ABSTRACT

CHENG, NI. Ceramic Microfiltration to Produce Dairy Ingredients for Milk Protein Beverages (Under the direction of Dr. MaryAnne Drake).

Membrane technology has delivered innovations to dairy research and the dairy industry for decades. A variety of membrane driven dairy ingredients and applications have contributed to market growth of the dairy industry. Increased consumer awareness of all natural, less processed ingredients and products, and consumer desires for nutritional beverages with flavorful sensory characteristics provide an opportunity for ceramic microfiltration membrane driven dairy protein ingredients for milk based protein beverages. The objectives of this dissertation were to determine the impact of casein as a percentage of true protein (CN%TP) and true protein level on the physical and sensory properties of skim, 1% and 2% fat milk protein beverages. Achieving this goal will generate a platform for fluid milk and beverage innovation.

The first study determined the differences in the sensitivity of Hunter and CIE color systems at two different viewer angles (2 and 10 degrees) for measurement of whiteness, red/green, and blue/yellow color of milk based beverages over a wide range of compositions. The Hunter system of color measurement was more sensitive to differences in whiteness while the CIE system was more sensitive to differences in yellowness for milk based beverages. There was no difference between the Hunter and CIE system in sensitivity to green/red color of milk based beverages. Temperature of color measurement had a large impact on color measurement in both the Hunter and CIE measurement systems with the largest for skim milk and the least for 2% fat milk.

The second study had two objectives: 1) to determine the impact of pasteurization-homogenization, fat and protein concentration, casein to true protein ratio, and temperature on sensory and instrumental measures of viscosity and color of milk based beverages, 2) to use

instrumental measures of whiteness and yellowness to predict sensory measures of whiteness and yellowness. Variation in casein as a percentage of true protein (CN%TP) in the beverages had a large impact on beverage whiteness and yellowness and higher CN%TP resulted in beverages that were more white and less yellow. Multiple linear regressions of L, a and b* values produced more robust predictions for both sensory whiteness and yellowness than simple linear regression with L and b *values alone.

The objective of the third study was to determine the impact of systematically controlled variation in milk fat, true protein, casein, and serum protein concentrations on sensory color, flavor and texture properties, instrumental color and viscosity, and milk fat globule size distribution of milk based beverages and to provide guidance for milk protein beverage formulation. Formulating milk protein beverages to higher casein concentration increased instrumental viscosity and decreased flavor intensities while higher serum protein concentration increased aroma intensity, sweet aromatic, cooked/sulfur and cardboard/doughy flavors, and sensory yellowness scores within each fat level and across fat levels. Formulating milk protein beverages to higher true protein level increased astringency regardless of fat level.

These findings from this dissertation can be directly applied for fluid milk beverage product development/processing while introducing liquid micellar casein concentration (MCC) as a new dairy protein ingredient. This dissertation also calls for the further understanding of sensory and processing characteristics of liquid MCC and its applications to better position MCC as a value added ingredient in the market.

Ceramic Microfiltration to Produce Dairy Ingredients for Milk Protein Beverages

by
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APPROVED BY:

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DEDICATION

To my parents! Love you guys!

BIOGRAPHY

Ni Cheng was born in Chongqing, China on Dec. 29th 1988 to Boqing Cheng and Zhiping Li. She is one of the Dragon Year Babies. Ni enjoyed studying history and traveling with her parents since she was young. Ni finished her middle school (in 2004) and high school (in 2007) at Chongqing Yucai middle school. Ni graduated from Beijing Forestry University with a bachelor's degree in Food Science and Engineering in 2011. During college, she obtained a National College Student Innovation grant and did a summer intern at the Institution of Process Engineering, Chinese Academy of Science. Ni joined Dr. James Harper's lab at the Ohio State University in Sept 2011 and focused on quality control of Swiss Cheese by mid infrared spectroscopy. Ni graduated in May 2013 with her master in Food Science. Interestingly, Ni met Dr. MaryAnne Drake and Dr. David Barbano separately at the 2013 ADSA meeting in Indianapolis, IN. Ni still remembers when she asked Dr. Harper "Should I work for one of them? Are they good at what they do?" Dr. Harper laughed loudly and said "You will be lucky if you could work for one of them. Go learn from them!" Therefore, Ni joined Dr. Drake's lab at North Carolina State University as a research assistant in Jan. 2014 and then started her PhD. that fall. Ni had no idea that she would work with Dr. Barbano until she met him again when invited for a dinner at Dr. Drake's house. It turned out that Ni would work for both Dr. Drake and Dr. Barbano!

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1: LITERATURE REVIEW: SENSORY CHARACTERS OF MEMBRANE DRIVEN DAIRY INGREDIENTS AND PRODUCTS

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Abstract

Fluid milk sales and consumption have declined in the past several decades. However, milk and milk components provide a solid nutritional and functional profile. With the help of membrane technology, milk is a promising source for developing new ingredients, functional foods and beverages. A brief review of current milk consumption is presented, followed by an in-depth review of membrane technologies in the dairy industry (primarily microfiltration and ultrafiltration). Sensory and functional characters of membrane driven dairy ingredients are discussed in detail. Previous applications of those ingredients in dairy products will be briefly discussed. The outcome of this review is to provide options for innovation of dairy ingredients and beverages.

Current Fluid Milk Market

Milk is a rich source of quality protein, fat, mineral and vitamins. Milk is generally considered to have a positive effect on bone density, calcium, vitamin A and D consumption and body weight control. However, the decline of fluid milk consumption began after the WWII. The per capita fluid milk consumption per person decreased about 58.33% (from 0.96 to 0.61 cups per day) from 1970 to 2007 (Stewart, Dong, & Carlson, 2013). Although milk consumption for lower fat milk (skim milk, 1% fat milk and 2% fat milk) is increasing, this does not overcome the decrease in whole milk consumption and total fluid milk consumption (Stewart et al., 2013). A detailed paper on the past 100 years of the fluid milk market was summarized by Barbano (2017).

Several studies have discussed the drivers of decline for fluid milk consumption. 1) Change in eating habits. Americans consumed 48% of total food away from home in 2000 compared to 25% in the 1940s. Fast food was one popular choice for food away from home and it has had a negative effect on fluid milk consumption (Bowman, Gortmaker, Ebbeling, Pereira, & Ludwig, 2004). 2) Competition from other beverages. Milk seems less attractive compared to other beverages, including juice, coffee, soda and functional drinks. The shift to fast food also has lead consumers to a more diverse beverage menu, but with limited dairy products included (Stewart, Dong, & Carlson, 2012). Americans consumed less volume of milk and consumed milk less frequently in the 2000s compared to the 1970s. Meanwhile, people started to consume more sweetened beverages (Nielsen & Popkin, 2004; Stewart et al., 2013). 3) Cohort effect on fluid milk demand. Stewart et al. (2012) reported that the younger generation consumed less fluid milk compared to the older generation. As the younger generation will take over the older generation eventually, a further decrease in milk consumption can be expected. 4) Change in demographics.

Due to eating habits and lactose tolerance, Caucasian people traditionally consumed more milk and milk products than Hispanic people (Krebs, 2013). However, Caucasians had a lower birth rate compared to Hispanic and black populations, which could change the future demographic structure and total milk consumption (Monte & Ellis, 2014). 5) Incorrect consumer concerns. Although milk has showed several nutritional benefits for humans, concerns about milk consumption and health issues, for example, milk fat, hormones and animal welfare, still remain debatable topics on social media (McCarthy, Lopetcharat, & Drake, 2017).

Continuous effects have been spent on promoting milk and milk products for the past few decades. The Federal Milk Marketing Orders protected dairy farmers and indirectly optimized the usage of milk and milk products. To attract consumers back to milk, the "Got milk?" campaign and commercials were initiated in the 1990s. As the habit of milk drinking from childhood is essential for adult milk consumption (McCarthy et al., 2017; Stewart et al., 2012), nationwide nutritional programs such as the National School Lunch Program and Women, Infant, Children (WIC) might help to seed milk consumption in future generations. The Dairy Checkoff Program funded product research and brought innovations to the dairy industry. Membrane technology was one of the most important areas in dairy research. It extended the dairy categories with membrane driven dairy ingredients and products.

Membrane Technology in Dairy Industry

Introduction

The 1960s were marked as the beginning of modern membrane technology when Sourirajan and Loeb invented a reverse osmosis (RO) membrane for water desalination at the University of California in Los Angeles (Loeb & Sourirajan, 1963). The dairy industry has adopted membrane technology since the 1970s and continues to enrich its processing lines and

products categories with the developments of membrane technology. Membrane technology optimizes the utility of milk and minimizes processing waste (such as whey from cheese making).

Membrane technology is a screen separation of components under a mild or low processing temperature. Based on membrane modes, there are two types of membrane filtrations: deadend or conventional filtration (Figure 1) and cross flow-velocity filtration. (Figure 2) There are two major differences for these two modes. Firstly, the direction of flow. The flow direction of the feed is parallel as the flow direction of the permeate (stream went across the membrane) in deadend filtration while the flow direction of the feed was orthogonal to the flow direction of permeate in cross flow velocity filtration. As processing time increases, the membrane is gradually fouled by milk components, which precipitate on the membrane surface and plug in the pore of the membrane and accumulate on the membrane surface. Flux (permeate flow rate across the membrane) continues decreasing until no permeate flows through the membrane because of the gradient thick layer of foulant. In deadend filtration, the end of processing was when the layer was too thick and the flux became uneconomically low. The layer could only be removed after stopping processing. Fouling happens in all membrane filtrations and can only be controlled, not eliminated. In cross flow-velocity filtration, the membrane still fouls during processing, but as the flow directions of the feed and permeate are orthogonal, the feed washes off the foulant on the membrane during the processing, which helps to maintain a reasonable flux for a longer processing time. Milk is a protein, mineral and fat rich matrix, which can easily foul the membrane. To increase the efficiency of processing, cross flow-velocity mode filtration is the main stream in dairy industry. Processing temperature is controlled around 50-55°C during the

run to prevent protein denaturation and fouling, and to maintain a reasonable flux (Cheryan, 1998).

There are four major pressure driven membranes used in dairy processing. Based on pore size, these membranes are named Microfiltration (MF), ultrafiltration (UF), nanofiltration (NF) and RO (Figure 3). MF has the largest pore size (0.1-10um) and the lowest processing pressure (0.01-0.2Mpa). It is originally invented for bacterial removal for production of extended shelf life milk and pretreatment of cheese milk. MF has been applied in the dairy industry since the 1980s. When a 1.4um MF membrane is used, both bacteria (10-100 um) and milk fat globules (10um) are rejected by the membrane (called retentate) while micellar casein (50-500 nm) and whey proteins (3-6 nm), lactose (1 nm), minerals and water go across the membrane (called permeate) (Adams, 2012; Pouliot, 2008; Smith, 2013). When a 0.1 um MF is used, native micellar casein is retained while native whey protein (serum protein) passes through the membrane. UF was the first and most widely used membrane in dairy processing. It is a primary unit in whey protein processing. UF usually has a pore size of 10-100nm and processing pressure of 0.1-1.0Mpa (Adams, 2012; Pouliot, 2008; Smith, 2013). UF was first used for protein separation and purification in biology, and its molecular weight cut off (MWCF) (kDa) was used to differentiate UF membranes. Some membrane manufacturers still use the MWCF as a parameter for dairy UF membrane selection. In UF, only lactose, minerals and water pass through the membrane while all the other milk components (casein, whey proteins and fat) are rejected by the membrane. RO has the smallest pore size (<1nm) with the highest processing pressure (3.0-5.0Mpa) (Adams, 2012; Pouliot, 2008; Smith, 2013). RO only allows water to penetrate the membrane and is used to increase total solids content of milk or whey before further processing. The high pressure of RO limits further processing and NF was introduced as a

“loose” RO, with pore size 1-10 nm and lower processing pressure 1.5-3.0Mpa (Adams, 2012; Pouliot, 2008; Smith, 2013).NF membranes reject all milk components besides water and monovalent salts.

Besides size, membrane technology provides several other options for dairy processing. Membrane materials can be polymers (cellulose acetate, polysulphone/polyethersulphone, polyamide, polyvinylidene fluoride and polypropylene) or inorganic materials (ceramic, metallic) (Jensen & KØnigsfeldt, 2000). A more hydrophilic and negatively changed membrane is desired to avoid membrane fouling. The installation fee of the polymer membranes is reported as $1/10^{th}$ of ceramic membranes, while ceramic membranes have a wider pH and temperature range and a longer life (at least 10 years). Ceramic membranes are more brittle than polymer membranes and require extra attention to operate. Generally, polyamide is used for RO, polyvinylidene fluoride and polypropylene for MF and UF and ceramic for MF in dairy. Membrane technology also provides several modules to house membranes. Polymer membranes are commonly housed in plate and frame, spiral wound and hollow fiber modules and inorganic membrane have their own tubular designed module. Varied from case to case, different membrane systems can produce different or similar products. Therefore, selection of membranes is a balance between target products, raw material, processing efficiency, installation fee and maintenance cost. A detailed review of functionality and sensory properties of those membrane drive milk products is presented with a focus on MF and UF.

Microfiltration

There are two sizes of MF primarily used in the dairy processing: 1.4 um for bacterial removal and fat removal and 0.1 um of casein and serum protein separation. MF membranes are available in both ceramic and polymer spiral wound forms. As MF membranes have a larger pore

size or pore size distribution, this causes fast fouling on or in MF membranes. Generally, to prevent fouling, high cross flow velocity can be applied in a ceramic system while it could damage spiral wound membranes. To reduce fouling on ceramic MF, a higher cross flow velocity (5-7m/s) is applied on the retentate side by increasing retentate recirculation pump rate. This introduces high wall shear stress and washes away the foulant on the membrane surface. However, high cross flow velocity also causes pressure increases at the inlet on the retentate side and then decreases along the membrane length from inlet to outlet while pressure remains consistent on the permeate side (Figure 4).

The differences in trans-membrane pressure (TMP) from the inlet to the outlet of ceramic membranes increases the chance of fouling. To control the fouling on MF membranes at a higher cross flow velocity, the TMP needs to remain less than 0.1 Mpa uniformly across the membrane length. The uniform transmembrane pressure (UTP) concept was invented to generate a low TMP along the membrane to minimize fouling in ceramic MF by increasing the inlet pressure on the permeate side (Sandblom, 1978) (Figure 5.a).

There are two primary ways to achieve a UTP in reality, using a permeate recirculation pump (UTP system) (Figure 5.b) (Sandblom, 1978) or by modifying the properties of membranes to mimic the higher pressure at the inlet on the permeate side. Two modified membranes were used in recent studies: 1) Membralox Gradient Permeability (GP) membrane, changing the resistance of the supporting layer of the membrane to flux, with higher resistance at the inlet to provide more pressure and lower resistance at the outlet (Figure 6); 2) Isoflux membrane patented by TAMI Industries (France), gradually decreasing the thickness of the inner selective layer of membrane from inlet to the outlet. A thicker selective layer is used for more resistance obtained on the permeate side (Figure 6) (Grangeon, Lescoche, Fleischmann, &

Ruschel, 2002; Garcera & Toujas, 2002). Isoflux MF and GP MF can be more economically feasible compared with UTP-MF, which requires an extra permeate pump, consumes more energy and potentially increases production cost.

The following summarized MF based on different milk components separated by using 1.4um (milk fat, bacterial) or 0.1um MF membranes (casein and milk serum protein):

MF for Milk Fat

Milk fat content of fluid milk typically varies from 3.5 to 4.7%. Milk fat exists as milk fat globules (MFG) in milk and contains triacylglycerol, free fatty acids, phospholipids, sterols, protein and enzymes. MFG consists of a triacylglycerol core, which is naturally emulsified by a phospholipid-rich milk fat globule membrane (MFGM) on the outside (Figure 7). The size of native milk fat globules varies from 0.2 to 15 um, with an average of 4um (Huppertz & Kelly, 2006). The size of MFG decreases and the MFGM is partially damaged and replaced by proteins (casein and whey proteins) (Figure 8) during mechanical stress, such as homogenization or churning. The disruption of MFGM caused the destabilization of MFG and cream separation.

MF in fat removal/fractionation

Polymeric MF and ceramic cross-flow velocity MF have been studied for whole milk fat removal (Glimenius, Jansson, Kemi, & Sandblom, 1979). However, as milk fat fouls the membrane heavily and negatively impacts processing, skim milk was preferred as the MF feed (Cheryan, 1998). A higher installment cost was another reason that fat removal of whole fat milk by MF was not widely used. On the other hand, MF is a general unit in whey protein isolate (WPI) processing. MF is more efficient in residual fat removal than a separator in sweet whey processing (Surel & Famelart, 1995; Qiu, Smith, Foegeding, & Drake, 2015).

From an ingredient innovation and application standpoint, ceramic MF was studied for fractionation of milk fat based on MFG sizes. Goudédranche, Maubois, and Fauquant (1998) patented a process on size separation of MFG, using a UTP-ceramic MF membrane with a 2-12 um pore size distribution. Goudédranche et al. (1998) processed milk at 50°C and fractionated native milk fat globules into small fat globules (SFG) and larger fat globules (LFG) (Briard, Leconte, Michel, & Michalski, 2003). The z-potential values of microfiltrated SFG and LFG showed no significant changes and indicated that this MF processing had no damage on MFGM nor did it cause partial homogenization in MF permeate and MF retentate(Michalski et al., 2002; Michalski, Ollivon, Briard, Leconte, & Lopez, 2004b). Michalski et al.(2006) optimized the MFG size separation by using a 5um pore size UTP-MF membrane for whole milk and produced a SFG rich permeate with d4, 3=0.9-3.3 um and a LFG rich retentate with d4, 3=5-7.5um. A flow chart of their processing setup was shown in Figure 9. They also claimed that using different sizes of MF membranes could produce specific sizes of MFG (Michalski et al., 2006).

Different sizes of MFGs had different compositions. The triacylglycerol core of the SGM had more conjugated linoleic acids, medium-chain fatty acids and less stearic acid compared with the triacylglycerol core of LGM (Briard, Leconte, Michel, & Michalski, 2003; Briard-Bion, Juaneda, Richoux, Guichard, & Lopez, 2008.; Fauquant, Briard, Leconte, & Michalski, 2005; Lopez et al., 2011; Michalski, Briard, & Juaneda, 2005). Size specific MFGs influenced physical and sensory properties of dairy products. SFG and LFG showed difference in supercooling and their stabilized crystalline forms at 20 °C followed by subsequent cooling (Michalski, Ollivon, Briard, Leconte, & Lopez 2004b). For semi skim milk, yogurt, fresh cheese, sour cream and butter, SFG made the texture smoother and finer, while LGF samples were firmer and grainier (Goudédranche, Fauquant, & Maubois, 2000). Butter with more LFG had a

more yellow color (Goudédranche et al., 2000). Both Emmental and Camembert cheeses with SFG had higher moisture and more proteolysis during ripening compared with cheeses with LFG (Michalski et al., 2003, 2004a). For mini Swiss cheeses, cheese with SFG showed a 3% increase for yield due to more water remained in the cheese curd (Goudédranche et al., 2000). Both Emmental and Camembert cheeses with SFG had a higher melting temperature, elasticity and stretch compared with cheeses with LFG (Michalski et al., 2003, 2004a). MF driven MFGs could standardize milk fat by specific MFG sizes, and customize physical and sensory properties of products to meet certain consumer desires and to help maintain consistent product quality. More studies on MF driven MFG fractions and their applications are needed.

MF in milk fat globule membrane separation

It was possible to commercially produce MFGM from buttermilk by MF (Corredig, Roesch, & Dalgleish, 2003). Microfiltrating buttermilk for MFGM could turn buttermilk into more value added products, such as all natural food emulsifiers or nutritional and pharmaceutical raw ingredients. As sizes of the casein micelle and MFGM fragments (0.4-1.5 um) are close, MF was not able to obtain a good separation of MFGM by using regular buttermilk (Morin, Jiménez-Flores, & Pouliot, 2004). Both Corredig et al. (2003) and Rombaut, Dejonckheere, and Dewettinck (2006) added sodium citrate or sodium citrate and ethanol in buttermilk to disassociate casein before MF, while Rombaut, Dejonckheere, and Dewettinck (2007) and Sachdeva & Buchheim (1997) used casein precipitation to pretreat buttermilk and acid cheese whey. Using a 0.5 um UTP-MF at 50°C, Morin, Britten, Jiménez-Flores, and Pouliot (2007) found that washing cream buttermilk with skim milk ultrafiltrate increased flux and separation of casein and MFGM. Fresh buttermilk had a better separation of MFGM in MF compared with rehydrated buttermilk powder (Morin et al., 2004). Both polymer and ceramic MF were used in

the these studies, and ceramic membranes showed better phospholipid recovery from buttermilk compared with polysulfone spiral membrane (Sachdeva & Buchheim, 1997). Whey buttermilk was also studied as the feed to concentrate MFGM compounds (Morin, Pouliot, & Jiménez-Flores, 2006). However, the phospholipids content increased 50% when using whey buttermilk as feed for a 2x ceramic MF processing (pore size= 0.45 um), while doubled when using regular buttermilk as feed (Morin et al., 2006).

Limited work was done on the functional properties of MF processed buttermilk and whey buttermilk MFGM. Roesch, Rincon, and Corredig (2004) reported that MFGM isolate (obtained from a sodium citrate treated buttermilk) decreased the size of emulsion oil droplet. Good review papers on emulsification of MFGM have been published by Singh (2006), Spitsberg (2005) and Jiménez-Flores and Brisson (2008). Additionally, MFGM compounds had potential heath benefits and could add value for whey and milk processers. MFGM phospholipids and proteins have bioactivity for human health, including anticancer and age-related diseases (Fauquant, Briard-Bion, Leconte, Guichardant, & Michalski, 2007; Singh 2006; Spitsberg 2005). Sphingolipids were found to inhibit the early and late stages of colon carcinogenesis in mice tumors (Dewettinck et al., 2008; Schmelz, 2004).

MF for Bacterial Removal

Fluid milk

HTST pasteurized milk and even more so ultrapasteruized (UP) milk is characterized by “cooked flavor”, which is not preferred by some consumers (Lee, Barbano, & Drake, 2017). For fluid milk processing, microfiltration could be used for bacterial removal in fluid milk by processing at a lower temperature (50°C) and decreased cooked flavor (Chugh et al., 2014). A ceramic MF process for bacterial removal in whole milk was first patented by Holm, Malmberg, and Svensson (1986) (Figure 10). They called this processing Bactocatch and reported a 99.6%

bacterial removal from whole milk. In Bactocatch, the raw whole milk was centrifuged into cream and skim milk under 35-50°C, then raw skim milk was microfiltered into MF permeate (skim milk) and bacterial rich MF retentate. This retentate and the raw cream from the separator were mixed together for UHT treatment (130°C, 4s). Then this mix was added back to MF permeate-skim milk for a final HTST pasteurization. To prevent fouling and maintain a reasonable flux, a 1.4 um ceramic UTP MF and skim milk as feed was primarily used (Rysstad & Kolstad, 2006).

The total bacteria count from Bactocatch of raw skim milk was around 3.7 to 5.63 log reduction (Olesen & Jensen 1989, Pafylias, Cheryan, Mehaia, & Saglam, 1996; Guerra, Jonsson, Rasmussen, Nielsen, & Edelsten, 1997; Osterland, 1999; Saboya & Maubois, 2000, Binetti, Bailo, & Reinheimer, 2004; Elwell & Barbano, 2006). Reduction of spores was around 3.3 to 5 log reduction (Olesen & Jensen 1989; Kosikowski & Mistry, 1990; Trouvé et al., 1991; Guerra et al., 1997; Saboya & Maubois, 2000). The shelf life of the Bactocatch processed milk varied in the literature. Eino (1997) and Russel (1999) reported a 32 day shelf life while Malmberg and Holm (1998) reported a 16-21 day shelf life and García and Rodríguez (2014) reported a 30 day shelf life for Bactocatch processed skim milk. This could be due to differences in raw milk quality or processing and post processing hygiene (Schmidt, Kaufmann, Kulozik, Scherer, & Wenning, 2012). Limiting the somatic cell count in raw milk can help to extend the shelf life of Bactocatch milk (Elwell & Barbano, 2006). Considering both microbial and flavor quality, Elwell and Barbano (2006) pointed out that Bactocatch processed skim milk could have more than 92 day, 78 day, 46 day and 32 day shelf life under 0.1°C, 2°C, 4.6°C and 6°C storage temperatures, respectively. Some commercial products are available in UK, Canada, Austria and several European countries.

Indirect high temperature pasteurization (115-130°C, 2s) and direct steam injection high temperature pasteurization (115-130°C, 6s) were used to replace the HTST in Bactocatch by García and Rodríguez (2014). They reported a 70+day shelf life under ambient temperature for all indirect and direct high temperature skim milk treatments (García & Rodríguez, 2014). To further prevent cooked flavor from the final HTST, pulsed electric fields was used by Rodríguez-González, Walkling-Ribeiro, Jayaram, and Griffiths (2011).They claimed that the skim milk bacterial reduction obtained by Bactocatch and MF with pulsed electric fields treatments were similar (Rodríguez-González et al., 2011). Although Bactocatch studies claim that their milk products had less cooked flavor and longer shelf life in terms of bacteria count, the sensory profiling within this category is very limited.

Cheese making

Bactocatch was also studied as a pretreatment for cheese milk. Bactocatch processed milk not only had a high log reduction of total bacteria counts and spores, but also had a low content of non starter lactic acid bacteria (McSweeney, Fox, Lucey, Jordan, & Cogan, 1993). This process made a clearer milk for cheese making, which could eliminate or alleviate potential problems for certain types of cheese and perhaps be applied others cheeses. Kosikowski and Mistry (1990) reported that Cheddar and Emmental cheeses made from Bactocatch processed milk (with pasteurized retentate mixed back) had a higher quality. Reduction of spores can help to eliminate split defects in semi-hard and hard cheese, such as Swiss cheese. For Swiss-type cheeses, Beuvier et al. (1997) indicated that cheeses made from raw milk and Bactocatch processed milk (with pasteurized retentate mixed back) were similar in flavor and both showed higher overall aroma intensity compared to cheeses made from pasteurized milk and Bactocatch processed milk (without pasteurized retentate). They suggested that this flavor difference was

related to nonstarter bacteria: facultative heterofermentative *bactobacilli*, *propionibacteria* and *enterococci* (Beuvier et al., 1997). Swiss-type mini cheeses made from Bactocatch processed milk (without pasteurized retentate) showed a much lower intensity for buttery and nutty aromas (Demarigny, Beuvier, Buchin, Pochet, & Grappin, 1997). Different heat treatments of cheese milk (pasteurized or microfiltered) could influence the expression of lipolysis by propionic acid bacteria in Emmental cheese and affect cheese flavors (Chamba & Perreard, 2002). For Cheddar cheese, the sensory differences between Bactocatch processed milk cheese and pasteurized milk cheese might also be due to the composition of nonstarter lactic acid bacteria (Roy et al., 1997). Bactocatch processed milk cheese had a slightly higher Cheddar cheese flavor compared with regular Cheddar cheese (Roy et al., 1997). However, McSweeney et al. (1993) indicated that Cheddar cheese made from Bactocatch processed milk and pasteurized milk had no difference in sensory quality.

MF for Separation of Micellar Casein and Serum Protein

Early studies on separation of micellar casein and serum protein from skim milk by MF were conducted in the 1990s. The pore size of MF membrane was smaller; usually 0.1 or 0.14 um for ceramic MF and 0.5um for spiral wound MF. Based on the size of protein, micellar casein (50-500nm) was rejected by the MF membrane as retentate while serum protein (3-6nm) passed through the membrane as permeate.

A 70-80% serum protein transmission was achieved by a 0.1 um ceramic MF in skim milk (Le Berre & Daufin, 1996). Samuelsson, Dejmek, Trägårdh, & Paulsson (1997) reported that higher processing temperature and higher cross flow velocity favored serum protein removal rate in skim milk MF. An 88% serum protein removal was obtained at 55°C with circulation velocity of 8m/s by a 0.14um ceramic MF (Samuelsson et al., 1997). Several studies have

addressed micellar casein and serum protein separation by both ceramic (UTP, GP and Isoflux) and spiral wound (SW- with PVDF as membrane material) MF. In a single stage 3x (x=concentration factor= total volume/retentate volume, CF) MF of skim milk at 50°C, UTP MF had the highest serum protein removal (64.40%) followed by GP (61.04%) and SW was 38.62% (Zulewska, Newbold, & Barbano, 2009). The serum protein removal in skim milk was significantly increased in UTP (achieved 95%) and SW (achieved 70.3%) when engaging diafiltration (DF) (Beckman, Zulewska, Newbold, & Barbano, 2010). The low serum protein removal rate in SW could be due to fouling caused by the higher hydrophobicity of polymeric membranes and lower cross flow velocity (Beckman et al., 2010). SW could achieve a 95% serum protein removal by an 8 stage MF with 7 stages of DF (Beckman et al., 2010). The cumulative serum protein removal for a 3 stage 3x processing at 50°C using UTP, GP and Isoflux was 98.3%, 96.5% and 70.2%, respectively (Adams & Barbano, 2013; Beckman et al., 2010; Hurt, Zulewska, Newbold, & Barbano, 2010). The serum protein removal rate for Isoflux was much lower than its theoretical value (97%), which could be due to variations in distribution of pore size, modification of selective layer and channel geometric shape (Adams & Barbano, 2013; Adams, Hurt, & Barbano, 2015). The higher the serum protein removal in skim milk was, the more pure the micellar casein concentrate obtained in the MF retentate, and more serum protein was recovered from the MF permeate. MF permeate was concentrated by UF to produce lower protein concentration serum protein concentrate (eg. SPC34) or went through a 2 stage UF with DF to produce higher protein concentration serum protein concentrate/isolate (eg. SPC80, SPI) (Figure 11) (Bacher & Kønigsfeldt, 2000; Evans, Zulewska, Newbold, Drake, & Barbano, 2009; 2010).

Micellar Casein Concentrate

Micellar casein concentrate (MCC) and serum protein concentrate/isolate (SPC/SPI) obtained from MF were free from starter cultures, rennet, acid exposure and glycomacropeptide (GMP). Along with the development of MF membrane with better separation and less cost, MCC and SPC are relatively new ingredients. MF MCC was more heat stable then serum protein. However, liquid MCC aggregated and coagulated after retort and UHT process (Beliciu, Sauer, & Moraru, 2012). Two reasons could lead to this phenomena: 1) calcium phosphate was more dissoluble at higher temperature and it might deposit on the micellar casein surface to cause aggregation and coagulation; 2) κ-casein dissociation, less repulsion and steric blocking. Fresh liquid MCC was more heat stable than rehydrated micellar casein concentrate (RMCC) powder under UHT processing (Beliciu et al., 2012; Lu, McMahon, Metzger, Kommineni, & Vollmer, 2015). Increasing pH (>6.9) could help to stabilize RMCC under retort and UHT processing (Sauer & Moraru, 2012). Lower temperature, higher serum protein removal and higher protein concentration increased MCC apparent viscosity (Sauer, Doehner, & Moraru, 2012). MCC with 18% protein content was reported as a thermal reversible protein gel with being solid gel under 4°C and as liquid when heating from 4 to 20 °C (Amelia & Barbano, 2013). Similar phenomena were observed by Lu et al. (2015) that MCC with 23% protein content was gel when temperature was lower than 38°C.

MCC was also named native phosphocaseinate by some French research groups. They had done a series of studies on rehydration proprieties of native phosphocaseinate powder, also called micellar casein concentrate powder or micellar casein powders. MCC powder had a protein content of about 80% on dry basis and water transfer in MCC powder was lower compared with “low heat” milk powder (Schuck et al., 1994). Higher rehydration temperature

and faster agitation speed decreased the rehydration time of MCC (Jeantet, Schuck, Six, Andre, & Delaplace, 2010). Addition of UF permeate of the skim milk MF permeate (primarily lactose and minerals) and NaCl to MCC (liquid) before spray drying significantly helped MCC powder to rehydrate faster (Gaiani, Banon, Scher, Schuck, & Hardy, 2005; Schuck et al., 2002). This could be due to the hygroscopic nature of lactose and sodium (Shuck et al., 2002). Addition of $CaCl_2$ and sodium caseinate before spray drying also decreased rehydration time of MCC powder by influencing Ca balance and modifying micellar casein structure (Shuck et al., 2002; Schokker et al., 2011). Higher storage temperature and longer storage time negatively impacted rehydration of MCC (Gaiani et al., 2009). Temperature and time favored lipid migration to the MCC powder surface and formation of a hydrophobic barrier on the surface, which slowed water transfer during the rehydration (Gaiani et al., 2009). Lu et al. (2015) suggested that mixing at higher temperature (50°C) and adding 60 mM trisodium citrate enhanced the dispersion and solubility of highly concentrated-MCC in water. Non agglomerated MCC powder showed faster rehydration than agglomerated MCC powder which was the opposite for WPI powders (Gaiani et al., 2009).

Flavor studies on MCC are limited. Rehydrated MCC was characterized by sweet aromatic, cooked/milky and cardboard/doughy flavors and had a distinct corn chip flavor compared to liquid MCC (Smith, Campbell, Jo, & Drake, 2016c; Carter, Patel, Barbano, & Drake, 2016). Using liquid MCC as an ingredient, the 100% MCC direct steam injection UP processed ready to drink (RTD) beverages were reported for the highest in vanilla flavor intensity and no detection of sulfur/eggy flavor (Vogel, Carter, Barbano, & Drake, 2018). MCC has a decent bland flavor and is a good vehicle of hydrated calcium, which makes it a promising

ingredient for shelf stable high protein RDT milk protein beverage with naturally fortifying calcium.

Details about MCC as a beverage ingredient will be discussed later in this review. Bong and Moraru (2014) reported that MCC powder could be used to fortify Greek style yogurt protein content and eliminate the acid whey removal in conventional Greek style yogurt processing. However, they also reported differences in physicochemical properties between the MCC fortified and regular Greek style yogurt (Bong & Moraru, 2014). Further formulation and sensory work will be needed to validate this alternative processing. MCC formulated high protein nutritional bar were softer and less cohesive comparing with MPC ones (Banach, Clark, & Lamsal, 2014).

Serum Protein Concentrate/Isolate

Several studies have addressed the physical and functional properties of SPC/SPI. However, researchers named their MF driven serum protein products from skim milk differently. To make comparison easier in this paper, SPC and SPC powder were defined to have a protein content of 30-85% on dry basis and SPI and SPI powder were defined to have protein content no less than 90% on dry basis. SPC and SPI obtained from MF had less fat, less heat treatment, less protein denaturation and no GMP compared with whey protein concentrate (WPC) or whey protein isolate (WPI) (Evans, Zulewska, Newbold, Drake, & Barbano, 2009, 2010; Heino, Uusi-rauva, Rantamäki, & Tossavainen, 2007; Coppola, Molitor, Rankin, & Lucey, 2014; Qiu et al., 2015). However, SPI processed from lower temperature MF (7°C) contained a high amount of beta-casein while SPI processed from 50°C MF had none (Coppola et al., 2014). This could be due to the beta-casein self-dissociation at lower temperature and beta-casein passing through the MF membrane (Coppola et al., 2014; Rose, 1968).

The color of SPI (10.88% protein content on w/w) was reported as amber to reddish due to the high concentration of lactoferrin and lactoperoxidase (Misawa, Barbano, & Drake, 2016). Color of SPC34 powder and WPC34 powder were identical (Evans et al., 2009). Rehydrated SPC34 and SPC80 powders, both with a fat content of 0.02%, were clear while rehydrated WPC34 and WPC80 were opaque due to the presence of GMP and higher fat content (Evans et al., 2009, 2010).

The density and viscosity of a SPI that contained 23.7% protein (w/w) at 20°C was 1.11g*ml and 11.65 mPa*s (Marcelo & Rizvi, 2008). SPC34 and SPC80 powders' solubility were the same as WPC powder while rehydrated SPC34 and SPC80 powders were less turbid than rehydrated WPC34 and WPC80 powder (pH3-7)(Bacher & Kønigsfeldt, 2000; Heino et al., 2007; Luck et al., 2013). SPC34 and SPC80 showed better gelation and foaming compared with WPC due to lower GMP and fat content and heat induced protein denaturation (Heino et al., 2007; Luck et al., 2013; Morr & Ha, 1993; Mangino, Liao, Harper, Morr & Zadow, 1987). A serum protein solution (the skim milk MF permeate was concentrated and dialyzed to 5% protein content) showed an identical overrun volume as an egg white protein (5% protein content) at pH=7 (Punidadas & Rizvi, 1998). Coppola et al. (2014) reported that SPI processed from 7°C MF with a high amount of beta-casein produced a more stable foam than SPI processed from 50°C MF. The unfolding of beta-casein would expose more hydrophilic groups (Swaisgood, 2003). The unfolding and annealing of beta-casein on the interface of air and water could decrease the surface tension and stabilize the foam. Drying methods (spray dry and freezing dry) had no effect on turbidity, gelation, foaming of SPC34 and SPC80 (Luck et al., 2013). Regardless of bleaching agent (hydrogen peroxide or benzoyl peroxide), bleached SPC80 had a more stable foam than unbleached SPC80, which could be due to more sulfide-sulfide

configuration reactions during the foaming (Campbell, Adams, Drake, & Barbano, 2013; Schmidt, Packard, & Morris, 1984). The hydrogen peroxide treated SPC80 showed better heat stability than benzoyl peroxide treated one (Campbell et al., 2013). Benzoyl peroxide was more efficiently for Norbixin removal in SPC80 (Campbell et al., 2013).

Rehydrated SPC powders were bland in flavor (Coppola et al., 2014; Evans et al., 2009, 2010). The spray dried SPC34 powder had no buttery (diacetyl) or cardboard flavors compared to WPC34 powder (spray dried and freeze dried) and freeze dried SPC34 powder presumably due to less fat content, processing time and lipid oxidation compounds (Evans et al., 2009). Due to no starter culture and lower fat content in SPC80 powder, it was lower in lipid oxidation compounds compared to spray dried WPC80 and commercial WPC powders (Evans et al., 2010). Liquid SPI was characterized by sweet aromatic, cooked/milky and cardboard/doughy flavors (Cheng, Barbano, & Drake, 2017). Hydrogen peroxide treated SPC80 had higher aroma intensity and cardboard and fatty flavors and higher lipid oxidation compounds compared to the benzoyl peroxide treated SPIC80 and unbleached SPC80 (Campbell et al., 2013). Clear acidic protein beverages were formulated with commercial WPC80 powders and pilot plant spray dried SPC80 and WPC80 powders for a consumer acceptance test (Evans et al., 2010). Beverages made with SPC80 had equal or higher liking scores for aroma, appearance, and mouthfeel compared to the WPC80 formulated beverages. In contrast, SPC80 beverages had lower flavor and overall liking scores compared with beverages made with 3 of the 4 commercial WPC80 powders (Evans et al., 2010). Increases of serum protein to casein ratio in RTD beverages (processed by DSI-UP pasteurization) showed increases in the sulfur/eggy flavor and decreases in vanilla flavor (Vogel et al., 2018). Serum protein could be the source of sulfur/eggy flavor in higher heat processed

milks (such as UP) and vanilla flavor compounds binding (Jo, Barbano, & Drake, 2018 and Vogel et al., 2018).

Fluid milk protein standardization by MF

MF driven MCC and SPI pushed protein standardization of milk and milk protein beverages to a new level by controlling both true protein level and casein to true protein ratio. Misawa et al. (2016) formulated liquid MCC, SPI, UF permeate of MF permeate, lactose monohydrate and cream into 40 milk beverages (lactose-5.0%, fat-1% and 2%, protein-3.0, 3.76, 4.34 and 5.0%, casein as a percentage of true protein 5%, 25%, 50%, 75% and 80%). They found that as casein to true protein ratio increased, milk became more white and viscous due to higher content of micellar casein. Changes in casein as a percentage of true protein had more influence on appearance and mouthfeel/texture of milk compared with changes in true protein level. Trained panelists detected the effects of changing casein as a percentage of true protein on appearance and effects of changing true protein level on mouthfeel/texture. These results provided information on instrumental analysis and sensory properties of milks with different true protein level and casein as a percentage of true protein, which could help to develop milk protein beverages to meet certain consumer needs. Cheng, Barbano, and Drake (2018 a and b) extended their work into 3 fat levels (skim, 1 and 2% fat) and investigated the composition variations and heat treatment on the sensory properties, instrumental viscosity, color and partial size of milk protein beverages. They found that increases in casein concentration increased the instrumental viscosity and decreased flavor intensity and on the other hand, increases in serum protein concentration increased aroma intensity, sweet aromatic, cooked/sulfur, cardboard/doughy flavors, and sensory yellowness scores within each fat level and across fat levels. Previous researches on milk protein standardization showed that when lower fat content milk was

standardized to higher protein, it showed similar sensory properties to higher fat content milk due to increase of whiteness (Quiñones, Barbano, & Phillips, 1997, 1998). Additionally, regardless of what fat type of milk consumers claimed to consume, consumers preferred higher fat milks (McCarthy et al., 2017). Applying information from Misawa et al. (2016) and Cheng et al. (2018 a and b), it would be possible to develop a high protein, low fat milk protein beverage with similar sensory properties as a higher fat milk protein beverage. From a flavor stand point, MCC and SPI blend DSI-UP RTD beverages (6.3% and 10.5% protein) showed that increases in SPI decreased vanilla flavor and headspace vanillin while increased the sulfur/egg flavor and astringency (Vogel et al., 2018).

Cheese making protein standardization by MF

Before MF was studied in cheese making, UF was the main stream (which will be discussed more later). However, the UF process was not successful in high CF (5-8) and medium CF (3-5) UF Mozzarella and Cheddar cheese (Garrett, 1987; Swientek, 1984). Although the higher the CF was, the higher the cheese yield was, those cheeses had texture and flavor defects (Green, Glover, Scurlock, Marshall, & Hatfield, 1981). This could due to higher CF and higher retention of serum protein, which slowed down proteolysis during ripening (Creamer, Iyer, & Lelievre, 1987). MF could provide a chance to optimize cheese yield and assure sensory quality by standardizing casein and serum protein to a certain ratio. A study reported a 7.3+/-1.8% yield increase of Mozzarella by using a rehydrated serum protein reduced MF processed powder (Garem, Schuck, & Maubois, 2000).

Brandsma and Rizvi (2001) reported that the optimal manufacture parameters for using a CF=8x skim milk MF retentate for Mozzarella cheese making were: 80-100 uL rennet kg^{-1}, coagulation temperature 32-36°C and post-coagulation curd cutting time of 15 min. A Cheddar

cheese made from MF retentate with CF=1.82x had higher yield and better net revenue than regular milk cheeses (Neocleous, Barbano, & Rudan, 2002a, 2002b; Papadatos, Neocleous, Berger, & Barbano, 2003). Increase of CF (from 1x-1.82x) increased hardness of fresh cheese and decreased proteolysis (less cheese flavor intensity). Limited substrate for chymosin, inhibition of chymosin activity by serum protein and lower residual chymosin could slow down proteolysis in higher CF MF Cheddar cheese (Neocleous et al., 2002a, 2002b; Nelson & Barbano 2005b). Processing modifications (more rennet, less ripening time and shorter cooking) were made for the higher CF (1.82x) MF Cheddar cheese manufacturing, and no significant sensory differences were noticed between the higher CF (1.82x) MF processed Cheddar and CF=1x Cheddar cheese by a triangle test (n= 24, untrained panelists) (Neocleous et al., 2002b). To optimize the profit of MF cheese procedure and increase serum protein recovery in the MF permeate, Nelson and Barbano (2005a) made a 95% serum protein removed cheese milk by a 3 stage, 3x MF with 2 stage DF (MF DF source: UF permeate of the MF permeate of the cheese milk). They reported that this cheese milk had the same lactose, nonprotein nitrogen and soluble mineral content as skim milk and could be standardized to a desired lower CF for conventional Mozzarella and Cheddar cheese manufacture (Nelson & Barbano, 2005a).

Ultrafiltration

The dairy industry began to use UF to deal with whey, the by-product of cheese manufacture, since the 1970s. UF turned whey from a processing waste stream into a value added source of ingredients, such as WPC and WPI. A general flow chart of WPC and WPI processing was shown in Figure 12. WPC and WPI were produced from sweet whey (start culture and rennet cheeses) and acid whey (acidification cheeses and Greek yogurt). For lower protein content (<50%) products, UF was applied to concentrate protein to target levels. While

for higher protein concentrate and isolate (>50%) production, extra diafiltration was needed due to heavy fouling of minerals and fat on the UF membrane (Kelly, 2003). Extra fat was removed by centrifugation or MF before further UF in the process of higher protein concentrate and isolate. Due to shipping and storage cost consideration, UF retentate was spray dried into powder for further applications.

UF permeate was used as the feed for lactose production. UF was also used to produce high protein (without changing casein to true protein ratio), low lactose and low mineral milk protein products, such as milk protein concentrate (MPC) and milk protein isolate (MPI). The processing of milk protein products is similar to WPC/WPI processing but the UF feed is changed to skim milk and extra fat removal is optional (Figure 13).

Milk Protein Concentrate /Isolate

MPC and MPI have a similar composition profile as milk, except for higher protein, lower lactose and mineral content. MPC retains the same casein to serum protein ratio as milk. MPC and MPI are divided into 3 categories based on their protein content: low-protein MPC (<40%), medium-protein MPC (60-70%) and high-protein MPC (80%) (Sikand, Tong, Roy, Rodriguez-Saona, & Murray, 2011). No caking was observed for MPC powers after 1 year storage at 25 to 30°C due to low amount of lactose (Mistry 2002). MPC powders, especially higher protein content ones (MPC80, MPC85), had poor solubility at 20°C. Longer storage time and higher storage temperature negatively affected MPC powder solubility (Anema, Pinder, Hunter, & Hemar, 2006; Mimouni, Deeth, Whittaker, Gidley, & Bhandari, 2010; Smith et al., 2016c). Several studies were done to understand the insolubility of MPC powder. Havea (2006) indicated that the insoluble material was formed by hydrophobic protein-protein interactions and consisted of alpha and beta caseins. Fang, Rogers, Selomulya, and Chen (2012) also reported that

the insoluble material were primarily caseins. Like MCC powders, the poor solubility of MPC powder could also be caused by slow dissolution kinetics (Mimouni, Deeth, Whittaker, Gidley, & Bhandari, 2009; Schuck, et al., 2002). Along with decreased solubility during MPC powder storage, increase of the beta-sheet structure (unfolded-denatured) and decrease of the alpha-helix structure (folded-native) in the casein protein was noticed (Haque et al., 2010). This change of conformation exposed more hydrophobic regions and made the casein protein more hydrophobic, which favored the hydrophobic protein-protein interaction within and between micelles (Baldwin, 2010; Haque et al., 2010). The casein micelles were compacted and formed a porous, gel-like structure that limited the dispersion of individual micelles into water but allowed water to come into the particle and solubilize non-micellar components (Mimouni et al., 2010). This confirmed the previous finding that the release of micelles from the particle limited the fast rehydration of MPC powders (Mimouni et al., 2009). Figure 14 illustrates a hypothesis for the rehydration process of MPC85 powder.

Several studies showed that changes in mineral composition could help to solubilize MPC powder due to the hygroscopic nature of salts (Na, K) or changes in micellar structure (Ca). Monovalent salts (Carr, Bhaskar, & Ram, 2002) and sodium caseinate (Schokker et al., 2011) were added to UF milk retentate before drying and these process showed an improvement of MPC powder rehydration. Sikand et al. (2011) found a significant positive correlation between higher sodium and lower calcium, magnesium, and phosphorus content and higher solubility of MPC powders. NaCl and KCl were added in the diafiltration step in MPC processing and this caused fast rehydration of MPC powders (Gualco, 2011; Sikand, Tong, & Walker, 2013). Decreases in inlet temperature could increase the solubility of MPC particles due to less protein denaturation during the spray drying (Fang et al., 2012).

MPC had relatively lower emulsifying capability compared with WPC/WPI, which had a smaller particle size and made more stable emulsions (Ye, 2011). Dybowska (2008) stabilized an oil-in water emulsion with MPC. They found preheated oil at a lower temperature gave a more stable emulsion than preheated oil at a higher temperature. Ye (2011) found that the emulsifying capability and the adsorption behaviors of MPC were largely affected by the aggregation state of casein. Foaming ability of MPC depended on pH and increased with high pH (Mistry & Hassan, 1991). At higher pH (pH=10) , MPC showed better foaming with 941% overrun than at lower pH 7 and 8 with less than 500% for overrun (Mistry, 2002). Fat content in MPC powder could also negatively influence the foaming ability of rehydrated MPC.

Increase of MPC protein concentration caused a flavor profile shift. Lower or medium protein MPC (MPC 56 and MPC70) were characterized by cooked/milky, sweet aromatic, and cereal flavors and sweet taste, while high protein MPC(MPC70, MPC77, MPC80, MPC85 and MPI) had more tortilla, brothy, cardboard, soapy, fatty and animal flavors and astringency (Drake, Miracle, & Wright, 2014; Smith, Foegeding, & Drake, 2016d). Storage favored formation of furosine and hydroxymethylfurfural while increasing spray drying inlet temperature and feed solids concentration decreased cardboard flavor and lipid oxidation products in MPC powders (Smith et al., 2016c; Park, Stout, & Drake, 2016b). Due to Maillard reaction, MPC80 powder had a higher furosine, hydroxymethylfurfural and b* value after a 12 weeks' storage at 40°C (Le, Bhandari, & Deeth, 2011).

