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

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ABSTRACT

Our objectives were to determine the impact of fat (skim to whole milk) and protein (3.4 to 10.5%) concentration on the sensory and physical properties of milk beverage base that had lactose and other low molecular components removed by ultrafiltration. In experiment 1, a matrix of 16 treatments was produced to achieve 4 levels of lactose removal (0, 30, 70, and 97%) at each of 4 fat levels (skim, 1%, 2% and whole milk). In experiment 2, a matrix of 12 treatments was produced to achieve 4 levels of lactose removal (0, 30, 70, and 97%) at each of 3 protein concentrations (3.4, 6.5, and 10.5% protein). Physical and sensory properties of these products were determined. Removal of >95% of milk lactose by UF required a diafiltration volume of approximately 3 times the milk volume. Lactose and low molecular weight solute removal increased whiteness across the range from skim to whole milk while decreasing viscosity and making milk flavor more bland. In addition, lactose (and other low molecular weight solute) removal by UF decreased titratable acidity by more than 50% and increased milk pH at 20°C to > 7.0. Future work on milk and milk-based beverages with lactose removed by UF needs to focus on interaction of the remaining milk solids with added flavorings, changing casein to whey protein ratio before removal of lactose by UF, and the impact of lactose and low molecular weight solute removal on heat stability, particularly for neutral-pH, shelf-stable milk-based beverages.

Key Words: Lactose removal, ultrafiltration, lactose free milk

INTRODUCTION

Milk and dairy products are among the most versatile foods on the market, ranging from indulgent to nutritional depending on the many ways they can be processed. A major issue that limits the consumption of dairy products is lactose intolerance. Lactose intolerance occurs when a person is deficient in β-galactosidase in the small intestine which can lead to negative symptoms such as nausea, bloating, and abdominal pain (Storhaug et al., 2017). A 240 mL serving of fluid milk contains approximately 12 g of lactose. An average lactose intolerant individual can tolerate about 12 g of lactose per day (Dalal et al., 2016). The lactose is an issue for lactose intolerant individuals but also provides carbohydrate calories. The primary treatment of those impacted by lactose intolerance is a lactose-free diet (Vardhanabhuti and Wang, 2022). It is recommended that the majority of Americans consume 3 servings of dairy per day by The Dietary Guidelines for Americans 2020-25 (US Department of Agriculture and US Department of Health and Human Services, 2020). Instead of avoiding dairy completely, lactose-free dairy is a viable and established alternative. In 2020, the lactose-free milk market in the United States was estimated at $1.7 billion, with 201 million gallons sold (Gerdes, 2021).

Two approaches used in industry to reduce the sugar lactose to improve the healthiness of dairy products are lactose hydrolysis and lactose removal by ultrafiltration (Rehman, 2009). Lactose hydrolysis involves addition of the enzyme β-galactosidase (lactase) to milk which breaks down lactose into glucose and galactose. The hydrolysis of lactose into glucose and galactose also makes the product sweeter. The addition of β-galactosidase treatment increases costs of fluid milk by $0.06–$0.08/L (Rehman, 2009). For milk, UF concentrates protein and removes lactose, soluble minerals and water (Mistry and Maubois, 2004). In industry, a 3X concentration factor is most commonly used during ultrafiltration of milk in which 66–67% of lactose is removed from the milk (Rehman, 2009). The starting lactose content of 4.8 g/100 g cows’ milk decreases to 1.6 g/100 g during the 3X UF. As diafiltration is completed, the lactose of the milk is then only 1.6%. This remaining lactose can be hydrolyzed to get a 100% lactose free milk that will not have an elevated sweet taste as with lactose hydrolysis (Rehman, 2009). If diafiltration with water...
is done to remove nearly all lactose, soluble minerals and other low molecular weight components, then the resulting product is a milk beverage base (rather than milk or UF milk).

