Ultrafiltration: Impact of process temperature (7 and 50°C) on process performance and protein beverage physical, chemical, and sensory properties.

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ABSTRACT

Our objectives were to determine the impact of ultrafiltration (UF) of skim milk at 7 and 50°C on UF processing, lactose removal, mineral partitioning, and skim milk retentate physical, chemical, and sensory properties at 3 (3.4 7.5, and 10.5%) protein concentration with 2 different heat processing treatments high temperature short time (HTST) pasteurization and autoclave. Pasteurized skim milk was split into 2 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 s) 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 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 s) than skim milk based on both sensory scores and instrumental measures. A 98 to 99% removal of lactose from skim milk was achieved (final lactose concentration <0.06 g/100g) with a diafiltration ratio of water to milk of about 4 to 1 was used at both 7°C and 50°C. The processing time to achieve that lactose removal from the same starting weight 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 versus −0.525°C for skim milk) and the pH of the lactose-free milk at 20°C increased from about 6.5 for skim to about 7.33 and 7.46 for UF/DF skim milk at 7 and 50°C, respectively. Removal of compounds that absorb light (in the range of 360 to 500 nm) from milk in the permeate, increased light reflectance and whiteness and decreased yellowness relative to the starting skim milk.

Key Words: Lactose removal, ultrafiltration, milk minerals

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 with 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 2012).
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 in the retentate. The concentration of lactose, and soluble mineral decrease 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 concentration factor UF process (based on starting weight of skim milk and weight of permeate removed) 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). When using continuous DF of skim milk with permeate replacement with water, the starting skim milk had 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 UF/DF. With one 3X concentration stage, followed by 2 3X total weight 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 anhydrous 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. Pranata et al. (2024) reported that both protein, calcium, and phosphate concentration in the ultracentrifugation supernatants for 7.5% protein MPC and MCC beverages increased nearly 2 fold over 14 d of storage at 4°C, but the freezing point of the supernatants did not change with increasing total calcium and phosphate concentration, indicating that there was little if any re-equilibration of bound calcium and phosphate to soluble calcium phosphate during cold storage of beverages that had most of their lactose and soluble mineral removed by diafiltration. The calcium and phosphorous in the ultracentrifugation supernatant was still bound to protein. Hernandez et al. (2023) reported 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. When protein concentration was increased by UF from 3.4 to 10.5%, 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 milk serum phase 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 UF retentates. The composition of casein micelles including the hydration, calcium and casein content were altered to some extent by the temperature of UF (Liu et al., 2014).

Other than differences in composition of UF retentates, there are also differences in energy costs when operating UF at different temperatures. Méthot-Hains et al. (2016) reported that there was less thermal energy consumption at 10°C than at 50°C during UF 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 UF 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 was achieved 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 UF retentates.
from skim milk. 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 of milk protein beverages at 3 (3.4 7.5, and 10.5%) protein concentration with 2 different heat processing treatments (HTST and autoclave).

**MATERIALS AND METHODS**

**Experimental Design**

One batch of pasteurized skim milk was split into 2 portions (held at 4°C) 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 (Figure 1). 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 the weight of 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. Lactose removal was estimated during processing by infrared analysis of lactose in skim, retentate, and permeate. The final lactose removal was determined based on the mass of lactose in the starting skim milk and the mass of lactose removed in permeate measured using lactose analysis with a spectrophotometric lactose method (AOACI, 2019; method 2006.06) as described in detail by Lynch et al. (2006). The total weight of 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. The typical serving size for milk is 240 g and the recommended dietary intake of protein for an adult is 50 g/day. Typical skim milk (3.4% protein) delivers about 8 g of protein per serving. At 7.5 and 10.5% protein, one 240 g serving delivers about 1/3 and about one half of the daily requirement protein. The consumer preference for protein beverages is 15 to 25 g protein for an adult is 50 g/day. Typical skim milk (3.4% protein) delivers about 8 g of protein per serving. At 7.5 and 10.5% protein, one 240 g serving delivers about 1/3 and about one half of the daily requirement protein. The consumer preference for protein beverages is 15 to 25 g of protein per serving (Harwood and Drake, 2019) and our 7.5 and 10.5% protein span this range of g of protein per 240 g serving.

