ABSTRACT

TRUONG, TINA PHUONGTHY. Impact of Process Temperature (7 and 50°C) on Process Performance and Protein Beverage Physical, Chemical, and Sensory Properties. (Under the direction of Dr. MaryAnne Drake).

Our objectives were to determine the impact of UF of skim milk at 7 and 50°C on UF flux, lactose removal, mineral partitioning, and skim milk retentate physical, chemical, and sensory properties at three (3.4, 7.5, and 10.5%) protein concentration with two different heat processing treatments (HTST and autoclave). Pasteurized skim milk was split into two portions and the 7°C UF processing run was done on one day and the 50°C UF processing run was done on the next day. skim milk was ultrafiltered and diafiltered at 7 and 50°C and as permeate was removed, deionized water at 7 or 50°C was added in an equal amount by weight as permeate removed to maintain constant protein concentration in the retentate during UF until 98% or more of lactose and low molecular weight soluble milk components were removed. All skim milk-based beverage bases from the 7 and 50°C UF of skim milk were HTST (78°C for 15 sec) processed or autoclaved (116°C for 6 min). The physical, chemical, and sensory properties of all treatments were measured. This process was replicated twice with a new batch of pasteurized skim milk in a different week with the 7 and 50°C UF processing runs ran in reverse order. Overall, lactose-free milk skim milk at 3.4, 7.5, and 10.5% protein produced by UF with DF, was more bland, more white and less heat stable (i.e., stable to retorting but not direct steam injection at 142°C for 2 to 3 sec) than skim milk. A 98 to 99% removal of lactose was achieved with a diafiltration ratio of water to milk of about 4 to 1 achieved at both 7°C and 50°C. The processing time to achieve that removal from the same volume of milk was about twice as long when filtering at 7°C than 50°C because of the lower flux (23 versus 48 Kg/m²/h). The continuous DF at constant protein concentration maintained constant flux for a processing time of 4 and 8 h at 50 and 7°C,
respectively. The final freezing point of the lactose and soluble mineral reduced milk was close to that of water (-0.015°C) and the pH of the lactose free milk at 20°C increased from about 6.5 to about 7.33 and 7.46 for UF/DF at 7 and 50°C, respectively. Removal of compounds from milk in permeate that absorb light in the range of 360 to 500 nm, increased light reflectance and whiteness and decreased yellowness.

**Key Words:** Lactose removal, ultrafiltration, milk minerals
Impact of Process Temperature (7 and 50°C) on Process Performance and Protein Beverage Physical, Chemical, and Sensory Properties.

by
Tina Truong

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

Food Science

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APPROVED BY:

__________________________                                     __________________________
Dr. MaryAnne Drake                                                       Dr. Dana Hanson
Chair of Advisory Committee

__________________________
Dr. Clint Stevenson
BIOGRAPHY

Tina Truong was born in Santa Clara, California on September 6th, 1999. She graduated with a Bachelor of Science Degree in Food Science in 2020. To further her education, Tina began pursuing her Master of Science Degree at North Carolina State University with Dr. MaryAnne Drake in 2020. Upon entering the industry, she hopes to advocate for gender inclusion and diversity, specifically in the dairy processing space.
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CHAPTER 1: LITERATURE REVIEW: THE PRODUCTION, PHYSICAL PROPERTIES, AND HEAT STABILITY CHARACTERISTICS OF MILK PROTEIN BEVERAGES

Tina Truong and MaryAnne Drake
Department of Food, Bioprocessing and Nutrition Sciences, Southeast Dairy Foods Research Center, North Carolina State University, Raleigh, NC 27695

1Corresponding 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
E-mail : mdrake@ncsu.edu
Milk Introduction

Statistics and Decline in Fluid Milk Consumption

Beginning in the 1800’s in the United States, bovine milk and milk products were primarily produced for home or local use. During this time, important inventions such as commercial milk packaging, milking machines, pasteurization equipment, refrigerated trucks, homogenizers, and automatic bottling machines contributed to making milk a commercial product. These developments also made it easier to not only mass produce milk, but also to improve the quality and preservation of milk (Rankin et al., 2017). In 2020, worldwide milk production amounted to over 530 million metric tons. The European Union was the leader in milk production during this year, with 145.7 million metric tons produced while the United States was second with 102.6 million metric tons produced (Statista, 2021). Fluid milk and cream comprised 31 percent of the dairy products expenditure of households in the United States while the other 69 percent of expenditure is comprised of butter, ice cream, yogurt, and other dairy foods (Statista, 2021).

It is important to note that the American consumption of fluid bovine milk on a per person basis has decreased since the 1940’s (Stewart, 2020). According to the United States Department of Agriculture, the estimated sales of total fluid milk sales decreased by 0.8 percent from November 2020 through November 2021 (United States Department of Agriculture, 2022). Many factors such as animal rights activism, vegan diet education, the growing plant-based foods industry, and diagnosis of lactose intolerance may have influenced this decline (Zingone et al., 2017). Additionally, it has been reported in the media that lower milk consumption in the United States is also associated with allergies, gastrointestinal symptoms, eczema, carcinogenic characteristics, and more (Bahna, 2022). Plant-based milk alternative product sales have reached
20.9 billion dollars worldwide (Gerdes, 2022). There are currently a variety of fluid plant-based products available sourced from almond, oat, cashew, pea, soy, and rice, among others. For consumer perception of plant-based milk alternatives, the main drivers of preference include lactose intolerance, milk allergy, absence of cholesterol, and perceived healthiness and sustainability (Jeske et al., 2018). The popularity of plant-based milk alternatives is contributed by their marketing of being more environmentally friendly and ethical to consumers, but also similar in taste and use as fluid bovine milk (Fuentes and Fuentes, 2017).
According to the Economic Research Service food availability data, the increasing amount of fluid plant-based products negatively affect household purchase of gallons of bovine milk (Stewart, 2022). However, the sales of plant-based alternative beverages are not the primary factor of declining sales of fluid bovine milk. Change of dietary habits and product choices are the main contributors to lower consumption of milk as a beverage. From 2010 to 2019, the U.S. daily milk consumption decreased from 0.62 cups to 0.49 cups; this trend in consumption decrease has been prevalent since the 1970’s (Stewart and Kulcher, 2022). Diet soft drinks and water are chosen as substitutes over dairy beverages because consumers consider them to be healthier and lower in calories. Regardless, bovine milk remains a staple food item for

<table>
<thead>
<tr>
<th>Product Name</th>
<th>Sales(^1)</th>
<th>Change From:</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Month</td>
<td>Year to Date</td>
</tr>
<tr>
<td>Conventional Production Practice</td>
<td>(million pounds)</td>
<td>(percent)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole Milk</td>
<td>1,255</td>
<td>13,376</td>
<td>---</td>
<td>-5.7</td>
</tr>
<tr>
<td>Flavored Whole Milk</td>
<td>69</td>
<td>721</td>
<td>-5.8</td>
<td>4.9</td>
</tr>
<tr>
<td>Reduced Fat Milk (2%)</td>
<td>1,097</td>
<td>12,306</td>
<td>-10.3</td>
<td>-8.3</td>
</tr>
<tr>
<td>Low Fat Milk (1%)</td>
<td>412</td>
<td>4,631</td>
<td>-6.8</td>
<td>-6.1</td>
</tr>
<tr>
<td>Fat Free Milk (Skim)</td>
<td>204</td>
<td>2,279</td>
<td>-8.9</td>
<td>-12.7</td>
</tr>
<tr>
<td>Flavored Fat-Reduced Milk</td>
<td>333</td>
<td>2,986</td>
<td>46.0</td>
<td>16.0</td>
</tr>
<tr>
<td>Buttermilk</td>
<td>43</td>
<td>414</td>
<td>8.1</td>
<td>8.3</td>
</tr>
<tr>
<td>Other Fluid Milk Products</td>
<td>155</td>
<td>1,157</td>
<td>38.1</td>
<td>47.8</td>
</tr>
<tr>
<td>Total Fat Reduced Milk</td>
<td>2,046</td>
<td>22,203</td>
<td>-3.4</td>
<td>-5.7</td>
</tr>
<tr>
<td>Total Products Sold</td>
<td>3,568</td>
<td>37,872</td>
<td>-0.8</td>
<td>-4.3</td>
</tr>
</tbody>
</table>

\(^1\)Data may not add due to rounding (United States Department of Agriculture, 2022).

*Adapted from (United States Department of Agriculture, 2022).*
Americans and holds a large share in the grocery beverage category (Heng et al., 2017). However, the dairy industry should be prepared to have solutions to address the decline of bovine milk consumption (Stewart, 2022).

**Need to Increase Fluid Milk Consumption**

Nutrition and health policy researchers have stated that there are potential health implications of declining fluid milk consumption (Popkin, 2010). The increasing decline in bovine milk also negatively affects companies and programs supported by dairy farmers, milk processors, and the federal government (Kaiser and Dong, 2006). Bovine milk provides a variety of important nutrients including protein, calcium, potassium, phosphorus, and iodine, along with the vitamins B2 and B1. The milk of mammals contains the same principal components such as water, proteins, fats, carbohydrates, vitamins, and minerals (Claeys et al., 2014). The milk of different mammals is differentiated by the amount of casein and whey protein content. For example, 80 percent of bovine milk proteins are composed of casein, which is beneficial in providing all the essential amino acids necessary for body growth and repair (Malacarne et al. 2002). In human milk, the whey/casein ratio will fluctuate between 70/30 and 80/20 in early lactation and decreases to 50/50 in late lactation (Martin et al., 2016). The proportion of whey proteins is still significantly greater than that of cow’s milk. On the same topic, dairy protein is a complete protein, meaning that it contains all essential amino acids; vegetable proteins on the other hand lack one or more essential amino acids and are not considered as complete proteins by definition (Hoffman and Falvo, 2004).
### Table 1.2. Composition of bovine, buffalo, goat, and sheep milk. *

<table>
<thead>
<tr>
<th></th>
<th>Bovine Milk (%)</th>
<th>Buffalo Milk (%)</th>
<th>Goat Milk (%)</th>
<th>Sheep Milk (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Solids</td>
<td>11.8-13.0</td>
<td>15.7-17.2</td>
<td>11.9-16.3</td>
<td>18.1-20.0</td>
</tr>
<tr>
<td>Total Protein</td>
<td>3.0-3.9</td>
<td>2.7-4.7</td>
<td>3.0-5.2</td>
<td>4.5-7.0</td>
</tr>
<tr>
<td>Casein/Whey Ratio</td>
<td>4.7</td>
<td>3.1</td>
<td>3.5</td>
<td>4.6</td>
</tr>
<tr>
<td>Fat</td>
<td>3.3-5.4</td>
<td>5.3-9.0</td>
<td>3.0-7.2</td>
<td>5.0-9.0</td>
</tr>
<tr>
<td>Lactose</td>
<td>4.4-5.6</td>
<td>3.2-4.9</td>
<td>3.2-5.0</td>
<td>4.1-5.9</td>
</tr>
<tr>
<td>Ash</td>
<td>0.7-0.8</td>
<td>0.8-0.9</td>
<td>0.7-0.9</td>
<td>0.8-1.0</td>
</tr>
</tbody>
</table>

*Adapted from (Claeys et al., 2014).

Soy milk is the only dairy alternative that contains comparable amounts of nutrients such as protein, fat, and calories to bovine milk (Vanga and Raghavan, 2018). Soy milk, however, naturally only contains one-fifth of the calcium of cow’s milk and is deficient in the amino acid, methionine. Unlike dairy proteins, the main issue with other common plant-based milks such as almond and oat is the denaturation of proteins upon heat treatment. Globulins are a major class of proteins in plant-based milks which have low solubility and form crosslinks during heat-induced denaturation at sterilization temperatures of 121 °C (Nicolai and Chassenieux, 2019; Aydar et al., 2020). Additionally, although nut-based plant milks are rich in protein, dietary fiber, fatty acids, and vitamins, these bioactive compounds are lost because of thermal processing (Aydar et al., 2020). Fortification of plant-based milks are often done because the final products contain less protein and essential nutrients compared to cow’s milk (Sethi et al., 2016). Additionally, other issues in plant-based beverages that are not found in bovine milk include unconventional flavors and lower calorie density (Vanga and Raghavan, 2018). Previous studies have shown that most consumers (regardless of demographic) tend to have lower liking of plant-based milk alternatives, such as soy and almond, compared to cow’s milk (Cardello et al., 2022, McCarthy et al., 2017). For example, oat, rice, coconut, and cashew-based milk products were strongly associated with ‘weak/bland’, thin/watery’, and ‘cardboard like’ sensory terms whereas that was absent in cow’s milk products (Cardello et al., 2022). Consumers of both cow’s milk
and plant-based milks perceive cow’s milk for it being ‘a treat’ and for its flavor, whereas plant-based milk was only purchased if there was a desire to limit animal product consumption (McCarthy et al., 2017).

**Table 1.3.** Nutritional composition of plant-based and bovine milk per 240 mL serving. *

<table>
<thead>
<tr>
<th></th>
<th>Calories (g)</th>
<th>Protein (g)</th>
<th>Fat (g)</th>
<th>Carbohydrates (g)</th>
<th>Calcium (% daily value)</th>
<th>Iron (% daily value)</th>
<th>Vitamin A (% daily value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soy milk (Silk)</td>
<td>80</td>
<td>7</td>
<td>4</td>
<td>4</td>
<td>30</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>Oat milk (Oatly)</td>
<td>104</td>
<td>4.5</td>
<td>6</td>
<td>9</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Almond Milk (Silk)</td>
<td>40</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>20</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>Cow’s milk (UHT)</td>
<td>168</td>
<td>8</td>
<td>10</td>
<td>11</td>
<td>26</td>
<td>7</td>
<td>19</td>
</tr>
</tbody>
</table>

*Adapted from (Sethi et al., 2016).

Milk, tree nut, and soybean allergies are major issues associated with the proteins of cow’s milk and plant-based milk products. With cow’s milk allergy specifically, it is the most common food allergy among infants and young children and has a higher chance of persistence into adulthood compared to tree nut and soybean allergies (Sicherer, 2011). In developed countries, the estimated prevalence of cow’s milk allergy ranges between 0.5 to 3.0% in infants at one year of age (Flom and Sicherer, 2019). To date, cow’s milk allergy’s impact on adult populations in the United States and globally remains largely unknown, but studies suggest that a diet in children excluding cow’s milk may have deleterious effects on growth (Warren et al., 2022). However, consumers of only nondairy milks also frequently purchased these products over cow’s milk for other reasons including perceiving cow’s milk to be unhealthy, beliefs about animal mistreatment, and the belief plant-based milks are more environmentally friendly (McCarthy et al., 2017). Cow’s milk is proven to be beneficial in diets for people at all life stages as it contains vitamin D and magnesium to maintain bone health, as well as casein and calcium for bone development (Givens, 2020). In many cases involving growing children, individuals
have become seriously ill after only consuming diets with only plant-based milk alternatives to replace cow’s milk, causing deficiencies in micronutrients and protein (Forreau et al., 2013). A previous study also found that the lower protein content, lower calcium, and higher general intake requirements for soy, coconut, almond, rice, cashew, and hemp plant-based milks make them nutritionally inferior to cow’s milk (Chalupa-Krebzdak et al., 2018).

**Current Fluid Milk Market Trends and Products**

There is an increasing consumer demand for authenticity and transparency in food products. Currently, there is no legal or official definition of the term, “clean label” in use for describing food products. However, food companies face issues with consumer misinformation and confusion in regard to if their products are label compliant under descriptors such as “natural” or “artificial” (Shelke, 2020). It is estimated that the market for global clean label foods sales will be worth $180 billion USD in 2021, which is a dramatic increase from the total global market sales of $165 billion USD in 2015 (Shelke, 2020). There are common attributes that can be associated with products that fall into this description such as, minimally processed, sustainable, fewer ingredients, simple ingredients, organic, non-GMO and free from claims (Burrington, 2019). Because there is not an official definition for the term “clean label”, many factors influence consumer perception on whether a food product or company practices fulfill their “clean label” needs or not (Asioli et al., 2017).

There is currently a high demand for high-protein, functional, and “clean label” dairy products worldwide (Cornall, 2020). For example, Kraft Heinz removed artificial preservatives, flavors and dyes in their Kraft Macaroni and Cheese for natural spices that can fulfill those same ingredient purposes (Goldman, 2016). Arla Foods also launched a “clean label” stirred yogurt
product that contained only five all-natural ingredients and no artificial flavors or sweeteners (Cornall, 2020).

