to determine if SDS PAGE could be used to accurately determine MCC purity following UP heat treatment.

Materials and Methods

Experimental Design Overview

MCC was produced on three separate occasions from skim milk to a purity level of 95% MDWP reduced by using a ceramic MF process at 50 ^{o}C following a 3X, 3-stage, bleed-and-feed, process as prescribed in Cheng et al. (2018) and Zulewska and Barbano (2014). A ceramic membrane with a 0.1 µm pore size and graded permeability was used. For each experimental replication of MCC, nine MCC-based beverages were formulated at 15 g protein per 240 mL serving (i.e. about 6.3% (w/w) true protein) following a 3 x 3 factorial arrangement: 3 fat levels (0%, 1%, 2%) and 3 different MCC purity levels (95.2%, 83.2%, and 69.2% MDWP reduced). The MDWP removal percentages corresponded CN%TP of 93.8%, 92.2%, and 90.2%, respectively. Additionally, the percent MDWP removal also aligned with g of MDWP/100g of protein of 0.39g, 0.50 g, and 0.60 g, respectively. The experiment was designed to determine the role of fat content on sulfur-eggy flavors in UP MCC-based beverages at different levels of MCC purity. The experiment was replicated three times with different lots of milk. Each experimental replication consisted of two production days where MCC was manufactured from skim milk on d 1 and beverages were formulated and thermally processed on d 2.

Milk Based Ingredients

Raw Skim Milk Raw bovine skim milk (0.087% ± 0.03, SSC < 300,000) was secured from the North Carolina State University Dairy Enterprise System (Raleigh, NC) on the morning of each MCC processing run. Raw skim milk was collected into a glycol-chilled, jacketed storage tank, which was cleaned and sanitized immediately prior to milk receipt, and then held at 4°C until use (ca 2 h).

Micellar Casein Concentrate (MCC) Liquid MCC was made on d 1 of processing and made fresh each day before beverage formulation as described by Whitt et al. (2020). Briefly, raw skim milk (570 kg) was prefiltered at 4°C using a Nexis T-filter (NXT 10-30U-M7S, Pall Corp., Port Washington, N.Y.) and then HTST pasteurized (720 kg/h) with a plate heat exchanger (model T4 RGS- 16/2, SPX Flow Technology, Greensboro, N.C.) at 72°C for 16 s. De-ionized (DI) water was used to flush out the storage acid solution from the microfiltration apparatus system; after which, ~30 kg of the pre-filtered, HTST pasteurized skim milk was used to flush out the DI water from the ceramic microfiltration membrane system before collection began. MCC was processed following the process described by Zulewska and Barbano (2014), which followed a 3 stage, 3X, bleed-and-feed, ceramic microfiltration process. A final MCC product of 95% MDWP reduced and a true protein concentration between 7.3 to 7.5% was produced using a pilot scale MF system (Tetra Alcross MFS-7, TetraPak Filtration Systems) equipped with a 0.1-µm nominal pore diameter graded permeability ceramic Membralox (model EP1940GL0.1u, AGP1020, alumina, Pall Corp.) membrane. After filtration and confirmation of MDWP reduction using MIR (Lactoscope FTIR, Delta Instruments, Drachten, the Netherlands), the 95% MDWP reduced, ~7.3% TP (w/w) MCC was cooled in milk cans to 8°C using a glycol-chilled water bath immediately post-processing, and then stored at 4°C in a walk-in cooler until use (ca 12 h). The

MCC was used as a fresh liquid ingredient in beverage formulation the following day (d 2 processing) for each experimental replication.

Serum Protein Isolate (SPI) SPI was produced in a large batch at South Dakota State University (Brookings, SD) (Whitt et al., 2020). Briefly, SPI was produced by concentrating the MF permeate obtained from the production of MCC using ultra-filtration (UF) to a high protein (about 25% w/w) SPI, and then the concentrate was packaged into half gallon plastic jugs (Upstate Niagara Cooperative, Buffalo, N.Y.), frozen, and shipped overnight to N.C. State University where it was held frozen (-20°C) until used in the beverage study. Three days before d 2 of processing (beverage formulation and thermal processing), the frozen SPI containers were placed in a 4°C cooler to thaw prior to beverage formulation.

Beverage Formulation

Beverage formulations (15,000 g per batch) were calculated using the Microsoft Excel linear optimization solver function (Redmond, WA) as described by Misawa et al. (2016) and Cheng et al. (2018). Ninety five percent MDWP reduced, 6.3% TP MCC, SPI, DI water, and anhydrous milk fat (AMF) (Dairy Farmers of America, Kansas City, KS) were used as the raw ingredients. Other non-dairy ingredients in the beverage formulations for each 15,0000 g batch were: 2.5 g carrageenan (Ticaloid® 780 Stabilizer, Tic Gums, Belcamp, MD), 60 g of cellulose gel (Ticaloid Pro HC 988®, Tic Gums, Belcamp, MD), and 22.5 g dipotassium phosphate (CAS No. 7758-11-4, ICL Food Specialties, St. Louis, MO). All the beverage formulations were unsweetened, unflavored model beverage bases.

