ABSTRACT

HERNANDEZ, ALEX JORDAN. Milk Beverage Base with Lactose Removed with Ultrafiltration: Impact of Fat and Protein Concentration on Sensory and Physical Properties. (Under the direction of Dr. MaryAnne Drake).

Our objectives were to determine the impact of fat (skim to whole milk) and protein (3.4 to 10.5%) concentration on the sensory and physical properties of milk beverage base that had lactose and other low molecular components removed by ultrafiltration. In experiment 1, a matrix of 16 treatments was produced to achieve 4 levels of lactose removal (0, 30, 70, and 97%) at each of 4 fat levels (skim, 1%, 2% and whole milk). In experiment 2, a matrix of 12 treatments was produced to achieve 4 levels of lactose removal (0, 30, 70, and 97%) at each of 3 protein concentrations (3.4, 6.5, and 10.5% protein). Physical and sensory properties of these products were determined. Removal of > 95% of milk lactose by UF required a diafiltration volume of approximately 3 times the milk volume and increased whiteness of skim and whole milks while decreasing viscosity and making the flavor of milk more bland. In addition, lactose (and other low molecular weight solute removal) by UF decreased titratable acidity by more than 50% and increased milk pH at 20°C to >7. Future work on milk and milk-based beverages with lactose removed by UF needs to focus on interaction of the remaining milk solids with added flavorings, changing casein to whey protein ratio prior to UF removal of lactose, and the impact of lactose and low molecular weight solute removal on heat stability, particularly for neutral-pH, shelf-stable milk-based beverages.

Key Words: Lactose removal, ultrafiltration, lactose free milk
Milk Beverage Base with Lactose Removed with Ultrafiltration: Impact of Fat and Protein Concentration on Sensory and Physical Properties.

by
Alex Jordan Hernandez

A thesis submitted to the Graduate Faculty of North Carolina State University in partial fulfillment of the requirements for the degree of Master of Science in Food Science

Raleigh, North Carolina
2022

APPROVED BY:

_______________________________
Dr. MaryAnne Drake
Committee Chair

_______________________________
Dr. Clint Stevenson

_______________________________
Dr. Dana Hanson
DEDICATION

This thesis is dedicated to my family. Thank you mom and dad for teaching me to be a hard worker by your example. Without your encouragement and counsel I wouldn’t have realized my potential of what I can accomplish in this life. I want to thank my beautiful wife Vanessa for her constant love and support that I needed to move forward. Thank you for putting up with the many early mornings and long days spent in the pilot plant. Most importantly, thank you for making me a father to our beautiful daughter Alena and our little one on the way. You have all been my motivation to do the best I can in finishing my degree.
BIOGRAPHY

Alex Jordan Hernandez was born February 21, 1994 to his parents Samuel and Vicki Hernandez in Santa Maria, California. Alex moved and spent the rest of his younger years up until young adulthood in sunny south Florida. Upon graduating high school Alex served a two-year mission in San Diego, California for the Church of Jesus Christ of Latter-Day Saints in 2013. After returning home, Alex began his undergraduate studies at Brigham Young University Idaho where he met his future wife Vanessa. It was from Vanessa’s roommate that Alex learned about food science and once he took the introductory classes he was hooked. Alex graduated with his Bachelors of Science in Food Science in 2018. Upon hearing that there was an opening in Dr. MaryAnne Drake’s lab, Alex decided to further his education with a Masters in food science in 2019. With a master’s degree, Alex hopes to make an impact in other people’s lives by providing them with the benefits of dairy products, especially those who are unable to consume them.
ACKNOWLEDGMENTS

I want to first acknowledge my incredible advisors Drs. MaryAnne Drake and Dave Barbano for their guidance, knowledge, and constant support they have provided me. They have pushed me out of my comfort zone time and time again in which I am thankful. They have provided the perfect atmosphere for my growth as a student, researcher, and a team member of a pilot plant. I am grateful for the time this lab puts into not only learning but spent gaining hands-on experience with dairy processing and products.

I want to thank my fellow lab members for their time and effort put forth in helping me finish my research. The experiences we had and memories made will never be forgotten. Thank you Dr. Hanson and Dr. Stevenson for being on my committee and for all your help throughout my time here at NCSU.

I want to thank my wife Vanessa for her sacrifice these past few years. Being a wife and mother isn’t easy especially when your husband in graduate school. I appreciate the constant support you were to me and giving me the motivation to get through the long days spent in the pilot plant. I could not have done this without you.
TABLE OF CONTENTS

LIST OF FIGURES .............................................................................................................. vii

CHAPTER 1: LITERATURE REVIEW: LACTOSE FREE TREND AND THE PRODUCTION OF LACTOSE FREE PROTEIN BEVERAGES ........................................................................ 1
Introduction .......................................................................................................................... 2
Current Dairy Statistics ........................................................................................................ 2
Allocation of Milkfat and Milkfat Solids ............................................................................... 2
Lactose Free Trend ............................................................................................................... 3
Milk Composition ................................................................................................................ 5
Milkfat ................................................................................................................................... 5
Dairy Proteins ...................................................................................................................... 9
Lactose ............................................................................................................................... 11
Minerals ............................................................................................................................. 12
Membrane Separation Filtration ....................................................................................... 13
Microfiltration ................................................................................................................... 15
Ultrafiltration ...................................................................................................................... 15
Nanofiltration ..................................................................................................................... 16
Reverse Osmosis ............................................................................................................... 17
Dairy Ingredients and Ultrafiltered Milk Beverages .......................................................... 17
Relevant Research .......................................................................................................... 21
Conclusions ...................................................................................................................... 30
References ......................................................................................................................... 31

CHAPTER 2: MILK BEVERAGE BASE WITH LACTOSE REMOVED WITH ULTRAFILTRATION: IMPACT OF FAT AND PROTEIN CONCENTRATION ON SENSORY AND PHYSICAL PROPERTIES .................................................................. 44
Abstract ............................................................................................................................. 45
Introduction ....................................................................................................................... 46
Materials and Methods .................................................................................................... 48
Experimental Overview ................................................................................................. 48
Experiment 1 .................................................................................................................... 48
Experiment 2 .................................................................................................................... 49
Ultrafiltration for Lactose Removal ................................................................................. 49
Ultrafiltration for Experiment 1 ....................................................................................... 49
Beverage Formulation: Experiment 1 .............................................................................. 52
Ultrafiltration for Experiment 2 ....................................................................................... 52
Beverage Formulation: Experiment 2 .............................................................................. 53
Analysis Methods ............................................................................................................ 54
Lactose Determination .................................................................................................... 54
Freezing Point, Titratable Acidity, and pH ..................................................................... 54
Microbial Analysis .......................................................................................................... 54
Apparent Viscosity .......................................................................................................... 55
Color ................................................................................................................................. 55
Descriptive Analysis ...................................................................................................... 55
Statistical Analysis ......................................................................................................... 56
Results and Discussion ............................................................................................................. 57
Experiment 1 ................................................................................................................................. 57
  Processing Data ......................................................................................................................... 57
  Beverage Lactose, Freezing Point, pH, and TA ....................................................................... 57
  Beverage AV and Color ............................................................................................................. 59
  Beverage Sensory ....................................................................................................................... 60
Experiment 2 ................................................................................................................................. 62
  Processing Data ......................................................................................................................... 62
  Beverage Lactose, Freezing Point, pH, and TA ....................................................................... 62
  Beverage AV and Color ............................................................................................................. 63
  Beverage Sensory ....................................................................................................................... 65
Conclusions ................................................................................................................................. 66
Acknowledgments ....................................................................................................................... 67
References ..................................................................................................................................... 68
Figures .......................................................................................................................................... 72
LIST OF FIGURES

Figure 1 Experimental 1 overview: lactose and soluble mineral removal by UF at 4 different fat concentrations ................................................................. 72

Figure 2 Experimental 2 overview: lactose and soluble mineral removal by UF at 3 different protein concentrations ............................................................ 73

Figure 3 Experiment 1: percent lactose as a function of diafiltration volume. ............... 74

Figure 4 (A) Experiment 1: The impact of fat concentration on milk pH with increasing lactose removal. and (B) Experiment 2: The impact of protein concentration on milk pH with increasing lactose removal ........................................ 75

Figure 5 (A) Experiment 1: The impact of fat concentration on milk titratable acidity (TA) with increasing lactose removal and (B) Experiment 2: The impact of protein concentration on milk TA with increasing lactose removal .................... 76

Figure 6 (A) Experiment 1: The impact of fat concentration on milk apparent viscosity (AV) with increasing lactose removal and (B) Experiment 2: The impact of protein concentration on milk apparent viscosity (AV) with increasing lactose removal ........................................................................ 77

Figure 7 The impact of lactose removal on L-value (A – experiment 1; B – experiment 2), a-value (C – experiment 1; D – experiment 2), and b*-value (E – experiment 1; F – experiment 2) ................................................................................. 78

Figure 8 Reflectance curves. (A) – Experiment 1 – skim milk; (B) Experiment 1 – whole milk; (C) Experiment 2 – 3.4% protein, (D) Experiment 2 – 10.5% protein ........................................................................................................ 81

Figure 9 Principal component biplot of sensory attributes of milks with variable lactose and fat: experiment 1 ................................................................................. 83

Figure 10 Principal component biplot of sensory attributes of milks with variable lactose and protein: experiment 2 .............................................................................. 84
CHAPTER 1: LITERATURE REVIEW: LACTOSE FREE TREND AND THE PRODUCTION OF LACTOSE FREE PROTEIN BEVERAGES

A.J. Hernandez, and M.A. Drake
Department of Food, Bioprocessing and Nutrition Sciences, Southeast Dairy Foods Research Center, North Carolina State University, Raleigh, NC 27695

*Corresponding Author: MaryAnne Drake
Box 7624, Department of Food, Bioprocessing and Nutritional Sciences
North Carolina State University
Raleigh, NC 27695-7624
Phone: 919-513-4598
Fax: 919-513-0014
Email: maryanne_drake@ncsu.edu
Introduction

Current Dairy Statistics

Of all the iconic food goods on the market, dairy products have been at the forefront in both sales and popularity (Jerome, 2020). Throughout the years, technological advances have provided dairy companies with new possibilities in processing which has allowed them to modify their products to meet shifting social trends. Milk production in the United States has increased 14 percent over the past 10-year period (USDA, 2021a). Not only has the production of milk increased, but the density of nutrients of milk has grown. In 2000, milk contained an average of 3.68 percent milkfat and 8.72 percent skim solids (Teran and Cessna, 2021). In 2020, the average milkfat percentage was 3.97 percent and the skim solids was 8.94 percent (Teran and Cessna, 2021). The rising milkfat percentage and skim solids can be linked to a response to the increase in demand for products with higher milkfat content (Teran and Cessna, 2021). Farmers selectively bred cows for improved milkfat output. This trend came from new information regarding the lessened health risk in milkfat consumption and the nutritional benefits of milkfat (Thorning et al., 2016). Although the production of milk has increased, fluid milk sales decreased by 15 percent between 2010 and 2020 (Cessna and Teran, 2021a). Another report estimated that sales of total conventional fluid milk products decreased 5.7 percent from January 2020 to January 2021 (USDA, 2021b). Changes in consumer behavior and beverage competition have led to a decline in dairy sales (Adams et al., 2019).

Allocation of Milk Fat and Milk Skim Solids

The U.S. consumption per capita of dairy products has increased 6 percent over the past 5 years with the cheese, butter, and yogurt categories driving this growth (Jerome, 2020). The two largest dairy product production categories are cheese and fluid milk (Teran and Cessna, 2021).
Since most dairy products contain both milkfat and skim solids it is important to know where these are being allocated. Cheese is the dairy product category that accounts for the largest percentage of the U.S. milkfat supply, 42 percent, and that percentage has been growing (Cessna and Teran, 2021b). Fluid milk is the dairy product that accounts for the largest percentage of the US skim solids supply, 21.7 percent, but that percentage has been declining (Cessna and Teran, 2021b). The decline of the US skim solids supply allocated to fluid milk is due to an increase in the cheese and whey industry. With the consumption of dairy products in general on the rise, the number of gallons of fluid milk consumed in a household per week has decreased by 12.2% from 2013 to 2017 (Stewart, 2020). There is a need to utilize fluid beverage milk in a way that will make it viable again in the market.