Addition of MPC to yogurt could improve the texture and reduce syneresis, which was well adapted by the industry (Mistry & Hassan, 1992). An increases of 2.4 kg per 100 kg cheese milk was reported by adding 2% of MPC power to the Gouda cheese processing (Mistry & Pulgar, 1995). MPC/MPI was also used for process cheese production (Blazey, Dybing, Knights,

& Huang, 2001; Moran, Dever, Miller, Silver, & Hyde, 2001). Formulating high protein bar with MPC/MPI faced a shorter shelf life due to hardening (Loveday, Hindmarsh, Creamer, & Singh, 2009; Imtiaz, Kuhn-Sherlock & Campbell, 2012). Banach et al. (2014) used extruded the MPC80 for bar formulation and reported a reduction of hardening and extension of shelf life. MPC/MPI can also be a promising ingredient for "low carb" products due to its higher protein and lower lactose content, such as ice cream (Alvarez, Wolters, Vodovotz, & Ji, 2005) and beverages.

Whey Protein Concentrate/Isolate

Many functional studies on WPC and WPI have been done since the 1970s and good review papers were published by Schmidt, Packard, and Morris (1984), Morr and Ha (1993) and Foegeding, Davis, Doucet, and McGuffey (2002). WPC and WPI composition varied based on whey source, seasons, heat treatment, fractionation methods (filtration or participation) and storage and their composition differences caused variations in whey product functional properties (Schmidt et al., 1984; Morr & Ha, 1993). Functionality can only be applied when WPC and WPI are solubilized in solution. Generally, mild heating, adding sugar and monovalent mineral ions increased WPC and WPI solubility while adjusting pH close to isoelectric point, higher concentrations of salt and heating to about 70°C decreased WPC and WPI solubility (Morr & Ha, 1993). Bleaching decreased solubility of WPC80 due to protein degradation (Smith et al., 2015a), but hydrogen peroxide bleached WPC80 was more heat stable than benzoyl peroxide bleached WPC80 (Jervis et al., 2012).

Foaming and emulsification of WPC and WPI solutions depended on the absorbing/diffusing, unfolding and annealing of whey proteins on the air/water and oil/water interfaces, which reduced the surface tension (Morr & Ha, 1993). Calcium ions were essential for both foaming and emulsification. Increase of total solids to 10% increased foaming while the

present of fat and sugar decreased overrun (Schmidt et al., 1984). Mild heating of WPC and WPI solution before whipping increased the overrun due to partial denaturation and unfolding of whey protein (Morr 1987; Foegeding, Luck, & Davis, 2006). Heating to higher temperature (80°C) and alkaline conditions decreased foaming due to protein aggregation (Morr, 1987; Richert, Morr, & Cooney, 1974). Oxidizing agents, such as hydrogen peroxide and benzoyl peroxide, were reported to increase foaming due to sulfhydryl disulfide mediated protein conformation changes during whipping (Richert, 1979). Similar to foaming, heating WPC solution to 70°C also decreased emulsification. pH and ionic composition of the WPC solution affected emulsification properties of protein by altering their charges and size of the emulsified oil droplets (Morr & Ha 1993).

Lower whey protein concentration (3-5%) , heating temperature (55-70°C) and lower ionic strength (Ca^{+2}) formed a translucent gel due to small protein aggregates due to low number of intermolecular protein bonds while higher whey protein concentration (10%), heating temperature (above 90°C) and higher concentration of Ca^{+2} generated a more opaque gel due to more cross linked structure due to disulfide interchange and more hydrophobic protein bonds (Morr & Ha 1993; Schmidt et al., 1984; Shimada & Cheftel, 1989). Morr and Ha (1993) summarized pH effects on the gel proprieties of whey protein. When heating a whey protein solution with pH near isoelectric point, a weaker gel was produced as strong attractive forces between proteins caused protein aggregates. When the pH of whey solution was between 6-7, heating made a normal gel due to more net electrostatic charges repulsion (Morr & Ha, 1993). However, whey protein gels became weaker as the pH became more alkaline. This could be explained by enhancement of disulfide interaction under alkaline conditions, which favored formation of larger protein aggregates and affected gel firmness.

Several studies were done to understand flavors sources in WPC and WPI products. Ideal whey protein products have a delicate and bland flavor (Drake, Karagul-Yuceer, Cadwallader, Civille, & Tong, 2003). However, different processing initiate and enhance lipid oxidation and Maillard reactions and result in off-flavors in whey protein products (Smith et al., 2016c). Cardboard flavor is a common off flavor for whey protein products and is associated with lipid oxidation (Whitson, Miracle, & Drake, 2010; Whitson, Miracle, Bastian, & Drake, 2011). Diversity of whey sources can also cause flavor variations in liquid whey. Generally, fresh sweet wheys were characterized by cooked milk, buttery, milky, caramelized , cardboard, musty, metallic, sweet aromatic, and sour aromatic flavors and sweet taste while acid whey had stale, dirty, pungent, rancid, chalky, metallic and chemical flavors and salty and bitter tastes (Whetstine, Croissant, & Drake, 2005; Gallardo-Escamilla, Kelly, & Delahunty, 2005; Campbell, Miracle, Gerard, & Drake, 2011; Smith, Smith, & Drake, 2016b). Rennet cheese whey had lower overall aroma intensity, sweet aromatic, and sour aromatic flavors compared to Cheddar and Mozzarella cheese whey which were characterized by cooked milky, sweet aromatic, potato/brothy and cardboard flavors (Campbell et al., 2011; Smith et al., 2016b). Additionally, flavor variations in liquid whey were present in powder products (Drake, Miracle, & Wright, 2014). Rehydrated WPC80 was characterized by sweet aromatic, cardboard, brothy and cooked/milky flavors and astringency (Whetstine et al., 2005). WPI had a similar flavor profile as WPC80 with the addition of animal/wet dog, soapy and cucumber flavors and bitter taste (Whetstine et al., 2005). Higher protein concentration whey products had lower intensities of sweet aromatic and milky flavors (Drake et al., 2014). WPI from Cheddar and Cottage cheese whey had the highest cardboard flavor while WPI from Mozzarella whey had the highest sweet aromatic flavor, and rennet casein WPI had the lowest overall flavor and aroma (Smith et al.,

2016c). WPI from Cottage cheese whey had a distinct sour taste and brothy/potato flavor (Smith et al., 2016c).

Bleaching of liquid whey is used to reduce or remove residual annatto colorant from Cheddar cheese whey, but bleaching enhanced off flavors and lipid oxidation compounds in WPC and WPI products (Croissant, Kang, Campbell, Bastian, & Drake, 2009; Campbell, Kang, Bastian, & Drake, 2012; Jervis et al., 2012; Smith, Li, & Drake, 2014). Hydrogen peroxides (HP) and benzoyl peroxide (BP) are the major bleaching agents allowed in the US. Both HP and BP destroy the residual annatto colorant in Cheddar cheese whey. Both lipid oxidation and protein degradation compounds were found in HP, BP and lactoperoxidase bleached WPC80 (Jervis et al., 2012). A darker color, higher cardboard and fatty flavors and higher lipid oxidation compounds were present in HP bleached whey compared with BP bleached whey (Croissant et al., 2009; Jervis et al., 2012). BP was also more effective in color removal compared with HP in WPC34 powder processing (Listiyani, Campbell, Miracle, Dean, & Drake, 2011). HP bleached WPC80 was characterized by fatty and cardboard flavors while lactoperoxidase bleached WPC80 had both cabbage and cardboard flavors (Campbell et al., 2012). HP bleached WPI had higher aroma intensity, cardboard, cabbage, and fatty flavors compared with BP bleached WPI (Smith, Gerard, & Drake, 2015b).

Other unit operations also influenced liquid oxidation and cardboard flavors in whey proteins. Increase of storage time of WPC80/WPI liquid retentate prior to spray drying increased off-flavors and lipid oxidation compounds in WPC80 and WPI (Whitson et al., 2011). Park, Parker, and Drake (2016a) suggested that bleached WPC80 should be stored as liquid whey to minimize off-flavors. Spray drying and freezing drying of WPC resulted in increased cardboard flavors in the final powders (Evans et al., 2009). Increase in spray dry inlet temperature (from

180°C to 220°C) and total solids in feed (from 10%-25%) decreased volatile lipid oxidation compounds in WPC80 powder (Park, Bastian, Farkas, & Drake, 2014a.b). Agglomerated WPI/WPC80 powder had higher concentration and faster formation of lipid oxidation flavors (cardboard, raisin/brothy, cucumber, and fatty) and higher concentration of aldehydes and ketones compared to non agglomerated powder after a 18 month storage (Wright, Zevchak, Wright, & Drake, 2009). Adjusting pH of WPC to 3.5 before spray drying decreased cardboard flavor in spray dried WPC80 (Park et al., 2014b). Heating and acidification processing in beverage formulations increased cardboard, potato/brothy and malty flavors in WPI beverages (White, Fox, Jervis, & Drake, 2013). WPC/WPI had been widely accepted for yogurt, cheese, ice cream, bar and beverage applications for better performance of sensory, nutrition and yield.

UF in Protein Standardization

Fluid milk

Milk composition varies with cow breed, feed, lactation stage, and cow health, which also causes variations in dairy ingredients and products. Milk fat was standardized for years by centrifugation for economical and nutritional reasons. Milk protein content varied from 2.80-4.22% with the average 3.3-3.4% (Rattray & Jelen, 1996a). UF could be used to achieve protein standardization of milk. This could help to ensure the consistence of functionality of dairy ingredients and products. For a specific application, milk protein can be standardized to a lower level (downstream) or a higher level (upstream) (Rattray & Jelen, 1996a). For fluid milk, upstream protein standardization was more accepted by market and regulation due to higher protein content (Jelen, 2011), while downstream protein standardization of milk protein could increase freezing point and cause false adulteration (Rattray & Jelen, 1996c; Jelen, 2011). The acceptable protein level for downstream protein standardization of fluid milk remains under

negotiation. Milk protein content was required to be not less than 2.8% by the European Union and 3% was being discussed as a minimum requirement for fluid milk protein content internationally (Jelen, 2011). The benefit of downstream milk protein standardization was that the extra milk protein could be separated and used to produce higher value-added dairy products, such as cheese, yogurt, beverages and dairy powders.

A few studies discussed using UF to standardize protein in fluid milk. Poulsen (1978) standardized skim milk, half-skim milk and whole milk using skim milk UF permeate and conducted triangle tests (n=6, trained panelists) to detect potential differences in those protein standardized milks. They claimed a wide protein range (skim milk: 3.1-6.4%, half-skim milk: 1.75-6.5% and whole milk: 1.5-6.5%) could be applied to fluid milk protein standardization without causing significant sensory differences. Poulsen (1978) pointed out that sensory differences of standardized skim milks came more from surface gloss and translucency than taste and flavor. However, Rattray and Jelen (1996c) reported a narrower protein range (2.4-3.44%) for skim milk protein standardization based on their triangle test (n=15, untrained panelist) results. This conflict could be due to differences in protein test range and size of panelists. Both Poulsen and Rattry and Jelen's tests utilized a small group of trained/untrained panelists, which were not statistically strong enough for convincing results.

Quiñones et al. (1997, 1998) used skim milk UF permeate to standardize the protein content of skim milk (0.1% fat), 1% fat milk, 2.2% fat milk and 3.3% fat whole milk from 0.9/1.0% to 4.6/4.8%). They used a trained panel to profile those protein standardized milks and reported no flavor/aroma changes were detected in the protein range at each fat level. However, appearance and/or texture differences were noticed when increasing protein content by 0.9% in skim milk and in 1% fat milk (Quiñones et al., 1997). No appearance and/or texture changes

were recorded within 1.8%-2.8% protein for 2% fat milk and 1.8-3.7% protein for 3.3% fat milk (Quiñones et al., 1998). They also noticed that sensory differences of UF protein standardized milk primarily came from appearance and texture, and changes in protein had greater impact on lower fat level milk than on whole milk (Quiñones et al., 1997; 1998). Interestingly, when lower fat content milk was standardized to higher protein, it showed similar sensory properties to the higher fat content milk due to increased whiteness (Quiñones et al., 1997; 1998). This could be due to more protein in lower fat milk blocked the pathway of light going into the milk and reflected more light back to make the milk more white while in higher fat content milk, milk fat globules reflected light and made the milk white.

Different sources of UF permeates were studied to determine the freezing points of UF protein standardized milks after HTST, direct and indirect UHT processing (Rattray, Gallmann, & Jelen, 1997b; Rattray & Jelen, 1996c). Regardless of different thermal processing, it was reported that the freezing point of skim milk with added UF permeates from skim milk and sweet whey increased while added UF permeates from acid whey and fermented milk decreased the freezing point (Rattray et al., 1997b; Rattray & Jelen, 1996c). Rattray and Jelen (1996c) proposed that a false freezing point result could be avoided by standardizing skim milk with appropriate ratios of different sources of UF permeate. This group further studied the heat stability of protein standardized skim milk (protein range: 3.4-2.6/2.4%) using different UF permeate sources (Rattray, Gallmann, & Jelen, 1997a; Rattray & Jelen, 1996b). Although the heat stability of standardized skim milks increased with increasing amount of skim milk UF permeate and sweet whey permeate at 140°C, only skim milk standardized with skim milk UF permeate was stable during and after UHT processing (Rattray & Jelen, 1996b, 1997a). Triangle tests (n=12, 6 trained panelists and 6 untrained panelists) showed no differences in sensory

quality between skim milk standardized with skim milk UF permeate and regular UHT skim milk (Rattray et al., 1997a).

A process of UF coupled with DF and lactase for the production of lactose free high protein milks was patented by Tossavainen and Sahlsten (2013). Commercially available products have been launched by Valio in Finland and Fairlife in the US, such as flavored and non-flavor fluid products and milk powders. This process may help to increase the fluid milk products sale and consumption in areas with a high population of lactose intolerance consumers.

Cheese making

UF was used for milk for cheese making and many studies had been done in this area since the 1970s. The earliest patented method is the Maubois Macquot Vassal process, in which the milk was ultrafiltered to CF=5-7 before cheese making (Maubois, Mocquot, &Vassal, 1969). Mistry and Maubois (2017) published a good review paper of UF in cheese making. A table of UF in cheese making is summarized below (Table 1).

UF cheese making studies were conducted in fresh cheeses, soft cheeses, semi-hard cheese and hard cheeses, by using low CF (1-2), medium CF (2-5) and high CF (5-8) at normal or adjusted pH. UF was also used in cream cheese. Mistry (2013) summarized the benefits of UF membrane for cheese making: consistence of milk composition, firmer curd, and better recovery of fat and protein and yield increase. For higher CF UF cheese, it was possible to minimize the whey drainage (Mistry & Maubois, 2017). Conventional cheese making equipment could be used in lower CF (1-2) UF cheese milk while higher CF (>2) required specific equipment or modifications. UF cheese making was most successful in fresh and soft cheese manufacture in European and Middle East markets, such as Feta cheese (Horton, 1997). However, UF cheese was not successful in all markets. For example, Camembert was the first commercially produced

UF cheese in France in the 1970s. Regardless of the decent flavor it delivered, this cheese failed on the market due to the noticeable texture difference compared with regular Camembert cheese (Horton, 1997). Medium CF Cheddar and Mozzarella did not gain popular in US due to flavor and texture defects (Swientek, 1984, Garrett, 1987). This could be due to the slower proteolysis in the ripening due to higher retention of whey protein (Mistry & Maubois, 2017; Creamer et al., 1987). Considering both yield and sensory quality, low CF was suggested for Cottage (CF=1.2-1.7), Mozzarella (CF=1.75), Cheddar and hard cheese (CF=1.5-1.7) making (Mistry & Maubois, 2017).

In the US, UF for cheese making was restricted. The FDA approved application of cold (<7°C) UF of milk for Cheddar and Mozzarella cheese making in 1996 (Code of Federal Regulations, 2003). The FDA recommended labeling of UF milk in standardized cheese and related products since Aug. 2017 (FDA, 2017). Govindasamy-Lucey and others (2004, 2005, 2011) extended the lower concentrated retentate (obtained from cold UF) application to Parmesan Cheese, Pizza cheese and Swiss cheese manufacture. Their data showed yield increases for all three types of cheeses and no sensory differences were detected between UF milk cheeses and regular cheeses (Govindasamy-Lucey, Jaeggi, Bostley, Johnson, & Lucey, 2004; Govindasamy-Lucey, Jaeggi, Johnson, Wang, & Lucey, 2005; Govindasamy-Lucey, Jaeggi, Martinelli, Johnson, & Lucey, 2011). Clotting time was reduced in Pizza cheese and Swiss cheese making (Govindasamy-Lucey et al., 2005; Govindasamy-Lucey et al., 2011).

Nanofiltration

NF is primarily used for demineralization of whey and whey permeates. Demineralized whey was used in infant formula to modify the casein to serum protein ratio to 60:40 or in other workout or medical protein drinks to enhance protein load. Demineralized UF permeate was

further processed into lactose powder, which served as a sweetener (infant formula, confection, bakery and tablet making), or substrate for ethanol fermentation or lactose derivatives production (Siso, 1996; Paterson, 2009).

To demineralize whey, traditionally, whey was firstly subjected to evaporation then electrodialysis or ion exchange. However, NF could demineralize and concentrate whey simultaneously, which could reduce energy consumption and gross processing cost. Kelly and Kelly (1995b) suggested that NF was the cheapest option for demineralization of whey. NF rejects lactose in retentate and allows monovalent ions (Na+, Cl-) and water to pass through the membrane. Several studies were done to investigate monovalent ion removal or passage in NF. The present of multivalent ions (such as Ca^{2+}, Mg^{2+}) or trisodium citrate increased the passage of monovalent ions in NF processing of whey (Kelly & Kelly 1995a; Suárez, Lobo, Álvarez, Riera, & Álvarez, 2006; Van der Horst, Timmer, Robbertsen, & Leenders, 1995). Van der Horst et al. (1995) also found that lower flux favored the permeation of monovalent cations while at higher flux, rejection reaches a maximum constant value. Changing pH of cottage cheese whey to 5.5 was reported to achieved the most efficient mineral removal by NF (Barrantes & Morr, 1997). Cuartas-Uribe, Alcaina-Miranda, Soriano-Costa, and Bes-Pia (2007) reported 70-80% monovalent ion removal and 96% lactose retention for UF sweet whey when transmembrane pressure was above 1.5 Mpa. Due to calcium and phosphate fouling on the surface of NF membrane, multiple stages of diafiltration could be applied to achieve a higher mineral removal rate in NF whey processing (Kelly & Kelly, 1995b). Over 90% of the monovalent ions could be removed in cottage cheese whey NF processing by engaging of DF (Barrentes & Morr, 1997; Román, Wang, Csanádi, Hodúr, & Vatai, 2009). Sensory work of the impact of different NF processing conditions on lactose products was not well documented.

Reverse osmosis

RO produces a protein, fat, lactose and mineral enhanced retentate and a disposable and environmentally friendly permeate. Most studies of RO were focused on on-farm milk concentration to reduce transport costs in the 1980-1990s. A promising profit of on-farm milk concentration could be achieved for larger farms (Slack, Amundson, & Hill, 1982; Zall, 1987). Large on-farm RO facilities were built in Texas and New Mexico with processing milk from 2000 cows every day (Fleming & Area, 1999).

RO can also be used to increase total solids of retentate before drying or other processing. RO is applied in whole and skim milk powder processing to primarily replace the evaporator and produces identical products as the commercial evaporated powders (Abbot, Glover, Muir & Skudder, 1979; Park et al., 2014b). RO cheese milk had higher lactose content, which required 50% less starter culture and 60% less rennet compared with regular cheese milk (Agbevavi, Rouleau, & Mayer, 1983). RO milk produced a good quality aged Cheddar (Bynum and Barbano, 1985). They observed a 2-3% cheese yield increase when milk volume was reduced about 20% compared to the theoretical yield. Increased retention of whey solids and milk fat recovery was reported as the cause of yield increase (Barbano & Bynum, 1984). RO concentrated whey and skim milk UF permeates were used as a salt substitute (Smith, Metzger, & Drake, 2016a).

Conclusions

Membrane technology provides a wide range of dairy ingredients with diverse functional and sensory properties. It makes milk protein standardization to specific true protein levels and even specific casein as a percentage of true protein levels possible, which could open new

avenues for fluid milk innovation. To generate a platform for future milk protein beverage application, from the manufacturing to the consumer end, the objectives of this dissertation are:

1) to determine the differences in sensitivity of Hunter and CIE methods at two different viewer angles (2 and 10 degrees) for measurement for whiteness, red/green, and blue/yellow color of milk based beverages over a range of composition;

2) to determine the impact of pasteurization-homogenization, fat and protein concentration, the proportion of milk protein that is casein and serum protein, and temperature on sensory and instrumental measures of viscosity and color of milk based beverages and to use instrumental measures of whiteness and yellowness to predict sensory measures of whiteness and yellowness.

3) to determine the impact of systematically controlled variation in milk fat, true protein, casein, and serum protein concentrations on the sensory color, flavor and texture properties, instrumental color and viscosity, and milk fat globule size distribution of milk based beverages.

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Table 1. UF in cheese making (modified from Jenson and KØnigsfeldt (2000).

Cheeses type	UF processing-concentration factor			
	(1-2)	(2-5)	(5-8) normal pH	(5-8) normal pH around 4.5
Fresh cheeses	Cottage		Ricotta	Quarg, Cream cheese, Fromage frais
Soft cheeses	Camebert, Brie, Mozzarella	Blue cheese, Blue& White, Camebert, Brie, Mozzarella		
Semi-hard cheeses	Danbo, Feta, structured white cheeese,Colby, Edam cheese	Havarti, Gouda, Danbo, Feta, structured white cheese	Feta, Quartirolo	
Hard cheeses	Cheddar	Cheddar	Cheddar	

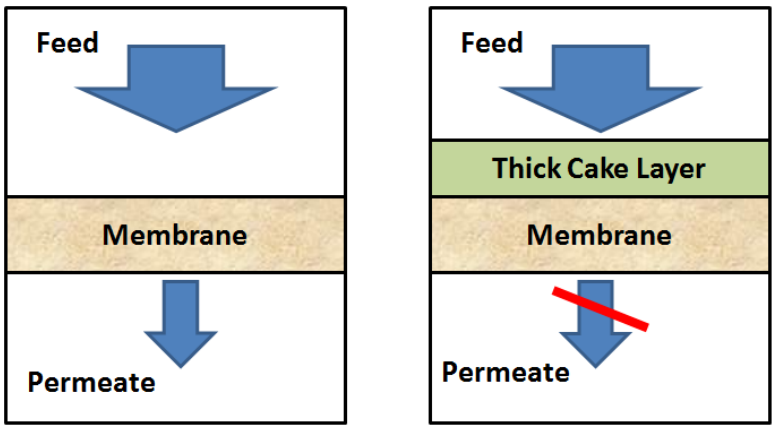

Figure.1 Diagram of deadend filtration.

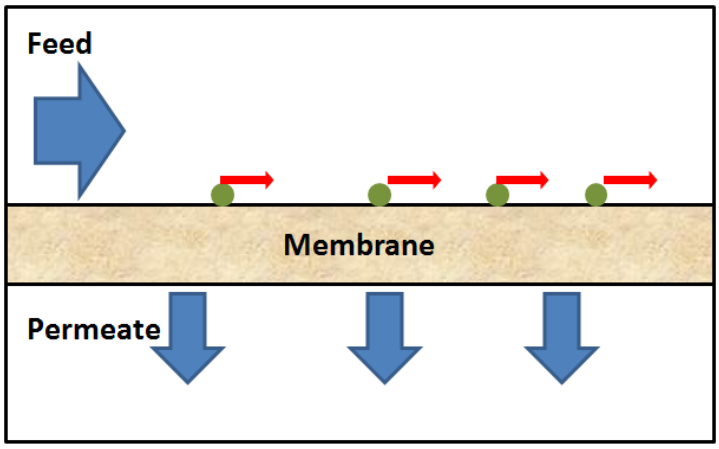

Figure.2 Diagram of cross flow velocity filtration.

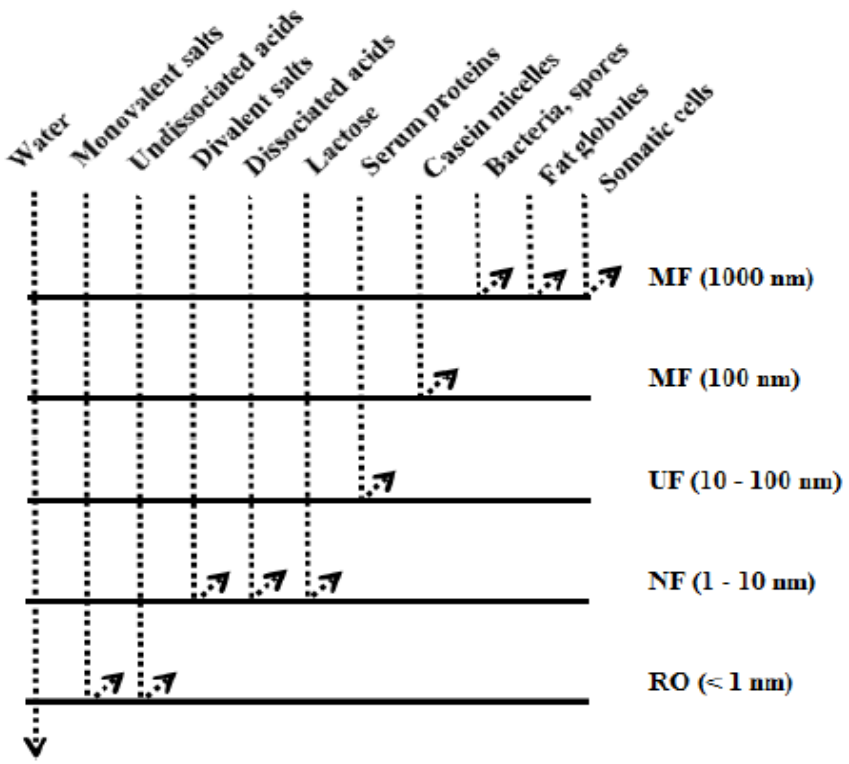

Figure.3. Passed and rejected dairy components based on membrane pore size (modified from Adams, 2012).

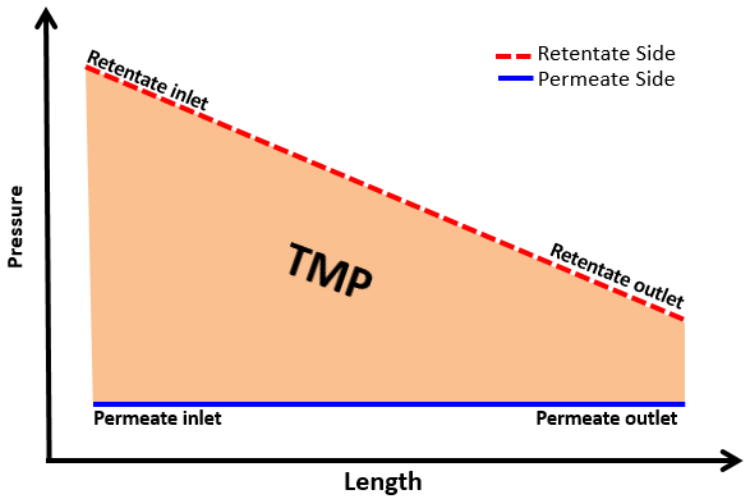

Figure. 4. Pressure difference on the retentate and permeate sides at inlet and outlet in ceramic MF membrane (TMP= Trans-Membrane Pressure) (Modified from Adams, 2015).

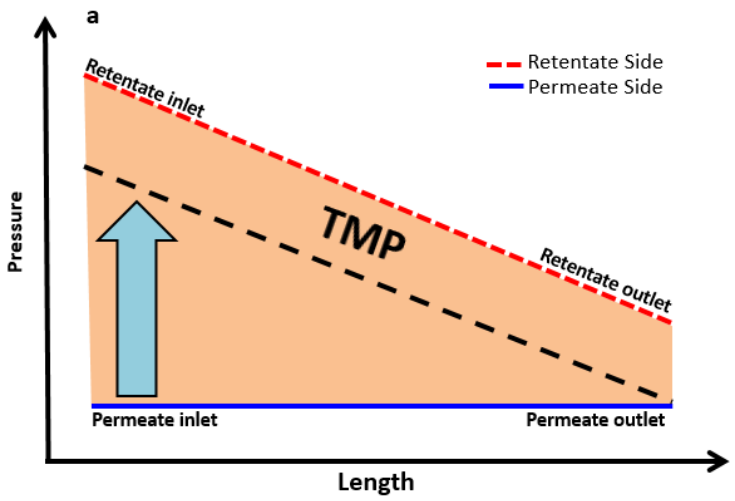

Figure.5 a) Uniform Transmembrane Pressure (UTP) concept of ceramic membrane (TMP= Trans-Membrane Pressure) (Modified from Adams, 2015).

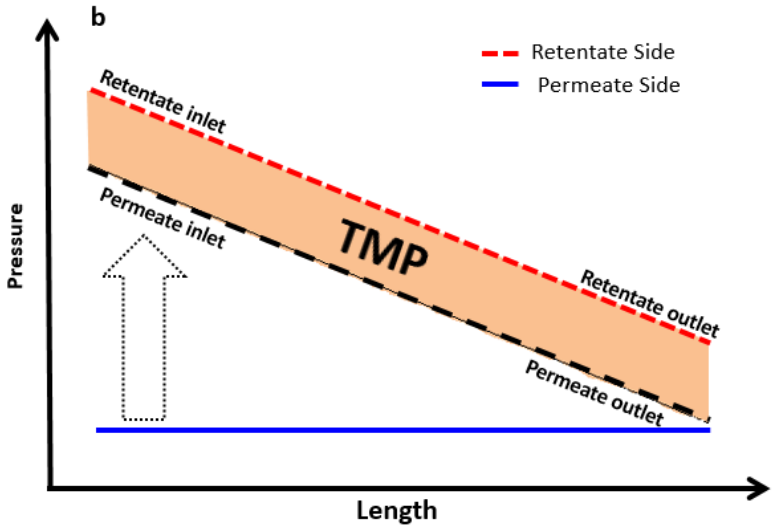

Figure.5 b) Uniform Transmembrane Pressure (UTP) MF membrane pressure diagram (TMP= Trans-Membrane Pressure) (Modified from Adams, 2015).

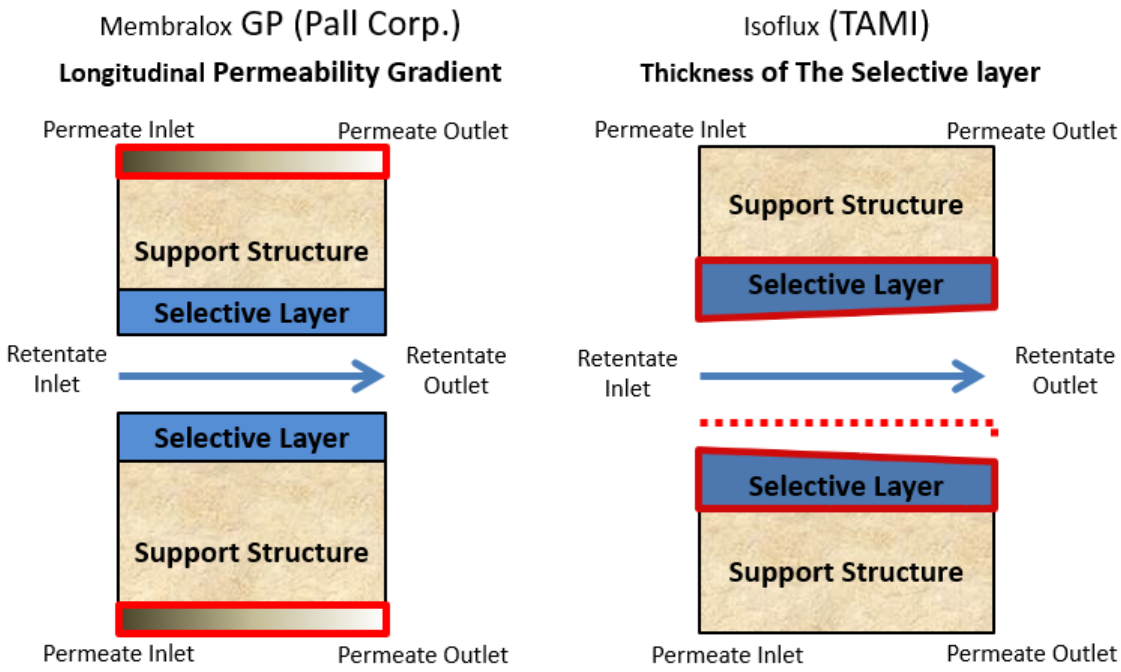

Figure 6. Membrane structure diagram of GP MF and Isoflux (Modified from Adams, 2015).

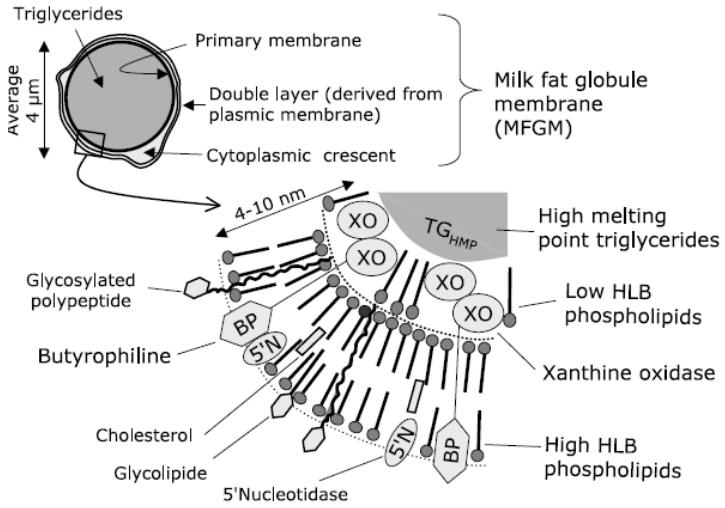

Figure.7. Native milk fat globule structure (Michalski, Michel, Sainmon, & Briard, 2002).

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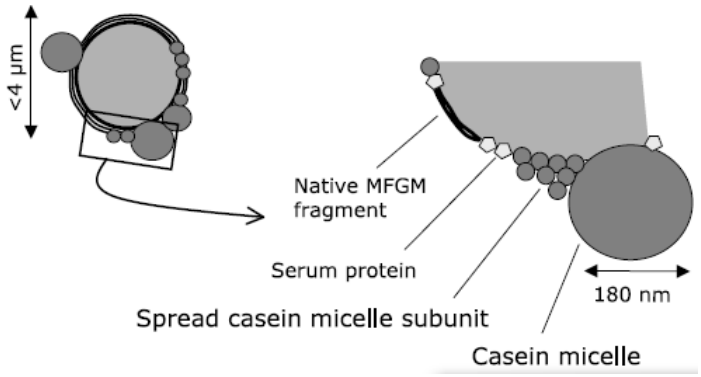

Figure.8. Mechanical disruption of MFGM (Michalski et al., 2002).

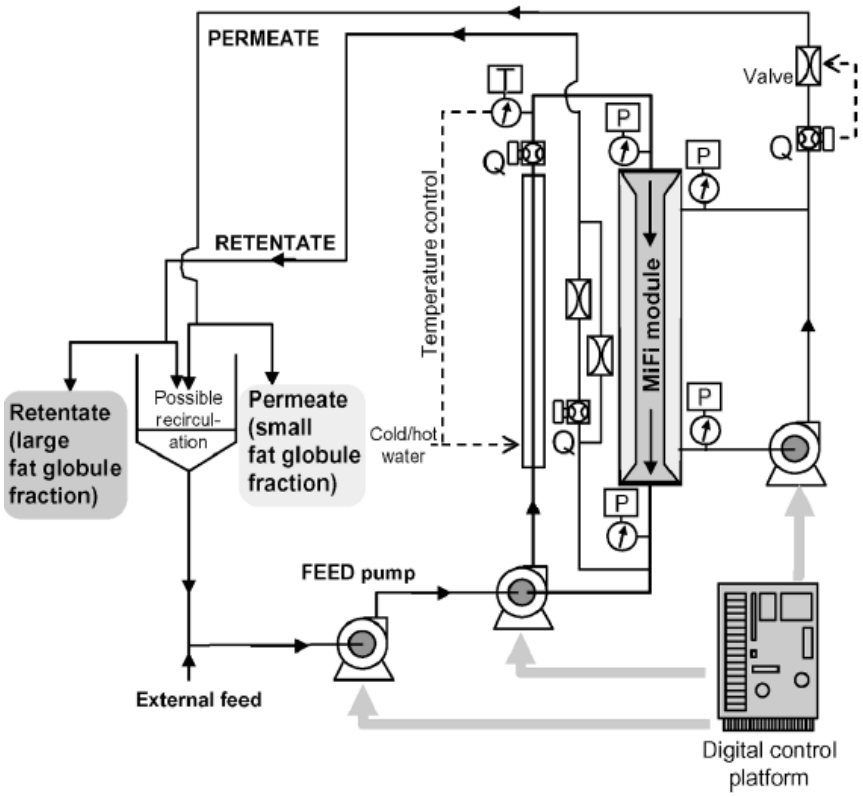

Figure.9. Set-up of the microfiltration system for SFG and LFG production (Michalski et al., 2006).

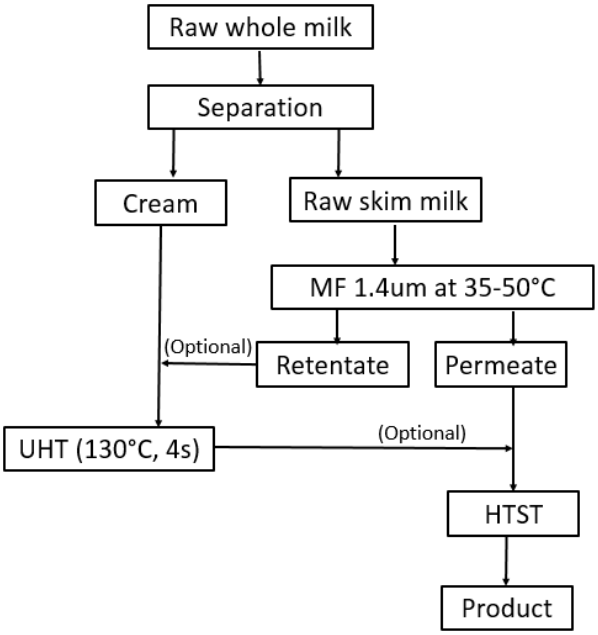

Figure.10. Process chart of MF bacterial reduction (Modified from Holm et al., 1986).

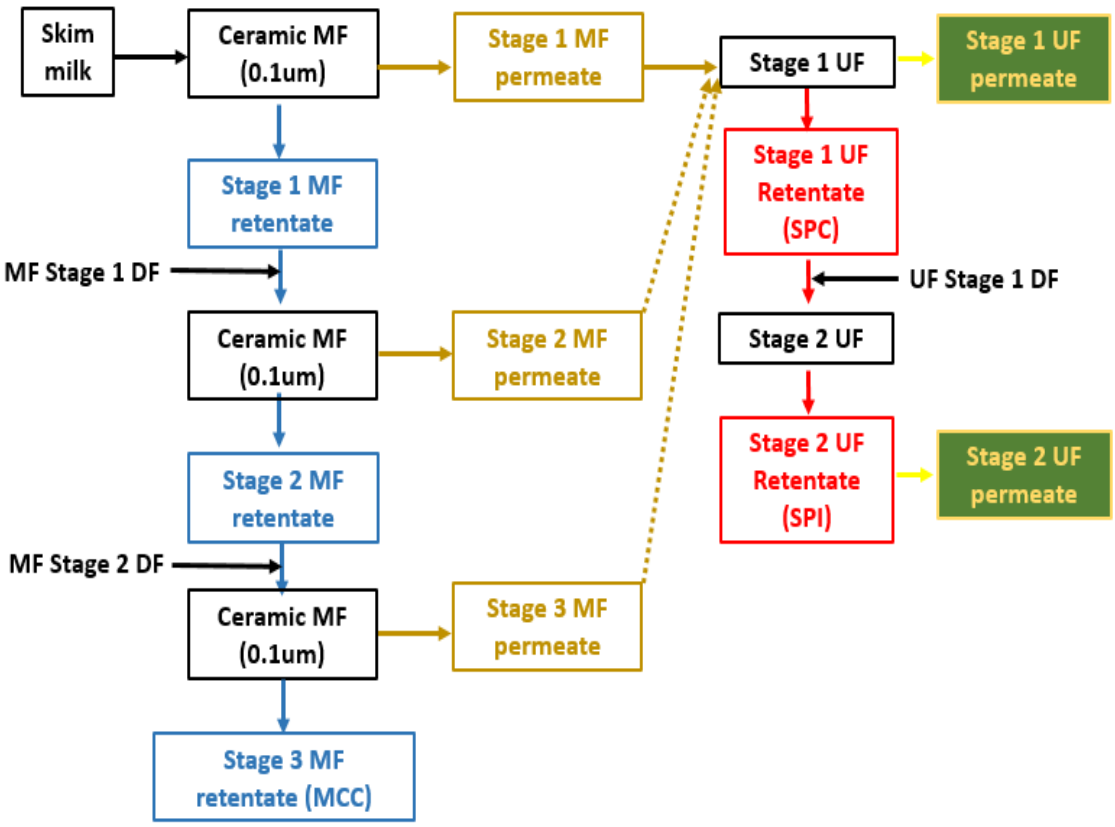

Figure.11. Processing flow chart for a 3stage, 3x MF for MCC production and 2 stage UF for SPI production (a. MF DF source could be: Deionized water, RO water or Stage 1 UF permeate. b. UF DF source could be: Deionized water or RO water. c. Stage 2 MF permeate and stage 3 MF permeate as Stage 1 UF feed was optional).

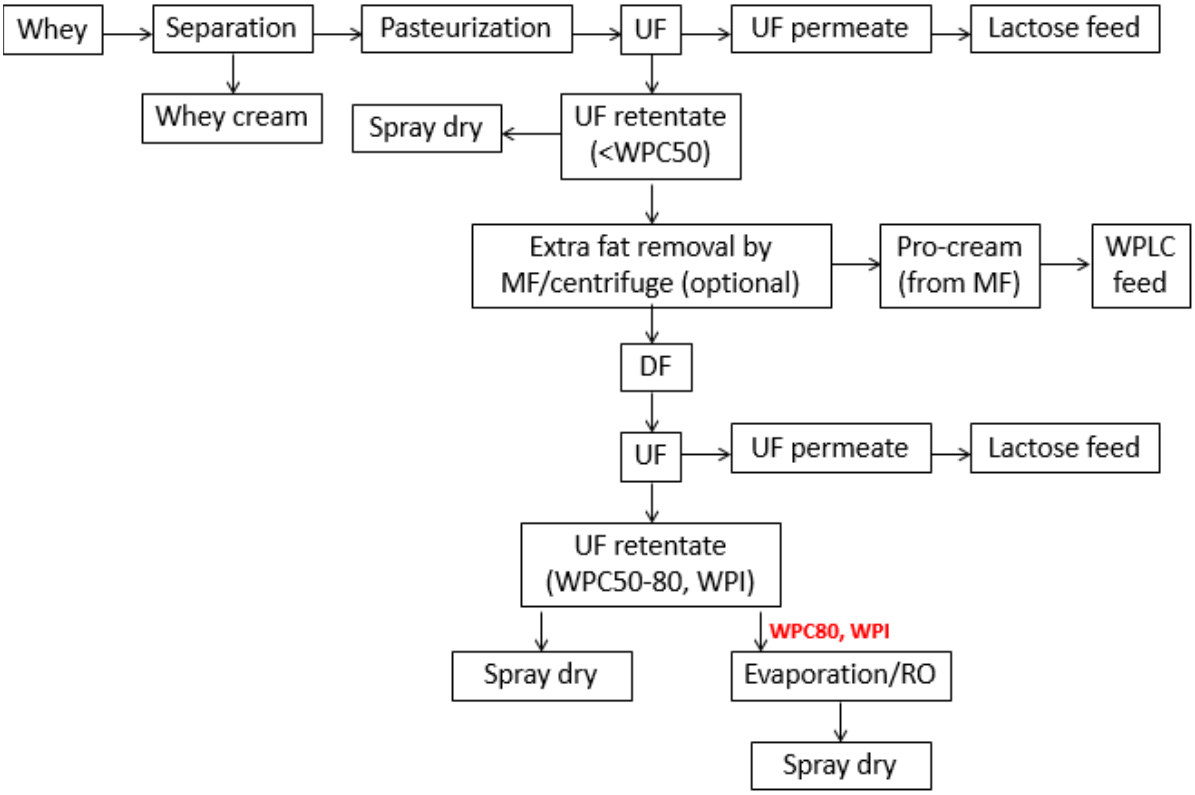

Figure. 12 Processing of WPC and WPI powders.

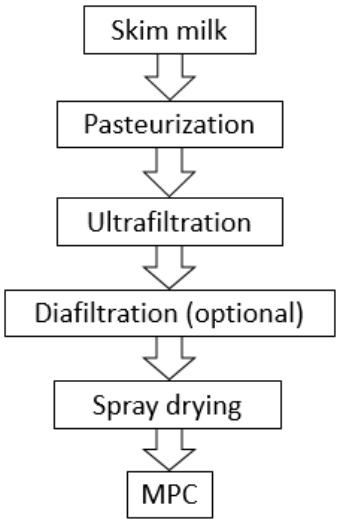

Figure.13. Milk protein concentrate processing flow chart. (Also applied in MPI production, modified from Patel & Patel, 2014).

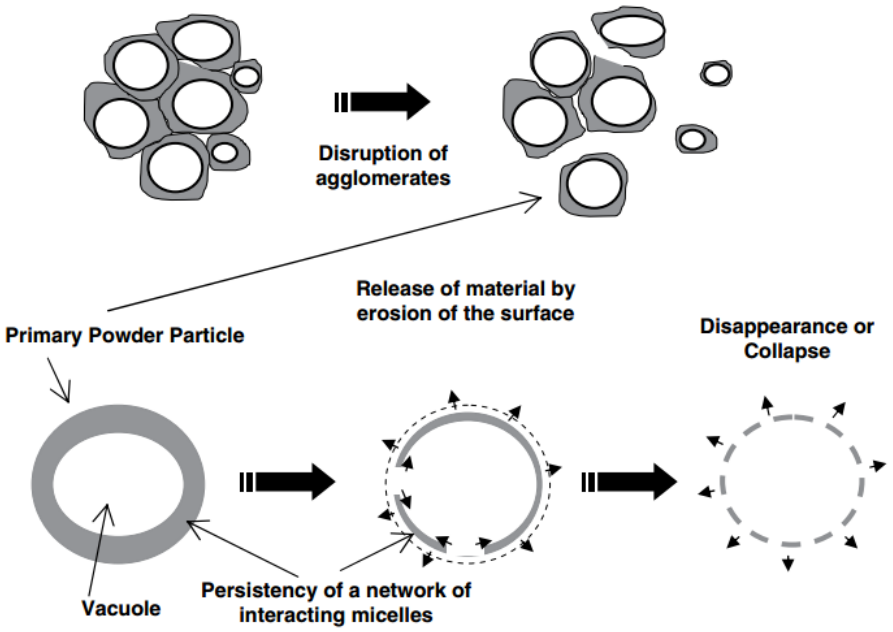

Figure. 14. Hypothesis for the rehydration process of MPC85 powder (Mimouni et al., 2009).

CHAPTER 2: HUNTER VERSUS CIE COLOR MEASUREMENT SYSTEMS FOR ANALYSIS OF MILK BASED BEVERAGES

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* Use of names, names of ingredients, and identification of specific models of equipment is for scientific clarity and does not constitute any endorsement of product by authors, Cornell University, or the Northeast Dairy Foods Research Center.

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Abstract

The objective of our work was to determine the differences in sensitivity of Hunter and CIE methods at two different viewer angles (2 and 10 degrees) for measurement for whiteness, red/green, and blue/yellow color of milk based beverages over a range of composition. Sixty combinations of milk based beverages were formulated (2 replicates) with a range of fat level from 0.2 to 2%, true protein level from 3 to 5%, and casein as a percent of true protein from 5 to 80% to provide a wide range of milk based beverage color. In addition, commercial skim, 1 and 2% fat high temperature short time pasteurized fluid milks were analyzed. All beverage formulations were HTST pasteurized and cooled to 4^{o}C prior to analysis. Color measurement viewer angle (2 versus 10 degree) had very little impact on objective color measures of milk based beverages with a wide range of composition for either the Hunter or CIE color measurement system. Temperature (4, 20, and 50^{o}C) of color measurement had a large impact on the results of color measurement in both the Hunter and CIE measurement systems. The effect of milk beverage temperature on color measurement results was the largest for skim milk and the least for 2% fat milk. This highlights the need for proper control of beverage serving temperature for sensory panel analysis of milk based beverages with very low fat content and for control of milk temperature when doing objective color analysis for quality control in manufacture of milk based beverages. The Hunter system of color measurement was more sensitive to differences in whiteness among milk based beverages than the CIE system, while the CIE system was much more sensitive to differences in yellowness among milk based beverages. There was little difference between the Hunter and CIE system in sensitivity to green/red color of milk based beverages. In defining milk based beverage product specifications for objective color measures

for dairy product manufacturers, the viewer angle, color measurement system (CIE versus Hunter) and sample measurement temperature should be specified along with type of illuminant.