Nine beverage formulations were replicated three times, starting with freshly produced MCC from d 1 processing for each replicate. The MCC was diluted with DI water to a final protein concentration of 6.3% TP (w/w), which was measured and confirmed using MIR

(Lactoscope FTIR). The beverages were formulated using a factorial arrangement of 3 added fat levels (0 added (0.15% fat overall), 1%, and 2% added AMF) and 3 different MCC purity levels (95.2%, 83.2%, and 69.2% MDWP removed). The percent MDWP reduced values corresponded to the following values for CN%TP, respectively: 93.8%, 92.2%, and 90.2%. AMF was (stored at 4°C) was melted on the day of processing using a 70°C water bath. Beverages were formulated at 50°C in a 50°C waterbath by slowly mixing in each ingredient using an immersion blender until homogenous. The liquid AMF (50°C) was then mixed into the beverage formulations, which remained inside the 50°C waterbath. After all the ingredients had been added, the beverage formulations were removed from the waterbath and homogenized using a 2-stage homogenizer at 60°C (model NS2006H, GEA, Niro Soave, Parma, Italy) with 20.7 MPa total pressure and 3.4 MPa on the second stage to ensure homogeneity of all formulations. Beverages were then cooled to 4°C until UP processing (ca 2 h).

Beverage Processing

UP and Homogenization For each experimental replication, 15,000 g of each homogenized beverage formulation was hand mixed individually with a hand immersion blender and then continuously fed through a Microthermics EHVH pasteurization unit (Microthermics, Raleigh, N.C.) at a flow rate of 1.4 g/min and then processed under DSI-UP conditions: preheated to 90°C, pasteurized at 140°C for 2.3 s under 33 kPa of pressure with direct culinary steam injection (model LG-30, Electro-Steam Generator Corp., Alexandria, V.A.), cooled under 1,040 mmHg of pressure by a vacuum chamber, and homogenized by a 2-stage in-line homogenizer—20.7 MPa total pressure and 3.4 MPa on the second stage (GEA Niro Soavi) (Whitt et al., 2020). Beverages were packaged under a clean fill hood (model EL422TT-ST, HEPA filter, 0.3 micron size filter) into 473 mL high-density polyethylene (HDPE) dairy bottles (VWR®, Radnor, PA)

and stored at 4°C in a walk-in cooler until analysis (ca 24 h). Beverage were evaluated at d 1 post-processing following conclusions drawn by Jo et al. (2018): sulfur volatile compounds diminish over time in refrigerated storage. The objectives of this study required that the beverage formulations be evaluated at their highest possible volatile sulfur compound concentration.

Analysis Methods

Chemical Composition of Beverages MCC-based beverage formulations and ingredients were analyzed in triplicate using a variety of analytical testing methods. The tests performed: fat by ether extraction (AOACI 2016, method number 989.05), total nitrogen (TN) (AOACI 2016, method number 990.20), total solids was analyzed by direct forced-air method (AOACI 2016, method number 990.20), lactose by an enzymatic method (AOACI, 2016; method number 2006.06), nonprotein nitrogen (NPN) (AOACI, 2016; method number 990.21), noncasein nitrogen (NCN) AOACI, 2016; method number 998.05). True protein (TP) was calculated as TN minus NPN multiplied by 6.38, MDWP content was calculated by subtracting NPN from NCN and multiplying by 6.38, and CN was calculated as TN minus NCN multiplied by 6.38, (Carter et al, 2018). During the production of milk protein ingredients, the following ingredients were monitored on a consistent basis by mid-infrared (MIR) spectrophotometry (Lactoscope FTA, Delta Instruments, Drachten, Netherlands): the skim milk using for MCC production, MF retentate and permeate, MCC for beverage formulations, UF permeate and retentate composition (i.e., fat, protein, and lactose concentration g/100 g milk). The MIR milk product group was calibrated prior to ingredient testing as described by Kaylegian et al. (2006).

Measurement of CS_2 and H_2S The relative abundance of H_2S and CS_2 was measured the beverage formulations as outlined by Jo et al. (2019) and modified by Whitt et al. (2020). The

two sulfur compounds were extracted by SPME followed by gas chromatography triple quadrupole mass spectrometry using an Agilent 7890B GC applied to an Agilent 7000C triple quad mass spectrophotometer (GC-MC/MS) and sulfur selective flame photometric detector (FPD; Agilent Technologies Inc., Santa Clara, CA) equipped with a ZB-5ms column (30 m length x 0.25 mm i.d. x 0.25 µm film thickness; Phenomenex, Torrance, CA). Following procedures reported by Whitt et al. (2020), five g of each beverage formulation and 20 µL of internal standard (ethyl methyl sulfide in ethyl ether at 1.65 mg/kg; Sigma-Aldrich) were weighed into a 20-mL amber glass autosampler vial with a steel screw top containing silicone septa faced in Teflon (Microliter Analytical, Suwannee, GA). The analytical conditions and the multiple reaction monitoring transition for the selected compounds were followed as prescribed in Jo et al. (2019). Each beverage formulation from each experimental replication was evaluated in triplicate. Data analysis was done using MassHunter Qualitative and Quantitative Analysis software (Agilent Technologies Inc.). The relative abundance calculation of each compound for each beverage formulation was based on the response ratio of each quantified ion to that of the internal standard.