*Lactose Free Trend*

One primary reason why fluid milk consumption has seen a decline in recent decades, especially in developed countries is due to the general public perception that lactose intolerance is widespread (Zingone et al., 2017). Lactose is the major sugar found in milk (Portnoy and Barbano, 2021) and comprises 4.8% of milk solids (Fox, 2011). Once humans are weaned from complete reliance on milk as babies, the enzyme that is used to digest lactose – lactase -- decreases in some people which leads to a trait known as lactase non-persistence (Anguita-Ruiz et al., 2020). Lactase non-persistence can lead to lactose malabsorption and when it is combined with negative symptoms such as nausea, bloating, and pain, it is normally referred to as lactose intolerance (Storhaug et al., 2017). It is estimated that 68% of the world’s population and 36% of people in the United States have lactose intolerance (Storhaug et al., 2017). Many of these consumers avoid dairy milk and have switched to other beverages including lactose free plant-based alternatives.
When it comes to lactose intolerance, it is difficult to know how much of the population are actually lactose intolerant and not just under the belief that they are. Self-reported cases of lactose intolerance skews the statistics found in the United States (Keith et al., 2011). In a study conducted of lactose intolerance among African Americans, the self-reported occurrence of lactose intolerance was 24% compared to the 80% estimate found in literature (Keith et al., 2011). In another study evaluating self-reported lactose intolerance, 20% of African Americans, 10% of Hispanic Americans, and 8% of European Americans were reported to actually have lactose intolerance (Wilt et al., 2010). There are dangers that come with incorrect self-diagnosis of lactose intolerance. Without a doctor’s confirmation of lactose intolerance, other diseases could remain undiagnosed (Szab et al., 2021). Also, if dairy is avoided altogether in the diet this could lead to more health complications in the future like osteoporosis or cardiovascular disease if proper supplementation is not achieved (Hodges et al., 2019; Thorning et al., 2016). This situation not only affects the health of the individual but could also completely change their lifestyle which in some cases is not practicable for everyone (Suri et al., 2019).

Dairy companies are now starting to focus research and development on lactose-free products to meet the rise in demand for lactose free products (Mintel, 2021). Pasteurized milk is currently the largest category in the lactose-free dairy and represents two-thirds (~70%) of the market (Dekker et al., 2019). In 2020, lactose-free milk had a growth of 19% in both sales and volume (Gerdes, 2021). When considering manufacture of a lactose-free dairy product, it is important to understand the standards and regulations that need to be met. When it comes to a “lactose free” or a “low lactose” claim, there is no specific FDA definition (Suri et al., 2019; National Dairy Council, 2018). This means that a “lactose free” or “low lactose” milk could contain low levels of lactose (Idaho Milk Products, 2022). There is a need for a specific labeling
policy to establish a specific cut-off value for the “lactose-free” label in the United States and European Union (Facioni et al., 2020).

There are two primary ways to remove lactose from milk: lactose hydrolysis and membrane filtration (Paige et al., 1975; Kosikowski, 1979; Gerdes, 2021). These processes can be used separately or together in order to achieve the desired amount of lactose reduction. Adding the lactose hydrolysis enzyme β-galactosidase (lactase) breaks down lactose into galactose and glucose (Harju et al., 2012). The hydrolysis process can be added pre or post heat treatment (Dekker et al., 2019). When done prior to processing, hydrolysis is done in a batch process and when post process, hydrolysis is done in an aseptic manner to prevent post process contamination (Harju et al., 2012). The batch process includes adding the lactase to the raw milk for incubation, pasteurization (care must be taken to ensure that the pasteurization time/temperature inactivates the lactase enzyme in addition to meeting legal pasteurization), packaging, and then storage (Dekker et al., 2019). The aseptic process differs in that the milk is first heat treated (UHT; 2-4 seconds 142° C), then the lactase is added aseptically, packaged, quarantined, and then stored (Dekker et al., 2019). The hydrolysis process occurs in the packaged milk prior to sale. The results of lactose hydrolysis are an increase in carbohydrate solubility, higher sweetness, and the availability of the product to those who are lactose intolerant (Skryplonek et al., 2017).

**Milk Composition**

*Milkfat*

It is important to understand the different properties of milk in order to fully grasp how milk can be manipulated by processing to get desirable products. Milk, especially milkfat, provides a significant source of nutrition and energy for humans (Dhankhar et al., 2016). Milk is
an oil-in water emulsion where milk fat is suspended as small droplets, known a milkfat globules, in the milk serum (Dhankhar et al., 2016). The diameters of these droplets can range from 0.1 to 20 µm and are stabilized by the milkfat globule membrane, which consists of polar lipids, proteins, and enzymes (Byllund, 2003) (Logan et al., 2014). The fat droplet is mainly comprised of glycerides with cholesterol, free fatty acids, and phospholipids also being present (Walstra et al., 1999).

There are a series of conditions and factors that can vary the amount of fat found in milk. Milk composition varies throughout lactation which is caused by the changes that occur physiologically in the cow (Auldis et al., 1995). The fat amount is highest at beginning of lactation in the colostrum and decreases as time goes on (Heinrichs et al., 2005). The amount of fat in milk can also change according to the season. This is due to the changes in feed that shift from indoor housing during winter or dry lot and pasture-based systems (Pacheco-Pappenheim et al., 2021). Milk fat can also vary within and across the different breeds of cows (Soyeurt et al., 2006) The average milk fat percentages of milk producing cows are: Jersey cow 4.6%, Ayrshire 3.86%, Brown Swiss 4.04%, Guernsey 4.51%, and Holstein 3.65% (Heinrichs et al., 2016). The breed of cow that is the backbone of the dairy industry is the Holstein. Even though it has the lowest fat content of the breeds, it produces more milk which leads to higher total yield of fat (Heinrichs et al. 2016). Infections and cow health can vary the amount of fat in milk as well (Heinrichs et al., 2005). Mastitis is the most common infection that can affect lactating cows. The infection damages the secretory cells of the cow’s udder and results in the reduced synthesis of milk components which leads to a decrease of fat content in the milk (Heinrichs et al., 2005). Hydrolytic enzymes which can break down triglycerides can also be secreted and can change milk composition by the breakdown of milkfat (Auldis et al., 1995).
Fat globules are the largest particles in milk and are also the least dense (Bylund, 2003). This leads to a layer of cream on raw milk that comes from the fat globules rising to the surface. Geer et al. (2014) conducted a study on gravity separation and found that both somatic cells and immunoglobulins needed to be present in milk in order for normal gravity separation (rising to the top) of fat, bacteria, and spores in whole milk to take place. The exact mechanism of the gravity separation needs more research but immunoglobulins alone without the presence of somatic cells were enough to cause the bacteria, fat, and spores to rise to the top. The fat content of milk is standardized in industrial processing by using the densities of cream (fat) and skim in the milk. Standardization starts with first separating the fat and skim mechanically with the use of centrifugation (Kosikowski and Mistry, 1990). Depending on the target amount of milkfat, these two streams can then be added back together in order to form the final product composition. This process is referred to as standardization which blends the starting milk base to many different types of dairy products (Ma and Barbano, 2000).

In order to incorporate milkfat into a stable solution in milk, a process called homogenization is conducted. Homogenization is normally described as the process of mixing two immiscible liquids, in this case fat and water, so they can be in the same phase (Loi et al., 2019). The process of homogenization involves heating the milk to 55°C to liquefy the milkfat and inactivate serum phase lipase followed by shearing the milk under pressure in a homogenizer. The process decreases the fat globule size to stabilize the milk emulsion (Floury et al., 2000). This process eliminates the rising of the cream to the surface as the fat globules are broken down to smaller sizes by the homogenization process and the fat globule surface is coated in protein which act as a barrier (Rosenberg and Lee, 1993). The process of homogenization with fluid milk is effective and established. Homogenization can also be applied to other dairy...
products and there are two mechanisms that can occur in addition to stable suspension of fat: coalescence and aggregation (Rosenberg and Lee, 1993). Coalescence is when fat globules merge together to form a larger fat globule when there is a break of the liquid film between the emulsion droplets (Huppertz and Kelly, 2006). Aggregation is when there is a bridge in the protein coated fat globules which causes them to join together (Huppertz and Kelly, 2006).

Milkfat contributes many properties to the physical and sensory properties of dairy products. The higher the amount of milkfat in a product, the higher the viscosity (Mulder and Walstra, 1974). The viscosity of skim milks was lower ($P \leq 0.05$) than reduced fat and whole milks (Adhikari et al., 2010; Chapman et al., 2001). Milkfat level has a stronger effect on color parameters (Hunter L, a, and b values) than heat treatment. Hunter color L, a, and b values were higher ($P < 0.05$) for 2% milk than skim which indicate higher whiteness, higher yellowness, and less greenness due to higher amounts of milkfat globules that mask the reflection of light (Lee et al., 2017). Whole milks scored higher ($P \leq 0.05$) than skim milks for fatty mouthfeel which is described by descriptive analysis panels as, “the intensity of the oily feeling in the mouth when the product is manipulated between the tongue and the palate” (Adhikari et al., 2010). In milk, differences in visual appearance are detected at a smaller variation in milk fat than flavor and aroma attributes (McCarthy et al., 2017). Also, fat plays a big role in masking flavors in food products (Bennet et al., 2012). Skim milks score higher on “lack of freshness” than reduced-fat and whole milks. This “lack of freshness” can be attributed to increased perception of light oxidized flavor that is masked in higher fat milks and lower intensities of dairy-related flavor due to the lower amounts of fat in skim milk (Adhikari et al., 2010). Skim milk and low fat milk drinkers prefer slightly more fat in their milk than they are normally used to with aftertaste being the deciding factor in preferences of milk (McCarthy et al., 2017).
Dairy proteins

Cow’s milk normally contains approximately 3.2 percent protein (Corredig, 2009). Just like with milk fat, protein content of fluid milk can vary for a variety of reasons. Stage of lactation, age, the season of the year, infections, and genetics all play a role in the variation of protein content (Heinrichs et al., 2005; Auldis et al., 1995). There are two main types of proteins found in milk. Casein comprises approximately 80 percent of the protein and whey proteins comprise the other 20 percent (Lara-Villoslada et al., 2005). There are four main casein components in milk: A1-, A2-, and B-, and k-casein (Swaisgood, 1993). These casein components are present in the casein micelle, which are large colloidal suspensions of protein that exist in the serum phase of milk (Fox and Brodkorb, 2008). The casein micelles are aggregated caseins that have a stabilizing surface layer that keeps them all together (Dalgleish, 1998). This is different than that of the whey proteins which are dissolved in the serum phase of milk (Fox et al., 2015). The casein micelle is 50-600 nm in size and have an average diameter of 200 nm (Glantz et al., 2010; Kruif, 1998; Fox and Brodkorb, 2008). The casein micelle structure/composition are influenced by hydrophobic interactions and calcium phosphate solubility and both are sensitive to temperature (Ng et al., 2018). A casein micelle is made up of submicelles that are linked together by hydrophobic bonds between the submicelles and electrostatic bonds (salt bridges) with calcium phosphate (Walstra 1990). Micellar calcium phosphate is distributed throughout the protein matrix of casein micelles and the micellar calcium phosphate particles are several nanometers in size that are formed from amorphous hydrated calcium phosphate linked to casein phosphate centers (Holt, 2004). Calcium phosphate helps with the structure and stability of the casein micelle because they form linkages between the protein molecules (Tsioulpas et al., 2007).
The main function that calcium phosphate provides is to act as a buffer to protect the casein micelle against heat (Omoarukhe et al., 2010). As heat increases, the soluble calcium decreases due to its migration into the casein micelles as colloidal calcium phosphate (Omoarukhe et al., 2010; Karlsson et al., 2019). When temperatures are lower, refrigeration temperatures (1.7 to 3.3 C), calcium phosphate becomes more soluble which leads to the migration of calcium ions out of the micelle and the hydrophobic interactions between the submicelles become weaker (Creamer et al., 1977; Karlsson et al., 2019). This process leads to the solubilization of micellar calcium phosphate (Creamer et al., 1977). The isoelectric point for casein is around pH 4.6, when the pH is below 4.6, gelation or precipitation occur due to the loss of electrostatic repulsion between micelles (Agarwal et al., 2015).

The whey proteins found in milk are β-lactoglobulin, α-lactoglobulin, immunoglobulins, bovine serum albumin, bovine lactoferrin, and lactoperoxidase (Madureira et al., 2007). Most whey proteins, mainly β-lactoglobulin and α-lactoglobulin, are globular proteins that have high hydrophobicity and compactly folded peptide chains (Walstra et al., 1999). Unlike casein that are colloidal dispersions, whey proteins are found in serum phase (Fox et al., 2015). β-lactoglobulin is the most dominant whey protein comprising 58% w/w of the whey protein, consists of 162 amino acid residues, and it is made out of primarily β-sheet secondary protein structure (Madureira et al., 2007). A-lactoglobulin comprises 20% of the whey proteins and its globular structure is stabilized by four disulphide bonds (Madureira et al., 2007). Unlike caseins, whey proteins don’t precipitate when the pH is adjusted to 4.6. Whey proteins have an isoelectric point ranging from 4.8 to 5.3 (Burrington, 2018). Unlike casein, which is generally heat stable, whey proteins when exposed to temperatures between 70 to 100 C will denature while the structure of the casein micelle remains intact (Fang et al., 2017; Fox et al., 2015). As heat treatment takes
place, both β-lactoglobulin and α-lactoglobulin proteins are denatured, forming aggregates and these aggregates form complexes with one another and casein (Al-Saadi, 2013). The net result can be loss of solubility and visible precipitation or particulates (aggregates). There are multiple interactions that can occur during this denaturation process which include complex formation between β-lactoglobulin and k-casein, interaction of β-lactoglobulin with itself, complex formation between β-lactoglobulin aggregates and k-casein, and interactions between β-lactoglobulin with α-lactoglobulin and subsequently with k-casein (Kessler and Beyer, 1991).