Previous work has addressed the use of UF to increase the concentration of protein in milk but data is limited on the influence of lactose removal on UF retentates. Research into how manipulation of lactose, fat, and protein impact sensory and physical properties by ultrafiltration of milk is important to establish baseline properties. Our objectives were to determine the impact of fat (skim to whole milk) and protein (3.4 to 10.5%) concentration on the sensory and physical properties of milk beverage base that had lactose and other low molecular components removed by ultrafiltration.

MATERIALS AND METHODS

Experimental Overview

The experiment consisted of 2 distinct experiments: the role of fat and lactose removal and the role of protein and lactose removal. These experiments were conducted and replicated on separate weeks from each other and were analyzed as separate objectives.

Experiment 1

In experiment 1, pasteurized skim milk was ultrafiltered and as permeate was removed, deionized water was added in an equal amount by weight to maintain constant protein concentration in the retentate until lactose and low molecular weight soluble milk components were removed. Second, pasteurized whole milk was ultrafiltered as was done for skim milk until lactose and low molecular weight soluble milk components were removed. This process produced 4 batches of product, skim milk and whole milk with full lactose and skim milk with lactose removed. About 97% of the lactose was removed. From these 4 ingredients, a matrix of 12 treatments (Figure 1) was formulated to achieve 4 levels of lactose removal (0, 30, 70, and 97%) at each of 3 protein concentrations (3.4, 6.5, and 10.5% protein). Physical and sensory properties of these products were determined. This process was replicated twice starting with different batches of skim.

Experiment 2

In experiment 2, skim milk was ultrafiltered and as permeate was removed deionized water was added in an equal amount by weight to maintain constant protein concentration in the UF retentate until lactose and low molecular weight soluble milk components were removed. Half of the lactose-removed skim milk (UF retentate) was collected and the other half of the UF retentate continued with UF to concentrate the protein to 10.5%. Second, another portion of skim milk was UF without diafiltration to produce a 10.5% protein concentration without lactose removal with diafiltration. This process produced 4 batches of product, skim milk at 3.4% and 10.5% protein with full lactose and skim milk at 3.4% and 10.5% protein with lactose removed. About 96% of the lactose was removed. From these 4 ingredients, a matrix of 12 treatments (Figure 2) was formulated to achieve 4 levels of lactose removal (0, 30, 70, and 97%) at each of 3 protein concentrations (3.4, 6.5, and 10.5% protein). Physical and sensory properties of these products were determined. This process was replicated twice starting with different batches of skim.

Ultrafiltration for Lactose Removal.

Ultrafiltration for Experiment 1. The overall ingredient production by UF is provided in Figure 1. Four batches (Batches 1, 4, 13, and 16) were the prime ingredients produced. The inset table in Figure 1, provides the full formulation matrix of 16 batches made from these 4 base ingredients. Batches 1 and 4 were the original full lactose skim and whole milk before UF and batches 13 and 16 were the skim and whole milk with the lactose removed by filtration.

The UF unit was assembled and cleaned the day before milk processing. Briefly, the UF unit was rinsed twice with DI water at 50°C for 5 min each, followed by a recirculation wash with an alkaline cleaner (76 kg of 50°C DI water, 302 mL Ultrasil 110 and 480 mL of Ultracil 01, Ecolab) at 50°C for 30 min. The system was then flushed twice with DI water at 50°C for 5 min each, followed by an acid recirculation wash at 50°C (76 kg of 50°C DI water, 226 mL Ultrasil 76, Ecolab) for 30 min. The system was then rinsed twice with 50°C DI water for 10 min. Next, clean membrane water flux was measured at 50°C at 124 kPa with DI water in full recirculation mode for 3 min by collecting water for from the permeate line for 1 min and recording the weight collected. After a clean membrane water flux was measured, the UF was sanitized by recirculation (76 kg of 50°C DI water, 240 mL of XY-12 Liquid Sanitizer, Ecolab) at 21°C for 10 min, followed by 2 5 min DI water rinses and then recirculation for 10 min at 21°C with membrane storage solution (76 kg of 50°C DI water, 200 mL Ultracil MP, Ecolab). At the beginning of the processing the next day, 50°C DI water was used to flush the membrane storage solution out of the UF
system before milk processing. Start of day clean water flux at 50°C was about 140 kg/m²/h.