Cow’s milk is traditionally known for its healthy nutrition label and simple, natural, and wholesome ingredient label. However, present day consumers consider dairy products with label statements such as organic, antibiotic-free, hormone-free, grass-fed, and pasture-raised to be “clean label” (Glanbia Nutritionals, 2021). Consumers also consider products to be “clean label” if there are no additives, artificial colors, flavors, and preservatives (Bearth et al., 2014). Concern for ‘health’ was also a major driver in the need for “clean label” products (Asioli et al., 2017). Specific health concerns driving the change to “clean label” foods include diseases such as diabetes, obesity, cardiovascular disease, and cancer (Kearney, 2010). Milk protein beverages are often not perceived as “clean label” due to most containing ingredients such as hydrocolloids and calcium chelators to improve beverage viscosity and heat stability, respectively (Fallourd and Viscione, 2009). However, functional ingredients in dairy protein beverages have long or chemically sounding names, creating a longer product ingredient list and makes them likely to not be perceived as “clean label” (Aschemann-Witzel et al., 2019). Further research is needed to determine if there are alternative ways to achieve the same physical and functional characteristics of dairy protein beverages without the addition of ingredients that are not perceived as “clean label”.

Climate change and sustainability concerns are other factors that influence consumer purchase habits of clean-label products. Meat and dairy alternatives often have issues with taste expectations, texture expectations, or ingredient lists that are too long (Brewster, 2021). Six percent of the total greenhouse gas emissions in the United States are emitted from livestock and manure sources (Rotz, 2018). However, approximately 25% of the world’s greenhouse gas
emissions come from crop production and fertilization, products that could be used to create plant-based alternative milks (Kanyama et al., 2021). In a study focused on the consumer perception of dairy and plant-based products, consumers associated minimal carbon footprints, low greenhouse gas emissions, and few/no preservatives, animal welfare, and minimal ingredients for a product to be sustainable (Schiano et al., 2020). In the same study by Schiano (2020), consumers perceived plant-based alternatives to be more sustainable to fluid milk and protein powders with package type and organic status having the largest influences. Overall, continuous improvement should be taken towards practices of dairy farmers and product design to cater to changing consumer ideologies to meet the clean label product demands.

**Protein Beverage Ingredients**

The functionality and choice of use of dairy protein ingredients is crucial in the final protein beverage quality and stability. Properties of casein, whey, and milk protein ingredients in beverage applications will be discussed in this review.

**Casein Ingredients**

Caseins have high stability at high temperatures and under severe heat and pressure treatments, they undergo dephosphorylation, proteolysis, and polymerization (Sadiq et al., 2021). Although there is currently no standard of identity for micellar casein concentrate (MCC), it is a relatively new ingredient that is typically over 85% total casein protein and manufactured from the microfiltration of skim milk (Carter et al., 2021). To make MCC, whey and serum proteins, lactose, and mineral are fractionated into the permeate, resulting in a higher casein-to-whey ratio in the retentate (Singh et al., 2022). The microfiltration process to make MCC can utilize either
ceramic or polymeric membranes, however, Zulewska et al. (2009) reported that ceramic membranes are more efficient at removing serum proteins compared to spiral wound polymeric membranes (i.e., 95% and 70% serum protein reduction, respectively). Higher removal of whey and serum proteins will require additional diafiltration or a larger membrane surface area, which will increase operational costs (Carter et al., 2021). Among MCC, other casein ingredients like caseinates, sodium, and calcium caseinates are also used in high protein neutral pH protein beverages because of their heat stability (Singh et al., 2022). Previous studies by Hoyt et al. (2023) and Vogel et al. (2021) demonstrated that liquid MCC used to formulate 7.5% and 6.3% protein neutral pH beverages, respectively, as heat stable by ultra-high temperature (UHT) processing conditions. Sauer and Moraru (2012) found that protein beverages made from reconstituted MCC with a pH less than 6.7 aggregated and coagulated when subjected to ultra-high (135 or 142°C) and retort (115.6 or 121°C) temperature conditions. Only after pH adjustments to 7.1 and 7.3 were there few or no changes in the particle size after the same sterilization heats treatment of the reconstituted MCC beverages (Sauer and Moraru, 2012). In terms of sensory characteristics, beverages made with rehydrated MCC had sweet aromatic, cooked, cardboard, and dough flavors when compared to beverages made with liquid MCC (Smith et al., 2016; Carter et al., 2018). When comparing liquid MCC beverages to liquid MPC beverages, only the MCC based beverages had a distinct tortilla flavor (Carter et al., 2018). Depending on the amount or lack of whey and serum proteins in MCC based beverages made for sterilization processing, sulfur and cooked flavors may be decreased (Jo et al., 2019).

Caseinates are made through pH adjustment of milk to induce isoelectric precipitation of casein proteins, casein and whey protein separation, addition of hydroxide, and drying (Singh et al., 2022). During the acidification step of this process, all of the colloidal calcium phosphate...
contributing to the assembly of casein micelles is dissolved, which causes casein micelles to
dissociate into smaller sub-micelles (Lin et al., 2017). Because caseinates typically fall in a pH
range of 6.5 to 7.0 and the difference in their protein structures, they are more appropriate to
stabilize beverages containing milk fat over other milk protein ingredients, (Lin et al., 2017). For
example, calcium caseinates have a turbid and milky appearance in dispersions due to their
ability to form aggregates in water (Singh et al., 2022). Although there is little research on the
heat stability of caseinate ingredients, O’Mahony and Fox (2014) found sodium caseinate was
able to withstand heating at 140°C for hours in a pH 7 solution without visible changes, which
well exceeds normal UHT processing conditions.

Whey Ingredients

There are three types of commercial forms of whey protein: whey protein concentrate
(WPC), whey protein isolate (WPI), and whey protein hydrolysate (WPH). Commercial whey
protein concentrate (WPC), which has 30 to 85% protein and whey protein isolate (WPI), which
has over 90% protein, are products that are made from liquid whey by ultrafiltration with
diafiltration followed by drying into powder form (Luck et al., 2013). WPH is typically made by
enzyme-catalyzed hydrolysis of liquid whey protein that has undergone the filtration process,
followed by drying into powder form (Ghosh et al., 2017). The hydrolysis process with WPH
manufacture results in new amino groups that are formed. One amino group for each broken
peptide bond is formed, meaning there is still bioavailable protein (Ghosh et al., 2017). Whey
protein ingredients inevitably contain residual fat in them. However, the amount of fat will
increase with increasing protein concentration. For example, WPC at 34% protein is expected to
have about 2% fat while WPC at 80% protein is expected to have about 7% fat (Zadow, 2003).
Both WPC and WPI are high in manufacture demand due to their nutritional values and physical properties including solubility, emulsification, and foaming (Luck et al., 2013). WPC at 34% is generally used in the industry as a cost-effective replacement for skim milk while WPC at 80% is used for its gelation properties because of its higher protein content (Zadow, 2003). The primary use of WPH are in infant formula or human milk substitutes because the process of hydrolysis removes intact protein molecules, particularly β-lactoglobulin that may cause allergic reactions (Boland, 2010). Not only are WPHs used for their lack of milk allergy, but they are also high in nutritional value and are more rapidly digested than intact proteins (Ghosh et al., 2017). They can be used in nutritional drinks and bars. In protein bars, they can reduce bar hardening that occurs with storage time. Whey proteins are also generally found in high-acid, low-pH clear acidic RTD beverages because they have poor solubility at neutral pH upon heating, but are quite acid stable (Singh et al., 2022). Whey proteins are also prevalent in ready to mix (RTM) protein beverages. Previous research had found that reconstituted WPC dispersions (2% protein) at neutral pH had poor stability and caused severe fouling at UHT processing (145°C for 5 s) (Singh et al., 2019). In RTD beverage applications, WPC at 34% would have more turbidity and precipitation due to fewer purification steps in its production compared to WPC at 80% or WPI (Liu and Zhong, 2014). The acid stability of whey protein in clear acidic RTD beverages will be further discussed later in the review.
**Milk Protein Ingredients**

Milk protein concentrate (MPC) and milk protein isolate (MPI) contain both whey and casein proteins in their natural ratio and native state (Singh et al., 2022). MPCs have a protein content ranging between 35 to 85% on a dry basis while MPIs have a protein content over 90% on a dry matter basis (Suthar et al., 2017). There are three main types of categories of milk protein powders: low protein which are less than 40 percent protein, medium which is between 60 to 70 percent protein, and high protein which is above 80 percent protein (Sikand et al., 2011). Milk protein products are made by applying ultrafiltration with diafiltration to skim milk and are followed by drying into powder form (Sikand et al., 2011). Protein content concentration in these ingredients is inversely proportional to the amount of lactose and mineral salt content that is removed (Meena et al., 2017). MPCs have relatively more undenatured proteins and severe heat treatment is not required for manufacture (Meena et al., 2017). MPCs and MPIs provide important functional properties including solubility, viscosity building, emulsification, and heat stability (Suthar et al., 2017). However, studies have shown that MPC with over 80% protein have poor reconstitution properties, lower solubility compared to lower protein MPCs, and higher levels of sedimentation during storage over time (Sikand et al., 2011). MPC with 40% protein was observed to be more soluble than MPC with 80% protein due to the lower level of κ-casein and calcium, and higher level of monovalent salt in MPC40 (Sikand et al., 2011). It has been found that to achieve better solubility in MPC, lower spray drying temperatures as well as more comprehensive contact of sprayed particles with the drying air for optimal moisture removal should be done (Fang et al., 2012). Calcium amount also influences the heat stability of high protein beverages made with reconstituted MPC undergoing UHT or retort thermal processing (Sunkesula et al., 2021). For example, high calcium ion activity through the addition
of calcium salt lowered heat stability, heat coagulation, and dropped the pH below 6.75 for unconcentrated and concentrated milk systems (Philippe et al., 2003). Sunkensula et al. (2021) found an increase in heat stability in high protein beverages with the use of MPC powder at 80% protein once the amount of calcium was reduced by 30% and the pH was above or at least 7. Another study found that more diafiltration during MPC manufacture also strongly and negatively affected the heat coagulation time of reconstituted beverages, indicating that lowering serum protein and mineral composition have major effects on increasing heat stability (Eshpari et al., 2014). Neutral pH RTD beverages can be made with either liquid milk protein retentate or rehydrated MPC powder, but their sensory profiles will be different (Singh et al., 2022). When comparing RTD high protein beverages made with varying levels of MPC and MCC, sulfur and eggy flavors were highest in beverages with higher proportions of serum proteins (Vogel et al., 2021). Dispersions made from MPCs with 70, 80, and 85% and MPI were high in astringency, low sweet aromatic flavor, and had off-flavors described as tortilla, brothy, cardboard, and animal (Smith et al., 2016).

**Protein Beverage Type**

Protein beverages are continually growing in popularity due to consumer association with the products to greater general health and wellness (Cornall, 2021). As a result of the COVID-19 pandemic, consumers have become more proactive in understanding the connection between their food intake and overall health. Applications of protein beverages include convenient snacking, meal replacement, satiety, or sports nutrition recovery (Harwood and Drake, 2021). According to a survey conducted by Kerry in 2020, consumers associated claims on protein beverages such as “high protein”, “natural”, “grass-fed” and “sustainable” to be healthy and
products without these claims to not be health (Innova Market Insights, 2021). When consumers were asked about their beliefs on the protein content of dairy or plant-based products, of those who preferred plant-based products, 86% were aware that the protein content of plant-based products was not greater or equal to that of dairy products (Schelepphorst et al., 2023). In addition to protein type, consumer preferences in RTD dairy or plant-based protein beverages were also dependent on the natural label, carbohydrate content, and sweetener type (Liu et al., 2021).

According to the Food and Drug Administration, the acceptable macronutrient distribution range for protein for adults is 10 to 35 percent of the daily energy and 50 grams of protein as the daily value when consuming a 2,000-calorie diet (Center for Food Safety and Applied Nutrition, 2021). Recent studies have found that consumers preferred protein beverages that provided 20 to 29 grams per serving (Harwood and Drake, 2021). Beverages that were within this protein content range can legally include “high”, “rich in”, or “excellent source” statements on their product packaging for means of marketing (CFR Title 101 Section 101.54, 2022). In 2020, the average protein content per serving in grams (g) of foods and beverages with a high protein claim was 17.8 grams (Innova Market Insights, 2021). Thus, innovation in higher protein contents, new protein sources, sustainability, clean label, and simpler processing technologies are necessary in propelling protein beverage market growth (Cornall, 2021).

Common types of protein beverages include ready-to-drink, ready-to-mix, and clear acidicics. Ready-to-drink (RTD) protein beverages refer to packaged beverages that are sold in a prepared form and can be consumed immediately upon purchase (Market Research, 2021). Ready-to-mix (RTM) refers to packaged powder protein ingredients that need to have additional ingredients, such as water added to make a drinkable beverage (Cornall, 2021). Clear acidic or high acid
protein beverages such as sports and energy drinks have acids added to them to provide a distinctive flavor, refreshment, and longer shelf-life (Li et al., 2022).

**Neutral pH Ready-to-Drink**

The RTD protein beverage market was valued at $1.56 billion USD in 2023 and expected to grow by 7.7% between 2023 through 2028 (Mordor Intelligence, 2023). RTD protein beverages that utilize dairy proteins are the most desirable and predominant in this market, with those having ≥15 g of protein per serving being favored (Oltman et al., 2015). Although dairy proteins have outperformed plant proteins in flavor and functionality in protein beverages, there are still many areas of improvement for dairy based protein beverages (Burrington, 2021). For example, the amount and type of dairy protein in RTD beverages will have different effects on flavor in the final product. Higher protein concentration and higher serum protein in RTD beverages increased sensory astringency and viscosity, while beverages with higher proportions of serum protein had higher levels of sulfur compounds after thermal processing (Vogel et al., 2021).

There are two types of RTD beverages: neutral pH which have a pH between 4.6 and 7.5 and acidified beverages, which have a pH between 2.5 and 3.0 (Vogel et al., 2021). Determining the pH when developing a protein beverage is crucial as the pH will dictate all aspects of the beverage manufacture. For example, whey protein ingredients such as whey protein isolate or concentrate function best in high acid beverages while milk protein ingredients, which typically contain 80% casein protein and 20% whey protein function best in low acid or neutral pH beverages (Singh et al., 2022). Caseins aggregate and precipitate at or below their isoelectric point of pH 4.6, thus, applications of MCC, non-fat dry milk, MCC in acidified beverages are
very limited (Li et al., 2022). In contrast, neutral pH RTD protein beverages with milk protein ingredients containing casein have higher heat stability and facilitate applications of UHT and aseptic processing (Singh et al., 2022). High acidic or clear acidic RTD beverages are only able to undergo hot-fill-hold processing, which operates at temperatures between 90–95°C while UHT and aseptic processing operate at temperatures above 138°C and 115°C, respectively (Singh et al., 2022). The thermal processes used for neutral pH RTD beverages must ensure an $F_0$ value at a temperature of 121.1°C (250°F) (time in minutes of heat treatment) and a minimum of a 12-decimal reduction in *Clostridium botulinum* because they are regulated as “low acid canned foods” in the United States (CFR 21 Title 101 Section 101.13, 2022). To produce low-acid foods, a minimum thermal process equivalent of $F_0$ of 3 (121.1°C for 3 minutes) is required to achieve a 12-decimal reduction in *Clostridium botulinum* (Singh et al., 2022). To reduce thermophilic spore-forming spoilage bacteria in low-acid beverages in countries with hot climates, an $F_0$ greater than 10 is recommended (Stannard, 1997). For more detail, the isoelectric point and charge of a protein is important for its interaction with ingredients and solubility in the neutral pH RTD protein beverage matrix (Khaldi and Shields, 2011). For example, whey protein isolate is a common ingredient in RTD clear acidic and high acid protein beverages that have a pH below 3.0 (Choi et al., 2021). At pH range of 3.5 to 4.5, whey proteins are close to their isoelectric point and an additional stabilizer may be necessary to prevent the whey proteins from aggregating during thermal processing (Kotchabhakdi and Vardhanabhuti, 2020). However, at neutral pH or in high concentration, whey proteins are denatured and can form gels, which is an unfavorable physical characteristic of protein beverages (Chantrapornchai and McClements, 2002). Studies found a rapid decrease in heat coagulation time with milk protein powders made from milk acidified to pH 6 prior to ultrafiltration (Eshpari et al., 2014). At pH values below 6.7,
interactions between whey proteins and caseins will occur on the surfaces of casein micelles in milk (Singh et al., 2022). In beverages made with MPC80 at pH 6.9, calcium ions in the serum phase promote interactions between whey proteins and the surfaces of casein micelles, thus providing more steric hindrance to casein micelles (Sunkesula et al., 2021). In the same study, it was hypothesized that at pH 7.1, κ-casein has moved out of the micelle, into the serum phase, and interacting with whey protein, thereby lowering heat coagulation time and heat stability (Sunkesula et al., 2021).