The whey proteins interact with k-casein at the exterior of the casein micelle through thiol group-disulfide bond exchange reactions (Al-Saadi, 2013). As the whey proteins interact with casein and form complexes, it will impact the process of curd (gel) formation in products such as yogurt or cheese (Asaduzzaman et al., 2021; Giroux et al., 2020).

**Lactose**

Lactose is the main carbohydrate that is found in milk and the most abundant milk solid (Portnoy and Barbano, 2021). It is a disaccharide made up of glucose and galactose and it comprises 4.8 percent of milk solids (Walstra et al., 2006). Lactose exists in two different forms α and β, when in aqueous solutions at equilibrium, with 37.3% being in α form and 62.7% being in β form (Gänzle et al., 2008). The equilibrium ratio between these two forms are affected by temperature, pH, other sugars and salts (Portnoy and Barbano, 2021). The α –lactose is more soluble at ambient conditions while the β – lactose is more soluble at temperatures 93.5° C (Thorning et al., 2016). As such, lactose is more soluble at higher temperatures rather than at ambient temperatures.

Lactose has a lot of functionality in milk and dairy products. Lactose helps the growth of beneficial bacteria which promote a healthy intestine, provides sweetness or sweetness
enhancement, supports protein stabilization, and aids in emulsifying properties of foods (Corredig, 2009; Wong and Hartel, 2014). In terms of sweetness, lactose is about 5 times lower than that of sucrose (Skryplonek et al., 2017). At ambient temperature, lactose has about 10 times lower solubility than that of sucrose (Skryplonek et al., 2017). This can lead to lactose crystallization which is a two-step process where lactose crystals are formed from the processes of nucleation and growth of the nucleus (Gänzle et al., 2008). As supersaturation occurs, the rate of crystal growth increases (Wong and Hartel, 2014). The problem with lactose crystallization is that large lactose crystals can leave the product with a rough or gritty sensation known as sandiness which is undesirable in dairy products (Gänzle et al., 2008; Johnson, 2014).

Minerals

The minerals that are found in milk encompass 0.7-0.8 percent of milk solids but are extremely important to the conformation and stability of milk proteins (Fox et al., 2008). The minerals found in milk are calcium, magnesium, sodium, potassium, chloride, and inorganic phosphate (Gaucheron, 2005). These minerals can be divided into diffusible (soluble) and non-diffusible (colloidal phase) fractions (de La Fuente, 1998). Potassium, sodium, and chloride are considered diffusible while calcium, inorganic phosphate and magnesium are partly bound to the casein micelles (Gaucheron, 2005). A third of calcium, half of the inorganic phosphate, two-thirds of magnesium, and most of the citrate can be located in the aqueous phase of milk (McSweeney, 2010). The diffusible fraction does not have ions that are totally free in solution because these associations that occur depend on the affinity between cations and anions while also taking into account the solubilities of the salts (Holt et al., 1981). The colloidal calcium in milk is composed of the mixture of calcium caseinate and calcium phosphate (Koutina et al., 2015). Calcium helps the internal structure and stability of the casein micelle with the colloidal
calcium phosphate in the casein micelle and with the formation of calcium bridges that are formed between negatively charged residues of the caseins (Karlsson et al., 2019). Calcium is a very important mineral that helps with heat stability in milk and dairy products (Tessier and Rose, 1961). When it comes to heat stability, the main drivers behind stability are temperature and pH (Ma and Barbano, 2003). At refrigeration temperatures, calcium phosphate dissolves into the serum of milk while at higher temperatures, calcium phosphate migrates into the micelle (Karlsson et al., 2019). This migration of calcium phosphate into the micelle helps stabilize the casein micelle against high processing temperatures of 140-150° C (Datta and Deeth, 2001; Douglas et al., 1981). This is important to know because as temperature rises in milk it lowers the pH (Ma and Barbano, 2003; Omoarukhe et al., 2010). As pH is lowered, the calcium equilibrium is shifted which leads to an increase of soluble calcium levels in the serum phase and less colloidal calcium phosphate in the micelles. Less colloidal calcium phosphate destabilizes the casein micelles, promotes aggregation, and forms sediment (Walstra, 1990; Karlsson et al., 2019; Chow and Eanes, 2001). Understanding the properties of milk minerals allows for the modification of the physico-chemical conditions during the manufacture of dairy products to get different end products. Factors such as acidification, heat treatment, cooling, adding chelators or sodium chloride can be manipulated to create new products or to improve existing milk products (McSweeney, 2010).

**Membrane Separation filtration**

One of the ways milk can be processed is with the use of filtration. Filtration utilizes a membrane process in which a solution is passed through a semipermeable membrane (Walstra et al., 1999). The filtration works by the use of a pressure differential over the membrane that separates molecules depending on their molecular weight (Scott, 1998). The liquid, or filtrate,
that passes through the membrane is called the permeate. The liquid, or concentrate, that is retained in the membrane is called the retentate (Mistry and Maubois, 2017). There are a few factors that are important to successful membrane fractionation. They include operating pressure, temperature, feed flow velocity, and the feed concentration (Hu and Dickson, 2015). Filtration is also affected by changes in the ionic strength, thermal pretreatment, and modifications in pH (Ng et al., 2018). Diafiltration can also be used if needed. Diafiltration consists of adding a solvent, usually water, to the feed in order to either to help lessen the problem of lower fluxes that comes with higher concentrations or to get a better removal of the smaller solutes through the permeate (Renhe et al., 2019). The diafiltration can be done continuously or in stages, depending on what the desired final product composition is (Hu and Dickson, 2015).

Filtration has many advantages when it comes to processing. Since there are many different membranes and filtration techniques that can be utilized, filtration can remove fractions and co-fractions from the initial products (Mohammad et al., 2012). The operations are done under milder temperatures which can reduce the risk of damage to the product (Walstra et al., 1999). There are a few different types of filtration units that can be used for processing including plate units, tubular membranes, or spiral wound membranes (Scott, 1998). Depending on the project, the choice of the right filtration unit is essential. The main difference between these membranes are the way that they are cleaned or replaced. For plate units, the whole plate and frame membrane unit needs to be dismantled completely in order to be cleaned, tubular membranes are easily cleaned and can be replaced without great effort. Spiral wound membranes are hard to inspect and if leaking occurs, the complete cartridge needs to be replaced (Walstra et al., 1999). There are 4 main membrane separation techniques that are used in the
dairy industry which consist of microfiltration, ultrafiltration, nanofiltration, and reverse osmosis (Chen et al., 2018).

**Microfiltration**

For microfiltration, the pore size that is used is $10^{-1}$-$10^1$ μm (Rosenberg, 1995). In the dairy industry, microfiltration can be used to reduce the amount of microbes in milk without disrupting the functionality of milk proteins or affecting the taste of the milk (Fernández García et al., 2013). Microfiltration can also be used to concentrate the caseins in milk into a product called micellar casein concentrate (MCC) that can be further processed into a micellar casein powder that can be used to fortify products, like cheese milk (Saboya et al., 2000; Carter et al., 2021). This filtration method can be ran in either dead-end mode or crossflow of operation. For dead-end mode operation, the flow of the feed is perpendicular to the surface of the membrane and this is an issue because as the flow continues, the particles of the product that aren’t filtered through can accumulate on the surface of the membrane that can create a solid cake (van der Bruggen, 2018). To combat this occurrence, cross flow is used for the feed to flow mostly parallel along the membranes surface so it reduces the buildup of the fouling layer (Scott, 1998; Carter et al., 2021). The flow is important to know because the biggest issue found during filtration is fouling. This occurs as the flux rate declines due to the accumulation of material on the surface of the membrane (van der Bruggen, 2018).

**Ultrafiltration**

For ultrafiltration, the pore size that is used is $10^{-2}$-$10^{-1}$ μm (Rosenberg, 1995). This leads to bacteria, fat, and proteins remaining in the retentate while lactose, minerals, and water leave through the permeate (Bylund, 2003). As milk is processed with ultrafiltration, water, soluble minerals, and lactose permeate through the membrane while the proteins become concentrated in
the retentate (Mistry & Maubois, 2004). This kind of processing is normally done to standardize milk for a variety of dairy products like cheese and various powders (Dairy Management Inc., 2005). When it comes to ultrafiltration, having a cross flow or tangential flow is the best way to get the most efficient separation and flux in the system (Snyder Filtration, 2020). This can also improve the membrane life. Ultrafiltration processing can be used in the food and beverage industry by gelatin concentration/purification, plant extract processing, and fruit juice concentration (McHugh and Avena-Bustillos, 2020). In the dairy industry it is mostly used for concentrating whey protein isolates, concentrating milk protein, standardizing, clarifying brine, and fractionation of casein/whey proteins (Snyder Filtration, 2020). Ultrafiltration is not only used in the food, beverage, and dairy industry but can also be used in the biotech/pharmaceutical industry. It can be utilized to concentrate enzymes/proteins, the production of antibiotics, and to process blood plasma (McHugh and Avena-Bustillos, 2020).

*Nanofiltration*

Nanofiltration uses pore sizes of $10^{-3}$-$10^{-2}$ during processing (Rosenberg, 1995). Just like with ultrafiltration and microfiltration it is a pressure driven process which separates ions from solutes. It delivers a partial desalination process to a product. The characteristics that stand out for nanofiltration membranes are that they have a higher flux, a higher rejection of divalent ions, and low rejection of monovalent ions (Mohammad et al., 2015). Nanofiltration is mostly used for treating wastewater. In the dairy industry, nanofiltration can be used to separate lactose from whey or milk (Harju et al., 2012). Nanofiltration can also be used to separate and concentrate minerals simultaneously, remove salt from salty whey, partial removal of acid from acid whey, and the partial demineralization of sweet whey in the production of lactose or demineralized whey (Rosenberg, 1995).
Reverse Osmosis

Reverse osmosis uses pore sizes of $10^{-4}$-$10^{-3}$ during processing (Rosenberg, 1995). It can separate both inorganic and organic solute species from the solution being processed (Mohammad et al., 2015). Reverse osmosis is mainly used for removing water from a solution to get a more concentrated product or a pure water stream (van der Bruggen, 2018). Unlike ultrafiltration or microfiltration that target mainly the physical process of removal by size, reverse osmosis focuses the removal by size and shape but also on the ionic charge and the interactions with the membrane (Scott, 1998). The main processing that occurs for reverse osmosis is desalination of water or sea water, production of pure water, concentration of solutions of food products, pharmaceutical solutions, and chemical streams, and wastewater management (Mohammad et al., 2015). In the dairy industry, reverse osmosis can be used as a way of concentrating raw milk on-site at the farm level which is cost-effective in that it diminishes the initial bulk and lowers the cost involved with cooling, heating, further transportation, and overall handling of the milk (Deshwal et al., 2021). Reverse osmosis can be used as well to concentrate whey to recover its valuable components such as protein and lactose (Yorgun et al., 2008). This in turn reduces the waste disposal problem that comes from separating whey from milk. There are 3 types of membranes that are used in reverse osmosis which are spiral wound (cellulose acetate, polymide), hollow fibre (polymide), and thin film composites (Scott, 1998).

Dairy ingredients and ultrafiltered milk beverages

There are many dairy ingredients that can be obtained through the use of the various membrane filtration technologies. These ingredients are in high demand because they are used to obtain the preferred functional and nutritional characteristics in food products, especially in high
protein beverages (Renhe et al., 2019). Ultrafiltration can used to reduce lactose while increasing protein and calcium content of dairy products (Ng et al., 2017). The main types of dairy products obtained by the use of ultrafiltration would be whey protein concentrate/isolate, milk protein concentrate/isolate, and ultrafiltered milk.

Whey protein concentrate (WPC) and whey protein isolate (WPI) can be produced by ultrafiltration to concentrate and purify whey protein from cheese whey (Snyder Filtration, 2020). Whey that comes from cheese manufacture was first thought of as a by-product and considered a waste stream. Modern technologies have allowed for both the utilization of this discarded whey and the decrease of whey disposal with the use of processing or treatment of this whey to be used in other food or feed products (Macwan et al., 2016). Whey protein ingredients are not only used in the food industry but in pharmaceutical manufacture, and in biomedical or personal care products (Smithers, 2008). Now instead of whey being seen as a by-product of cheese production it can be utilized as a co-product to be used in a more sustainable way. The whey is first concentrated by ultrafiltration and then spray dried in order to create whey protein concentrates. Microfiltration can be applied in addition to UF to produce whey protein isolate powders (Chegini and Taheri, 2013). These products can be used as dietary protein supplements and can also offer antimicrobial activity, modulation of the immune system, and help prevent cardiovascular disease and osteoporosis (Mollea et al., 2013).

Milk protein concentrate (MPC) and milk protein isolate (MPI) can be produced from the use of ultrafiltration to concentrate and purify milk proteins from fluid milk (Sikand et al., 2011). Using ultrafiltration to process milk, mass ratios can be adjusted without negatively affecting their physicochemical characteristics (Rosenberg, 1995). The total levels of milk proteins, lactose, and minerals can be adjusted (Kumar et al., 2013; Rosenberg, 1995). These standardized
milk powders or liquids can be used in various fermented products like cheese or yogurt and can reduce the requirement of processing equipment, increase yield, and increase the quality of the final product (Gujar and Dhangare, 2022).