Sensory Descriptive Analysis Sensory descriptive analysis was conducted as described by Whitt et al. (2020) and in accordance with the North Carolina State University Institutional Review Board for the Protection of Human Subjects in Research regulations. The nine MCC-based beverage formulations were for overall aroma, sulfur/eggy, cooked, and milkfat flavor by 7 panelists (3 males, 4 females, ages 24-50 y) at d 1 post processing. Each panelist had a minimum of 80 h of experience evaluating flavors of milk and dairy protein beverages using the Spectrum™ descriptive analysis method with a 0 to 15 point universal intensity scale (Meilgaard

et al., 2007). Samples were prepared by pouring 30 mL of each beverage into a 59 mL souffle cup, which was then lidded (Dart Container Corp.), and labeled with a randomized 3-digit blinding code. Samples were prepared with overhead lights turned off to prevent light oxidation. Beverages were tempered to 15°C for evaluation. Data was collected on paper ballots.

SDS-PAGE Analysis The polyacrylamide gels used to determine the trace quantity (mm x OD) of all protein bands (i.e., intact milk casein, casein proteolysis products, and milk serum protein), were made following the procedure described by Verdi et al. (1987) using the gel unit Protean™ II and the power unit 3000/300 from Bio-Rad (Bio-Rad Laboratories Inc., Hercules, CA) gel electrophoresis system. The preparation of the samples for SDS-PAGE was done by diluting each milk with 0.9 g of a buffer that consisted of 10 mM Tris-HCl, 1.0% SDS, 20% glycerol, 0.02% bromophenol blue tracking dye. Samples were prepared with, and without 45 mM dithiothreitol (**DTT**). DTT disrupts intermolecular disulfide bonds that can be produced as a result of exposing milk serum proteins to high heat in the presence of casein micelles during direct steam injection pasteurization.

The weight of milk used for the dilution was calculated based on Kjeldahl TP content of the milk sample to achieve a loading of approximately 28 μg of true protein loaded per slot. Each milk plus buffer mixture was placed in a sealed glass vial (Target DP Vials C4000-1W, National Scientific Co., Rockwood, TN), heated to 100°C using steam, held at 100°C for 3 min and stored frozen at -80°C. On the day of the SDS-PAGE run, diluted milks were thawed using steam heated to 100°C for 3 min, and then cooled to about 25°C. Each milk plus buffer mixture was loaded into a different well of the gel. The volume loaded was chosen to achieve a maximum peak OD in the range of 1.0 to 1.4 for the most intense protein band [i.e., α_s-caseins (**α_s-CN**)] in the sample to

avoid overloading and nonlinear response in quantitation. One raw milk was used as a marker in one lane of each gel for evaluation of loading consistency and comparison of resolution of the proteins from gel-to-gel. Three SDS-PAGE runs were performed, each with 2 gels. All gels were scanned with USB GS 800 Densitometer (Bio-Rad Laboratories Inc., Hercules, CA) and quantified using Quantity 1 1-D Analysis software. For quantitative analysis, a center line for each lane on each gel was created during the scanning of the gel. The lines were adjusted individually to match the middle section of the bands in each lane. Next, each lane background was adjusted using the rolling disk method of subtraction to obtain a straight baseline for each lane. The detection of bands was done by setting the bracket width of the bands and then by adjusting each band in each lane by moving the brackets up or down based on visual observation.

Statistical Analysis A two way generally linear model (GLM) procedure was used (SAS, version 9.4, SAS Institute Inc., Cary, N.C.). The ANOVA model included category variables for fat level (n =3 levels), and percent MDWP removal (n=3 levels). Replicate (n=3) for chemical and sensory data were also included in the model.

Results and Discussion

Beverage Composition

The composition of the nine formulated beverages and their MCC purity levels are shown in Table 1. The compositional analysis data of the nine formulated beverages show that the crude (i.e. total) protein (CP) values were statistically equivalent, but casein (CN), MCC purity (CN%TP), and residual MDWP content were distinct within a fat content. The beverages were formulated as expected and the only statistically significant difference among beverages within

the same fat content category was MCC purity. Additionally, MCC purity across the different fat content categories were similar according to their percent MDWP class (i.e. 95% MDWP reduced samples were all similar across 0.1%, 1%, 2% fat content categories). Measured MCC purity decreased across similar fat content categories as the MDWP removal decreased in unheated beverages. These results are consistent with previous research that MCC purity acts as a function of % MDWP removal (Carter et al., 2020; Whitt et al., 2020).

Measurement of MCC Beverage Purity Before and After UP Treatment

Kjeldahl Measurements Consistent with that of previous research, MCC purity cannot be accurately measured by Kjeldahl analysis post-thermal processing (Table 2, Whitt et al., 2020). Analyses done using Kjeldahl on the nine formulated MCC-based beverages show that there is a statistically significant increase in the MCC purity of the same beverage when analyzed before and after UP processing. When exposed to high temperatures, whey proteins will denature and form disulfide bonds with κ-caseins (Jang and Swaisgood, 1989; Dalgleish, 1990, Sharma and Dalgleish, 1993; Walstra et al., 1999; Whitt et al., 2020). The bonding between κ-caseins and whey proteins makes the measurement of MCC purity by Kjeldahl inaccurate following high heat treatment processes because the method is incapable of distinguishing between the two types of proteins in their bonded state. Consequently, the inability for this analysis to distinguish between the bonded proteins will lead to an overestimation of the amount of casein due to the mistaken attribution of bonded whey protein as casein.