Thus, 3.4, 7.5, and 10.5% protein lactose reduced beverages were produced by UF/DF running the system at 7°C and the same products were produced running the UF/DF process at 50°C. Each of these 6 products received either no heat treatment, HTST (78°C for 15 s), or an autoclave (116°C for 6 min) heat treatment (Figure 1). This was replicated twice starting with a different batch of skim milk. 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 UF processing runs at 7 and 50°C run 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 before milk processing. Start of day clean water flux at 50°C was about 133.5 ± 8.8 kg/m²/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 stainless-steel feed tank (Meyer-Blank Company, St Louis, MO) jacketed with a recirculating 50°C to heat the milk to from 4°C 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 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). Biomax 10 kDal polyether-sulfone (PES) membranes with a 10 kilodalton (kDal) cut-off with a surface area of 0.5 m² per plate were used in the UF system. For optimal pressure, the UF 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 processing runs and stored in membrane soak solution at 4°C. The skim milk feed tank was connected to the membrane stack by a centrifugal pump (Baldor Industrial Motor, single phase, 1.5 H.P., 3450 RPM, 60hz, Baldor Electric Co., Ft. Smith, AR). The UF plate system 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 retentate recirculation loop and a flux of about 23.3 ± 1.4 kg/m²/h at 7°C and 47.7 ± 3.7 kg/m²/h at 50°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 (wt/wt) in final DF skim milk retentate. The retentate pH was taken at the beginning and end of processing run. The UF processing was oper-
ated in a continuous DF mode 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 equal the weights of permeate removed to maintain constant true protein concentration in the UF feed tank. This ensured that the correct amount of lactose removal was achieved without altering the starting milkfat and protein percentages in the UF retentate. The amount of water added with time of processing was recorded and reported as the DF ratio and was the total weight of added water divided by the total starting weight of milk processed. The anhydrous 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 spectrophotometric method analysis for anhydrous lactose (Lynch et al., 2006). Permeates and retentates were analyzed by MIR along with a flux measurement after each DI water addition (about every 10 min for the 50°C run and every 20 min for the 7°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 was increased to produce a 10.5% protein, lactose-free high protein UF milk protein beverage base. When the UF of the milk was complete, the UF plate unit was flushed with DI water (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. 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 base produced by either a 7 or 50°C UF processing run was used to create 15,000 g batches of 7.5% and 3.4% protein lactose-free 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% true protein content was achieved. 5,000 g of each of the unheated high-protein lactose-free beverages at the 3.4%, 7.5%, and 10.5% protein levels were collected and not thermally processed as controls without heat treatment.

**HTST Thermal Processing**

A Microthermics EHVH pasteurization unit (Microthermics, Raleigh, NC) 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, lactose-free beverages of different protein concentrations 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 retentate beverages were collected in commercial light shielded 2 L milk containers and cooled in an ice batch to 4°C.

**Autoclave Thermal Processing**

For autoclave heat treatment, lactose-free beverages at different protein concentrations were filled into 6 500 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): 116°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 s. After bottles were removed from the autoclave (when the autoclave air temperature was < 100°C), the beverages were cooled in an ice bath to approximately 4°C and then stored 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 30 s intervals. The probes were placed at the center of the bottle. All treatments were analyzed on the next day.

**Analysis Methods**

**Chemical Analyses.** Duplicate analysis for total solids (TS), fat, total N (TN), and nonprotein nitrogen (NPN) content were done using forced-air oven drying (AOAC, 2019; method 990.20), Kjeldahl TN (AOAC, 2019; method 991.20), and Kjeldahl NPN (AOAC, 2019; method 991.21), respectively. The non-casein nitrogen (NCN) content was determined using Kjeldahl (AOAC, 2019; method 998.05). The TP was calculated by subtracting NPN from TN and multiplying by 6.38; casein nitrogen (CN) was calculated by subtracting the NCN from TN and multiplying by 6.38; and whey protein content was calculated by subtracting NPN from NCN and multiplying by 6.38. 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 7 and 50°C UF runs using an Advanced Instruments milk cryoscope (Model 4250, Norwood, MA, USA). The final pH of each beverage 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 at 20°C 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) were conducted (Petrifilm Aerobic Count Plate, 3M ID 7100039310 and Petrifilm Coliform Count Plate, 3M ID 7100039392, 3M Food Safety, Maplewood, MN).