During the manufacture of MPC and MPI powders, there are changes to the composition, heat stability, and rheological characteristics (Sikand et al., 2011). If incorrectly handled, the main problem that arises is the insolubility of the powders. Strategies such as modification of composition, physical treatments, optimization of the manufacturing conditions, and optimization of storage conditions are all utilized to reduce the insolubility of MPC and MPI (Khalesi and FitzGerald, 2021). The protein powder composition can be modified with addition of salts or calcium chelators (Sikand et al., 2011). The temperature of the ultrafiltration (10-50 C) and spray drying process (65-90 C) need to be kept in a certain range in order to avoid protein denaturation (Fang et al., 2012; Khalesi and FitzGerald, 2021). Physical treatments such as high pressure processing or microfluidation, are used prior to spray drying in order to modify the structure of the casein micelle by loosening the non-covalent bonds between submicelles to promote disaggregation of the casein proteins (Cadesky et al., 2017; Augustin et al., 2012).

To optimize storage of MPC and MPI powders, both temperature and relative humidity need to be monitored in order to decrease the insolubility. Less than 40 C and a relative humidity of 44% will increase or optimize powder solubility (Anema et al., 2006; Khalesi and FitzGerald 2021). Combining ultrafiltration with microfiltration along with adjusting pH and temperature conditions can lead to an MPC or MPI with unique functional properties for food or beverages (Hallstrom and Dejmek, 1988). These functional properties include water-holding, solubility, fat-binding, whippability, emulsification characteristics, and heat stability that these proteins can add to food or other applications (Rosenberg, 1995). Also MPC and MPI nutritionally provide a
concentrated source of high quality protein and can fortify foods with the contribution of minerals such as calcium, magnesium, and phosphorous (Agarwal et al., 2015).

Ultrafiltered fluid milk can be found on the market with the same fat percentages as regular milk: skim, 1%, 2%, and whole. As milk goes through the ultrafiltration process, protein is accumulated while sugars are reduced in the final retentate stream. Lactose can be reduced up to 76% or even as high as 84-89% during an ultrafiltration processing run on milk (Vyas and Tong, 2003; Srilaorkul et al., 1989). Protein content also increases as milk is ultrafiltered (Misty and Maubois, 2017). Ultrafiltration can be done in either a batch or a continuous mode of operation. For a batch process, retentate is recirculated back to the feed tank once going through the membrane while permeate is removed (Gabelman, 2017). The volume of the feed of this process gradually decreases as the concentration of the retained components increase in the retentate. The use of concentration factors can be used in order to concentrate the components that are retained in the retentate (serum proteins, casein micelles, fat globules). As milk is processed with ultrafiltration in a batch operation, water, soluble minerals, and lactose permeate through the membrane while the proteins become concentrated in the retentate (Mistry and Maubois, 2004). Diafiltration can be done at the end of the stage in order to flush out more of the permeable components of the retentate through the permeate stream.

In a continuous mode of operation, diafiltration takes place where water is added at the same rate that permeate is removed so the feed volume remains constant (Gabelman, 2017). Using ultrafiltration coupled with constant diafiltration, lactose can be reduced significantly (Srilaorkul et al., 1989). Since it can take a considerable amount of time and energy to produce lactose free milk by ultrafiltration alone, ultrafiltration can be applied in combination with the addition of lactase in order to produce lactose free milk (Werley, 2018). Ultrafiltration can also
be used to increase protein percentage in fluid milk for fortification purposes. Fortification allows for the flavor and mouthfeel to be enhanced without adding extra ingredients (Twiford, 2004). Ultrafiltered milk can also be used to make other dairy products like cheese, creamer, cottage cheese, yogurt, and ice cream (Dekker et al., 2019).

**Relevant Research**

Research has addressed how to process fluid milk in different ways to change flavor profiles and appearance. Membrane separation technology has allowed the dairy industry to be able to control composition of fat, protein, and lactose levels in fluid milk beverages (Berk, 2013). One of these research pathways involves removing lactose from milk. Since acceptance of dairy products is mainly sensory driven, it is essential to understand the sensory characteristics of lactose-free milk compared to normal milk (Claassen and Lawless, 1992; Suri et al., 2019; Adhikari et al., 2010; Rizzo et al. 2020). Much research has been done on the physical properties of ultrafiltered milk with some efforts to address sensory effects. Much of this research is done to understand and improve physical and sensory properties such as color, viscosity, and flavor. Other research has been done to focus on processing parameters such as flux rates, concentration factors, the effects of temperature on processing, efficiency of the processing, and calcium retention.

As discussed earlier, diafiltration can be coupled with filtration during dairy processing in order to more fully wash out smaller solutes through a permeate stream (Hu and Dickson, 2015). Renhe et al. (2019) studied the heat stability of milk protein concentrates to document the impact of changes during membrane filtration obtained by microfiltration, ultrafiltration, and diafiltration. The treatments analyzed were ultrafiltered retentate (UF), microfiltered retentate (MF), microfiltered retentate diafiltered with UF permeate (PF), and microfiltered retentate
diafiltered with water (DF). Diafiltration concentrates had a higher pH due to the ions (inorganic phosphate) that were removed from the serum phase. There were no significant differences in the amount of total calcium in the UF, MF, and PF samples but the DF retentate had lower levels of total calcium. Soluble, diffusible, and total phosphate ions were significantly lower for the DF retentates compared to UF, MF, or PF. Heat coagulation time (HCT), in minutes, was measured via oil bath at 120° C. There was no significant difference between the coagulation times of UF (28 ±1 minutes), MF (33 ±2 minutes), or PF (31 ±5 minutes) milks. The DF concentrate had a higher coagulation time of 49 ± 1 minutes. The higher HCT of the DF retentates can be attributed to the removal of calcium and phosphate from the soluble phase and the higher pH (Crowley et al., 2014; Singh, 2004). This study is important because it shows that alterations in milk filtered by either UF or MF coupled with DF can improve heat stability. Gavazzi-April et al. (2018) varied the amount of diafiltration water (5 x -0.8 diavolume vs 3.5 x -.2 diavolume) to see how it would affect the overall efficiency of ultrafiltration of skim milk. The results from the two diafiltration sequences showed there was a higher amount of total solids, ash, and lactose content in retentates in the 5 x -0.8 diavolume (DV) sequence. A diavolume is defined as the total added volume introduced to the operation during diafiltration divided by the initial retentate volume (Millipore, 2003). The total flux incline increased to 89% for the 5 x -0.8 DV versus 58% for the 3.5 x -2 DV. For each kilogram of lactose removed from the feed for the 3.5 x -.2 DV condition, 85% was removed through the UF step and 7% through the DF step. The 5 x -0.8 DV condition showed that, 93% of the lactose was removed through the UF step and 7% was removed through the DF step. More energy was consumed for the 3.5 x -2 DV during the diafiltration step since it took a larger volume of water and more time. Although the 3.5 x -2 DV sequence had higher permeate flux values, it required a larger volume of water for the DF step and produced more
permeate than the $5 \times 0.8DV$. This information is important to know when considering the amount of water to be used during diafiltration and how that can impact energy costs.

Temperatures and the use of a concentration factor are essential when it comes to filtration processing. Liu et al. (2014) investigated how the casein micelle and retentate serum were altered during ultrafiltration depending on the concentration factor (2, 3, and 4) and temperature ($10^\circ C$ vs $40^\circ C$). The concentration of soluble casein increased and there was a net shift of casein from the serum to the micelles during ultrafiltration at both temperatures but it was higher at $10^\circ C$ retentate as shown in previous studies (Creamer et al., 1977; Liu et al., 2013). Calcium removal was apparent in the micelles of the $10^\circ C$ retentate but not in the $40^\circ C$. Another characteristic that was measured was the hydration of the casein micelle. For $10^\circ C$, the micelle hydration of the starting skim milk to a 4-fold concentration went from $2.8 \text{ g}_{\text{water}} \text{ g}^{-1}_{\text{solids}}$ to approximately $3.2 \text{ g}_{\text{water}} \text{ g}^{-1}_{\text{solids}}$. For $40^\circ C$, the micelle hydration of the starting milk to a 4-fold concentration went from $2.0 \text{ g}_{\text{water}} \text{ g}^{-1}_{\text{solids}}$ to approximately $2.2 \text{ g}_{\text{water}} \text{ g}^{-1}_{\text{solids}}$. The differences of temperature on the hydration of casein micelles is consistent with Snoeren & Damman, 1984. When ultrafiltration was performed at different temperatures, it changed the final calcium content in the retentate. The proportion of the soluble casein was higher at $10^\circ C$ than the $40^\circ C$. This study showed that temperature has an effect on the final calcium content of UF retentates and the hydration of casein micelles. Ng et al. (2018) evaluated ultrafiltration of skim milk at different temperatures ($10^\circ C$, $30^\circ C$, and $50^\circ C$) and the influence of the processing temperature on flux decline. Declining flux can be attributed to two occurrences, retained particles that accumulate at the membrane surface (concentration polarization) and deposition of particles on the membrane surface (fouling), which increase permeation resistance (Gavazzi-April et al., 2018). There were no variations observed in concentration polarization resistance as
a function of temperature and time. Fouling increased with both the filtration duration and temperature. The rate of fouling resistance at 50°C was greater than 30°C and 10°C. A minor presence of mineral fouling occurred. The authors attributed the main cause of the flux decline to protein fouling which was also observed by James et al. (2003) and Ng et al. (2017). Understanding how fouling occurs can help optimize both filtration and cleaning. In order to get a membrane fully clean, it takes a significant amount of water, chemicals, and downtime (Shi et al., 2014). Knowing that it is mainly protein that is fouling on the membrane, cleaning procedures can focus more on alkaline cleaners that remove protein rather than the acid cleaners that remove minerals (Rabiller-Baudry et al., 2008).

Vyas and Tong (2003) evaluated calcium retention during ultrafiltration of skim milk. As the process of ultrafiltration takes place, minerals are lost in the permeate stream and one such mineral that is important to milk is soluble calcium (de La Fuente, 1998). This study used a cross-flow unit with a 10 kDa flat sheet spiral wound membrane for ultrafiltration at 10°C at a concentration factor of 4. The permeate that was collected was evaluated by three different methods of precipitation to test the prospect of recovering calcium from the permeate of skim milk. The three methods consisted of a pH adjustment to 8, heat treatment at 63°C for 30 min, and a pH adjustment to 8 and then a heat treatment at 63°C for 30 minutes. Once put through these three processes, the permeates were re-filtered using a clean 10 kDa membrane to test for calcium retention. The most calcium retention was found from the permeate adjusted to pH of 8 and then put under heat treatment. Approximately 70% of the calcium found in the permeate was recovered. The pH adjusted permeate had approximately 50% calcium retention and the heat treated permeate had approximately 42% calcium retention. The authors concluded that a
combination of a pH adjustment and heat treatment of the permeate is the optimal way to recover calcium that can be either reincorporated in the milk or to other products.

Studies on processing parameters of ultrafiltered milk are important to know in order to get the best results from a processing run. The next section of this review will be on the different studies that focused on the sensory and physical properties of ultrafiltered milk in which fat content and protein content are varied.

Quiñones et al. (1997) investigated the possibility of making the sensory properties of skim and 1% milks more similar to higher fat milks while preserving nutritional characteristics. HTST pasteurized milk was processed by UF at 50° C using a plate-and-frame UF system. The retentate, permeate, and cream were combined to create two milks at fat concentrations of 0.1 and 1.0% and two milks at two protein concentrations (1.0 and 4.8%) at each fat concentration. The 1.0 and 4.8% protein milks were then mixed to make milks at five different protein concentrations within each fat concentration. Sensory properties, total solids, fat, total protein, NPN contents, L value, a value, b value, and relative viscosity were all measured in this study. As protein content increased in the skim and 1% milks, the L value increased (whiter), b value increased (less blue), and the a-value increased (less green). The relative viscosity, appearance scores, and opacity scores of the skim and 1% milk increased as protein increased. As the protein content increased in the skim and 1% milks, the perceived thickness of increased. When the protein concentration was increased by 0.9% the panelists could detect a change in sensory characteristics in the skim and 1% milks. All of the appearance descriptors (center color, edge color, opacity, and visual hang up), three of the five texture descriptors (mouth coating, residual mouth coating, and thickness) and one of the six flavors descriptors (residual flavor) were significantly affected by changes in protein. Increasing the protein concentration by UF in skim
and 1% milk can be used to make their sensory characteristics more similar to those of milks that have higher fat contents.