SDS-PAGE In contrast to Kjeldahl, measurements of MCC purity before and after UP by SDS-PAGE were not different ($P>0.05$, Figure 1, Table 3). During sample preparation for SDS-

PAGE, samples are heated and dithiothreitol is added to denature disulfide bonds to eliminate secondary protein structure. As anticipated, whey proteins bonded to casein are disassociated by this process and residual MDWP is distinguished from casein with or without previous UP processing. The data from this measurement confirmed that SDS-PAGE is a viable method for determining the purity of MCC after a thermal process. Similar results were demonstrated in a study conducted by Jovanovic et al. (2007) who evaluated skim milk subjected to varying temperatures (75°C, 80°C, and 90°C for 20 minutes) followed by SDS-PAGE with and without reducing agent (β mercaptoethanol) to evaluate the milk composition post-thermal processing. They also demonstrated that SDS-PAGE with a reducing agent was capable of separating bonded whey proteins from casein to measure the two proteins individually.

Trained Panel Profiling of Beverages

Overall aroma intensity increased with decreased % MDWP removal within the same fat content level (Table 4). Cooked/milky flavor was not different among beverages ($P>0.05$), and perceived milkfat flavor increased as expected with added milkfat. Percent MDWP removal and fat content impacted sensory perception of sulfur/eggy flavor ($p<0.05$). Sulfur/eggy flavor was not detected in beverages with 95% MDWP at any fat content level. At MDWP reductions of 83.3% and 69.2% MDWP, sulfur/eggy flavor increased accordingly but was modulated by the presence of milkfat. Beverages with 2% added milkfat at 69.2% MDWP had lower sulfur/eggy flavor than 1% milkfat 69.2% MDWP beverages and sulfur/eggy flavor in these beverages was lower than that of 0.1% milkfat 69.2% MDWP beverages. The presence of fat had a masking effect on the sensory detection of sulfur/eggy flavor in UP processed MCC-based high protein beverages. Fat solubilizes sulfur volatile compounds and limits their concentration in the

headspace, which may lead to lower detection by sensory analysis. In contrast, in the absence of fat, vapor pressure of volatile sulfur compounds can increase (Hatchwell, 1996), leading to a higher intensity and higher scores by the trained sensory panel. Previous studies with dairy products have also documented the impact of fat on sensory perception. Chocolate flavor intensity was higher in lower fat ice creams compared to those with higher fat content (Prindiville et al., 1999) while Zheng et al. (1999) documented temporal differences in vanillin flavor with differences in fat content of vanilla ice cream. Drake et al. (2010) demonstrated that fat and its impact on sensory thresholds and subsequently flavor was the primary source of flavor differences between full fat and reduced fat cheese rather than a difference in specific volatile compounds.

Sulfur Volatile Analysis of Beverages

Consistent with sensory results, both % MDWP removal and fat content impacted relative abundance of hydrogen sulfide and carbon disulfide (Figures 2, 3). Percent MDWP removal had a greater impact than fat content on hydrogen sulfide and carbon disulfide relative abundance. The relative abundance of both volatile sulfur compounds increased with decreased % MDWP removal. In the presence of fat, both sulfur compounds decreased although this decrease was not as large as the impact of % MDWP removal. Past research has shown that fat binds with fat-soluble volatile compounds and limits their relative abundance in the headspace, which leads to lower detection by gas chromatography (Hatchwell, 1996). Lowering the concentrations of sulfur volatiles in the headspace by increasing the fat content will lead to a decreased intensity of perceived sulfur flavor as demonstrated by the sensory results in this study. Keršiene et al. (2008) made model custards from skim or full fat milk powder in an effort to evaluate the effect that milk fat on the flavor and rheological properties of model deserts. They

concluded similar findings in that an increased amount of fat resulted in a significant decrease in the headspace concentration of volatile flavor compounds through hydrophobic interactions. Solubility appears to be a likely reason for decreased sulfur volatiles when fat is added, but it is possible that another mechanism is responsible or plays a role. The process of homogenization stabilizes suspension of fat globules by decreasing globule size and by shearing proteins onto the surface of the globules to assist with stabilization. Perhaps the shearing of proteins onto the fat globule surface binds MDWP and prevents oxidation of cysteine/cysteine.