Apparent Viscosity

Apparent viscosity (AV) was measured using a rotational Brookfield viscometer (LV-DV2T, Brookfield Engineering Laboratories Inc., Middleboro, Massachusetts) with the water (4°C) 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 AV of lactose-free beverages at different protein concentration 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 to 90%. All batches that were not thermally processed and HTST processed beverage formulations were measured at 30 RPM. All autoclave beverage formulations were measured at 8 RPM.

Color

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 (PolyScience, 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 beverage color was 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 µm. The Malvern multiple narrow mode model for spherical particles was used. The measurement 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 min into a 40°C water bath, then mixed gently by inversion for homogeneity before 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 (Fisher). 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 2 stages: Stage one had a 10 min ramp to 135°C and held for 3 min at 1500W. Stage 2 had a 12 min ramp to 200°C and held for 15 min 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 were collected electronically on the NCSU secure network.

Statistical Analysis.

Data were analyzed using the General Linear Models ANOVA procedure of SAS version 9.4 (Cary, NC). The original skim milk was compared with the 3 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 6 UF products (3 protein levels produced by UF at 7°C and 3 protein levels produced by UF at 50°C) were each given 3 different heat treatments (none, HTST, autoclave). The statistical model included UF process temperature (7 and 50°C), heat treatment (none, HTST, autoclave), 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. No differences (P > 0.05) in composition were detected (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. The microbial quality of the retentate was excellent (<260 cfu/mL) at both processing temperatures at the end of the processing runs. 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.

Processing Data

The mean clean water flux was 130 +/- 8.2 kg/m²h before and after the final cleaning. The mean flux during processing of skim milk at 7°C was 23.3 +/- 1.4 kg/m²h and at 50°C was 46.05 +/- 1.76 kg/m²h. Inlet pressure was 138 kPa and outlet pressure for permeate and retentate were 0 kPa, retentate cross flow velocity was 22 kg/min/m², for processing at both 7 and 50°C with 10 kDal PES membranes. The 7°C UF/DF run was about 9.5 h in length and a flux of about 23 kg/m²h was maintained constant for 9.5 h, while the processing run at 50°C was about 4 h and a flux of about 46 kg/m²h was maintained 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 true protein concentration in the retentate 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/m²h, while at 7°C it took 1.45 h and the flux declined from 23 to 13 kg/m³.

The higher flux at 50°C versus 7°C in our study was consistent with a report by Kapsimalis and Zall (1981) where they found the flux at 45°C was higher 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 with ultrafiltering at a cold temperature such as 10°C (Méthot-Hains et al., 2016).

Before 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

### 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>Protein¹</th>
<th>Lactose¹</th>
<th>Calcium²</th>
<th>Phosphorous²</th>
<th>Potassium²</th>
<th>Ca/P ratio</th>
<th>pH at 20°C</th>
<th>Freezing point³</th>
</tr>
</thead>
<tbody>
<tr>
<td>7°C</td>
<td>3.34⁴</td>
<td>4.78⁴</td>
<td>1376⁴</td>
<td>1108⁴</td>
<td>1612⁴</td>
<td>1.24⁴</td>
<td>6.670⁴</td>
<td>-0.530⁴</td>
</tr>
<tr>
<td>50°C</td>
<td>3.34⁴</td>
<td>4.78⁴</td>
<td>1360⁴</td>
<td>1104⁴</td>
<td>1601⁴</td>
<td>1.23⁴</td>
<td>6.685⁴</td>
<td>-0.528⁴</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>

¹ Values shown are grams/100 g 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.
temperature runs. The percent lactose reduction and DF ratio 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 min ±/−25. The average time to remove the lactose at 50°C took 251 min ±/−13. The 98 to 99% lactose removal was achieved by using 4 times more DF water than the starting weight of skim milk at both 7°C (Figure 2a) and 50°C (Figure 2b). Processing the skim milk at 50°C required approximately half the amount of time (Figure 2b) compared with 7°C (Figure 2a) to remove over 98% of the lactose. The freezing point of the UF retentate increased as skim milk was DF at 7°C (Figure 3a) and 50°C (Figure 3b). 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°H to about −0.013°H, even though the true protein concentration remained constant about 3.4% (Figures 3a and 3b). 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 3a), than at 50°C (pH 7.43; Figure 3b). The higher freezing point and higher pH with increasing lactose removal are consistent with results of 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 rapidly estimate lactose removal during UF or other filtration processing techniques.