Key words: Color, milk, whiteness

Introduction

Color influences sensory perception and consumer preference of food products. A review by Cyldesdale (1993) summarized studies focusing on color effects on taste thresholds, sweetness perception, pleasantness, salt perception, preference and acceptability and perception in an elderly population of food and beverages. More recently, Chung (2009) studied consumer preference of full fat, low fat and lactose free milk pasteurized by ultra high temperature (UHT) or low temperature long time (LTLT) and milks with higher whiteness and lower yellowness were perceived as having higher flavor intensity. McCarthy et al. (2017) conducted a fat threshold test in fluid milk with a preference follow-up interviews and reported better color or color/whiteness was an important reason that skim milk, 2% fat milk and whole milk drinkers all preferred higher fat level milks. Further, consumers were less sensitive to differences in fat concentration when correlated appearance differences were removed, confirming the role of appearance and color on sensory properties of fluid milk.

Phillips et al. (1995a, 1997) and Misawa et al. (2016) concluded that objective measurement of whiteness showed correlations with several appearance, texture and flavor sensory descriptors for lowfat milks. Phillips et al. (1995a) found that increasing milk fat level (0.06 to 2.0%) increased L-value (whiteness) and decreased a-value (greenness to redness) and b-value (blueness to yellowness) of milk, which were well correlated with perceived color of lowfat milk by sensory analysis with trained panels. Philips et al. (1995a) also reported using trained panelists that milk appearance largely influenced the perceived mouthfeel of milk. This confirmed the results from Pangborn et al. (1985) that visual cues were important for milk differentiation based on fat level. McCarthy et al. (2017) confirmed the importance of color with consumer panelists. Addition of nonfat dry milk (0 to 2%) to lowfat milk showed no difference

in color and sensory perception of milk (Phillips et al., 1995b) while addition of titanium dioxide made skim milk whiter and increased the trained panel sensory scores for milk appearance descriptors (Phillips et al., 1997). Increasing true protein level (Quiñones et al., 1997, 1998) and casein as a percentage of true protein (Misawa et al., 2016) increased whiteness of low fat milks and caused the sensory perception of these low fat milks to be more similar to higher fat level milks.

Hunter (L, a, b) and International Commission on Illumination (**CIE**) L*, a* and b* color measurement systems or color space are primarily used in food, textile and pharmacy industries. Both color measurement systems were developed based on the CIE (x, y, z) 1931 system to generate a more uniform color space for color measurement with specific calculation equations for L/L*, a/a* and b/b* (Hunter and Harold, 1987). The primary difference in these two color system is the Hunter (L, a, b) equations use the square root of CIE (x, y, z) for L, a, b calculation while the CIE (L*, a* and b*) equations use the cubic root (Hunter and Harold, 1987). However, both color systems are not ideally uniform. The Hunter (L, a, b) system is more sensitive in measuring blueness while the CIE (L*, a*, b*) system is more sensitive in measuring yellowness and dark colors (Hunter Associates Laboratory, 2012; CIE 1978; Hunter and Harold 1987). Today, the L/L*, a/a* and b/b* values can all be obtained easily from colorimeter software and the most repeatable and sensitive metrics can be selected for use by an analyst. However, it is not always clear if the Hunter or CIE color measurement system is the best for analysis of milk based beverages. The objective of our work was to determine the differences in sensitivity of Hunter and CIE methods at two different viewer angles for measurement for whiteness, red/green, and blue/yellow color of milk based beverages over a range of composition.

Materials and Methods

Experimental Design

Population of Beverage Formulations and Commercial Milks.

Beverage formulations were based on a complete balanced 3 factor (fat, true protein and casein as a percentage of true protein) design with 3 fat levels (0.2, 1.0 and 2.0%), 4 true protein (**TP**) levels (3.00, 3.67, 4.34 and 5.00%) within each fat level and 5 casein as a percentage of true protein (**CN%TP**) levels (5, 25, 50, 75 and 80%) within each protein level as shown in Table 1. The formulation, sensory and analytical work was done in one week for each fat level and there was a replication of the formulation, processing, and analysis for all the treatments within each fat level in a second week, for a total of 6 weeks of processing. Color of commercial skim, 1% and 2% fat milks was also evaluated using the same analysis methods. There were 2 replicates of the commercial milk at all 3 fat levels for color analysis by both methods.

Comparison of Hunter versus CIE color metrics.

The goal of this data collection was to determine the impact of viewer angle (2 vs 10 degrees) and color measurement system (Hunter vs CIE) for evaluation of milk based beverage color and was determined by the following comparison. For a color term, within each color metrics, the value differences observed at 2 and 10 degree viewer angles in response to changes in beverage composition were compared; while within each viewer angles, the value differences obtained from Hunter and CIE systems in response to changes in beverage compositions were compared to determine if the color differences among treatments were different under different objective color measurement approaches.

Impact of Processing and Composition Parameters on Milk Beverage Color

It is known that thermal processing causes protein-protein interactions (Corredig and Dalgleish, 1999; Donato and Guyomarc'h, 2009; Singh 2004) and thermal degradation of milk

proteins (Meltretter et al., 2007; Van Boekel, 1998) and lactose (O'Brien, 2009) and these degradation products and interactions may influence color and viscosity of milk-based beverages. Caseins and milk serum proteins differ in their heat denaturation and heat induced interaction characteristics. The effects of heat on milk proteins may influence temperature dependent dissociation of casein and milk minerals in and out of casein micelles (Downey and Murphy, 1970; Rose, 1968) and this may influence observed color. The inclusion of these composition (fat, protein, and casein as a percent of true protein) and processing (raw and pasteurized) parameters in the population of milk based beverages in the current study will provide a robust test of the factors influencing results from the Hunter versus CIE color measurement systems at different milk testing temperatures and help to determine when one or the other of the color measurement systems should be in combination with a properly selected analysis temperature should be used for analysis of milk based beverages.

Analysis Methods

Chemical Composition.

Milk based 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.

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 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 Kaylegain et al. (2009).

Color. Milk protein beverages (raw and pasteurized) and commercial skim, 1%, and 2% fat HTST milks were tested in duplicate using an Ultra Scan Pro Spectrophotometer (Hunter Associates Laboratory, Inc., Reston, VA) at 4, 20 and 50°C. A water bath was used to maintain sample temperature at 4 or 20 or 50°C. Milk beverages were measured in reflectance mode, wavelength from 360 to 750 nm with a 5 nm resolution, Illuminant A at both 2 and 10 degree viewer angles. The cuvette was glass with a 20 mm path length. Color data were collected using both Hunter L, a, and b-values and CIE L*, a* and b*-values.

Milk Based Ingredients

Milk Micellar Casein Concentrate. Liquid MCC was made the day before beverage formulation. 530 kg of raw skim milk was obtained from North Carolina State University Dairy and HTST pasteurized (11.34 kg /min) using a plate heat exchanger (model T4 RGS- 16/2, SPX

Flow Technology, Greensboro, NC) at 72 °C with a hold time of 16 s. The pasteurized skim milk was cooled to 4°C, prefiltered by a Nexis T Filter (NXT 10-30U-M7S, Pall Corporation, NY) and stored at 4°C in a 1200 kg jacketed stainless steel tank (type A & G, Chester-Jensen Company, Chester, PA). The pasteurized and pre-filtered skim milk was heated using a plate heat exchanger (Serial No.G201400849, Plate ID: SR1, SPX Flow Technology, Greensboro, NC) to 50°C, weighed and poured into the MF balance tank (190 kg, NUVAT for cheese making. Meyer-Blank Company. St Louis. MO) jacketed with 50°C water.

A 3 stage, 3x microfiltration process described by Zulewska and Barbano (2014) was used to produce a 95% serum protein removed MCC with true protein concentration between 8.4 to 8.6% with the following differences in procedure. The cleaning before and after processing, clean water flux and warm up procedure was done as described by Zulewska et al. (2009). The MF was started by filling the flow system with deionized (DI) water and the retentate and permeate removal rates were set to 48.0L/h and 110L/h respectively to achieve a 3x MF mass/mass concentration factor and cross-flow velocity was kept around 7.0 m/s. It is very important that all air is removed from the system on both the retentate and permeate side of the membranes. Ceramic membrane modules are mounted vertically. If there is a head space of air at the top (retentate inlet end) of the membrane module on the permeate side, then when the pumps are started permeate will flow through that area of the ceramic stick very fast where there is no liquid providing back pressure. The will cause an immediate fouling of the membrane and a shortened processing run.

Once switching to milk, the MCC production began and all retentate and permeate were collected and weighed and CF was controlled based on these weights. The pasteurized and pre-filtered warm skim milk was microfiltered at 50°C with a 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 (surface area of 1.68 m^2, membrane length of 1.02 m). The MF retentate and permeate removal rates were set to theoretical target values to achieve a 3x MF mass/mass concentration factor with the retentate removal rate was set to 48.0, 54.0, and 52.8 L/h and the permeate removal rate at 110, 120, and 120L/h for stages 1, 2 and 3, respectively. The retentate recirculation pump rate was increased gradually to maintain the cross-flow velocity around 7.0 m/s during processing. The retentate removal rate was adjusted during the run to control the concentration factor based on flux and infrared composition data every 15 min.

The first 160 kg of milk taken into the MF system were used to flush water out of the system and both the retentate and permeate collected during this time were discarded. At that point the flush ended and MF stage 1 started. Next, 370 kg more milk at 50^{o}C was used as the feed for the first stage. The second and third stages were diafiltration stages with same weight of DI water added to the retentate as permeate removed in the previous stage. The MF system was run continuously and not stopped and restarted from stage to stage as in Zulewska and Barbano (2014). The stage one 3X retentate plus DI water was heated to 50°C and fed into the MF for stage 2 of the process. At beginning of stage 2, the stage 1 retentate plus water mixture MF was recycled for 15min with both the retentate and permeate going back into the feed tank. The protein concentration of MF retentate was checked using the MIR milk analyzer and once a protein concentration of 8.4 to 8.6% was achieved, the collection of stage 2 MF retentate and permeate started. The same diafiltration process was done for stage 3. At end of MF stage 3, all retentate was mixed, cooled to 4°C, bottled into half gallon light shielding milk jugs (Upstate Niagara Cooperative, Baffulo, NY) and stored in 4°C walk-in cooler.

Serum Protein Isolate (SPI). To produce enough SPI for the study, several batches of SPI were produced and combined prior to the beverage study to obtain a large batch of liquid SPI. For production of each SPI batch, 1150 kg or 460 kg of raw skim milk was obtained from North Carolina State University Dairy. The raw skim milk was processed using the same procedure as it was in the MCC production before feeding to the MF. The MF processing of pasteurized and pre-filtered skim milk was a single stage process with the purpose of collecting stage 1 MF permeate with a high SP concentration. The stage 1 MF permeate was collected and fed into the UF. A 50°C, 2-stage UF process was used to produce liquid SPI with a protein concentration of about 25%. Two UF membrane systems (model Pellicon 2, Millipore Inc., Billerica, MA) were setup in parallel with each one of them equipped with 5 polyethersulfone cartridge membrane filters (model P2B010V05, nominal separation cutoff = 10,000 kDa, surface area = 0.5 m^2 per cartridge). A variable speed peristaltic pump (model 77410–10, Cole-Palmer, Vernon Hills, IL) equipped with 2 model 77601–00 pump heads (Cole-Palmer, Vernon Hills, IL) with silicone tubing (model 96440–73, Cole-Palmer, Vernon Hills, IL) was used. The 1% sodium hydroxide (Food Grade, Sigma-Aldrich, St. Louis, MO) membrane storage solution in the UF unit was flushed out with 50°C DI water for 20 min. This was done to ensure that residuals from cleaning and sanitizing compounds did not contaminate the product produced by the batch process. A UF clean water flux was taken by collecting flow from both permeate outlets for 30 sec. The UF clean water flux was 1300 to 1500 g/30sec, at 50°C with no back pressure. During the UF processing, about 124 to 138 kpa back pressure was applied on the UF retentate side of the membranes. The retentate outlets were put into UF balance tank for recirculation in a batch concentration mode for each stage. UF permeate was collected, weighted and discarded. UF retentate and permeate were collected to check flux and composition every 30

min. The stopping point of UF stage 1 was defined as when the of retentate protein as a percentage of lactose plus protein plus fat of UF retentate reached 41%. The second stage of the UF process was a DF stage. The diafiltration water was HTST pasteurized (11.34 kg /min) with a plate heat exchanger (model T4 RGS- 16/2, SPX Flow Technology, Greensboro, NC) at 72 °C for 16s and cooled to 50°C. The DI water added for stage 2 was the same amount as the permeate removed in UF stage 1. The UF stage 2 was stopped when the protein concentration in the UF retentate researched 28% measured using the MIR milk analyzer. Both retentate and permeate of UF stage 2 were collected and weighted. The SPI (i.e., second retentate) was mixed and bottled into half gallon milk jugs, and stored at -18°C. For cleaning after processing, the 50°C DI water was used to flush UF unit for 30 min. A 50°C, 1.0 % Hydroflux NP No.366 (Manufacture No. FP036601, Hydrite Chemical Co., Brookfield, WI) was used for alkaline cleaning and recirculated for 25 min. Next, 50°C DI water was used to flush UF unit for 25 min. A 50°C, 0.5% Reflux No.193 (Manufacture No. FP019301, Hydrite Chemical Co., Brookfield, WI) was used for acid cleaning and recirculated for 20 min. Next, 50°C DI water was used to flush for 20 min. The UF unit was disassembled and the UF membranes and gaskets were stored in a 1% sodium hydroxide solution at 4°C until their next use. Prior to the beginning of the beverage formulation portion of the project, all the batches of frozen SPI were thawed by moving the frozen containers of SPI into a 4ºC cooler for a 3-d thawing. During a couple of the UF processing runs for SPI production, cleaning problems were encountered. Each SPI bottle was screened for potential microbial off flavors by trained panelists (n=3) and only SPI bottles that passed the screening were mixed in cheese vat (230kg, Kusel Equipment Co. Watertown, WI) and was tested for protein content by MIR. The target protein content of the liquid SPI was about 25 to 28%.

Cream. A cold bowl separator (model 590, Separators Inc., Indianapolis, IN) was used for raw cream separation and the pasteurized (80^{o}C for 25 sec) cream containing about 41% fat was obtained from North Carolina State University Dairy and stored at 4°C.

Lactose and water. Lactose monohydrate (Hilmar^{TM} 5120 if Refined Edible Lactose 200 mesh, 25kg/bag) was donated by Hilmar Ingredients (Hilmar Ingredients, Hilmar, CA). Potable water was processed to produce DI water using a deionizing water system (unit number: 1933-2**,** Mar Cor, Raleigh, NC).

Beverage Formulation

Formulations (9,500 g per batch) were calculated using Microsoft Excel linear optimization solver function (Redmond, WA) as described by Misawa et al. (2016) with the following differences. MCC, SPI, cream, lactose and DI water were used as the raw ingredients. Within each fat level (0.2, 1, or 2% fat), the TP and CN%TP were targeted at 4 TP levels (3.00, 3.67, 4.34 and 5.00%) and 5 CN%TP (5, 25, 50, 75 and 80%), respectively. Anhydrous lactose concentration was standardized to 4.65% for all formulations. In total, there were 20 combinations of TP and CN%TP for each fat level in one replicate. The experiment with 20 formulations was done twice at each fat level starting with freshly produced MCC and cream. Two processing runs for skim were complete in two different weeks and the same was done for the formulations within the 1% and 2% fat levels for a total of six different formulation and sensory sessions.

On the formulation day, MCC, SPI, cream, lactose and DI water were kept in a 4°C walk-in cooler before use. As weights of ingredients varied from one formula to the next, a 620 g max weight balance (model: MS603TS/00, Mettler Toldedo, Switzerland), a 3200g max weigh balance (model: MS3002S/03, Mettler Toldedo, Switzerland), and a 32200g max weight balance

(model: MS32001L/03, Mettler Toledo, Switzerland) were calibrated with standard weights (Serial No. B628758127, Mettler Toledo, Columbus, OH) to check for the accuracy and linearity of each balance. A specific balance was selected for weighing an ingredient in a formulation so that the weight of each ingredient was within the linear weight range of the balance. Translucent round food storage containers (11.36L, Manufacture No. FG572624, Rubbermaid, Atlanta, GA) with yellow lids (Manufacture No. 1980386, Rubbermaid, Atlanta, GA) were used for formulation. Beverages were formulated one by one with a specific ingredient adding order (DI water, lactose, MCC, SPI and cream). Lactose was completely dissolved in DI water before adding other ingredients. Weights of each ingredient were recorded and checked to make sure within a tolerance range of +/-0.1g and stored at 4°C. MIR was used to verify the fat, lactose and protein concentration in the milk based beverages before and after pasteurization.

Pasteurization and Homogenization

Prior to the beginning of the first processing trial practice runs were done with the system in the same configuration as would be used in the study to determine the dead volume of the processing system and the amount of liquid that needed to run to purge water (at start up) out of the system. This was done by measuring the freezing point (Cryoscope, Model No.4250, Advanced Instruments. Inc., Norwood, MA) and the fat, protein, and lactose concentration (MIR analysis) to determine how much product (and time in seconds) needed to purge through the processing system before collecting product for sensory and chemical analyses.

Within a fat level for each replicate, 9,500 grams each of 20 raw milk beverages were well mixed individually and continuously fed to a Microthermics EHVH pasteurization unit (Microthermics, Raleigh, NC) with a 2-stage homogenizer (GEA Niro Soavi, Parma, Italy) at flow rate 2L/min. Each beverage formulation was preheated to 60°C, homogenized (1st stage at

17.3 MPa and 2nd stage at 3.4 MPa), HTST pasteurized (73°C for 15 s) and cooled to 10°C. A timer was started once switching from water to the first beverage or from the previous beverage to the next one. Once the timer reached 4 min, pasteurized beverage was collected into a labeled half-gallon milk jug. The end of the previous beverage collection and the switching point to the next beverage happened at 4 min 45 sec. Once a collection was done, the milk jug was sealed with a sanitized lid and stored in crushed ice temporally. Pasteurized milk beverages were stored at 4°C after sampling.

Statistical Analysis

Milk Based Beverages: Viewer Angle and Color Measurement System. The general linear models (**GLM**) procedure of SAS (version 9.4, SAS Institute Inc., Cary, NC) was used to determine the effect of viewer angle of color measurement, fat (0.2, 1, and 2%), TP (3.0, 3.67, 4.34, 5.0%) , CN%TP (5, 25, 50, 75, and 80%), heat treatment (raw and pasteurized), measurement temperature (4, 20, and 50^{o}C) of color of milk protein beverages. All interactions of these parameters were included in the model. Viewer angle, fat, TP, heat treatment and temperature were treated as categorical variables while CN%TP was handled as a continuous variable. The CN%TP was mean-center (Misawa et al., 2016) transformed to avoid co-linearity effects on statistical analysis (Glantz and Slinker, 2001) and named as CNTPT in models. The CN%TP was transformed by subtracting the mean CN%TP from each of the individual CN%TP and using these “mean-centered” data in the statistical analysis to provide a better point of reference in parsing the relative strength of main effects and interaction effect of factors in the model. If the F-value for the full model was significant ($P < 0.05$), then significance ($P < 0.05$) of each factor and their interactions was determined. The category effects of viewer angle, fat, TP, heat treatment, temperature of color measurement, and replicate and their interactions were

tested for significance using the interaction term of viewer angle*fat*heat*prot*temp*rep, while the effects (linear and quadratic) of the continuous variable of CN%TP and their interactions of the categorical variable were tested for significance using the full model error. The analysis of the color data for all factors and their interactions was done first, if the F-value for the full model was < 0.05, then a stepwise process was done to remove all non-significant terms from the model to produce a final reduced model. The Type III sum of squares table was produced and the r-squared for the reduced model was reported. To better understand the relative impact of each factor or interaction of factors on each color parameter, the Type III sum of squares value for each term was divided by the total Type III sum of squares of the significant terms in the model to determine the relative impact of each factor and interactions of factors.

The effect of system of color measurement (Hunter versus CIE), fat (0.2, 1, and 2%), TP (3.0, 3.67, 4.34, 5.0%) , CN%TP (5, 25, 50, 75, and 80%), heat treatment (raw and pasteurized), measurement temperature (4, 20, and 50°C) of color of milk protein beverages were determined by using the same ANOVA model described above with replacing the viewer angle term with system term.

Commercial Milks with Different Fat Levels. The GLM procedure of SAS (version 9.4, SAS Institute Inc., Cary, NC) was used to determine the effect of color measurement system, fat level (skim, 1, and 2%), and measurement temperature (4, 20, and 50^{o}C) of color of commercial HTST pasteurized milks. All interactions of these parameters were included in the model. Color measurement system, fat and temperature were treated as categorical variables. The same stepwise process was done as described previously to produce a final reduced model and a Type III sum of squares table. R-squared for the reduced model was reported. The Type III sum of squares value for each term was divided by the total Type III sum of squares of the

significant terms in the model to clarify the relative impact of each factor and interactions of factors. Additionally, a similar GLM procedure was applied on milk color data within each fat level and within each color measurement system to determine the impact of measurement temperature, using the lsmeans procedure of GLM if the F-value for the model had a $P < 0.05$.

Results and Discussion

Evaluation of Methods of Color Measurement

Viewer Angle. The Hunter L, a, and b-values and CIE a*, b* and L*-values for a wide range of milk based beverage compositions for a viewer angle of 10 and 2 degrees are shown in Table 2. Most of the variation in instrumental measurement of color for the beverages in this study was explained by variation in fat, CN%TP, and thermal treatment of the beverages (Table 3). The variation in CN%TP (5 to 80% of true protein) had a much larger (Table 3) impact on color than variation in true protein (3 to 5%). Variation in fat had a larger impact on a and b values than on L-value, while variation in CN%TP had much large impact on L-value than fat for the combination of raw and pasteurized beverages. The impact of component variation and processing on instrumental and sensory measures of color will be discussed in more detail in a separate paper.

No significant effect ($P > 0.05$) of viewer angle or interactions of viewer angle (10 versus 2 degrees) were detected on either the Hunter L-value or CIE L*-value measurements (Table 3). There was only a small impact of viewer angle (terms for angle and CNTPT*viewer angle) on the Hunter a and b-values and the CIE a* and b*-values. In the current study, the viewer angle explained less than 3% of the total type III sum of squares variation (Table 3) in results for a wide range of milk based beverage composition. No significant impact ($P > 0.05$) of any other interaction terms with viewer angle were detected (Table 3). Therefore, in subsequent analyses to

determine the impact of color measurement system, only data from a viewer angle of 10 degrees was used.

Hunter versus CIE Color Measurement. Data for both color measurement systems at a viewer angle of 10 degrees for raw and pasteurized milk based beverages ranging from 0.2 to 2% fat, 3 to 5% protein, and 5 to 80% CN%TP are shown in Table 2. The relative proportion of variation explained by color measurement system and other beverage composition factors, thermal processing conditions, and temperature at which color was measured for a viewer angle of 10 degrees is shown in Table 4.

L/L* Values. Choice of color measurement system explained about 7.17% of the total variation in L/L* value measurement (Table 4) with CIE system reporting higher L-values (Table 2) than the Hunter system. However, the interactions of measurement system with other factors in the model were mostly not significant ($P > 0.05$) or only explained a very small percentage (e.g, system*fat, system*heat, etc.) of the variation (Table 4). The Hunter L-value was more sensitive than CIE L*-value to differences in whiteness among beverages differing in CN%TP at all 3 fat levels as shown by the larger differences between to 80 and 5% CN%TP levels (Table 2). Thus, the difference between Hunter and CIE measures of whiteness (L-value) are primarily a bias difference with the CIE system yielding higher values for whiteness than Hunter when fat (Figure 1), CN%TP (Figure 2), and true protein (Figure 3) concentrations vary in milk based beverages.

a/a* Values. Color measurement system only explained a small percentage (0.01%) of the total variation in a/a* (Table 4) with CIE and Hunter systems producing similar a/a* values (Table 2). Interaction of color measurement system with other factors in the model were mostly not significant ($P > 0.05$) or only explained a very small percentage (system*fat*heat,

CNTPT*system*fat, CNTPT*system*heat) of the variation (Table 4). Therefore, CIE and Hunter system differ very little in redness-greeness (a/a* values) measurements of milk protein beverages in the present study.

b/b* Values. System alone explained 14.84% of the total variation in b/b* values (Table 4) with Hunter system indicating lower b-values (Table 2) than the CIE system. Interactions of system and other terms in the models were all significant for b/b* values (Table 4). System*fat, CNTPT*system and CNTPT*system*fat counted for 2.68%, 2.26% and 0.89% of the total variation in b/b* values respectively, while all the other system involved interactions explained 3.73% of the total variation in b/b* values with each one counted less than 0.75% (Table 4). The Hunter system is less sensitive to measuring differences in yellowness of milk based beverages than the CIE system as fat content (Figure 4) and as CN%TP content (Figure 5) change (Hunter Associates Laboratory, 2012; CIE 1978; Hunter and Harold, 1987).

Objective Color Measures for Commercial Skim, 1% and 2% Fat Fluid Milks

Data for Hunter and CIE color measurement systems at a viewer angle of 10 degrees for commercial HTST pasteurized milks from skim to 2% fat and 4 to 50°C are shown in Table 5. The relative proportion of variation explained by color measurement system, fat and temperature at which color was measured for a viewer angle of 10 degrees is shown in Table 6.

L/L* Values. Color measurement system explained about 29.77% of the total variation in L/L* value measurement (Table 6) for commercial skim, 1 and 2% fat milks versus 7.17% of total variation (Table 4) for beverages that vary in CN%TP. CIE system reported higher L-values (Table 5) than the Hunter system. But the interactions of system with other factors in the model were mostly not significant ($P > 0.05$) or only explained a very small percentage (system*fat, system*temp, system*fat*temp and system*fat*rep) of the variation (Table 6).

Therefore, the difference between Hunter and CIE measures of whiteness (L-value) are primarily a bias difference with the CIE system yielding higher values for whiteness than Hunter when fat (Figure 6), and milk measurement temperature (Figure 7) varies among in commercial HTST pasteurized milks.

a/a* Values. Color measurement system only explained a small percentage (0.05%) of the total variation in a/a* (Table 6) with CIE and Hunter systems producing similar a/a* values (Table 5). Interaction of color measurement system with other factors in the model were mostly not significant ($P > 0.05$) or only explained a very small percentage (system*fat, system*temp, system*fat*temp and system*fat*rep) of the variation (Table 6). CIE and Hunter system differ very little in redness-greeness (a/a* values) measurements of commercial HTST pasteurized milks.

b/b* Values. System alone explained 62.91% of the total variation in b/b* values (Table 6) with CIE system reporting higher b-values (Table 5) than the Hunter system. Interactions of system and other terms in the models were all significant for b/b* values (Table 6). System*fat explained 2.97 % of the total variation in b/b* values, while all the other system involved interactions only explained 0.32% of the total variation in b/b* values (Table 6). The CIE system is more sensitive to measuring differences in yellowness of commercial HTST pasteurized milks than the Hunter system as fat level (Figure 8) changes.

Sensitivity of Color Measurement to Milk Temperature

L/L* values Temperature contributed to about 6.55% of the total variation of L/L* values (Table 6) with 50°C skim milk having higher ($P < 0.05$) L/L*values than 4°C skim milk, respectively (76.91 versus 82.36 and 81.38 versus 85.92 for Hunter and CIE, respectively). All temperature related interaction terms in the model were significant for L/L* values with fat*temp

accounting for 8.27% of the total variance (Table 6). Increasing temperature of color measurement from 4 to 50^{o}C increased L/L* values less (i.e., lower slope Figure 9) as fat level increased in commercial HTST pasteurized milks. The large decrease in whiteness of skim milk with decreasing color measurement temperature (Figure 9) was probably due to low temperature induced migration of monomeric casein and calcium phosphate out of the casein micelles into the milk serum phase (Downey and Murphy, 1970; Rose, 1968) and a reduction of light scattering by the changed casein micelles at 4 versus 50^{o}C. This change in the casein micelles happens in the milk containing 1 and 2% fat with change in milk temperature, but there was a smaller effect of measurement temperature on the L-value of 1 and 2% milk than skim milk. This was because the fat globules are larger than casein micelles and the fat globules are reflecting most of the light at all 3 temperatures. While the fat within fat globules at 4 versus 50°C was probably very different with respect to the proportion of liquid versus solid fat within the fat droplets, this did not have an effect on the reflection of all wavelengths of light.

a/a* values. About 0.26% of the total variation of a/a* values was explained by temperature (Table 6) with 50°C skim milk had a higher a/a* values than 4°C skim milk (P<0.05) while 50°C 1% and 2% fat milks had a lower (P<0.05) a/a* values than 4°C (Table 5). The fat*temp interaction explained 5.41% of the total variation of a/a* values with respect to color measurement temperature (Table 6 and Figure 10). Increasing color measurement temperature increased a/a* values at lower fat levels commercial HTST milks while a/a* values decreased in higher fat milks (i.e., crossing of the linear regression lines in Figure 10). If more fat levels (i.e., > 3) were tested then it would probably become more clear that as fat level increases, change in a-value with increasing fat level is a quadratic relationship.

b/b* values. The effect of measurement temperature explained 0.49% of the total variation of b/b* values (Table 6) with 50°C skim milk and 4°C 1% and 2% fat milks having about 0.5 to 1 unit higher ($P < 0.05$) b/b* values than 4°C skim milk and 50°C 1% and 2% fat milk, respectively (Table 5). The interaction of fat*temp explained 1.98% of the total variation of b/b* values while other temperature related interaction terms (system*temp, temp*rep, system*fat*temp and fat*temp*rep) within the model only explained a small percentage of the variation (Table 6). Decreasing temperature increased b/b* values in higher fat level commercial HTST milks while it decreased b/b* values in lower fat levels (Figure 11). If more fat levels (i.e., > 3) were tested then it would probably become more clear that as fat level increases, the change in a-value with increasing fat level is a quadratic relationship.

Reflectance curves. The mean reflectance curves for the skim and 2% fat commercial milks measured at 3 different milk temperatures (4, 20, 50^{o}C) are shown in Figure 12. The total amount of reflected light was higher at all wavelengths for 2% fat milk than skim milk. Milk temperature had very little effect on the amount of light reflected at all wavelengths from 2% fat milk but had a large impact on the amount of light reflected from skim milk. For skim milk, the influence of milk temperature was much larger at wavelengths from 550 to 750 nm than at wavelengths <550 nm. For 2% fat milk, the reflectance from 550 to 750 nm was very similar at all 3 measurement temperatures. As color measurement temperature increased the the solution would expand. The approximate coefficients of expansion for water, fat, protein, and carbohydrate are about 0.000214, 0.000727, 0.000115, and 0.000110 per degree C, respectively. Fat expands more with increased temperature than water and protein and carbohydrate less than water. The total expansion of the solution going from 4 to 50^{o}C would be expected to increase the solution volume by about 1%. This expansion of the solution would be expected decrease

the light scattering particle density at the surface of the cuvette window and produce a decrease in L-value with increasing temperature if all other factors were equal. The opposite was actually observed (Table 5 and Figure 7).

When particles are present in a liquid and reflect light of all wavelengths uniformly, the solution will appear white. The reflective particles in the commercial milks analyzed in our study were primarily casein micelles and homogenized fat droplets. The fat droplets in homogenized milk typically have a mean particle diameter of about 0.6 to 0.8 microns, while casein micelles are about 0.15 to 0.2 microns in diameter. Larger particles tend to more effectively reflect light. In skim milk almost all of the reflected light would be from casein micelles while in 2% fat milk most of the reflected light would be from fat globules with some small contribution from casein micelles. It is clear from the reflectance curves for skim milk that temperature of milk during objective color measurement had a large impact on the ability of casein micelles to reflect light with much less light reflected at 4 than at 20 and 50^{o}C. This may be due to the dissociation of casein monomers and calcium phosphate from the casein micelles into the milk serum phase at low temperature (Downey and Murphy, 1970; Rose, 1968). In 2% fat milk this same dissociation of casein micelles was happening as a function of temperature. However, the larger milk fat globules reflected most of the light and therefore the temperature dependent casein micelle dissociation had minimal impact on total light reflection for 2% fat milk. It is interesting to note that over this wide range of temperature the status of the fluidity of the milk fat within fat globules differs greatly with most of the fat being solid at 4^{o}C while most of the fat would be liquid at 50^{o}C. In the 2% homogenized milk, the fat globules at 4°C reflected more light than at 50°C with a higher reflectance at higher wavelength range (Figure 12). This could be due to milk fat crystallization at 4°C made the fat globules less spherical and thus

reflect more light (Mulder and Walstra, 1974); while at 50°C, the fat globules become more spherical and may lead to less light reflectance (Mulder and Walstra, 1974). The difference in reflectance curves of skim versus 2% fat milk indicates that to make skim milk appear more white and improve consumer perception of skim milk, any processing treatment or additive would need to increase reflected light much more in the range of 550 to 750 nm than at shorter wavelengths.

Conclusions

Color measurement viewer angle (2 versus 10 degree) had very little impact on objective color measures of milk based beverages with a wide range of composition for either the Hunter or CIE color measurement system. Temperature (4, 20, and 50^{o}C) of color measurement had a large impact on the results of color measurement in both the Hunter and CIE measurement systems. The effect of milk beverage temperature on color measurement results was the largest for skim milk and the least for 2% fat milk. This highlights the need for proper control of beverage serving temperature for sensory panel analysis of milk based beverages with very low fat content and for control of milk temperature when doing objective color analysis for quality control in manufacture of milk based beverages. The Hunter system of color measurement was more sensitive to differences in whiteness among milk based beverages than the CIE system, while the CIE system was much more sensitive to differences in yellowness among milk based beverages. There was little difference between the Hunter and CIE system in sensitivity to green/red color of milk based beverages. In defining milk based beverage product specifications for objective color measures for dairy product manufacturers, the viewer angle, color measurement system (CIE versus Hunter) and sample measurement temperature should be specified along with type of illuminant.

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Table 1. Formulation design for 0.2%, 1.0% and 2.0% fat level milk protein beverages with variation in casein as a percentage of true protein (CN%TP) of 5, 25, 50, 75 and 80% at true protein (TP) levels of 3.00, 3.67, 4.34 and 5.00% within each fat level.

Fat (%)	TP (%)				
	3	3.67	4.34	5	
0.2	5	5	5	5	CN%TP
	25	25	25	25	CN%TP
	50	50	50	50	CN%TP
	75	75	75	75	CN%TP
	80	80	80	80	CN%TP
1.0	5	5	5	5	CN%TP
	25	25	25	25	CN%TP
	50	50	50	50	CN%TP
	75	75	75	75	CN%TP
	80	80	80	80	CN%TP
2.0	5	5	5	5	CN%TP
	25	25	25	25	CN%TP
	50	50	50	50	CN%TP
	75	75	75	75	CN%TP
	80	80	80	80	CN%TP

Table 2. Mean and difference [80 minus 5% casein as a percentage of true protein (CN%TP)] of Hunter (H) L, a, b values and CIE (C) L*, a*, b* values at viewer angles of 10 and 2 degrees for raw and pasteurized milk protein beverages with CN%TP of 5, 25, 50, 75 and 80% within fat levels of 0.2% , 1% and 2%.

	Viewer angle (degrees)	**10**	**10**	**2**	**2**	**10**	**10**	**2**	**2**	**10**	**10**	**2**	**2**	**10 -2**	**10 -2**	**10 -2**	**10 -2**	**10 -2**	**10 -2**
Fat level	CN%TP	LH	L*C	LH	L*C	aH	a*C	aH	a*C	bH	b*C	bH	b*C	LH	L*C	aH	a*C	bH	b*C
0.2%	5	53.85	59.45	53.84	59.44	-2.22	-2.42	-2.39	-2.62	0.63	1.32	0.49	1.02	0.01	0.01	0.17	0.20	0.14	0.30
0.2%	25	70.86	76.10	70.85	76.09	-3.11	-3.18	-3.39	-3.48	0.75	1.44	0.58	1.10	0.01	0.01	0.28	0.30	0.16	0.33
0.2%	50	76.57	81.07	76.57	81.07	-3.00	-2.97	-3.32	-3.30	0.92	1.77	0.78	1.48	0.00	0.00	0.31	0.33	0.14	0.29
0.2%	75	80.21	84.13	80.20	84.12	-3.14	-3.05	-3.48	-3.40	0.62	1.18	0.51	0.96	0.01	0.01	0.34	0.35	0.11	0.22
0.2%	80	81.37	85.09	81.36	85.09	-3.00	-2.90	-3.35	-3.25	0.72	1.36	0.62	1.16	0.00	0.00	0.35	0.35	0.10	0.20
0.2%	80 minus 5	27.52	25.65	27.52	25.65	-0.78	-0.48	-0.96	-0.63	0.10	0.05	0.13	0.14						
Fat level	CN%TP	LH	L*C	LH	L*C	aH	a*C	aH	a*C	bH	b*C	bH	b*C	LH	L*C	aH	a*C	bH	b*C
1%	5	69.91	75.12	69.99	75.18	-0.24	-0.22	-0.47	-0.45	3.13	6.53	2.98	6.17	-0.07	-0.06	0.23	0.24	0.15	0.36
1%	25	76.94	81.36	77.00	81.40	-1.01	-0.99	-1.27	-1.26	2.49	4.88	2.35	4.57	-0.05	-0.04	0.27	0.27	0.14	0.30
1%	50	80.58	84.44	80.63	84.48	-1.28	-1.24	-1.59	-1.54	2.19	4.22	2.08	3.98	-0.05	-0.04	0.30	0.30	0.11	0.24
1%	75	83.32	86.71	83.37	86.75	-1.53	-1.46	-1.87	-1.80	1.88	3.56	1.80	3.39	-0.04	-0.04	0.34	0.33	0.08	0.17
1%	80	83.70	87.02	83.74	87.06	-1.55	-1.48	-1.89	-1.82	1.84	3.48	1.77	3.32	-0.04	-0.04	0.34	0.34	0.07	0.16
1%	80 minus 5	13.79	11.90	13.76	11.87	-1.31	-1.26	-1.42	-1.36	-1.29	-3.05	-1.21	-2.85						
Fat level	CN%TP	LH	L*C	LH	L*C	aH	a*C	aH	a*C	bH	b*C	bH	b*C	LH	L*C	aH	a*C	bH	b*C
2%	5	75.84	80.37	75.94	80.46	0.46	0.47	0.20	0.21	3.81	7.74	3.68	7.41	-0.10	-0.09	0.26	0.26	0.13	0.33
2%	25	79.80	83.77	79.88	83.84	-0.16	-0.16	-0.44	-0.43	3.15	6.16	3.04	5.90	-0.08	-0.07	0.28	0.28	0.11	0.26
2%	50	82.36	85.92	82.44	85.98	-0.53	-0.51	-0.84	-0.81	2.75	5.28	2.66	5.07	-0.07	-0.06	0.31	0.30	0.09	0.20
2%	75	84.35	87.56	84.42	87.61	-0.81	-0.77	-1.16	-1.10	2.43	4.61	2.37	4.46	-0.07	-0.05	0.34	0.33	0.06	0.14
2%	80	84.89	88.00	84.96	88.05	-0.92	-0.88	-1.28	-1.22	2.34	4.42	2.29	4.30	-0.07	-0.05	0.36	0.35	0.05	0.12
2%	80 minus 5	9.05	7.63	9.02	7.60	-1.38	-1.34	-1.48	-1.43	-1.47	-3.32	-1.39	-3.11						

Table 3. Percentage of type III sum of squares variation explained by model factors (angle= viewer angle, fat = fat level, heat = pasteurization and homogenization, prot = milk true protein, temp = temperature of color measurement, rep = replicate, CNTPT = casein as a percentage of true protein transformed) for Hunter L, a, b and CIE L*, a* and b* values for milk protein beverages.

Factor						
	L	L*	a	a*	b	b*
Angle	NS[2]	NS	2.72[1]	2.86[1]	0.57[1]	0.65[1]
Fat	2.19[1]	1.57[1]	49.04[1]	50.39[1]	39.52[1]	33.37[1]
Heat	1.61[1]	1.13[1]	5.84[1]	6.21[1]	6.98[1]	5.97[1]
Prot	0.21[1]	0.18[1]	2.73[1]	2.93[1]	2.17[1]	1.86[1]
Temp	0.67[1]	0.60[1]	0.24[1]	0.12[1]	0.80[1]	0.75[1]
Rep	0.01[1]	0.02[1]	0.01[1]	NS	0.02[1]	0.13[1]
fat*heat	0.22[1]	0.28[1]	0.34[1]	0.48[1]	1.45[1]	1.42[1]
fat*prot	0.20[1]	0.18[1]	0.94[1]	1.12[1]	1.83[1]	1.63[1]
fat*temp	0.42[1]	0.39[1]	2.37[1]	2.81[1]	1.95[1]	1.93[1]
fat*rep	0.03[1]	0.01	0.05[1]	0.05[1]	0.26[1]	0.23[1]
heat*prot	0.06[1]	0.06[1]	0.10[1]	0.14[1]	0.21[1]	0.18[1]
heat*temp	0.13[1]	0.13[1]	0.09[1]	0.07[1]	NS	NS
heat*rep	0.02[1]	0.02[1]	NS	NS	0.05[1]	0.03[1]
prot*rep	0.05[1]	0.04[1]	0.11[1]	0.13[1]	0.08[1]	0.05[1]
fat*heat*prot	0.02[1]	NS	0.44[1]	0.46[1]	0.27[1]	0.24[1]
fat*heat*temp	0.06[1]	0.06[1]	0.09[1]	0.10[1]	0.17[1]	0.10[1]
fat*heat*rep	0.03[1]	0.03[1]	NS	NS	NS	NS
fat*prot*rep	0.08[1]	0.07[1]	0.21[1]	0.24[1]	0.16[1]	0.14[1]
fat*temp*rep	NS	NS	0.09[1]	NS	NS	NS
angle*fat*heat*prot*temp*rep	0.12	0.13	0.30	0.41	0.34	0.32
CNTPT	44.81[1]	41.77[1]	14.21[1]	10.66[1]	15.29[1]	18.37[1]
CNTPT*angle	NS	NS	0.07[1]	0.06	0.03[1]	0.05[1]
CNTPT*fat	7.29[1]	8.07[1]	1.87[1]	3.37[1]	7.89[1]	8.79[1]
CNTPT*heat	19.35[1]	19.13[1]	3.29[1]	2.30[1]	0.50[1]	1.28[1]
CNTPT*prot	NS	NS	0.13[1]	0.18[1]	0.42[1]	0.50[1]
CNTPT*temp	0.33[1]	0.29[1]	1.75[1]	1.95[1]	0.93[1]	1.01[1]
CNTPT*rep	NS	NS	0.10[1]	0.11[1]	NS	0.02[1]
CNTPT*fat*heat	4.33[1]	5.12[1]	0.68[1]	1.35[1]	8.01[1]	8.53[1]
CNTPT*heat*prot	0.07[1]	0.06[1]	0.23[1]	0.22[1]	0.09[1]	0.04[1]
CNTPT*heat*temp	0.06[1]	0.05[1]	0.26[1]	0.29[1]	0.78[1]	0.87[1]
CNTPT*fat*temp	0.04[1]	0.04[1]	0.54[1]	0.62[1]	0.63[1]	0.75[1]
CNTPT*fat*prot	0.09[1]	0.08[1]	NS	NS	0.10[1]	0.09[1]
CNTPT*temp*rep	NS	NS	0.04[1]	0.05[1]	0.04[1]	0.04[1]
CNTPT*prot*rep	0.03[1]	0.02[1]	0.05[1]	0.06[1]	0.05[1]	0.06[1]
CNTPT*CNTPT	5.69[1]	6.43[1]	2.45[1]	2.27[1]	0.15[1]	0.55[1]
CNTPT*CNTPT*fat	2.60[1]	3.40[1]	0.17[1]	0.09[1]	1.97[1]	2.32[1]
CNTPT*CNTPT*heat	5.83[1]	6.52[1]	6.40[1]	5.75[1]	4.72[1]	5.79[1]
CNTPT*CNTPT*prot	0.04[1]	0.04[1]	NS	NS	NS	NS
CNTPT*CNTPT*temp	0.39[1]	0.38[1]	0.06[1]	0.13[1]	0.27[1]	0.31[1]
CNTPT*CNTPT*fat*heat	2.51[1]	3.30[1]	1.31[1]	1.03[1]	0.07[1]	0.20[1]
CNTPT*CNTPT*fat*prot	0.07[1]	0.07[1]	0.08[1]	0.08[1]	0.07[1]	0.08[1]
CNTPT*CNTPT*fat*temp	0.03[1]	0.04[1]	0.38[1]	0.61[1]	0.79[1]	1.05[1]

Table 3 (continued).

CNTPT*CNTPT*fat*rep	0.02[1]	NS	NS	NS	NS	NS
CNTPT*CNTPT*heat*prot	0.12[1]	0.11[1]	0.17[1]	0.21[1]	0.20[1]	0.13[1]
CNTPT*CNTPT*heat*temp	0.18[1]	0.18[1]	NS	0.04[1]	0.10[1]	0.16[1]
CNTPT*CNTPT*prot*rep	0.02[1]	NS	0.05[1]	0.07[1]	0.05[1]	NS
Sum (%)	100.00	100.00	100.00	100.00	100.00	100.00
R^2	0.98	0.98	0.95	0.95	0.96	0.96

[1] means $P < 0.05$.
[2] NS = not significant, means $P > 0.05$.

Table 4. Percentage of type III sum of squares variation explained by model factors (system= color system, fat = fat level, heat = pasteurization and homogenization, prot = milk true protein, temp = temperature of color measurement, rep = replicate, CNTPT = casein as a percentage of true protein transformed) for L/L*, a/a* and b/b* values (Hunter, CIE) for milk protein beverages at 10 degree viewer angle.

Factor	L10	a10	b10
System	7.17[1]	0.01[1]	14.84[1]
Fat	1.71[1]	52.54[1]	25.60[1]
Heat	1.27[1]	5.74[1]	4.72[1]
Prot	0.18[1]	2.85[1]	1.51[1]
Temp	0.59[1]	0.18[1]	0.60[1]
Rep	0.01[1]	NS	0.10[1]
fat*heat	0.23[1]	0.44[1]	1.07[1]
fat*prot	0.17[1]	1.11[1]	1.26[1]
fat*temp	0.37[1]	2.73[1]	1.42[1]
fat*rep	0.03[1]	0.06[1]	0.18[1]
heat*prot	0.05[1]	0.14[1]	0.14[1]
heat*temp	0.12[1]	0.05[1]	NS
heat*rep	0.02[1]	NS	NS
prot*rep	0.04[1]	0.12[1]	0.04[1]
temp*rep	0.01	NS	NS
system*fat	0.06[1]	NS	2.68[1]
system*heat	0.11[1]	NS	0.51[1]
system*prot	NS[2]	NS	0.46[1]
system*temp	NS	NS	0.07[1]
fat*heat*prot	NS	0.46[1]	0.19[1]
fat*heat*temp	0.06[1]	0.10[1]	0.08[1]
fat*heat*rep	0.03[1]	NS	0.11[1]
fat*prot*rep	0.07[1]	0.23[1]	NS
fat*temp*rep	NS	0.09[1]	NS
system*fat*heat	NS	0.08[1]	0.64[1]
system*fat*prot	NS	NS	0.26[1]
system*fat*temp	NS	NS	0.06[1]
system*heat*temp	NS	NS	0.04[1]
system*fat*heat*prot*temp*rep	0.14	0.27	0.38
CNTPT	40.13[1]	11.69[1]	14.80[1]
CNTPT*system	0.20[1]	0.09[1]	2.26[1]
CNTPT*fat	7.06[1]	2.83[1]	6.47[1]
CNTPT*heat	17.81[1]	2.88[1]	0.77[1]
CNTPT*prot	NS	0.17[1]	0.37[1]
CNTPT*temp	0.28[1]	1.87[1]	0.81[1]
CNTPT*rep	NS	0.10[1]	NS
CNTPT*system*fat	0.06[1]	0.07[1]	0.89[1]
CNTPT*system*heat	NS	0.03[1]	0.27[1]
CNTPT*system*prot	NS	NS	0.06[1]
CNTPT*system*temp	NS	NS	0.11[1]
CNTPT*fat*heat	4.33[1]	1.17[1]	6.39[1]
CNTPT*heat*prot	0.06[1]	0.23[1]	0.04[1]

Table 4 (continued).