In a subsequent study, Quiñones et al. (1998) investigated how decreasing the protein content of 2 and 3.3% fat milks impacted sensory properties (Quiñones et al., 1998). Pasteurized milk was ultra-filtered by a batch process at 50° C at a 2x concentration factor using a plate-and-frame UF system. The retentate, permeate, and cream were then used to create two milks at two fat concentrations (2.0 and 3.3%) and two milks at two protein concentrations (0.9 and 4.6). The milks at 0.9 and 4.6% protein were mixed to make 5 different protein concentrations at the two fat concentrations. Sensory properties, total solids, fat, total protein, NPN contents, L, a and b values, and relative viscosity were measured. As protein content decreased in both 2 and 3.3% fat milks, L, a and b values decreased (less white, more green, less blue). The decrease in protein content also had a very large impact on relative viscosity. As protein decreased, the relative viscosity of 2 and 3.3% fat milks decreased. A one-unit decrease in protein content influenced RV more than a one-unit decrease in fat content. Trained panelists were unable to detect the removal of up to 32% of the protein from both the 3.3 and 2% fat milks. This shows that there is a way to decrease the protein amount in 3.3 and 2% fat milks, using UF permeate, without detectable changes in sensory properties of the milks. The findings of this study demonstrate the feasibility of a milk standardization process that takes protein from higher milkfat milks (2.0, 3.3) to use in lower fat milks (1% and skim) to improve their sensory characteristics.

Color of milk can be influenced by many different factors such as temperature, milkfat level, protein percentage, and casein as a percentage of true protein (CN%TP) as found by Cheng et al. (2018). This study found that the modification in CN%TP (5 to 80% of TP) had a greater effect on L-value than fat and CN%TP had a greater effect on color than the variation in total
protein (3.0 to 5.0). The increase of temperature from 4 to 50 C increased L/L* (more white), while decreasing a/a* (more green) and b/b*(less yellow) values as fat level (0.2 to 2) increased. These results of milkfat on color depending on the temperature of skim milk indicated that the reflected light would come from casein micelles, while at the higher fat (2%) content, the majority of the light reflected would be from fat globules with a lesser contribution coming from casein micelles. The decrease in color measurement with temperature would lead to the dissociation of casein monomers and calcium phosphate into the milk serum phase from the casein micelles which leads to the decrease of whiteness of skim milk (Rose, 1968; Downey and Murphy, 1970). The same effect happens in 2% fat milk but the larger milk fat globules are reflecting most of the light so the temperature dependent casein micelle dissociation has a minimal effect. In a subsequent study, Cheng et al. (2019a) found that in unpasteurized milk protein beverages, changes in CN%TP at 3 fat levels (0.2, 1, and 2%) had the largest effect on L values, sensory whiteness, opacity, color intensity, and yellowness. As the CN%TP increased from 5 to 80%, it increased L-values, opacity, and sensory whiteness, while decreasing sensory color intensity and yellowness. As fat concentration increased so did the a and b* values. In a later study, Cheng et al. (2019b) found that L-value, sensory whiteness, and sensory opacity all increased with increasing fat level and CN%TP. The a value increased with increased fat concentration but decreased with increased CN%TP. Greenness decreased by increasing fat and increased as CN%TP increased. It was reported that the impact of increased fat on a values was greater for the pasteurized/homogenized beverages than on the same unpasteurized ones (Cheng et al., 2019a). This impact is due to the homogenization process that produces a larger number of smaller sized increased surface area fat globules that contribute to more light reflection than just the unhomogenized fat and casein micelles in the unpasteurized milk beverages. This can all be
attributed to the riboflavin that doesn’t get to absorb as much light since more of the light is reflected by the milk fat (Misawa et al., 2016; Cheng et al., 2019a). The b* value increased with increased fat concentration but decreased with increased CN%TP. The increased fat concentration will have more compounds that absorb light, like carotenoids, that are dissolved in milk fat which yield yellowness in the beverages (Johnson, 1974).

Sensory flavor and texture properties in milk can be influenced by components in milk such as protein, milkfat, and lactose and heat treatment. Chapman et al. (2001) and Adhikari et al. (2010) demonstrated differences in sweet taste and more heat treatment related flavors in lactose free milks and attributed these flavors to lactose hydrolysis. More recently, Rizzo et al. (2020) conducted a large sensory study on lactose free milks which included descriptive sensory analysis. Of the 9 lactose free milks in the study, 8 were traditional lactose hydrolysis milks while one lactose free milk, LFM 2, was processed by ultrafiltration followed by lactose hydrolysis. Sensory profiles of the traditional lactase-alone milks were distinct from the commercial UF processed lactose free milk. The lactose free milk by UF did not have a sweet taste and was instead characterized by cooked/eggy flavor and lower basic taste intensities compared with traditional fluid milk. Cheng et al. (2019b) varied the amount of fat, total protein, and casein as a percentage of true protein (CN%TP) of milk protein beverages to evaluate sensory flavor and texture properties. The milk protein beverages were evaluated at 3 levels of fat content (0.2, 1.0, and 2.0%), 4 levels of total protein (3.00, 3.67, and 4.34%), and 5 CN%TP levels (5, 25, 50, 75, and 80). As the CN%TP increased in the beverages, aroma intensity, sweet aromatic, cooked/sulfur flavors, and cooked/milky flavors decreased. As true protein concentration increased in the beverages, astringency increased at all fat levels regardless of type of milk protein. Cooked/sulfur flavor decreased as CN%TP increased because serum protein
content decreased. Serum proteins contain sulfur containing amino acids, such as cysteine in beta lactoglobulin, that contribute to cooked/sulfur flavor in milk (Zweig and Block, 1952, Jo et al., 2019). The increase in serum protein content also increased cardboard flavor which is normally noted in dried whey protein products (Drake et al., 2003; Wright et al., 2009). Vogel et al. (2019) also studied milk protein beverages but at higher protein percentages (6.3 and 10.5%) and varying degrees of dairy protein ingredient compositions (100% MCC, 100% MPC, 18:82 SPI:MCC, 50:50 SPI:MCC, or 50:50 SPI:MCC). The beverages at the lower protein percentage and those with lower quantities of serum protein as a percentage of true protein scored higher (P < 0.05) in sweet aromatic/vanillin than those with higher protein percentage or higher quantities of serum protein as a percentage of true protein. Sulfur/eggy flavor was not affected by protein amount but increased with a higher amount of serum protein as a percentage of true protein. Astringency also increased as the beverages increased in protein percentage and serum protein as a percentage of true protein. Beverages with higher protein and beverages with higher amounts of serum protein had a larger increase in sensory viscosity than the lower protein beverages or the beverages with lower amounts of serum protein as a percentage of true protein. This increase in viscosity with higher protein was also observed in whey protein/pectin dispersions by Wagoner and Foegeding (2017). Descriptive analysis results from a trained panel conducted by McCarthy et al. (2017) showed that as milkfat content increased in milk, milkfat/lactone flavor, viscosity, and yellow color increased. Sweet aromatic flavor and sweet taste increased with higher fat content but cooked flavor was not different between the milks and aroma intensity was only lower in the skim milk (P < 0.05). The opacity of the milk increased and astringency decreased with increased fat content (P < 0.05).
Viscosity of milk, just like with other sensory attributes, can be affected by changes in milk composition and temperature. Sensory viscosity of skim milk is lower ($P \leq 0.05$) than reduced fat and whole milks (Adhikari et al., 2010; Chapman et al., 2001). Cheng et al. (2019a) found that instrumental viscosity decreased as temperature was decreased from 50° C to 4° C. Sensory viscosity was not measured. The impact of temperature masked the viscosity changes by the composition differences within the formulas (fat, total protein, and casein as a percentage of protein). At 4° C, the CN%TP increased viscosity more compared to an increase in protein. Misawa et al. (2016) also found that an increase in CN%TP and CN concentration increased relative viscosity in milk protein beverages. Sensory texture results showed that an increase in CN%TP increased mouthcoating and throat cling in 2% fat milks. For 1% milks the throat cling score containing 3% total protein was significantly lower than those at 3.67, 4.43, and 5% total protein.

**Conclusions**

As the protein beverage category continues to grow, consumers are seeking variety in order to satisfy both their health and indulgence. Ultrafiltered milk has the potential to redirect the decline of fluid beverage milk sales by expanding the utility of fluid milk to a variety of protein beverages. Research into how manipulation of milk lactose, fat, and protein impact sensory and physical properties is important to establish baseline properties. There were two objectives for this study. First, to determine the impact of lactose (0, 30, 70, 97%) and soluble mineral/NPN removal by ultrafiltration on the sensory properties of unflavored skim, 1%, 2%, and whole milk. Second, our objective was to determine the impact of the protein concentration (3.4, 6.5, and 10.5%) on sensory properties of lactose and soluble mineral/NPN (0, 30, 70, 97%) reduced skim milk.
References

Adams, C., I. Torres, M.L. Meilhac, M. Ramirez, and R. Uchoa De Paula. 2019. A winning growth formula for dairy Modest growth forecasts, shifting consumer tastes, and increased competition at home will force executives to look to a new model for growth domestically, and beyond their borders, for more attractive opportunities. McKinsey & Company


CHAPTER 2: Milk Beverage Base with Lactose Removed with Ultrafiltration: Impact of 
Fat and Protein Concentration on Sensory and Physical Properties.

Alex H. Hernandez\textsuperscript{1}, Tina Truong\textsuperscript{1}, David M. Barbano\textsuperscript{2}, and MaryAnne Drake\textsuperscript{1}

\textsuperscript{1}Department of Food, Bioprocessing and Nutrition Sciences, Southeast Dairy Foods Research Center, North Carolina State University, Raleigh NC 27695 
\textsuperscript{2}Cornell University, Department of Food Science, Northeast Dairy Foods Research Center, Ithaca, NY

\textsuperscript{1}Corresponding Author: 
MaryAnne Drake 
Box 7624, Department of Food Science 
North Carolina State University 
Raleigh, NC 27695-7624 
Phone: 919-513-4598 
Fax: 919-513-0014 
E-mail: maryanne_drake@ncsu.edu
Abstract

Our objectives were to determine the impact of fat (skim to whole milk) and protein (3.4 to 10.5%) concentration on the sensory and physical properties of milk beverage base that had lactose and other low molecular components removed by ultrafiltration. In experiment 1, a matrix of 16 treatments was produced to achieve 4 levels of lactose removal (0, 30, 70, and 97%) at each of 4 fat levels (skim, 1%, 2% and whole milk). In experiment 2, a matrix of 12 treatments was produced to achieve 4 levels of lactose removal (0, 30, 70, and 97%) at each of 3 protein concentrations (3.4, 6.5, and 10.5% protein). Physical and sensory properties of these products were determined. Removal of > 95% of milk lactose by UF required a diafiltration volume of approximately 3 times the milk volume and increased whiteness of skim and whole milks while decreasing viscosity and making the flavor of milk more bland. In addition, lactose (and other low molecular weight solute removal) by UF decreased titratable acidity by more than 50% and increased milk pH at 20°C to >7. Future work on milk and milk-based beverages with lactose removed by UF needs to focus on interaction of the remaining milk solids with added flavorings, changing casein to whey protein ratio prior to UF removal of lactose, and the impact of lactose and low molecular weight solute removal on heat stability, particularly for neutral-pH, shelf-stable milk-based beverages.
Introduction

Milk and dairy products are among the most versatile foods on the market, ranging from indulgent to nutritious depending on the many ways they can be processed. A major issue that limits the consumption of dairy products is lactose intolerance. Lactose intolerance occurs when a person is deficient in β-galactosidase in the small intestine which can lead to negative symptoms such as nausea, bloating, and pain (Storhaug et al., 2017). The primary treatment of those impacted by lactose intolerance is a lactose-free diet (Vardhanabhuti and Wang, 2022). Milk is nutrient rich, high in complete protein and essential amino acids compared to non-dairy plant or nut alternatives (Paul et al., 2020). The nutrient density of milk plays an important role in healthy human nutrition and development throughout life, especially in childhood (Wijesinha-Bettoni and Burlingame, 2013). It is recommended that the majority of Americans consume three servings of dairy per day by The Dietary Guidelines for Americans 2020-25 (US Department of Agriculture and U.S. Department of Health and Human Services, 2020). Instead of avoiding dairy completely, lactose-free dairy is a viable and established alternative. The global lactose free market is growing with an estimated value at $12.1 billion in 2020 and a projected value of $18.4 billion by 2025 (Markets and Markets, 2020). In 2020, the lactose-free milk market in the United States was estimated at $1.7 billion, with 201 million gallons sold (Gerdes, 2021).

During the past 2 decades, consumers have focused more on health and industry has responded with healthier food selections (Küster and Vila, 2017). Parents desire reduced-sugar products for their children and the importance of development of healthy eating habits at a young age is emphasized (Bryd-Bredbenner et al., 2016). A 240 ml serving of fluid milk contains approximately 12 g of lactose. The lactose is an issue for lactose intolerant individuals but also provides carbohydrate calories.
Two approaches used in industry to reduce the sugar lactose to improve the healthiness of dairy products are lactose hydrolysis and ultrafiltration. Lactose hydrolysis involves addition of the enzyme β-galactosidase (lactase) to milk which breaks down lactose into glucose and galactose (Harju et al., 2012). The hydrolysis of lactose into glucose and galactose also makes the product sweeter (Vardhanabhuti and Wang, 2022). The addition of β-galactosidase treatment increases costs of fluid milk by $0.06–$0.08/L (Rehman 2009). Ultrafiltration (UF) utilizes pressure over a membrane in order to separate compounds by molecular weight (Scott, 1998). For milk, UF concentrates protein and removes lactose, soluble minerals and water (Mistry and Maubois, 2017). Ultrafiltration is commonly coupled with diafiltration to remove more lactose in the milk which also increases protein content (Renhe et al., 2019). In industry, a 3X concentration factor is most commonly used during ultrafiltration of milk in which 66-67% of lactose is removed from the milk (Rehman, 2009). The starting lactose content of 4.8 g/100 g cows milk decreases to 1.6 g/100 g during the 3X UF. As diafiltration is completed, the lactose of the milk is then only 1.6%.