Previous work by Whitt et al. (2020) established the role of MCC purity on the relative abundance of volatile sulfur compounds. The lower the percent MDWP removal (i.e. the lower the MCC purity), the higher the relative abundance of hydrogen sulfide and carbon disulfide in the MCC or MCC product following UP. In the previous study, Whitt et al. (2020) concluded that a minimum of 91% MDWP reduction was necessary to reduce volatile sulfur compounds to a level that would not be detected by consumers following UP. In the current study, the role of percent MDWP removal on volatile sulfur compounds and sulfur/eggy flavor was also observed. However, the presence of fat modulated the headspace concentration of these compounds and sensory perception. These data suggest that a lower purity MCC could be utilized in a product if fat is added to help modulate the undesirable sulfur-eggy off flavors. For example, data from this study suggests that 2% fat content in a 6.3% protein MCC-based beverage would lower the detectable sulfur-eggy off flavor to be at or below the detectable threshold limit of the average consumer using an 83% MDWP reduced MCC as opposed to 91% MDWP if no fat was added (Whitt et al., 2020).

Conclusions

SDS-PAGE is a viable method to determine the purity of an MCC-based product after a high heat treatment process, like UP. The fat content of a high protein beverage produced with MCC has impacts on sensory and instrumental analysis of sulfur/eggy flavor; however, this impact is less than the impact of % MDWP removal from MCC on sulfur/eggy flavor. These results provide valuable insight into the practical application of using MCC in a flavored beverage for manufactures by providing additional insight to reduce/eliminate sulfur/eggy flavor using a lower purity MCC in high protein MCC-based beverages processed under UP conditions.

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References

Agarwal, S., R.L.W. Beausire, S. Patel, and H. Patel. 2015. Innovative uses of milk protein concentrates in product development. J. Food Sci. 80:A23-A29.

Beckman, S. L., J. Zulewska, M. Newbold, and D.M. Barbano. 2010. Production of efficiency of micellar casein concentrate using polymeric spiral-wound microfiltration membranes. J. Dairy Sci. 93: 4506-4517.

Carter, B.G., Cheng, N., Kapoor, R., Meletharayil, H.G., and Drake, M.A. 2020. Microfiltration derived casein and whey proteins from milk. J. Dairy Sci. In Press.

Cheng, N., D.M. Barbano, and M.A. Drake. 2018. Hunter versus CIE color measurement systems for analysis of milk-based beverages. J. Dairy Sci. 75: S513-521.

Cheng, N., D.M. Barbano, and M.A. Drake. 2019a. Effect of pasteurization and fat, protein, casein to serum protein ration, and milk temperature on milk beverage color and viscosity. J. Dairy Sci. 102: 2022-2043.

Cheng, N., D.M. Barbano, and M.A. Drake. 2019b. Effects of milk fat, casein, and serum protein concentrations on sensory properties of milk based beverages. J. Dairy Sci. 102: 8670-8690.

Christensen, K.R., and G.A. Reineccius. 1992. Gas chromatographic analysis of volatile sulfur compounds from heat milk used static headspace sampling. J. Dairy Sci. 75:2098-2104

Dalgleish, D. D. 1990. Denaturation and aggregation of serum proteins and caseins in heated milk. J. Agric. Food Chem. 38:11.

Drake, M.A., Miracle, R.E., and McMahaon, D.J. 2010. Impact of fat reduction on flavor and flavor chemistry of cheddar cheeses. J. Dairy Sci. 93:5069-5081.

Hatchwell, L.C. 1996. Implications for fat on flavor. Pages 14-23. Flavor-food interactions. R.J. McGorrin, J.V. Leland, ed. American Chemical Society, Washington D.C.

Hoffmann, J.R., and M.J. Falvo. 2004. Protein-which is best? J. Sports Sci. Med. 3:118-130

Jang, H. D., and H. E. Swaisgood. 1989. Disulfide bond formation between thermally denatured b-lactoglobulin and k-casein in casein micelles. J. Dairy Sci. 73:900-904.

Jo, Y., B.G. Carter, D.M. Barbano, and M.A. Drake. 2019. Identification of the source volatile sulfur compounds produced in milk during thermal processing. J. Dairy Sci. 102:8658-8669.

Jovanovic, S., M. Barac, O. Macej, T. Vucic, and C. Lacnjevac. 2007. SDS-PAGE analysis of soluble proteins in reconstituted milk exposed to different heat treatments. J. Sens. 7:371-383.

Kaylegian, K. E., G. E. Houghton, J. M. Lynch, J. R. Fleming, and D. M. Barbano. 2006. Calibration of infrared milk analyzers: Modified milk versus producer milk. J. Dairy Sci. 89:2817–2832.

Kaylegian, K. E., J. M. Lynch, J. R. Fleming, and D. M. Barbano. 2009. Influence of fatty acid chain length and unsaturation on mid-infrared milk analysis. J. Dairy Sci. 92:2485–2501. doi:10.3168/jds.2008-1910.

Keršiene, M., A. Adams, A. Dubra, N. De Kimpe, D. Leskauskaite. 2008. Interactions between flavour release rheological properties in model custard desserts: effect of starch concentration and milk fat. Food Chem. 108:1183-1191.

Lee, A.P., D.M. Barbano, and M.A. Drake. 2017. The influence of ultra-pasteurization by indirect heating versus direct steam injection on skim and 2% fat milks. J. Dairy Sci. 100:1688-1701.

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

McCarthy, K.S., K. Lopetcharat, and M.A. Drake. 2017. Milk fat threshold determination and the effect of milk fat content on consumer preference for fluid milk. J. Dairy Sci. 100:1702-1711.