To produce lactose-free skim milk and lactose-free whole milk, pasteurized skim milk (195 kg) and pasteurized (HTST 78°C for 28s) and homogenized whole milk (120 kg) with no added vitamins were received from the North Carolina State University commercial Dairy Enterprise System on the morning of the processing run (Keefer et al., 2022). Pasteurized skim (95 kg) was weighed and poured into a jacketed stainless-steel feed tank (Meyer-Blank Company, St Louis, MO) with a recirculating 50°C water jacket to heat the milk to 50°C. The remaining pasteurized skim milk (100 kg) for batch 1 was stored at 4°C to be used for processing later on and batching the following day. On d 1, the ultrafiltration (UF) processing of skim milk was carried out by using a stainless-steel sanitary design plate Pellicon® 2, 10K plate ultrafiltration apparatus (Millipore Sigma, Burlington, Massachusetts). The plate UF system uses Biomax 10k polyethersulfone plates with a 10,000 Dalton cut-off with a surface area of 0.5 m² per plate. For optimal pressure, the ultrafiltration unit was assembled with 5 plates in the stack. The feed tank was connected to the membrane stack by a feed pump (Baldor Industrial Motor, single phase, 1.5 H.P., 3450 RPM, 60hz, Baldor Electric Co., Ft. Smith, AR). The plate stack was operated with an inlet pressure of 124 kPa and an outlet pressure of 0 kPa during processing with a permeate removal rate that achieved a 2X concentration factor in the recirculation loop and a flux of about 45 kg/m²/h throughout the processing run for both skim and whole milks. The known weight of the starting milk and protein content were used to create a permeate weight removal goal to achieve the 0.1% lactose (wt/wt) in both the pasteurized skim and whole milk. The pH was taken at the beginning and end of processing at 50°C. The UF processing was operated in a constant diafiltration configuration in which the retentate was recirculated back into the UF feed tank and as a bucket of permeate (~18 kg) was removed, roughly the same amount of deionized (DI) water was added to the UF feed tank to balance the weights of permeate removed to maintain protein concentration constant in the UF feed tank. This ensured that the correct amount of lactose removal was achieved without altering the starting milkfat and protein percentages. The lactose

![Figure 1](image-url). Experiment 1 overview: lactose and soluble mineral removal by UF at 4 different fat concentrations with batch #2, 3, 5, 6, 7, 8, 9, 10, 11, 12, 14 and 15 formulated from batches 1, 4, 13, and 16.

<table>
<thead>
<tr>
<th>% Lactose Removal</th>
<th>Batch #</th>
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<tr>
<td>Skim</td>
<td>1%</td>
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<tr>
<td>0</td>
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<td>5</td>
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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).

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

Figure 2. Experiment 2 overview: lactose and soluble mineral removal by UF at 3 different protein concentrations, with batch number 2, 4, 5, 6, 7, 8, 9, and 11 formulated from batches 1, 3, 10, and 12.
run. All other stainless steel and plastic parts were disassembled and hand cleaned.

**Beverage Formulation: Experiment 1.** The formulations of the milk beverages were calculated based on 4 base products: 1) the original skim milk (full lactose), 2) the original whole milk (full lactose), 3) the skim milk with “complete” (0.1%) lactose removal, and 4) the whole milk with “complete” (0.1%) lactose removal. The milks were formulated in 4000 g batches (as blends using 4 base products listed above) with varying percentages of lactose reduction (0%, 30%, 70%, 100%) and milkfat [Skim (0.1%, 1%, 2%), Whole (3.45%)] to make 16 formulated milk beverages in total, as shown in the Figure 1.