CNTPT*heat*temp	0.05[1]	0.29[1]	0.63[1]
CNTPT*fat*temp	0.03[1]	0.61[1]	0.54[1]
CNTPT*fat*prot	0.08[1]	NS	0.07[1]
CNTPT*temp*rep	NS	0.04[1]	NS
CNTPT*prot*rep	0.02[1]	0.06[1]	0.07[1]
CNTPT*CNTPT	5.59[1]	2.40[1]	0.27[1]
CNTPT*CNTPT*system	NS	NS	0.10[1]
CNTPT*CNTPT*fat	2.73[1]	0.10[1]	1.74[1]
CNTPT*CNTPT*heat	5.70[1]	6.04[1]	4.05[1]
CNTPT*CNTPT*prot	0.03[1]	NS	NS
CNTPT*CNTPT*temp	0.35[1]	0.10[1]	0.22[1]
CNTPT*CNTPT*system*fat	NS	NS	0.23[1]
CNTPT*CNTPT*system*heat	NS	NS	0.71[1]
CNTPT*CNTPT*system*temp	NS	NS	0.03[1]
CNTPT*CNTPT*fat*heat	2.65[1]	1.11[1]	0.13[1]
CNTPT*CNTPT*fat*prot	0.06[1]	0.08[1]	0.06[1]
CNTPT*CNTPT*fat*temp	0.03[1]	0.53[1]	0.72[1]
CNTPT*CNTPT*fat*rep	0.02[1]	NS	NS
CNTPT*CNTPT*heat*prot	0.11[1]	0.19[1]	0.11[1]
CNTPT*CNTPT*heat*temp	0.16[1]	0.03	0.11[1]
CNTPT*CNTPT*prot*rep	0.01[1]	0.06[1]	NS
Sum (%)	100.00	100.00	100.00
R^2	0.98	0.95	0.96

[1] means $P < 0.05$.
[2] NS = not significant, means $P > 0.05$.

Table 5. Mean of Hunter (H) L, a, b values and CIE (C) L*, a*, b* values for commercial HTST pasteurized skim, 1% and 2% fat milks with temperature of 4, 20 and 50°C at 10 degree viewer angle.

Fat level	Temperature (°C)	LH10	L*C10	aH10	a*C10	bH10	b*C10
Skim	4	76.91[c]	81.38[c]	-4.26[c]	-4.21[c]	1.93[b]	3.76[b]
Skim	20	79.83[b]	83.82[b]	-3.59[b]	-3.50[b]	2.31[a]	4.45[a]
Skim	50	82.36[a]	85.92[a]	-3.06[a]	-2.94[a]	2.36[a]	4.51[a]
SE[1]		0.111	0.094	0.006	0.007	0.039	0.019
Skim	50 minus 4	5.44	4.54	1.21	1.27	0.43	0.75

Fat level	Temperature (°C)	LH10	L*C10	aH10	a*C10	bH10	b*C10
1% fat	4	83.39[c]	86.77[c]	-1.20[a]	-1.15[a]	3.80[a]	7.35[a]
1% fat	20	83.77[b]	87.08[b]	-1.46[b]	-1.40[b]	3.55[b]	6.84[b]
1% fat	50	84.49[a]	87.67[a]	-1.53[b]	-1.46[b]	3.27[c]	6.24[c]
SE		0.082	0.068	0.082	0.025	0.017	0.032
1% fat	50 minus 4	1.10	0.90	-0.33	-0.31	-0.54	-1.11

Fat level	Temperature (°C)	LH10	L*C10	aH10	a*C10	bH10	b*C10
2% fat	4	85.92[a]	88.84[a]	-0.30[a]	-0.28[a]	4.02[a]	7.71[a]
2% fat	20	85.51[b]	88.50[b]	-0.53[b]	-0.50[b]	3.84[b]	7.36[b]
2% fat	50	85.80[a]	88.74[a]	-0.73[c]	-0.69[c]	3.59[c]	6.86[c]
SE		0.085	0.070	0.023	0.022	0.019	0.039
2% fat	50 minus 4	-0.12	-0.10	-0.43	-0.41	-0.43	-0.86

[a, b, c] Means within the same column and the same fat level that do not share a common superscript differ ($P < 0.05$).
[1] SE – standard error

Table 6. Percentage of variation explained by model factors (system= color system, fat = fat level, temp = temperature of color measurement, rep = replicate) for L/L*, a/a* and b/b* values (Hunter, CIE) for commercial HTST pasteurized milks at 10 degree viewer angle.

Factors	L10	a10	b10
System	29.77[1]	0.05[1]	62.91[1]
Fat	54.28[1]	94.01[1]	30.75[1]
Temp	6.55[1]	0.26[1]	0.49[1]
Rep	0.01[1]	0.04[1]	NS
system*fat	0.49[1]	0.01[1]	2.97[1]
system*temp	0.06[1]	NS	0.07[1]
fat*temp	8.27[1]	5.41[1]	1.98[1]
fat*rep	0.31[1]	0.18[1]	0.55[1]
temp*rep	0.19[1]	0.02[1]	0.01[1]
system*fat*temp	0.07[1]	NS	0.19[1]
system*fat*rep	NS[2]	NS	0.06[1]
fat*temp*rep	0.01[1]	0.02[1]	0.03[1]
Sum (%)	100.00	100.00	100.00
R^2	1.00	1.00	1.00

[1] means $P < 0.05$.
[2] NS = not significant, means $P > 0.05$.

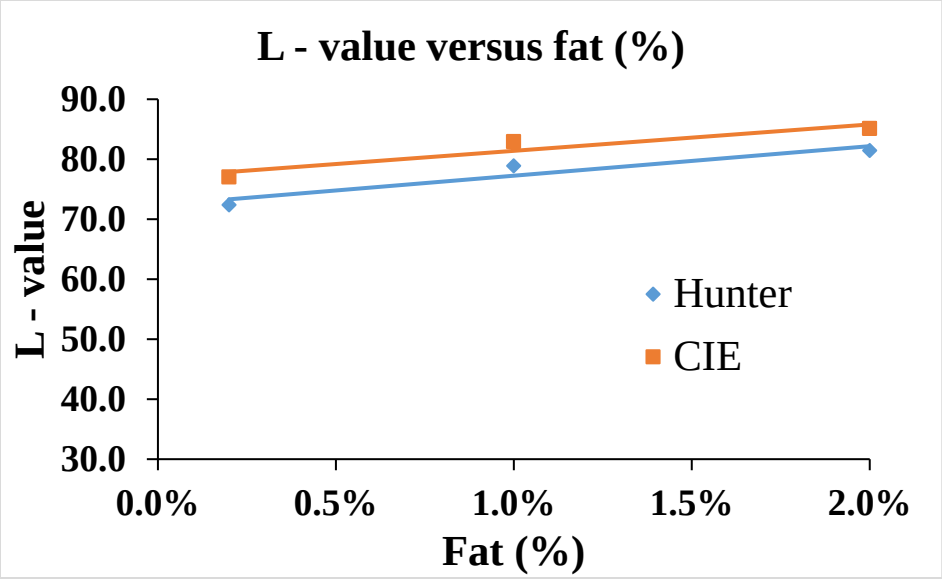

Figure 1. L-values for milk protein beverages with CIE and Hunter color measurement systems as a function of fat percent at 10 degree viewer angle. N = 120 per each fat level for a total of 360 for each color measurement method and 720 for the full experiment.

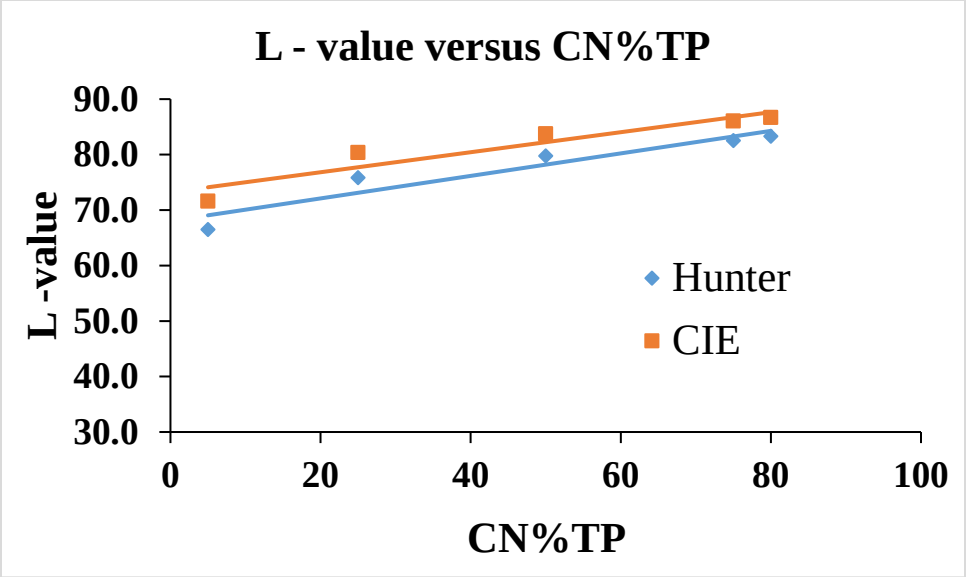

Figure 2. L-values for milk protein beverages with CIE and Hunter color measurement systems as a function of casein as a percentage of true protein (CN%TP) at 10 degree viewer angle. N=72 at each CN%TP level for a total of 360 for each color measurement method and 720 for the full experiment.

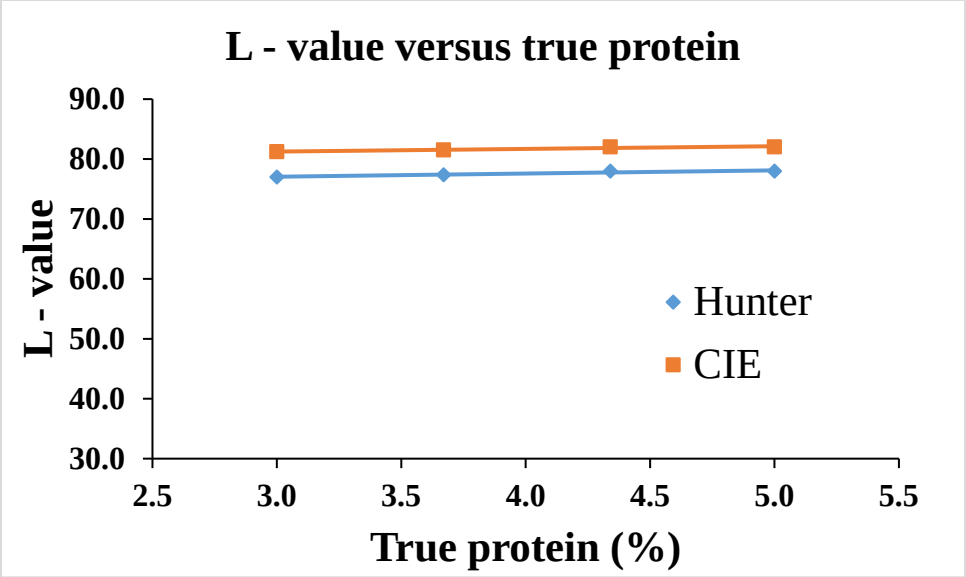

Figure 3. L-values for milk protein beverages with CIE and Hunter color measurement systems as a function of true protein percent at 10 degree viewer angle. N = 90 at each true protein level for a total of 360 for each color measurement method and 720 for the full experiment.

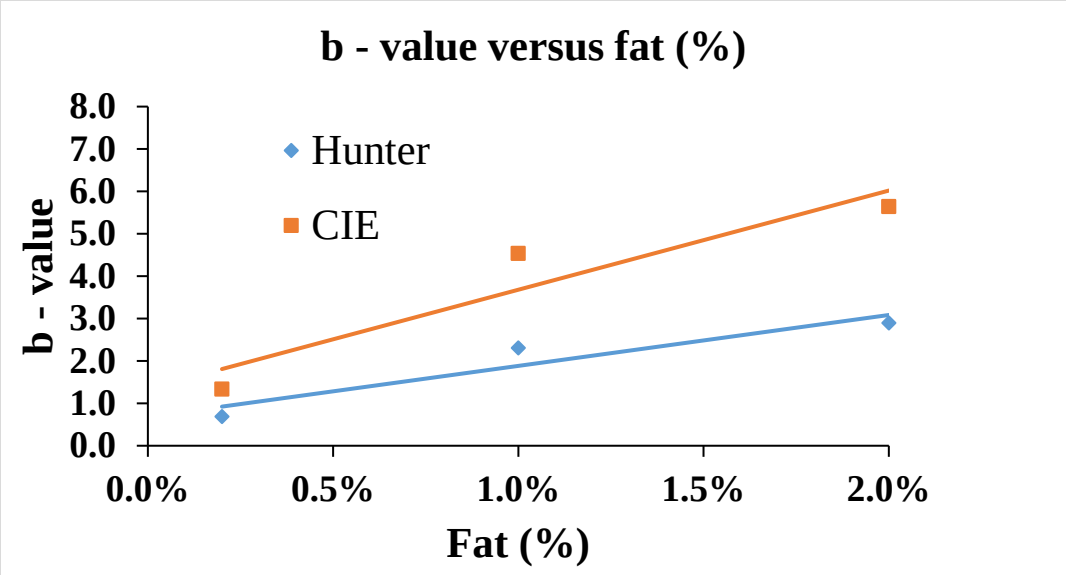

Figure 4. b-values for milk protein beverages with CIE and Hunter color measurement systems as a function of fat percent at 10 degree viewer angle. N = 120 at each fat level for a total of 360 for each color measurement method and 720 for the full experiment.

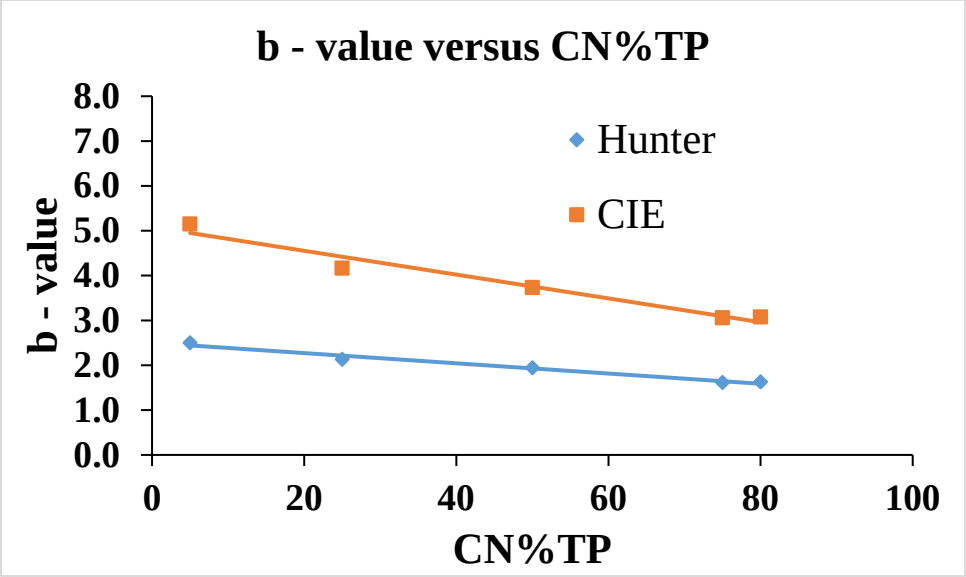

Figure 5. b-values for milk protein beverages with CIE and Hunter color measurement systems as a function of casein as a percentage of true protein (CN%TP) at 10 degree viewer angle. N = 72 at each CN%TP level for a total of 360 for each color measurement method and 720 for the full experiment.

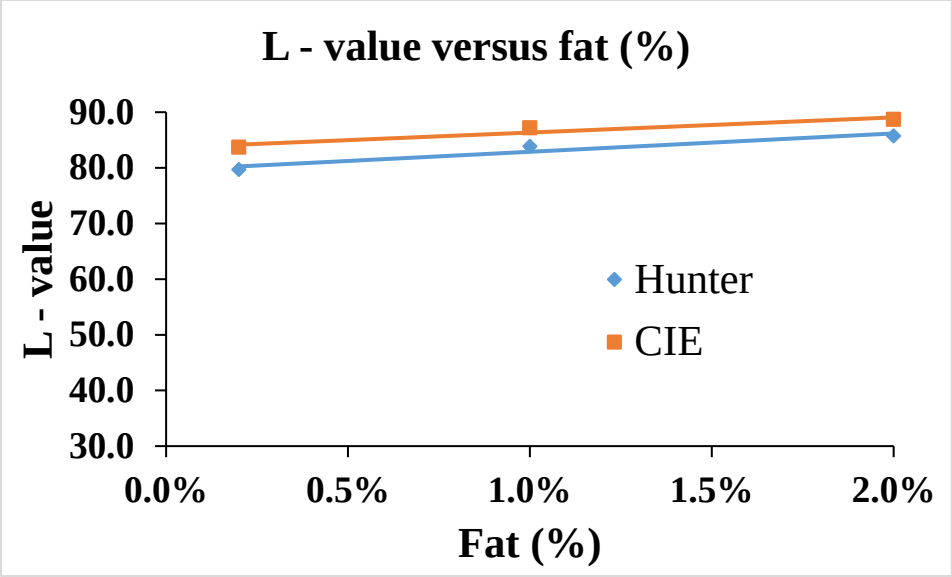

Figure 6. L-values for commercial HTST pasteurized milks with CIE and Hunter color measurement systems as a function of fat percent at 10 degree viewer angle. N = 3 at each fat level for a total of 9 for each color measurement method and 18 for the full experiment.

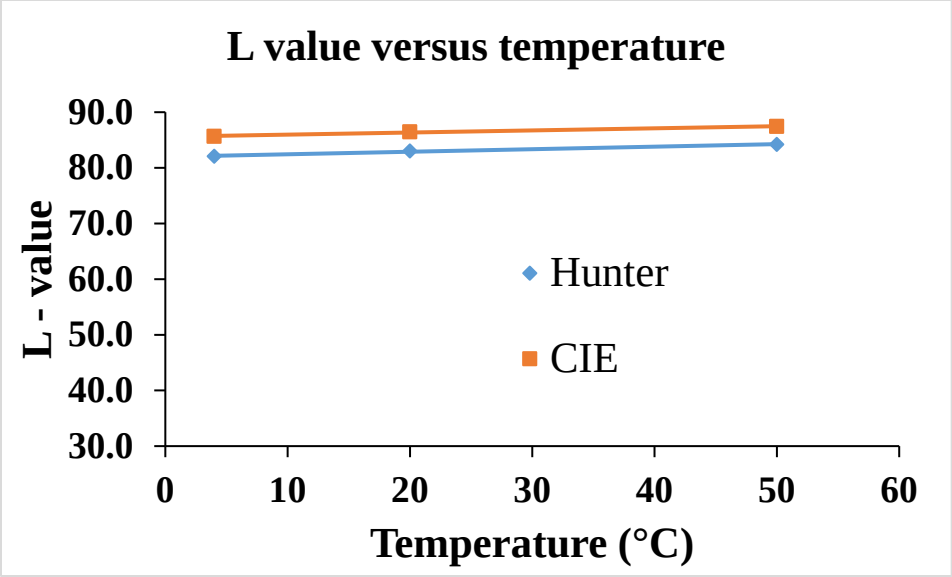

Figure 7. L-values for commercial HTST pasteurized milks with CIE and Hunter color measurement systems as a function of temperature at 10 degree viewer angle. N = 3 at each fat level for each color measurement method for a total of 9 and 18 for the full experiment.

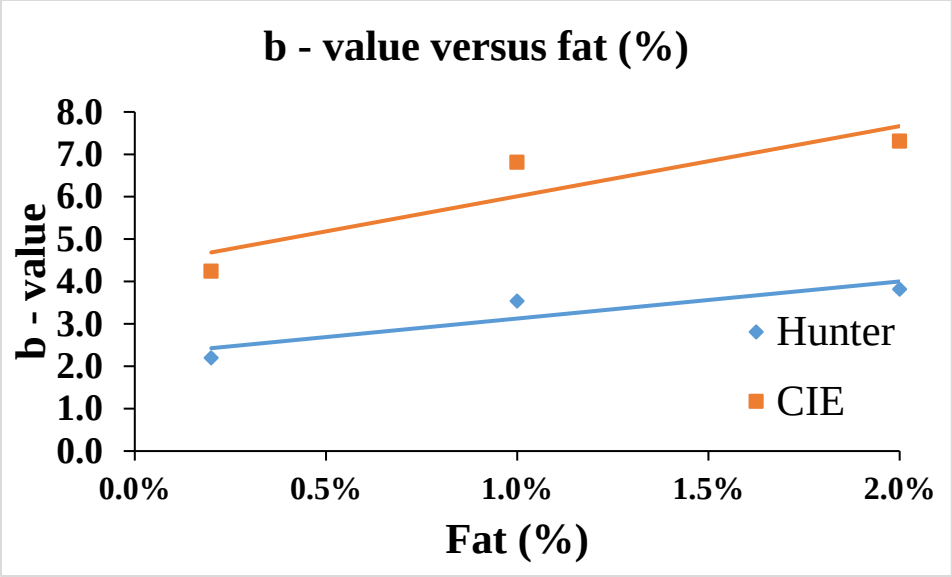

Figure 8. b-values for commercial HTST pasteurized milks with CIE and Hunter color measurement systems as a function of fat percent at 10 degree viewer angle. N = 3 at each fat level for a total of 9 for each color measurement method and 18 for the full experiment.

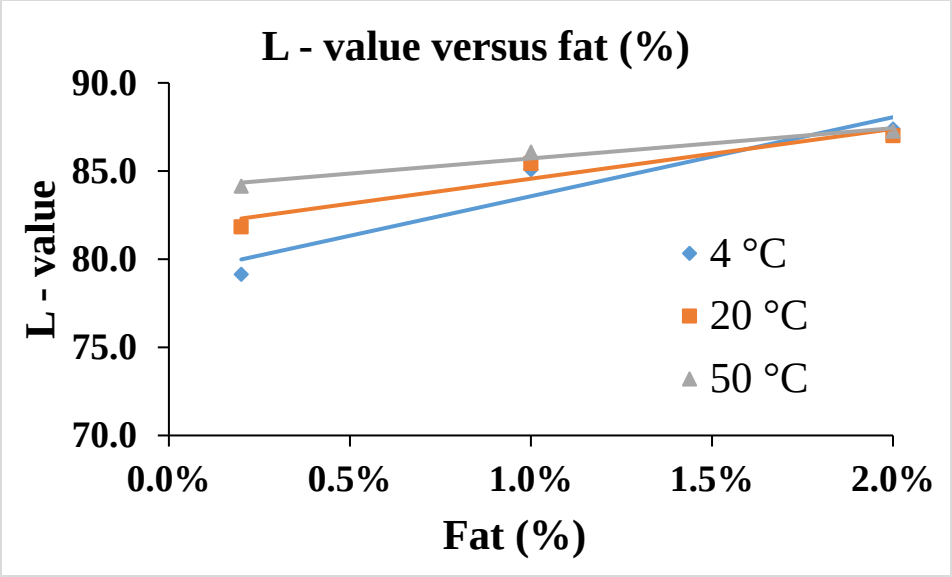

Figure 9. L-values for 4, 20 and 50°C commercial HTST pasteurized milks as a function of fat percent at 10 degree viewer angle. N = 2 at each fat level for a total of 6 for each color measurement method and 12 for the full experiment.

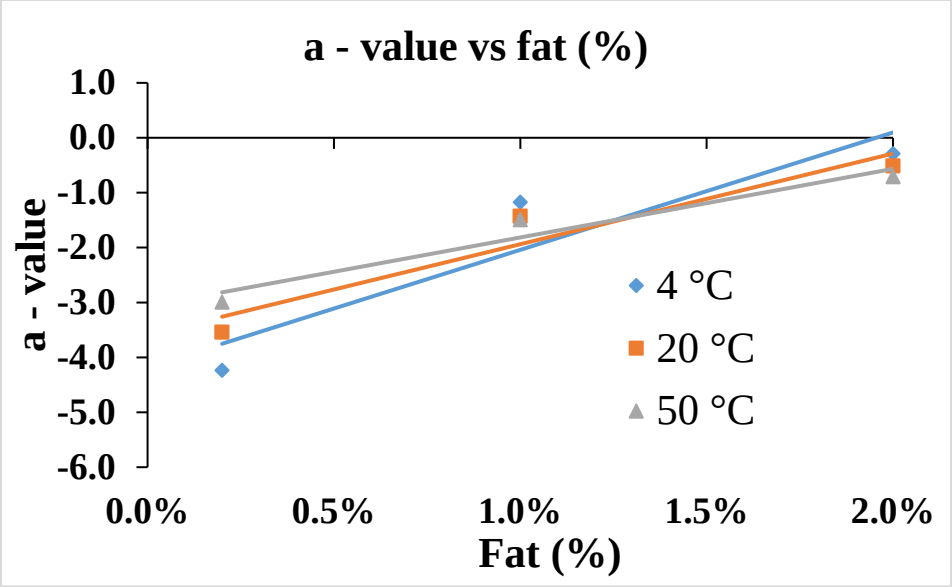

Figure 10. a-values for 4, 20 and 50°C commercial HTST pasteurized milks as a function of fat percent at 10 degree viewer angle N = 2 at each fat level for a total of 6 for each color measurement method and 12 for the full experiment.

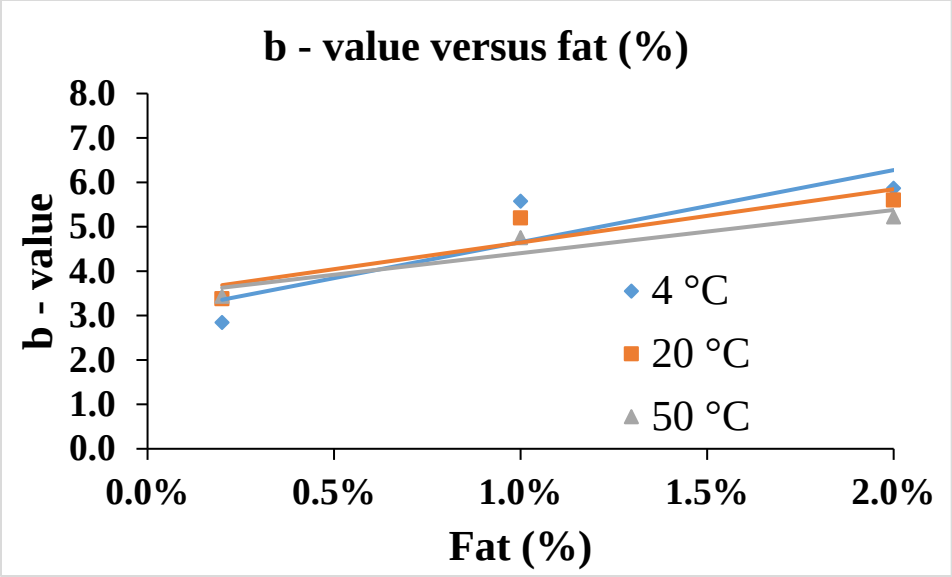

Figure 11. b-values for 4, 20 and 50°C commercial HTST pasteurized milks as a function of fat percent at 10 degree viewer angle. N = 2 at each fat level for a total of 6 for each color measurement method and 12 for the full experiment.

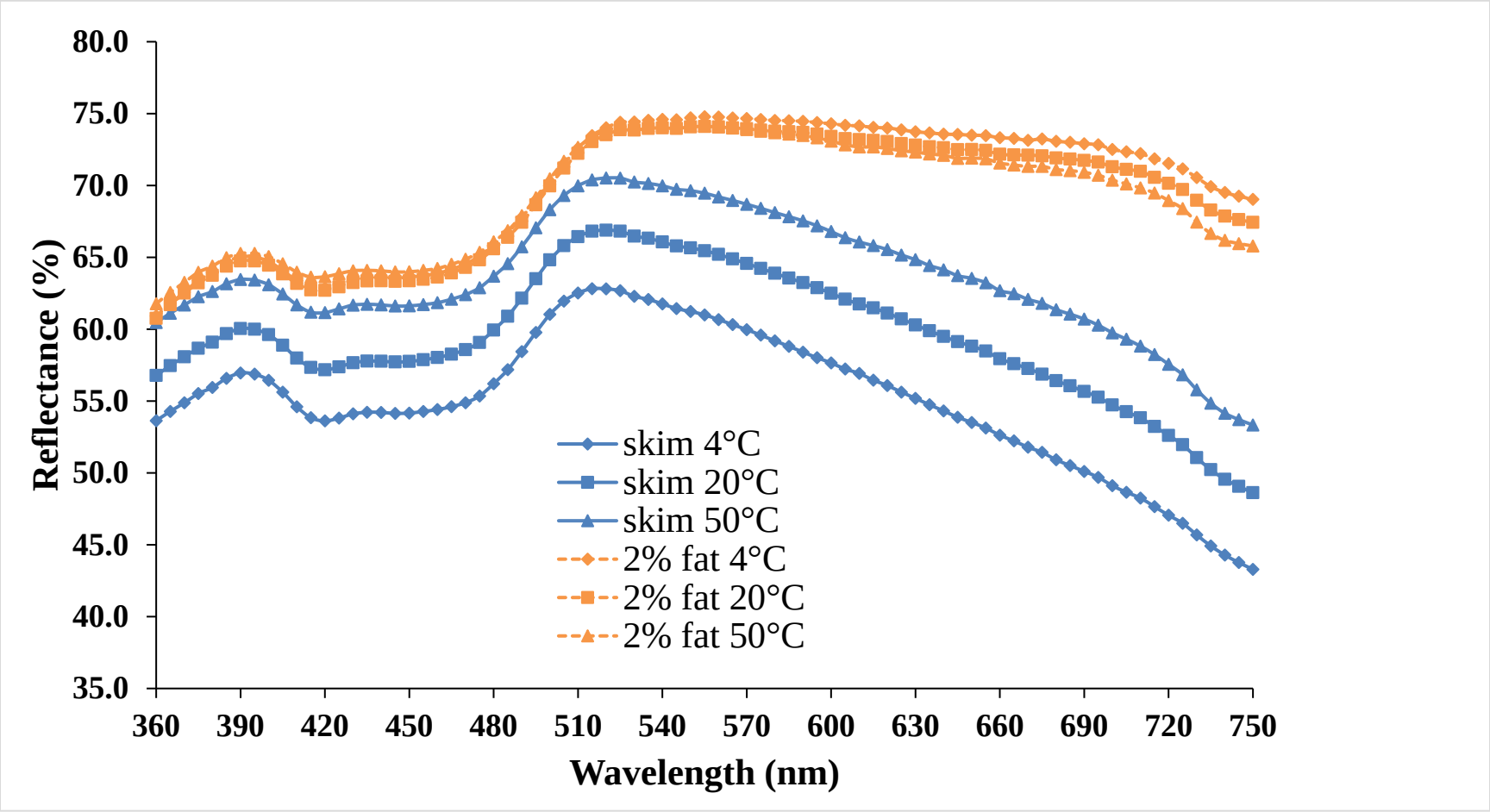

Figure 12. Mean diffuse reflectance (%) for commercial HTST pasteurized skim and 2% fat milks at 4, 20 and 50°C. N=1 for each temperature within skim and within 2% fat.

CHAPTER 3: IMPACT OF PASTEURIZATION AND FAT, PROTEIN, CASEIN TO SERUM PROTEIN RATIO, AND MILK TEMPERATURE ON MILK BEVERAGE COLOR AND VISCOSITY

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Abstract

Our goal was to determine the impact of pasteurization-homogenization, fat and protein concentration, the proportion of milk protein that is casein and serum protein, and temperature on sensory and instrumental measures of viscosity and color of milk based beverages. A second goal was to use instrumental measures of whiteness and yellowness to predict sensory measures of whiteness and yellowness. A complete balanced 3 factor (fat, true protein and casein as a percentage of true protein) design was applied with 3 fat levels (0.2, 1.0 and 2.0%), 4 true protein (TP) levels (3.00, 3.67, 4.34 and 5.00%) within each fat level and 5 casein as a percentage of true protein (CN%TP) levels (5, 25, 50, 75 and 80%) within each protein level for beverage formulation. Instrumental color and viscosity, and visual sensory color analyses were done on each beverage formulation. For unpasteurized beverages across 3 fat levels (0.2, 1, and 2%), changes in CN%TP had the largest impact on L values, sensory whiteness, opacity, color intensity and yellowness while changes in fat concentration had a stronger influence on a and b* values. Increasing CN%TP from 5 to 80% increased L values, sensory whiteness and opacity and decreased sensory color intensity and yellowness. The a and b* values increased with increasing fat concentration. For unpasteurized milk protein beverages within each fat level, variation in CN%TP dominated the changes in L values, sensory whiteness and opacity and decreased a and b* values, sensory color intensity and yellowness. The effect of heat (pasteurization and homogenization) and its interaction terms had the second largest impact on color of milk protein beverages with respect to instrumental color data and sensory appearance attributes. Heat increased L values, sensory whiteness and opacity and decreased a and b* values, sensory color intensity and yellowness. Increases in temperature decreased instrumental viscosity and changes in protein concentration and CN%TP had more impact on instrument viscosity data within each

temperature (4, 20, and 50^{o}C) than fat. Sensory perception of yellowness was not highly correlated with b* values. Multiple linear regressions of L, a and b* values produced more robust predictions for both sensory whiteness and yellowness than simple linear regression with L and b *values, alone and may be a useful instrumental approach for quality control of sensory whiteness and yellowness of milk protein beverages.

Key words: Casein as a percent of true protein, protein beverages, sensory whiteness and yellowness

Introduction

Hunter (L, a, b) and International Commission on Illumination (CIE) L*, a* and b* color measurement systems are widely used in color measurement of dairy and dairy products. The intent of development of these two color measurement systems was to generate a uniform color space for color measurement. The Hunter (L, a, b) system is more sensitive in measuring blueness while the CIE (L*, a*, b*) system is more sensitive in measuring yellowness and dark colors (Hunter Associates Laboratory, 2012; CIE 1978; Hunter and Harold 1987). Cheng et al. (2018) recently applied both systems for color measurement of milk beverages and they concluded that for best sensitivity in instrumental color analysis of dairy beverages. Hunter L values for whiteness and CIE b* values for yellowness were recommended as the most sensitive while there was little difference between the selection of Hunter a and CIE a* values at a viewer angle of 10 degree using illuminant A. Measurement temperature, viewer angle and illuminant also need to be reported for reproducibility of instrumental color measurements.

The relationship between visual instrumental data and sensory attributes of dairy products have been investigated by several researchers. Guinard and Mazzucchelli (1999) found a high correlation (R=0.98) between instrumental color lightness (L values) and sensory darkness in nine milk chocolate with varying sugar and cocoa butter levels. Tárrega and Costell (2007) observed strong correlations between instrumental color measurement (CIE color system, at 10 degree viewer angle) and sensory ranking of yellow color of seven dairy desserts. Hetherington and MacDougall (1991) used principle component analysis to understand the inter-relationship between visual instrumental (CIE color system, at 10 degree viewer angle) data and sensory data for whole milk, and they also built regression models to predict sensory data by using multiple visual instrumental variables. The multiple correlation coefficient they reported for color and

lightness of whole milk were both 0.80 (Hetherington and MacDougall, 1991). It is possible to use the visual instrumental data to generate a prediction model for sensory attributes. Sensory color attribute prediction from instrumental data could help dairy product manufactures to better control the quality of products in real time to meet consumers expectations for color of milk protein beverages.

Several studies have reported milk color and its effects on perception and preferences for milk and milk protein beverages. Pangborn et al. (1985) pointed out that visual cues were important for milk differentiation based on fat level. Phillips et al. (1995a) reported that milk appearance also influenced the perceived mouthfeel of low fat milk. Milk appearance was correlated with color perception of low fat milk when increasing fat from 0.06 to 0.2% and addition of titanium dioxide also increased the sensory scores for low fat milk appearance (Phillips et al., 1995a; Phillips et al., 1997). Increases in true protein level (Quiñones et al., 1997, 1998) and casein as a percentage of true protein (Misawa et al., 2016) increased sensory whiteness of low fat milks. McCarthy et al. (2017) conducted consumer interviews with consumers and concluded that better color or whiteness was one reason that milk drinkers, regardless of what type of milk they regularly consumed, preferred higher fat level milks when the fat content was not disclosed. Currently, consumers are interested in high protein food products and high protein milk-based beverages deliver high quality protein in a ready to consume form that needs to look and taste great. Today, commercial membrane filtration technology offers milk processing approaches to concentrate and fractionate milk proteins for use as fresh liquid ingredients in high protein milk based beverages. Different combinations of milk protein concentration and protein types (native casein micelles versus milk serum proteins) can produce different flavor and functionalities in high protein beverages. Data is needed to

understand the impact of these ingredients on high protein dairy beverage flavor and functionality. One objective of our work was to determine the impact of pasteurization-homogenization, fat and protein concentration, the proportion of milk protein that is casein and serum protein, and sample temperature on sensory and instrumental measures of viscosity and whiteness, red/green, and blue/yellow color of milk based beverages. A second objective was to use instrumental measures of whiteness and yellowness to predict sensory measures of whiteness and yellowness in unpasteurized milk protein beverages.

Materials and Methods

Experimental Design

Beverage formulations were based on a complete balanced 3 factor (fat, true protein and casein as a percentage of true protein) design with 3 fat levels (0.2, 1.0 and 2.0%), 4 true protein (TP) levels (3.00, 3.67, 4.34 and 5.00%) within each fat level and 5 casein as a percentage of true protein (CN%TP) levels (5, 25, 50, 75 and 80%) within each protein level (for a total of 60 formulations within each replicate) (Table 1). The formulation, sensory and analytical work was done in one week for each fat level and there was a replication of the formulation, processing, and analysis for all the treatments within each fat level in a second week, for a total of 6 weeks of processing. Instrumental measures of L, a, and b*-values, instrumental viscosity, and visual sensory analysis of color were done on each unpasteurized and pasteurized/homogenized beverage formulation. The impact of unpasteurized beverage composition on instrumental and sensory measures of appearance was analyzed separately and then together with the same formulation after pasteurization/homogenization to determine the impact of processing on appearance over the range of fat, protein, and CN%TP in the group of formulations.

Milk Based Ingredients and Beverages Processing

The ingredients used for beverage formulation and pasteurization-homogenization processing were as described by Cheng et al. (2018). The relative weight percentage of usage of each ingredient in each formulation was listed in Table 1.

Milk Micellar Casein Concentrate (MCC). Liquid MCC was made the day before beverage formulation as described by Cheng et al. (2018). A 3 stage, 3x microfiltration (MF) process described by Zulewska and Barbano (2014) was used to produce a 95% serum protein removed MCC with true protein concentration between 8.4 to 8.6% (Cheng et al., 2018) using an 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 MCC was used as a fresh liquid ingredient in beverage formulation produced immediately prior to each replication of the experiment.

Serum Protein Isolate (SPI). To produce enough SPI for the study, several batches of SPI were produced and combined prior to the beverage study to obtain a large batch of liquid SPI as described by Cheng et al. (2018). Briefly MF permeate from production of MCC was concentrated using UF to a high protein (about 25%) SPI, the concentrate was packaged in half gallon plastic jugs (Upstate Niagara Cooperative, Baffulo, NY), and placed in -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.

Cream. A cold bowl separator (model 590, Separators Inc., Indianapolis, IN) was used for raw cream separation and then pasteurized (80^{o}C for 25 s) cream containing about 41% fat was obtained the day before formulation (North Carolina State University Dairy Enterprise System) and stored at 4°C.

Lactose and water. Lactose monohydrate (Hilmar™ 5120 if Refined Edible Lactose 200 mesh, 25kg/bag) was donated by Hilmar Ingredients (Hilmar Ingredients, Hilmar, CA). Potable water was processed to produce DI water using a deionizing water system (unit number: 1933-2, Mar Cor, Raleigh, NC).

Beverage Formulation

Formulations (9,500 g per batch) were calculated using Microsoft Excel linear optimization solver function (Redmond, WA) as described by Misawa et al. (2016) and Cheng et al. (2018). MCC, SPI, cream, lactose and DI water were used as the raw ingredients. Within each fat level (0.2, 1, or 2% fat), the TP and CN%TP were targeted at 4 TP levels (3.00, 3.67, 4.34 and 5.00%) and 5 CN%TP (5, 25, 50, 75 and 80%), respectively. Anhydrous lactose concentration was standardized to 4.65% for all formulations. In total, there were 20 combinations of TP and CN%TP for each fat level in one replicate, as described by Cheng et al. (2018). The experiment with 20 formulations was replicated twice at each fat level starting with freshly produced MCC and cream. Two processing runs for skim were complete in two different weeks and the same was done for the formulations within the 1% and 2% fat levels for a total of six sets (2 replications of each fat level with 20 formulations per replication).

Pasteurization and Homogenization

Within a fat level for each replicate, 9,500 grams each of 20 unpasteurized milk beverages were well mixed individually and continuously fed to a Microthermics EHVH pasteurization unit (Microthermics, Raleigh, NC) with a 2-stage homogenizer (GEA Niro Soavi, Parma, Italy) at flow rate 2L/min. Each beverage formulation was preheated to 60°C, homogenized (1st stage at 17.3 MPa and 2nd stage at 3.4 MPa), HTST pasteurized (73°C for 15

s) and cooled to 10°C, as describe by Cheng et al. (2018). Pasteurized milk beverages were stored at 4°C.

Analysis Methods

Chemical Composition and Color. Chemical composition analysis during processing, of ingredients, and calculation of formulations based on ingredient composition were conducted as described by Cheng et al. (2018). Milk protein beverages (unpasteurized and homogenized/pasteurized) were tested in duplicate using an Ultra Scan Pro Spectrophotometer (Hunter Associates Laboratory, Inc., Reston, VA) at 4 and 20°C. A water bath was used to maintain sample temperature at 4 or 20°C. Milk beverages were measured in reflectance mode, wavelength from 360 to 750 nm with a 5 nm resolution, Illuminant A at a 10 degree viewer angle. Color data were collected using both Hunter L, a, and b-values and CIE L*, a* and b*-values (Cheng et al., 2018). Based on a previous color methodology study (Cheng et al., 2018), Hunter L, a and CIE b* values were reported in current paper.

Viscosity. Milk protein beverages (unpasteurized and pasteurized/homogenized) were tested in duplicate at 4, 20 and 50°C using a rotational viscometer (LV-DV2T, Brookfield Engineering Laboratories Inc., Middleboro, MA) with a jacketed enhanced UL Adapter (Brookfield Engineering Laboratories Inc.) as described by Adams et al. (2015).

Descriptive Analysis of Appearance. 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 appearance within 96 h after processing. Appearance profiling was conducted in separate sessions with six of trained panelists (2 males, 4 females, ages 24-52yr). Each panelist had a minimum of 80 h of prior descriptive analysis training on food appearance attributes using the Spectrum™ method

with a 0 to 15 point intensity scale (Meilgaard et al., 2007), and at least 40 h of prior experience with the sensory profiling of fluid milk and dried dairy ingredients using established sensory languages (Croissant et al., 2007; Drake et al., 2003; McCarthy et al., 2017). Compusense Cloud (Guelph, Canada) was used for data collection.

Appearance profiling consisted of unpasteurized beverages at 4°C, unpasteurized beverages at 20°C, pasteurized/homogenized beverages at 4°C and pasteurized/homogenized beverages at 20°C. Appearance attributes (color intensity, whiteness, yellowness and opacity) were adopted from previous research (Lee et al., 2017; McCarthy et al., 2017; Misawa et al., 2016; Phillips et al., 1995b). Samples (10ml) was poured into covered 60mm*15ml transparent petri dishes (Sigma-Aldrich, St. Louis, MO) with random 3-digit codes. Unpasteurized beverages were gently inverted before pouring and gently swirled before evaluation to help better distribute the fat due to the lack of homogenization. A 25.4 mm wide black stripe was printed horizontally across the middle of a blank piece of copy paper (92 bright, paper weight: 75g/m2, size: 216 mm* 279mm, Staples, Inc., Framingham, MA) by a HP LaserJet Enterprise P3015dn Printer equipped with a HP 55A Black Toner Cartridge (CE255A) (HP Inc., Palo Alto, CA) and used as the background for the appearance evaluation. For 20°C sessions, samples were tempered to 20°C and placed on the middle of the black stripe. The evaluation of the 4°C sessions were the same as the 20°C, except the 4°C beverages were stored in a 4°C walk-in cooler before evaluation and a three-layer ice box was made to maintain the low temperature during evaluation. Crushed ice was the bottom layer, a plastic wrap was the mid layer and the black strip paper was the top layer. Before each session, panelists were calibrated with appearance properties of water, heavy cream, MCC and SPI ingredients using the same light source (20W, F20T12 warm white fluorescent tube light bulb, General Electric., Boston, MA). Each panelist

evaluated each sample in duplicate in a randomized design. A 10 min rest was enforced following every 10 samples.

Statistical Analysis

Unpasteurized Beverages. The GLM procedure of SAS (version 9.4, SAS Institute Inc., Cary, NC) was used to determine the effect of fat (0.2, 1, and 2%), TP (3.0, 3.67, 4.34, 5.0%), CN%TP (5, 25, 50, 75, and 80%), measurement temperature of instrumental color (4 and 20^{o}C) and replicate on beverage color measured instrumentally. The effect of the same composition parameters at appearance evaluation temperatures of 4 and 20^{o}C on sensory appearance was also determined. All interactions of these parameters were included in the model. Fat, TP, and temperature were treated as categorical variables while CN%TP was handled as a continuous variable. The CN%TP data was transformed (named CNTPT) by mean-centering (Misawa et al., 2016) to avoid co-linearity effects on statistical analysis (Glantz and Slinker, 2001). If the F-value for the full model was significant ($P < 0.05$), then significance ($P < 0.05$) of each parameter and their interactions was determined. The effects of fat, TP, heat treatment, temperature of color measurement, and replicate and their interactions were tested for significance using the interaction term of fat*prot*temp*rep as the error term, while the effects (linear and quadratic) of the continuous variable of CN%TP and their interactions with the categorical variables were tested for significance using the full model error. A stepwise process was done to remove all non-significant terms from the model to produce a final reduced model and Type III sum of squares table. To understand the relative amount of total variation explained by each parameter or interaction of parameters on each instrumental color and each sensory appearance metric, the Type III sum of squares value for each term was divided by the total Type III sum of squares of the significant terms in the model and multiplied by 100. The term for fat

and its related interaction terms in the model were removed from the model when analyzing data to determine the effect of each parameter within each fat level.

Unpasteurized and Pasteurized Beverages. The SAS models (across all fat levels) described above were modified by adding a term of heat treatment (homogenized and pasteurized) and its interaction terms with composition parameters to determine the effect of heat treatment on instrumental color, instrumental viscosity (4, 20 and 50°C), and sensory appearance for unpasteurized and pasteurized/homogenized milk protein based beverages.

Principle component analysis (**PCA**) and cluster analysis (using k-means) were also conducted using XLSTAT (Addinsoft, Paris, France) on both instrumental and sensory measures of beverage color. The goal of the cluster analysis was to determine if there were subpopulations of formulations that were distinct from each other. This result would guide how subpopulations of beverage formulations would be grouped for development of within subpopulation specific regression (linear, quadratic, and multiple linear) models to predict of sensory whiteness and sensory yellowness from instrumental color measures. When a very different subpopulation of formulations was identified using the biplot analysis, that group was removed and the bipolt was redone for the subpopulation group of formulations. Linear, quadratic and multiple linear regressions were also conducted using Excel (Redmond, WA) data analysis to generate the prediction models of sensory whiteness and sensory yellowness by using single instrumental parameters (L or b *values) and multiple instrumental parameters (L, a and b* values).

Results and Discussion

Unpasteurized Beverage Formulations

All fat levels

L values, Sensory Whiteness and Opacity

L values (whiteness). Whiteness of milk based beverages is produced by reflectance of all wave lengths of light back to the observer by particles in a beverage such as fat droplets and casein micelles (Walstra 1965; Holt 1975). The linear component of CNTPT explained about 65.50% of the total variation of L values followed by the interaction of CNTPT*fat (15.22%), the quadratic component of CNTPT*CNTPT (9.51%) and the interaction of CNTPT*CNTPT*fat (5.80%) across fat levels (Table 2) with 96.03% of the total variation explained by the model. Increasing CN%TP from 5% to 80% increased the L values more in the lower fat level unpasteurized beverages than it did at higher fat level (Figure 1.A) as indicated by the CNTPT*fat and CNTPT*CNTPT*fat interactions ($P < 0.05$, Table 2). Change in CN%TP had a more quadratic impact on the 0.2% fat unpasteurized beverages than on the 1% and 2% ones (Figure 1.A). This finding confirmed the results from Misawa et al. (2016) that L values increased as the CN%TP increased due to the increase of concentration of light scattering casein micelles in beverages. The effect of CN%TP on L values was more profound in the lower fat level unpasteurized beverages because the light scattering in 0.2% fat unpasteurized beverages primarily came from the casein micelles, while the light scattering in 1 and 2% fat beverages came from two non additive sources: casein micelles and fat globules (Quiñones et al., 1997, 1998 and Misawa et al., 2016).

Sensory Whiteness. Across fat levels, the majority of the total variation of sensory whiteness was explained by variation in CNTPT (87.07%) while 7.05% was explained by

variation in protein and an additional 3.45% from all the fat related terms (Table 2) with 97.62% of the total variation explained by the model. Increasing CN%TP from 5 to 80% increased sensory whiteness in a similar pattern regardless of the fat content in the raw products (Figure 1.B). This result was consistent with the report by Misawa et al. (2016) for pasteurized milk protein beverages. In contrast, the quadratic effect of increasing CN%TP and the interaction with fat was not as strong for sensory whiteness as seen in the instrumental L value data. In the unpasteurized beverages in the present study, increasing CN%TP over the range from 5 to 80% had a larger impact on sensory whiteness than increasing fat content from 0.2 to 2%, particularly in the lower fat beverages (Table 2 and Figure 1.B).