The pH of a beverage impacts astringency. Acidic solutions (with no protein) are astringent, but clear acidic whey protein beverages exhibit high intensities of astringency beyond that contributed by the lower pH itself (Carter et al., 2020). Milk proteins are astringent as well, but astringency is amplified in clear acidic protein beverages. For example, a neutral pH protein beverage made with MPC at pH 6.8 had a maximum sensory astringency score of 1.2 on 15-point scale, whereas a pH 3.4 beverage had an 8.8 score on the same scale; astringency increased with decrease in pH (Beecher et al., 2008). The sensation of astringency is due to the interactions of the positively charged whey proteins with the negatively charged saliva proteins, resulting in loss of lubricating saliva on the tongue (Carter et al., 2020). Astringency is not limited to dairy proteins and has been documented in RTD made from dairy and plant-based proteins (Liu et al., 2021). Astringency increases with protein amount and with heat treatment (Vogel et al., 2021; Lee et al., 2017). Increased consumption (e.g., a small volume versus a larger volume) also increases sensory perception of astringency (Withers et al., 2014).
Ready-to-Mix

The ready-to-mix (RTM) protein beverage market was valued at $11.1 billion USD in 2021 and is expected to grow by 8.1% by 2024 (Cornall, 2021). Considering this large market, whey protein powder is the most profitable animal-based protein contained in RTM products while pea protein powder is the most profitable plant-based protein (Myers, 2019). For RTM products, areas of innovation as expressed by consumers include sugar reduction, clean label, new flavors, and simpler processing technologies (Myers, 2019). Personalized protein powders are another strategy companies have been taking to cater to individual consumer nutrient or flavor needs (Cornall, 2021). Based on age demographics, millennials are the most frequent customer of RTM protein products and males are more frequent consumers than females. Overall, millennial concerns of health, weight loss or maintenance, energy levels, and muscle building are factors that contribute to the growth of the RTM industry (Grand View Market Research, 2021).

As with RTD protein beverages, RTM protein beverages having ≥15 g of protein per serving are favored (Oltman et al., 2015; Harwood and Drake, 2019). While the desire to increase protein content and healthfulness drives purchase intent for RTM products, the flavor, texture, mouthfeel, and mixing experience are important characteristics of the consumer usage experience (Zhang et al., 2020). Studies suggest that for neutral pH RTM whey-based protein beverages, consumers desire vanilla-flavored protein beverages that have strong dairy/milky flavor, low aftertaste, low astringency, and low cardboard flavor (Zhang et al., 2020). In the same study, Zhang et al. (2020), found that those same consumers preferred lower bitterness, metallic flavor, and off-flavors from nonnutritive alternative sweeteners in their neutral pH RTM
whey protein beverages. These preferences, especially for astringency are consistent with previous studies focused on RTD whey protein beverages (Beecher et al., 2008).

**Clear Acidic beverages**

Clear acidic whey protein beverages are of great interest due to their mild heating requirements to achieve shelf stability and their lack of need of refrigeration (Carter et al., 2020). The dairy industry generally conducts hot fill processing equivalent to about 88°C for 2 min in order to achieve shelf stability at ambient conditions (Etzel, 2004). Like RTD and RTM beverage products, clear acidic protein beverages fall into the global sports nutrition products market, which was valued at over $38 billion USD in 2022 (Global Newswire, 2023). Whey protein concentrates and isolates are the most utilized dairy protein ingredients used for clear acidic dairy protein beverages due to their acid stability and solubility at pH < 3.5 (Mueller, 2021).

Unlike neutral pH RTD and RTM beverages, cloudiness in what is supposed to be a clear beverage, or perception of a turbid appearance is the main formulation challenge in these beverages (Carter et al., 2020). Whey protein ingredients are least soluble at their isoelectric point, which is around pH 4.5, but, solubility of whey protein will increase as pH decreases (Pelegrine and Gasparetto, 2005). As noted previously, astringency is amplified in these beverages. In some foods such as coffee, tea, and wine, astringency is desirable, but studies suggest that consumer liking is higher in clear acidic dairy protein beverages if astringency is minimal (Childs and Drake, 2010). High intensities of astringency are associated with higher levels of turbidity or cloudiness in clear acidic dairy protein beverages due to the presence of larger whey protein aggregates interacting with saliva proteins on the tongue (Beecher et al., 2008). Because of the sourness that is inherently associated with clear acidic beverages, fruity
flavors are more commonly used compared to flavors like vanilla in chocolate used in neutral pH RTD and RTM beverages (Carter et al., 2020). To manage the sourness and acidity of clear acidic dairy protein beverage, acidulants such as phosphoric acid or organic acids (citric, lactic, and malic) are used (Singh et al., 2022). Considering the challenges of cloudiness and astringency in clear acidic dairy protein beverages, choice in acidification source is vital and it is recommended to choose acidulants that will contribute the smallest effect in the final product (Carter et al., 2020).

**Milk Composition**

Bovine milk is a complex mixture of water, carbohydrates, lipids, minerals, proteins, and vitamins. Because of the high amount of essential nutrients bovine milk contains, it is used to promote nourishment and growth in the human diet (Willett and Ludwig, 2020). Variations can occur in the composition of bovine milk through facts such as breed, genetic variation, health environment, management practices, and diets (Laben, 1963). Bovine milk is typically composed of 85-87% water with the remaining composed macronutrients and micronutrients (Foroutan et al. 2019). In commercial production of milk, milk fat and solids non-fat are typically standardized. In terms of fat, flavored and unflavored fluid milk is available as skim (less than 0.5), 1%, 2%, or whole or 3.25% fat milk. Lactose is the main carbohydrate and major non-fat milk solid in milk, comprising about 5% (Costa et al., 2019). Fluid cow’s milk contains on average 3.5% protein, with 80% of that amount consisting of casein proteins and the latter consisting of whey proteins (Vincent et al. 2016). Minerals make up the smallest percentage of non-fat solids in milk at 0.8 to 1% of the total composition (Gaucheron, 2005). Like other milk components, studies have found that mineral concentration of milk such as calcium, potassium,
magnesium, sodium, and phosphorus are influenced by the animal feed or breed of the cow (Soyeurt et al. 2009).

**Dairy Proteins**

**Casein Proteins**

Proteins in bovine milk are present in a colloidal solution or suspension. Casein proteins are one of the two main proteins found in bovine milk and are present in a colloidal suspension due to their relatively larger size (Bylund, 2003). Casein accounts for approximately 80 percent of the total protein and the white color of milk (Hoffman and Falvo, 2004). There are four types of casein proteins: $\alpha_{s1}$-caseins, $\alpha_{s2}$-caseins, $\beta$-caseins, and $\kappa$-caseins. $\alpha_{s1}$-caseins, $\alpha_{s2}$-caseins, and $\beta$-caseins proteins are classified as calcium sensitive while $\kappa$-caseins are considered as calcium insensitive (Perinelli et al., 2018). Casein proteins in milk can take the form of colloidal aggregates and are approximately 150 to 200 nanometers in diameter and are known as casein micelles (Fox and Brodkorb, 2008). These casein micelles exist in the serum phase of milk and are the prime nutritional source of calcium, phosphate, and amino acids in bovine milk (Horne, 2002). The casein micelle is comprised of submicelles that are bonded by hydrophobic interactions and electrostatic interactions with calcium phosphate (Walstra, 1990). Not to mention, casein micelle’s structure is highly hydrated and the high concentration of steric groups on the surface provides stability in its structure (McMahon and Brown, 1984). Understanding how casein micelles will react in different food environments is crucial in dairy processing operations. Calcium phosphate, a soluble mineral in milk also acts as buffer to protect casein micelles against high temperatures of thermal processing (Sadiq et al., 2021). As the temperature
of milk increases, the soluble calcium in the serum phase migrates into the casein micelle, aiding in maintaining the integrity of the colloidal casein micelle (Sadiq et al., 2021).

Milk is typically consumed at cold temperatures around 4°C or less, the pH of milk is on average around 6.7 (M’Hamdi et al., 2018). Specifically at refrigerated temperatures, the solubility of calcium phosphate in the milk serum phase increases, which also increases the solubility of casein micelles present due to the bonding between the submicelles becoming weaker (Schiffer et al., 2021). On the contrary, if the pH of milk is at 4.6 or below, gelation or precipitation of casein proteins will occur due to the loss of electrostatic interactions within the micelle (Li et al., 2022). There have been various models proposed to explain the supramolecular structure of casein micelles and there is not one unanimous model that has been confirmed. The ‘classical model’ of the casein micelle has several important elements (Phadungath, 2005):

1) The casein micelle is spherical but does not have a smooth surface.

2) It is built of smaller units called sub-micelles, which contain primarily casein.

3) Sub-micelles that form a casein micelle can have two types. One is composed of mainly \( \alpha_s \)-caseins and \( \beta \)-caseins while the other type is composed mainly of \( \alpha_s \)-caseins and \( \kappa \)-caseins.

4) Through bonding of calcium phosphate groups, the sub-micelles aggregate until they form a casein micelle.

5) \( \kappa \)-casein, which is calcium insensitive, will protrude outside from the casein micelle surface to prevent further aggregation of the sub-micelles through electrostatic and steric repulsion.
Whey Proteins

Whey proteins are the other one of the two main proteins found in bovine milk and are present in a compact, globular form due to their smaller size (Bylund, 2003). Whey proteins account for approximately 20 percent of the total protein content of milk (Hoffman and Falvo, 2004). Unlike casein proteins, whey proteins are dissolved in the serum phase of milk (Fox et al., 2015).

Table 1.1. Major types of whey proteins and characteristics. *

<table>
<thead>
<tr>
<th>Proteins</th>
<th>Concentration (gL⁻¹)</th>
<th>Molecular Weight (kDa)</th>
<th>Isoelectric Point (pI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-lactoglobulin</td>
<td>3-4</td>
<td>18.4</td>
<td>5.2</td>
</tr>
<tr>
<td>α-lactalbumin</td>
<td>1.5</td>
<td>14.2</td>
<td>4.7-5.1</td>
</tr>
<tr>
<td>Bovine Serum Albumin</td>
<td>0.3-0.6</td>
<td>69</td>
<td>4.7-4.9</td>
</tr>
<tr>
<td>Immunoglobulins</td>
<td>0.6-0.9</td>
<td>150-1000</td>
<td>5.5-8.3</td>
</tr>
<tr>
<td>Lactoperoxidase</td>
<td>0.006</td>
<td>89</td>
<td>9.6</td>
</tr>
<tr>
<td>Lactoferrin</td>
<td>0.05</td>
<td>78</td>
<td>8.0</td>
</tr>
<tr>
<td>Protease-peptone</td>
<td>0.5</td>
<td>4-20</td>
<td>unknown</td>
</tr>
</tbody>
</table>

*Adapted from (Tavares and Malcata, 2013).

Whey protein compounds have several functional, nutritional, and physiological functions. β-lactoglobulin and α-lactalbumin are the most prominent of the whey proteins, accounting for approximately 80 percent of all whey proteins in bovine milk (Madureira et al., 2007). β lactoglobulin, the predominant protein, comprises about 58% w/w of the whey protein in milk, and primarily has a β-sheet secondary protein structure (Madureira et al., 2007). Due to the low thermal stability of profiles of β-lactoglobulin and α-lactalbumin, these proteins have the largest influence on the structural changes of bovine milk during heating (Ryan et al., 2012). Thermal stability is the ability of proteins to withstand thermal processing without detrimental changes (Horne and Muir, 1990). Factors that will influence whey protein aggregation following thermal denaturation include the protein type, concentration, pH of the environment, ionic strength, heating temperature, and type of heat treatment (Kehoe and Foegeding, 2011). At
temperatures higher than 80°C, α-lactalbumin refolds into its native structure and aggregates with other unfolded monomers or protein molecules at the natural pH of milk (Akkerman et al., 2016). Studies have shown that β-lactoglobulin shows thermal denaturation and unfolding of its structure at 70°C at the pH of milk (de Wit and Swinkels, 1980). The unfolding of β-lactoglobulin will expose its thiol groups, increase its potential to form disulfide bonds, and lead to irreversible denaturation (Qian et al., 2017). Newly formed whey protein aggregates resulting from thermal denaturation can also lead to changes in viscosity, turbidity, particle size, and gel formation in bovine milk (Ryan et al., 2012).

Milk serum proteins, such as lactoferrin and immunoglobins, are also types of soluble whey proteins, but classified as such because they are present outside of the colloidal dispersion of casein micelles. However, due to their molecular form similar to counter ions and bound water, they are easily denatured at temperatures used for the commercial processing of milk and milk-based products (Larson and Rolleri, 1955). Because of the irreversible damage to serum protein conformations after thermal treatment, there is often extreme decrease or complete disappearance of their antimicrobial, anti-inflammatory, and immune-modulatory properties in the final milk product (Brisson et al., 2007). Lactoferrin is a serum protein that is becoming a further area of study due to potential antimicrobial activity that could be contributed to its iron- and lipopolysaccharide-binding properties (Farnaud and Evans, 2003). However, processing milk at a long hold time pasteurization treatment of 65°C for 30 minutes followed by acid precipitation of casein, showed a significant decrease in total native protein content of the fluid whey compared to unheated whey; this decrease was due to the denaturation of serum proteins such as lactoferrin (Xiong et al., 2020). In a separate study, native lactoferrin and iron-saturated lactoferrin in 36 mg/ml solutions were both completely denatured at a UHT processing treatment
of 90°C for 15 seconds (Paulsson et al., 1993). Immunoglobulin activity in cow’s milk decreased by 40% after application of a long hold long time pasteurization treatment of 65°C for 30 minutes and complete disappearance was observed after heating at 75°C and above for 30 minutes (Elgamy, 2000).

**Milkfat**

Milkfat makes up on average three to four percent of the total composition of milk and is present in the form of an oil-in-water emulsion (Bylund, 2003). It is naturally present in milk as a colloid and a spherical globular form and are the largest particles in milk, with an approximate diameter ranging between 0.1 to 20 micrometers (Dos Santos Morais et al., 2022). Milkfat is mostly composed of triglycerides and some diglycerides, monoglycerides, fatty acids, and sterols (Jensen et al., 1991). There is a complex membrane called the milkfat globule membrane, which consists of phospholipids, lipoproteins, proteins, nucleic acids, trace metal elements, and bound water (Lee et al., 2018). Saturated fatty acids make up 60 to 70 percent (w/w) of the total fatty acids and unsaturated fatty acids make up the 30 to 40 percent (Jensen et al., 1991). The diet, health, and environment of cows are some of the main influences on total milkfat production (Chilliard, 1993). From a nutrient perspective, milkfats provide high energy to calves in the early period of development and growth (Galli and Risé, 2017).

From a production perspective, milkfat can be utilized in the form of cream, butter, cheese, infant formula, yogurt, or ice cream products. Milkfat aids in the structure, flavor, mouthfeel, and texture of the previously mentioned products. These particles can play different and significant roles in different products depending on the amount, distribution, temperature, and other ingredients in the product (Waldron et al., 2020). Homogenization of milk causes a
reduction of the globule size and as a result, increases the stability of milk fat and the amount of surface area that is taken up (Ye et al., 2016). Inactivation of lipase, which is an enzyme in the serum phase of milk that breaks down fat, is crucial in milk because it can breakdown milkfat and release free fatty acids and subsequent hydrolytic rancidity. Studies have found that a heating temperature of 142°F was sufficient to prevent the development of rancid flavor and inactivate the lipase enzyme (Hetrick and Tracy, 1948).

Milk fat has the largest effect in the instrumental a (i.e., redness) and b* (i.e., yellowness) color values of fluid milk due to the fat globules on the surface of pasteurized milk reflecting more light at all wavelengths and less light being absorbed by green compounds in milk’s serum phase (Misawa et al., 2016). In unpasteurized milk beverages ranging from 0.2 to 1%, increasing milk fat had the largest effect in decreasing the a and b* instrumental values (Cheng et al., 2019). Milk sensory appearance, perceived mouthfeel, and perceived creaminess are also largely influenced by the fat level (McCarthy et al., 2017). Specifically, it was found that increasing levels of milk fat in milk resulted in a whiter sensory appearance, higher thickness, and higher residual mouth coating (McCarthy et al., 2017, Phillips et al., 1995).