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

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

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

**Beverage Formulation: Experiment 2.** The formulations of the milk beverages were calculated based on 4 base products: 1) the original skim milk (full lactose) 3.4% protein, 2) the original skim milk (full lactose) concentrated by UF to 10.5% protein, 3) the skim with “complete” (0.1%) lactose removal at 3.4% protein, and 4) the skim with “complete” (0.1%) lactose removal concentrated to 10.5% protein. The milks were formulated into 4000 g batches (as blends using 4 base products listed above) with varying percentages of lactose reduction (0%, 30%, 70%, 100%) and protein (3.4%, 6.5%, 10.5%) to make 16 formulated milks in total, as shown in Figure 2.

**Analysis Methods**

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

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

**Microbial Analysis** The microbial quality of the retentate through the processing and final UF retentates were determined by aerobic plate counts (APC) (Laird et al., 2004; 6.040) and coliform counts (Davidson et al., 2004; 7.071) (Petrifilm Aerobic Count Plate, 3M ID 7100039392, 3M Food Safety, Maplewood, Minnesota). The APC was determined using an Advanced Instruments milk cryoscope (Model 4250, Norwood, MA, USA).

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

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

**Descriptive Analysis.** Descriptive analysis was conducted in accordance with the North Carolina State University Institutional Review Board for the Protection of Human Subjects in Research (NCSU IRB) regulations. Thirty mL of each beverage was poured into 59-mL souffle cups, capped (Dart Container Corp.), and labeled with a randomized 3-digit blinding code. Samples were prepared with overhead lights off to prevent light oxidation. Beverages were evaluated at 4°C.

Milk beverages were evaluated by 7 trained and experienced panelists (3 males, 4 females, ages 22 to 48 y). Each panelist had a minimum of 80 h of prior descriptive analysis experience documenting flavors of milks and dairy protein beverages using the Spectrum method with a 0 to 15 point intensity scale (Meilgaard et al., 2007). Panelists evaluated each treatment in duplicate in a randomized balanced order of presentation. No more than 6 samples were evaluated in a session and a minimum 2 min rest was enforced between samples. Panelists expectorated samples and rinsed their palates with bottled spring water. The milk protein beverages were evaluated the week of processing for appearance/whiteness, opacity, sweet aromatic, papery, cooked/milky, sweet taste, astringent mouthfeel, and viscosity. These attributes were previously established for fluid milk (McCarthy et al., 2017) with the exception of the attribute papery. This flavor attribute was defined as the aromatics associated with wet white paper and was considered distinct from cardboard flavor which is also a defined trained panel sensory attribute for dairy products and dairy ingredients (Smith et al., 2016). Compusense Cloud (Compusense, Guelph, Canada) was used for data collection.

**Statistical Analysis.**

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

**RESULTS AND DISCUSSION**

**Experiment 1**

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

**Processing data** The mean clean water flux at the beginning of the run and after being cleaned at the end of the run were 144.2 +/- 20 Kg/m²h before the start of the UF processing run and 142.05 +/- 21 Kg/m²h after the final cleaning. The UF processing time of running milk for both skim milk and whole milk UF (Experiment 1) were 3 h +/- 10 min starting with the same weight of milk. The mean flux did not differ (P > 0.05) between skim milk (47.0 Kg/m²h +/- 3 Kg/m²h) and whole milk (46.5 Kg/m²h +/- 3 Kg/m²h). The mean fouled water flux at the end of the milk processing run was 58.45 +/- 12 Kg/m²h. The mean anhydrous lactose for the skim milk was 4.75 +/- 0.04% and the ending lactose was 0.18 +/- 0.01% for a 96.2% lactose removal by UF of skim milk. The mean anhydrous lactose for whole milk was 4.44 +/- 0.12% and the ending lactose was 0.18 +/- 0.02% for a 96% lactose removal during the UF of whole milk. The amount of DF to achieve >=96% lactose removal was approximately 3 times the starting volume of milk for both skim and whole milk (Figure 3).