The anhydrous lactose concentration of the high-protein beverages produced by UF at 7°C and 50°C was determined by enzymatic assay (AOAC, 2019). No difference in the percentage of anhydrous lactose removed by UF/DF at 7°C versus 50°C (P > 0.05) was detected and no difference in lactose removal (P > 0.05) among the 3 different protein concentrations was detected (Table 2).

The chemical compositions of the UF beverages at the 3 protein levels before heat treatment from both 7°C and 50°C UF are shown in Table 3. No differences in the chemical components listed in Table 3 (P > 0.05) between UF/DF at 7°C compared with 50°C were detected (data not shown). As expected, the percent protein, noncasein nitrogen, casein and total solids increased (P < 0.05) with increased removal of permeate by UF, but as expected 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 (P < 0.05) in NPN than the starting skim milk. The NPN 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 3 protein levels before thermal processing are shown in Table 4. A lactose intolerant individual can tolerate the consumption of approximately 12 g 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 g per 240 g serving) and decreased (P < 0.05) with decreasing protein concentration (Table 4). Both total calcium and phosphorous concentrations (Table 4) in the DF beverages 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 calcium phosphate to milk serum soluble calcium phosphate in skim milk shifts to decrease the amount of calcium and phosphorous bound in the casein micelles at 7°C with an increased concentration of soluble calcium in the serum phase of milk (Davies and White, 1960) making more soluble calcium available to pass through a UF membrane at 7°C than 50°C. The lower amount of bound calcium and phosphorous at 7°C than 50°C before UF allowed more calcium and phosphorous to be removed in the permeate during UF at 7°C versus 50°C (Table 4). It is apparent that during UF/DF at constant temperature, there was not a continuous re-equilibration of mineral from bound to soluble mineral during the UF/DF process as soluble mineral was removed at constant low temperature. The UF retentate post process did not decrease in freezing point (i.e., mineral shifting from protein bound to soluble) when held at a constant temperature. Soluble calcium and phosphorous are lower in molecular weight than lactose and are removed in proportion to lactose removal. The freezing point of the aqueous phase of the

### Table 2. Mean percentage of lactose removed in lactose-free milk protein beverages at 3.4, 7.5, and 10.5% protein produced by ultrafiltration with diafiltration at 7°C and 50°C

<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*</td>
</tr>
<tr>
<td>7.5%</td>
<td>99.6*</td>
</tr>
<tr>
<td>10.5%</td>
<td>98.7*</td>
</tr>
</tbody>
</table>

*a Numbers not sharing a common superscript within a column are different (P < 0.05).

*Values shown are percentages based on spectrophotometric enzymatic lactose assay.
Table 3. Mean chemical composition of lactose-free milk protein 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>TN2</th>
<th>NCN3</th>
<th>NPN4</th>
<th>CN3</th>
<th>CN/TP5</th>
<th>TS7</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.4</td>
<td>3.60a</td>
<td>0.50a</td>
<td>0.031a</td>
<td>3.10a</td>
<td>86.81a</td>
<td>4.10a</td>
</tr>
<tr>
<td>7.5</td>
<td>7.85b</td>
<td>1.02b</td>
<td>0.046b</td>
<td>6.83b</td>
<td>87.42b</td>
<td>8.78b</td>
</tr>
<tr>
<td>10.5</td>
<td>10.81c</td>
<td>1.44c</td>
<td>0.058c</td>
<td>9.37c</td>
<td>87.12c</td>
<td>12.03c</td>
</tr>
</tbody>
</table>

1 Numbers not sharing a common superscript within a column are different (P < 0.05).
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.

UF retentates after filtration did not change with time of storage at 4°C. 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 with 40°C. The calcium and phosphorous concentration in the lactose-free beverages at 3.4% protein (Table 4) were lower (P < 0.05) 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 (P < 0.05) 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 were 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). In the starting milk, the calcium to phosphorous ratio in the soluble mineral fraction of the milk is lower than the calcium to phosphorous ratio in the bound mineral fraction. Because only soluble calcium and phosphorous are being removed and bound calcium and phosphorous are not being removed, the ratio of calcium to phosphorous changes from 1.24 to about 1.66 which represents the Ca/P ratio in the protein bound mineral fraction of the milk. The Ca/P ratio was higher (P < 0.05) when milk was ultrafiltered at 50°C than 7°C (Table 4).