Milkfat is mainly present in the form of triglycerides and contains about 400 different fatty acids and is derived from the feed and the microbial activity of the cow (Parodi, 2004). Myristic, palmitic, stearic, and oleic acids are the most prominent fatty acids in milk (Jensen et al., 1994). The variation in the amounts of different fatty acids can vary considerably and affect the physical, chemical, and sensory properties of dairy products. For example, myristic, palmitic, and stearic acid are saturated fats and will mainly affect the hardness of a dairy product (Bylund, 2003). Oleic acid is an unsaturated fat and will mainly influence the melting properties of a dairy product due to its long-chain saturated fat structure (Bylund, 2003). Because of the large
diversity of milk fatty acids present, milk fat does not have a true melting point, but instead, a melting range that spans about -40 to 40°C (Lopez et al., 2006). Triglyceride classes of milk fat are classified by their melting points and could fall in three categories: low, medium, or high melting point classes (Lopez et al., 2001). The low melting class makes up the majority of the total fat in milk at about 65 to 70% of fat while the medium melting class makes up about 25% and the high melting class makes up about 5 to 10% (Shi et al., 2001). These three main fractions of milk triglycerides would also often crystallize separately and at different temperatures in milk due to differences in their chain structures (Lopez et al., 2001).

**Standardization**

If milk is left to stand without homogenization, the fat will rise and form a layer of cream at the surface (Bylund, 2003). According to Stoke’s law, smaller milk fat globule size will decrease the cream separation rate due to the density difference between milk fat and the aqueous phase (Michalski, 2006). Milkfat content of fluid milk is standardized according to Food and Drug Administration regulations.

### Table 1.2. Standards for fluid milk and milk products based on 240 mL serving size as defined by the Food and Drug Administration. *

<table>
<thead>
<tr>
<th>Product</th>
<th>Standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole Milk</td>
<td>(21 CFR § 131.110) Not less than 3.25 percent total milkfat</td>
</tr>
<tr>
<td>Acidified Buttermilk</td>
<td>(21 CFR § 131.111) Not less than 3.25 percent total milkfat</td>
</tr>
<tr>
<td>Cultured Buttermilk</td>
<td>(21 CFR § 131.112) Not less than 3.25 percent total milkfat</td>
</tr>
<tr>
<td>Reduced Fat Milk</td>
<td>(21 CFR § 101.62) At least 25 percent less total fat</td>
</tr>
<tr>
<td>Low-fat Milk</td>
<td>(21 CFR § 101.62) Maximum of 3 g or less total fat</td>
</tr>
<tr>
<td>Nonfat, skim, or fat-free milk</td>
<td>(21 CFR § 101.62) Less than 0.5 g of total fat</td>
</tr>
</tbody>
</table>

*Adapted from (CFR Title 101 Section 131.110, 2022).*
Lactose

Lactose is the most abundant solid non-fat component of milk and comprises approximately 3.6 to 5.5 percent of the total composition of bovine milk (Bylund, 2003). It is a water-soluble disaccharide consisting of the monomers, glucose, and galactose, linked by a β-glycosidic bond. For human consumption of bovine milk, lactose composes 30 to 40 percent of the total calorie content in milk, as it is an abundant source of carbohydrates (Shendurse and Khedkar, 2016). Lactose can be derived from large-scale whey or permeate production, but oftentimes, these product streams are disposed of due to the difficulty of handling (Zadow, 1984). There are many advantages in utilizing the lactose of milk in commercial food applications due to the flavors and colors it carries (Zadow, 1984). For example, lactose can be used in baked goods because can provide browning and caramel flavors in the products (Nickerson, 1975). Lactose that has been separated from milk or whey can also be manufactured into derivatives such as lactulose, lactitol and galacto-oligosaccharides ingredients for other sweetener ingredient applications (Schaafsma, 2008). Like other reducing sugars, lactose exists in a partially open-chain form with an aldehyde that can form a hemiacetal and ring structure. Reducing sugars with this property creates a chiral center and may exist as α-lactose, β-lactose, or a mixture of α. and β- isomeric forms (López-Pablos et al., 2018). The form that lactose takes is dependent on the temperature of the milk or the processing method applied. The ability to change between these different anomer forms in a solution is referred to as mutarotation and has a significant role in lactose crystallization of bovine milk (Sánchez-García et al. 2021). The solubility of α-lactose is 70 g1⁻¹ and the solubility of β-lactose is 500 g1⁻¹ (Schaafsma, 2008). In fluid cow’s milk at room temperature, 63% of the lactose is in the β-lactose form due to β-lactose having higher solubility (Zadow, 1984). However, at temperatures higher than 93.5°C, β-lactose
is less soluble than α-lactose and has the ability to form crystals (Schaafsma, 2008). Regarding
the functionality of lactose in spray-dried milk, crystallization is unable to occur because of the
rapid increase in viscosity of milk needed to prepare the final product (Aguilar, 1993). This
results in lactose to exist as a mix of both α- and β- isomeric forms and is resistant to air but is
extremely hygroscopic due to the β-lactose content (Aguilar, 1993).

When compared to other reducing sugars at the same concentration, lactose is much less
sweet than that of glucose, galactose, and sucrose (Zadow, 1984). However, when lactase
enzymes, specifically β-galactosidase, hydrolyze lactose in milk in the human digestive system,
lactose is digested as individual monomers of α-D-glucose and β-D-galactose (Romero-Velarde et
al., 2019). In lactose-free milk products that are made through lactase hydrolysis, the sweet taste
is a driver of liking among consumers who purchase these products (Rizzo et al., 2020).
However, studies have found that consumers who consume only traditional milk have indicated
that the sweetness in lactose-free milk is a driver of disliking (Adhikari, 2010). On the other
hand, the hydrolysis of lactose through acid or heat in milk can result in the formation of
discoloration and off-flavors in the final product (Zadow, 1984). Some undesirable sensory
attributes that can result from the mentioned processes include cooked flavor, lack of freshness,
astringency, and chalkiness (Adhikari, 2010). These attributes are specifically a result of a series
of Maillard browning and the denaturation of whey proteins in milk (Messia et al., 2007).

Minerals

Minerals in milk include cations such as calcium, magnesium, sodium, and potassium
and anions such as phosphorus, citrate, and chloride. The minerals in milk have a crucial role in
the structure and stability of casein protein micelles (Gaucheron, 2005). Nutritionally, the
minerals in milk also play significant roles in the biochemical functions of the human body. There are also different recommended daily intake amounts and health benefits for all the essential macronutrients, micronutrients, and trace elements found in milk (Kandhro et al., 2022). The mineral content in milk is influenced by factors such as the stage of lactation, health status of the cow, environment, and genetics (Cashman et al., 2006). Calcium is the most prominent mineral in milk and typically makes up 23 to 28 percent of the total mineral concentration of milk (Gaucheron, 2005). Balancing the amount of cationic and anionic minerals heavily influences the physical state and stability of the casein milk proteins (de la Fuente et al., 2021). The acidity and temperature of the environment will affect the solubility of the proteins, which is the balance between attractive and repulsive intermolecular forces.

Depending on the type of the overall mineral content in milk or the mineral salt added to milk, stabilization or unfolding of the milk proteins may occur (Augustin, 2000). For example, in milk at temperatures below 15°C, there is an increased release of casein monomers that solubilize into the serum phase of milk (Schiffer et al., 2021). Even with induced heating, acidification, or mineral adjustment in milk, studies have found that the reversibility of casein protein changes in milk are related to the ability to re-form colloidal calcium phosphate (Gebhardt et al., 2023). Others have found that higher levels of ionic calcium in the milk serum are related to lower heat stability in milk and higher intensities of fouling (Singh et al., 2021). Colloidal calcium phosphate is the main ligand that the micronutrient zinc has the highest affinity to bind with. The binding of zinc to colloidal calcium phosphate at temperatures between 20 to 40°C results in phase separation and precipitation of calcium phosphate (de la Fuente et al., 2021). Chelants or chelators, are chemical compounds that will form a water-soluble complex between itself and metal ions, such as minerals (de Kort et al., 2012). Adding anionic chelants
such as phosphorous and citrate will act as chelants and reduce the calcium activity in milk or concentrated milk products and in turn, improve its heat stability (Singh et al., 2021). In relation to UHT treatments of concentrated milk protein systems, the precipitation of calcium phosphate during processing was found to be one of the factors that caused heat-induced aggregation of casein micelles (Dumpler et al., 2020). It was also found that excessively high calcium activity in concentrated milk beverages resulted in larger denatured whey protein aggregate sizes after heating to 120°C (Crowley et al., 2015).

**Water**

Water is a chemical compound of hydrogen and oxygen and makes up about 86 to 90 percent of the total composition of milk (Bylund, 2003). The interaction between water and milk protein is considered as its water-holding capacity, retention, or hydration (Kneifel and Seiler, 1993). Depending on what is desired, the water content of fluid milk can be altered through different food processing techniques to produce different final products such as milk powder, condensed milk, evaporated milk, milk protein beverages, and more.

**Milk pH**

In chemistry, pH refers to the function of free hydrogen ions in a solution and its scale is used to specify the acidity or basicity of aqueous solutions. The range is from zero to 14. A pH value of seven is neutral, because pure water has a pH value of exactly seven. Values lower than seven are categorized as acidic and values greater than seven are categorized as basic or alkaline (Rodrigues, 2023). Milk has an expected pH of 6.8 and any variance may be an indication of contamination or infection (Hage et al., 1998). In cultured dairy products, there is lactic acid,
which increases the acidity of the product and can also inhibit the growth of pathogenic organisms (Steinkraus, 1992). pH gel electrode instruments are used to give accurate and quick results of the hydrogen ion concentration in dairy products. Instruments are designed based on the production environment and the specific type of dairy product. For example, many have electrodes capable of being inserted into semi-solid matrices such as cheese (Rodrigues, 2023). The pH is temperature sensitive so careful attention to temperature is needed. Both the instrumental calibration and the samples to be measured must be at the same temperature.

**Filtration Processing**

**Types of Filtration**

Microfiltration, ultrafiltration, nanofiltration, and reverse osmosis are all separation technologies that utilize a membrane, and the differences between the technologies for separation are based on the molecular pore size (Bylund, 2003). All these processes are pressure driven, temperature driven, used to maintain the functional properties of milk, and beneficial for commercializing lactose-free milk and increased protein products (Reig et al., 2021).

![Illustration of filtration membranes: microfiltration (MF), ultrafiltration (UF), nanofiltration (NF), reverse osmosis (RO) taken directly from Saboya and Maubois (2000).](image)

**Figure 1.1.** Illustration of filtration membranes: microfiltration (MF), ultrafiltration (UF), nanofiltration (NF), reverse osmosis (RO) taken directly from Saboya and Maubois (2000).
Figure 1.2. Illustration of components of milk removed through membrane processes and size indication: microfiltration (MF), ultrafiltration (UF), nanofiltration (NF), reverse osmosis (RO) taken direction from Saxena et al., (2009).

In all these filtration processes, milk or whey is separated into permeate and retentate portions. The “permeate” is the portion of milk or whey that passes through the membrane and “retentate” is the portion of milk or whey that does not pass through the membrane. The permeate portion may contain lactose, minerals, water, and water-soluble vitamins which are lost with water. The retentate portion contains all the milk protein components (Tremblay-Marchand et al. 2016). Depending on the membrane used, whey and serum proteins can also be separated from fluid milk into the permeate while casein remains in the retentate (Saboya and Maubois, 2000). Diafiltration is also a technique applied during filtration processes to obtain better purification of the casein proteins while washing out residual components of the permeate (Bylund, 2003). Typically, diafiltration is introduced in the filtration process when the protein concentration in the retentate reaches as high as possible while membrane fouling and water consumption are minimized (Gavazzi-April et al., 2018).
Components of a Membrane Filtration System

All separation techniques that utilize a type of membrane will have its pores designed based on the molecular size and/chemical composition of what needs to be separated (Daufin et al., 2001). The configuration of membranes is classified as either flat, spiral wound, or tubular. The material of membranes are classified as polymeric, liquid, solid (i.e. ceramics), or ionic exchange categories (Wenten, 2005). Ultimately, the design of the membrane configuration and materials should aim to maximize the water flux, which is also known as the selectivity of solutes that are removed into the permeate (Harder et al., 2009). In all membrane filtration systems, pressure support systems, feed inlets, retentate concentration outlet ports, and permeate outlets are required (Wenten, 2005) The main challenge with filtration processes is membrane fouling, which is the build-up of milk proteins, milkfat, and bacteria inside the porous support layer of the membrane (Guerra et al, 1997). Fouling causes a decline in flow and processing parameters such as equipment design, temperature, feed concentration, flow, and pressure can contribute to its occurrence. There are two types of fouling that can occur 1) internal fouling, which is the constriction of pores by feed particles and 2) the deposition of rejected solutes and particles on the membrane surface (Gesan-Guiziou, 2013). To minimize fouling, the process cannot stop and must have constant shear stress applied in an axial direction with the feed flow. Chemical cleaning is the only solution to remove fouling, thus, the materials used to create membranes must be durable to cleaning agents used in the dairy industry (Guerra et al, 1997).

Microfiltration

Microfiltration is a membrane-based filtration process used to separate particles suspended in milk that fall within a 0.1 to 10 um size range (Saboya and Maubois, 2000). With a
pore size of 0.1 um, casein proteins are retained while fat globules, serum proteins, lactose, minerals, water, and water-soluble vitamins are separated into the permeate (Bylund, 2003). This process produces MCC (Tremblay-Marchand et al., 2016). One of the barriers in commercial production of MCC is the risk of high fouling and incompatible flow of the retentate across the membrane (Gesan-Guiziou, 2013). When retentate is too high in protein and fouls a membrane due to build up on the surface, it becomes difficult to achieve the appropriate level of applied pressure and cross flow velocity to continue with the filtration process (Gesan-Guiziou, 2013). In the dairy industry, polymeric spiral-wound membranes are more commonly used because of their low capital and replacement costs (Meyer et al., 2015). Ideally, microfiltration can be conducted with tubular ceramic membranes because their design allows for more turbulent conditions and achievement of higher flux, lowering the risk of irreversible fouling compared to polymeric membranes (Zulewska et al., 2009). Use of ceramic membranes is advantageous because it minimizes the amount of permeate produced and diafiltration required, however, the benefits come at the expense of higher capital and operational energy costs (Hurt and Barbano, 2010).

**Ultrafiltration**

Ultrafiltration membrane separation is used to separate particles suspended in milk that fall within a 0.01 to 0.1 um size range (Saboya and Maubois, 2000). With a pore size of 0.01 um, casein and serum proteins can be retained in the milk while lactose, minerals, water, and water-soluble vitamins are separated into the permeate (Bae and Tak, 2005). Unlike microfiltration, UF membranes are conventionally polymeric and can take on a wider variety of forms such as hollow-fiber, spiral wound, plate-and-frame, or tubular (Kammakakam and Lai, 2023). Ultrafiltration is the main filtration process used for milk prior to cheese production to optimize
the production capacity of cheese plants (Lauzin et al., 2020). The increase in cheese yield is attributed to the higher concentration of whey proteins in the aqueous phase of the final cheese product (Lauzin et al., 2020). Studies have also found that ultrafiltration of milk for cheese manufacture resulted in considerably less whey expelled compared to milk that did not undergo ultrafiltration (Yan et al., 1979).

Ultrafiltration was first applied to fluid whey to concentrate whey protein when new pollution abatement regulations made it more challenging for dairy processors to discard fluid whey into water streams (Melachouris, 1984). Because of the higher protein contents associated with the production of whey protein concentrate (WPC) or whey protein isolate (WPI), diafiltration is used during ultrafiltration to prevent fouling and facilitate lactose and mineral removal (de la Fuente et al., 2002). Ultrafiltration with diafiltration and no protein concentration is also used to reduce the lactose content in milk before it is spray dried to make MPC powders (Rupp et al., 2018). Both liquid and powder MPC produced by ultrafiltration are popular ingredients that can be used for neutral pH RTD protein beverages because of their high solubility, high protein, and low lactose content.

Nanofiltration

Nanofiltration is used to separate particles suspended in milk that are as small as 0.001 um or less. The typical pore size of nanofiltration membranes falls between 0.4 to 2.0 nanometers (Saboya and Maubois, 2000). The advantages of nanofiltration include relatively low initial investment, low operational costs, achievement of high flux, and the ability to operate at low temperatures (Cao et al., 2015). The solutes that are removed through nanofiltration are present in the form of a true solution, such as organic compounds and monovalent salts and
typically fall into a molecular weight range of 200 to 1000 g mol\(^{-1}\) (Peeva et al., 2010). The pH and processing temperature of the feed stream can be altered to selectively remove charged minerals present in milk while retaining lactose (Saboya and Maubois, 2000). Nanofiltration is commonly used in the dairy industry for partial demineralization of monovalent salts, such as NaCl, in fluid whey that could negatively affect product quality (Horst et al., 1995). Divalent salts with high nutritional value such as calcium, magnesium, and phosphorus, along with protein and lactose, are retained in fluid whey after nanofiltration (Suárez et al., 2006). Nanofiltration can be used as an additional non-thermal processing step on milk retentate that has undergone ultrafiltration to completely remove residual salts and organic molecules (Cao et al., 2015).