Previous work has addressed the use of UF to increase the concentration of protein in milk, but data is limited on how protein, lactose/minerals, and fat influence the sensory and physical properties of milk across a wide protein or lactose concentration. Quinones et al. (1997, 1998) evaluated the sensory properties of skim milk, 1%, and 2% fat milk across a protein range of 1 to 4.8%. Protein concentrations greater than 4.8% were not evaluated nor was lactose removal. More recently, Cheng et al. (2019b) varied the amount of fat, total protein, and casein as a percentage of true protein (CN%TP) of milk protein beverages to evaluate sensory flavor and texture properties. The milk protein beverages were evaluated at 3 fat levels (0.2, 1.0, and 2.0%), 4 levels of total protein (3.00, 3.67, and 4.34%), and 5 CN%TP levels (5, 25, 50, 75, and 80). Once again, the role of lactose/minerals and protein concentrations > 4.5% were not evaluated. The
protein trend continues globally and the role of UF to adjust lactose/minerals and protein in conjunction with varying fat content to create milk/milk beverages with a range of sensory and physical properties is important to create a platform for milk beverage innovation. The objective of this study was to progressively remove lactose and other low molecular weight soluble compounds from milk (ca. 0, 30, 70 and 98% removal) using UF in combination with diafiltration to determine the impact of 4 fat levels (skim, 1%, 2% and 3.5% fat) and 3 protein levels (3.3, 6.5 and 10.5% true protein) on chemical, physical, and flavor of milk-based beverages.

**Materials and Methods**

**Experimental Overview**

The experiment consisted of two distinct experiments: the role of fat and lactose removal and the role of protein and lactose removal. These experiments were conducted and replicated on separate weeks from each other and were analyzed as separate objectives.

**Experiment 1**

In experiment 1, pasteurized skim milk was ultrafiltered and as permeate was removed deionized water was added in an equal amount by weight to maintain constant protein concentration in the retentate until lactose and low molecular weight soluble milk components were removed. Second, pasteurized whole milk was ultrafiltered as was done for skim milk until lactose and low molecular weight soluble milk components were removed. This process produced 4 batches of product, skim milk and whole milk with full lactose, skim milk with lactose removed, and whole milk with lactose removed. About 97% of the lactose was removed. From these 4 ingredients, a matrix of 16 treatments (Figure 1) was formulated to achieve 4 levels of lactose removal (0, 30, 70, and 97%) at each of 4 fat levels (skim, 1%, 2%, and whole milk). Physical and
sensory properties of these products were determined. This process was replicated twice starting with a different batch of skim and whole milk.

**Experiment 2**

In experiment 2, skim milk was ultrafiltered and as permeate was removed deionized water was added in an equal amount by weight to maintain constant protein concentration in the UF retentate until lactose and low molecular weight soluble milk components were removed. Half of the lactose removed skim milk (UF retentate) was collected and the other half of the UF retentate continued with UF to concentrate the protein to 10.5%. Second, another portion of skim milk was UF without diafiltration to produce a 10.5% protein concentration without lactose removal with diafiltration. This process produced 4 batches of product, skim milk at 3.4% and 10.5% protein with full lactose and skim milk at 3.4% and 10.5% protein with lactose removed. About 97% of the lactose was removed. From these 4 ingredients, a matrix of 12 treatments (Figure 2) was formulated to achieve 4 levels of lactose removal (0, 30, 70, and 97%) at each of 3 protein concentrations (3.4, 6.5, and 10.5% protein). Physical and sensory properties of these products were determined. This process was replicated twice starting with different batches of skim.

**Ultrafiltration for Lactose Removal**

*Ultrafiltration for Experiment 1*

The overall ingredient production by UF is provided in Figure 1. Four batches (Batches 1, 4, 13, and 16) were the prime ingredients produced. The inset table in Figure 1, provides the full formulation matrix of 16 batches made from these 4 base ingredients. Batches 1 and 4 were the original full lactose skim and whole milk before UF and batches 13 and 16 were the skim and whole milk with virtually all the lactose removed by filtration.
The UF unit was assembled and cleaned the day before milk processing. Briefly, the UF unit was rinsed twice with DI water at 50°C for 5 min each, followed by a recirculation wash with an alkaline cleaner (76 kg of 50°C DI water, 302 mL Ultrasil 110 and 480 ml of Ultracil 01, Ecolab) at 50°C for 30 min. The system was then flushed twice with DI water at 50°C for 5 min each, followed by an acid recirculation wash at 50°C (76 kg of 50°C DI water, 226 mL Ultrasil 76, Ecolab) for 30 min. The system was rinsed twice with 50°C DI water for 10 min. Next, clean membrane water flux was measured at 50°C at 124 kPa with DI water in full recirculation mode for 3 min by collecting water for from the permeate line for 1 min and recording the weight collected. After a clean membrane water flux was measured, the UF was sanitized by recirculation (76 kg of 50°C DI water, 240 mL of XY-12 Liquid Sanitizer, Ecolab) at 21°C for 10 min, followed by two 5 min DI water rinses and then recirculation for 10 min at 21°C with membrane storage solution (76 kg of 50°C DI water, 200 mL Ultracil MP, Ecolab). At the beginning of the processing the next day, 50°C DI water was used to flush the membrane storage solution out of the UF system prior to milk processing. Start of day clean water flux at 50°C was about 140 kg/m²/h.

To produce lactose-free skim milk and lactose-free whole milk, pasteurized skim milk (195 kg) and pasteurized (HTST 78°C for 28s) and homogenized whole milk (120 kg) with no added vitamins were received from the North Carolina State dairy on the morning of the processing run. Pasteurized skim (95 kg) was weighed and poured into a jacketed stainless-steel feed tank (Meyer-Blank Company, St Louis, MO) with a recirculating 50°C water jacket to heat the milk to 50°C. The remaining pasteurized skim milk (100 kg) for batch 1 was stored at 4°C to be used for processing later on and batching the following day. On day 1, the ultrafiltration (UF) processing of skim milk was carried out by using a stainless-steel sanitary design plate Pellicon® 2, 10K plate ultrafiltration apparatus (Millipore Sigma, Burlington, Massachusetts). The plate UF system uses
Biomax 10k polyethersulfone plates with a 10,000 Dalton cut-off with a surface area of 0.5 m² per plate. For optimal pressure, the ultrafiltration unit was assembled with 5 plates in the stack. The feed tank was connected to the membrane stack by a feed pump (Baldor Industrial Motor, single phase, 1.5 H.P., 3450 RPM, 60hz, Baldor Electric Co., Ft. Smith, AR). The plate stack was operated with an inlet pressure of 124 kPa and an outlet pressure of 0 kPa during processing with a permeate removal rate that achieved a 2X concentration factor in the recirculation loop and a flux of about 45 kg/m²/h throughout the processing run for both skim and whole milks. The known weight of the starting milk and protein content were used to create a permeate weight removal goal to achieve the 0.1% lactose (w/w) in both the pasteurized skim and whole milk. The pH was taken at the beginning and end of processing at 50°C. The UF processing was operated in a constant diafiltration configuration in which the retentate was recirculated back into the UF feed tank and as a bucket of permeate (~18 kg) was removed, roughly the same amount of deionized (DI) water was added to the UF feed tank to balance the weights of permeate removed to maintain protein concentration constant in the UF feed tank. This ensured that the correct amount of lactose removal was achieved without altering the starting milkfat and protein percentages. The lactose percentage was monitored throughout the processing with the use of a mid-infrared (MIR) spectrophotometer (Lactoscope FTA, Delta Instruments, Drachten, Netherlands) and verified in the final product with a reference method analysis for lactose. Permeates and retentates were analyzed by MIR along with a flux measurement after each DI water addition (about every 10 minutes).

After processing skim milk the UF plate system was switched directly to feed tank that contained pasteurized (HTST 78°C for 28s) homogenized whole milk (90 kg) that was weighed into a jacketed stainless-steel tank recirculating with 50°C water near the end of processing of the skim. Once the lactose percentage reached 0.1% in the skim, the flow into the UF was switched
over to the whole milk in the tank without stopping or cleaning the UF. The feed tank containing
the lactose free skim milk was then disconnected from the UF and the UF skim retentate was
collected. The pasteurized whole milk underwent the same UF processing as the skim, as described
above. Once the whole milk lactose percentage reached 0.1%, the UF whole retentate was
collected. The UF plate unit was flushed (no recirculation) with both retentate and permeate lines
open with 38 kg of 50°C DI water to remove residual milk from the UF system. The UF feed tank
was refilled with 76 kg of 50°C DI water, the water was run in full recycle for 3 min and then
permeate was collected for 1 min and weighed. The typical fouled membrane water flux was about
60 kg/m²/h. Following the fouled water flux measurement, the long clean cycle was done
described above, except for the final soak solution. After the sanitation cycle and water rinse, the
UF plate was disassembled and the membrane plates were stored in a soak solution (Ultrasil MP
Ecolab) at 4°C until the next processing run. All other stainless steel and plastic parts were
dissembled and hand cleaned.

*Beverage Formulation: Experiment 1*

The formulations of the milk beverages were calculated based on four base products: 1) the
original skim milk (full lactose), 2) the original whole milk (full lactose), 3) the skim milk with
“complete” (0.1%) lactose removal, and 4) the whole milk with “complete” (0.1%) lactose
removal. The milks were formulated in 4000 gram batches with varying percentages of lactose
reduction (0%, 30%, 70%, 100%) and milkfat [Skim (0.1%, 1%, 2%, Whole (3.45%))] to make
sixteen formulated milk beverages in total, as shown in the Figure 1.

*Ultrafiltration for Experiment 2*

The overall ingredient production by UF is provided in Figure 2. Four batches (Batches 1, 3, 10, and 12) were the prime ingredients produced. The inset table in Figure 2, provides the full
formulation matrix of 12 batches made from these 4 base ingredients. Batches 1 and 3 were the original full lactose 3.4% protein skim milk before UF and the full lactose skim milk concentrated to 10.5% protein. Batch 10 was the 3.4% protein skim milk with virtually all the lactose removed and batch 12 was the 10.5% protein skim milk with virtually all the lactose removed by filtration.

The UF unit was assembled and cleaned the day before milk processing as previously described for Experiment 1. At the beginning of the processing the next day, 50°C DI water was used to flush the membrane storage solution out of the UF system prior to milk processing. Start of day clean water flux at 50°C was about 140 kg/m²/h.

Pasteurized (HTST 78°C for 28 s) skim milk (430 kg) with no added vitamins was received from the North Carolina State dairy and was split into two batches (200 kg and 230 kg) on the morning of the processing run. A portion of the pasteurized skim milk was saved as Batch 1 at full lactose with no increase in protein concentration (Figure 2). Pasteurized skim (200 kg) was weighed and poured into a jacketed stainless-steel tank with a recirculating 50°C water jacket to heat the milk to 50°C and that milk was used to produce (Figure 2) 3.4% protein skim milk with virtually all the lactose removed by ultrafiltration/diafiltration (Batch 10) and skim milk at 10.5% protein with virtually all the lactose removed (Batch 12) was produced from (Batch 10) by UF with no diafiltration. The protein concentration in the UF retentate was monitored using MIR and the UF process was stopped when the protein concentration reached 10.5%. When production of batches 10 and 12 were complete, another portion of 50°C pasteurized skim milk was UF without diafiltration to concentrate the protein from 3.4% to 10.5% (Batch 3).

**Beverage Formulation: Experiment 2**

The formulations of the milk beverages were calculated based on four base products: 1) the original skim milk (full lactose) 3.4% protein, 2) the original skim milk (full lactose)
concentrated by UF to 10.5% protein, 3) the skim with “complete” (0.1%) lactose removal at 3.4% protein, and 4) the skim with “complete” (0.1%) lactose removal concentrated to 10.5% protein. The milks were formulated into 4000 g batches with varying percentages of lactose reduction (0%, 30%, 70%, 100%) and protein (3.4%, 6.5%, 10.5%) to make twelve formulated milks in total, as shown in figure 2.

**Analysis Methods**

**Lactose Determination**

Lactose concentration in milks and UF retentate was determined using a spectrophotometric method (AOAC, 2019; method 2006.06) as described in detail by Lynch et al. (2006).