Meilgaard, M., G.V. Civille, B.T. Carr. 2007. Descriptive analysis techniques, Chapter 10. In: Sensory Evaluation Techniques, 4th ed. Boca Raton, Fla.: CRC Press.

Misawa, N., D. M. Barbano, and M. A. Drake. 2016. Influence of casein as a percentage of true protein and protein level on color and texture of milks containing 1 and 2% fat. J. Dairy Sci. 99:5284–5304.

Prindiville, E.A., Marshall, R.T., and Heymann, H. 1999. Effect of milkfat on sensory properties of chocolate ice cream. J. Dairy Sci. 82:1424-1432.

Queiros, M.S., R.L.S. Viriato, D.A. Veja, A.O.B. Ribeiro, and M.L. Gigante. 2020. Milkfat nanoemulsions stabilized by dairy proteins. J. Food Sci. Tehcn. 57:3295-3304.

Rutherford, S.M., A.C. Fanning, B.J. Miller, and P.J. Moughan. 2015. Protein digestibility-corrected amino acid scores and digestible indispensable amino acid acid scores differentially describe protein quality in growing male rates. J. Nutr. 145:372-379.

Sharma-Shri, K., and D. G. Dalgleish. 1993. Interactions between milk serum proteins and synthetic fat globule membrane during heating of homogenized whole milk. J. Agric. Food Chem. 41:1407-1412.

Walstra, P., T.J. Geurts, A. Noomen, A. Jallema, and M.A.J.S. Van Boekel. 1999. 2.4.1.5 Denaturation. Page 77 in Dairy Technol: Principles of Milk Properties and Processes. Marcel Dekker Inc., New York, NY.

Wojciechowski, K. L., C. Melilli, and D. M. Barbano. 2016. A proficiency test system to improve performance of milk analysis methods and produce reference values for component calibration samples for infrared milk analysis. J. Dairy Sci. 99:6808–6827. http://dx.doi.org/10.3168/jds.2016-10936

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

Zhang, Y., and C.-T. Ho. 1991. Comparison of the volatile compounds formed from the thermal reaction of glucose with cysteine and glutathione. J. Agric. Food Chem. 39: 760-763.

Zheng, Li, R. Marshall, H. Heymann, and L. Fernando. 1997. Effect of milk fat content on flavor perception of vanilla ice cream. J. Dairy. Sci. 80: 3133-3141.

Zulewska, J., and D. M. Barbano. 2014. The impact of linear velocity and flux on performance of ceramic graded permeability membranes when processing skim milk at 50°C. J. Dairy Sci. 97:2619– 2632.

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

Table 3.1 Mean (n=3) MCC beverage composition (%) with differing target milk derived whey protein (MDWP) removal percentages at about 6.3% true protein at targets of 0.1, 1.0, 2.0% fat formulated to 15 g per 240 mL serving prior to ultrapasteurization.

Target MDWP removal %	Target fat content	CP[1] (TN x 6.38)	NPN[2] (NPN x6.38)	CN[3] (TN-NCN)	MCC Purity (CN%TP)[4]	Residual MDWP[5]
95.20	0.1	6.69[a]	0.06[a]	6.16[a]	92.87[a]	0.473[c]
83.30	0.1	6.67[a]	0.07[a]	5.98[b]	90.44[b]	0.631[b]
69.20	0.1	6.74[a]	0.07[a]	5.85[c]	87.68[c]	0.822[a]
95.20	1.0	6.66[a]	0.06[a]	6.12[a]	92.77[a]	0.477[c]
83.30	1.0	6.68[a]	0.06[a]	5.98[b]	90.39[b]	0.635[b]
69.20	1.0	6.68[a]	0.06[a]	5.80[c]	87.58[c]	0.822[a]
95.20	2.0	6.56[a]	0.07[a]	6.03[a]	92.82[a]	0.466[c]
83.30	2.0	6.70[a]	0.07[a]	6.02[a]	90.82[b]	0.609[b]
69.20	2.0	6.71[a]	0.07[a]	5.87[c]	88.30[c]	0.777[a]
Standard Error		0.02	0.0008	0.021	0.091	0.0056
R^2		0.79	0.99	0.927	0.995	0.996

[a-c] Means in the same column followed by a different superscript differ ($P < 0.05$).
[1]CP = crude protein
[2]NPN = Non-protein nitrogen
[3]Casein – total nitrogen (TN) minus nonprotein nitrogen (NPN) * 6.38
[4] MCC Purity = Casein as a percentage of true protein (CP minus NPN).
[5] Residual milk derived whey protein (MDWP)

Table 3.2 Mean (n=3) micellar casein concentrate (MCC) purity measurement by Kjeldahl before and after ultra-pasteurization (UP) processing of differing MCC at about 6.3% total protein formulated to 15 g per 240 mL serving.