**Reverse Osmosis**

Reverse osmosis is the smallest of the membrane pore sizes and is typically less than 0.001 um (Saboya and Maubois, 2000). The process is typically practiced in the dairy industry alone or in combination with other filtration or multi-stage evaporation processes to concentrate solids in milk (Stabile, 1983). The permeate of reverse osmosis will only contain water and small amounts of organic solids, such as vitamins, making it an energy efficient concentration process to use in place of thermal evaporation prior to spray drying because it does not require heating (Beldie and Moraru, 2021). Reverse osmosis is also used on fluid whey streams resulting from cheese make or ultrafiltration in order to save costs in transportation (Hiddink and Boer, 1979). Using solely reverse osmosis to concentrate the solids in milk only has a maximum practical solids concentration is between 25 to 30%, however, using reverse osmosis in combination with multi-stage evaporation allows for a maximum solids concentration of about 45% (Stabile, 1983). Reverse osmosis can be used in conjunction with evaporation in place of evaporation
alone to concentrate solids and achieve a non-fat dry milk with lower cardboard and higher sweet aromatic sweet flavors compared to the flavor of product produced using vacuum evaporation alone (Park and Drake, 2016).

**Lactose-Free Milk and Beverages**

In the food industry, new technologies are being created to produce a variety of milk products with higher protein, higher calcium, and lower lactose content (Prosch, 2020). Lactose-free dairy products have a growing appeal as they provide all the essential nutrients present in milk, but also meet the needs of individuals who are lactose intolerant (Suchy et al., 2010).

**Definition of Lactose-Free Milk and Beverages**

In order to make “lactose-free” label claims, although there is no specific definition defined by the Food and Drug Administration, the product must be specially processed to not have the expected nutrient (CFR 21 Title 101 Section 101.65, 2022). Manufacturers must ensure that information on their food labels and nutrition labels are truthful and not misleading (CFR 21 Title 101 Section 101.13, 2022). Since there is no specific legislation or restriction, most manufacturers have different options to make claims such as “absence of lactose”, “lactose-free”, “lactose reduced”, “low lactose”, or “100% lactose-free” (Gerdes, 2021). It is important to note that the Food and Drug Administration does not have a specific definition for “dairy-free” products. However, it is important to note that the term “dairy-free” is different from “lactose-free”. The use of “dairy-free” on a label indicates that the product does not contain any ingredients sourced from milk and no form of milk allergen whatsoever (Food and Drug Administration, 2022). In the case of “non-dairy” products, they can contain 0.5 percent of milk
by weight, only caseinate ingredients can be used, and must indicate on the label that the product contains “milk” (CFR 21 Title 101 Section 101.4, 2022). Since casein milk proteins and ingredients do not contain lactose, it is also likely for “non-dairy” products to also be referred to as “lactose-free” (Food and Drug Administration, 2022).

**Increasing Lactose Intolerance in Consumers**

One of the largest reasons consumers avoid the consumption of dairy is an increase of diagnosis or perception of lactose intolerance (Zingone et al., 2017). It is estimated that 70% of the world’s population is afflicted with some degree of lactose intolerance (Storhaug et al., 2017). Lactose intolerance is a clinical syndrome where the small intestine of individuals lacks the lactase enzyme, which is necessary for digesting lactose. Common symptoms of lactose intolerance include diarrhea, nausea, stomach cramps, bloating, and gas (Deng et al., 2015). The dairy industry is one of the largest food industry sectors despite the high number of cases of lactose intolerance due to most people learning to consume milk within their physiological limitations (Silanikove et al., 2015). Many consumers believe the main advantage of plant-based milk alternatives is that they are lactose-free (Adamczyk et al., 2022).

**Current Lactose-Free Milk and Beverage Market**

In 2021, the global lactose-free dairy market was valued at $11.5 billion USD and is projected to reach $24.5 billion USD by 2031 (Allied Market Research, 2022). Lactose-free milk can also be found in the form of other dairy products such as coffee creamers, protein shakes, and ice cream (Gerdes, 2021). The top claim consumers look for in plant-based milk beverages are low/no allergens, dairy-free, and kosher (Buchet, 2022). To compete with the plant-based
milk market, lactose-free milk beverages with label claims such as “high protein”, “high calcium” and “organic” are most sought after (Rizzo et al., 2020). Understanding consumer preferences in lactose-free dairy milk products is necessary for further market growth. With the large and significant market potential of lactose-free milk products, beverage innovation needs to appeal to the key liking factors found in previous studies for further growth. In the dairy industry, enzymatic hydrolysis of lactose and filtration processing are common technologies used to produce lactose-free dairy milk (Li et al., 2023). One strategy for removing lactose is by adding soluble lactase to milk followed by incubation (hot or cold) before pasteurization. This method typically results in an increase in sweetness in milk due to the higher availability of monosaccharides (Dekker et al., 2019). Another strategy is to first pasteurize the milk and proceed with a filtration process to physically remove all the lactose (Dekker et al., 2019). One company, Fairlife, utilizes membrane processing in combination with lactase addition to produce milk that contains 50% more protein than traditional fluid milk and is lactose-free (Prosch, 2020).

**Heat Stability**

After the commercial sterilization of milk was developed in the early 20th century, many heat stability studies have been motivated by availability of new technologies, legislation, and consumer needs (Dumpler et al., 2020). Heat stability refers to the ability of milk to withstand heat treatment for a duration of time without coagulation or gelation. The optimization of thermal processes must be performed to maintain the desirable aspects of milk while also preventing undesirable physical, chemical, and sensory changes (Dumpler, 2017).
Coagulation

Coagulation is characterized by the visible formation of proteinaceous flocs, or large, dense particles (Dumpler et al., 2020). When milk is heated to temperatures that are too high (i.e., ultra-high pasteurization temperatures), there are changes in the mineral equilibrium between the serum and colloidal phases that lead to heat-induced coagulation (Pandalaneni et al., 2018). Previous studies suggest heat-induced coagulation occurs due to swelling of casein micelles and submicelles caused by adsorption of positively charged precipitated calcium phosphate that has dispersed into the serum phase upon heat (O’Connell and Fox, 2000). A sensorial indication of coagulation in fluid dairy milk or concentrated milk systems may involve a coarse or sandy mouthfeel (Dumpler, 2017). Floc formation due to heat-induced coagulation will also separate milk into serum, protein precipitation or sedimentation, and cream layer phases (Dumpler et al., 2020). To prevent coagulation in dairy protein beverages undergoing UHT processing, salts with heavily charged negative ions are added to stabilize calcium bound to the casein micelle surface (Webb and Holm, 1932).

Aggregation

Protein aggregation can occur in milk through exposure to high heat, high pressure, low pH, and the addition of salts (Zhou et al., 2008). Milk protein aggregates formed from heat can either be soluble and remain dispersed, or insoluble and lead to precipitation (Singh et al., 2022). Native whey proteins can easily form aggregates high acid or salt environments at temperatures between a range of 20 to 45°C, which is far below what is required for pasteurization (Ju et al., 1999). Studies have found that at ultra-high heating temperatures, aggregates are composed of mainly κ-caseins, β-lactoglobulin, and other whey proteins linked through thiol-disulfide and
hydrophobic bonds (Li et al., 2021). Carroll et al. (1971) found that casein micelles in concentrated milk system and skim milk become larger than their original size after UHT thermal processing; aggregates are formed with fragments of soluble κ-casein that has moved out of the micelle upon cooling. Considering the sensitivity of whey proteins, at ultra-high processing temperatures starting at 90°C, substantial aggregation of whey proteins occurs due to loss of their folded structures, but there are only minor charges in the physical dimensions of casein micelles (Morr, 1969). Buggy et al. (2018) determined that whey protein isolate solutions at pH 6.2 had greater proportions of insoluble aggregates, higher particle size, and higher turbidity compared to solutions at pH 6.7 and 7.2. In a study focused on the heat stability of 5 to 10% w/w reconstituted and liquid MCC protein beverages, UHT processing of the beverages resulted in visible particle aggregation and coagulant structure formed due to calcium phosphate precipitation and heat-induced dissociation of κ-casein (Beliciu et al., 2012). To determine the state or type of protein aggregation in milks or milk powders, sulfhydryl determination, surface hydrophobicity, mid and near infrared measurement, electron microscopy, capillary electrophoresis, and polyacrylamide gel electrophoresis methods can be applied (Li et al., 2021; Patel et al., 2007).

**Denaturation**

Denaturation in milk is the unfolding of proteins and the loss of native structure; this causes proteins to lose or unfold their native structure into a disordered, random structure (Qian et al., 2017). The principal effect of thermal processing is protein denaturation. Thermally denatured milk proteins, predominantly involving β-lactoglobulin, can form complexes through either disulfide, hydrophobic, or covalent bonding (Considine et al., 2007). Whey proteins such as lactoferrin denature at temperatures of about 65°C, while β-lactoglobulin and α-lactalbumin
Denature at temperatures between 70 to 75°C, temperatures that are much lower than what is required for sterilization of milk (Vasbinder and de Kruif, 2003). When the thiol groups of β-lactoglobulin are exposed and form disulfide bonds with other whey proteins or κ-casein, the denaturation at this point is considered to be irreversible (Qian et al., 2017). Studies have also found that the size of aggregate particles formed from denatured β-lactoglobulin and other serum proteins is limited to the amount of κ-casein present on the surfaces of casein micelles (Dalgleish, 1990). Higher serum protein denaturation was found to increase casein micelle particle size and increase the L-values, or whiteness values of direct and indirect ultrapasteurized milks (Lee et al., 2017). “Cooked” flavors, also distinct to ultrapasteurized milks, are derived from serum protein denaturation and the formation of volatile sulfur compounds upon high temperature treatment (Jo et al., 2019). In liquid MPC beverages with no buffering salts, the casein percentage of true protein amount increased compared to non-heated beverages after ultrapasteurization due to thermal denaturation of milk serum proteins (Hoyt et al., 2022). Lee et al. (2017) demonstrated that indirect and direct ultrapasteurized skim milk had significantly higher furosine concentrations (mg/100g) compared to high temperature short time pasteurized milk, indicating more severe thermal damage to milk proteins in the ultrapasteurized skim milks. Identification of denatured proteins in reconstituted milk powders and fluid milk products can also be characterized through polyacrylamide gel electrophoresis methods (Li et al., 2021). The degree of denaturation can also be indicated with whey protein nitrogen content, which is a measure of the undenatured whey protein nitrogen level (Patel et al., 2007).
**Relevant Ultrafiltration Processing Temperature Research**

As mentioned previously, there is a growing need for production of nutritious low lactose and lactose-free dairy products with desirable sensory properties due to the high prevalence of lactose intolerance in the world’s population (Suri et al., 2019; Storhaug et al., 2017). Studies have found that consumers diagnosed with lactose intolerance can consume up to 10 g /day lactose and have no observable adverse reactions to 2 g /day lactose (Li et al., 2023). Although there is no standard on the lactose threshold to label a product as “low lactose” or “lactose-free”, most commercial products are within 1 g/100 g or 10 mg/ 100g, respectively (CFR 21 Title 101 Section 101.65, 2022; Li et al., 2023). The retentate produced by ultrafiltration of skim milk is lower in calories compared to lactose-hydrolyzed milk due to the physical removal of lactose and minerals (Kosikowski, 1979). After enzymatic lactose hydrolysis, the total carbohydrate and calorie contents remain constant due to lactose being broken down into monosaccharides, which also make the milk sweeter (Zadow, 1984). In the industry, ultrafiltration of skim milk is typically conducted at about 10 or 50°C; each operational temperature comes with its own advantages and disadvantages (Ng et al., 2018). Operation at higher and hotter temperatures (40 to 50°C) can achieve higher flux of milk resulting from lower permeate viscosity, however, growth of thermophilic microorganisms is promoted (Kapsimalis and Zall, 1981). On the contrary, Schiffer and Kulozik (2020) found high bacterial growth during microfiltration of skim milk at 10 and 20°C, with the cause presuming to be remaining psychrotolerant bacteria that survived the pasteurization process. The rate of flux decline was found to be significantly higher (P<0.05) during a processing temperature at 50°C compared to temperatures of 15 and 30°C (Luo et al., 2015). In that same study, Luo et al. (2015) determined that operating ultrafiltration at 15°C took less time to reach 5x concentration and had less membrane fouling compared to
operating at 50°C. Permeate flux will occur in the early stages of ultrafiltration at hotter temperatures and is associated with irreversible fouling, increased cleaning time, labor, energy costs, and shorten membrane life (Tong et al., 1988). Studies have also shown that performing ultrafiltration on skim milk beginning at 50°C is more prone to fouling on the surface of a polymeric spiral wound membrane, predominantly caused by proteins and minerals (Bégoin et al., 2005). Ng et al. (2018) found higher magnitudes of irreversible fouling and pore blockage by α-lactalbumin and β-lactoglobulin on the membrane surface when conducting ultrafiltration at 50°C compared to lower temperatures. Thermophile growth was inhibited at colder temperatures (10°C or less), but there was a lower flux in the permeate viscosity (Ng et al., 2018). Kapsimalis and Zall (1981) found that decreasing the temperature of ultrafiltered milk resulted in a decrease in flux (ml/minute) averaging about 3 percent per °C reduction. In ultrafiltration at 15°C, deposit formation still occurred due to dissociation of β-casein from the casein micelles; increasing membrane pore size also did not increase milk flux at low temperatures (Kapsimalis and Zall, 1981). Lee and Merson (1974) also determined that the deposit throughout the membrane resulting from ultrafiltration conducted at 23°C contained high casein content and precipitates of calcium phosphate.

Ultrafiltration a common process in fluid milk industrial manufacture but is run at either a cold (10°C or less) or hot temperature (45°C or more) in different factories (Kapsimalis and Zall, 1981). Understanding the effects of ultrafiltration processing parameters on casein micelles and mineral composition in milk could lead to improvements in processing and heat stability (David et al., 2008; Dumpler et al., 2020). Previous studies have found that when milk is below 7°C, calcium and phosphate are more soluble and move out the casein micelle (Koutina et al., 2014). Increased solubility of calcium and phosphate from the casein micelle will also increase
the pH (Schiffer et al., 2021). The formation of casein micelles is strongly dependent on pH and ionic calcium available in milk, meaning that heat stability of ultrafiltered milk will be dependent on these factors as well (Dumpler, 2017). Liu et al. (2014) found that when ultrafiltration temperature was at 10°C, the proportion of soluble casein and calcium removed in skim milk was higher than at 40°C. Through polyacrylamide gel electrophoresis analysis, Liu et al. (2014) determined that β-caseins were the predominate protein to solubilize into the serum phase, while α-caseins and κ-caseins were relatively stable. Other than differences in compositional properties, there were also differences in water and energy costs when operating ultrafiltration at a cold or hot temperature. For example, Méthot-Hains et al. (2016) found that there was less thermal energy consumption at 10°C than at 50°C during ultrafiltration of skim milk to produce 3.6x milk retentate on a pilot scale, however, operating at 10°C did require 2.3 times more pumping energy than operating at 50°C. In that study, skim milk ultrafiltration would have the highest processing efficiency with maximized permeation flux (kg/h) at low transmembrane pressure (465 kPa) and 50°C operating conditions (Méthot-Hains et al., 2016). The volume of diafiltration water used in an ultrafiltration run can be determined by multiplying the water flow rate (kg/hour⁻¹) by the duration of diafiltration (hours) (Gavazzi-April et al., 2018). While there are many studies on the differences in milk flux, mineral composition, and casein micelle behavior at cold and hot processing temperatures, there is a lack of research on the retentate produced at different temperatures in ready to drink protein beverage applications. Ultimately, studies focused on the comparison of mineral, physical, and sensory properties of ultrafiltered milk made at different processing temperatures will allow manufacturers to make the best choice for product development of low lactose and lactose-free high protein beverages.
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CHAPTER 2: LITERATURE REVIEW: THE PRODUCTION, PHYSICAL PROPERTIES, AND HEAT STABILITY CHARACTERISTICS OF MILK PROTEIN BEVERAGES

Tina Truong, Alex Hernandez, David Barbano¹, and MaryAnne Drake²

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

²Corresponding 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
E-mail : mdrake@ncsu.edu
**Interpretive Summary**

Removal of 99% of lactose to make a high-protein, high-calcium lactose-free skim milk beverages produced beverage bases that were more bland, more white, and contained 2 to 2.5 times more calcium per serving than skim milk.
**Introduction**

The global lactose-free dairy products market is currently valued at $13.5 billion and expected to reach $23.9 billion by 2033 (Future Market Insights, 2023). Lactose-free dairy beverages are the most common form available and held a larger market share compared to lactose-free yogurt, cheese, and other dairy products (Allied Market Research, 2021). The pathology of lactose intolerance has been reviewed by Li et al. (2023). Storhaug et al. (2017) provided national, regional, and global estimates in a meta-analysis of lactose malabsorption assessed by a combination of methods, lactose malabsorption has an estimated global prevalence of 68%, indicating that lactose malabsorption is widespread in most of the world. Zingone et al. (2017) reported that consumers replace cow’s milk with plant-based alternative milks in their diets when they think they are lactose intolerant. However, plant-based milks are not nutritionally comparable or equivalent to cow’s milk (Vanga and Raghavan, 2018). Dairy protein is a complete protein, meaning that it contains all essential amino acids while individual plant-based proteins sources lack one or more essential amino acids and are not considered complete proteins (Hoffman and Falvo, 2004). The dairy industry has made lactose reduced and lactose-free milk available by either hydrolyzing lactose using lactase or partially removing lactose (to reduce calories) and hydrolyzing the remaining lactose, while providing the original or increased concentration of calcium, phosphorous, and protein in various fat-free, or low-fat beverage milks.