The potassium concentration in the lactose-free beverages was much lower (Table 4) than the concentration in skim milk (Table 1). Potassium in the skim milk is not protein bound 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 (Table 4). France et al. (2021) reported higher amounts of ionic calcium concentration (P < 0.05) were found in UF permeate 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 lactose-free milk protein beverages was much lower than that of skim milk.

In the case of exhaustive diafiltration, the milk protein is in solution in nearly pure water (i.e., freezing point close to 0°C) and as a result, the mixture of milk proteins with bound mineral approaches a pH of its isoionic point (Bryan, 1978). Under the condition of a protein or mixture of proteins in solution in pure water, the pH of the solution will approach the isoionic point of the pure protein, or the mixture of proteins in solution. The isoionic point is the point at which all dissociable groups in the substance combine equally and only with hydrogen and hydroxyl ions (Sookne and Harris, 1939; Sorenson et al., 1927). For a protein with no bound substances, the isoelectric point and isoionic point will be essentially equal. However, in the case of milk proteins, particularly caseins, bound minerals are interacted with some of the charged side groups in the protein chain. Thus, the isoionic point of the same protein, or mixture of proteins, with a different amount of electrostatically bound mineral will be different. At low protein concentration in pure water, the pH of the solution will approach the isoionic pH point. The isoionic points of the milk protein produced by UF at 7°C vs UF at 50°C were different and this can be seen by the comparison (Table 4) of the pH of the protein solutions at 3.4% protein concentration (pH 7.33 and 7.46, respectively).

The isoionic point of a protein, or protein with some bound mineral, will change as a function of protein concentration in solution and it can be seen that both the mixtures of proteins (i.e., proteins isolated by UF at 7 vs 50°C) with bound minerals decreased in pH by about the same amount (i.e., about 0.23 pH units) with an increase in protein concentration from 3.4 to 10.5% (Table 4). The milk protein/bound mineral mixture in the retentate produced by UF at 7°C had a lower isoionic point than that produced at by UF at 50°C due to the lower bound mineral content per unit of protein in the protein produced at 7°C.

Hernandez et al. (2023) also reported that when milk was ultrafiltered at 50°C to achieve progressively increased removal of lactose and soluble mineral from milks of different fat contents, the pH of the UF retentate increased progressively at all fat contents as percent lac-
tose and soluble mineral removal approached 98 to 99% removal. In addition, Hernandez et al. (2023) reported that as protein concentration was increased by UF for the lactose removed milks at different fat contents, the pH of the 98 to 99% lactose removed milks was higher as the protein concentration was lower (i.e., 3.3 vs 10.5% protein).

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

The mean chemical composition of the UF beverages averaged across all 3 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).

This may indicate that there is some thermal degradation of protein structures that releases TCA soluble nitrogen (Table 2). The chemical nature of these heat formed 12% TCA soluble 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 4 and 5. Compounds in the aqueous phase of milk absorb light in the 360 to 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 360 to 500 nm range for the lactose-free beverages ultrafiltered at either temperature increased (Figures 4 and 5). 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 4 and 5). 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 and autoclave thermal treatments at all protein concentration for lactose-

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**Table 4. Mean anhydrous lactose, mineral composition, pH (measured at 20°C), and freezing point (FP) of 7°C and 50°C lactose-free milk protein beverages produced by ultrafiltration (UF) with diafiltration (DF) at 7°C and 50°C before thermal processing**