**Freezing Point, Titratable Acidity and pH**

Milk and beverage freezing points were measured using an Advanced Instruments milk cryoscope (Model 4250, Norwood, MA, USA). Titratable acidity was measured by titration of 9 mL of test portion of milk beverage base with 0.1N NaOH (Fisher Chemical SS276-1, Fairlawn, NJ, USA) using a NAFIS titration apparatus with 3 drops of 1% phenolphthalein in ethanol added as an indicator. The percent acidity of the test portion was expressed as percent lactic acid. The pH of each formulation was determined at 20°C using a pH meter (Fisher Scientific, Accument, Model 915) and gel filled electrode (Mettler-Toledo HA-405 DXK-S8/120, Columbus, OH). The pH meter was calibrated using a pH 7 and 4 buffers (Fisher Scientific).

**Microbial Analysis**

The microbial quality of the retentate through the processing and final UF retentates were determined by aerobic plate counts (APC) (Laird et al., 2004; 6.040) and coliform counts (Davidson et al., 2004; 7.071) (Petrifilm Aerobic Count Plate, 3M ID 7100039310 and Petrifilm Coliform Count Plate, 3M ID 7100039392, 3M Food Safety, Maplewood, Minnesota)
Apparent Viscosity

Apparent viscosity (AV) was measured on all batches using a rotational Brookfield viscometer (LV-DV2T, Brookfield Engineering Laboratories Inc., Middleboro, Massachusetts) with the jacketed cup-and-bob attachment (Enhanced UL Adapter, Brookfield Engineering Laboratories Inc.) in accordance to the procedure identified by Adams and Barbano (2016) with a few modifications. The various beverage formulations for both experiments were measured at a constant temperature of 4°C. In order to get the best results from the viscometer, the RPM that was chosen needed to fall within a torque range of 10-100%. For experiment 1, all beverage formulations, 16 in total, were measured at 60 RPM. For experiment 2, beverage formulations 1, 2, 4, 5, 7, 8, 10, and 11 were measured at 60 RPM. Beverage formulations 3, 6, 9, and 12 were measured at 8 RPM due to their higher viscosity.

Color

Color of the milk beverages was measured within the week of processing using an Ultra Scan Pro Spectrophotometer (Hunter Associates Laboratory, Inc., Reston, VA) at 4°C. To maintain the samples at 4°C a water bath (PloyScience, SD7LR, Warrington, PA) was used. The color data that was collected was the Hunter L, a, and CIE b*-values for each of the milk beverages. As described by Cheng et al (2018), the beverages were measured in reflectance mode using wavelengths between 360-750 nm with a 5 nm resolution using Illuminant A at 10 degree viewer angle.

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 (NCSU IRB) regulations. Thirty mL of each beverage was poured into 59-mL souffle cups, capped (Dart
Container Corp.), and labeled with a randomized 3-digit blinding code. Samples were prepared with overhead lights off to prevent light oxidation. Beverages were evaluated at 4°C.

Milk beverages were evaluated by seven trained and experienced panelists (3 males, 4 females, ages 22 to 48 y). Each panelist had a minimum of 80 h of prior descriptive analysis experience documenting flavors of milks and dairy protein beverages using the Spectrum™ method with a 0 to 15 point intensity scale (Meilgaard et al., 2007). Panelists evaluated each treatment in duplicate in a randomized balanced order of presentation. No more than six samples were evaluated in a session and a minimum 2 min rest was enforced between samples. Panelists expectorated samples and rinsed their palates with bottled spring water. The milk protein beverages were evaluated the week of processing for appearance/whiteness, opacity, sweet aromatic, papery, cooked/milky, sweet taste, astringent mouthfeel, and viscosity. These attributes were previously established for fluid milk (McCarthy et al., 2017) with the exception of the attribute papery. This flavor attribute was defined as the aromatics associated with wet white paper and was considered distinct from cardboard flavor which is also a defined trained panel sensory attribute for dairy products and dairy ingredients (Smith et al., 2016). Compusense Cloud (Compusense, Guelph, Canada) was used for data collection.

Statistical Analysis

Experiments 1 and 2 were analyzed separately. Two-way analysis of variance (ANOVA) with means separation (Fisher’s LSD) was performed on sensory and instrumental data from each experiment (experiment 1: lactose x fat; experiment 2: lactose x protein). Analyses were performed with XLSTAT (version 2019.3.1, Addinsoft, Boston, USA) at 95% confidence (P <0.05).
Results and Discussion

Experiment 1

Processing data

Samples were taken at the start and end of the UF run of the pasteurized skim and whole milks in order to measure the microbial quality by aerobic plate counts (APC) and coliform counts. No coliforms were detected by direct plating of 1 mL of sample. The average CFU/mL for APC started at 20 +/- 10 at the start of the skim milk UF run and ended at 37.5 +/- 12 CFU/mL. For the whole milk, the average whole milk CFU/mL was 27.5 +/- 10 CFU/mL at the beginning of the run and 40 +/- 8 at the end.

The mean clean water flux at the beginning of the run and after being cleaned at the end of the run were 144.2 +/- 20 Kg/m²·h before the start of the UF processing run and 142.05 +/- 21 Kg/m²·h after the final cleaning. The mean fouled water flux at the end of the milk processing run was 58.45 +/- 12 Kg/m²·h.

The mean anhydrous lactose for the skim milk was 4.75 +/- 0.04% and the ending lactose was 0.18 +/- 0.01% for a 96.2% lactose removal by UF of skim milk. The mean anhydrous lactose for whole milk was 4.44 +/- 0.12% and the ending lactose was 0.18 +/- .02% for a 96% lactose removal during the UF of whole milk. The amount of DF to achieve 96% lactose removal was approximately 3 times the starting volume of milk for both skim and whole milk (Figure 3).

Beverage lactose, freezing point, pH and TA.

Lactose content of the 4 main batches of beverage was measured by the enzymatic lactose spectrophotometric method. Batches 1, 4, 13, and 16 (Figure 1) contained 4.79, 4.54, 0.14, and 0.13% anhydrous lactose, respectively, and had freezing points of -0.529, -0.511, -
0.018, and -0.017°C, respectively. When lactose and low molecular weight soluble minerals were removed, the freezing point of those batches (i.e., 13 and 16) were close to that of pure water. It is interesting to note that when samples from batches 13 and 16 were held refrigerated for one week, the freezing point did not decrease with time of cold storage indicating that mineral bound to protein was not becoming soluble in the aqueous phase around the casein micelles with time of storage at 4°C. Lactose and soluble mineral removal influenced both beverage pH and TA (Figures 4a and 5a). Beverage pH at 20°C increased progressively ($P < 0.05$) from about 6.6 to 7.4 as lactose and soluble mineral were removed (Figure 4a), while no effect ($P > 0.05$) of change in fat content on pH from skim to whole milk was detected. In contrast to pH, beverage TA decreased ($P < 0.05$) with increasing removal of lactose and soluble mineral (Figure 5a), while no effect of variation in fat content was detected ($P > 0.05$). The magnitude of change in pH at 20°C and TA were larger than expected. Change in lactose concentration should have little impact on beverage pH and TA, however soluble mineral removal was the likely reason for the large decrease (from 0.18 to 0.04% expressed as lactic acid) in milk TA. These changes in beverage pH and TA with lactose and soluble mineral removal may impact the heat stability of milk protein beverages. High quality raw milk has a TA value between 0.14 to 0.17% (expressed as lactic acid) (Schmidt, 1996). The TA of raw milk increases with age (i.e., developed acidity due to microbial growth, Santoso, 2018). Proteins, minerals, and dissolved gasses all attribute base acid content of milk (Schmidt, 1996) with TA increasing with protein content. The factors that affect the variation of soluble mineral in cows are the breed, stage of lactation, infection of the udder, and the feed (Fox et al., 2008; Zwierzchowski and Ametaj, 2019). Data is limited for pH and TA of UF retentates.
Milk pH changes reversibly as a function of temperature due to temperature dependent migration of milk mineral in and out of casein micelles. Ma and Barbano (2003a) reported that milk with a normal casein and serum protein content at about 0°C had a pH of about 6.97, and separately Ma and Barbano (2003b) reported that milk pH decreased linearly from 40°C to 80°C, from 6.57 to 6.25, respectively.

*Beverage AV and color*

The AV of the beverages increased ($P < 0.05$) with increasing fat content and decreased ($P < 0.05$) with increasing removal of lactose and soluble minerals (Figure 6a). Phillips et al. (1995a) and Quiñones et al. (1997, 1998) reported that relative viscosity of milk increased with increasing fat content and Cheng et al. (2019a) reported AV of milk-based beverages increased with increasing fat content at 4°C. Data is limited on the influence of lactose removal on AV of UF retentates.

L-value (whiteness) increased as expected with increasing fat content (Figure 7a) but also increased with increasing lactose and soluble mineral removal. The increase in L-value was largest from skim to 1% fat. The a-value and b*-values both increased ($P < 0.05$) with increasing fat content and the largest absolute increase was between skim to 1% fat milk (Figures 7c and d). Similarly, Phillips et al. (1995a) reported that as milk fat content increased from 0.6 to 2%, the L, a, and b* values increased, which indicated a whiter, less blue, and less green beverage. We found both an effect of lactose removal ($P < 0.05$) and the interaction of fat x lactose removal ($P < 0.05$) on both a-value and b*-values. The effects of light scattering and removal of light absorbing low molecular weight chromophores on changes in L, a, and b* values can be more clearly seen from the reflectance curves (Figures 8a and b). As lactose and other low molecular weight compounds (e.g., riboflavin) that absorb light are removed, the amount of reflected light
from 360 to 510 nm increases causing an increase in whiteness and a decrease in greenness and blueness. As fat content increased, light scattering by fat globule increases (increased whiteness) and that increased the reflect light from 510 to 750 nm. A comparison of reflectance curves of skim and 2% fat milk at 4, 20, and 50°C was reported by Cheng et al. (2018) and they found that in the range of wavelengths from 360 to 510 nm, the reflectance of light was increased uniformly at all wavelengths, while in the range from 510 to 750 nm, the amount of reflected light increased with increasing wavelength. Increased total light reflected (or in the case of lactose removal, less light absorbed) makes the percentage of light reflected from 360 to 750 nm more uniform, which will increase perceived whiteness (L-value). The total amount of light reflected was affected more by milk temperature for skim than 2% milk with more light be reflected as milk temperature increased.

*Beverage sensory*

A summary of the descriptive analysis sensory results for experiment 1 is presented as a PCA biplot in Figure 9. Interactions between lactose and fat were evident for all sensory attributes (p<0.05) so a PCA biplot is used to visualize these results. As lactose and soluble mineral were removed, beverage whiteness increased. Increased sensory whiteness was consistent with the increase in L-value as lactose was removed (Figure 7a). Previous studies have determined that milk or milk beverage whiteness increased with increased protein concentration (Quinones et al., 1997, 1998; Cheng et al., 2018). Sensory yellowness decreased with increased lactose removal concurrent with b*-values (Figure 7d). Sensory viscosity and opacity increased with increasing fat concentration. Phillips et al. (1995) and McCarthy et al. (2017) reported that as milkfat content increased in milk, sensory viscosity and yellow color
increased. Cheng et al. (2019b) also found that as fat level increased from 0.2 to 2% in milk protein beverages sensory whiteness and sensory opacity increased.

Sensory viscosity decreased as lactose and low molecular weight solutes were removed. As lactose was removed, the milk became very bland/neutral in flavor, consistent with a decrease in cooked/milky, sweet aromatic, sweet taste, and salty taste decrease (Figure 9). Previous work to our knowledge, has not addressed aromatic flavor properties of milks with lactose removed by UF. Rizzo et al. (2020) utilized a trained panel to document sensory properties of commercial lactose free milks as part of a larger study on consumer acceptance. One of the commercial lactose free milks that they evaluated had the lactose removed by a combination of UF and lactose hydrolysis. This UF milk was lower in sweet aromatic flavor and sweet and salty tastes compared to the other milks. Milk protein concentrates and isolates are manufactured from skim milk by UF. When milk protein powders are rehydrated at 10% solids (w/v) or liquid retentates are diluted to 10% solids (w/v), these rehydrated proteins are characterized by lower intensities of traditional milk flavors and undetectable intensities of sweet and salty tastes (Smith et al., 2016, Carter et al., 2018). Milk fat flavor did not change with lactose removal and increased as expected with increasing fat concentration.

Astringency increased with decreased milkfat and increased lactose removal. Previous work has established that increased heat treatment or increased protein concentration as well as decreased milkfat increase astringency (Vogel et al., 2021; Lee et al., 2017). Skim milk had higher astringency intensity than whole milk (Lee et al., 2017). Liquid and rehydrated whey and milk protein powders at 10% (w/v) solids, which also have little to no lactose or fat, are also astringent (Carter et al., 2018; Smith et al., 2016) and the astringency intensities are higher than fluid skim milk.
**Experiment 2**

**Processing data**

Samples were taken of the starting pasteurized skim of the two tanks (pasteurized skim 1 and pasteurized skim 2), low lactose skim at 3.3% protein, low lactose skim at 10.5% protein, and full lactose skim at 10.5% protein to measure the microbial quality of the products of the UF process. No coliforms were detected by direct plating of 1 mL of sample.