Sensory Opacity. Variation in CNTPT, fat and CNTPT*fat explained 77.67%, 9.17% and 3.13% of the total variation in sensory opacity across all fat levels (Table 2) with 89.97% of the total variation explained by the model. Increasing CN%TP from 5 to 80% increased the sensory opacity more in the lower fat level unpasteurized products while increasing fat from 0.2 to 2% increased the sensory opacity less in the higher CN%TP in unpasteurized products (Figure 1.C) than in lower CN%TP unpasteurized beverages. Increases in fat levels that increased sensory opacity were also reported by Misawa et al. (2016). The quadratic effect of CN%TP had a stronger influence on skim beverages than 1% and 2% fat beverages (CNTPT*fat interaction $P < 0.05$, Table 2 and Figure 1.C).

a values, b* values, Sensory Color Intensity and Yellowness

a values (redness – greenness). The sensory perception of redness and greenness in milk based beverages occurs when light travels through the skim portion of milk and the concentration of light scattering particles is low (e.g., low fat and low casein). The perception of redness or greenness is due to compounds in the skim milk portion of milk that absorb light at specific

wavelengths allowing reflectance of green or red light. Typical light absorbing compounds in the skim portion of the milk based beverages are lactoferrin (redness) and riboflavin (greenness). Positive a values indicate redness and negative a values indicate greenness, and a values near zero indicate that perceived redness or greenness will be low. In general, a values for all beverages were near zero (i.e., not strongly green or red), with the skim beverages having a tendency to be more green than those with 1 or 2% fat (Figure 2.A).

Variation in fat, CNTPT and CNTPT*CNTPT respectively contributed to 48.84%, 28.77% and 11.01% of the total variation of a values for the unpasteurized products across fat levels (Table 3) with 88.62% of the total variation explained by the model. Increases in fat level from 0.2% to 1% increased the a values (i.e. redness) of the unpasteurized beverages more than increases in the fat level from 1% to 2% did (Figure 2.A). Fat had the largest impact on a values because increases in fat level presented more fat globules on the surface of the beverages and more light at all wavelengths was reflected back and less light was absorbed by green compounds (such as: riboflavin) in the serum phase (Misawa et al., 2016). The reflection of light at all wavelengths was already relatively high in the 1% fat beverages and increasing fat to 2% did not increase the light reflection to the extent as increasing fat from 0.2% to 1% [i.e., interactions (P <0.05) of fat with linear and quadratic terms for casein and protein Table 3]. The linear and quadratic effects of increasing CN%TP caused a values of the unpasteurized beverages to decrease (Figure 2.A) due to the increases in contribution of green components in the beverages (Misawa et al., 2016).

b* values (yellowness – blueness). The perception of yellowness or blueness is primarily due to the presence or absence of compounds in the fat portion of milk that absorbed light at specific wavelengths allowing reflectance of yellow light. Typical light absorbing compounds in

the fat portion of the milk based beverages are carotenoids that will produce a yellow/orange color perception. Positive b* values indicate yellowness and negative b* values indicate blueness, and b* values near zero indicate that perceived yellowness or blueness will be low. In general, b* values for all beverages increased by large amounts when fat concentration in the beverages increased, with yellowness of skim milk being low (Figure 2.B).

Across all fat levels, 38.35%, 29.86%, 14.73% and 8.91% of the total variation of b* values was explained by the effects of fat, CNTPT, CNTPT*fat and CNTPT*CNTPT, respectively in unpasteurized beverages (Table 3) with 91.85% of the total variation explained by the model. Increasing fat level from 0.2% to 1% increased the b* values more than increasing fat level from 1% to 2% did (Figure 2.B) in unpasteurized beverages. The reason for the increases in fat level causing the increases in b* values could be due to the presence of carotenoids in milk fat (Johnson 1974; Misawa et al., 2016). b* values decreased with increasing CN%TP and the decrease in b* values was larger in the higher fat unpasteurized beverages (Figure 2.B). CN%TP had a quadratic effect on b* values for unpasteurized beverages with increasing CN%TP decreasing b*value more in 1 and 2% fat unpasteurized beverages than in skim beverages (Figure 2.B). The decrease of b* values as CN%TP increased could have been caused by increased casein micelle concentration in the skim portion of unpasteurized beverages causing more light at all wavelengths to be reflected before it had a chance to enter fat globules and be absorbed by carotenoids to produce a perception of yellow color. This assumption is supported by the fact that when the CN%TP becomes very low (i.e., going from 25 to 5% CN%TP), yellowness of 1 and 2% fat beverages increased quickly due to much more light penetrating fat droplets (Figure 2.B, unpasteurized treatments).

Sensory Color Intensity. The effects of CNTPT, fat and CNTPT*fat explained 65.77%, 9.92% and 8.54% of the total variation of color intensity across fat levels for the unpasteurized beverages (Table 3) with 84.23% of the total variation explained by the model. As CN%TP increased color intensity decreased and the decrease in color intensity was large in higher fat unpasteurized beverages than in lower unpasteurized beverages (Figure 2.C). Color intensity increased with increasing fat content in unpasteurized beverages (Figure 2.C).

Sensory Yellowness. Variation in CNTPT explained 90.1% of the total variation of sensory yellowness, while fat level explained 4.5% of variation (Table 3) in unpasteurized beverages with 94.6% of the total variation explained by the model. Unpasteurized beverages with lower fat content were less yellow (Figure 2.D). This could due to the decreases of fat soluble carotenoids absorbing light (Misawa et al., 2016). As CN%TP increased, sensory yellowness decreased for all fat levels in unpasteurized beverages (Figure 2.D), which showed the same trend in the b*values (Figure 2.B).

Within Fat Levels

L values, Sensory Whiteness, and Opacity.

L – values (whiteness). The linear effect of CNTPT explained 79.34%, 89.38% and 92.82% of the total variation of L values for the 0.2%, 1% and 2% fat raw products respectively and its quadratic term (CNTPT*CNTPT) contributed to 18.17%, 7.79% and 3.91% of the total variation of L values in unpasteurized beverages, respectively (Table 4). L values increased with increasing CN%TP at each fat level, but the increase was larger in lower CN%TP level unpasteurized beverages (Figure 1.A). This could be due to the fact that the concentration of light scattering casein micelles was relatively low and a slight increase in casein micelle concentration could cause a rapid increase in light scattering and L values, while in higher

CN%TP unpasteurized beverages, the light scattering was already high and increases in casein micelle concentration still increased the L values, but not to the extent as in the beverages with lower CN%TP.

Sensory Whiteness. Variation in CNTPT explained 87.31%, 92.59% and 89.05% of the total variation of sensory whiteness for 0.2, 1 and 2% fat unpasteurized beverages, respectively, and protein explained 9.92%, 5.33% and 6.96% of variation in sensory whiteness, respectively (Table 4). Sensory whiteness increased linearly with increasing CN%TP within each fat level in the unpasteurized beverages (Figure 1.B). Misawa et al. (2016) also reported that an increase in CN%TP had a strong effect to increase sensory whiteness in 1 and 2% pasteurized milk protein beverages.

Sensory Opacity. Variation in CNTPT and CNTPT*CNTPT explained 85.18% and 7.71% of the total variation of opacity for the 0.2% fat, 86.04% and 3.68% for the 1% fat, and 93.47% and 3.36% for the 2.00% fat unpasteurized beverages (Table 4). As CN%TP increased, sensory opacity within each fat level increased for unpasteurized beverages (Figure 1.C), which also confirmed results from Misawa et al. (2016).

a and b* Values, Sensory Color Intensity and Yellowness

In general, the r-squared values for ANOVA models to determine the impact of variation in composition parameters on a and b* values and sensory color intensity and yellowness were lower than those for instrument and sensory metrics of whiteness and opacity. When r-squared values for the total model are lower, the ability of the ANOVA to correctly partition variation and assign that variation gets weaker. This was the case for models in this section that ranged in r-squared values from 0.68 to 0.99.

a values (redness-greenness). Within the skim (0.2% fat) unpasteurized beverages, the effects of CNTPT, CNTPT*CNTPT, and protein explained 19.88%, 54.08%, and 15.12% of the total variation of a values, respectively (Table 5) with a r-squared value of 0.80. When the r-squared value for the model was low, the model had a more difficult time separating the impact of the effect of variation in protein concentration and CN%TP on a values. While within the 1% fat unpasteurized beverages, CNTPT explained 76.56% of the total variation of a values followed by CNTPT*CNTPT (15.54%) and protein (5.68%) with a r-squared value of 0.96 (Table 5). Within the 2% fat unpasteurized beverages, CNTPT explained 91.08% of the total variation of a values followed by CNTPT*CNTPT (5.01%) with an r-squared value of 0.99 (Table 5). Generally, a values decreased with increasing CN%TP within each fat level in unpasteurized beverages, which could be due to increased absorption of light by riboflavin or other green appearing components in the serum phase of the milk protein beverages (Misawa et al., 2016).

b* values (yellowness – blueness). Again, for the 0.2% fat level unpasteurized beverages, the r-squared value for the ANOVA model was low (i.e., 0.72) and the model had a difficult time separating the effect of variation of protein level and variation in CN%TP on b* value. In the 1 and 2% fat level unpasteurized beverages, the r-squared values were higher (0.97 and 0.99, respectively) and there was a strong effect of the linear and quadratic terms for CN%TP on b* value (Table 5). As CN%TP in the unpasteurized beverages increased, b* value decreased, with larger decreases in the 1 and 2% fat unpasteurized beverages (Figure 2.B). Increases in CN%TP resulted in more casein micelles at the interface of the milk fat globule, and blocked the light path into the fat globule and decreased the b* values.

***Sensory Color Intensity*.** Variation in CNTPT explained 82.58%, 69.86%, and 83.99% of the total variation of color intensity for the 0.2, 1, and 2% fat unpasteurized beverages, respectively (Table 5). As CN%TP increased, sensory color intensity decreased with a larger decrease from 5 to 25% CN%TP than from 25 to 80% in unpasteurized beverages (Figure 2.C).

***Sensory Yellowness*.** Within each fat level, variation in CNTPT explained 94.0%, 94.89% and 92.82% of the total variation of yellowness for the 0.2, 1 and 2% fat unpasteurized beverages, respectively (Table 5). As previously reported by Misawa et al. (2016), as CN%TP increased, sensory yellowness decreased at all fat levels (Figure 2.D) in unpasteurized milk based beverages.

Combined Unpasteurized and Pasteurized Beverage Formulations

All fat levels

L values, Sensory Whiteness and Opacity

***L values* (*whiteness*).** The effect of heat of pasteurization and homogenization on the total variation of L values is reflected by the sum of all significant terms in the model that include heat. The most important of these terms heat, CNTPT*heat, CNTPT*fat*heat, CNTPT*CNTPT*heat, and CNTPT*CNTPT*fat*heat contributed to the total variation of L values across fat levels in unpasteurized and pasteurized beverages (Table 6). Heat treatment (homogenization and pasteurization) increased the L values more in the products with lower fat levels and lower CN%TP levels than in products with higher CN%TP (Figure 1.A). This may be due to the heat induced denaturation of noncasein proteins that interact with casein micelles to build larger light scattering protein aggregates in the pasteurized, homogenized skim milk beverages.

Sensory *Whiteness.* Variation in CNTPT, heat and CNTPT*heat had the largest impact on sensory whiteness in unpasteurized and pasteurized milk based beverages (Table 6). Heat treatment increased the whiteness of all beverages at all fat levels and the effect was larger at low CN%TP than at high CN%TP (Figure 1.B). The impact of heat and homogenization increased the sensory whiteness more for beverages containing 1 or 2% fat than in beverages containing 0.2% fat (Figure 1B).

Sensory Opacity. Variation in fat (21.88%), heat (15.98%), CNTPT (36.51%), and CNTPT*heat (8.89%) explained most of the variation in opacity among unpasteurized and pasteurized milk beverages (Table 6). Heat treatment increased the sensory opacity more in the products with higher fat levels and lower CN%TP (Figure 1.C).

Generally, heat (pasteurization and homogenization) increased the L values, sensory whiteness and sensory opacity more in the skim beverages than it did in the 1 and 2% fat beverages (Figure 1). More specifically, pasteurization caused the denaturation and attachment of serum protein to the casein micelles, and homogenization generated a more uniform fat distributed emulsion with a larger surface area and more casein micelles at the interface of the fat globule and the serum phase of the milk beverages. The effects on L values of increasing CN and fat content at the same time were not additive (Quiñones et al., 1998, Misawa et al., 2016). The pasteurization derived denaturation of milk serum proteins may have impacted light scattering of the skim beverages more, while both pasteurization and homogenization could influence whiteness in the 1 and 2% fat beverages.

a values, b* values, Color Intensity and Yellowness

***a values* (*redness-greenness*).** Variation in fat content explained more than 50% of the variation in a value (Table 7). The effects of heat, CNTPT*heat and CNTPT*CNTPT*heat

explained 4.96%. 3.83% and 5.01% of the total variation of a values across fat levels (Table 7 and Figure 2.A).

b* values (yellowness-blueness). Variation in fat content explained about 38% of the variation in b* values (Table 7), with lower fat content beverages having lower b* values (Figure 2B). Heat, CNTPT*heat and CNTPT*CNTPT*heat, fat*heat and CNTPT*fat*heat contributed to 5.28%, 3.11% and 6.49%, 2.29% and 6.48%, in total 23.65% of the total variation of b* values (Table 7). Heat treatment decreased the b* values in the 5%-25% range of CN%TP for the 1% and 2% fat beverages, while it showed little impact on b*values in the 0.2% fat products and higher range of CN%TP for the 1% and 2% fat beverages (Figure 2.B).

Color Intensity. Variation in fat, CNTPT, CNTPT*fat, and CNTPT*heat, explained 81% of the total variation of color intensity, with CNTPT having the largest impact (Table 7). Heat treatment decreased the color intensity of the 1% and 2% products more than the skim products in the lower CN%TP range (Figure 2.C).

Sensory *Yellowness.* Variation in heat, fat*heat, CNTPT, and CNTPT*heat explained 94.75% of the total variation of color intensity, with CNTPT and heat having the largest impacts (Table 7). Heat treatment plus homogenization decreased the yellowness more in the higher fat level products than in low fat products (Figure 2.D). Overall, heat (pasteurization and homogenization) decreased sensory color intensity and sensory yellowness (Figures 2.C and D). Changes in a and b* values with increasing CN%TP were more complex (Figures 2.A and B).

The impact of heat (pasteurization plus homogenization) caused more light of all wavelengths to be reflected back from the surface of milk protein beverages and increased L values (Figure 1.A) and sensory whiteness (Figure 1.B). After pasteurization plus homogenization, the amount of light that penetrated into the serum phase and into the fat

globules decreased (more casein micelles surround the fat globule) more than the light absorbed by the serum phase soluble compounds (such as riboflavin for a values) and fat soluble compounds (such as carotenoids for b* values) increased (Misawa et al., 2016) resulting in overall decreases in sensory color intensity and sensory yellowness (Figures 2C and D). However, overall fat concentration had more impact on a and b*values than heat (pasteurization plus homogenization) (Table 7, Figures 2.A and B) and CNTPT plus the interactions of CNTPT with fat and heat (Table 7) had more impact on sensory color intensity and sensory yellowness than both fat and pasteurization plus homogenization.

Instrumental Viscosity Heat (i.e., pasteurized versus unpasteurized) and heat related terms explained < 0.25% of the total variation of instrumental viscosity across fat levels while temperature (i.e., temperature of measurement of viscosity) explained 83.85% of the variation in measured viscosity (Table 6). When measuring the viscosity of these beverages at 4°C, the effects of CNTPT, protein, CNTPT*prot and fat explained 65.35%, 16.19%, 5.55% and 4.54% of the total variation of instrumental viscosity, respectively, with a r squared value of 0.96 (Table 8). Variation in protein, CNTPT and fat explained 48.85%, 27.50% and 10.94% of the total variation of instrumental viscosity, respectively, when testing at 20°C with a higher r squared value of 0.88 for the model, while they contributed to 35.44%, 7.96 and 16.95% of the total variation of instrumental viscosity at 50°C with a lower r square value of 0.68 for the model (Table 8). When the r-squared value for the model was low (such as at 50°C), the model had a more difficult time separating the impact of the effect of variation in protein concentration, CN%TP and fat on instrumental viscosity value. Increasing testing temperature from 4°C to 50°C decreased instrumental viscosity and masked the viscosity changes caused by composition differences within the formulas (Figure 3). Increases in CN%TP increased viscosity more than

increases in protein did, when measuring at 4°C (Table 8 and Figure 4), while the impact of changes in protein and CN%TP at 20 and 50°C were small (data not shown). Increases in CN%TP and CN concentration were reported to increase the relative viscosity of milk protein beverages (Misawa et al., 2016). Temperature dependent disassociation of beta-casein out of micelles at 4°C increased the protein concentration in the serum phase and may have increased the viscosity for the milk protein beverages.

Prediction of sensory whiteness and yellowness with instrumental color measures

Preliminary PCA runs for the sensory whiteness data and the sensory yellowness data (biplots not shown) indicated that all the 5% CNTP formulations were a different cluster of formulations due to the non-linear changes in color with the change in CN%TP (Tables 4 and 5; Figure 1 and 2). Removal of the 5% CN%TP formulations also caused major changes in the PCA vectors and their interrelationships for the subpopulation of formulations, with principle component 1 explaining most of the variation in sensory whiteness and yellowness. Once the 5% CNTP formulations were removed from the population and new biplots were constructed with skim, 1% and 2% formulations together as one population (not shown), it was determined that the skim unpasteurized beverages were clustered separately from the unpasteurized 1 and 2% fat beverages with respect to whiteness. Separate PCA plots were done for skim (Figure 5) and 1 plus 2% fat beverages (Figure 6) whiteness which represented more homogeneous subpopulations of beverage population (with most of the variation explained by principle component 1 for prediction of sensory whiteness from instrumental color measurements. When only one principle component explains most of the variation in sensory whiteness and the vectors for whiteness opacity and L values are of similar magnitude and direction (Figure 5 and 6), it is likely that L values alone may be a reasonably good predictor of sensory whiteness.

Similar PCA biplots were made for skim yellowness (Figure 7) and 1 plus 2% fat beverage (Figure 8) yellowness. In these PCA biplots, the vector for sensory yellowness and b* value do not project in the same direction for skim (Figure 7) or for 1 plus 2% fat (Figure 8). In addition, the relationship of the vectors to principle components 1 and 2 are different in skim (Figure 7) versus 1 plus 2% fat (Figure 8). It is possible that there may be a more complex relationship between instrumental measures of color and sensory color perception for yellowness. This approach was designed to select subpopulations of beverages to achieve better predictive power for the instrumental whiteness and yellowness that will match sensory.

Regression statistics of linear, quadratic and multiple linear regression (MLR) for the whiteness group (L values, or L, a and b* values) and for the yellowness group (b* values or L, a and b* values) were summarized in Tables 9 and 10, respectively. The higher the R squared that a model has, the better the fit or prediction. Using the L values, the linear regression prediction of sensory whiteness produced a R squared of 0.71 for the skim beverages and 0.76 for the 1 and 2% fat beverages and the quadratic regression prediction of sensory whiteness had an R squared of 0.77 for the skim beverages and 0.80 for the 1 and 2% fat beverages (Table 9). Other researchers have found that Hunter L or CIE L* values had a higher correlation with sensory whiteness or lightness of milk chocolate (Guinard and Mazzucchelli, 1999) and dairy desserts (Tárrega and Costell, 2007) by using a single instrumental parameter for the prediction of whiteness/lightness. MLR was used in the current study to generate a more powerful prediction model for sensory whiteness of unpasteurized milk protein beverages than linear or quadratic regression. MLR prediction for sensory whiteness including L, a and b* values, produced a higher R squared (0.84 for skim beverages and 0.87 for 1 and 2% fat beverages) than the linear and quadratic regression predictions (Table 9). Only using the b* values, the linear regression

prediction of sensory yellowness showed a low R squared (0.02) for the skim beverages and 0.6347 for the 1 and 2% fat beverages and the quadratic regression prediction of sensory yellowness had a R squared of 0.005 for the skim beverages and 0.65 for the 1 and 2% fat beverages (Table 10). These lower R squared values for linear regression b values and sensory yellowness (Table 10) versus the higher values for L value and sensory whiteness (Table 9) were consistent with the visual relationships in the PCA biplots for L values and sensory whiteness (Figure 5 and 6), while for sensory yellowness and b* values there was no visual relationships (Figures 7 and 8). This was in agreement with results from Misawa et al. (2016) that the instrumental b* value and sensory yellowness of milk protein beverages were different. Thus, MLR prediction for sensory yellowness used L, a and b* values, produced a much higher R squared with 0.92 and 0.84 for skim, 1 and 2% fat beverages respectively (Table 10) than linear or polynomial regression. MLR using L, a and b* values together generated a more robust prediction of sensory whiteness and sensory yellowness of the unpasteurized milk protein beverages than using a single instrumental parameter (L or b* values) and this may be a useful approach for using instrumental color measurements for quality control testing and for control of the consistency of color of milk based beverages in a beverage manufacturing plant. Instrumental predictions of sensory color may be useful for exploring beverage reformulation with different ingredients to control raw material cost while determining if the formulation change is like to cause a perceived difference in sensory properties of the beverage by consumers**.**

Conclusions

For unpasteurized milk protein beverages across 3 fat levels (0.2, 1, and 2%), change in CN%TP had the largest impact on L values, sensory whiteness, opacity, color intensity and yellowness while changes in fat concentration had a stronger influence on a and b* values.

Increasing in CN%TP from 5 to 80% increased L values, sensory whiteness and opacity and decreased sensory color intensity and yellowness. The a and b* values increased with increasing fat concentration. For unpasteurized milk protein beverages within each fat level, the impact of variation in CN%TP dominated the changes in L values, sensory whiteness and opacity and decreased a and b* values, sensory color intensity and yellowness. The effect of heat (pasteurization and homogenization) and its interaction terms were the second largest impact on color of milk protein beverages with respect to instrumental color data and sensory appearance attributes. Heat increased L values, sensory whiteness and opacity and decreased a and b* values, sensory color intensity and yellowness. Increases in temperature dramatically decreased instrumental viscosity and changes in protein concentration and CN%TP had more impact on instrument viscosity data within each temperature (4, 20, and 50^{o}C) than fat. Sensory perception of yellowness was not highly correlated with b* values. Multiple linear regressions of L, a and b* values produced more robust predictions for both sensory whiteness and yellowness than simple linear regression with L and b* values, alone and may be a useful instrumental approach for monitoring processing and quality control of sensory whiteness and yellowness of milk protein beverages.

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Table 1. Formulation design and relative weight percentage use of ingredients for 0.2%, 1.0% and 2.0% fat level with a range of casein as a percentage of true protein (CN%TP) of 5, 25, 50, 75 and 80% and true protein (TP) levels of 3.00, 3.67, 4.34 and 5.00% within each fat level.

Fat (%)	Protein		Formulation code		MCC[1]	SPI[1]	Lactose[1]	Heavy cream	DI water
	CN%TP	TP (%)	Unpasteurized	Pasteurized					
0.2 (skim)	5	3.00	R1	P1	1.04	12.18	4.82	0.47	81.49
	25	3.00	R2	P2	9.00	9.38	4.78	0.42	76.42
	50	3.00	R3	P3	18.95	5.88	4.72	0.36	70.09
	75	3.00	R4	P4	28.89	2.38	4.67	0.30	63.75
	80	3.00	R5	P5	30.88	1.68	4.66	0.29	62.48
	5	3.67	R6	P6	1.29	14.90	4.80	0.47	78.54
	25	3.67	R7	P7	11.03	11.48	4.75	0.41	72.34
	50	3.67	R8	P8	23.20	7.19	4.69	0.34	64.58
	75	3.67	R9	P9	35.37	2.91	4.62	0.26	56.84
	80	3.67	R10	P10	37.80	2.06	4.61	0.24	55.29
	5	4.34	R11	P11	1.54	17.62	4.79	0.47	75.58
	25	4.34	R12	P12	13.05	13.57	4.73	0.40	68.25
	50	4.34	R13	P13	27.45	8.51	4.65	0.31	59.09
	75	4.34	R14	P14	41.84	3.45	4.57	0.22	49.92
	80	4.34	R15	P15	44.72	2.43	4.56	0.20	48.09
	5	5.00	R16	P16	1.79	20.30	4.77	0.47	72.67
	25	5.00	R17	P17	15.05	15.64	4.70	0.39	64.23
	50	5.00	R18	P18	31.63	9.80	4.61	0.28	53.67
	75	5.00	R19	P19	48.21	3.97	4.52	0.18	43.11
	80	5.00	R20	P20	51.53	2.80	4.51	0.16	41.00
1.0	5	3.00	R21	P21	0.69	12.17	4.77	2.44	79.94
	25	3.00	R22	P22	8.65	9.36	4.72	2.39	74.87
	50	3.00	R23	P23	18.60	5.87	4.67	2.32	68.54
	75	3.00	R24	P24	28.55	2.37	4.62	2.26	62.21
	80	3.00	R25	P25	30.54	1.67	4.61	2.25	60.94
	5	3.67	R26	P26	0.94	14.89	4.75	2.43	76.99
	25	3.67	R27	P27	10.68	11.46	4.70	2.37	70.79
	50	3.67	R28	P28	22.85	7.18	4.63	2.30	63.04
	75	3.67	R29	P29	35.02	2.90	4.57	2.22	55.29
	80	3.67	R30	P30	37.46	2.04	4.56	2.20	53.74
	5	4.34	R31	P31	1.19	17.61	4.73	2.43	74.03
	25	4.34	R32	P32	12.71	13.56	4.67	2.36	66.70
	50	4.34	R33	P33	27.10	8.49	4.59	2.27	57.54
	75	4.34	R34	P34	41.49	3.43	4.52	2.18	48.38
	80	4.34	R35	P35	44.37	2.42	4.50	2.16	46.55
	5	5.00	R36	P36	1.44	20.29	4.72	2.43	71.12
	25	5.00	R37	P37	14.71	15.62	4.65	2.35	62.68
	50	5.00	R38	P38	31.29	9.79	4.56	2.24	52.12
	75	5.00	R39	P39	47.87	3.96	4.47	2.14	41.57
	80	5.00	R40	P40	51.19	2.79	4.45	2.12	39.45
2.0	5	3.00	R41	P41	0.20	12.14	4.72	4.89	78.04
	25	3.00	R42	P42	8.16	9.34	4.68	4.84	72.97
	50	3.00	R43	P43	18.11	5.84	4.63	4.78	66.64
	75	3.00	R44	P44	28.06	2.34	4.58	4.72	60.30
	80	3.00	R45	P45	30.05	1.64	4.56	4.70	59.04
	5	3.67	R46	P46	0.45	14.86	4.71	4.89	75.09
	25	3.67	R47	P47	10.19	11.44	4.65	4.83	68.89
	50	3.67	R48	P48	22.36	7.16	4.59	4.75	61.14
	75	3.67	R49	P49	34.53	2.88	4.53	4.67	53.39
	80	3.67	R50	P50	36.97	2.02	4.51	4.66	51.84
	5	4.34	R51	P51	0.70	17.59	4.69	4.89	72.13
	25	4.34	R52	P52	12.22	13.53	4.63	4.82	64.80
	50	4.34	R53	P53	26.61	8.47	4.55	4.72	55.64
	75	4.34	R54	P54	41.01	3.41	4.48	4.63	46.47

Table 1 (continued).

2.0	80	4.34	R55	P55	43.89	2.40	4.46	4.61	44.64
	5	5.00	R56	P56	0.95	20.27	4.67	4.89	69.22
	25	5.00	R57	P57	14.22	15.60	4.60	4.80	60.78
	50	5.00	R58	P58	30.80	9.77	4.51	4.70	50.22
	75	5.00	R59	P59	47.38	3.93	4.43	4.59	39.66
	80	5.00	R60	P60	50.70	2.77	4.41	4.57	37.55

[1] micellar casein concentrate = MCC, serum protein isolate = SPI, lactose = anhydrous lactose.

Table 2. Hunter L values at a 10 degree viewer angle (LHA10) using illuminant A, sensory whiteness and sensory opacity of unpasteurized milk protein beverages: relative percentage of Type III sum of squares (for factors with $P < 0.05$) explained by model factors (fat = fat level with 0.2, 1 and 2%, prot = milk true protein with 3.00, 3.67, 4.34 and 5.00%, temp = temperature of color measurement with 4°C and 20°C, rep = replicate, CNTPT = casein as a percentage of true protein transformed with 5, 25, 50, 75 and 80%).

Factors	LHA10	Whiteness	Opacity
fat	1.34	2.76	9.17
prot	0.33	7.05	2.63
temp	0.23	NS	NS
rep	0.08	NS	NS
fat*prot	0.35	0.22	0.65
fat*temp	0.36	NS	0.07
fat*rep	0.09	0.02	0.02
prot*temp	0.10	0.01	0.10
prot*rep	NS	0.01	0.05
fat*prot*temp	NS	NS	0.05
fat*prot*rep	0.18	0.03	0.04
fat*prot*temp*rep	0.01^{NS}	NS	0.09
CNTPT	65.50	87.07	77.67
CNTPT*fat	15.22	0.17	3.13
CNTPT*prot	0.11	0.14	0.10
CNTPT*temp	0.18	NS	NS
CNTPT*rep	NS	0.01	0.03
CNTPT*fat*prot	0.09^{NS}	NS	NS
CNTPT*fat*temp	NS	0.11	0.31
CNTPT*CNTPT	9.51	1.53	4.50
CNTPT*CNTPT*fat	5.80	0.06	0.72
CNTPT*CNTPT*prot	0.18	0.68	0.10
CNTPT*CNTPT*temp	0.21	NS	NS
CNTPT*CNTPT*fat*prot	0.14	0.08	0.43
Sum (%)	100.00	100.00	100.00
R^2	0.98	0.97	0.96
N	480	2880	2880

NS = not significant ($P > 0.05$).

Table 3. Hunter a value (aHA10) and CIE b* value (bCA10) at a 10 degree viewer angle using illuminant A, sensory color intensity and sensory whiteness of unpasteurized milk protein beverages: relative percentage of Type III sum of squares (for factors with $P < 0.05$) explained by model factors (fat = fat level with 0.2, 1 and 2%, prot = milk true protein with 3.00, 3.67, 4.34 and 5.00%, temp = temperature of color measurement with 4°C and 20°C, rep = replicate, CNTPT = casein as a percentage of true protein with 5, 25, 50, 75 and 80% transformed).

Factors	aHA10	bCA10	Color Intensity	Yellowness
fat	48.84	38.35	9.92	4.50
prot	2.11	1.72	0.11^{NS}	2.16
temp	0.06	0.20	0.08^{NS}	0.18
rep	NS	NS	0.01^{NS}	NS
fat*prot	1.21	2.20	0.60	0.33
fat*temp	1.32	0.84	0.50	NS
fat*rep	NS	0.15	0.17	0.22
prot*temp	NS	NS	0.19	0.03^{NS}
prot*rep	NS	NS	0.44	NS
temp*rep	NS	NS	0.06	0.05
fat*prot*temp	NS	NS	0.18	0.06
fat*prot*rep	0.44	0.37	0.19	NS
fat*temp*rep	NS	NS	0.35	0.06
prot*temp*rep	NS	NS	0.12	0.10
fat*prot*temp*rep	0.06^{NS}	0.02^{NS}	0.28	0.15
CNTPT	28.77	29.86	65.77	90.10
CNTPT*fat	1.90	14.73	8.54	0.59
CNTPT*prot	NS	0.22	0.15	0.08
CNTPT*temp	0.36	0.41	0.32	NS
CNTPT*rep	NS	NS	0.55	0.04
CNTPT*fat*prot	NS	NS	0.21	0.10
CNTPT*fat*temp	0.20	0.21	0.50	0.10
CNTPT*fat*rep	NS	NS	1.48	NS
CNTPT*prot*temp	NS	NS	0.10	NS
CNTPT*temp*rep	NS	NS	0.10	NS
CNTPT*prot*rep	NS	NS	0.19	NS
CNTPT*CNTPT	11.01	8.91	3.30	0.88
CNTPT*CNTPT*fat	2.98	0.38	3.79	NS
CNTPT*CNTPT*prot	0.14^{NS}	0.15	0.24	0.08
CNTPT*CNTPT*temp	NS	NS	NS	0.07
CNTPT*CNTPT*rep	NS	NS	0.23	NS
CNTPT*CNTPT*fat*prot	0.59	0.92	0.52	0.11
CNTPT*CNTPT*fat*temp	NS	0.35	0.04	NS
CNTPT*CNTPT*fat*rep	NS	NS	0.12	NS
CNTPT*CNTPT*prot*temp	NS	NS	0.21	NS
CNTPT*CNTPT*prot*rep	NS	NS	0.42	NS
Sum (%)	100.00	100.00	100.00	100.00

Table 3 (continued).

R^2	0.96	0.98	0.91	0.90
N	480	480	2880	2880

NS = not significant ($P > 0.05$).

Table 4. Hunter L values at a 10 degree viewer angle (LHA10) using illuminant A, sensory whiteness and sensory opacity of unpasteurized milk protein beverages: relative percentage of Type III sum of squares (for factors with $P < 0.05$) explained by model factors (prot = milk true protein with 3.00, 3.67, 4.34 and 5.00%, temp = temperature of sensory appearance evaluation with 4°C and 20°C, rep = replicate, CNTPT = casein as a percentage of true protein with 5, 25, 50, 75 and 80% transformed) at 3 fat levels (0.2, 1 and 2%).

	LHA10			Whiteness			Opacity		
Factors									
Fat (%)	0.2	1	2	0.2	1	2	0.2	1	2
prot	0.83	0.26	0.07^{NS}	9.92	5.33	6.96	4.30	9.54	1.67
temp	0.71	0.16	NS	NS	NS	NS	0.06^{NS}	NS	0.03^{NS}
rep	0.01	0.71	0.69	NS	0.08	NS	0.04^{NS}	0.02	NS
prot*temp	NS	NS	NS	NS	0.06	0.06	0.45	NS	0.09
prot*rep	0.35	NS	NS	NS	0.13	0.02	0.25	NS	0.10
temp*rep	NS	NS	NS	NS	NS	0.03	NS	NS	NS
prot*temp*rep	NS	NS	0.13	0.03^{NS}	0.02^{NS}	NS	0.14	0.08^{NS}	0.07
CNTPT	79.34	89.38	92.82	87.31	92.59	89.05	85.18	86.04	93.47
CNTPT*prot	NS	0.31	0.60	0.36	0.34	0.04	0.40	0.63	0.45
CNTPT*temp	NS	0.63	1.14	NS	NS	NS	NS	NS	0.02
CNTPT*rep	NS	NS	NS	NS	NS	0.01	0.05	NS	0.08
CNTPT*prot*temp	NS	NS	0.04	NS	NS	0.03	NS	NS	0.07
CNTPT*temp*rep	NS	NS	NS	NS	NS	NS	NS	NS	0.05
CNTPT*prot*rep	NS	NS	NS	NS	0.09	NS	NS	NS	NS
CNTPT*CNTPT	18.17	7.79	3.91	1.22	1.01	2.85	7.61	3.68	3.36
CNTPT*CNTPT*prot	0.29	0.33	0.33	1.16	0.35	0.83	0.94	NS	0.48
CNTPT*CNTPT*temp	0.30	0.37	0.28	NS	NS	0.01^{NS}	NS	NS	NS
CNTPT*CNTPT*prot*temp	NS	NS	NS	NS	NS	0.05	0.41	NS	NS
CNTPT*CNTPT*temp*rep	NS	NS	NS	NS	NS	0.02	NS	NS	NS
CNTPT*CNTPT*prot*rep	NS	NS	NS	NS	NS	0.02	0.18	NS	0.07
Sum (%)	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
R^2	0.97	1.00	1.00	0.96	0.96	0.99	0.95	0.96	0.97
N	160	160	160	960	960	960	960	960	960

NS = not significant ($P > 0.05$).

Table 5. Hunter a value (aHA10) and CIE b* values at a 10 degree viewer angle (bCA10) using illuminant A, sensory color intensity and sensory yellowness of unpasteurized milk protein beverages: relative percentage of Type III sum of squares (for factors with $P < 0.05$) explained by model factors (prot = milk true protein with 3.00, 3.67, 4.34 and 5.00%, temp = temperature of sensory appearance evaluation with 4°C and 20°C, rep = replicate, CNTPT = casein as a percentage of true protein with 5, 25, 50, 75 and 80% transformed) at 3 fat levels (0.2, 1 and 2%).

	aHA10			bCA10			Color Intensity			Yellowness		
Factors												
Fat (%)	0.2	1	2	0.2	1	2	0.2	1	2	0.2	1	2
prot	15.12	5.68	0.76	45.38	2.31	0.05^{NS}	5.37^{NS}	0.84^{NS}	0.01^{NS}	2.99	3.88	3.90
temp	3.12	1.09	2.03	2.91	2.58	1.77	0.13^{NS}	2.06^{NS}	0.03^{NS}	0.45	0.08	0.23^{NS}
rep	NS	0.10	0.26	0.57	0.36	0.08	NS	0.49^{NS}	0.08^{NS}	0.39	NS	0.30^{NS}
prot*temp	NS	NS	NS	NS	NS	NS	0.55	3.03	0.06	NS	NS	0.19
prot*rep	1.92	NS	NS	6.80	NS	NS	NS	3.19	0.19	NS	NS	0.00
temp*rep	NS	NS	NS	NS	NS	NS	NS	1.40	0.06	NS	NS	0.27
prot*temp*rep	0.25^{NS}	0.06^{NS}	0.06^{NS}	0.09^{NS}	0.02^{NS}	0.06^{NS}	0.51	0.79	0.31	0.22^{NS}	0.07^{NS}	0.51
CNTPT	19.88	76.56	91.08	0.01	75.69	87.43	82.58	69.86	83.99	94.00	94.89	92.82
CNTPT*prot	NS	NS	0.25	NS	0.52	0.57	1.31	0.20	0.82	NS	0.29	0.13
CNTPT*temp	2.33	0.75	0.07	6.09	0.43	NS	0.61	1.74	NS	NS	0.08	0.05
CNTPT*rep	NS	NS	NS	NS	NS	NS	1.81	6.24	0.32	NS	NS	0.10
CNTPT*prot*temp	NS	NS	NS	NS	NS	NS	NS	0.54	0.17	NS	NS	0.24
CNTPT*temp*rep	NS	NS	NS	NS	NS	NS	NS	1.38	NS	NS	NS	NS
CNTPT*prot*rep	NS	NS	NS	NS	NS	NS	1.09	1.67	0.42	NS	NS	0.16
CNTPT*CNTPT	54.08	15.54	5.01	22.34	18.09	9.51	2.42	0.01	12.09	1.36	0.55	0.96
CNTPT*CNTPT*prot	3.29	NS	0.29	9.15	NS	0.32	1.41	NS	1.06	0.41	0.16	NS
CNTPT*CNTPT*temp	NS	0.22	0.20	3.47	NS	0.20	NS	0.12	NS	0.18	NS	0.14
CNTPT*CNTPT*rep	NS	NS	NS	NS	NS	NS	NS	0.77	0.25	NS	NS	NS
CNTPT*CNTPT*prot*temp	NS	NS	NS	NS	NS	NS	1.17	3.20	0.05	NS	NS	NS
CNTPT*CNTPT*prot*rep	NS	NS	NS	3.21^{NS}	NS	NS	1.04	2.49	0.08	NS	NS	NS
Sum (%)	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
R^2	0.80	0.96	0.99	0.72	0.97	0.99	0.68	0.83	0.98	0.85	0.89	0.92
N	160	160	160	160	160	160	960	960	960	960	960	960

NS = not significant ($P > 0.05$).

Table 6. Hunter L values at a 10 degree viewer angle (LHA10) using illuminant A, sensory whiteness, sensory opacity and instrumental viscosity of unpasteurized and homogenized/pasteurized milk protein beverages: relative percentage of Type III sum of squares (for factors with $P < 0.05$) explained by model factors (fat = fat level with 0.2, 1% fat and 2% fat, heat=pasteurization and homogenization, prot = milk true protein with 3.00, 3.67, 4.34 and 5.00%, temp = temperature of sensory appearance evaluation with 4°C and 20°C, rep = replicate, CNTPT = casein as a percentage of true protein with 5, 25, 50, 75 and 80% transformed).

Factors	LHA10	Whiteness	Opacity	Viscosity[1]
fat	3.97	7.40	21.88	1.13
heat	2.58	26.93	15.98	0.08
prot	0.23	4.47	2.25	4.24
temp	0.18	NS	0.01[NS]	83.85
rep	NS	NS	NS	0.46
fat*heat	0.20	1.23	5.78	NS
fat*prot	0.35	0.42	1.11	NS
fat*temp	0.24	0.01	NS	0.53
fat*rep	0.04	0.01	NS	1.46
heat*prot	0.09	0.62	0.03	NS
heat*temp	0.05	NS	NS	NS
heat*rep	0.05	NS	NS	NS
prot*temp	NS	NS	0.02	NS
prot*rep	0.11	NS	0.02	NS
temp*rep	NS	0.01	NS	0.75
fat*heat*prot	NS	0.21	0.95	NS
fat*heat*temp	0.04	NS	0.03	NS
fat*heat*rep	0.04	0.01	0.01	NS
fat*prot*temp	NS	NS	0.01	NS
fat*prot*rep	0.18	0.02	0.02	NS
heat*prot*temp	NS	NS	0.01	NS
prot*temp*rep	NS	NS	0.01	NS
heat*prot*rep	NS	0.01	0.01	NS
fat*heat*prot*temp	NS	NS	0.02	NS
fat*heat*prot*rep	NS	0.02	0.01	NS
fat*heat*prot*temp*rep	0.09[NS]	0.04	0.07	0.47
CNTPT	43.15	44.08	36.51	3.36
CNTPT*fat	8.81	0.12	2.53	0.14
CNTPT*heat	19.26	12.47	8.89	0.11
CNTPT*prot	NS	0.01	NS	0.27
CNTPT*temp	0.18	NS	NS	2.89
CNTPT*rep	NS	NS	NS	NS
CNTPT*fat*heat	5.36	0.03	0.07	NS
CNTPT*heat*prot	0.10	0.20	0.09	NS
CNTPT*heat*temp	0.02	NS	NS	NS
CNTPT*heat*rep	NS	0.01	0.02	NS
CNTPT*fat*prot	0.09	0.14	0.24	NS

Table 6 (continued).

CNTPT*fat*rep	NS	NS	0.02	NS
CNTPT*prot*temp	NS	NS	NS	0.24
CNTPT*temp*rep	NS	0.01	NS	NS
CNTPT*CNTPT	3.94	0.67	1.53	NS
CNTPT*CNTPT*fat	2.60	NS	0.04	NS
CNTPT*CNTPT*heat	4.79	0.28	0.87	0.03
CNTPT*CNTPT*prot	0.06	0.16	0.04	NS
CNTPT*CNTPT*temp	0.13	NS	NS	NS
CNTPT*CNTPT*fat*heat	2.72	0.04	0.59	NS
CNTPT*CNTPT*fat*prot	0.09	0.08	0.25	NS
CNTPT*CNTPT*fat*temp	0.03	0.01	NS	NS
CNTPT*CNTPT*heat*prot	0.13	0.29	0.02	NS
CNTPT*CNTPT*heat*temp	0.07	NS	NS	NS
CNTPT*CNTPT*prot*temp	NS	NS	0.02	NS
CNTPT*CNTPT*temp*rep	NS	NS	NS	NS
CNTPT*CNTPT*prot*rep	0.04	NS	0.01	NS
Sum (%)	100.00	100.00	100.00	100.00
R^2	0.99	0.98	0.98	0.94
N	960	5760	5760	1440

[1] instrumental viscosity included data from 4°C, 20°C and 50°

[2] NS = not significant ($P > 0.05$).

Table 7. Hunter a (aHA10) and CIE b* values at a 10 degree viewer angle (bCA10) using illuminant A, sensory color intensity and sensory yellowness of unpasteurized and homogenized/pasteurized milk protein beverages: relative percentage of Type III sum of squares explained by model factors (fat = fat level with 0.2, 1% fat and 2% fat, heat=pasteurization and homogenization, prot = milk true protein with 3.00, 3.67, 4.34 and 5.00%, temp = temperature of sensory appearance evaluation with 4°C and 20°C, rep = replicate, CNTPT = casein as a percentage of true protein with 5, 25, 50, 75 and 80% transformed).

Factor	aHA10	bCA10	Color intensity	Yellowness
fat	54.72	38.09	11.64	0.14
heat	4.96	5.28	1.44	31.88
prot	1.85	1.59	0.35	1.09
temp	0.13	0.18	0.01^{NS}	0.06
rep	0.02	0.02	0.03^{NS}	0.01^{NS}
fat*heat	0.57	2.29	0.63	9.45
fat*prot	1.81	1.67	0.48	0.50
fat*temp	1.15	0.49	0.40	NS
fat*rep	NS	0.25	0.42	0.02
heat*prot	0.07	0.16	0.12	0.67
heat*temp	NS	NS	0.11	0.06
heat*rep	NS	NS	0.09	0.03
prot*temp	NS	NS	0.06	0.03
prot*rep	0.12	0.09	0.14	0.03
fat*heat*prot	0.17	0.12	1.07	0.36
fat*heat*temp	0.08	0.16	0.39	NS
fat*heat*rep	NS	NS	1.01	0.07
fat*prot*temp	NS	NS	0.11	0.04
fat*prot*rep	0.37	0.26	0.18	0.08
fat*temp*rep	NS	NS	0.15	NS
heat*prot*temp	NS	NS	0.10	NS
heat*prot*rep	NS	NS	0.25	0.03
heat*temp*rep	NS	NS	0.06	0.03
fat*heat*prot*temp	NS	NS	0.19	NS
fat*heat*prot*rep	NS	NS	0.14	0.05
fat*prot*temp*rep	NS	NS	0.26	0.05
fat*heat*temp*rep	NS	NS	0.15	0.05
heat*prot*temp*rep	NS	NS	0.13	0.04
fat*heat*prot*temp*rep	0.12^{NS}	0.10^{NS}	0.12	0.05^{NS}
CNTPT	18.13	24.92	46.97	46.19
CNTPT*fat	0.70	4.86	10.33	NS
CNTPT*heat	3.85	3.11	12.01	7.23
CNTPT*prot	0.12	0.38	0.24	0.04
CNTPT*temp	0.28	0.18	0.15	NS
CNTPT*rep	NS	NS	0.02	0.02
CNTPT*fat*heat	0.58	6.48	0.31	0.79

Table 7 (continued).

CNTPT*heat*prot	0.16	NS	0.21	NS
CNTPT*heat*temp	NS	0.17	0.15	0.02
CNTPT*heat*rep	NS	NS	0.63	NS
CNTPT*fat*temp	0.23	0.25	0.15	0.03
CNTPT*fat*prot	NS	NS	0.23	0.13
CNTPT*fat*rep	NS	NS	0.76	NS
CNTPT*prot*temp	NS	NS	0.08	NS
CNTPT*temp*rep	NS	NS	0.02	NS
CNTPT*prot*rep	NS	0.08	0.20	NS
CNTPT*CNTPT	2.59	1.31	0.63	0.37
CNTPT*CNTPT*fat	0.64	0.50	0.87	NS
CNTPT*CNTPT*heat	5.01	6.49	2.30	0.11
CNTPT*CNTPT*prot	NS	0.07	0.09	0.06
CNTPT*CNTPT*temp	NS	NS	NS	0.05
CNTPT*CNTPT*rep	NS	NS	0.06	NS
CNTPT*CNTPT*fat*heat	1.46	0.10	2.40	NS
CNTPT*CNTPT*fat*prot	NS	0.11	0.40	NS
CNTPT*CNTPT*fat*temp	NS	0.17	NS	NS
CNTPT*CNTPT*fat*rep	NS	NS	0.03	0.05
CNTPT*CNTPT*heat*prot	0.13	0.09	0.19	NS
CNTPT*CNTPT*heat*rep	NS	NS	0.14	0.03
CNTPT*CNTPT*prot*temp	NS	NS	0.07	NS
CNTPT*CNTPT*prot*rep	NS	NS	0.13	0.02
Sum (%)	100.00	100.00	100.00	100.00
R^2	0.97	0.97	0.88	0.90
N	960	960	5760	5760

NS = not significant ($P > 0.05$).

Table 8. Instrumental viscosity of milk protein beverages: relative percentage of Type III sum of squares explained by model factors (fat= fat level with 0.2, 1% and 2% fat, heat= pasteurization and homogenization, prot = milk true protein with 3.00, 3.67, 4.34 and 5.00%, temp = temperature of sensory appearance evaluation with 4°C and 20°C, rep = replicate, CNTPT = casein as a percentage of true protein with 5, 25, 50, 75 and 80% transformed) at 3 measurement temperatures (4, 20 and 50°C).