Ultrafiltration (UF) is a physical membrane filtration process that physically removes lactose, minerals, other low molecular weight water soluble compounds, and water from milk as filtrate, while retaining protein, protein bound minerals, and fat as retentate. The concentration of lactose and soluble mineral decreased slightly in the UF retentate due to the increase in fat and
protein concentration (Hernandez et al. 2023). In skim milk, about 66% of the total calcium is bound to casein and about 34% is soluble in the UF permeate portion of the skim milk (Rehman, 2009). In a 3X UF concentration process for skim milk, protein concentration is increased from about 3.4 to 10.5% while lactose concentration only decreases from about 4.7 to 4.2% (Hernandez et al. 2023), even though 66% of the lactose has been removed. Diafiltration (DF) is a technique of adding solvent (i.e., water) to the UF retentate (either continuously or discontinuously) during the UF of milk or whey to wash out low molecular weight soluble milk components in the permeate (Cheryan, 1998) and this process greatly reduces the concentration of lactose and soluble mineral concentration in the UF retentate. When a UF concentration factor of 3X is used without DF, about 66 to 67% of the lactose removed in the permeate and 33 to 34% is retained in the retentate (Rehman, 2009). For commercial high protein UF milk, residual lactose is hydrolyzed to form glucose and galactose with the added enzyme β-D-galactosidase to make low-lactose (less than 1g/100g) or lactose-free (less than 10 mg/100 g) products (Li et al., 2023). With continuous diafiltration and permeate replacement of skim milk starting with a mean anhydrous lactose content of 4.75 +/- 0.04%, Hernandez et al. (2023) was able to achieve a mean ending lactose content at 0.18 +/- 0.01% for a 96.2% lactose removal by continuous ultrafiltration. With one 3X concentration stage, followed by two 3X total volume replacement DF stages in a 3-stage process to produce 7.5% protein milk protein concentrate, Hoyt et al. (2023) achieved a mean ending UF retentate lactose content of about 0.64%. In terms of mineral composition of UF retentate after UF, the bound calcium has been concentrated in direct proportion to the increase in final protein concentration. Hernandez et al. (2023) found a large decrease in milk titratable acidity (TA) from 0.18 to 0.04% due to the removal of low molecular
weight soluble components (e.g., mineral, citrate, etc.) even though the protein concentration remained the same, while when protein concentration was increased by UF, the TA increased.

Liu et al. (2014) reported that during UF concentration of skim milk the progressive removal of calcium was affected by the temperature dependent partitioning of calcium between the micelles and the serum with more calcium removed in permeate at 10°C than 40°C. Performing UF at different temperatures therefore altered the final calcium content of the retentates. The composition of casein micelles including the hydration, calcium and casein content were all altered to some extent by the temperature of UF (Liu et al., 2014).

Other than differences in compositional properties of UF retentates, there are also differences in energy costs when operating UF at different temperatures. Méthot-Hains et al. (2016) found that there is less thermal energy consumption at 10°C than at 50°C during ultrafiltration of skim milk to produce 3.6x milk retentate on a pilot scale, however, operating at 10°C did require 2.3 times more pumping energy due to higher retentate viscosity than operating at 50°C. Skim milk UF had the highest processing efficiency with maximized permeation flux (kg/h) at low transmembrane pressure (465 kPa) and 50°C operating conditions (Méthot-Hains et al., 2016). Gavazzi-April et al. (2018) determined that the sequence of operation and different concentration factors during UF of skim milk at 50°C also influenced the amount of membrane surface area to process the same volume of skim milk. A higher efficiency of removal of water-soluble milk components and reduced membrane surface area were required at 50°C which minimized energy consumption. Membranes with different molecular weight cut-off had little impact.

Previous work has addressed the processing efficiency and physical properties of UF retentate of skim milk produced at low and high process temperature, but data is limited on the
comparison and influence on the heat stability and sensory characteristics of ultrafiltered retentate. Our objectives were to determine the impact of UF of skim milk at 7 and 50°C on UF flux, lactose removal, mineral partitioning, and skim milk retentate physical, chemical, and sensory properties at three (3.4 7.5, and 10.5%) protein concentration with two different heat processing treatments (HTST and autoclave).

**Materials and Methods**

**Experimental Design**

One batch of pasteurized skim milk was split into two portions and the 7°C UF processing run was done on one day and the 50°C UF processing run was done on the next day. skim milk was ultrafiltered at 7 and 50°C and as permeate was removed, deionized (DI) water at 7 or 50°C was added in an equal amount by weight as permeate removed to maintain constant protein concentration in the retentate during UF until 95% or more of lactose and low molecular weight soluble milk components were removed. The total amount of diafiltration (DF) water added at 7 or 50°C was the same for processing runs at both processing temperatures. Once at least 98% lactose and soluble low molecular weight soluble milk components were removed, the UF process was continued with permeate removal, but with no addition of DF water until the protein concentration in the retentate reached 10.5%. The 10.5% protein retentate was diluted to 7.5% and 3.4% protein with DI water. All skim milk-based beverage bases from the 7 and 50°C UF of skim milk were HTST (78°C for 15 sec) processed or autoclaved (116°C for 6 min). The physical, chemical, and sensory properties of all treatments were measured. This process was replicated twice with a new batch of pasteurized skim milk in a different week with the cold and hot UF processing runs ran in reverse order.
**Ultrafiltration for Lactose Removal**

*Filtration at 7 and 50°C*

The UF unit was assembled and cleaned the day before milk processing, as described by Hernandez et al. (2023). 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 $133.5 \pm 8.8 \text{ kg/m}^2/\text{h}$.

To produce lactose-free skim milk, pasteurized skim milk (about 300 kg) with no added vitamins was received from the North Carolina State dairy on the morning of the processing run. Pasteurized skim milk (125 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 for the UF run. The remaining pasteurized skim milk (125 kg) was stored at 4°C to be used for UF processing the next day at 7°C in the first replicate. The order of the running temperatures was reversed in the second replicate. The ultrafiltration (UF) processing of skim milk was carried out with a stainless-steel sanitary design plate Pellicon® 2, 10K plate ultrafiltration apparatus (Millipore Sigma, Burlington, Massachusetts). The plate UF system uses Biomax 10 kDal polyethersulfone (PES) plates with a 10 kilodalton (kDal) 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 membrane plates used in this study have been used on multiple milk UF studies with milk over a period of 3 years and have maintained their clean water flux across time. The membrane plates were removed from the membrane holder between studies and held in membrane soak solution at 4°C between uses. 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 2.5X concentration factor in the recirculation loop and a flux of about $23.3 \pm 1.4 \text{ kg/m}^2\text{/h}$ at $7^\circ\text{C}$ and $47.7 \pm 3.7 \text{ kg/m}^2\text{/h}$ at $50^\circ\text{C}$ throughout the processing run. 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 final diafiltered skim milk. The retentate pH was taken at the beginning and end of processing run. The UF processing was operated in a continuous diafiltration configuration in which the retentate was recirculated back into the UF feed tank and as permeate (~18 kg) was removed, roughly the same amount of 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 amount of added water with time of process run added with time was recorded and reported as the diafiltration ratio (DFR) and was the weight added water divided by the starting weight of milk processed. 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 min for the $50^\circ\text{C}$ run and every 20 min for the $7^\circ\text{C}$ run).

After over 98% of the lactose from the skim was removed by DF at constant protein concentration, the UF retentate was concentrated by UF without DF until the protein percentage had increased to produce a 10.5% protein, lactose-free UF skim retentate. When the UF of the milk was complete, the UF plate unit was flushed (no recirculation) with both retentate and permeate lines open with 38 kg of $50^\circ\text{C}$ DI water to remove residual milk from the UF system. A long clean cycle was done after processing as described by Hernandez et al. (2023).
**Beverage Formulation for Thermal Processing**

The original batch of 10.5% protein UF retentate beverage produced from either 7 or 50°C UF run was used to create 15,000g batches of 7.5% and 3.4% protein UF retentate beverages. DI water at 21°C was added to the original batch of 10.5% protein UF retentate until the 3.4% and 7.5% protein content was achieved. 5,000 grams of unheated MPC beverages at the 3.4%, 7.5%, and 10.5% protein levels were collected and not thermally processed.

**HTST Thermal Processing**

A Microthermics EHVH pasteurization unit running T12B software (10.11.12.90, v6.0, build 104) with a 2-stage homogenizer (model NS2006H, GEA Niro Soavi, Parma, Italy) was used to process the milks. For the HTST treatment, UF retentate beverages were processed at a flow rate of 2.0 L/min. Following preheating to 60°C, the milks were homogenized and pasteurized at 78°C for 15 s before cooling to 10°C. UF retentates beverages were collected and cooled in an ice batch to 4°C.

**Autoclave Thermal Processing**

For autoclave heat treatment, UF beverages were filled into six 500 mL screw cap glass bottles at 350 g per bottle. All bottles were thermally processed using an autoclave (Consolidated Sterilizer Systems, Model SR-24C Sterilizer Autoclave, Billerica, MA): 121°C for 6 min. Beverage temperature and air temperature were monitored using high temperature data loggers throughout the heating and cooling process at every 30 seconds. After bottles were removed from the autoclave (when the autoclave air temperature was 100°C), the beverage was cooled in an ice bath to approximately 4°C and then stored in the fridge at 4°C. Beverage temperature and
air temperature were monitored using high temperature data loggers (MadgeTech HiTemp140-FR 2in probe, Warner, NH) throughout the heating and cooling process at every 30 seconds. The probes were placed at the center of the bottle. All treatments were subjected to analyses on the next day.

**Analysis Methods**

**Lactose Determination**

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

**Freezing Point and pH**

Milk and beverage freezing points were measured throughout the 10 and 50°C UF runs using an Advanced Instruments milk cryoscope (Model 4250, Norwood, MA, USA). 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 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 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 with 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. The viscometer rotation speed was adjusted to an RPM that achieved a torque in the range of 10-100%. All batches that were not thermally processed and those that were HTST processed beverage formulations were measured at 30 RPM. All autoclave beverage formulations were measured at 8 RPM.

**Color**

The color of the milk beverages was measured 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. The beverages were measured in reflectance mode using wavelengths between 360 and 750 nm with a 5 nm resolution using Illuminant A at 10 degree viewer angle (Cheng et al. 2018).

**Particle Size Analysis**

Beverages were analyzed using a Malvern Mastersizer 2000 (Malvern Instruments Ltd., Enigma Business Park, Malvern, Worcestershire, UK, software version 5.4) as described by Di Marzo et al. (2016). Refractive indices of 1.57 for the protein particles and 1.33 for the water
(42°C) suspending liquid were used for a range of particle size from 0.02 to 2000 micrometers. The Malvern multiple narrow mode model for spherical particles was used. The measure time for sample and background was set at 5 s with 5,000 snaps. A light obscuration range limit was set to fall with a range of 7 to 9%, with 3 measurement cycles per sample with zero time delay between measurements.

**Calcium, Phosphorous, Potassium**

Calcium, potassium, and phosphorus were measured in duplicate by DairyOne Laboratory (Warren Road, Ithaca, NY) on beverage samples at the beginning of storage. Samples were held at -80°C for storage then moved to a -20°C freezer the day before analysis. Samples were removed from the -20°C freezer and placed for 30 minutes into a 40°C water bath, then mixed gently by inversion for homogeneity prior to weighing for analysis. Samples were digested using CEM Microwave Accelerated Reaction System (MARS6) with MarsXpress Temperature Control using 50 mL calibrated Xpress Teflon PFA vessels with Kevlar/fiberglass insulating sleeves then analyzed by Inductively Coupled Plasma (ICP) using a Thermo iCAP 6300 or iCAP Pro XP Inductively Coupled Plasma Radial Spectrometer. Samples (about 5 g) were first pre-digested at ambient temperature for 10 min with 8 mL nitric acid (HNO₃) and 2 mL hydrochloric acid (HCl) and then an additional 10 min with 1 mL 30% hydrogen peroxide (H₂O₂). After pre-digestion was completed, samples were digested in two stages: Stage one had a 10 min ramp to 135°C and held for 3 min at 1500W. Stage two had a 12 min ramp to 200°C and held for 15 minutes at 1600W. Vessels were brought to 50 mL volume, with an aliquot used for ICP analysis. The following calibration reference standards (Assurance Spex Certiprep Stock Standards 203 Norcross Avenue Metuchen, NJ 08840) were used: Calcium 10,000 µg/mL in 5% HNO₃ –
Catalog# PLCA2-3X; Phosphorous 10,000 µg/mL in H₂O – Catalog# PLP9-3X: Potassium 10,000 µg/mL in 5% HNO₃ – Catalog# PLK2-3X.

**Descriptive Analysis**

Descriptive analysis was conducted in accordance with the North Carolina State University Institutional Review Board for the Protection of Human Subjects in Research regulations. The milk protein beverages were evaluated for the following attributes: whiteness, yellowness, opacity, overall aroma intensity, sulfur/eggy, cooked/milky, and papery flavors, viscosity, and astringency (Jo et al., 2018, 2019; Hernandez et al., 2023), by 7 panelists (3 males, 4 females, ages 23- 50 y) one day post processing. Each panelist had a minimum of 80 h of prior descriptive analysis experience documenting flavors of milks and dairy protein beverages using the Spectrum™ method with a 0- to 15- point intensity scale (Meilgaard et al., 2007). Thirty mL of each beverage was poured into 59-mL souffle cups, capped (Dart Container Corp.), and labeled with a randomized 3-digit blinding code. Samples were prepared with overhead lights off to prevent light oxidation. Beverages were evaluated at 4°C. Data was collected electronically on the NCSU secure network.

**Statistical Analysis**

Data were analyzed using the General Linear Models ANOVA routine of SAS version 9.4. The original skim milk was compared to the three UF lactose free beverages (3.4, 7.5, and 10.5% protein) produced at 7 and at 50°C for composition, functional, and sensory parameters. The statistical model included UF process temperature (7 and 50°C), protein concentration (3.4, 7.5, and 10.5%), and replicate (n=2) as category variables and their interactions. The composition,
functional and sensory properties of the six UF products (3 protein levels produced by UF at 7°C and 3 protein levels produced by UF at 50°C) were each given three different heat treatments (none, HTST, DSI-UP). The statistical model included UF process temperature (7 and 50°C), protein concentration (3.4, 7.5, and 10.5%), panelist (n=7) and replicate (n=2) as category variable and their interactions.

**Results and Discussion**

*Skim Milk Composition*

The compositions of the skim milk used for the 7°C and 50°C UF runs are shown in Table 1. There were no differences ($P > 0.05$) in the composition (Table 1).