**Beverage lactose, freezing point, pH and TA.** Lactose content of the 4 main batches of beverage was measured by the enzymatic lactose spectrophotometric method. Batches 1, 4, 13, and 16 (Figure 1) contained 4.79, 4.54, 0.14, and 0.13% anhydrous lactose, respectively, and had freezing points of -0.529, -0.511, -0.018, and -0.017°C, respectively. When lactose and low molecular weight soluble minerals were removed, the freezing point of those batches (i.e., 13 and 16) were close to that of pure water. It is interesting to note that when samples from batches 13 and 16 were held refrigerated for one week, the freezing point did not decrease with time of cold storage indicating that
mineral bound to protein did not become soluble in the aqueous phase around the casein micelles with time of storage at 4°C. The mean anhydrous lactose content (g/100 g milk) of all the treatments were: for skim milk: 4.78, 3.39, 1.53, and 0.14; for 1% fat milk: 4.66, 3.31, 1.51, and 0.135; for 2% fat milk: 4.55, 3.23, 1.46, 0.13, and for whole milk: 4.45, 3.17, 1.43, 0.13, for a percent lactose removal of 0, 29, 68, and ≥96%, respectively. The treatments listed above with ≥96% removal would contain about 0.5 g of lactose per 240 g serving, which is well below the 12 g per day that a lactose intolerant individual can tolerate (Dalal et al., 2016).

Lactose and soluble mineral removal influenced both beverage pH and TA (Figures 4a and 5a). Beverage pH at 20°C increased progressively ($P < 0.05$) from about 6.6 to 7.4 as lactose and soluble mineral were removed (Figure 4a), while no effect ($P > 0.05$) of change in fat content on pH from skim to whole milk was detected. In contrast to pH, beverage TA decreased ($P < 0.05$) with increasing removal of lactose and soluble mineral (Figure 5a), while no effect of variation in fat content was detected ($P > 0.05$). The magnitude of change in pH at 20°C and TA were larger than expected. Both the proteins and low molecular weight soluble milk components (e.g., minerals, citrate, etc.) in milk contribute to the titratable acidity. In experiment 1, there was very little difference in protein concentration (3.2 for whole to 3.4% for skim) of the beverages at different fat contents. Change in lactose concentration should have little impact on beverage pH and TA, however low molecular weight soluble milk component (e.g., mineral, citrate, etc.) removal was the likely reason for the large decrease (from 0.18 to 0.04% expressed as lactic acid) in milk TA.

These changes in beverage pH and TA with lactose and soluble mineral removal may impact the heat stability of milk protein beverages. High quality raw milk has a TA value between 0.14 to 0.17% (expressed as lactic acid) (Schmidt, 1996). The TA of raw milk increases with age (i.e., developed acidity due to microbial growth, Santoso, 2018). Proteins, minerals, and dissolved gasses all contribute to the base acid content of milk (Schmidt, 1996) with TA increasing with protein content. The factors that affect the variation of soluble mineral in cows are the breed, stage of lactation, infection of the udder, and the feed (Fox et al., 2008; Zwierzchowski and Ametaj, 2019). Data is limited for change in pH and TA of UF retentates as lactose and soluble minerals are removed by UF.

Milk pH changes reversibly as function of temperature due to temperature dependent migration of milk mineral in and out of casein micelles. Ma and Barbano (2003a) reported that milk with a normal casein and serum protein content at about 0°C had a pH of about 6.97, and separately Ma and Barbano (2003b) reported that milk pH decreased linearly from 40°C to 80°C, from 6.57 to 6.25, respectively.