<table>
<thead>
<tr>
<th>7°C UF</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>3.4</td>
<td>0.025&lt;sup&gt;a&lt;/sup&gt;</td>
<td>977&lt;sup&gt;f&lt;/sup&gt;</td>
<td>602&lt;sup&gt;i&lt;/sup&gt;</td>
<td>57&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1.62&lt;sup&gt;d&lt;/sup&gt;</td>
<td>7.33&lt;sup&gt;b&lt;/sup&gt;</td>
<td>−0.009&lt;sup&gt;e&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>7.5</td>
<td>0.052&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2181&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1354&lt;sup&gt;e&lt;/sup&gt;</td>
<td>125&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.61&lt;sup&gt;e&lt;/sup&gt;</td>
<td>7.15&lt;sup&gt;c&lt;/sup&gt;</td>
<td>−0.017&lt;sup&gt;d&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>10.5</td>
<td>0.064&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2716&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1711&lt;sup&gt;i&lt;/sup&gt;</td>
<td>168&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.59&lt;sup&gt;f&lt;/sup&gt;</td>
<td>7.09&lt;sup&gt;d&lt;/sup&gt;</td>
<td>−0.025&lt;sup&gt;e&lt;/sup&gt;</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>50°C UF</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>3.4</td>
<td>0.024&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1074&lt;sup&gt;e&lt;/sup&gt;</td>
<td>640&lt;sup&gt;d&lt;/sup&gt;</td>
<td>39&lt;sup&gt;f&lt;/sup&gt;</td>
<td>1.68&lt;sup&gt;e&lt;/sup&gt;</td>
<td>7.46&lt;sup&gt;c&lt;/sup&gt;</td>
<td>−0.009&lt;sup&gt;d&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>7.5</td>
<td>0.044&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2341&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1406&lt;sup&gt;e&lt;/sup&gt;</td>
<td>86&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.67&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.30&lt;sup&gt;c&lt;/sup&gt;</td>
<td>−0.014&lt;sup&gt;e&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>10.5</td>
<td>0.057&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3048&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1853&lt;sup&gt;e&lt;/sup&gt;</td>
<td>112&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1.64&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.22&lt;sup&gt;c&lt;/sup&gt;</td>
<td>−0.019&lt;sup&gt;d&lt;/sup&gt;</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>

<sup>a-c</sup> Numbers not sharing a common superscript within a column within UF processing temperature are different ($P < 0.05$).

<sup>1</sup> Values for protein and lactose are g/100 g beverage (wt/wt); n = 2.

<sup>2</sup> Values shown are mg/liter of milk.

<sup>3</sup> Values shown are °H.
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 beverages through a direct steam injection (DSI) thermal process, all of the lactose-free products gelled during the UHT-DSI process (regardless of protein concentration) and were not heat stable under direct steam injection UHT conditions with a holding tube temperature of 142°C. Peak temperatures at steam injection were in the range of 150 to 160°C depending on the steam injection flow pressure when a holding tube temperature of 142°C is 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 116°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) 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 4 and 5). There were effects (P < 0.05) of temperature of UF, thermal 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 increasing protein concentration on AV 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 s 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 (data not shown). The lactose-free beverages were more opaque, white, astringent, and less yellow, and had lower cooked/milky and sweet aromatic flavors than the starting pasteurized skim milk (Table 8). Sensory opacity, cooked/milky flavor, sensory viscosity, and astringent mouthfeel increased (P < 0.05) with increased protein concentration. Increasing severity of heat treatment increased cooked/milky flavor, opacity, sulfur/eggy flavor, and astringent mouthfeel (Figure 6, 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 6. The sensory properties of skim milk are not shown on the biplot to more easily visualize the differ-

### 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 (g x 100 g beverage)</th>
<th>NCN (g x 100 g beverage)</th>
<th>NPN (g x 100 g beverage)</th>
<th>TP (g x 100 g beverage)</th>
<th>CN (g x 100 g beverage)</th>
<th>CN/TP (%)</th>
<th>TS (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No heat</td>
<td>7.36a</td>
<td>1.25b</td>
<td>0.033a</td>
<td>6.11c</td>
<td>83.27a</td>
<td>8.24b</td>
<td></td>
</tr>
<tr>
<td>HTST</td>
<td>7.35b</td>
<td>1.09b</td>
<td>0.028c</td>
<td>6.27b</td>
<td>85.27b</td>
<td>8.25b</td>
<td></td>
</tr>
<tr>
<td>Autoclave</td>
<td>7.38a</td>
<td>0.51c</td>
<td>0.084a</td>
<td>7.07a</td>
<td>94.23a</td>
<td>8.47a</td>
<td></td>
</tr>
</tbody>
</table>

- **Table 6.** The effect of UF processing temperature on least squares means of apparent viscosity (AV) (mPa·s), whiteness (L), redness-greenness (a), and yellowness-blueness (b*) values of lactose-free milk protein beverages.