	Viscosity		
Factors	4°C	20°C	50°C
fat	4.54	10.94	16.95
heat	0.56	1.92	0.54^{NS}
prot	16.19	48.85	35.44
rep	0.08	0.77	0.09^{NS}
fat*heat	NS	0.95	NS
fat*prot	0.94	NS	NS
fat*rep	0.61	NS	NS
heat*prot	0.43	0.35	3.69
heat*rep	NS	0.03^{NS}	5.09
prot*rep	0.53	0.37	1.55
fat*heat*rep	NS	0.33	5.17
fat*prot*rep	0.74	1.15	6.66
heat*prot*rep	NS	0.20^{NS}	1.47^{NS}
fat*heat*prot*rep	0.27^{NS}	0.34^{NS}	4.93
CNTPT	65.35	27.50	7.96
CNTPT*fat	1.70	1.47	2.69
CNTPT*heat	1.19	1.62	NS
CNTPT*prot	5.55	2.33	NS
CNTPT*heat*prot	0.18	NS	NS
CNTPT*fat*prot	NS	NS	5.06
CNTPT*fat*rep	0.12	NS	NS
CNTPT*prot*rep	0.15	NS	NS
CNTPT*CNTPT	0.08	0.04^{NS}	NS
CNTPT*CNTPT*fat	0.12	NS	NS
CNTPT*CNTPT*heat	0.27	0.85	NS
CNTPT*CNTPT*prot	0.20	NS	NS
CNTPT*CNTPT*heat*prot	NS	NS	2.72
CNTPT*CNTPT*prot*rep	0.19	NS	NS
Sum (%)	100.00	100.00	100.00
R^2	0.96	0.88	0.68
N	480	480	480

NS = not significant ($P > 0.05$).

Table 9. Linear, quadratic and multiple linear regression statistic summary by using Hunter L, a and CIE b* values at 10 degree viewer angles (LHA10, aHA10 and bCA10) as indicators to predict sensory whiteness for unpasteurized skim, 1 and 2% fat milk protein beverages with excluding beverages of 5% casein as a percentage of true protein.

Fat level (%)	0.2			1 and 2		
Indicator /predictor	LHA10/ sensory whiteness		LHA10, aHA10 and bCA10/sensory whiteness	LHA10/ sensory whiteness		LHA10, aHA10 and bCA10/sensory whiteness
Regression type	Linear	Quadratic	Multi linear	Linear	Quadratic	Multi linear
Multiple R	0.8455		0.9194	0.8738		0.9311
R Squared	0.7149	0.7738	0.8453	0.7635	0.8044	0.8670
Adjusted R Square	0.6945		0.8066	0.7556		0.8528
Standard Error	0.7091		0.5641	0.5811		0.4510
Observations	16	16	16	32	32	32

Table 10. Linear, quadratic and multiple linear regression statistic summary by using Hunter L, a and CIE b* values at 10 degree viewer angles (LHA10, aHA10 and bCA10) as indicators to predict sensory yellowness for unpasteurized skim, 1 and 2% fat milk protein beverages with excluding beverages of 5% casein as a percentage of true protein.

Fat level (%)	0.2			1 and 2		
Indicator /predictor	bCA10/ sensory yellowness		LHA10, aHA10 and bCA10/sensory yellowness	bCA10/ sensory yellowness		LHA10, aHA10 and bCA10/sensory yellowness
Regression type	Linear	Quadratic	Multi linear	Linear	Quadratic	Multi linear
Multiple R	0.1270		0.9612	0.7967		0.9173
R Squared	0.0161	0.0500	0.9238	0.6347	0.6455	0.8415
Adjusted R Square	-0.0541		0.9048	0.6225		0.8245
Standard Error	0.5446		0.1637	0.3676		0.2506
Observations	16		16	32		32

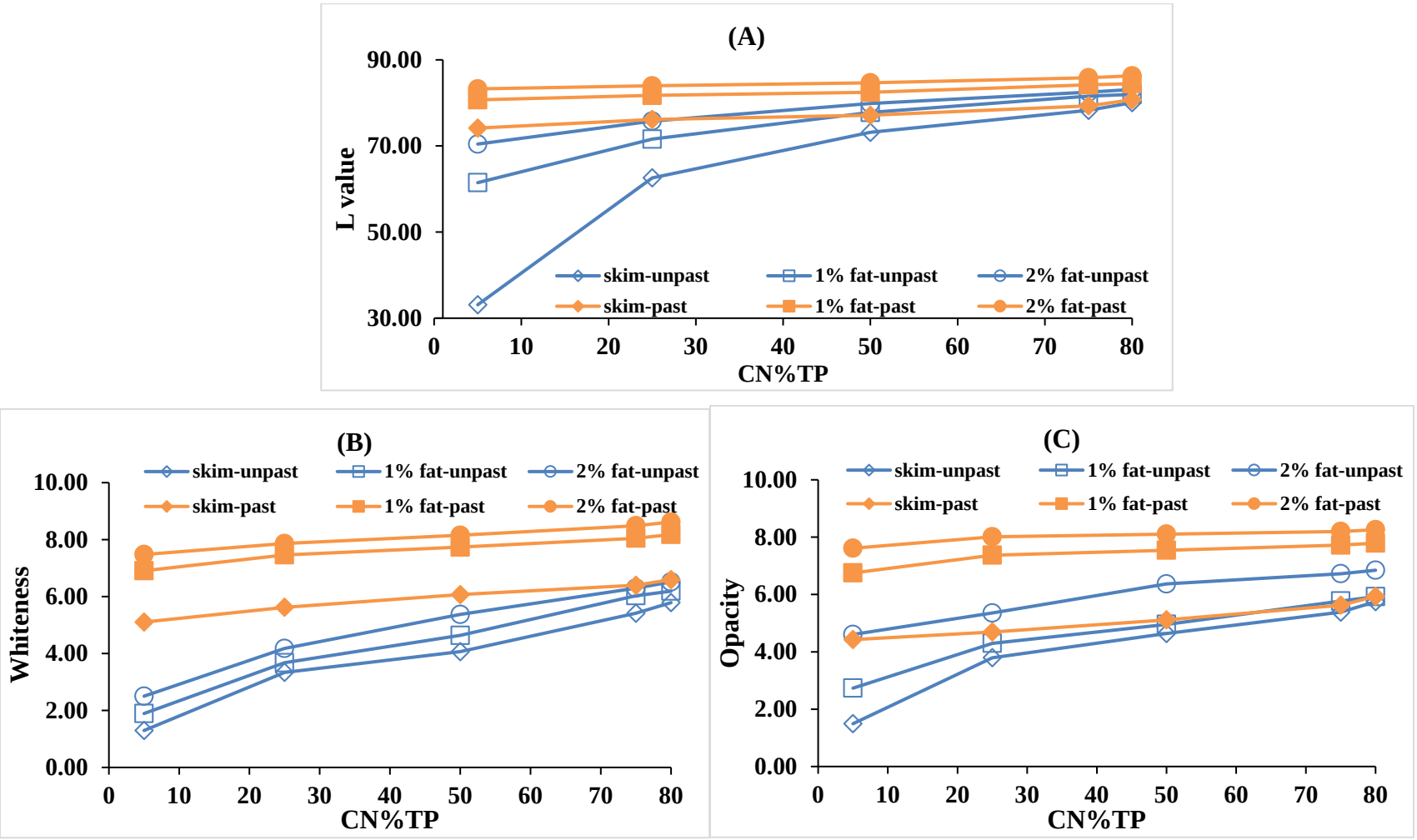

Figure 1. (A) Hunter L values (4°C and 20°C) at 10 degree viewer angle using illuminant A, (B) sensory whiteness and (C) sensory opacity for unpasteurized (unpast) and pasteurized (past) milk protein beverages with different fat levels (skim, 1% fat and 2% fat) as a function of casein as a percentage of true protein (CN%TP).

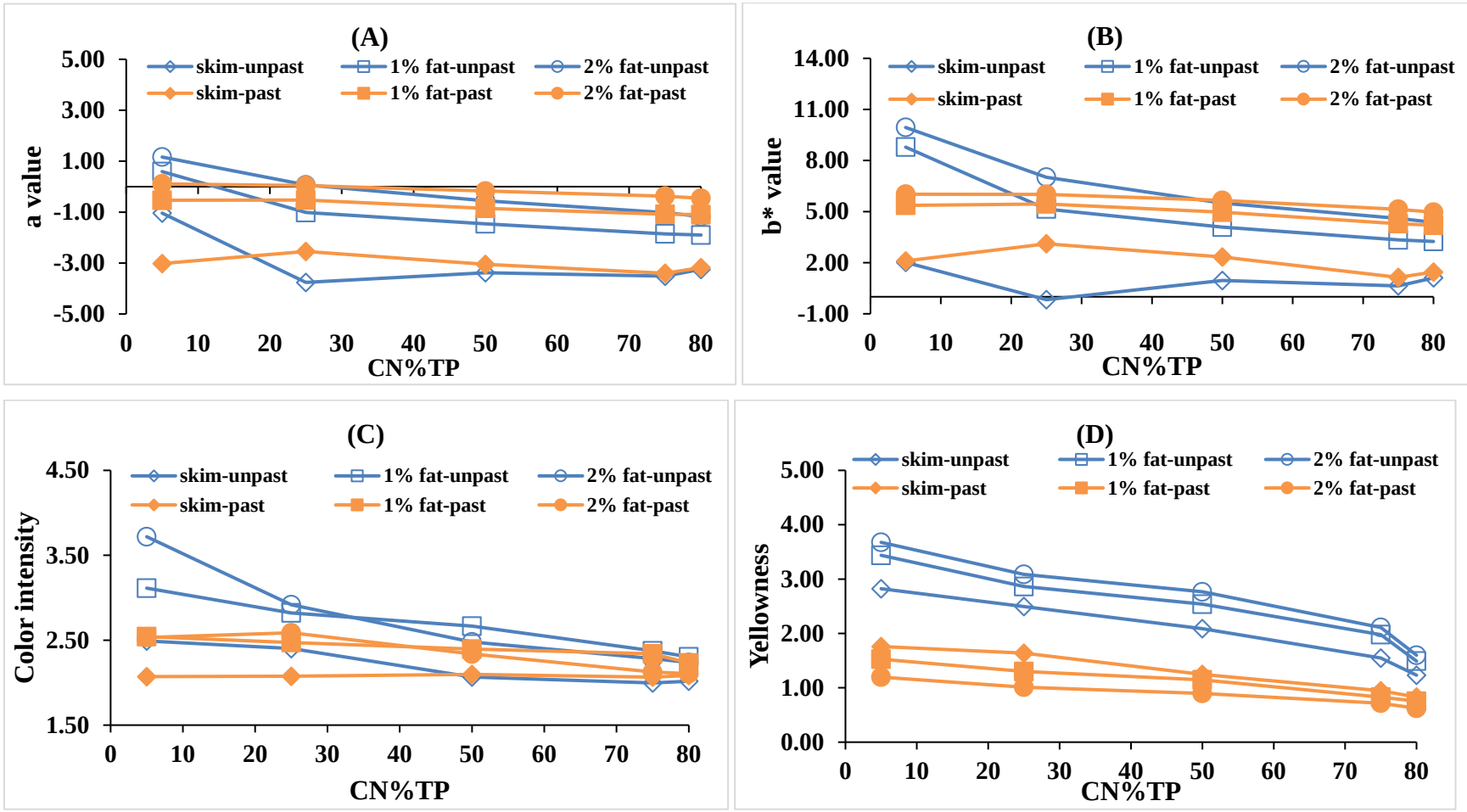

Figure 2. (A) Hunter a values (4°C and 20°C) at 10 degree viewer angle using illuminant A, (B) CIE b* values (4°C and 20°C) at 10 degree viewer angle using illuminant A, (C) sensory color intensity, and (D) sensory yellowness for unpasteurized (unpast) and pasteurized (past) milk protein beverages with different fat levels (skim, 1% fat and 2% fat) as a function of casein as a percentage of true protein (CN%TP).

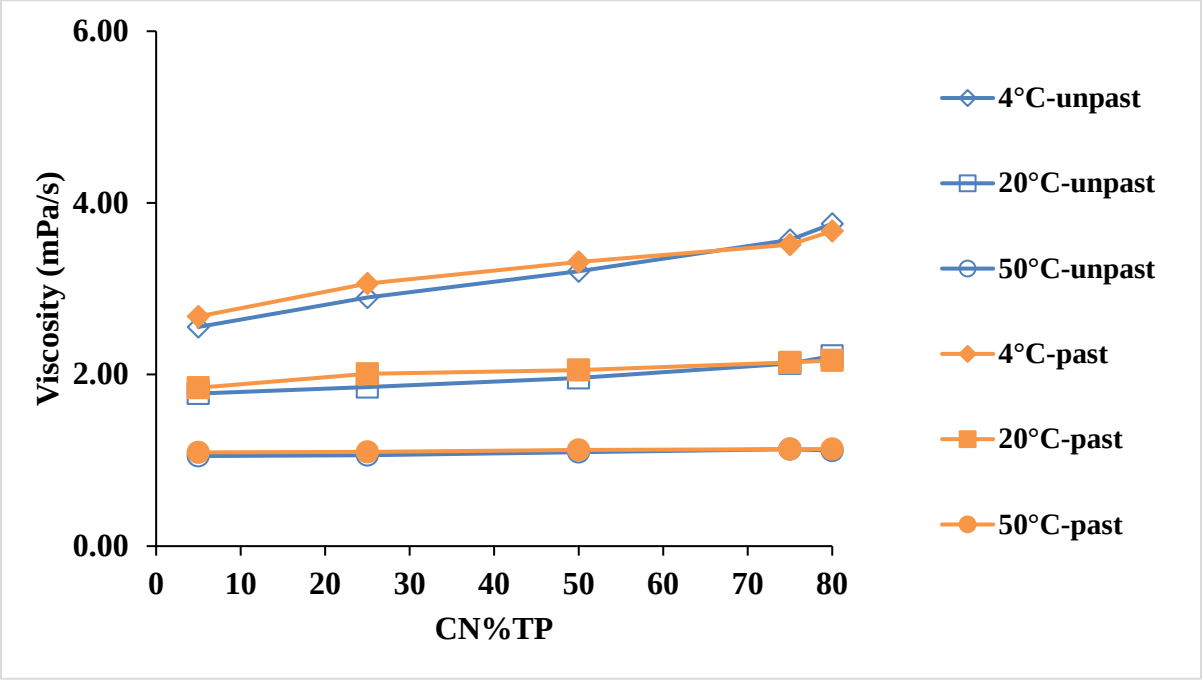

Figure 3. Instrumental viscosity for unpasteurized (unpast) and pasteurized (past) milk protein beverages with different temperatures (4, 20 and 50°C) as a function of casein as a percentage of true protein (CN%TP).

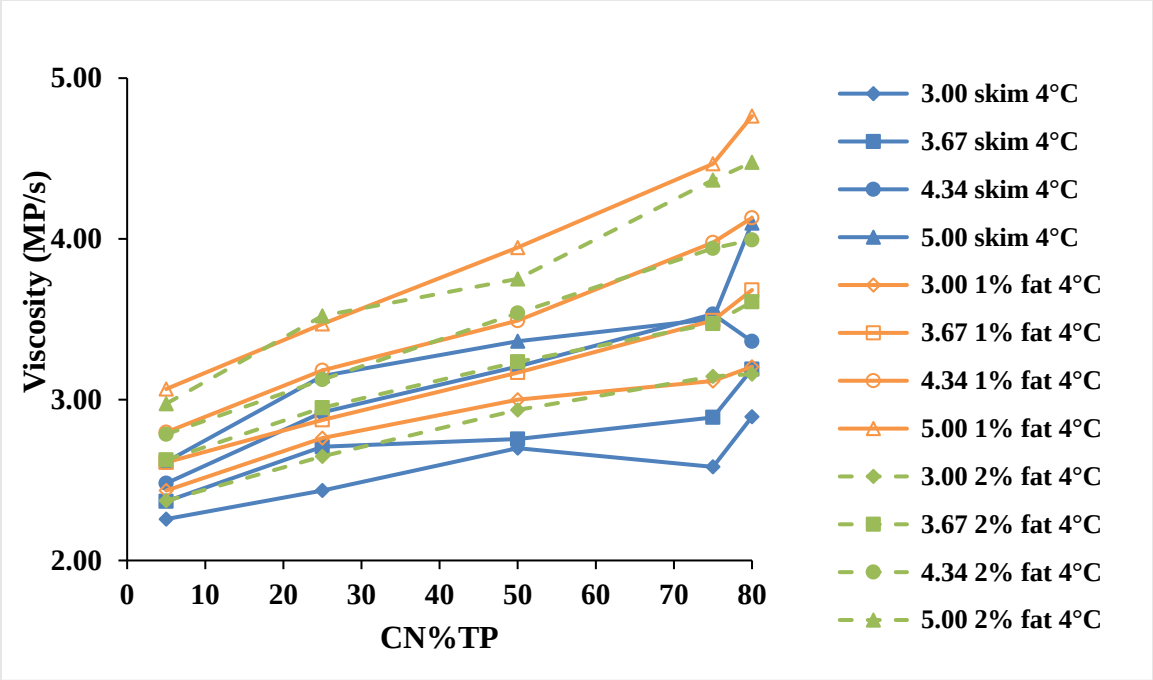

Figure 4. Instrumental viscosity for milk protein beverages with different true protein levels (3.00, 3.67, 4.34 and 5.00%) as a function of casein as a percentage of true protein (CN%TP) at 4°C.

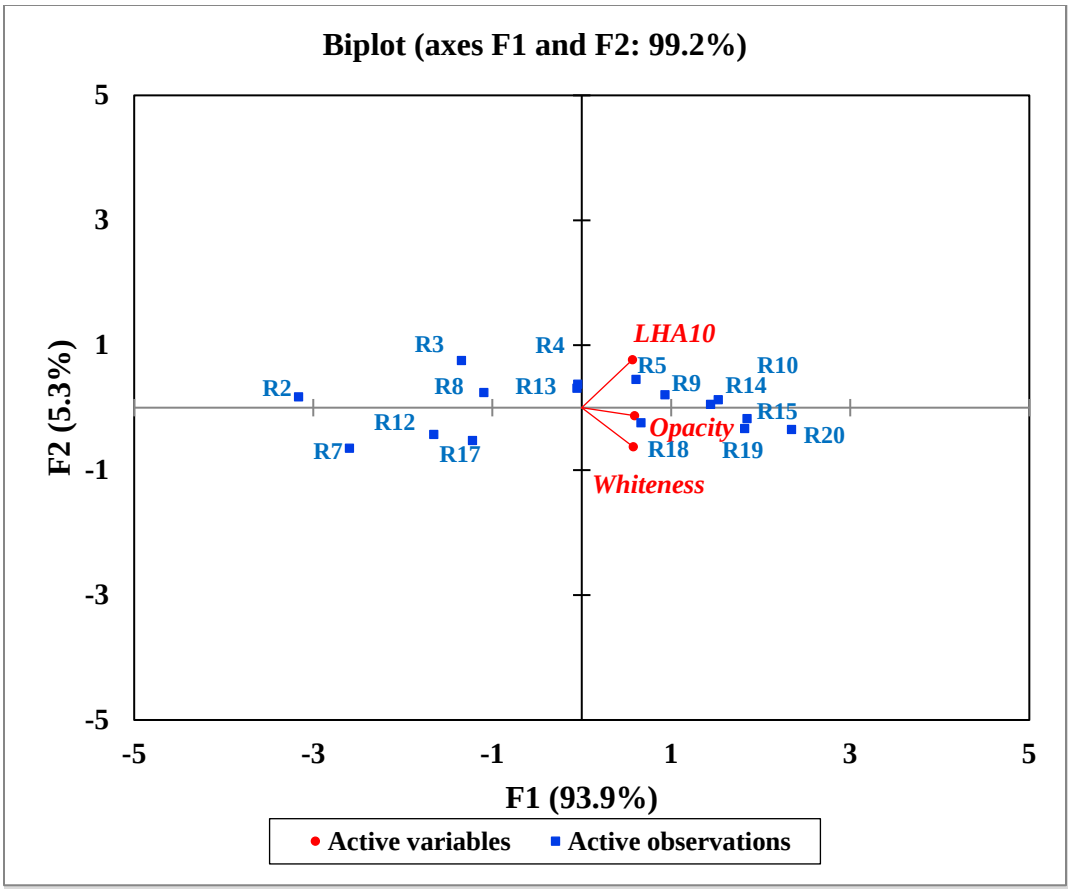

Figure 5. Principle component analysis (PCA) biplot for Hunter L values at 10 degree viewer angle (LAH10), sensory whiteness and sensory opacity of unpasteurized skim beverages with removing 4 beverages with 5% casein as a percentage of true protein.
"R" represents unpasteurized. The number next to each letter represents a specific formula in Table 1.

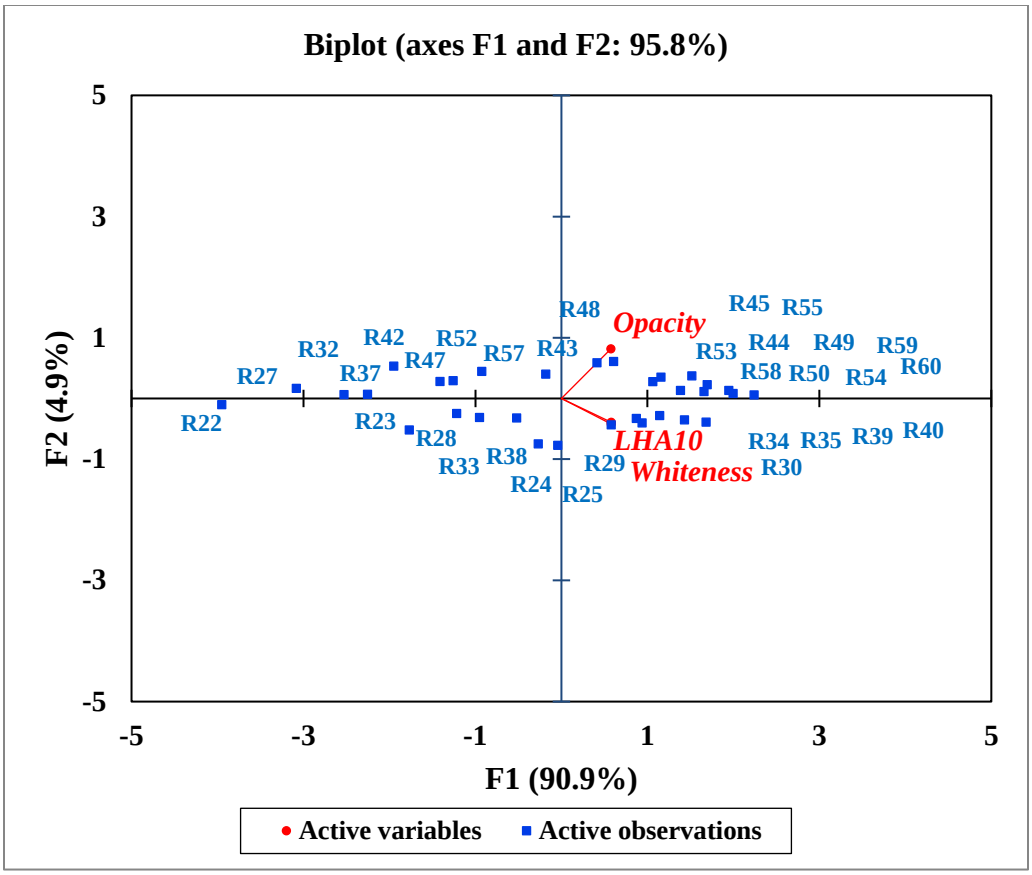

Figure 6. Principle component analysis (PCA) biplot for Hunter L values at 10 degree viewer angle (LAH10), sensory whiteness and sensory opacity of unpasteurized 1% and 2% fat beverages with removing 8 beverages with 5% casein as a percentage of true protein. "R" represents unpasteurized. The number next to each letter represents a specific formula in Table 1.

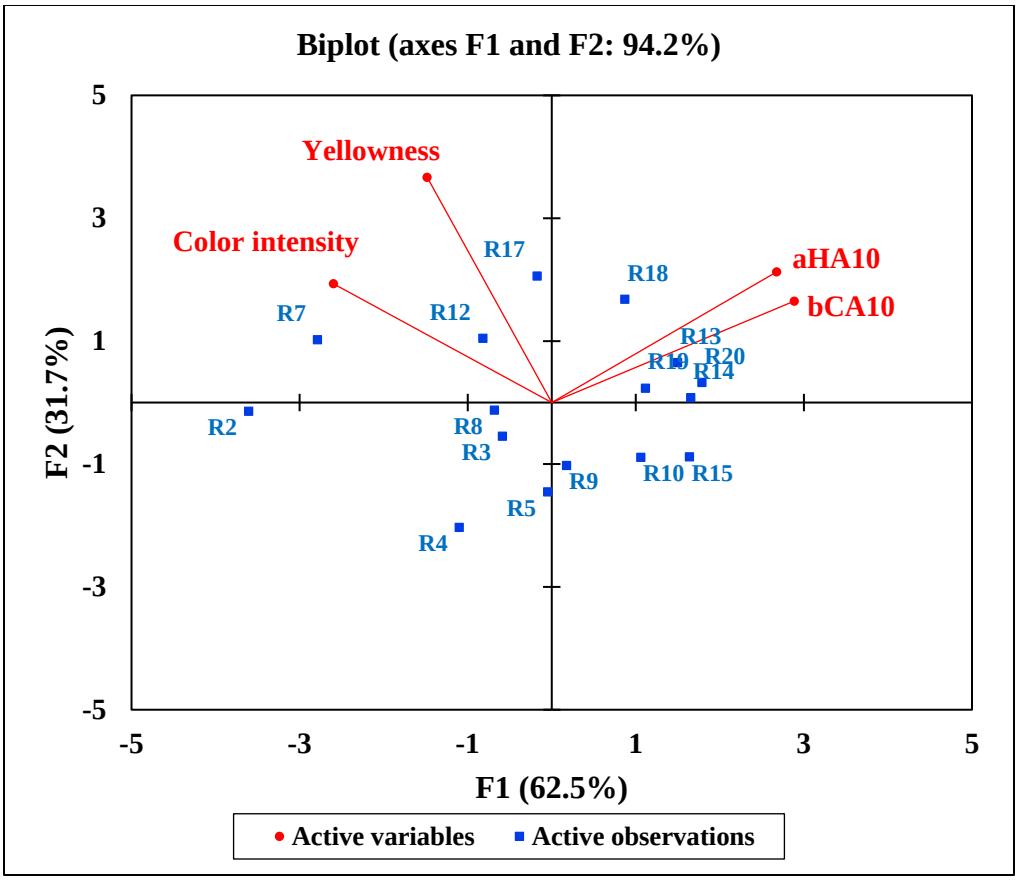

Figure 7. Principle component analysis (PCA) biplot for Hunter a and CIE b *values at 10 degree viewer angle (aHA10 and bCA10), sensory color intensity and sensory yellowness of unpasteurized skim beverages with removing 4 beverages with 5% casein as a percentage of true protein.
"R" represents unpasteurized. The number next to each letter represents a specific formula in Table 1.

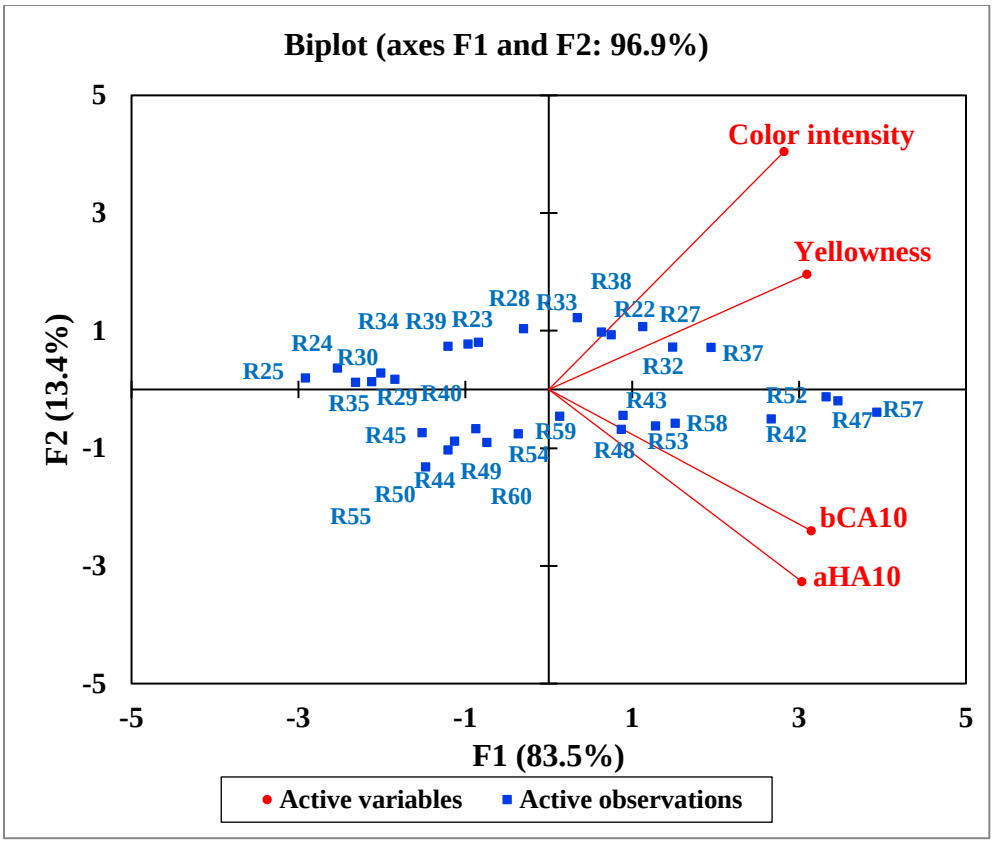

Figure 8. Principle component analysis (PCA) biplot for Hunter a and CIE b *values at 10 degree viewer angle (aHA10 and bCA10), sensory color intensity and sensory yellowness of unpasteurized 1% and 2% beverages with removing 8 beverages with 5% casein as a percentage of true protein.
"R" represents unpasteurized. The number next to each letter represents a specific formula in Table 1.

CHAPTER 4: IMPACT OF MILK FAT, CASEIN AND SERUM PROTEIN CONCENTRATIONS ON SENSORY PROPERTIES OF MILK BASED BEVERAGES

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* Use of names, names of ingredients, and identification of specific models of equipment is for scientific clarity and does not constitute any endorsement of product by authors, Cornell University, or North Carolina State University, or the Northeast or Southeast Dairy Foods Research Center.

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Abstract

Our goal was to determine the impact of systematically controlled variation in milk fat, true protein, casein, and serum protein concentrations on the sensory color, flavor and texture properties, instrumental color and viscosity, and milk fat globule size distribution of milk based beverages. Beverage formulations were based on a complete balanced 3 factor (fat, true protein and casein as a percentage of true protein) design with 3 fat levels (0.2, 1.0 and 2.0%), 4 true protein (TP) levels (3.00, 3.67, 4.34 and 5.00%) within each fat level and 5 casein as a percentage of true protein (CN%TP) levels (5, 25, 50, 75 and 80%) within each protein level (for a total of 60 formulations within each of 2 replicates). Instrumental measures of Hunter L, a, and CIE b*-values, instrumental viscosity, particle size, flavor, sensory texture and sensory appearance evaluation were done on each pasteurized/homogenized beverage formulation. Within each of the 3 fat levels higher SP concentration drove higher aroma intensity, sweet aromatic, cooked/sulfur, cardboard/doughy flavors, and sensory yellowness scores, while higher casein concentration drove higher instrumental viscosity in milk protein beverages. Increasing serum protein concentration increased yellowness, sweet aromatic, aroma intensity, cooked/sulfur, cardboard/doughy flavors across all fat levels and also had the largest impact on L, a and b* values, sensory whiteness and opacity within each fat level. Increases in true protein increased throat cling and astringency intensities. Increases in fat concentration were correlated with higher L, a, and b*values, larger particle size, and increased sensory whiteness, mouth coating, cooked/milky and milkfat flavors. Multiple linear regression of L, a and b* values produced better predictions of sensory whiteness and yellowness of pasteurized milk protein beverages than simple linear regression of L or b* values, respectively. Formulating milk protein beverages to higher casein concentration drove higher instrumental viscosity and more bland

flavor while higher serum protein concentration increased higher aroma intensity, sweet aromatic, cooked/sulfur, and cardboard/doughy flavors and sensory yellowness scores within each fat level and across fat levels. Formulating milk protein beverages to higher true protein level increased astringency regardless of fat level.

Key words: casein, serum protein, milk based beverage

Introduction

Consumer preference and liking can be influenced by the sensory properties (appearance, flavor, texture) of fluid milk and milk based protein beverages. Qualitative consumer interviews on fluid milk indicated that better color or color/whiteness was an important reason that skim milk, 2% fat milk and whole milk drinkers all preferred higher fat level milks (McCarthy et al., 2017). Lee et al. (2017) reported that heat treatment (indirect ultra pasteurization (**UP**), direct UP and HTST) induced appearance differences (i.e. whiteness) in skim and 2% fat milk that could impact consumer liking. Furthermore, Chung (2009) reported that Korean consumer liking of milks was positively correlated with sweetness, sweet cream flavor, and smooth texture. Grassy odor, raw milk flavor, artificial milk flavor, and rancid flavor were negatively correlated with liking. Consumer acceptance studies of fluid milks by Deane et al. (1967), Chapman and Boor (2001), Gandy et al. (2008) and Lee et al. (2017) indicated that heat treatment related flavors (such as: cooked/sulfur or eggy) were disliked by American kids and adults, and that the flavor of HTST milk was generally accepted in the US market, while flavor of milks produced with higher temperature thermal treatments were not.

Several studies have demonstrated the effects of fat and protein standardization on sensory properties of fluid milk and milk based protein beverages. Pangborn et al. (1985), Pangborn and Dunkley (1964a and b), Phillips et al. (1995a), Misawa et al. (2016), McCarthy et al. (2017) and Cheng et al. (2018b) indicated that the addition of milkfat impacted sensory appearance (i.e. whiteness, yellowness, opacity), texture (i.e. mouth coating, viscosity, thickness and astringency) and flavor (i.e. milkfat) of fluid milk and milk protein beverages. However, visual cues (i.e., whiteness) were the most important factor for differentiation of milks with different fat levels (Phillips et al., 1995a). Phillips and Barbano (1997) reported that suspension

of titanium dioxide as a fat substitute in fat free milk made the milk whiter, which improved perceived sensory appearance, creamy aroma, and texture of fat free milk.

Protein standardization of fluid milk and milk protein beverages can be divided into two approaches: changing true protein (**TP**) concentration by UF and changing casein as a percentage of true protein (**CN%TP**) and true protein concentration by microfiltration (**MF**) and UF. Poulsen (1978) reported no significant sensory changes were detected for whole milk (3.5% fat); for half-skimmed milk (1.5 to 1.8% fat) and for skim milk with a range of protein from 1.5-6.4% but a triangle test with 6 panelists was used to make these conclusions. Rattray and Jelen (1996) conducted triangle sensory tests with 15 panelists and reported that skim milk downward standardized in milk protein concentration with addition of UF permeate from either UF of milk or sweet whey to not lower than 2.4% (w/w) protein was indistinguishable from normal skim milk, again suggesting that these types of permeates would be suitable for downward standardization of protein concentration of fluid milk. However, appropriate sensory methodology was not applied in either of these studies which would necessitate additional work to confirm their conclusions. A trained descriptive sensory analysis panel was used to determine the effect of milk protein standardization of skim and 1% fat milk (Quiñones et al., 1997) and 2 and 3% fat milks (Quiñones et al., 1998) on sensory properties of protein standardized fluid milks. Increases in true protein concentration increased appearance scores (center color, edge color, opacity and visual hang-up) and mouthcoating, residual mouthcoating and thickness (Quiñones et al., 1997, 1998). Standardizing the lower fat milk to a higher protein content made the lower fat milks similar to the higher fat milks from a sensory standpoint due to increases in whiteness due to higher concentration of light scattering casein micelle (Quiñones et al., 1997, 1998).

Misawa et al. (2016) standardized TP to 4 levels (3.00, 3.67, 4.34 and 5.00%) and CN%TP to 5 levels (5, 25, 50, 75 and 80%) within each TP level for pasteurized 1 and 2% fat milk protein beverages, and Cheng et al. (2018b) extended their work to unpasteurized skim, 1 and 2% fat milk protein beverages. The changes in CN%TP had more impact than TP on the appearance of unpasteurized and pasteurized milk protein beverages and the texture of the pasteurized beverages (Misawa et al., 2016; Cheng et al., 2018b). Increases in CN%TP increased the sensory whiteness and opacity but decreased the sensory yellowness of milk protein beverages (Misawa et al., 2016; Cheng et al., 2018b). Increases in CN%TP increased throat cling and mouth coating perceived by trained panelists in 2% fat pasteurized beverages even when the visual cues were masked (Misawa et al., 2016).

Principle component analysis (**PCA**) is generally used for sensory or multidimensional data analysis and visualization (Wold et al., 1987). Mean sensory attributes can be used to calculate the principle components (**PC**) and the first two PC that contributed to the most of the variability are typically used to generate PCA biplots (Drake et al., 2003; Drake et al., 2010). The correlation coefficient of each sensory attribute (the active variables) to the first two PC can be calculated and the sensory attributes can be projected on the biplot. Supplementary variables are the additional objective data measurements (such as compositions and volatile compounds) that can be included or brought into a PCA biplots to better explain the active variables. Pagès and Husson (2001) and Pagès (2005) demonstrated that the introduction of supplementary variables onto the PC based sensory biplot didn't interfere the biplot PC, but their correlation coefficient to the PC were calculated and then projected on the biplot in the sensory evaluation of wines and chocolates. This approach puts the active variables and the supplementary variables onto the same data space and can help the interpretation of sensory or multidimensional data. In

the current study of standardization of fat, protein, and CN%TP of milk beverages, the idea of bringing milk composition data as the supplementary variables for sensory data (the active variables) may help to better visualize the relative relationships among beverage composition parameters and instrumental and sensory data for milk protein beverages. This novel approach could produce a more clear and concise understanding for milk protein beverages formulation to achieve milk based beverage sensory properties while controlling ingredient and processing costs.

Our first objective was to determine the impact of systematically controlled variation in milk fat, true protein, casein, and serum protein concentrations on the sensory color, flavor and texture properties, instrumental color and viscosity, and milk fat globule size distribution of milk based beverages. Our second objective was to use PCA biplots with supplemental composition variables superimposed on the PCA biplot to provide a visual overview of how composition of milk protein beverages influences sensory properties as a potential guide for beverage formulation.

Materials and Methods

Experimental Design

Beverage formulations were based on a complete balanced 3 factor (fat, true protein and casein as a percentage of true protein) design with 3 fat levels (0.2, 1.0 and 2.0%), 4 true protein (TP) levels (3.00, 3.67, 4.34 and 5.00%) within each fat level and 5 casein as a percentage of true protein (CN%TP) levels (5, 25, 50, 75 and 80%) within each protein level (for a total of 60 formulations within each replicate) as described by Cheng et al. (2018b). The formulation, sensory and analytical work were conducted in one week for each fat level and there was a replication of the formulation, processing, and analysis for all the treatments within each fat level

in a second week, for a total of 6 weeks of processing (Cheng et al., 2018b). Instrumental measures of L, a, and b*-values, instrumental viscosity, particle size, flavor, sensory texture and sensory appearance evaluation were done on each pasteurized/homogenized beverage formulation. The impact of composition, specifically fat, true protein, CN%TP on pasteurized beverage on instrumental color and viscosity and sensory properties were determined.

Milk Based Ingredients, Beverage Formulation, and Processing

The ingredients used for beverage formulation and pasteurization-homogenization processing conditions were as described by Cheng et al. (2018a). The relative weight percentage of usage of each ingredient in each formulation was presented by Cheng et al. (2018b).

Analysis Methods

Chemical Composition, Instrumental Color and Viscosity. Chemical composition analysis during processing, of ingredients, and calculation of formulations based on ingredient composition, and instrumental color measurement and viscosity at 4°C and 20°C on pasteurized milk protein beverages were conducted as described by Cheng et al. (2018b).

Particle Size. Fat globule particle size of the pasteurized milk protein beverages was measured using a Mastersizer 2000 (Malvern Instrument Ltd., Worchestire, UK) as described by DiMarzo et al. (2016) and d(0.9) was reported.

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 appearance within 96 h after processing. Sensory appearance, flavor and texture/mouthfeel profiling were conducted in separate sessions and two different cohorts of trained panelists were used. One panel conducted appearance attributes (n=6, 3 males, 3 females, ages 24-54yr) and one panel conducted flavor

and texture (n=8, 3 males, 5 females, ages 23-54yr). Each panelist had a minimum of 80 h of prior descriptive analysis training on food appearance attributes using the Spectrum™ method with a 0 to 15 point intensity scale (Meilgaard et al., 2007), and at least 40 h of prior experience with the sensory profiling of fluid milk and dried dairy ingredients using established sensory languages (Croissant et al., 2007; Drake et al., 2003; McCarthy et al., 2017). Compusense Cloud (Guelph, Canada) was used for data collection.

Appearance. Sensory appearance profiling of pasteurized beverages was done at 4 and 20°C. Appearance attributes (whiteness, yellowness, and opacity) were consistent with those used in previous research (Lee et al., 2017; McCarthy et al., 2017; Misawa et al., 2016; Phillips et al., 1995b) and the conditions of the appearance evaluation procedure was described by (Cheng et al., 2018b). The panel was trained for color, opacity, and visual viscosity attributes as described by McCarthy et al. (2017). Briefly, 80 mL of milk was dispensed into 100 × 10 mm clear, plastic Petri dishes (Thermo Scientific, Waltham, MA) and placed onto a white paper background. Paint chips were used as references for white and yellow color [Behr "Ultra Pure White" PPU18–06 = 0 (L = 97.68, a = −0.84, b = 2.22) and "Glass of Milk" P260–1u = 3.5 (L = 96.21, a = −0.05, b = 11.03); Behr Process Corp., Santa Ana, CA]. For opacity, milks were dispensed into 118-mL black soufflé cups with random 3-digit blinding codes. Water was used as a reference of 0 and whole-fat, HTST milk was assigned a 12 for opacity. Visual viscosity was determined by determining the amount of force needed to slurp 1 teaspoon of milk from a spoon. The references for viscosity were water = 0 and heavy cream = 3.2 (Meilgaard et al., 2007). Samples were evaluated in duplicate in a randomized order by each panelist. A 5 min rest was enforced after every 10 samples.

Flavor. Sensory flavor profiling was conducted on the pasteurized milk protein beverages at 20°C. Aroma intensity, sweet aromatic, cooked / milky, cooked / sulfur, cardboard, and milkfat flavors were the selected flavor attributes. Reference materials were described by Schiano et al. (2017).

Samples (30 ml) were dispensed into lidded 59-mL plastic soufflé cup - translucent (Stock Number: P200N, Dart Container Crop., Mason, MI) with random 3-digit blinding codes. Beverages were prepared with overheard lights off to prevent light oxidation. Samples were tempered to room temperature (20°C) before evaluation. Each panelist evaluated each sample in duplicate in a randomized design. A 1 min rest was enforced by a timer on iPads after each sample and panelists were instructed to rinse their mouths with spring water. An enforced rest of 10 min followed every 5 samples.

Texture/mouthfeel. Sensory texture/mouthfeel profiling of pasteurized beverages was done at 4 and 20°C. Texture attributes: sensory in mouth viscosity**,** mouth coating, throat cling (also called residual smoothness of mouth coating) and astringency of milk were adopted from previous work (Foegeding and Drake, 2007; Schiano et al., 2017).

Samples (30 mL) were dispensed into lidded 59-mL plastic soufflé cup - translucent (Stock Number: P200N, Dart Container Crop., Mason, MI) with random 3-digit codes. Samples were tempered to room temperature for the 20°C session. Samples were kept in the 4°C walk-in cooler during the 4°C session and only when needed, a sample was taken out to serve. Each panelist evaluated each sample in duplicate in a randomized design. A 2 min rest was enforced by a timer on iPads after each sample and panelists were instructed to rinse their mouths with spring water. An enforced rest of 15 min was applied following every 5 samples.

Statistical Analysis

The GLM procedure of SAS (version 9.4, SAS Institute Inc., Cary, NC) was used to determine the effect of fat (0.2, 1, and 2%), TP (3.0, 3.67, 4.34, 5.0%) , CN%TP (5, 25, 50, 75, and 80%), measurement temperature of instrumental color (4 and 20^{o}C) of milk protein beverages, and replicate on beverage color and viscosity measured instrumentally. The effect of the same composition parameters temperatures of 4 and 20^{o}C on sensory appearance and sensory texture was determined. All interactions of these parameters were included in the model. Fat, TP, and temperature were treated as categorical variables while CN%TP was handled as a continuous variable. The CN%TP data was transformed (named CNTPT) by mean-centering (Misawa et al., 2016) to avoid co-linearity effects on statistical analysis (Glantz and Slinker, 2001). If the F-value for the full model was significant ($P < 0.05$), then significance of each parameter ($P < 0.05$) and their interactions was determined. The effects of fat, TP, heat treatment, temperature of color or viscosity measurement, and replicate and their interactions were tested for significance using the interaction term of fat*prot*temp*rep as the error term, while the effects (linear and quadratic) of the continuous variable of CN%TP and their interactions with the categorical variables were tested for significance using the full model error. A stepwise process was done to remove all non-significant terms from the model to produce a final reduced model and Type III sum of squares table. To understand the relative amount of total variation explained by each parameter or interaction of parameters on each instrumental color, instrumental viscosity, each sensory appearance metric and each sensory texture metric, the Type III sum of square value for each term was divided by the total Type III sum of squares of the significant terms in the model and multiplied by 100. The term for fat and its related interaction terms in the model were removed from the model when analyzing data to determine

the effect of each parameter within each fat level for instrumental color and sensory appearance data. The term temperature and its interaction terms in the model were removed from the model when analyzing data to determine the effect of each parameter for flavor and particle size data because those measurements were done at only one temperature.

Linear and multiple linear regressions were conducted using Excel (Microsoft, Redmond, WA) data analysis to generate the prediction models of sensory whiteness and sensory yellowness by using single instrumental parameters (L or b *values) and multiple instrumental parameters (L, a and b* values).

Principle component analysis (PCA) was conducted using XLSTAT (Addinsoft, Paris, France) on the active variables (L, a and b* values, instrumental viscosity, particle size (d(.9)), sensory whiteness, opacity, yellowness, mouth coating, throat cling, astringency, sweet aromatic, aroma intensity, cooked/milky, cooked/sulfur, cardboard/doughy and milkfat) for all fat levels to produce PCA vector biplots. The supplementary variables (fat, TP, casein concentration (CN) and serum protein concentration (SP)) were projected onto the PCA vector biplots. The supplementary variables have no impact on the PCA vector biplots as their correlation coefficient with the PC are calculated and represented in the PC biplot. The inclusion of these data assist with interpretation of the PCA biplot, and may provide a clearer path for beverage formulations, as has been done for other foods (Pagès and Husson, 2001; Pagès, 2005).

Results and Discussion

ANOVA was used to determine the relative effect of variation in fat concentration, true concentration protein, CN%TP on the sensory properties of HTST pasteurized and homogenized formulated milk based beverages. The R-squared values for ANOVA models to determine that impact of variation in composition parameters on instrumental color and viscosity, and sensory

whiteness and opacity were generally higher than those for other sensory color and flavor attributes. The lower the R-squared values for the total models, the lower the power of the ANOVA to correctly quantifying the relative contribution of each model factor in explaining variation in sensory parameters, particularly when there were interaction effects.

Instrument and Sensory Color

Across All Fat Levels

L Values (Whiteness). The L values of pasteurized and homogenized milk protein beverages increased ($P < 0.05$) with increased fat concentration and increased CN%TP (Table 1 and Figure 1A). Fat, CNTPT and CNTPT*fat explained 48.75, 39.38 and 3.53%, respectively, of the total variation of L values for the pasteurized milk protein beverages across fat levels with 91.66% of the total variation in L values explained by these terms in the model (Table 1). Increasing fat level increased the L values more from 0.2 to 1.0% fat than from 1.0 to 2.0% fat and increased CN%TP increased the L value more in the lower fat content pasteurized milk protein beverages ($P< 0.05$, CNTPT*fat interaction in Table 1, Figure 1A). This result confirmed previous studies that light scattering (whiteness) from casein micelles and fat globules were non additive, and casein micelles were the major source for whiteness of skim beverages while both casein micelles and fat globules increased whiteness of 1 and 2% fat beverages (Quiñones et al., 1997, 1998; Misawa et al., 2016; Cheng et al., 2018b). The effect of beverage temperature on instrumental and sensory evaluations of color parameters explained only a very small amount of variation in color (Tables 1, 2, 3, and 4), therefore data for 4 and 20^{o}C were averaged for presentation in Figures 1 and 2.

Sensory Whiteness. The sensory whiteness of pasteurized and homogenized milk protein beverages increased ($P < 0.05$) with increased fat concentration and increased CN%TP (Table 1

and Figure 1B). Variation in fat, CNTPT and true protein explained 92.89% of the total variation of sensory whiteness, with 51.04, 35.30 and 6.55%, respectively, of the pasteurized beverages across fat levels (Table 1). Increased fat level increased the sensory whiteness more from 0.2 to 1.0% fat than from 1.0 to 2.0% fat for the pasteurized milk protein beverages (Figure 1B). Increased CN%TP increased the sensory whiteness (Figure 1B), which was consistent with previous studies by Misawa et al. (2016) and Cheng et al. (2018b).