Target % MDWP removal	MCC Purity (CN%TP)[1] Before UP	MCC purity (CN%TP)[1] after UP	Apparent increase in MCC purity
95.20	92.28^{bA}	94.03^{aA}	1.75^{C}
83.30	90.55^{bB}	93.57^{aA}	3.02^{B}
69.20	87.85^{bC}	92.29^{aA}	4.44^{A}

[a-b]Means in the same row followed by a different superscript differ ($P < 0.05$).
[A-C] Means in the same column followed by a different superscript differ ($P < 0.05$).
Standard error = 0.096 and $R^2 = 0.992$.
[1] MCC Purity = casein as a percentage of true protein (CN%TP)

Table 3.3 Mean (n=3) micellar casein concentrate (MCC) purity measurement by electrophoresis before and after ultrapasteurization (UP) processing of differing MCC purity percentages at about 6.3% total protein formulated to 15g per 240 mL serving.

Target % MDWP removal	MCC purity (CN%TP)[1] before UP	MCC purity (CN%TP)[1] after UP	Apparent increase in MCC purity
95.20	94.07^{aA}	94.65^{aA}	0.58^{A}
83.30	91.89^{aB}	92.47^{aB}	0.58^{A}
69.20	89.15^{aC}	89.16^{aC}	0.01^{A}

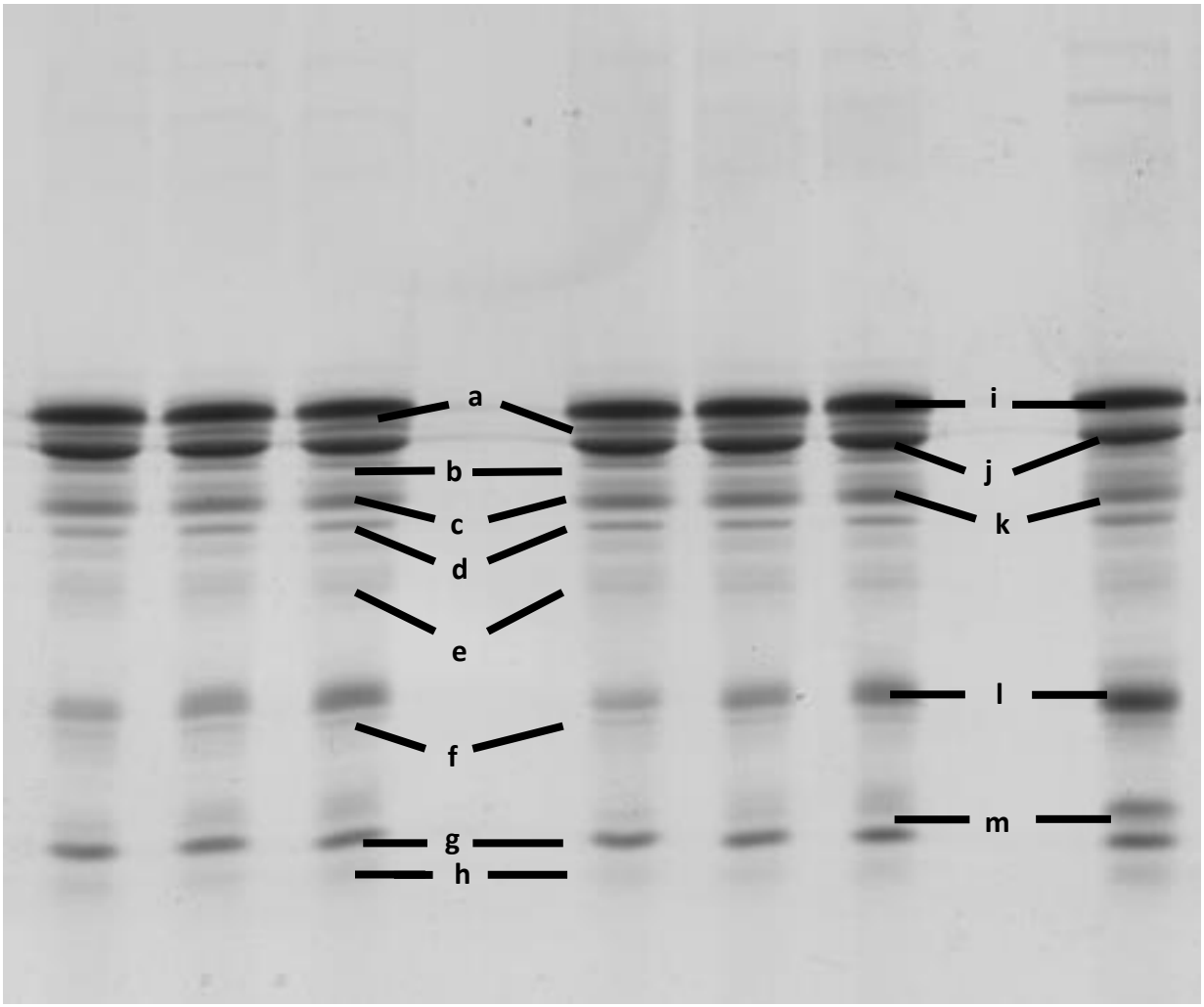
$^{a-b}$Means in the same row followed by a different superscript differ ($P < 0.05$).
$^{A-C}$ Means in the same column followed by a different superscript differ ($P < 0.05$).
Standard error = 0.014 and $R^2 = 0.995$.
1 MCC Purity = casein as a percentage of true protein (CN%TP)

Table 3.4 Mean (n=3) sensory intensity scores for overall aroma, cooked/milky, sulfur/eggy, milk fat aroma of ultrapasteurized milk protein MCC beverages with differing target milk derived whey protein (MDWP) removal percentages (95.2, 83.3, and 69.3%) at about 6.3% true protein at 0.1, 1.0, or 2.0% fat formulated to 15 g per 240 mL serving.