<table>
<thead>
<tr>
<th>UF Temperature</th>
<th>AV (mPa·s)</th>
<th>L</th>
<th>a</th>
<th>b*</th>
</tr>
</thead>
<tbody>
<tr>
<td>7°C</td>
<td>3.35a</td>
<td>78.43a</td>
<td>-4.10c</td>
<td>3.03a</td>
</tr>
<tr>
<td>50°C</td>
<td>3.21a</td>
<td>78.07a</td>
<td>-3.96c</td>
<td>3.11a</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>

- 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 milk protein 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>8.76</td>
<td>80.02</td>
<td>−2.54</td>
<td>−0.81</td>
</tr>
<tr>
<td>HTST</td>
<td>8.31</td>
<td>80.62</td>
<td>−2.63</td>
<td>−0.71</td>
</tr>
<tr>
<td>Autoclave</td>
<td>11.45</td>
<td>81.04</td>
<td>−2.41</td>
<td>−0.45</td>
</tr>
<tr>
<td>Target protein</td>
<td>3.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.4</td>
<td>2.88</td>
<td>79.66</td>
<td>−3.32</td>
<td>−1.76</td>
</tr>
<tr>
<td>7.5</td>
<td>6.91</td>
<td>81.65</td>
<td>−2.20</td>
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</tr>
<tr>
<td>10.5</td>
<td>18.72</td>
<td>80.37</td>
<td>−2.06</td>
<td>−0.02</td>
</tr>
</tbody>
</table>

abc Numbers not sharing a common superscript within a column are different (P < 0.05).

Table 8. Mean sensory intensity scores (0 to 15 point intensity scale) for lactose-free milk protein 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>11.8a</td>
<td>13.0b</td>
<td>0.5a</td>
<td>1.9b</td>
<td>2.0b</td>
<td>0.8b</td>
<td>0.5b</td>
<td>2.3b</td>
</tr>
<tr>
<td>7.5</td>
<td>12.5b</td>
<td>12.6b</td>
<td>0.5b</td>
<td>2.2b</td>
<td>2.2b</td>
<td>0.7b</td>
<td>1.0b</td>
<td>2.5b</td>
</tr>
<tr>
<td>10.5</td>
<td>13.0a</td>
<td>12.7b</td>
<td>ND</td>
<td>2.5a</td>
<td>2.6a</td>
<td>ND</td>
<td>1.1a</td>
<td>2.7a</td>
</tr>
<tr>
<td>Heat treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NH</td>
<td>11.7c</td>
<td>12.7b</td>
<td>0.5b</td>
<td>2.1b</td>
<td>2.1b</td>
<td>0.5b</td>
<td>ND</td>
<td>2.4b</td>
</tr>
<tr>
<td>HTST</td>
<td>12.6b</td>
<td>12.7b</td>
<td>0.5b</td>
<td>2.2b</td>
<td>2.3b</td>
<td>0.6b</td>
<td>ND</td>
<td>2.5b</td>
</tr>
<tr>
<td>Autoclave</td>
<td>13.0b</td>
<td>12.9b</td>
<td>ND</td>
<td>2.2b</td>
<td>2.4b</td>
<td>ND</td>
<td>2.6</td>
<td>2.7b</td>
</tr>
<tr>
<td>Skim Milk</td>
<td>8.0</td>
<td>8.1</td>
<td>2.4</td>
<td>1.9</td>
<td>3.1</td>
<td>1.9</td>
<td>ND</td>
<td>2.1</td>
</tr>
</tbody>
</table>

abc Numbers not sharing a common superscript within a column are different (P < 0.05). ND – not detected.

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 a 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 protein beverages at 3.4, 7.5, and 10.5% protein produced by UF with DF, were 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 s) 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. No sensory differences due to UF at 7°C versus 50°C were detected. However, all the lactose-free beverages were more opaque, white, astringent, and less yellow, and had lower cooked/milky and sweet aromatic flavors than the lactose-free beverages due to 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 4 and 5). 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 water soluble 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).
starting pasteurized skim milk. Sensory opacity, cooked/milky flavor, sensory viscosity, and astringent mouthfeel increased with increased protein concentration. Increasing severity of heat treatment increased cooked/milky flavor, opacity, sulfur/eggy flavor, and astringent mouthfeel.

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**REFERENCES**


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