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

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

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

Sensory viscosity decreased as lactose and low molecular weight solutes were removed \( (P < 0.05) \). As lactose was removed, the milk became very bland/neutral in flavor, consistent with a decrease in cooked/milky and sweet aromatic flavors and sweet and salty tastes (Figure 9). Previous work to our knowledge, has not addressed aromatic flavor properties of milks with lactose removed by UF. Rizzo et al. (2020) utilized a trained panel to document sensory properties of commercial lactose free milks as part of a larger study on consumer acceptance. One of the commercial lactose free milks that they evaluated had the lactose removed by a combination of UF and lactose hydrolysis. This UF milk was lower in sweet aromatic flavor and sweet and salty tastes compared with the other milks. Milk protein concentrates and isolates are manufactured from skim milk by UF. When milk protein powders are rehydrated at 10% solids (wt/vol) or liquid retentates are diluted to 10% solids (wt/vol), these rehydrated proteins are characterized by lower intensities of traditional milk flavors and undetectable intensities of sweet and salty tastes (Smith et al., 2016, Carter et al., 2018). Milk fat flavor did not change with lactose removal and increased as expected with increasing fat concentration.

Astringency increased with decreased milkfat and increased lactose removal \( (P < 0.05) \). Previous work has established that increased heat treatment or increased protein concentration as well as decreased milkfat increase astringency (Vogel et al., 2021; Lee et al., 2017). Skim milk had higher astringency intensity than whole milk (Lee et al., 2017). Liquid and rehydrated whey and milk protein powders at 10% (wt/vol) solids, which also have little to no lactose or fat, are also astringent (Carter et al., 2018; Smith et al., 2016), and the astringency intensities are higher than fluid skim milk.

**Experiment 2**

**Bacteria counts.** Samples were taken of the starting pasteurized skim of the 2 tanks (pasteurized skim 1 and pasteurized skim 2), low lactose skim at 3.3% protein, low lactose skim at 10.5% protein, and full lactose skim at 10.5% protein to measure the microbial quality of the products of the UF process. No coliforms were detected by direct plating of 1 mL of sample.
The average for APC for the pasteurized skim 1 started at 25 $\pm$ 10 cfu/mL, pasteurized skim 2 started at 15 $\pm$ 7 cfu/mL, and the low lactose skim at 3.3% ended at 25 $\pm$ 7 cfu/mL. The low lactose skim at 10.5% protein ended at 100 $\pm$ 12 cfu/mL and the full lactose skim at 10.5% protein ended at 775 $\pm$ 15 cfu/mL.

**Processing data.** The mean clean water flux at the beginning of the run and after being cleaned at the end of the run were 121 $\pm$ 5 Kg/m²h before the start of the UF processing run and 128 $\pm$ 12 Kg/m²h after the final cleaning. The mean fouled water flux at the end of the milk processing run was 62 $\pm$ 4 Kg/m²h. The mean anhydrous lactose for the skim milk was 4.71 $\pm$ 0.06% and the ending lactose was 0.24 $\pm$ 0.02% for a 95% lactose removal by UF of skim milk. The amount of DF to achieve 96% lactose removal was approximately 3 times the starting volume of milk (Figure 3).