Sensory Opacity. Sensory opacity of pasteurized and homogenized milk protein beverages increased ($P < 0.05$) with increased fat concentration and increased CN%TP (Table 1 and Figure 1C). The variation in fat, CNTPT and CNTPT*fat explained 72.88, 14.93 and 3.06%, respectively, of the total variation of sensory opacity for the pasteurized beverages across fat levels with 90.87% of the total variation explained by these 3 parameters in the model (Table 1). Decreases in fat concentration decreased the sensory opacity more from 0.2 to 1.0% fat than from 1.0 to 2.0% fat and increased CN%TP increased the sensory opacity more in the lower fat pasteurized milk protein beverages compared with the higher fat ones ($P<0.05$, CNTPT*fat interaction in Table 1, Figure 1C). Both Misawa et al. (2016) and Cheng et al. (2018b) reported that fat and CN%TP increased sensory opacity.

a Values (Redness – Greenness). The a values of pasteurized and homogenized milk protein beverages increased ($P < 0.05$) with increased fat concentration, but a-value decreased with increased CN%TP (Table 2 and Figure 2A), which confirmed previous work on CN%TP by Misawa et al. (2016) and Cheng et al. (2018b). Increased fat concentration levels increased the a values more from the skim to 1.0% fat beverages than it did from the 1.0% to 2.0% fat beverages (Figure 2A). Increased fat made the milk less green and increased CN%TP made the milk more green. Variation in fat and CNTPT explained 80.99 and 9.51%, respectively, of the total

variation of a values for pasteurized milk protein beverages across fat levels with 90.50% of total variation explained by these two terms in the model (Table 2). The effect of increasing concentration of fat on a values was much larger in pasteurized/homogenized beverages than reported for the same unpasteurized beverages (Cheng et al., 2018b). The larger impact of fat in pasteurized/homogenized beverages than unpasteurized beverages of the same composition was due to the smaller size, larger number, and increased surface area of the fat globules that was produced by homogenization and caused more light reflection than reported for the unhomogenized fat and casein micelles in the unpasteurized beverages. When more light was reflected by fat, light traveled a shorter distance into the milk before being reflected and that decreased the amount of light absorbed by riboflavin (Misawa et al., 2016; Cheng et al., 2018b).

b* Values (Yellowness – Blueness). The b* value of pasteurized and homogenized milk protein beverages increased ($P < 0.05$) with increased fat concentration, but b-value decreased with increased CN%TP (Table 2 and Figure 2B). Increased fat made the milk more yellow and increased CN%TP made the milk less yellow. Variation in fat, linear and quadratic effects of CNTPT explained 56.09, 26.38 and 4.98%, respectively, of the total variation in b* values of the pasteurized beverages with 87.45% of the total variation in b* value explained by these 3 terms in the model (Table 2). Increased fat concentration increased the b* values more from 0.2% to 1.0% fat beverages than it did from the 1.0% to 2.0% fat beverages (Figure 2B). Compounds that absorb light and produced the perception of yellowness (such as carotenoids) are dissolved in milk fat and will increase with increasing fat concentration (Johnson 1974; Misawa et al., 2016; Cheng et al., 2018b). Changes in CN%PT caused smaller changes in b* values than changes due to fat concentration of milk protein beverages (Figure 2B).

Sensory Yellowness. The sensory yellowness of pasteurized and homogenized milk protein beverages increased ($P < 0.05$) with increased fat concentration, but sensory yellowness decreased with increased CN%TP for all fat levels (Table 2 and Figure 2C). The effect of CNTPT (72.73%), fat (9.55%) and CNTPT*fat (4.16%) explained 86.44% of the total variation of sensory yellowness for the pasteurized milk protein beverages, but the R squared (0.55) was relatively low (Table 2). As CN%TP increased, sensory yellowness scores decreased for beverages of all fat concentrations, but sensory yellowness scores decreased more for skim beverages than beverages with 1 and 2% fat ($P < 0.05$, CNTPT*fat interaction in Table 2, Figure 2C). This is interesting because b*-values increased with increasing fat (Figure 2B) while sensory yellowness decreased with increasing fat content (Figure 2C). Cheng et al. (2018b) reported previously that b-values were not well correlated with yellowness of milk based beverages and that a combination of L, a, and b* values using MLR was a much better predictor of sensory yellowness. Increases in CN%TP also decreased the sensory yellowness scores in unpasteurized beverages (Cheng et al., 2018b). When CN%TP is low, then casein micelles are not scattering light in the aqueous phase (i.e., liquid phase around the fat globules). If the perceived yellowness is being produced by soluble components in the aqueous phase of the beverage that are absorbing light, then it would be expected that removing casein micelles from the skim phase would allow light to travel in the skim (i.e., aqueous) phase and soluble components would absorb wavelengths as a function of their structure. When the casein micelle concentration is high, light would be reflected by the casein micelles and the beverage would appear more white (Figure 1A and B) and less yellow (Figure 2C).

Within Fat Levels

Statistical analysis of the instrumental and sensory measures of beverage color within each fat concentration may provide a more clear understanding of the separate effects of protein concentration versus proportion of true protein that is casein. Microfiltration of skim milk separates milk casein and milk serum proteins, the use of MCC and SPI as separate ingredients is a new possibility for formulation of beverages with different sensory characteristics.

L Values (Whiteness). The L values of pasteurized and homogenized milk protein beverages increased ($P < 0.05$) with increased CN%TP for all fat levels (Figure 1A). The linear effect of CNTPT explained 77.36, 96.24 and 94.06% of the total variation of L values for 0.2, 1 and 2% fat pasteurized beverages, respectively (Table 3 and Figure 1A). The effect of protein and CNTPT*prot explained 4.79% and 3.37% of the total variation of L values for 0.2% fat pasteurized beverages, respectively (Table 3), and less in the 1% and 2% fat beverages. Thus within each beverage fat level, the impact of CN%TP on beverage L value was much larger than the effect of protein. This result was consistent with work on unpasteurized milk protein beverages by Cheng et al. (2018b). With constant fat concentration, the casein micelle was the major particle that reflected light in milk protein beverages (Misawa et al., 2016; Cheng et al., 2018). Therefore, to increase beverage whiteness within fat level, increasing casein concentration is more important than increasing protein concentration. Increasing CN%TP may decrease yellowness and greenness because light will be reflected by casein micelles and not be absorbed by soluble components in the aqueous phase of the beverage.

Sensory Whiteness. The sensory whiteness of pasteurized and homogenized milk protein beverages increased ($P < 0.05$) with increased CN%TP for all fat levels (Figure 1B) within each fat level (Table 3). Variation in CNTPT explained 60.98, 83.08, and 87.89% of the total variation

(Table 3) in sensory whiteness for 0.2, 1 and 2% fat pasteurized products, respectively, and was consistent with the CN%TP on L values. This confirmed previous work that increases in CN%TP increased sensory whiteness of 1 and 2% fat pasteurized, and skim, 1 and 2% fat unpasteurized milk protein beverages (Misawa et al., 2016; Cheng et al., 2018b).

Sensory Opacity. The sensory opacity of pasteurized and homogenized milk protein beverages increased ($P < 0.05$) with increased CN%TP for all fat levels (Figure 1C) and was consistent with the effect of increasing CN%TP on L values (Figure 1A) and sensory whiteness (Figure 1B), which confirmed results from Misawa et al. (2016). Increased CN%TP also increased sensory opacity of unpasteurized skim, 1 and 2% fat milk protein beverages (Cheng et al., 2018b). The linear plus quadratic effects of CNTPT explained 62.81, 93.40, and 81.74% of the total variation in sensory opacity for the 0.2, 1.0 and 2% fat pasteurized beverages, respectively (Table 3).

a Values (Redness – Greenness) and b* Values (Yellowness – Blueness). Both a and b* values decreased slightly with increased CN%TP, but the effect of CN%TP on a and b* values was small relative to the effect of fat (Figures 2A and B). The linear plus quadratic effects for CNTPT explained most of the variation in a and b* values within each fat level (Table 4) but the changes were small (Figure 2A and B) relative to the impact of fat (Table 2) on a and b values. This result was consistent with the data for the 1 and 2% fat pasteurized beverages work by Misawa et al. (2016). Increases in CN%TP decreased a and b* values within fat levels was also documented in the unpasteurized milk protein beverages (Cheng et al., 2018b).

Sensory Yellowness. In general, the R-squared values for the ANOVA models for yellowness were lower than for instrumental a and b* values (Table 4), but both sensory parameters decreased with increased CN%TP for 1 and 2% fat beverages (Figure 2C). Misawa

et al. (2016) also reported that increases in CN%TP decreased yellowness in 1 and 2% fat pasteurized beverages.

MLR Prediction of Sensory Whiteness and Yellowness

Summary of regression statistics of linear and multiple linear regression (MLR) for the whiteness group (L values, or combined L, a and b* values) and for the yellowness group (b* values or combined L, a and b* values) of pasteurized milk protein beverages are presented in Tables 5 and 6. Using the L values, the linear regression prediction of sensory whiteness produced an R squared of 0.75 for the skim, 0.68 for the 1 and 2% fat combined, 0.63 for 1% fat and 0.67 for 2% fat pasteurized/homogenized beverages (Table 5). The MLR prediction for sensory whiteness including L, a and b* values, produced a higher R squared with 0.78 for skim, 0.75 for 1 and 2% fat, 0.87 for 1% fat and 0.89 for 2% fat pasteurized beverages than the linear regression predictions (Table 5). This result was consistent with the linear and MLR prediction statistics for sensory whiteness of unpasteurized milk protein beverages that MLR using L, a, and b-values together produced a better prediction of sensory whiteness than L values alone (Cheng et al., 2018b).

Using the b* values alone, the linear regression prediction of sensory yellowness showed an R squared of 0.15 for the skim, 0.21 for the 1 and 2% fat 0.86 for 1% fat and 0.72 for 2% fat pasteurized beverages (Table 6). Cheng et al. (2018b) also previously documented hat instrumental b* values and sensory perception of yellowness were not well correlated for unpasteurized beverages. The MLR prediction for sensory yellowness using L, a and b* values, produced a much higher R squared with 0.92 for skim, 0.94 for 1 and 2% fat combined, 0.95 for 1% fat and 0.92 for 2% fat pasteurized beverages, respectively, (Table 6) than linear regression. Cheng et al. (2018b) also reported that using L, a and b* values together in an MLR produced a

better prediction of sensory yellowness for unpasteurized beverages (Cheng et al., 2018b). For pasteurized milk protein beverages, MLR (L, a and b* values) achieved a more robust prediction of sensory whiteness and sensory yellowness than simple linear regression (L or b* values). This could be a helpful tool for the use of instrumental color measurements as indicators for sensory color prediction of milk based beverages.

Across All Fat Levels: Instrumental Viscosity, Sensory Mouth Coating, Sensory Throat Cling, Sensory Astringency and Particle Size

Instrumental Viscosity. Variation in fat concentration in the pasteurized milk protein beverages in the current study explained <3% of the observed differences in instrumental viscosity (Table 7). The effect of temperature of instrumental viscosity measurement, variation in true protein concentration and CNTPT explained 67.33. 14.68, and 8.00% of the total variation of instrumental viscosity for the pasteurized beverages across fat levels with 90.01% of the total variation explained by the model (Table 7). Viscosity of liquids (including water) increases with decreasing temperature (Figura and Teixeira, 2007). Instrumental viscosity of pasteurized milk based beverages was higher at 4 than at 20^{o}C (Figure 3), as expected. When solutes and colloidal particles are dispersed in water (as in milk), temperature dependent of viscosity changes may be different than pure water. Increased concentration of true protein had a larger impact on instrumental viscosity than increased CN%TP (Figure 3) and the impact of protein concentration on viscosity was greater at 4 than at 20^{o}C.

Sensory Mouth Coating and Sensory Throat Cling. Unlike instrumental viscosity, observed differences in mouth coating and throat cling of the milk protein beverages were not greatly influenced by temperature (4 versus 20^{o}C) of sensory evaluation (Table 7). Fat, true protein and CN%TP explained 66.56, 12.94 and 3.52% of the total variation of mouth coating

across fat levels (Table 7). True protein, fat and CN%TP contributed to 38.74. 32.43 and 3.33% of the total variation of throat cling across fat levels (Table 7). Higher fat concentration produced more mouth coating and throat cling (Table7, Figure 4A and B). Higher true protein concentration in the beverages also produced more mouth coating and more throat cling (Figure 4A and B), while the effect of CN%TP on sensory mouth coating and throat cling was much smaller (Table 7). Thus, both higher fat and protein both contribute to mouth coating and throat cling, but fat explained more of variation in mouth coating while protein explained more of the variation in throat cling. Misawa et al. (2016) also reported increased CN%TP and true protein increased both mouth coating and throat cling (P <0.05) for 1 and 2% fat pasteurized beverages.

Sensory Astringency. Sensory astringency of tartaric acid, tannic acid, and alum produces a mouth drying sensation (Lee and Lawless, 1991). Sano et al. (2005) reported that sensory astringency of whey protein isolate at pH 3.5 increased linearly with increasing protein concentration in the range from 0 to 5% protein. Sensory astringency values ranged from 2 to 2.75 on a 0 to 15 point scale in our study (data not shown), so the beverages in the true protein range from 3 to 5% in the present study were not highly astringent. More severe heat treatments (such as UP) increased the astringency of both skim and 2% fat milks from a score of 1.8 to 2.5 on the same 0 to 15 point scale (Lee et al., 2017). Astringency increased from scores of about 2.2 to 2.75 with increased true protein concentration (from 3 to 5% true protein) for beverages at all fat levels (data not shown), with variation in protein concentration explaining about 43% and a fat * protein interaction explaining 21% of the variation in sensory astringency (Table 7) .

Particle Size. Laser light scattering particle volume distribution size analysis results were expressed as d(0.9) in microns and reflect the fat globule diameter below which 90% of the volume of fat is contained. Larger fat droplets are likely to contribute more to mouth feel of

milk. Jackson and Brunner (1960) found that casein was associated with the milk fat globule membrane after milk was homogenized. In the present study, as fat concentration in the milk protein beverages increased, d(0.9) increased (Figure 5). Fat concentration alone explained 76% of the variation in particle size in the beverages (Table 7), but both protein concentration and CN%TP also had an influence (P <0.05) on particle size. Higher true protein concentration and higher CN%TP decreased particle size for all fat levels (Figure 5), but the magnitude of the effect of change in protein and CN%TP on d(0.9) increased as fat content decreased (P < 0.05, fat*prot and CNTPT*fat interactions in Table 7, Figure 5). Misawa et al. (2016) reported similar results for the impact of fat, protein, and CN%TP on fat globule size in milk. Casein had stronger emulsifying capability than serum protein (i.e., d(0.9) increase as CN%TP decreased (Figure 5), and homogenization helped to deposit more casein micelles on the interface of milk fat and the serum phase of the beverages, and decreased d(0.9) (P < 0.05, fat*prot and CNTPT* fat interactions in Table 7).

Sensory Flavor

Aroma Intensity, Sweet Aromatic, Cooked/Sulfur, and Cardboard/doughy Flavors. Overall, there was not a high level of these flavors in the milk based beverages in our study, with all mean scores < 2.2 on a 15 point scale. These flavor intensities are consistent with previous studies on fluid milk and whey proteins (McCathy et al., 2017; Lee et al., 2017; Wright et al., 2009; Oltman et al., 2015). Dairy flavors generally fall between 0 and 4 on this sensory intensity scale (Schiano et al., 2017; Drake, 2007). All of the scores for these flavor descriptors decreased (P < 0.05) with increased CN%TP (Figure 6A, B, C, and D), while differences in fat and protein concentration had little effect on these sensory scores (Table 8 and Figure 6). Variation in CNTPT, CNTPT*fat and fat explained 63.40, 17.05, and 2.71% of the total variation of aroma

intensity for the pasteurized milk protein beverages, respectively, across fat levels with a R-squared value of 0.68, while variation in CNTPT (71.31%), CNTPT*fat (12.78%) and fat (3.77%) explained most of the variation in sweet aromatic for the pasteurized milk protein beverages across fat levels, with a R-squared value of 0.61 (Table 8). Aroma intensity was the overall perception of beverage flavors and higher sweet aromatic flavor may be related with Maillard reactions and lactose degradation compounds and further work is needed to measure Maillard reaction products in these milk based beverages. Increases in CN%TP decreased aroma intensity and sweet aromatic flavor may be due to the Maillard reaction related compounds bonding to the casein micelles resulting in a cleaner, less intense flavor profile for neutral pH milk based beverages. The linear and quadratic effects of CNTPT explained 83.89% and 7.00% of the total variation of cooked/sulfur flavor for pasteurized milk protein beverages, respectively, across fat levels with 90.89% of the total variation explained by these terms in the ANOVA model (Table 8). Cooked/sulfur flavors increased as CN%TP decreased (i.e., when serum protein concentration increased). Serum proteins, particularly β-lactoglobulin, have been reported as the source of cooked/sulfur flavor in milks due to sulfur containing amino acids (such as cysteine), which was the precursor of hydrogen sulfide and carbon disulfide (Hutton and Patton, 1952; Al-Attabi et al., 2008).

Cardboard/doughy flavor increased as CN%TP decreased (Figure 6D). Changes ($P < 0.05$) in cardboard/doughy flavors were observed and were related to differences in CN%TP associated with lower proportion of CN%TP or higher concentration of milk whey proteins. Cardboard flavor has been more frequently studied in dried whey protein products and has been sourced to volatile lipid oxidation products (Drake et al., 2003; Wright et al., 2009; Whitson et al., 2010). In the present study, all of the milk protein ingredients were liquid concentrates

isolated directly from milk and not cheese whey. Variation in CNTPT (79.17%), prot (4.20%), CNTPT*prot (3.36%) explained about 86.73% of the total variation explained by the model for cardboard/doughy flavor intensity in the pasteurized milk protein beverages across fat levels in the present study (Table 8).

Cooked/milky. Increasing fat level from 0.2% (skim) to 1% produced a large increase ($P < 0.05$) in cooked/milky flavor intensity but there was little increase going from 1 to 2% fat (Figure 7A) in pasteurized milk protein beverages. Increasing CN%TP also caused cooked/milky flavor intensity to increase ($P < 0.05$) by about the same amount within each fat level (Figure 7A). Variation in fat (55.59%), CNTPT (21.89%) and prot (7.13%) explained in total 84.61% of the total variation of cooked/milky flavor for pasteurized milk protein beverages across fat levels (Table 8).

Milkfat. The variation in fat concentration explained 94.68% of the total variation of milkfat flavor for the pasteurized milk protein beverages (Table 8), while no impact of variation in CN%TP on milkfat flavor (Figure 7B) was detected ($P > 0.05$). Increasing fat increased the milkfat flavor (Figure 7B). Higher milkfat flavor intensity could be due to higher fat content and associated higher concentration of lactones which contribute to milkfat flavor (Drake et al., 2010).

Biplot Analysis

Prior to the biplot analysis, the instrumental and sensory measures for color, texture, and flavor were reviewed and sorted. First, all the instrument and sensory metrics from Tables 1, 2, 5, and 6 for the ANOVA across all fat levels that had an R-squared > 0.6 were identified and then split into two groupings. One group was the parameters where fat concentration explained the highest proportion of variation in the data and the other group was where CN%TP or TP

concentration explained the highest proportion of variation in the data. The same sorting of measures by composition parameters that explained the percentage of total variation was done using a criteria of the R-squared > 0.50% for the ANOVA data for the analysis done within each fat concentration (i.e., 0.2, 1.0, and 2.0%). Thus, the results of the ANOVA was used as a sorting tool to form the basic grouping of parameters in the biplot analysis of the data.

Biplots were used in the current study to provide a novel approach to visualize relationships among beverage composition variables and sensory outcomes. Vector biplots were used to overlay the vectors for the milk component (e.g. fat, true protein, casein and serum protein) concentrations as supplemental variables on the vector space for instrumental and sensory data vectors for the population of milk beverages for all fat levels. This allows visualization of relationships of the variation in beverage composition parameters and the dependent sensory parameters. For example, if two composition parameters (e.g., fat and casein) both produce increased beverage whiteness, then a product developer has the opportunity to achieve the same visual whiteness perception with different product compositions and different ingredients choices or concentrations. This provides an opportunity for the product developer to prioritize ingredients that may minimize cost or maximize a particular nutritional attribute, while achieving the same perceived whiteness.

Vector Biplots: Within Fat Levels

Separate vector biplots are presented for skim (Figure 8), 1% (Figure 9), and 2% fat (Figure 10) beverages. Within each of the 3 fat levels, higher SP concentration drove higher aroma intensity, sweet aromatic, cooked/sulfur, and cardboard/doughy flavors, and sensory yellowness intensity scores, while higher casein concentration drove higher instrumental

viscosity. Formulating the beverages to a higher true protein levels, regardless of type of milk protein, increased the astringency within each fat level.

Vector Biplots: Among Fat Levels

Separate vector biplots are presented for the sensory parameters from all fat levels combined that were influenced the most based on the ANOVA analysis (Tables 1, 2, 5, and 6) by casein/protein (Figure 11) and by fat (Figure 12). As expected, the effects of CN and SP concentration on sensory parameters shown within fat levels (Figure 8, 9, 10) were similar and clear when data from all fat levels were combined (Figures 11, 12, 13). The parameters that were influenced the most (Tables, 1, 2, 5, and 6) by protein type were instrumental viscosity, astringency, aroma intensity, cardboard/doughy, cooked/sulfur and sweet aromatic flavors and yellowness (Figure 11). Instrumental viscosity was increased by increased casein and fat concentration while astringency was increased by true protein concentration. All of other parameters increased with increased milk serum protein concentration. The sensory parameters that were influenced the most (Tables 1, 2, 5 and 6) by difference in fat concentration (i.e., milkfat and cooked/milky flavors, throat cling, mouth coating, instrumental color parameters L, a, and b* values, opacity, and whiteness) follow the direction of the fat supplemental factor in the vector biplot (Figure 12). In Figure 13, all sensory measures with an R-squared > 0.6 are shown for all formulations to provide a complete view of the matrix of 60 formulated milk based beverages in the current study. Increasing fat concentration drove increased intensity scores for whiteness/color and mouth feel parameters, while increasing SP concentration drove higher yellowness and heat derived flavor intensity scores in the beverages. Higher CN concentration produced higher sensory whiteness, L values, cooked/milky flavors and higher instrumental measures of viscosity.

Conclusions

Within each of the 3 fat levels, higher SP concentration drove higher aroma intensity, sweet aromatic, cooked/sulfur, and cardboard/doughy flavors, and sensory yellowness scores, while higher casein concentration drove higher instrumental viscosity in milk protein beverages. Increasing serum protein concentration increased yellowness, aroma intensity, sweet aromatic, cooked/sulfur, and cardboard/doughy flavors across all fat levels and also had the largest impact on L, a, and b* values, sensory whiteness and opacity within each fat level. Increases in true protein increased sensory score for throat cling and astringency. Increases in fat concentration were correlated with higher L, a, and b*values, larger particle size, increased sensory whiteness, mouth coating, cooked/milky and milkfat flavor. Multiple linear regression of L, a, and b* values produced better predictions of sensory whiteness and yellowness of pasteurized milk protein beverages than simple linear regression of L or b* values, respectively. Formulating milk protein beverages to higher casein concentration increased instrumental viscosity and lowered overall flavor intensities while higher serum protein concentration increased aroma intensity, sweet aromatic, cooked/sulfur, and cardboard/doughy flavors, and sensory yellowness scores within each fat level and across fat levels. Formulating milk protein beverages to higher true protein level increased astringency regardless of fat level.

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Table 1. Pasteurized milk protein beverages: relative percentage of Type III sum of squares (for factors with $P < 0.05$) explained by model factors (fat = fat level with 0.2, 1% and 2%, prot = milk true protein with 3.00, 3.67, 4.34 and 5.00%, temp = temperature of color measurement with 4°C and 20°C, rep = replicate, CNTPT = casein as a percentage of true protein with 5, 25, 50, 75 and 80% transformed) for Hunter L values at a 10 degree viewer angle (LHA10) using illuminant A, sensory whiteness and sensory opacity.

Factors	LHA10	Whiteness	Opacity
fat	48.75	51.04	72.88
prot	0.31	6.55	2.87
temp	0.31	0.04	NS
rep	0.04	NS	0.02 [NS]
fat*prot	1.41	2.84	3.44
fat*temp	1.35	0.03	0.02
fat*rep	NS	0.05	0.03
prot*rep	0.37	NS	NS
temp*rep	NS	0.01	NS
fat*prot*temp	NS	NS	0.04
fat*prot*rep	0.73	0.16	NS
fat*temp*rep	NS	0.02	NS
prot*temp*rep	NS	NS	0.06
fat*prot*temp*rep	0.10[NS]	0.11	0.10
CNTPT	39.38	35.30	14.93
CNTPT*fat	3.53	0.40	3.06
CNTPT*prot	0.48	0.97	0.44
CNTPT*temp	0.63	NS	NS
CNTPT*rep	NS	0.04	NS
CNTPT*fat*prot	0.67	NS	NS
CNTPT*fat*temp	0.20	1.05	0.88
CNTPT*fat*rep	0.23	0.03	0.03
CNTPT*prot*temp	NS	0.05	NS
CNTPT*temp*rep	NS	0.03	0.01
CNTPT*prot*rep	0.34	0.04	NS
CNTPT*CNTPT	0.34	0.31	0.15
CNTPT*CNTPT*fat	NS	0.06	0.79
CNTPT*CNTPT*prot	0.25	0.36	0.04
CNTPT*CNTPT*temp	0.08	NS	NS
CNTPT*CNTPT*rep	NS	0.01	NS
CNTPT*CNTPT*fat*prot	0.33	0.43	0.20
CNTPT*CNTPT*fat*temp	NS	0.03	NS
CNTPT*CNTPT*fat*rep	0.07	NS	NS
CNTPT*CNTPT*prot*rep	0.10	0.02	NS
Sum (%)	100.00	100.00	100.00
R^2	0.98	0.97	0.97
N	480	2880	2880

NS = not significant ($P > 0.05$).

Table 2. Pasteurized milk protein beverages: relative percentage of Type III sum of squares (for factors with $P < 0.05$) explained by model factors (fat = fat level with 0.2, 1% and 2%, prot = milk true protein with 3.00, 3.67, 4.34 and 5.00%, temp = temperature of color measurement with 4°C and 20°C, rep = replicate, CNTPT = casein as a percentage of true protein with 5, 25, 50, 75 and 80% transformed) for Hunter a (aHA10) and CIE b* (bCA10) values at a 10 degree viewer angle using illuminant A and sensory yellowness.

Factors	aHA10	bCA10	Yellowness
fat	80.99	56.09	9.55
prot	2.27	2.10	1.71
temp	0.33	0.93	0.03^{NS}
rep	0.07	0.21	0.21^{NS}
fat*prot	1.07	2.06	5.80
fat*temp	1.19	0.42	NS
fat*rep	0.25	0.72	0.56
prot*temp	NS	NS	NS
prot*rep	0.17	NS	0.30
temp*rep	NS	NS	NS
fat*prot*temp	0.43	NS	NS
fat*prot*rep	NS	0.48	1.04
prot*temp*rep	NS	NS	NS
fat*prot*temp*rep	0.12^{NS}	0.08^{NS}	1.08^{NS}
CNTPT	9.51	26.38	72.73
CNTPT*fat	NS	0.38	4.16
CNTPT*prot	0.90	1.15	NS
CNTPT*temp	0.23	NS	0.16
CNTPT*rep	0.09	NS	NS
CNTPT*fat*temp	0.57	1.05	NS
CNTPT*fat*prot	0.36	0.66	0.63
CNTPT*fat*rep	NS	NS	NS
CNTPT*prot*temp	NS	NS	0.34
CNTPT*prot*rep	0.15	0.23	NS
CNTPT*CNTPT	0.71	4.98	0.33
CNTPT*CNTPT*fat	0.32	1.55	NS
CNTPT*CNTPT*prot	0.12	0.18	0.32
CNTPT*CNTPT*temp	NS	NS	0.16
CNTPT*CNTPT*rep	NS	NS	0.18
CNTPT*CNTPT*fat*prot	0.15	0.33	NS
CNTPT*CNTPT*fat*rep	NS	NS	0.73
CNTPT*CNTPT*prot*rep	NS	NS	NS
Sum (%)	100.00	100.00	100.00
R^2	0.99	0.98	0.55
N	480	480	2880

NS = not significant ($P > 0.05$).

Table 3. Pasteurized milk protein beverages: relative percentage of Type III sum of squares (for factors with P < 0.05) explained by model factors (prot = milk true protein with 3.00, 3.67, 4.34 and 5.00%, temp = temperature of sensory appearance evaluation with 4°C and 20°C, rep = replicate, CNTPT = casein as a percentage of true protein with 5, 25, 50, 75 and 80% transformed) for Hunter L values at a 10 degree viewer angle (LHA10) using illuminant A, sensory whiteness and sensory opacity at 3 fat levels (0.2, 1 and 2%).

	LHA10			Whiteness			Opacity		
Factors									
Fat (%)	0.2	1	2	0.2	1	2	0.2	1	2
prot	4.79	0.02^{NS}	1.49	29.60	11.12	4.10	29.00	0.58	13.61
temp	2.83	0.07^{NS}	0.90	0.08^{NS}	0.01^{NS}	0.05^{NS}	0.01^{NS}	NS	0.77
rep	0.28	0.05^{NS}	0.06^{NS}	0.12^{NS}	0.02^{NS}	0.20	0.03^{NS}	0.87	0.06^{NS}
prot*temp	NS	0.33	0.28	NS	NS	NS	0.19	1.18	NS
prot*rep	2.78	NS	0.30	NS	NS	1.12	NS	NS	NS
temp*rep	0.00	NS	NS	NS	NS	0.07^{NS}	NS	NS	0.43
prot*temp*rep	0.09^{NS}	0.15^{NS}	0.18	0.68	0.11^{NS}	0.07	0.61	0.09^{NS}	1.08^{NS}
CNTPT	77.36	96.24	94.06	60.98	83.08	87.89	61.66	82.62	70.80
CNTPT*prot	3.37	0.02	NS	5.38	0.70	5.08	6.30	NS	NS
CNTPT*temp	1.62	1.31	1.18	NS	NS	NS	NS	NS	NS
CNTPT*rep	0.58	0.44	NS	0.10	NS	0.49	NS	0.86	NS
CNTPT*prot*temp	NS	NS	NS	0.52	NS	NS	NS	0.78	NS
CNTPT*temp*rep	NS	0.08	0.17	0.34	NS	NS	0.17	NS	NS
CNTPT*prot*rep	2.85	NS	NS	0.32	NS	0.12	NS	NS	NS
CNTPT*CNTPT	0.54	0.86	1.15	0.54	1.74	0.13	1.15	10.78	10.94
CNTPT*CNTPT*prot	1.67	0.09	0.22	1.23	3.22	0.59	0.69	1.48	1.85
CNTPT*CNTPT*temp	0.30^{NS}	0.14	NS	0.12	NS	NS	NS	NS	0.47
CNTPT*CNTPT*rep	NS	NS	NS	NS	NS	0.07	NS	0.20	NS
CNTPT*CNTPT*prot*temp	NS	NS	NS	NS	NS	NS	0.20	0.57	NS
CNTPT*CNTPT*prot*rep	0.94	0.20	NS	NS	NS	NS	NS	NS	NS
Sum (%)	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
R^2	0.93	0.99	0.98	0.92	0.90	0.94	0.93	0.75	0.62
N	160	160	160	960	960	960	960	960	960

NS = not significant ($P > 0.05$).

Table 4. Pasteurized milk protein beverages: relative percentage of Type III sum of squares (for factors with $P < 0.05$) explained by model factors (prot = milk true protein with 3.00, 3.67, 4.34 and 5.00%, temp = temperature of sensory appearance evaluation with 4°C and 20°C, rep = replicate, CNTPT = casein as a percentage of true protein with 5, 25, 50, 75 and 80% transformed) for Hunter a (aHA10) and CIE b * (bCA10) values at a 10 degree viewer angle using illuminant A and sensory yellowness at 3 fat levels (0.2, 1 and 2%).

	aHA10			bCH10			Yellowness		
Factors									
Fat (%)	0.2	1	2	0.2	1	2	0.2	1	2
prot	27.78	6.21	3.57	15.58	1.23	0.40	0.51	3.94	26.70
temp	3.00	11.05	16.89	0.01^{NS}	8.12	5.75	0.02^{NS}	0.15^{NS}	0.55^{NS}
rep	2.41	0.82	0.23	2.58	0.53	1.72	1.82	0.15^{NS}	0.02
prot*temp	NS	NS	NS	NS	NS	NS	NS	0.54	NS
prot*rep	5.77	NS	NS	NS	0.12	NS	0.95	NS	NS
temp*rep	NS	1.01	0.16	NS	0.41	NS	NS	0.26	NS
prot*temp*rep	0.44^{NS}	0.27^{NS}	0.19^{NS}	1.83^{NS}	0.09^{NS}	0.23	0.15^{NS}	0.26^{NS}	6.28^{NS}
CNTPT	27.48	74.15	76.44	44.70	79.85	83.60	93.15	88.70	66.46
CNTPT*prot	12.57	2.66	0.67	7.96	1.30	0.19	NS	0.88	NS
CNTPT*temp	5.41	0.44	0.13	1.98^{NS}	0.35	1.56	NS	0.35	NS
CNTPT*rep	NS	0.93	0.10	NS	0.33	NS	0.22	NS	NS
CNTPT*prot*temp	NS	NS	NS	NS	NS	NS	NS	0.37	NS
CNTPT*temp*rep	NS	NS	NS	NS	0.05	0.08	NS	0.17	NS
CNTPT*prot*rep	4.16	0.54	NS	2.82	0.49	0.12	NS	0.63	NS
CNTPT*CNTPT	8.69	1.51	1.40	20.63	6.83	6.29	0.76	0.43	NS
CNTPT*CNTPT*prot	2.29	0.41	0.21	1.91	0.16	0.08	0.30^{NS}	0.52	NS
CNTPT*CNTPT*temp	NS	NS	NS	NS	NS	NS	NS	0.70	NS
CNTPT*CNTPT*rep	NS	NS	NS	NS	NS	NS	1.81	0.22	NS
CNTPT*CNTPT*prot*temp	NS	NS	NS	NS	NS	NS	NS	0.52	NS
CNTPT*CNTPT*temp*rep	NS	NS	NS	NS	NS	NS	NS	0.36	NS
CNTPT*CNTPT*prot*rep	NS	NS	NS	NS	0.12	NS	0.30^{NS}	0.85	NS
Sum (%)	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
R^2	0.85	0.97	0.98	0.88	0.99	0.99	0.74	0.77	0.21
N	160	160	160	160	160	160	950	960	960

NS = not significant ($P > 0.05$).

Table 5. Linear and multiple linear regression prediction of sensory whiteness for pasteurized/homogenized skim, 1% and 2% fat milk protein beverages from Hunter L, a and CIE b* values at 10 degree viewer angles (LHA10, aHA10 and bCA10 values).

Fat level (%)	0.2		1 and 2		1		2	
Indicator/predictor	LHA10/ sensory whiteness	LHA10, aHA10 and bCA10/ sensory whiteness	LHA10/ sensory whiteness	LHA10, aHA10 and bCA10/ sensory whiteness	LHA10/ sensory whiteness	LHA10, aHA10 and bCA10/ sensory whiteness	LHA10/ sensory whiteness	LHA10, aHA10 and bCA10/ sensory whiteness
Regression type	Linear	Multi linear	Linear	Multi linear	Linear	Multi linear	Linear	Multi linear
Multiple R	0.8638	0.8810	0.8245	0.8668	0.7913	0.9324	0.8215	0.9468
R Square	0.7461	0.7761	0.6798	0.7513	0.6261	0.8694	0.6748	0.8964
Adjusted R Square	0.7320	0.7341	0.6714	0.7305	0.6053	0.8450	0.6568	0.8770
Standard Error	0.4294	0.4277	0.3292	0.2981	0.3680	0.2306	0.2781	0.1664
Observations	20	20	40	40	20	20	20	20

Table 6. Linear and multiple linear regression prediction of sensory yellowness for pasteurized/homogenized skim, 1% and 2% fat milk protein beverages from Hunter L, a and CIE b* values at 10 degree viewer angles (LHA10, aHA10 and bCA10 values).

Fat level (%)	0.2		1 and 2		1		2	
Indicator/predictor	bCA10/ sensory yellowness	LHA10, aHA10 and bCA10/ sensory yellowness	bCA10/ sensory yellowness	LHA10, aHA10 and bCA10/ sensory yellowness	bCA10/ sensory yellowness	LHA10, aHA10 and bCA10/ sensory yellowness	bCA10/ sensory yellowness	LHA10, aHA10 and bCA10/ sensory yellowness
Regression type	Linear	Multi linear	Linear	Multi linear	Linear	Multi linear	Linear	Multi linear
Multiple R	0.3810	0.9612	0.4601	0.9705	0.9286	0.9769	0.8498	0.9584
R Square	0.1451	0.9239	0.2117	0.9419	0.8622	0.9544	0.7221	0.9185
Adjusted R Square	0.0976	0.9096	0.1910	0.9371	0.8546	0.9458	0.7067	0.9032
Standard Error	0.3659	0.1158	0.2708	0.0755	0.1184	0.0723	0.1366	0.0784
Observations	20	20	40	40	20	20	20	20

Table 7. Pasteurized milk protein beverages: relative percentage of Type III sum of squares (for factors with $P < 0.05$) explained by model factors (fat = fat level with 0.2, 1% and 2%, prot = milk true protein with 3.00, 3.67, 4.34 and 5.00%, temp = temperature of color measurement with 4°C and 20°C, rep = replicate, CNTPT = casein as a percentage of true protein with 5, 25, 50, 75 and 80% transformed) for instrumental viscosity, sensory mouth coating, sensory throat cling, sensory astringency and particle size (d(0.9)).

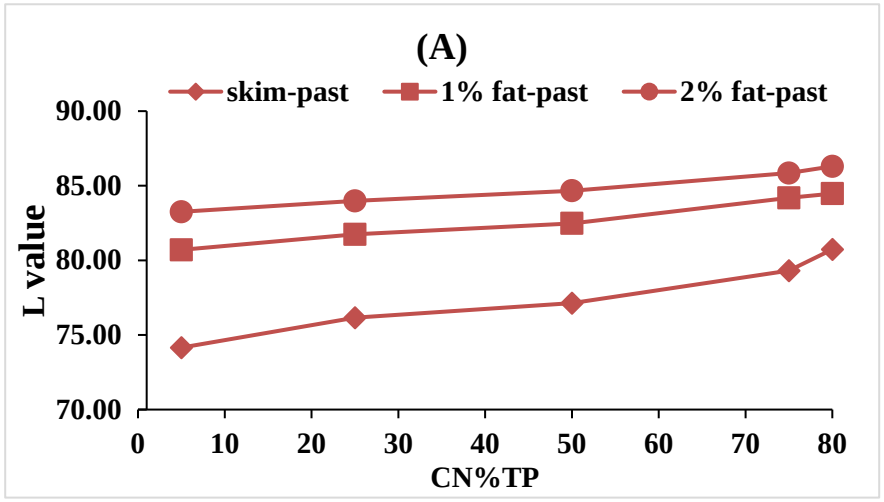
Factors	Instrumental viscosity	Mouth coating	Throat cling	Astringency	d(.9)[1]
fat	2.87	66.56	32.43	4.52	76.07
prot	14.68	12.94	38.74	42.74	9.26
temp	67.33	0.02[NS]	NS	0.55[NS]	-
rep	0.06	0.39[NS]	2.48	0.35[NS]	0.25
fat*prot	NS	2.40	2.33	21.19	0.95
fat*temp	0.77	0.14[NS]	0.19	0.30[NS]	-
fat*rep	NS	0.83	3.10	1.49	0.25[NS]
prot*temp	1.67	0.20	0.57	0.39	-
prot*rep	0.10	NS	0.52	0.50	-
temp*rep	NS	0.26	NS	1.14	-
fat*prot*temp	NS	0.63	0.71	1.30	-
fat*prot*rep	0.41	0.52	1.15	0.93	0.25
fat*temp*rep	0.06	0.82	1.78	2.83	-
prot*temp*rep	NS	0.31	NS	NS	-
fat*prot*temp*rep	0.08[NS]	0.76	0.55	1.51	-
CNTPT	8.00	3.52	3.33	NS	9.24
CNTPT*fat	0.30	2.67	5.77	9.21	1.98
CNTPT*prot	0.72	1.49	0.77	1.48	-
CNTPT*temp	2.59	0.16	0.33	NS	-
CNTPT*rep	NS	NS	0.14	NS	-
CNTPT*fat*prot	NS	0.41	0.43	0.67	0.58
CNTPT*fat*temp	0.06	1.98	1.14	1.13	-
CNTPT*fat*rep	0.00	0.52	0.31	0.56	-
CNTPT*prot*temp	0.16	0.33	0.70	NS	-
CNTPT*temp*rep	NS	NS	0.20	NS	-
CNTPT*prot*rep	NS	NS	0.40	1.08	-
CNTPT*CNTPT	0.14	0.09[NS]	0.03[NS]	0.10[NS]	0.28
CNTPT*CNTPT*fat	NS	0.87	0.78	0.55	0.90
CNTPT*CNTPT*prot	NS	NS	NS	1.84	-
CNTPT*CNTPT*fat*prot	NS	0.62	0.57	2.52	-
CNTPT*CNTPT*fat*temp	NS	NS	0.18	NS	-
CNTPT*CNTPT*fat*rep	NS	0.23	NS	0.77	-
CNTPT*CNTPT*prot*temp	NS	0.25	0.35	NS	-
CNTPT*CNTPT*temp*rep	NS	0.11	NS	0.33	-
Sum (%)	100.00	100.00	100.00	100.00	100.00
R^2	0.98	0.82	0.77	0.65	0.99
N	480	2880	2880	2880	120

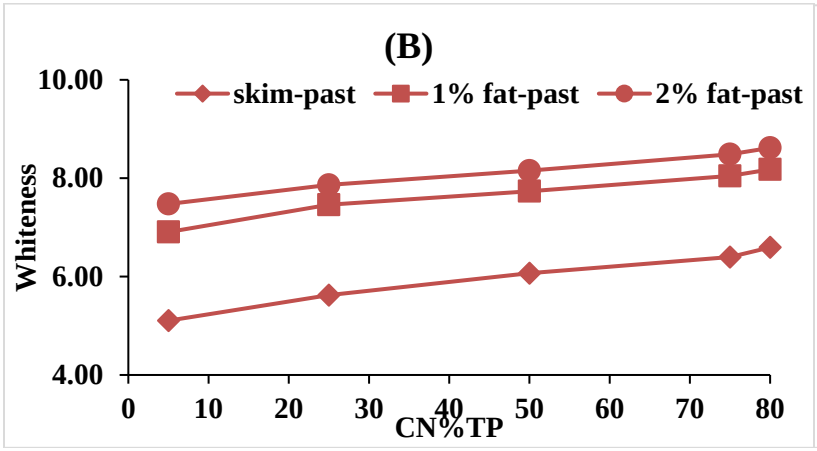
[1] d(.9) was measured at 40ºC.
[2]NS = not significant ($P > 0.05$).

Table 8. Pasteurized milk protein beverages: relative percentage of Type III sum of squares (for factors with P < 0.05) explained by model factors (fat = fat level with 0.2, 1% and 2%, prot = milk true protein with 3.00, 3.67, 4.34 and 5.00%, rep = replicate, CNTPT = casein as a percentage of true protein with 5, 25, 50, 75 and 80% transformed) for sensory flavors (aroma intensity, sweet aromatic, cooked/milky, cooked/sulfur, cardboard/doughy and milkfat).

Factors	Aroma intensity	Sweet aromatic	Cooked/milky	Cooked/sulfur	Cardboard/doughy	Milk fat
fat	2.71	3.77	55.59	0.13	2.94	94.68
prot	0.92	0.56^{NS}	7.13	0.70	4.20	0.15^{NS}
rep	1.04	0.16^{NS}	0.44^{NS}	NS	NS	0.85
fat*prot	2.58	2.26	4.51	1.01	2.53	NS
fat*rep	0.92	0.53	NS	0.24	0.04	1.92
prot*rep	NS	0.42	0.72	NS	0.10	NS
fat*pro*rep	0.45	1.09	1.37	0.11^{NS}	0.18	0.43
CNTPT	63.40	71.31	21.89	83.89	79.17	0.01^{NS}
CNTPT*fat	17.05	12.78	0.42	0.92	0.26	0.22
CNTPT*prot	1.56	0.88	1.92	NS	3.36	0.46
CNTPT*rep	0.88	0.19	0.49	NS	NS	0.17
CNTPT*fat*prot	0.97	0.78	1.56	5.38	2.95	NS
CNTPT*fat*rep	0.67	0.23	1.28	0.15	NS	0.20
CNTPT*prot*rep	0.75	NS	0.54	NS	0.10	0.10
CNTPT*CNTPT	1.68	0.07	0.07^{NS}	7.00	1.49	NS
CNTPT*CNTPT*fat	1.63	1.97	NS	NS	0.20	0.53
CNTPT*CNTPT*prot	0.89	NS	0.40	0.16	0.09	NS
CNTPT*CNTPT*rep	NS	NS	0.45	NS	NS	0.09
CNTPT*CNTPT*fat*prot	1.90	2.27^{NS}	NS	0.15	2.40	NS
CNTPT*CNTPT*fat*rep	NS	0.25^{NS}	0.44	0.15	NS	NS
CNTPT*CNTPT*prot*rep	NS	0.49^{NS}	0.77	NS	NS	0.18
Sum (%)	100.00	100.00	100.00	100.00	100.00	100.00
R^2	0.68	0.61	0.80	0.83	0.86	0.93
N	1920	1920	1920	1920	1920	1920

NS = not significant ($P > 0.05$).





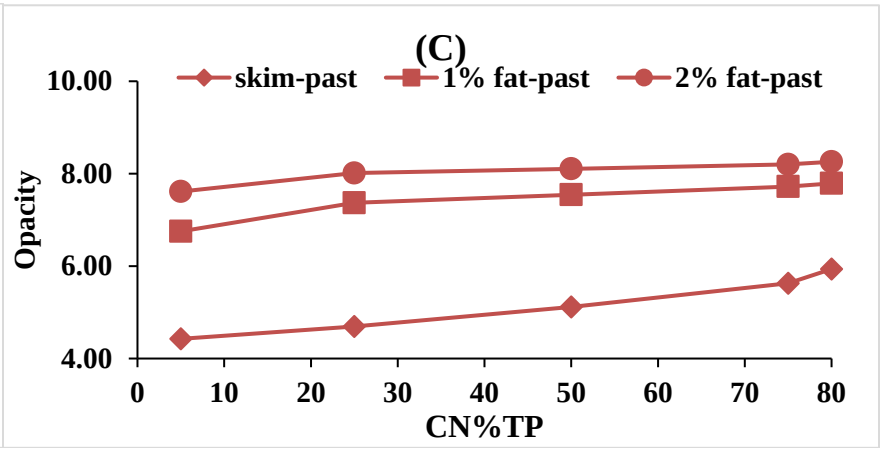


Figure 1. (A) Hunter L-values (4°C and 20°C) at 10 degree viewer angle using illuminant A, (B) sensory whiteness and (C) sensory opacity for pasteurized (past) milk protein beverages with different fat levels (skim, 1% fat and 2% fat) as a function of casein as a percentage of true protein (CN%TP).

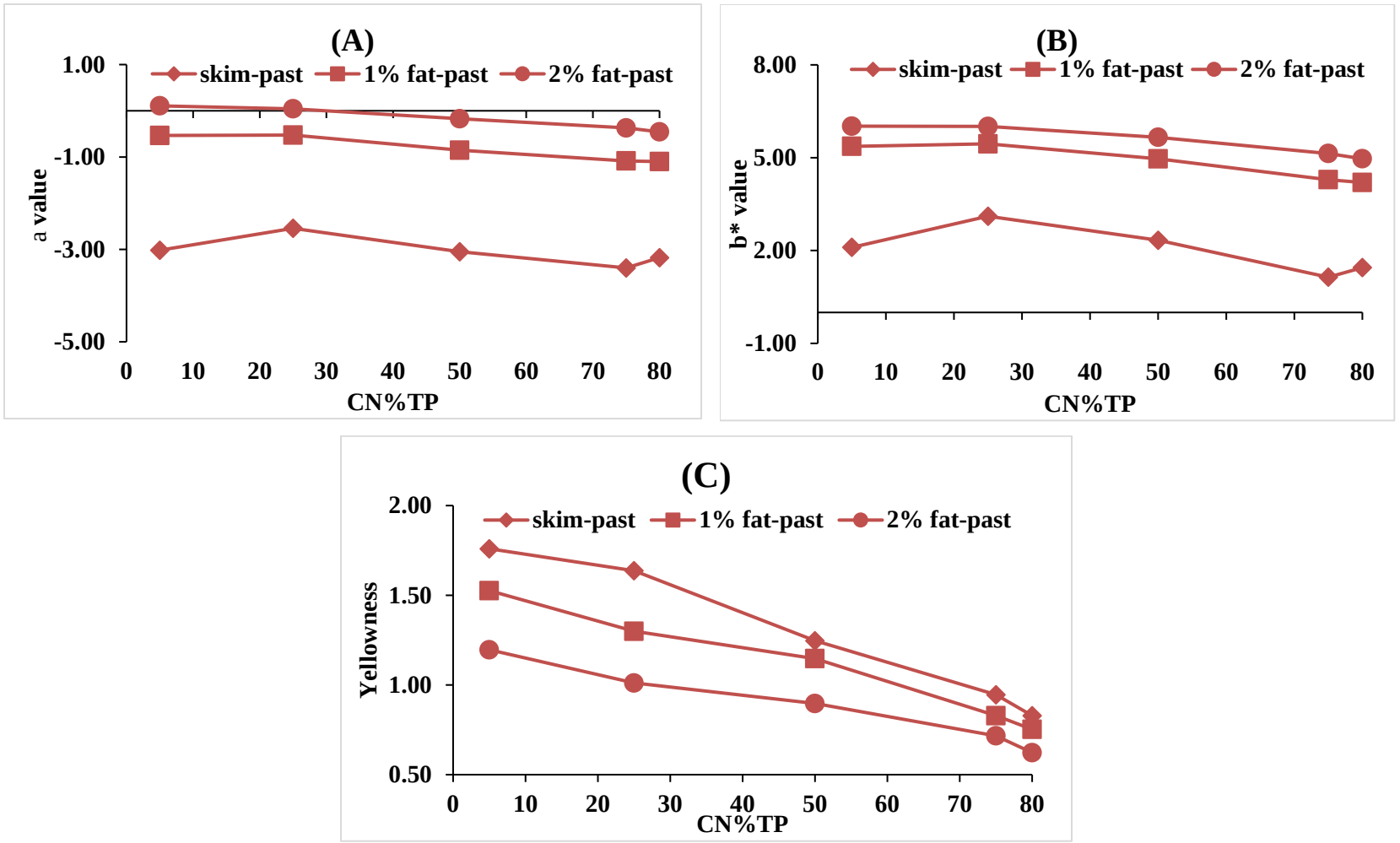


Figure 2. (A) Hunter a-values (4°C and 20°C) at 10 degree viewer angle using illuminant A, (B) CIE b*-values (4°C and 20°C) at 10 degree viewer angle using illuminant A, and (C) sensory yellowness for pasteurized (past) milk protein beverages with different fat levels (skim, 1% fat and 2% fat) as a function of casein as a percentage of true protein (CN%TP).