Target MDWP removal %	Target fat content %	Overall Aroma	Cooked/milky	Sulfur eggy	Milk fat	Residual MDWP[1]
95.20	0.1	1.8^{c}	4.0^{a}	ND	ND	0.473^{c}
83.30	0.1	2.7^{b}	4.2^{a}	2.1^{b}	ND	0.631^{b}
69.20	0.1	3.6^{a}	4.1^{a}	3.0^{a}	ND	0.822^{a}
95.20	1.0	2.0^{c}	4.2^{a}	ND	1.3^{b}	0.477^{c}
83.30	1.0	2.3^{b}	4.4^{a}	1.1^{d}	1.9^{a}	0.635^{b}
69.20	1.0	2.9^{a}	4.4^{a}	1.9^{b}	1.2^{b}	0.822^{a}
95.20	2.0	2.0^{c}	4.4^{a}	ND	2.0^{a}	0.466^{c}
83.30	2.0	2.2^{b}	4.4^{a}	0.4^{e}	1.9^{a}	0.609^{b}
69.20	2.0	2.8^{a}	4.4^{a}	1.6^{c}	1.9^{a}	0.777^{a}
Standard Error		0.04	0.45	0.04	0.02	0.0056
R^2		0.92	0.38	0.97	0.98	0.996

[a-c] Means in the same column followed by a different superscript differ ($P < 0.05$).
[1] Residual milk derived whey protein (MDWP)

Figure 3.1 SDS PAGE electrophoresis gel of liquid MCC with varying milk derived whey protein removal (MDWP) (rep 3). Lanes 1 – 3: 0.1% fat MCC with MDWP removal targets 95.2, 83.3, and 69.2% before ultrapasteurization. Lanes 4 – 6: 0.1% fat MCC with MDWP removal targets 95.2, 83.3, and 69.2% milk derived whey protein reduction after ultrapasteurization. Lane 7 – pasteurized milk standard.



[a]CNPP 1a
[b]CNPP 1b
[c]CNPP 2
[d]CNPP 3
[e]CNPP 4
[f]CNPP 5b
[g]CNPP 6
[h]CNPP 7
[i] α-CN
[j] β-CN
[k] K-CN
[l] β LG
[m] α LA

Figure 3.2 Hydrogen sulfide concentration (ug/kg) in ultrapasteurized milk protein (MCC) beverages with differing target milk derived whey protein (MDWP) removal percentages (95.2, 83.3, and 69.3%) at about 6.3% true protein with 0.1, 1.0, or 2.0% fat formulated to 15 g per 240 mL serving.

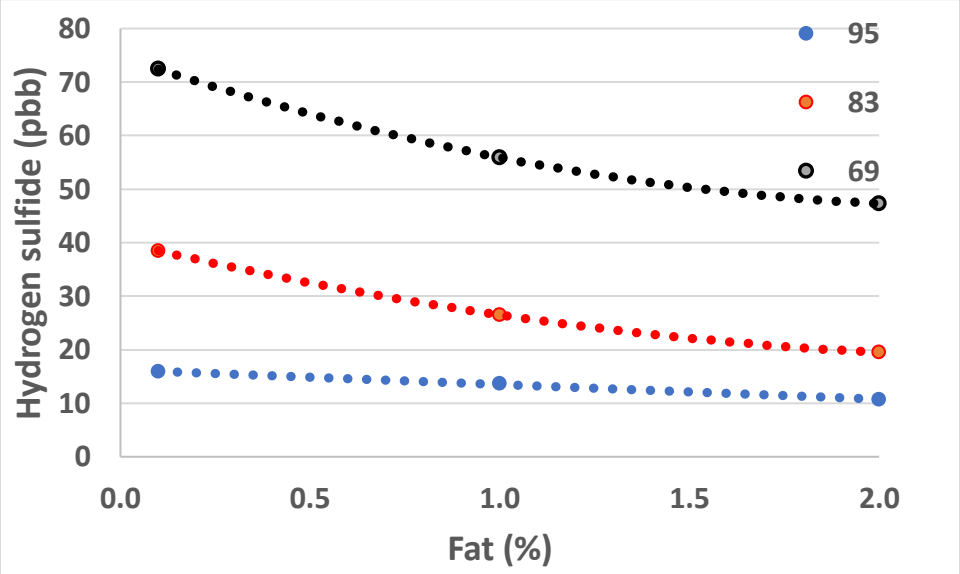


Figure 3.3 Carbon disulfide concentration (ug/kg) in ultrapasteurized milk protein (MCC) beverages with differing target milk derived whey protein (MDWP) removal percentages (95.2, 83.3, and 69.3%) at about 6.3% true protein with 0.1, 1.0, or 2.0% fat formulated to 15 g per 240 mL serving after ultrapasteurization (UP).