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Effects of micellar casein concentrate purity and milk fat on sulfur/eggy flavor in ultrapasteurized milk-based beverages

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ABSTRACT

Our objectives were to determine the level of milk-derived whey protein (MDWP) removal necessary to achieve no detectable sulfur/eggy flavor in ultrapasteurized fat-free micellar casein concentrate (MCC) beverages (6.5% protein) and in the same beverages containing 1 and 2% milk fat. Micellar casein concentrate with 95% MDWP removal was produced from skim milk (50°C) with a 3×, 3-stage ceramic microfiltration (MF) process using 0.1-μm pore size graded permeability membranes (n = 3). In experiment 1, MCC-based beverages at about 6.5% (wt/wt) true protein were formulated at a fat content of 0.15% fat (wt/wt) at 4 different levels of MDWP removal percentages (95.2%, 91.0%, 83.2%, and 69.3%). In experiment 2, a similar series of beverages at 3 MDWP removal percentages (95.2%, 83.2%, and 69.3%) with 0.1, 1, and 2% fat content were produced. The purity (or completeness of removal of whey protein by MF) of MCC was determined by the Kjeldahl method and sodium dodecyl sulfate (SDS)-PAGE. Sensory properties of beverages were documented by descriptive sensory analysis, and volatile sulfur compounds were evaluated using solid-phase microextraction followed by gas chromatography-triple quadrupole mass spectrometry. The purity of MCC measured by the Kjeldahl method (casein as a percentage of true protein) was higher after thermal treatment than before, whereas MCC purity evaluated by SDS-PAGE was unchanged by heat treatment. The purity of MCC had an effect on the flavor profile of thermally processed beverages at 6.5% protein made with fresh liquid MCC. No sulfur/eggy flavor was detected in MCC beverages when 95% of the MDWP was removed (MCC purity about 93 to 94%) from skim milk by microfiltration at 0.1, 1, and 2% fat. As the fat content of 6.5% protein beverages produced with MCC increased, sulfur/eggy flavor intensity and hydrogen sulfide concentration decreased. However, the effect of increasing milk fat on reducing sulfur/eggy flavor in MCC-based beverages at 6.5% protein was less than that of increasing MDWP removal from MCC. Sulfur off-flavors in neutral-pH dairy protein beverages can be mitigated by use of high-purity MCC or by incorporation of fat in the beverage, or both.
Key words: sulfur/eggy flavor, micellar casein concentrate, milk-derived whey protein

INTRODUCTION

Micellar casein concentrate (**MCC**) is made by removal of milk-derived whey protein (**MDWP**) from skim milk by means of microfiltration, using either ceramic or polymeric (spiral-wound) membranes (Zulewska et al., 2009; Carter et al., 2021). In the US dairy industry, the term "milk-derived whey proteins" has been recommended to describe whey proteins that have been removed directly from milk for the purpose of milk protein ingredient labeling on food products (ADPI, 2017). Zulewska and Barbano (2014) reported that ceramic membranes were more efficient at removing MDWP from skim milk than polymeric membranes. Zulewska and Barbano (2014) found that under similar processing conditions (i.e., at 50°C, crossflow filtration with 3 stages at 3× concentration factor), a microfiltration apparatus equipped with a ceramic membrane removed 95% of MDWP from skim milk, whereas the same microfiltration unit equipped with a polymeric membrane was only capable of achieving 70% MDWP removal.

Currently, no composition standards of identity for MCC or MCC purity exist (i.e., residual MDWP concentration; Carter et al., 2021). Recent studies have demonstrated that MDWP is the milk protein fraction responsible for undesirable sulfur/eggy flavor in ultrapasteurized (**UP**) milk (Lee et al., 2017; Jo et al., 2018, 2019). Vogel et al. (2021) reported that sulfur/eggy flavors were present in 6.3 and 10.5% milk protein beverages made from milk protein concentrate, but sulfur/eggy flavor was not detected in the same bev-