**Beverage lactose, freezing point, pH and TA.** Lactose content of the 4 main batches of beverage was measured by the enzymatic lactose spectrophotomet-
ric method. Batches 1, 3, 10, and 12 (Figure 2) contained 4.75, 4.27, 0.19, and 0.18% anhydrous lactose, respectively, and had freezing points of $-0.530$, $-0.550$, $-0.022$, and $-0.034^\circ$H, respectively. The mean anhydrous lactose content (g/100 g milk) of all the treatments were: for 3.4% protein: 4.75, 3.38, 1.54, and 0.19; for 6.5% protein: 4.51, 3.21, 1.48, and 0.185; and for 10.5% protein: 4.27, 3.04, 1.41, 0.18, for a percent lactose removal of 0, 29, 68, and 96%, respectively. When lactose and low molecular weight soluble minerals were removed, the freezing point of those batches (i.e., 10 and 12) were close to that of pure water. Lactose and soluble mineral removal influenced both beverage pH and TA (Figures 4b and 5b). Beverage pH at 20°C increased progressively ($P < 0.05$) from about 6.6 to 7.2 as lactose and soluble mineral were removed (Figure 4b), while there was small effect ($P > 0.05$) of change in protein content and the interaction of protein x lactose removal on pH. In contrast to pH, beverage TA ($P < 0.05$) increased with increasing protein concentration and decreased ($P < 0.05$) with increasing removal of lactose and soluble mineral (Figure 5b). Both proteins and low molecular weight soluble milk components contribute to milk TA. The stepwise increase in TA with increasing protein content (Figure 5b) coincides with previous research where 2.58% protein content raw milk had 0.15 TA and a 3.86% protein content raw milk had 0.18 TA (Schmidt et al., 1996). The magnitude of change in pH at 20°C and TA were larger than expected. Change in lactose concentration should have little impact on beverage pH and TA, however low molecular weight soluble component (e.g., mineral, citrate, etc.) removal was the likely reason for the large decrease in milk TA, within each of the 3 protein concentrations. These changes in beverage pH and TA with lactose and soluble mineral removal may impact the heat stability of milk protein beverages. Renhe and Corredig (2018) reported that UF retentates at 3.2%, 6.1%, 6.7%, and 12.8% protein (where lactose and soluble minerals in the permeate portion of the retentate was not changed greatly) had pH at 25°C of 6.67 ± 0.02, 6.67 ± 0.03, 6.67 ± 0.03, and 6.66 ± 0.05 respectively. The pH results for Renhe and Corredig (2018) coincides with the

Figure 8. Reflectance curves. (A) – Experiment 1 – skim milk; (B) Experiment 1 – whole milk; (C) Experiment 2 – 3.4% protein, (D) Experiment 2 – 10.5% protein.
pH of the UF retentates without the lactose removal in our study.

**Beverage AV and color.** The AV of the beverages increased ($P < 0.05$) with increasing protein concentration had higher AV ($P < 0.05$), while AV decreased ($P < 0.05$) slightly with increasing removal of lactose and soluble minerals (Figure 6b). Krishmankutty et al. (2013) and Dunn et al. (2021) also reported an increase in viscosity with increasing protein concentration and reported that viscosity increase was related to the increased volume fraction of casein micelles in solution with increasing protein concentration and results in the current paper are consistent with those observations. At 10.5% protein in skim milk the AV values were about 17 mPa·s. Studies conducted by Misawa et al. (2016), Cheng et al. (2019a), and Quiñones et al. (1997) reported that as protein content increased so did the viscosity of milk and milk beverages. Increased protein concentration increased AV more at low temperature (4°C) than at higher temperature (>20°C) Cheng et al. (2019a). Determination of AV of beverages ranging in fat from 0.2 to 2%, true protein from 3 to 5%, and casein as a percentage of true protein (CN%TP) from 5 to 80% at 4°C, revealed that CN%TP explained the majority of the variability (65%) in AV while protein, and fat explained 16.19 and 4.54% of the variability, respectively (Cheng et al., 2019a). Thus, increasing protein concentration and altering CN%TP in beverages may be an approach to increasing bever-
L-value (whiteness) increased as expected with increasing protein concentration (Figure 7b) but also increased slightly \((P < 0.05)\) with increasing lactose and soluble mineral removal. The a-value increased \((P < 0.05)\) with increasing protein concentration and lactose removal within each protein concentration (Figure 7d) resulting in less greenness. There was both an effect of lactose removal \((P < 0.05)\) and the interaction of protein x lactose removal \((P < 0.05)\) on a-value. The b*-value to decreased with increasing lactose removal (less yellow). The b*-value increased slightly \((P < 0.05)\) with increasing protein concentration. These findings were also demonstrated by Quiñones et al. (1997) who showed that increasing protein content in skim and 1% milks caused the L-value, a-value, and b-value to increase. Cheng et al. (2019b) found that changing not only the protein percentage but the type of protein, casein as a percentage of true protein (CN%TP), also altered color of milk protein beverages. As the CN%TP increased, the L-value increased and a and b* values decreased.