*Microbial Data*

Samples were taken at the start and end of the 7 and 50°C UF runs of the pasteurized skim milks to measure the microbial quality by aerobic plate counts (APC) and coliform counts (Petrifilm Aerobic Count Plate, 3M ID 7100039310 and Petrifilm Coliform Count Plate, 3M ID 7100039392, 3M Food Safety, Maplewood, Minnesota). No coliforms were detected by direct plating of 1 mL of sample. The average CFU/mL for APC started at 31 +/- 1 at the start of the 50°C UF run and ended (5 h run time) at 167 CFU/mL. The average CFU/mL for APC started at 29 +/- 1 at the start of the 7°C UF run and ended (9.5 h run time) at 253 CFU/mL. There were no coliforms detected. The microbial quality of the retentate was excellent (< 260 CFU/mL) at both processing temperatures at the end of the processing runs.
Processing Data

The mean clean water flux was 130 +/- 8.2 Kg/m$^2$h before and after the final cleaning. The mean flux during processing skim milk at 7°C was 23.3 +/- 1.4 Kg/m$^2$h and at 50°C was 46.05 +/- 1.76 Kg/m$^2$h. Inlet pressure was 138 kPa and outlet pressure for permeate and retentates were 0 kPa, retentate cross flow velocity was 22 Kg/min/m$^2$, for processing at both 7 and 50°C with 10 kDal PES membranes. The 7°C UF/DF run was about 8 h in length and a flux of about 23 Kg/m$^2$h was maintained constant for 8 h, while the processing run at 50°C was about 4 h and a flux of about 46 Kg/m$^2$h was maintained constant for 4 h at a constant protein concentration of about 3.4%. At the end of lactose removal by UF/DF, no additional DF water was added and permeate was removed until the protein increased from 3.4% to 10.5%. The protein concentration step took 1 h at 50°C and flux declined from 47 to 40 Kg/m$^2$h, while at 7°C it took 1.45 h and the flux declined from 23 to 13 kg/m$^2$h. The higher flux at 50°C would result in processing twice as much skim milk per hour at 50°C than at 7°C with the same membrane surface area, or the same amount of skim milk per hour with half the membrane surface area at 50°C versus 7°C. The higher flux of the skim milk at 50°C vs 7°C in our study is consistent with a report by Kapsimalis and Zall (1981) where they found the flux at 45°C was higher by a factor of 4 than at 15°C for both 10 and 50 kDal cellulose acetate UF membranes. It was predicted that ultrafiltering skim milk at 50°C requires more than 2.3 less pumping energy compared to ultrafiltering at a cold temperature such as 10°C (Méthot-Hains et al., 2016).

Prior to protein concentration of the skim milk at 7°C and 50°C, continuous diafiltration with permeate replacement was done to remove over 98% of the lactose. The same weight of 125 kg of skim milk was used for both temperature runs. The percent lactose reduction and diafiltration ratio (DFR) with time of UF processing was determined for both the 7°C and 50°C
UF runs. The average time to remove the lactose at 7°C took 481 minutes +/-25. The average time to remove the lactose at 50°C took 251 minutes +/-13. The 98 to 99% lactose removal was achieved by using 4 times more DF water than the starting volume of skim milk at both 7°C (Figure 1) and 50°C (Figure 2). Processing the skim milk at 50°C required approximately half the amount of time (Figure 2) compared to 7°C (Figure 1) to remove over 98% of the lactose. The freezing point of the UF retentate increased as skim milk was diafiltered at 7°C (Figure 3) and 50°C (Figure 4). Differences in UF processing temperature and time did not have an effect on the change in freezing point of the final UF retentate. When 98 to 99% of the lactose and soluble minerals were removed, the freezing point of the UF retentate had increased from a starting value of about -0.530°C to about -0.013°C, even though the protein concentration remained constant, about 3.4% (Figures 3 and 4). At both UF temperatures, the pH of the retentate increased with DF time but the final pH at the end of DF was lower ($P < 0.05$) at 7°C (pH 7.29; Figure 3), than at 50°C (pH 7.43; Figure 4). The higher freezing point and higher pH with increasing lactose removal are consistent with Hernandez et al., 2023. Milk freezing point measurement has been used as a near process line rapid method to measure the solids content of reverse osmosis concentrated skim milk (Barbano et al., 1983) and could be used to measure lactose removal during UF or other filtration processing techniques.

The lactose concentration of the MPC beverages from 7°C and 50°C was determined by enzymatic assay after batching to the target protein contents in the experimental design (AOACI, 2019). There was no difference in the percentage of lactose removed by UF/DF at 7°C versus 50°C detected ($P > 0.05$) and no difference ($P > 0.05$) among the three different protein concentrations (Table 2).
The chemical compositions of the UF beverages at the three protein levels prior to heat treatment from both 7°C and 50°C UF are shown in Table 3. There were no differences in the chemical components listed in Table 3 ($P > 0.05$) between UF/DF at 7°C compared to 50°C. As expected, the percent crude protein, noncasein nitrogen, casein and total solids increased ($P < 0.05$) with increased removal of permeate by UF, but casein as a percent of true protein did not differ ($P > 0.05$) among protein concentrations (Table 3). The NPN content of the starting skim milk was approximately 0.18% (N x 6.38) and the lactose-free UF produced beverages were lower in NPN than the starting skim milk. The non protein nitrogen compounds present in skim milk are primarily low molecular weight compounds that pass through the UF membrane into permeate and therefore, the lactose-free beverages contained less NPN than the starting skim milk.

**Beverage Composition Before Thermal Processing**

The lactose and mineral compositions of the 7°C and 50°C UF beverages at the three protein levels prior to thermal processing are shown in Table 4. A lactose intolerant individual can tolerate the consumption of approximately 12 grams of lactose per day (Dalal et al, 2016). The lactose concentrations in the beverages produced at 7°C and 50°C at all protein concentrations were low (< 0.5 grams per 240 gram serving) and decreased ($P < 0.05$) with decreasing protein concentration (Table 4). Both calcium and phosphorous concentrations (Table 4) were influenced by temperature of UF ($P < 0.05$), protein concentration ($P < 0.05$) and there was a protein concentration by UF temperature interaction ($P < 0.05$). Milk subjected to UF at 7°C had lower calcium and phosphorous concentrations than milk UF at 50°C. At 7°C versus 50°C, the equilibrium of protein bound to milk serum soluble calcium phosphate in skim milk
shifts to decrease the amount of calcium and phosphorous bound in the casein micelles with an increased concentration of soluble calcium in the serum phase of milk (Davies and White, 1960) making soluble calcium available to pass through a UF membrane. The decrease in bound calcium and phosphorus at 7°C allowed more calcium and phosphorus to be removed in the permeate during UF at 7°C versus 50°C (Table 4). Our results are consistent with Liu et al. (2014), who found a lower proportion of soluble calcium in ultrafiltered skim milk processed at 10°C compared to 40°C. The calcium and phosphorous concentration in the lactose-free beverages at 3.4% protein (Table 4) were lower than in the starting skim milk (Table 1), however at 7.5 and 10.5% protein, the calcium and phosphorous concentration (Table 4) were higher than the starting skim milk (Table 1). The increase in calcium and phosphorous concentration with increasing protein concentration during UF was due to calcium and phosphorous that are bound to protein in skim milk (Liu et al., 2014). The calcium to phosphorous ratio did not differ among protein concentrations in our study, but the ratio (about 1.6 to 1.7) was much higher in the lactose-free beverages (Table 4), than the original skim milk at a ratio of 1.24 (Table 1). The Ca/P ratio was higher ($P < 0.05$) when milk was ultrafiltered at 50°C than 7°C (Table 4). These results agree with Liu et al. (2014) as it is expected that there is a higher concentration of soluble calcium at lower temperatures of milk. The potassium concentration in the lactose-free beverages was much lower (Table 4) than the concentration in skim milk (Table 1). There is no bound potassium in the skim milk, and it was removed in the UF permeate like lactose and other soluble minerals. The pH of the lactose-free beverages was influenced ($P < 0.05$) by temperature of UF and protein concentration, with milk ultrafiltered at 7°C having lower pH than milk UF at 50°C. France et al. 2021 reported higher amounts of ionic calcium concentration ($P < 0.05$) were found in UF permeate streams when microfiltration of skim milk was conducted at 4°C than at 50°C.
and that is consistent with our observations at the same temperatures using UF. The freezing point of all lactose-free beverages increased relative to the starting skim milk and the freezing points of the lactose-free beverages were very close to that of water. This means that the ionic strength in the aqueous phase of the beverage was much lower than that of skim milk. Our observation is consistent with observations made by Hernandez et al. (2023) who also reported beverage freezing points at all milk protein concentrations up to 10.5% were near that of pure water. Despite more calcium deposit on the membrane surface for milk that has undergone ultrafiltration at 15°C compared to 50°C in a study done by Luo et al. (2015), similar solubility and heat stability percentages for freeze dried UF retentates produced by UF at 15°C and 50°C were reported, while emulsification index was higher for UF retentates UF at 15°C versus 50°C.

**Beverage Composition: No Heat, HTST, and Autoclave**

The mean chemical compositions of the UF beverages averaged across all three protein levels before and after thermal processing from both the 7°C and 50°C UF runs are shown in Table 5. There were differences detected in the NCN, NPN, CN, and CN/TP chemical components listed in Table 5 ($P < 0.05$) among heat treatments. The NCN, NPN, CN, and CN/TP concentrations were influenced ($P < 0.05$) by both protein concentration and heat treatment and there was a heat x protein concentration interaction for each parameter, but no influence of temperature of UF on these parameters. The total nitrogen-based protein concentration and total solids were higher ($P < 0.05$) for beverages that received the autoclave treatment than no heat or HTST because the bottles had to be left slightly open during the autoclave processing and that resulted in some loss of water due to evaporation (Table 5). Non-casein nitrogen content decreased and apparent casein increased as intensity of heat exposure increased due to the
severity of heat treatment (Table 5). Casein as a percent of true protein increased as intensity of heat treatment increased (Table 5). This was a result of milk derived whey protein being bound to the surface of casein micelles. Low molecular weight nitrogen containing compounds soluble in 12% TCA (i.e., NPN) was much higher in milk given an autoclave heat exposure than no heat and HTST. This may indicate that there is some thermal degradation of protein structures that releases TCA soluble nitrogen (Table 2). The chemical nature of these NPN compounds is not known.

**Beverage AV and Color Before Thermal Processing**

Mean Hunter L, a, and b* color measurements and apparent viscosity before heat treatment of the lactose-free beverages made by UF at 7°C and 50°C are shown in Table 6. The light reflectance curves for the unheated beverages produced by UF at 7 and 50°C are presented in Figures 5 and 6. Compounds in the aqueous phase of milk absorb light in the 360-500 nm region. During diafiltration, the light absorbing compounds in the skim milk were removed in the UF permeate at both the 7°C and 50°C processing temperatures. As a result, reflectance of light in the UF lactose-free beverages increased and this effect can be seen in the range of 360 to 500 nm for the skim milk beverages ultrafiltered at either temperature (Figure 5, Figure 6). In the absence of these light absorbing compounds, the UF retentates reflected more light in the range of 360 to 500 nm and as protein concentration of the retentate was increased from 3.4 to 7.5 and 10.5% protein, the total light reflected was increased (Figures 5 and 6). The effects of light scattering and removal of light absorbing low molecular weight chromophores are also seen in the reflectance curves of UF lactose-free beverages studied by Hernandez et al. (2023).
**Beverage Particle Size Before and After Thermal Processing**

Particle size distribution of all lactose free beverages was measured before and after HTST (about 78°C) and autoclave (about 121°C) thermal treatments at all protein concentration for lactose free beverages produced by UF/DF at 7 and 50°C. All particle size distributions had d(0.9) values of about 0.21 microns and no effect of heat treatment or protein concentration of particle size distribution (data not shown) were detected (P > 0.05). However, when we tried to run the lactose-free beverage through a direct steam injection UHT thermal process, all of the lactose-free products gelled during the UHT process and were not heat stable in that process where peak temperatures at steam injection are in the range of 150 to 160°C depending on the steam injection flow pressure when a holding tube temperature of 140°C was achieved. The lack of heat stability of lactose-free beverages in the UHT-DSI process was thought to be due to the low ionic strength of the beverages, however the beverages were heat stable in the autoclave treatment at 120°C. The impact of lactose and soluble mineral removal on heat stability of milk protein beverages warrants future research.

**Beverage AV and Color After Thermal Processing**

All lactose-free beverages after thermal processing (Table 7) were whiter (high L-value) and at higher protein concentration than the starting skim milks (Table 6). The reason for this was the removal of water-soluble light-absorbing compounds in the permeate at both temperatures of UF/DF (Figures 5 & 6). There were effects (P < 0.05) of temperature UF, processing temperature, protein concentration (relative % of type III sum of squares 0.25, 3.7 and 87%, respectively) and their interactions on AV, however the effect of protein concentration was the strongest and the effect of temperature of UF was the weakest based on relative Type III sum
of squares from the ANOVA. No effects ($P > 0.05$) of temperature of UF, heat treatment, or protein concentration on L-value (i.e., whiteness) were detected (Table 7). All lactose-free beverages (Table 7) were less blue (a value) and less yellow (b*-value) than the skim milks used to make the lactose free beverages (Table 6). There was very little impact of the high temperature of autoclave treatment on color of the lactose-free beverages (Table 7) because the compounds that are heat sensitive and reactive to heat (e.g., lactose and nonprotein nitrogen compounds) were removed during UF/DF.

The AV of the lactose-free beverages was increased ($P < 0.05$) by an increase in protein concentration and by the severity of the thermal treatment, but the effect of protein concentration was larger than thermal treatment (Table 7). Studies conducted by Misawa et al. (2016), Cheng et al. (2019), Quiñones et al. (1997), and Hernandez et al. (2023) reported that the instrumental viscosity of milk and milk beverages increased as protein content increased. The results from the current study are consistent with Ho et al. (2019) who found that MPC beverages heated at 120°C for 15 to 30 seconds had higher viscosity ($P <0.05$) than those heated at 85 or 100°C.

**Beverage Sensory Properties**

No differences in the descriptive sensory attributes evaluated were detected ($P >0.05$) due to cold (7°C) versus hot (50°C) UF. The lactose free beverages were more opaque, white, astringent, and less yellow, cooked-milky, and sweet aromatic than the starting pasteurized skim milk (Table 8). Sensory opacity, cooked milky flavor, sensory viscosity, and astringent mouthfeel increased with increased ($P <0.05$) protein concentration. Increasing severity of heat treatment increased cooked milky flavor, opacity, sulfur eggy flavor, and astringent mouthfeel (Figure 7, Table 8).
The descriptive sensory analysis results for the lactose-free skim milk beverages produced at 7 and 50°C at all protein concentrations are presented as a PCA biplot in Figure 7. The sensory properties of skim milk are not shown on the biplot to more easily visualize the differences among the lactose-free beverages due protein concentration and thermal process. The increased sensory whiteness values when lactose and soluble minerals were removed was consistent with instrumental changes in reflectance (Figures 5, 6). NH and HTST beverages were more bland (lower aromatics and basic tastes, \( P < 0.05 \)) than the starting skim milk. Removal of lactose and soluble minerals as well as volatile components by UF/DF would be expected to decrease overall flavor. Hernandez et al. (2023) observed similar effects when determining the effect of fat concentration on unheated lactose free beverages. Milk whiteness increased and aromatics and basic tastes decreased as lactose and soluble minerals were removed from milk, regardless of fat content.

Both heat treatment and protein concentration impacted sensory properties \( (P < 0.05) \). Previous studies have demonstrated that milk beverage whiteness increased with higher protein concentration, consistent with this study (Quiñones et al., 1997, 1998; Cheng et al., 2018; Hernandez et al., 2023). As the protein content in lactose free beverages increased, opacity, viscosity, papery flavor and astringency also increased \( (P < 0.05) \). Increased viscosity and astringency with higher protein concentration in milk and milk protein beverages were also observed in previous studies (Cheng et al., 2019; Vogel et al., 2021; Hernandez et al., 2023). Autoclaved beverages had higher aroma intensity than the NH or HTST beverages \( (P < 0.05) \). The autoclaved beverages were also distinguished by distinct sulfur/eggy flavor that increased with protein concentration. This flavor was not detected in NH or HTST beverages. Sulfur/eggy flavor is hallmark of ultrapasteurized milk (Lee et al., 2017) and is attributed to the denaturation
of serum proteins and the release of hydrogen sulfide (Jo et al., 2019). As such, sulfur/eggy flavor is also prevalent in milk protein beverages that have been heat processed at temperatures that denature serum proteins including ultrapasteurization and autoclaving (Vogel et al., 2021; Whitt et al., 2022).

**Conclusions**

Overall, lactose-free milk skim milk at 3.4, 7.5, and 10.5% protein produced by UF with DF, was more bland, more white and less heat stable (i.e., stable to retorting but not direct steam injection at 142°C for 2 to 3 sec) than skim milk. A 98 to 99% removal of lactose was achieved with a diafiltration ratio of water to milk of about 4 to 1 achieved at both 7°C and 50°C. The processing time to achieve that removal from the same volume of milk was about twice as long when filtering at 7°C than 50°C because of the lower flux (23 versus 48 Kg/m²/h). The continuous DF at constant protein concentration maintained constant flux for a processing time of 4 and 8 h at 50 and 7°C, respectively. The final freezing point of the lactose and soluble mineral reduced milk was close to that of water (-0.015°C) and the pH of the lactose free milk at 20°C increased from about 6.5 to about 7.33 and 7.46 for UF/DF at 7 and 50°C, respectively. Removal of compounds from milk in permeate that absorb light in the range of 360 to 500 nm, increased light reflectance and whiteness and decreased yellowness.
Acknowledgments

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REFERENCES


**Table 1.** Mean lactose and mineral composition of skim milk before ultrafiltration (UF) and diafiltration (DF) at 7°C and 50°C.