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erages made with 95% MDWP-removed MCC. Purity of MCC can be defined in 2 ways: (1) as casein as percentage of true protein (**CN%TP**) of the MCC, or (2) as the percentage of MDWP removed from starting skim milk. A summary of the approximate calculated relationship between MDWP removal and MCC purity expressed as CN%TP is shown in Table 1. Although the 2 definitions both relate to the purity of the MCC, percentage of MDWP removed refers to the percentage of MDWP removed from the starting skim milk. Skim milk is shown in Table 1 as a point of reference with no MDWP removed, and, by analysis of the protein fraction, skim milk has 82% casein purity. The purity of MCC (CN%TP) can be measured using the Kjeldahl method (Lynch et al., 1998) by determining the total nitrogen (**TN**) concentration in milk and then subtracting the noncasein nitrogen (**NCN**) in the filtrate after precipitating the casein at pH 4.6, using reagents and conditions described in the official method. However, heat treatment of the product has an influence on the casein determination. Lynch et al. (1998) provided data to demonstrate that casein determination in milk should be performed before thermal processing to obtain an accurate measurement of the NCN in milk and milk products. Whey proteins denature when exposed to high temperatures such as UP, and then bind to κ-casein through formation of disulfide bonds (Dalgleish, 1990; Jang and Swaisgood, 1990). This binding process makes measurement of the purity or CN%TP in MCC falsely elevated by Kjeldahl analysis following UP heat treatment or spray drying. This happens because once the MDWP binds to the surface of casein micelles, those bound MDWP precipitate with the casein micelles at pH 4.6 at 40°C. This causes an overestimation of the purity of thermally processed MCC.

Cooked flavor in milk and milk products has been long associated with the production of free sulfhydryl compounds by thermal processes (Hutton and Patton, 1952). The flavor and compositional profile of thermally processed MCC is dependent on its purity, because the residual MDWP is the main contributor to sulfur/eggy flavor in UP processed milk protein-based beverages (Lee et al., 2017; Jo et al., 2019). When subjected to high temperatures, MDWP denature due to breakage of intramolecular disulfide bonds between sulfur-containing amino acids (i.e., cysteine). Hydrogen sulfide and carbon disulfide have been documented as the volatile sulfur compounds responsible for undesirable sulfur/eggy off-flavor in UP milk products (Jo et al., 2019). Establishing a level of MCC purity necessary to avoid sulfur/eggy flavors in thermally processed MCC-based beverages would provide an operational target microfiltration (**MF**) process MCC purity to avoid sulfur/eggy off-flavors following thermal processing.

Fat and other compounds in foods can interact with volatile flavors and modulate their sensory effects (Hatchwell, 1996; Guichard, 2002). Fat in dairy products specifically has been demonstrated to mask the sensory influence of volatile flavors or to modulate sensory perception (Christensen and Reineccius, 1992; Cheng et al., 2019). Li. et al. (1997) reported that measured vanillin concentrations decreased as the fat content increased in vanilla ice creams, and reported differences by sensory analysis as well, demonstrating that fat modulated perception of vanilla flavor. Our objectives were to determine the level of MDWP removal necessary to achieve no detectable sulfur/eggy flavor in UP fat-free MCC-based beverages (6.5% protein) and in the same beverages containing 1 and 2% fat.

MATERIALS AND METHODS

Experimental Design

Micellar casein concentrate with 95% MDWP removal was produced from skim milk (50°C) with a 3×, 3-stage ceramic MF process using 0.1-μm pore size graded permeability membranes (n = 3 replications). In experiment 1, MCC-based beverages at about 6.5% (wt/wt) true protein (**TP**) were formulated at a fat content of 0.15% (wt/wt) at 4 different target levels of MDWP removal percentages (95.2%, 91.0%, 83.2%, and 69.3%). In experiment 2, a similar series of beverages at 3 target MDWP removal percentages (95.2%, 83.2%, and 69.3%) with 0.1, 1, and 2% fat content were produced. These MDWP removal percentages corre-

Table 1. Relationship between targeted percentage milk-derived whey protein (MDWP) removal and micellar casein concentrate (MCC) purity produced by microfiltration retentates of skim milk

Metric (%)	Skim milk[1]	Retentate 1	Retentate 2	Retentate 3	Retentate 4
MDWP removal[2]	0	69.3	83.3	91.0	95.0
MCC purity[3]	82	90.3	92.2	93.2	93.8

[1]Skim milk contains about 82% casein as a percentage of true protein without microfiltration.