The average for APC for the pasteurized skim 1 started at 25 +/- 10 CFU/mL, pasteurized skim 2 started at 15 +/- 7 CFU/mL, and the low lactose skim at 3.3% ended at 25 +/- 7 CFU/mL. The low lactose skim at 10.5% protein ended at 100 +/- 12 CFU/mL and the full lactose skim at 10.5% protein ended at 775 +/- 15 CFU/mL.

The mean clean water flux at the beginning of the run and after being cleaned at the end of the run were 121 +/- 5 kg/m²h before the start of the UF processing run and 128 +/- 12 Kg/m²h after the final cleaning. The mean fouled water flux at the end of the milk processing run was 62 +/- 4 Kg/m²h.

The mean anhydrous lactose for the skim milk was 4.71 +/- .06% and the ending lactose was 0.24 +/- .02% for a 95% lactose removal by UF of skim milk. The amount of DF to achieve 95% lactose removal was approximately 3 times the starting volume of milk (Figure 3).

**Beverage lactose, freezing point, pH and TA**

Lactose content of the 4 main batches of beverage was measured by the enzymatic lactose spectrophotometric method. Batches 1, 3, 10, and 12 (Figure 2) contained 4.75, 4.27, 0.19, and 0.18% anhydrous lactose, respectively, and had freezing points of -0.530, -0.550, -0.022, and -0.034°C, respectively. When lactose and low molecular weight soluble minerals were removed, the freezing point of those batches (i.e., 10 and 12) were close to that of pure
water. Lactose and soluble mineral removal influenced both beverage pH and TA (Figures 4b and 5b). Beverage pH at 20°C increased progressively ($P <0.05$) from about 6.6 to 7.2 as lactose and soluble mineral were removed (Figure 4b), while there was small effect ($P >0.05$) of change in protein content and the interaction of protein x lactose removal on pH. In contrast to pH, beverage TA ($P <0.05$) increased with increasing protein concentration and decreased ($P <0.05$) with increasing removal of lactose and soluble mineral (Figure 5b). The increase in TA with increasing protein content coincides with previous research where 2.58% protein content raw milk had 0.15 TA and a 3.86% protein content raw milk had .18 TA (Schmidt et al., 1996). The magnitude of change in pH at 20°C and TA were larger than expected. Change in lactose concentration should have little impact on beverage pH and TA, however soluble mineral removal was the likely reason for the large decrease in milk TA, within each of the 3 protein concentrations. These changes in beverage pH and TA with lactose and soluble mineral removal may impact the heat stability of milk protein beverages. Renhe and Corredig 2018, reported that UF retentates at 3.2%, 6.1%, 6.7%, and 12.8% protein (where lactose and soluble minerals in the permeate portion of the retentate was not changed greatly) had pH at 25°C of 6.67±0.02, 6.67±.03, 6.67±.03, and 6.66±.05 respectively. The pH results for Renhe and Corredig 2018 coincides with the pH of the UF retentates without the lactose removal in our study.

Beverage AV and color

The AV of the beverages increased ($P <0.05$) with increasing protein concentration had higher AV ($P <0.05$), while AV decreased ($P <0.05$) slightly with increasing removal of lactose and soluble minerals (Figure 6b). At 10.5% protein in skim milk the AV values were about 17 mPa's. Studies conducted by Misawa et al. (2016), Cheng et al. (2019a), and Quiñones et al. (1997) reported that as protein content increased so did the viscosity of milk and milk beverages.
Increased protein concentration increased AV more at low temperature (4°C) than at higher temperature (>20°C) Cheng et al. (2019a). Determination of AV of beverages ranging in fat from 0.2 to 2%, true protein from 3 to 5%, and casein as a percentage of true protein (CN%TP) from 5 to 80% at 4°C, revealed that CN%TP explained the majority of the variability (65%) in AV while protein, and fat explained 16.19 and 4.54% of the variability, respectively (Cheng et al., 2019a). Thus, increasing protein concentration and altering CN%TP in beverages may be an approach to increasing beverage viscosity without addition of nondairy ingredients to enhance viscosity of milk-based beverages.

L-value (whiteness) increased as expected with increasing protein content (Figure 7b) but also increased slightly ($P < 0.05$) with increasing lactose and soluble mineral removal. The a-value increased ($P < 0.05$) with increasing protein concentration and lactose removal within each protein concentration (Figure 7d) resulting in less greenness. There was both an effect of lactose removal ($P < 0.05$) and the interaction of protein x lactose removal ($P < 0.05$) on a-value. The b*-value decreased with increasing lactose removal (less yellow). The b*-value increased slightly ($P < 0.05$) with increasing protein concentration. These findings were also demonstrated by Quiñones et al. (1997) who showed that increasing protein content in skim and 1% milks caused the L-value, a-value, and b-value to increase. Cheng et al.(2019b) found that changing not only the protein percentage but the type of protein, casein as a percentage of true protein (CN%TP), also altered color of milk protein beverages. As the CN%TP increased, the L-value increased and a and b* values decreased.

The effects of light scattering and removal of light absorbing low molecular weight chromophores on changes in L, a, and b*values can be more clearly seen from the reflectance curves (Figures 8c and d). As lactose and other low molecular weight compounds (e.g.,
riboflavin) that absorb light are removed with lactose, the amount of reflected light from 360 to 510 nm increases causing an increase in whiteness and a decrease in greenness and blueness. As the protein concentration increases, light scattering by casein globule increases (increased whiteness) and that increases the reflect light from 510 to 750 nm, however the ability of casein micelles to scatter light is much less than the large milk fat globules.

**Beverage sensory**

A summary of the descriptive analysis sensory results for experiment 2 is presented as a PCA biplot in Figure 10. Interactions between lactose and fat were evident for all sensory attributes (p<0.05) so a PCA biplot is used to visualize these results. As lactose and soluble mineral were removed, beverage whiteness, opacity and astringency increased and sensory yellowness decreased, consistent with lactose and soluble mineral removal in experiment 1. Increased sensory whiteness was consistent with the increase in L-value (Figure 7b) as lactose was removed. Sensory yellowness decreased with increasing lactose removal as did b*-value (Figure 7f). Sensory viscosity did not change (p>0.05) as lactose and low molecular weight solutes were removed. As lactose was removed, sensory profiles of milks became very bland, concurrent with a decrease in cooked/milky and sweet aromatic flavors and sweet and salty tastes (Figure 10), also consistent with experiment 1.

Sensory viscosity increased with increasing protein concentration, consistent with instrumental AV (Figure 6b). Increasing protein concentration increases viscosity in fluid milk and milk beverages (Quiñones et al., 1997; Cheng et al., 2019b; Vogel et al., 2021). As protein percentage increased in the milk beverages so did sensory opacity, papery flavor, and astringency. Previous studies have demonstrated that increased protein content in milk and milk protein beverages increased opacity and astringency (Cheng et al., 2019b; Vogel et al., 2021).
Papery flavor, which increased with protein concentration, was described as the flavor of white paper. Sensory panelists agreed that the flavor was similar to cardboard flavor. Cardboard flavor is an established flavor in spray dried milk and whey proteins (Wright et al., 2008; Smith et al., 2016). Cardboard flavor increases with storage of dried proteins and is linked with neutral aldehydes and lipid oxidation and a loss of freshness (Wright et al., 2008; Whitson et al., 2010). Low intensities of cardboard flavor have been documented in liquid milk and whey protein (Carter et al., 2018; Oltman et al., 2015). The intensities of papery flavor were low (0.6, 1.2, 1.3 on a 0 to 15 point intensity scale for 3.3, 6.9 and 10.5% protein, respectively), consistent with previous studies with liquid milk proteins. Collectively, milk components had distinct contributions to milk/milk beverage sensory properties. Lactose and soluble mineral removal decreased overall flavor and taste of milk, decreased yellowness and increased opacity. Fat contributed milkfat flavor, opacity, viscosity and decreased astringency. Protein contributed opacity, viscosity and astringency with little impact on flavor. Future work on milk and milk-based beverages with lactose removed by UF needs to focus on interaction of the remaining milk solids with added flavorings, changing casein to whey protein ratio prior to UF removal of lactose, and the impact of lactose and low molecular weigh solute removal on heat stability, particularly for neutral-pH, shelf-stable milk-based beverages.

Conclusions

Previous research on UF of milk for beverage applications has focused on use of UF to increase the concentration of protein, while the current work has focused on use of UF to remove lactose from milk in combination with increasing protein concentration. Removal of > 95% of milk lactose by UF required a diafiltration volume of approximately 3 times the milk volume. In addition, lactose (and other low molecular weight solute removal) by UF decreased titratable
acidity by more than 50% and increased milk pH at 20°C to >7. Lactose and soluble mineral removal from milk increased whiteness of skim while decreasing viscosity and flavor. Fat contributed milkfat flavor, opacity, viscosity and decreased astringency. Protein contributed opacity, viscosity and astringency with little impact on flavor. Milk beverages with variable composition and sensory properties (appearance, flavor and viscosity) can be manufactured to meet consumer desires for beverage variety.

Acknowledgments

Funding was provided in part by Dairy West (Meridian ID), Dairy Management, Inc. (Rosemont, IL) and HP Hood (Boston, MA). The technical assistance of laboratory staff members Chassidy Coon, Michelle Bilotta, and Sara Hatch from the Department of Food Science at Cornell University (Ithaca, NY) with analytical testing was greatly appreciated.
References


Figures

Figure 1. Experimental 1 overview: lactose and soluble mineral removal by UF at 4 different fat concentrations

<table>
<thead>
<tr>
<th>% Lactose</th>
<th>Batch #</th>
</tr>
</thead>
<tbody>
<tr>
<td>Removal</td>
<td>Skim</td>
</tr>
<tr>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>30</td>
<td>5</td>
</tr>
<tr>
<td>70</td>
<td>9</td>
</tr>
<tr>
<td>97</td>
<td>13</td>
</tr>
</tbody>
</table>
Figure 2. Experimental 2 overview: lactose and soluble mineral removal by UF at 3 different protein concentrations.

<table>
<thead>
<tr>
<th>Batch #</th>
<th>% Lactose Removal</th>
<th>3.3%</th>
<th>6.5%</th>
<th>10.5%</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>30</td>
<td>30</td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>70</td>
<td>70</td>
<td>7</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>97</td>
<td>97</td>
<td>10</td>
<td>11</td>
<td>12</td>
</tr>
</tbody>
</table>
Figure 3. Experiment 1: percent lactose as a function of diafiltration volume.
Figure 4. (A) Experiment 1: The impact of fat concentration on milk pH with increasing lactose removal. and (B) Experiment 2: The impact of protein concentration on milk pH with increasing lactose removal.
Figure 5. (A) Experiment 1: The impact of fat concentration on milk titratable acidity (TA) with increasing lactose removal and (B) Experiment 2: The impact of protein concentration on milk TA with increasing lactose removal.
Figure 6. (A) Experiment 1: The impact of fat concentration on milk apparent viscosity (AV) with increasing lactose removal and (B) Experiment 2: The impact of protein concentration on milk apparent viscosity (AV) with increasing lactose removal.
Figure 7. The impact of lactose removal on L-value (A – experiment 1; B – experiment 2), a-value (C – experiment 1; D – experiment 2), and b*-value (E – experiment 1; F – experiment 2).
Figure 7. (Continued)
Figure 7. (Continued)

(E) Impact of lactose removal on b*-value

(F) Impact of lactose removal on b*-value
Figure 8. Reflectance curves. (A) – Experiment 1 – skim milk; (B) Experiment 1 – whole milk; (C) Experiment 2 – 3.4% protein, (D) Experiment 2 – 10.5% protein.
Figure 8. (Continued)

(C) Beverage reflectance for 3.4% protein

(D) Beverage reflectance for 10.5% protein
**Figure 9.** Principal component biplot of sensory attributes of milks with variable lactose and fat: experiment 1
**Figure 10.** Principal component biplot of sensory attributes of milks with variable lactose and protein: experiment 2

**Biplot (axes F1 and F2: 94.14 %)**

<table>
<thead>
<tr>
<th>% Lactose Removal</th>
<th>Batch #</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.3%</td>
<td>1</td>
</tr>
<tr>
<td>6.5%</td>
<td>2</td>
</tr>
<tr>
<td>10.5%</td>
<td>3</td>
</tr>
<tr>
<td>30</td>
<td>4</td>
</tr>
<tr>
<td>70</td>
<td>5</td>
</tr>
<tr>
<td>97</td>
<td>6</td>
</tr>
<tr>
<td>97</td>
<td>7</td>
</tr>
<tr>
<td>97</td>
<td>8</td>
</tr>
<tr>
<td>97</td>
<td>9</td>
</tr>
<tr>
<td>97</td>
<td>10</td>
</tr>
<tr>
<td>97</td>
<td>11</td>
</tr>
<tr>
<td>97</td>
<td>12</td>
</tr>
</tbody>
</table>

**Active variables**
- cooked / milky
- sweet taste
- salty taste
- sweet aromatic
- yellowness
- astringency
- papery
- opacity
- whiteness
- viscosity