<table>
<thead>
<tr>
<th>UF temperature</th>
<th>Protein $^1$</th>
<th>Lactose $^1$</th>
<th>Calcium $^2$</th>
<th>Phosphorous $^2$</th>
<th>Potassium $^2$</th>
<th>Ca/P ratio</th>
<th>pH at 20°C</th>
<th>Freezing point $^3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>7°C</td>
<td>3.34$^a$</td>
<td>4.78$^a$</td>
<td>1376$^a$</td>
<td>1108$^a$</td>
<td>1612$^a$</td>
<td>1.24$^a$</td>
<td>6.670$^a$</td>
<td>-0.530$^a$</td>
</tr>
<tr>
<td>50°C</td>
<td>3.34$^a$</td>
<td>4.78$^a$</td>
<td>1360$^a$</td>
<td>1104$^a$</td>
<td>1601$^a$</td>
<td>1.23$^a$</td>
<td>6.685$^a$</td>
<td>-0.528$^a$</td>
</tr>
<tr>
<td>All</td>
<td>3.34</td>
<td>4.78</td>
<td>1368</td>
<td>1106</td>
<td>1606</td>
<td>1.24</td>
<td>6.6775</td>
<td>-0.529</td>
</tr>
</tbody>
</table>

$^a$-$^c$ Numbers not sharing a common superscript within a column are different ($P < 0.05$).

$^1$Values shown are grams/100 grams milk (wt/wt); n = 2 for UF/DF at 7°C and n= 2 for UF/DF at 50°C.

$^2$Values shown are mg/liter of milk.

$^3$Values shown are °H.
Table 2. Mean percentage of lactose removed in lactose-free beverages at 3.4, 7.5, and 10.5% protein produced by ultrafiltration with diafiltration at 7°C and 50°C.\(^1\)

<table>
<thead>
<tr>
<th>Protein</th>
<th>Lactose removal (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skim Milk</td>
<td>0.0</td>
</tr>
<tr>
<td>3.4%</td>
<td>99.5(^a)</td>
</tr>
<tr>
<td>7.5%</td>
<td>99.0(^a)</td>
</tr>
<tr>
<td>10.5%</td>
<td>98.7(^a)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Protein</th>
<th>Lactose removal (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skim Milk</td>
<td>0.0</td>
</tr>
<tr>
<td>3.4%</td>
<td>99.5(^a)</td>
</tr>
<tr>
<td>7.5%</td>
<td>99.0(^a)</td>
</tr>
<tr>
<td>10.5%</td>
<td>98.7(^a)</td>
</tr>
</tbody>
</table>

\(^{a-c}\)Numbers not sharing a common superscript within a column are different (\(P < 0.05\)).

\(^1\)Values shown are percentages based on enzymatic assay results.

Table 3. Mean chemical composition of lactose free beverages produced by ultrafiltration with diafiltration at 7°C and 50°C before HTST or autoclave heat treatments.\(^1\)

<table>
<thead>
<tr>
<th>Protein target</th>
<th>TN(^2)</th>
<th>NCN(^3)</th>
<th>NPN(^4)</th>
<th>CN(^5)</th>
<th>CN/TP(^6)</th>
<th>TS(^7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.4</td>
<td>3.60(^c)</td>
<td>0.50(^c)</td>
<td>0.031(^c)</td>
<td>3.10(^c)</td>
<td>86.81(^a)</td>
<td>4.10(^c)</td>
</tr>
<tr>
<td>7.5</td>
<td>7.85(^b)</td>
<td>1.02(^b)</td>
<td>0.046(^b)</td>
<td>6.83(^b)</td>
<td>87.42(^a)</td>
<td>8.78(^b)</td>
</tr>
<tr>
<td>10.5</td>
<td>10.81(^a)</td>
<td>1.44(^a)</td>
<td>0.058(^a)</td>
<td>9.37(^a)</td>
<td>87.12(^a)</td>
<td>12.03(^a)</td>
</tr>
</tbody>
</table>

\(^{a-c}\)Numbers not sharing a common superscript within a column are different (\(P < 0.05\)).

\(^1\)Values shown are percentages (wt/wt); \(n = 3\). Kjeldahl measurements were taken from beverages prior to heat treatment for TN, NCN, NPN, TP, CN, and CN%TP.

\(^{2}\)TN = crude protein = total nitrogen x 6.38.
\(^{3}\)NCN = noncasein nitrogen x 6.38.
\(^{4}\)TP = true protein = (TN – NPN) x 6.38.
\(^{5}\)CN = casein = (TN – NCN) x 6.38.
\(^{6}\)CN/TP = casein as a percentage of true protein = (CN/TP) x 100.
\(^{7}\)TS = total solids.
Table 4. Mean anhydrous lactose, mineral composition, pH (measured at 20°C), and freezing point (FP) of 7°C and 50°C lactose-free beverages produced by ultrafiltration (UF) with diafiltration (DF) at 7°C and 50°C before thermal processing.

<table>
<thead>
<tr>
<th></th>
<th>Target protein</th>
<th>Lactose</th>
<th>Calcium</th>
<th>Phosphorous</th>
<th>Potassium</th>
<th>Ca/P ratio</th>
<th>pH</th>
<th>FP</th>
</tr>
</thead>
<tbody>
<tr>
<td>7°C UF 3.4</td>
<td>0.025</td>
<td>977f</td>
<td>602f</td>
<td>57e</td>
<td>1.62d</td>
<td>7.33b</td>
<td>-0.009a</td>
<td></td>
</tr>
<tr>
<td>7°C UF 7.5</td>
<td>0.052b</td>
<td>2181d</td>
<td>1354d</td>
<td>125b</td>
<td>1.61e</td>
<td>7.15e</td>
<td>-0.017c</td>
<td></td>
</tr>
<tr>
<td>7°C UF 10.5</td>
<td>0.064a</td>
<td>2716b</td>
<td>1711b</td>
<td>168a</td>
<td>1.59f</td>
<td>7.09f</td>
<td>-0.025c</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>7.13</td>
<td>0.05</td>
<td>1958</td>
<td>1222</td>
<td>117</td>
<td>1.61</td>
<td>7.19</td>
<td>-0.0167</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Target protein</th>
<th>Lactose</th>
<th>Calcium</th>
<th>Phosphorous</th>
<th>Potassium</th>
<th>Ca/P ratio</th>
<th>pH</th>
<th>FP</th>
</tr>
</thead>
<tbody>
<tr>
<td>50°C UF 3.4</td>
<td>0.024c</td>
<td>1074e</td>
<td>640e</td>
<td>39f</td>
<td>1.68a</td>
<td>7.46a</td>
<td>-0.009a</td>
<td></td>
</tr>
<tr>
<td>50°C UF 7.5</td>
<td>0.044b</td>
<td>2341c</td>
<td>1406c</td>
<td>86d</td>
<td>1.67b</td>
<td>7.30c</td>
<td>-0.014b</td>
<td></td>
</tr>
<tr>
<td>50°C UF 10.5</td>
<td>0.057a</td>
<td>3048a</td>
<td>1853a</td>
<td>112c</td>
<td>1.64c</td>
<td>7.22d</td>
<td>-0.019d</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>7.13</td>
<td>0.04</td>
<td>2155</td>
<td>1300</td>
<td>79</td>
<td>1.66</td>
<td>7.33</td>
<td>-0.014</td>
</tr>
</tbody>
</table>

\[a-c\] Numbers not sharing a common superscript within a column are different \((P < 0.05)\).

1 Values for protein and lactose are g/100 g milk percentages (wt/wt); \(n = 2\).

2 Values shown are mg/liter of milk.

3 Values shown are °H.
Table 5. Mean chemical composition of 7°C and 50°C lactose-free beverages across all protein concentrations (3.4, 7.5, and 10.5%) before (no heat) and after heat treatments [HTST, autoclave].

<table>
<thead>
<tr>
<th>Heat treatment</th>
<th>TN\textsuperscript{2}</th>
<th>NCN\textsuperscript{3}</th>
<th>NPN\textsuperscript{4}</th>
<th>CN\textsuperscript{5}</th>
<th>CN/TP\textsuperscript{6}</th>
<th>TS\textsuperscript{7}</th>
</tr>
</thead>
<tbody>
<tr>
<td>No heat</td>
<td>7.36\textsuperscript{b}</td>
<td>1.25\textsuperscript{a}</td>
<td>0.033\textsuperscript{b}</td>
<td>6.11\textsuperscript{c}</td>
<td>83.27\textsuperscript{c}</td>
<td>8.24\textsuperscript{b}</td>
</tr>
<tr>
<td>HTST</td>
<td>7.35\textsuperscript{b}</td>
<td>1.09\textsuperscript{b}</td>
<td>0.028\textsuperscript{c}</td>
<td>6.27\textsuperscript{b}</td>
<td>85.27\textsuperscript{b}</td>
<td>8.25\textsuperscript{b}</td>
</tr>
<tr>
<td>Autoclave</td>
<td>7.58\textsuperscript{a}</td>
<td>0.51\textsuperscript{c}</td>
<td>0.084\textsuperscript{a}</td>
<td>7.07\textsuperscript{a}</td>
<td>94.23\textsuperscript{a}</td>
<td>8.47\textsuperscript{a}</td>
</tr>
</tbody>
</table>

\textsuperscript{a-c}Numbers not sharing a common superscript within a column are different (P < 0.05).

\textsuperscript{1}Values shown are percentages (wt/wt); n = 3. Kjeldahl measurements were taken from beverages prior to heat treatment for TN, NCN, NPN, TP, CN, and CN%TP.

\textsuperscript{2}TN = total nitrogen x 6.38 (g x 100 g milk).

\textsuperscript{3}NCN = noncasein nitrogen x 6.38 (g x 100 g milk).

\textsuperscript{4}TP = true protein = (TN – NPN) x 6.38 (g x 100 g milk).

\textsuperscript{5}CN = casein = (TN – NCN) x 6.38(g x 100 g milk).

\textsuperscript{6}CN/TP = casein as a percentage of true protein = (CN/TP) x 100.

\textsuperscript{7}TS = total solids.

Table 6. Least squares means for apparent viscosity (AV) (mPa·s) for the effect of UF processing temperature on whiteness (L), redness-greenness (a), and yellowness-blueness (b*) values relative percentage of skim milk prior to UF.

<table>
<thead>
<tr>
<th>UF Temperature</th>
<th>AV \textsuperscript{a}</th>
<th>L \textsuperscript{a}</th>
<th>a \textsuperscript{a}</th>
<th>b* \textsuperscript{a}</th>
</tr>
</thead>
<tbody>
<tr>
<td>7°C</td>
<td>3.35\textsuperscript{a}</td>
<td>78.43\textsuperscript{a}</td>
<td>-4.10\textsuperscript{a}</td>
<td>3.03\textsuperscript{a}</td>
</tr>
<tr>
<td>50°C</td>
<td>3.21\textsuperscript{a}</td>
<td>78.07\textsuperscript{a}</td>
<td>-3.96\textsuperscript{a}</td>
<td>3.11\textsuperscript{a}</td>
</tr>
<tr>
<td>Mean</td>
<td>3.28</td>
<td>78.25</td>
<td>-4.03</td>
<td>3.07</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Numbers not sharing a common superscript within a column are different (P < 0.05).
Table 7. Least squares means for the effect of heat treatment (no heat, HTST, autoclave) and protein concentration (3.4, 7.5, and 10.5%) on apparent viscosity (AV) in mPa·s, whiteness (L), redness-greenness (a), and yellowness-blueness (b*) values of lactose-free beverages produced by ultrafiltration with diafiltration at 7°C and 50°C.

<table>
<thead>
<tr>
<th>Heat treatment</th>
<th>AV</th>
<th>L</th>
<th>a</th>
<th>b*</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Heat</td>
<td>8.76b</td>
<td>80.02a</td>
<td>-2.54b</td>
<td>-0.81c</td>
</tr>
<tr>
<td>HTST</td>
<td>8.31b</td>
<td>80.62a</td>
<td>-2.63c</td>
<td>-0.71b</td>
</tr>
<tr>
<td>Autoclave</td>
<td>11.45a</td>
<td>81.04a</td>
<td>-2.41a</td>
<td>-0.45a</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Target protein</th>
<th>AV</th>
<th>L</th>
<th>a</th>
<th>b*</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.4</td>
<td>2.88c</td>
<td>79.66a</td>
<td>-3.32c</td>
<td>-1.76c</td>
</tr>
<tr>
<td>7.5</td>
<td>6.91b</td>
<td>81.65a</td>
<td>-2.20b</td>
<td>-0.20b</td>
</tr>
<tr>
<td>10.5</td>
<td>18.72a</td>
<td>80.37a</td>
<td>-2.06a</td>
<td>-0.02a</td>
</tr>
</tbody>
</table>

a-c Numbers not sharing a common superscript within heat treatment or within protein concentration in a column are different ($P < 0.05$).
Table 8. Mean sensory intensity scores (0 to 15 scale) for lactose-free beverages produced by ultrafiltration (UF) with diafiltration (DF) at 7°C and 50°C before thermal processing at 3 different protein concentrations (3.4, 7.5, and 10.5%) and given three different thermal treatments: no heat (NH), high temperature short time pasteurization (HTST) and an autoclave treatment.

<table>
<thead>
<tr>
<th>Target protein</th>
<th>Opacity</th>
<th>Whiteness</th>
<th>Yellowness</th>
<th>Viscosity</th>
<th>Cooked milky</th>
<th>Sweet aromatic</th>
<th>Sulfur eggy</th>
<th>Astringent mouthfeel</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.4</td>
<td>11.8&lt;sup&gt;c&lt;/sup&gt;</td>
<td>13.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.9&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.3&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>7.5</td>
<td>12.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>10.5</td>
<td>13.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ND</td>
<td>1.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.6&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>Mean</td>
<td>12.4</td>
<td>12.8</td>
<td>0.46</td>
<td>2.2</td>
<td>2.3</td>
<td>0.7</td>
<td>0.9</td>
<td>2.5</td>
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<th>Opacity</th>
<th>Whiteness</th>
<th>Yellowness</th>
<th>Viscosity</th>
<th>Cooked milky</th>
<th>Sweet aromatic</th>
<th>Sulfur eggy</th>
<th>Astringent mouthfeel</th>
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<td>12.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.5&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>2.3&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>ND</td>
<td>2.5&lt;sup&gt;b&lt;/sup&gt;</td>
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Skim Milk
8.0 8.1 2.4 1.9 3.1 1.9 ND 2.1

<sup>a-c</sup> Numbers not sharing a common superscript within a column are different ($P < 0.05$).
**Figure 1.** The percent lactose reduction and diafiltration (DF) ratio with time of UF processing at a milk temperature of 7°C.

**Figure 2.** The percent lactose reduction and diafiltration (DF) ratio with time of UF processing at a milk temperature of 50°C.
**Figure 3.** Change in freezing point (°H) and pH (measured at 7°C) of the UF retentate with time of UF processing at a milk temperature of 7°C.

**Figure 4.** Change in freezing point (°H) and pH (measured at 50°C) of the UF retentate with time of UF processing at a milk temperature of 50°C.
**Figure 5.** Light reflectance curves (measured at 4ºC) for skim milk and reduced lactose UF retentates at 3.4, 7.5, and 10.5% protein produced by UF processing at a milk temperature of 7ºC with no post UF thermal treatment.

![Light reflectance curves for skim milk and reduced lactose UF retentates at 7ºC](image)

**Figure 6.** Light reflectance curves (measured at 4ºC) for skim milk and reduced lactose UF retentates at 3.4, 7.5, and 10.5% protein produced by UF processing at a milk temperature of 50ºC with no post UF thermal treatment.

![Light reflectance curves for skim milk and reduced lactose UF retentates at 50ºC](image)
Figure 7. Principal component analysis biplot of descriptive analysis results for skim milk ultrafiltered and diafiltered cold at 7°C (C) and hot 50°C (H) lactose reduced beverages with no heat (NH) treatment, high temperatures short time (HTST), and autoclaved (AC) at three different protein concentrations (3.4, 7.5, and 10.5%).