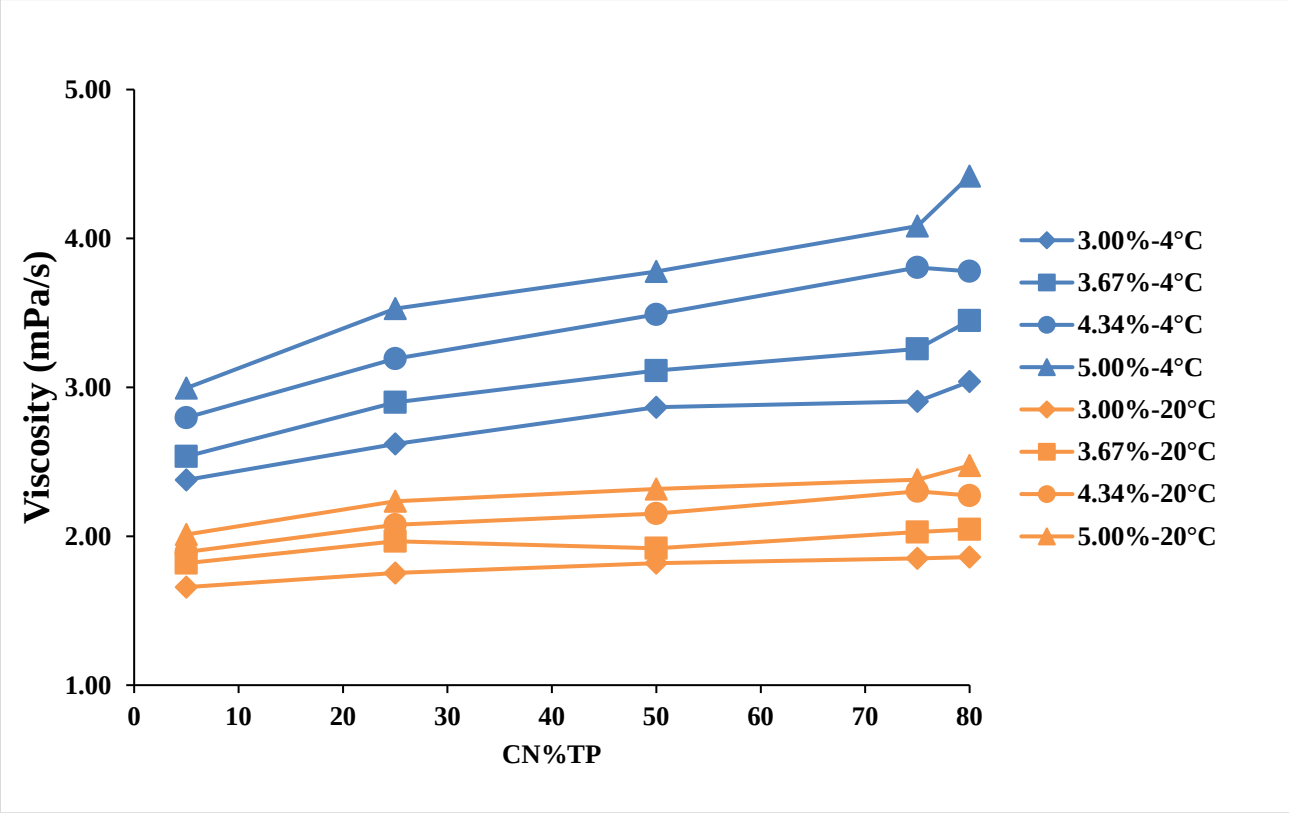


Figure 3. Pasteurized (past) milk protein beverages: instrumental viscosity with different temperature levels (4°C and 20°C) at different true protein levels (3.00, 3.67, 4.34 and 5.00%) as a function of casein as a percentage of true protein (CN%TP).

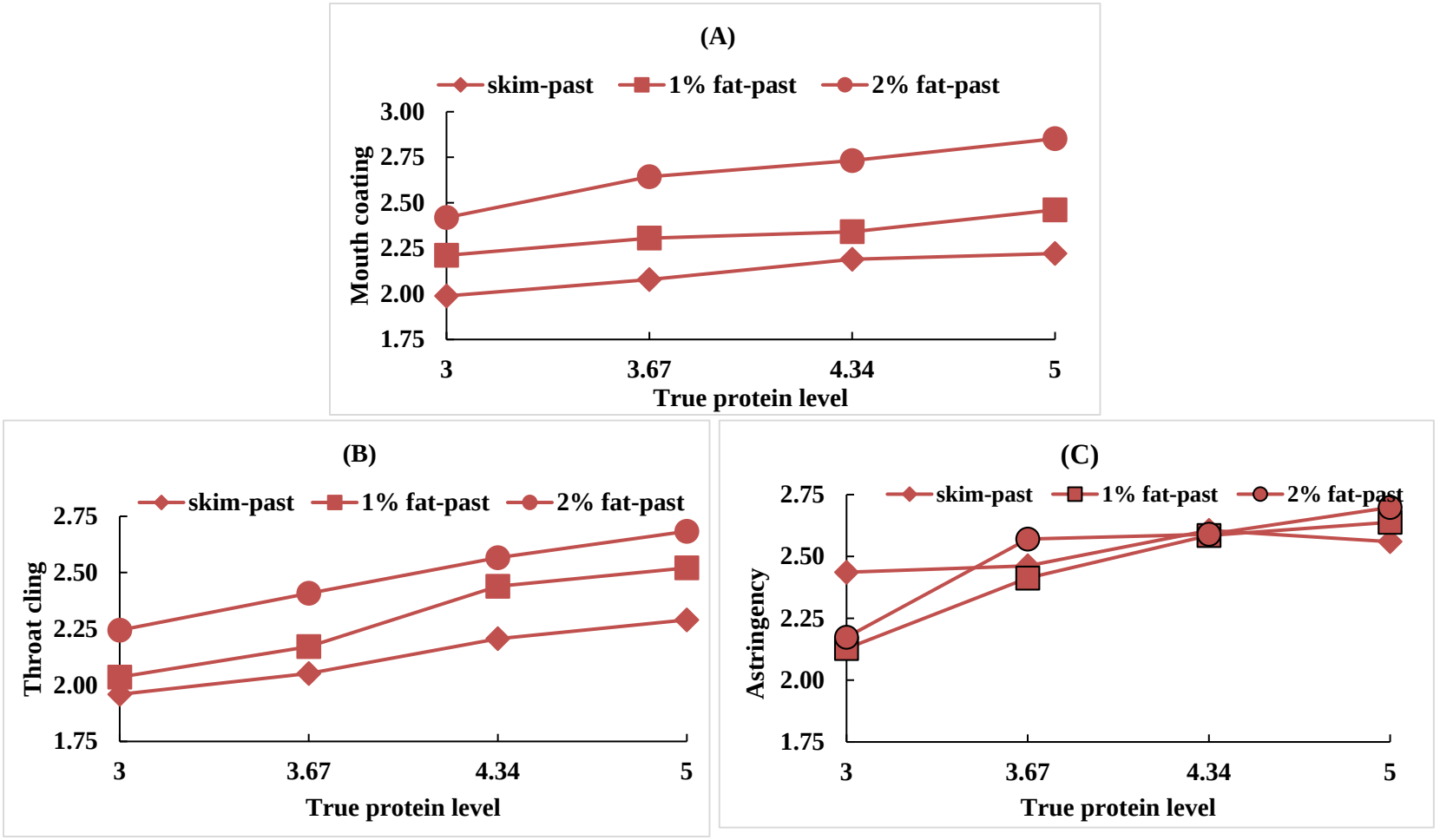


Figure 4. Pasteurized (past) milk protein beverages: (A) sensory mouth coating, (B) sensory throat cling, and (C) sensory astringency with different fat levels (skim, 1% fat and 2% fat) as a function of true protein.

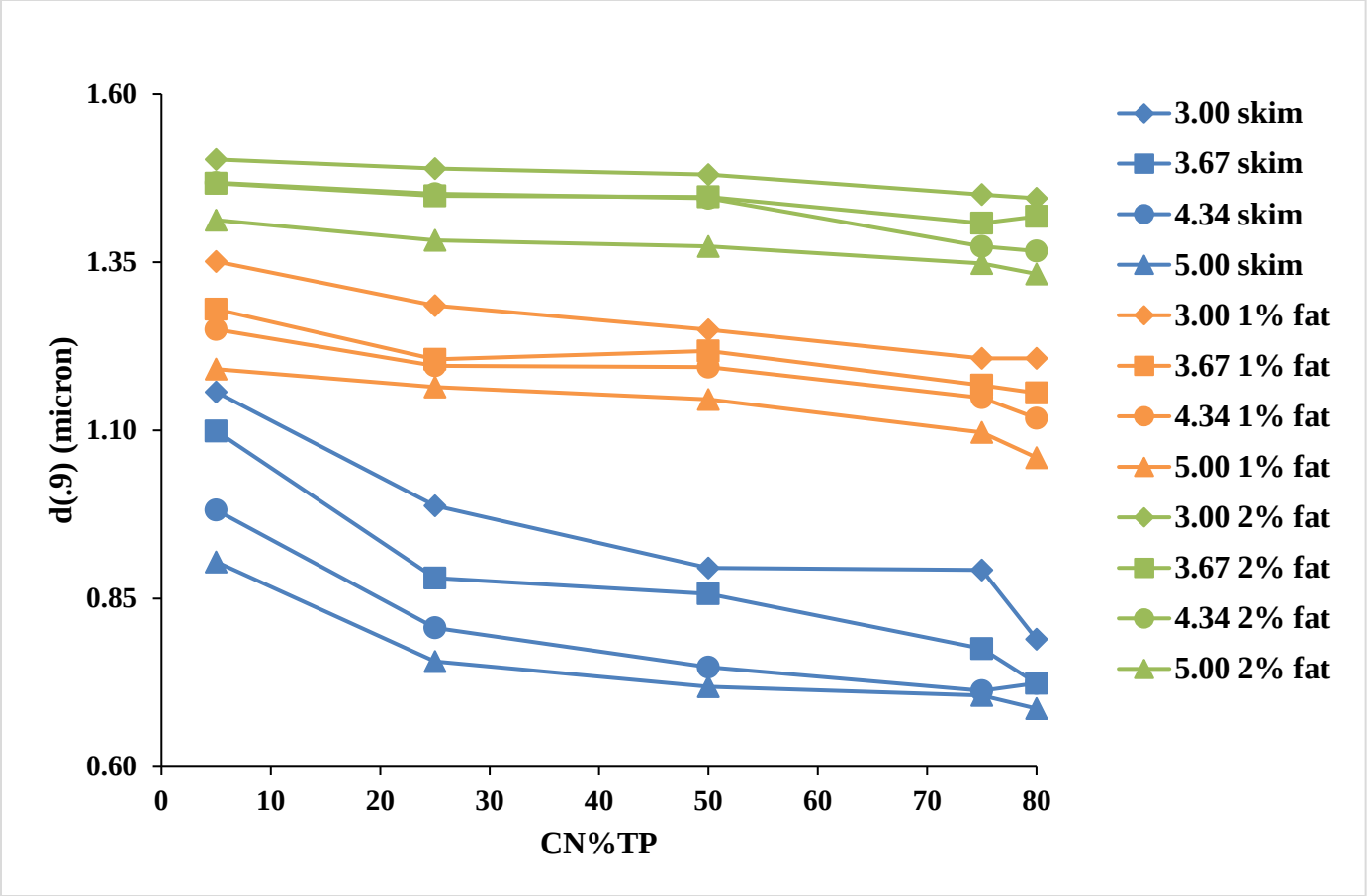


Figure 5. d(0.9) expressed in μ for pasteurized milk protein beverages with different true protein levels (3.00, 3.67, 4.34 and 5.00%) as a function of casein as a percentage of true protein (CN%TP) at different fat levels (skim, 1% and 2% fat).

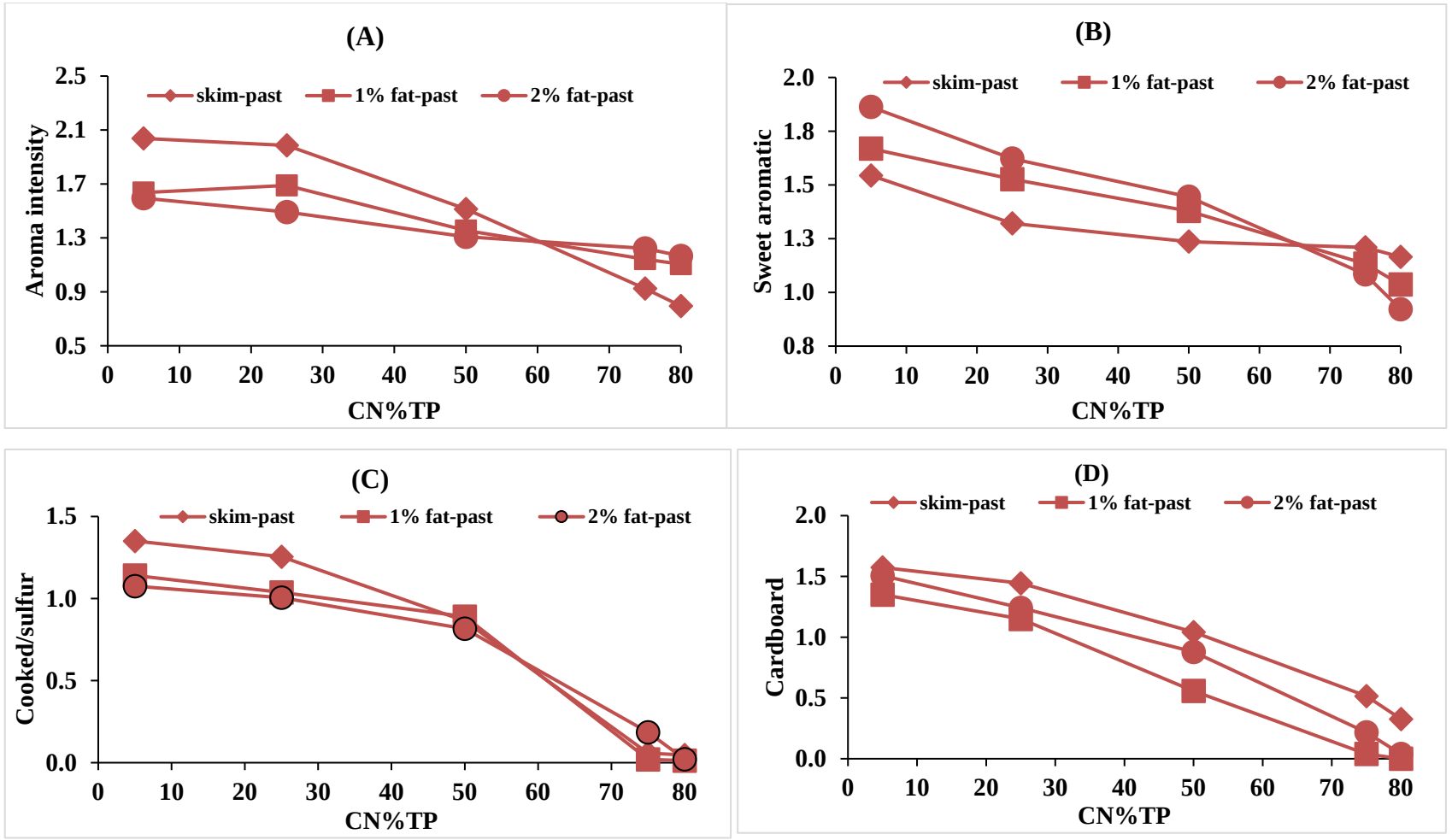


Figure 6. (A) Sensory aroma intensity, (B) sensory sweet aromatic, (C) sensory cooked/sulfur, and (D) sensory cardboard flavors for pasteurized (past) milk protein beverages with different fat levels (skim, 1% fat and 2% fat) as a function of casein as a percentage of true protein (CN%TP).

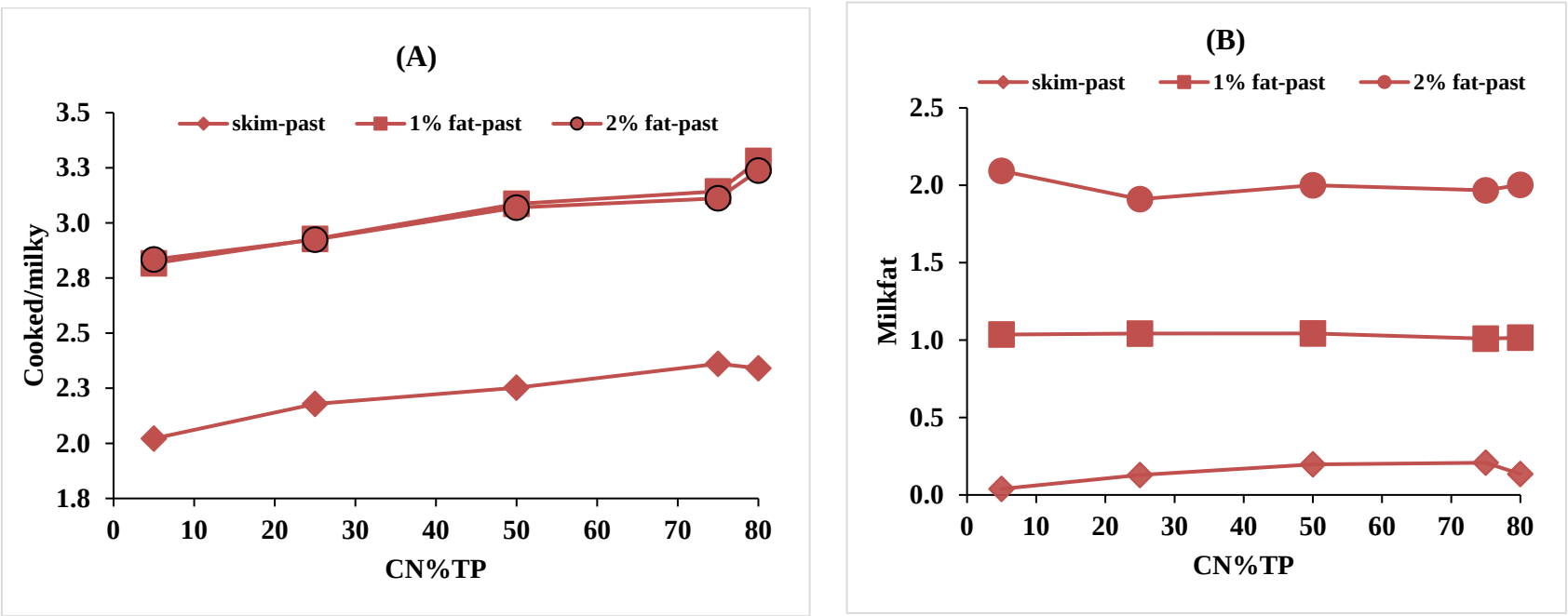


Figure 7. (A) Sensory cooked/milky and (B) sensory milkfat flavors for pasteurized (past) milk protein beverages with different fat levels (skim, 1% fat and 2% fat) as a function of casein as a percentage of true protein (CN%TP).

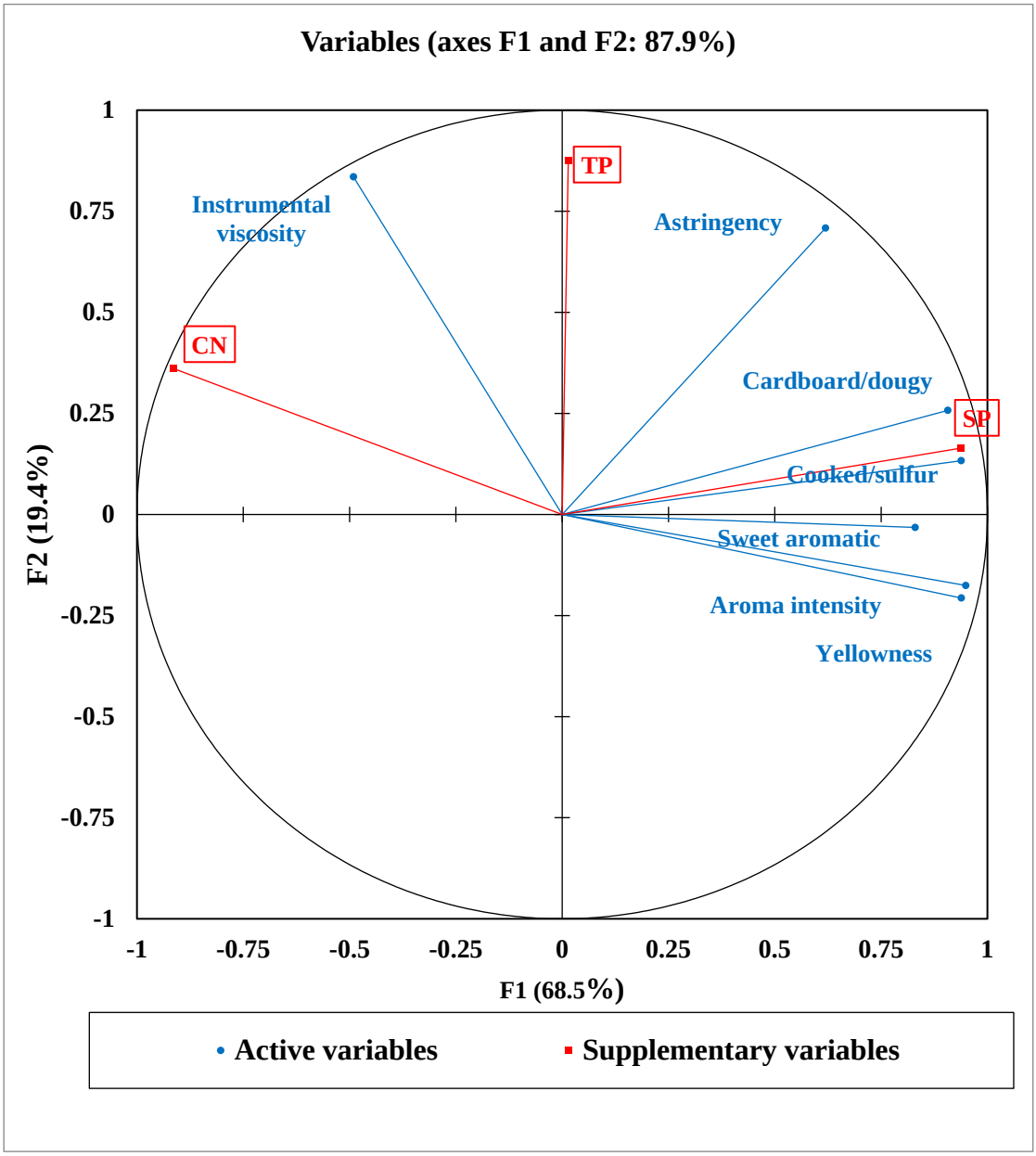


Figure 8. Skim milk pasteurized beverages: principle component analysis (PCA) vector plots for parameters where casein as a percentage of true protein contributed the most to the total variation of each parameter: instrumental viscosity, sensory yellowness (yellowness), astringency, sweet aromatic, aroma intensity, cooked/sulfur and cardboard/doughy with overlaying true protein (TP), casein protein (CN) and serum protein (SP) as supplementary variables.

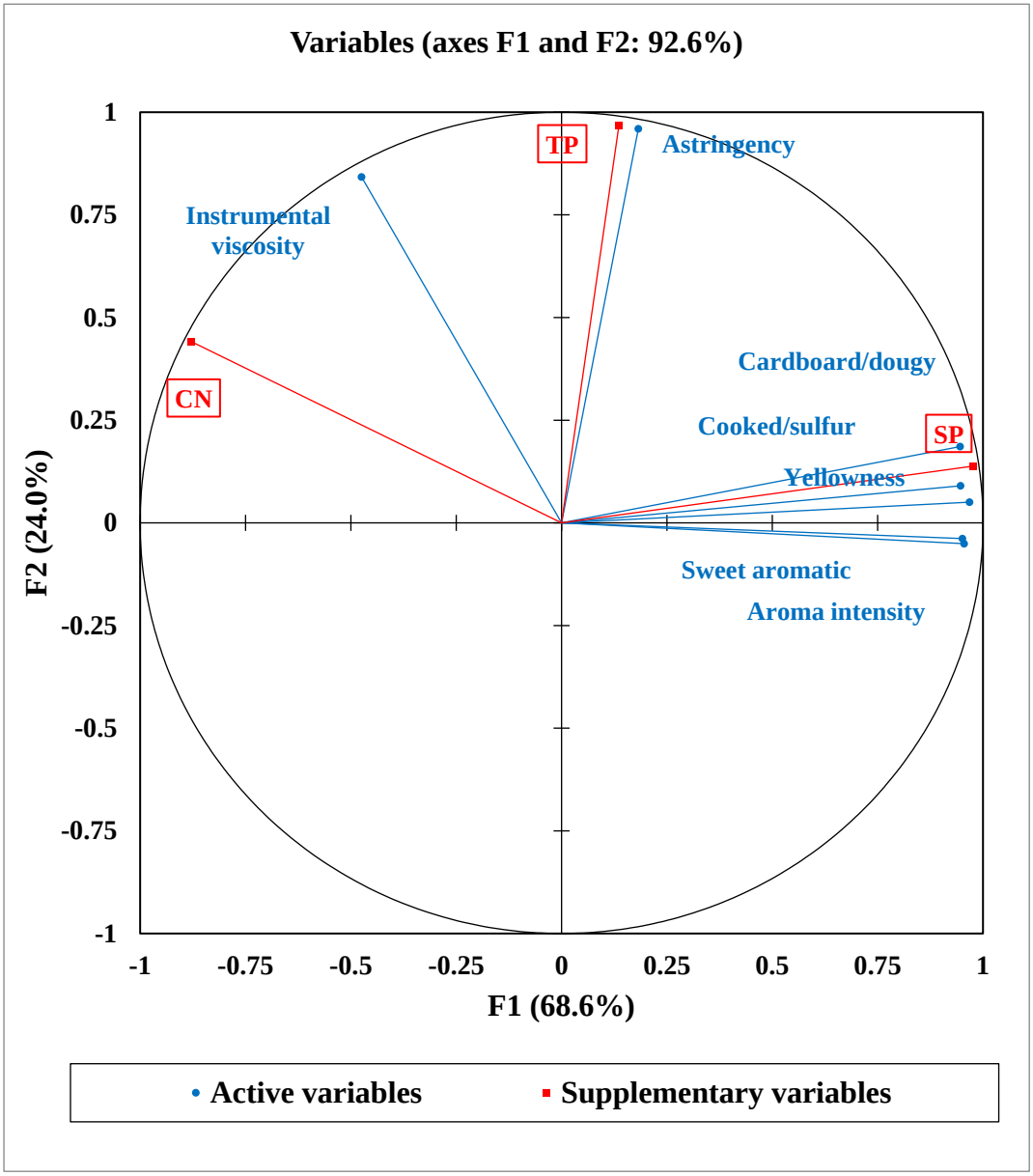


Figure 9. 1% fat pasteurized milk protein beverages: principle component analysis (PCA) vector plots for parameters where casein as a percentage of true protein contributed the most to the total variation of each parameter: instrumental viscosity, sensory yellowness (yellowness), astringency, sweet aromatic, aroma intensity, cooked/sulfur and cardboard/doughy with overlaying true protein (TP), casein protein (CN) and serum protein (SP) as supplementary variables.

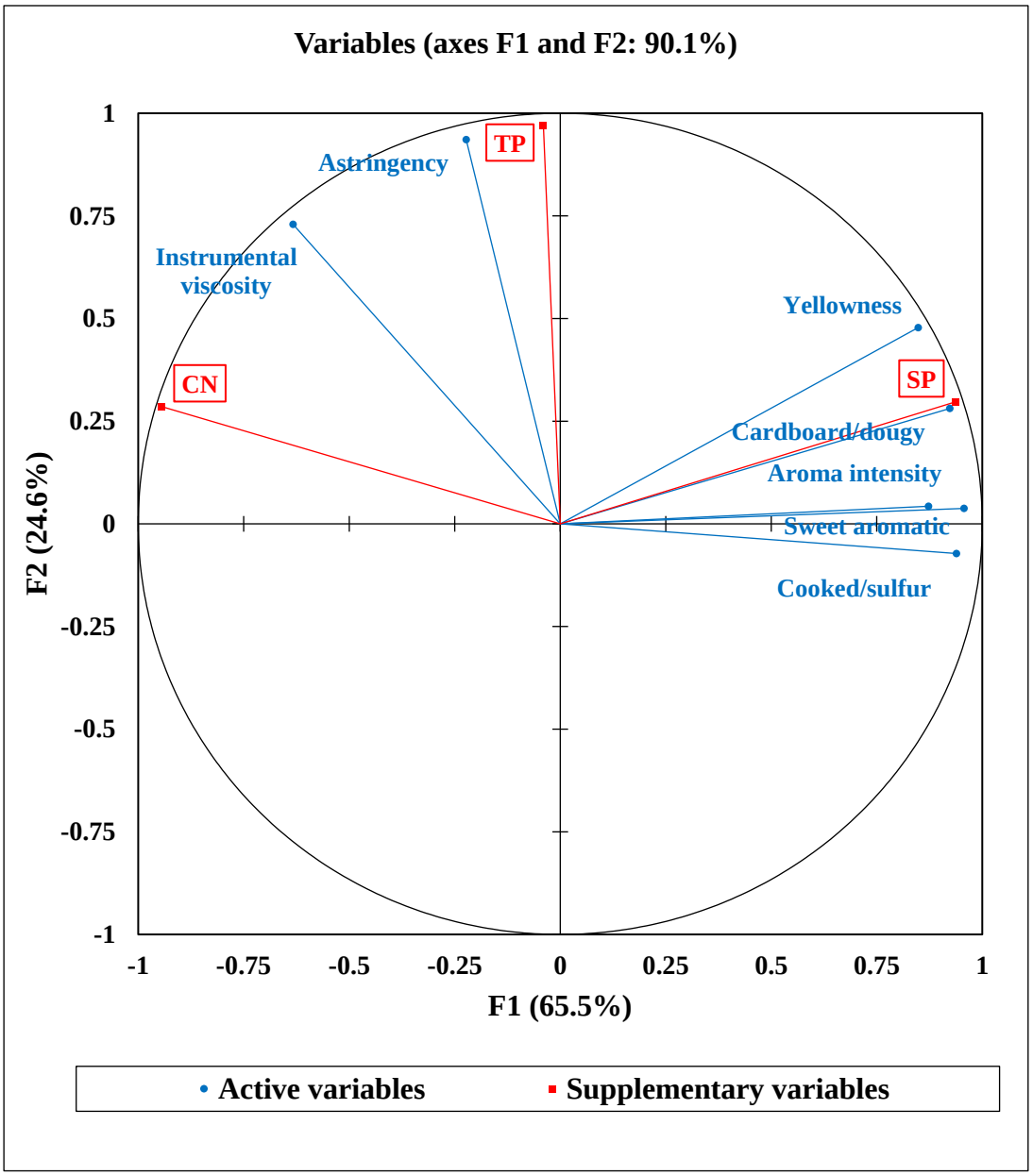


Figure 10. 2% fat pasteurized milk protein beverages: principle component analysis (PCA) vector plots for parameters where casein as a percentage of true protein contributed the most to the total variation of each parameter: instrumental viscosity, sensory yellowness (yellowness), astringency, sweet aromatic, aroma intensity, cooked/sulfur and cardboard/doughy with overlaying true protein (TP), casein protein (CN) and serum protein (SP) as supplementary variables.

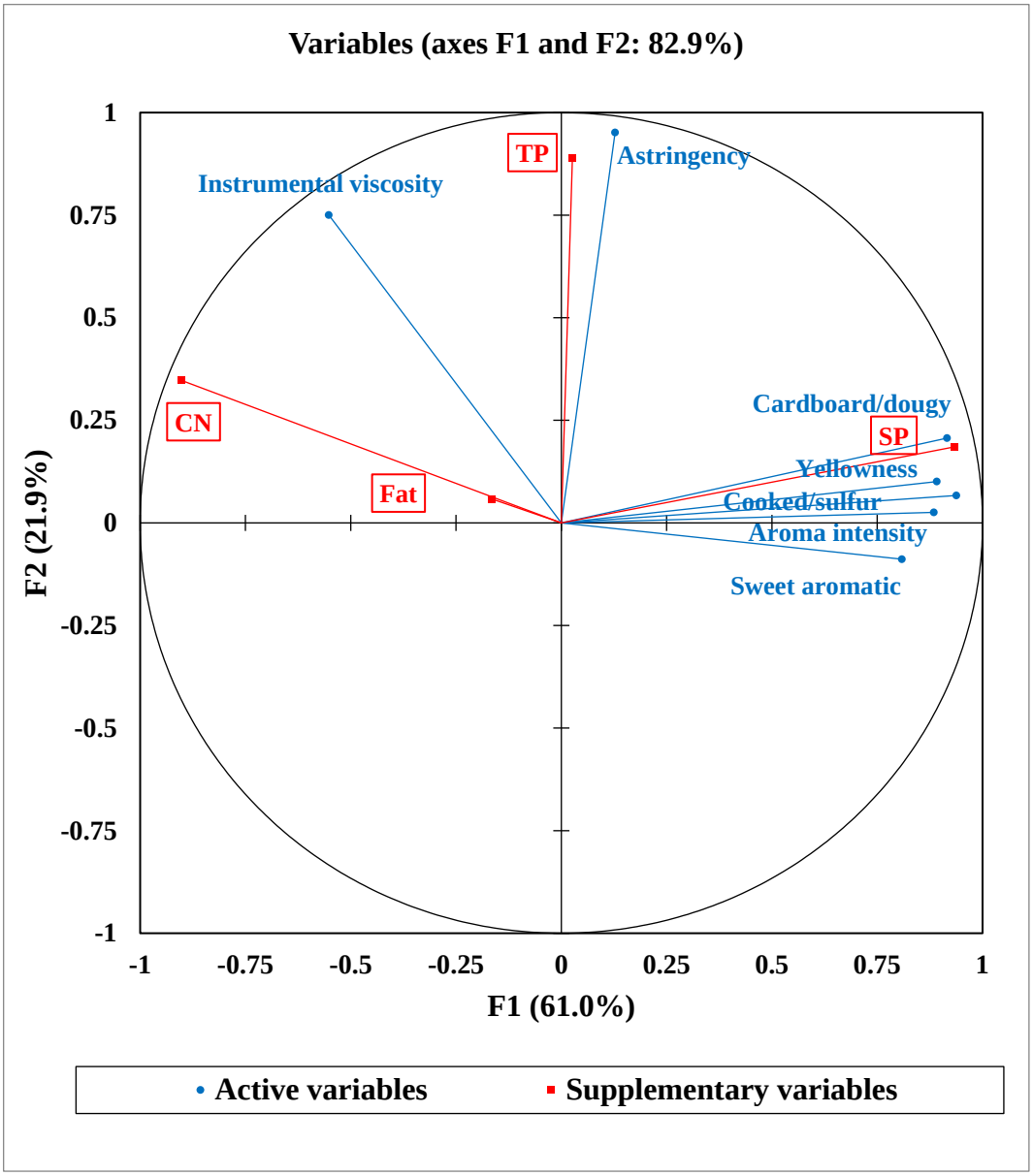


Figure 11. All fat levels for pasteurized milk protein beverages: principle component analysis (PCA) vector plots for parameters where casein as a percentage of true protein contributed the most to the total variation of each parameter: instrumental viscosity, sensory yellowness (yellowness), astringency, sweet aromatic, aroma intensity, cooked/sulfur and cardboard/doughy with overlaying fat, true protein (TP), casein protein (CN) and serum protein (SP) as supplementary variables.

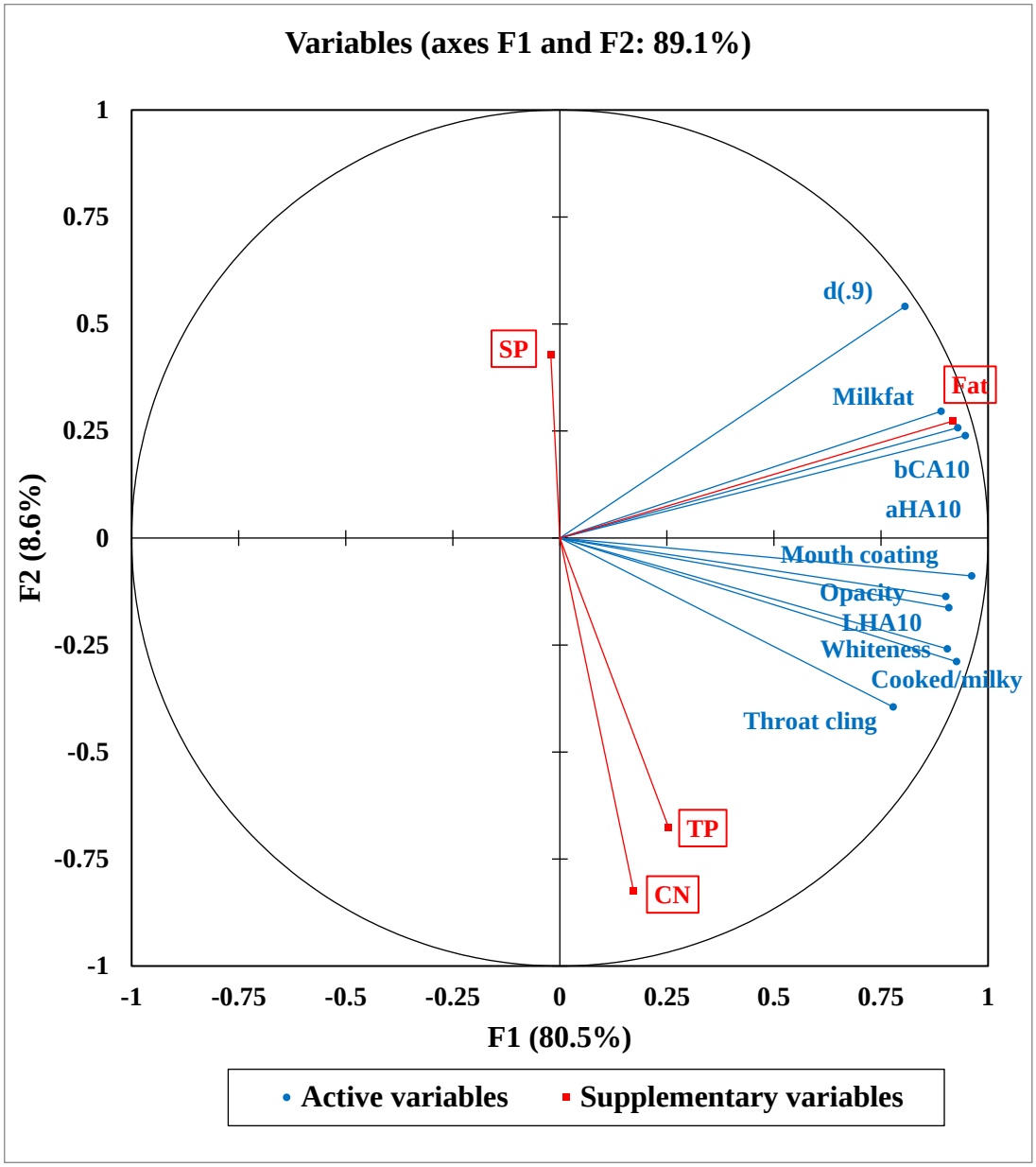


Figure 12. All fat levels for pasteurized milk protein beverages: principle component analysis (PCA) vector plots for parameters where fat contributed the most to the total variation of each parameter: Hunter L (LHA10), a (aHA10) and CIE b*(bCA10) at 10 degree viewer angles using illuminate A, particle size (d(.9)), sensory whiteness (whiteness), opacity, mouth coating, throat cling, cooked/milk and milkfat with overlaying fat, true protein (TP), casein protein (CN) and serum protein (SP) as supplementary variables.

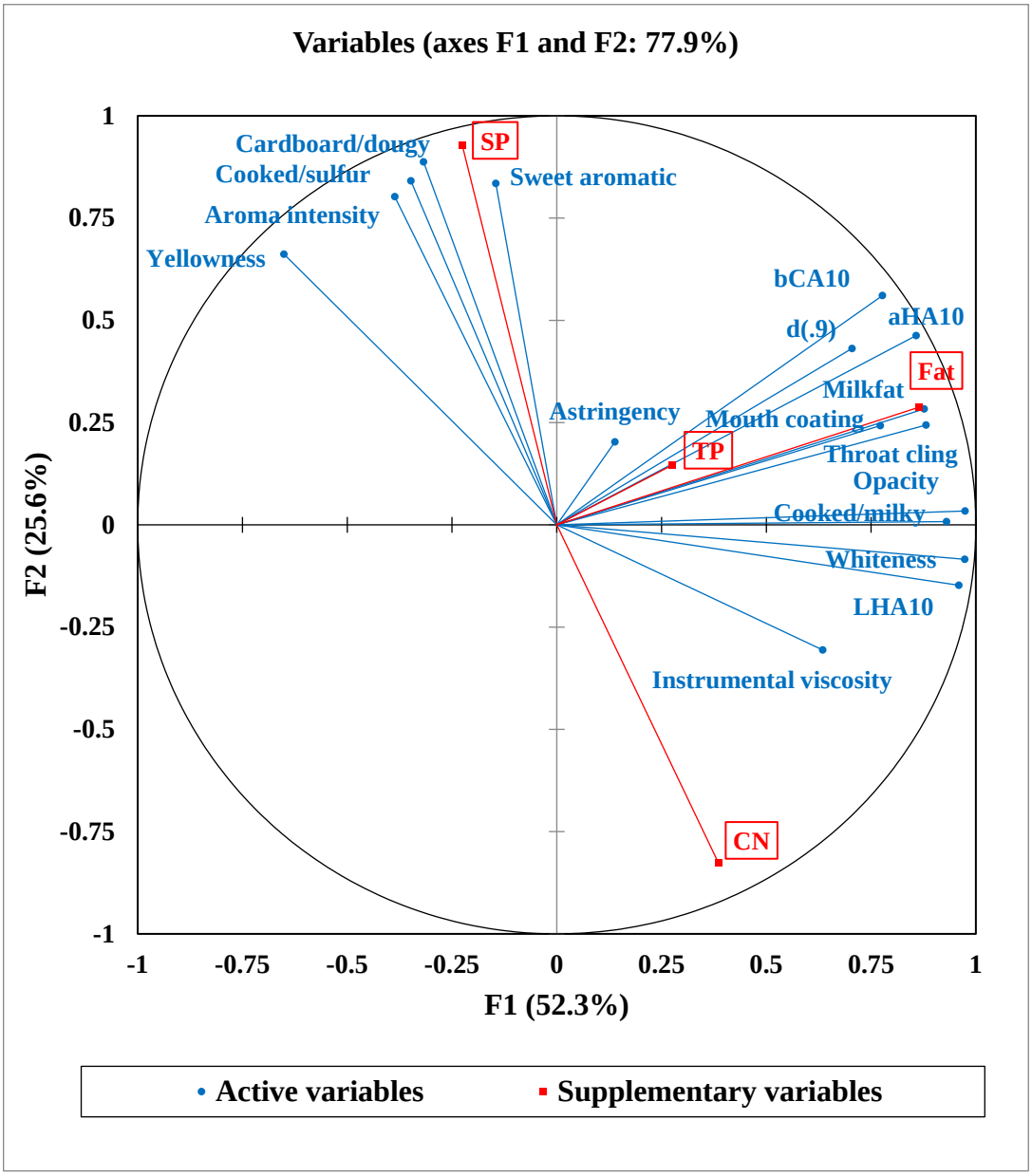


Figure 13. All fat levels and all sensory parameters for pasteurized milk protein beverages: Principle component analysis (PCA) vector plots for Hunter L (LHA10), a (aHA10) and CIE b*(bCA10) at 10 degree viewer angles using illuminate A, instrumental viscosity, particle size (d(.9)), sensory whiteness (whiteness), opacity, yellowness, mouth coating, throat cling, astringency, sweet aromatic, aroma intensity, cooked/sulfur, cooked/milk, cardboard/doughy and milkfat with overlaying fat, true protein (TP), casein protein (CN) and serum protein (SP) as supplementary variables.

CHAPTER 5: INTERPRETIVE SUMMARY

Previous studies have determined the possibility of milk components standardization by applying different processing units. My dissertation has determined the milk protein standardization by ceramic MF membrane technology for practical application in milk protein beverages. The effect of composition and heat treatment on instrumental and sensory properties of milk protein beverages were determined. This work is important to fluid milk new product development and manufacturers as its direct application would provide new opportunities to achieve milk protein standardization to specific true protein and casein as a percentage of true protein levels to provide insights for milk protein beverages sensory properties, to guide customization of milk protein beverages to meet specific consumer needs; and to balance fluctuations in milk supply/price, composition and quality.

Previous descriptive analysis and consumer studies have established the importance of appearance/color of fluid milk and dairy products, however, instrumental color measurement protocols vary in the literature. This variance creates difficulties for quality control and communication of fluid milk instrumental color across laboratories and countries. Hunter L values and CIE b* values should be used for whiteness and yellowness, respectively, of commercial HTST milk and milk protein beverage color measurement due to their higher sensitivities compared to one other. The selection of a/a* values and observer angles (2 vs 10 degree) were not different. Temperature impacted color measurement (Hunter and CIE) with its effect the largest for skim milk and the least for 2% fat milk.

The second and the third studies provide guidance for milk protein beverage formulation and quality control. Milk based beverages were produced with all combinations of 3 fat levels (0.2, 1.0 and 2.0%), 4 TP levels (3.00, 3.67, 4.34 and 5.00%) within each fat level and 5 CN%TP

levels (5, 25, 50, 75 and 80%) within each protein level for beverage formulation (60 beverages total). Variation in CN%TP in the unpasteurized beverages had a large impact on beverage whiteness and yellowness. Higher CN%TP will make the unpasteurized beverages more white and less yellow. For pasteurized beverages, higher fat concentration was correlated with higher L, a and b*values, larger particle size, increased sensory whiteness, mouth coating, cooked/milky and milkfat flavor. Higher casein concentration increased instrumental viscosity and decreased flavor intensities while higher serum protein concentration increased aroma intensity, sweet aromatic, cooked/sulfur and cardboard/doughy flavors, and sensory yellowness scores within each fat level and across fat levels. Controlling the level and ratio of casein and serum protein can be used to improve the sensory properties of value added high milk protein based nutritional beverages.

For future applications, liquid MCC as a new milk protein ingredient provides better functional and nutritional benefits than whey protein and adds more processing flexibility, which makes it a promising choice for shelf stable, neutral pH, high protein ready to drink milk protein beverages. Liquid MCC has been used more internally in cheese plants for cheese milk standardization, however, positioning it as an ingredient in the market may bring more added value. Therefore, future sensory and processing/formulation work are necessary. The flavor and functional characteristics of MCC after higher heat treatment (such as UP/UHT) in model beverages (without adding flavor agent) and formulated models (with adding flavor agent) will need to be further addressed. Off flavor (such as sulfur/eggy) prediction of MCC/SPI driven milk protein beverages by multiple linear regression models will be useful for product quality control. Processing line optimization to maximize whiteness while minimize cost of MCC beverages should be determined. Balancing cost of ingredients (based on dairy protein market prices) and

consumer perception of milk protein beverages should also be studied. Other product applications (ice cream, yogurt) could also be explored. This interpretive summary is intended to summarize the key messages from this dissertation. These findings have been presented and communicated with the industry at scientific and industry meetings and with peer reviewed publications. Information from this dissertation builds a foundation for fluid milk innovation.