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

**Beverage sensory.** The trained panel sensory results for experiment 2 are presented in Figure 10. Interactions between lactose and fat were evident for all sensory attributes \((P < 0.05)\) so a PCA biplot was used to visualize results. As lactose and soluble mineral were removed, beverage whiteness, opacity and astringency increased and sensory yellowness decreased \((P < 0.05)\), consistent with lactose and soluble mineral removal in experiment 1. Increased sensory whiteness was consistent with the increase in L-value (Figure 7b) as lactose was removed. Sensory yellowness decreased with increasing lactose removal as did b*-value (Figure 7f). Sensory viscosity did not change \((P > 0.05)\) as lactose and low molecular weight solutes were removed. As lactose was removed, sensory profiles of milks became very bland, concurrent with a decrease in cooked/milky and sweet aromatic flavors and sweet and salty tastes (Figure 10), also consistent with experiment 1. Sensory viscosity increased with increasing protein concentration \((P < 0.05)\), consistent with instrumental AV (Figure 6b). Increasing protein concentration increased viscosity in fluid milk and milk beverages (Quiñones et al., 1997; Cheng et al., 2019b; Vogel et al., 2021). As protein percentage increased in the milk beverages so did sensory opacity, papery flavor, and astringency \((P < 0.05)\). Previous studies have demonstrated that increased protein content in milk and milk protein beverages increased opacity and astringency (Cheng et al., 2019b; Vogel et al., 2021). Papery flavor, which increased with protein concentration, was described as the flavor of white paper. Sensory panelists agreed that the flavor was similar to cardboard flavor. Cardboard flavor is an established flavor in spray dried milk and whey proteins (Wright et al., 2008; Smith et al., 2016). Cardboard flavor increases with storage of dried proteins and is linked with neutral aldehydes and lipid oxidation and a loss of freshness (Wright et al., 2008; Whitson et al., 2010). Low intensities of cardboard flavor have been documented in liquid milk and whey protein (Carter et al., 2018; Oltman et al., 2015). The intensities of papery flavor were low (0.6, 1.2, 1.3 on a 0 to 15 point intensity scale for 3.3, 6.9 and 10.5% protein, respectively), consistent with previous studies with liquid milk proteins. Collectively, milk components had distinct contributions to milk/milk beverage sensory properties. Lactose and soluble mineral removal decreased overall flavor and taste of milk, decreased yellowness and increased opacity. Fat contributed milkfat flavor, opacity, viscosity and decreased astringency. Protein contributed opacity, viscosity and astringency with little impact on flavor. Future work on milk and milk-based beverages with lactose removed by UF should focus on interaction of the remaining milk solids with added flavorings, changing casein to whey protein ratio before UF removal of lactose, and the impact of lactose and low molecular weight solute removal on heat stability, particularly for neutral-pH, shelf-stable milk-based beverages.

**CONCLUSIONS**

Previous research on UF of milk for beverage applications has focused on use of UF to increase the concentration of protein, while the current work has focused on use of UF to remove lactose from milk in combination with increasing protein concentration. Removal of >95% of milk lactose by UF required a diafiltration volume of approximately 3 times the milk volume. In addition, lactose (and other low molecular weight solute removal) by UF decreased titratable acidity by more than 50% and increased milk pH at 20°C to > 7.0. Lactose and soluble mineral removal from milk
increased whiteness of skim while decreasing viscosity and flavor. Fat contributed milkfat flavor, opacity, viscosity and decreased astringency. Protein contributed opacity, viscosity and astringency with little impact on flavor. Milk beverages with variable composition and sensory properties (appearance, flavor and viscosity) can be manufactured to meet consumer desires for beverage variety.

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