[2]MDWP removal = (weight of MDWP removed in microfiltration permeate from skim milk in a 3×, 3-stage process/total weight of MDWP in the starting skim milk) × 100%.

[3]MCC purity is the casein as a percentage of true protein of the MCC measured by Kjeldahl analysis.

sponded to target MCC purities (CN%TP) of 93.8, 93.2, 92.2, and 90.2% before thermal processing, respectively. At about 6.5% protein in the MCC-based beverages, the measured residual MDWP concentrations were about 0.40, 0.44, 0.51, and 0.63 g, respectively, per 100 g of beverage. The 2 experiments were designed to determine the level of MDWP removal necessary to achieve no detectable sulfur/eggy flavor following UP thermal treatment. Each experiment was replicated 3 times with different lots of milk in 3 different weeks. Each experimental replication comprised 2 production days. Micellar casein concentrate was manufactured from skim milk on d 1 and beverages were formulated and UP processed on d 2 of processing.

Milk-Based Ingredients

Raw Skim Milk and Anhydrous Milk Fat. Raw bovine skim milk (0.087% ± 0.03 fat, SCC < 300,000) was obtained from the North Carolina State University Dairy Enterprise System (Raleigh, NC) on the morning of each processing run. Raw skim milk was collected and held at 4°C in a glycol-chilled, jacketed storage tank until use (~2 h). Commercial anhydrous milk fat (**AMF**) was purchased from Dairy Farmers of America (Kansas City, KS).

Micellar Casein Concentrate. Liquid MCC was made the day before beverage formulation, as described by Zulewska and Barbano (2014) and modified by Cheng et al. (2018). Raw skim milk (570 kg) was prefiltered before pasteurization at 4°C using a Nexis T-filter (NXT 10-30U-M7S, Pall Corp.), pasteurized (720 kg/h) with a plate heat exchanger (model T4 RGS-16/2, SPX Flow Technology) at 72°C for 16 s. About 30 kg of pre-filtered, HTST-pasteurized skim milk was used to flush out the deionized (**DI**) water from the membrane system before MCC collection began. A 3-stage, 3× MF process described by Zulewska and Barbano (2014) was used to produce a 95% MDWP reduced MCC with a TP concentration between 7.3 to 7.5% using a pilot scale MF system (Tetra Alcross MFS-7, TetraPak Filtration Systems) equipped with 0.1-μm nominal pore diameter graded permeability ceramic Membralox (model EP1940GL0.1u, AGP1020, alumina, Pall Corp.) membranes. The 95% MDWP-reduced 7.3% TP (wt/wt) MCC was immediately cooled in milk cans to 10°C using a glycol-chilled water bath and then stored at 4°C in a walk-in cooler until use (about 12 h).

Milk Serum Protein Isolate. To produce enough milk serum protein isolate (**SPI**; i.e., milk-derived whey proteins) for the study, the SPI was produced in a large batch at South Dakota State University (Brookings, SD), frozen, and shipped overnight to NC State University and held frozen (−20°C) until used in the beverage study. Briefly, MF permeate from the production of MCC was concentrated to a high protein (about 25% wt/wt) SPI by UF, and the concentrate was packaged in 2-L plastic jugs (Upstate Niagara Cooperative) and placed in a −20°C freezer for storage. Three days before beverage formulation, the frozen SPI containers were placed at 4°C to thaw slowly before formulation of beverages.

Beverage Formulation

Formulations (15,000 g per batch) were calculated using the Microsoft Excel linear optimization solver function (Microsoft Corp.) as described by Misawa et al. (2016) and Cheng et al. (2018). The raw ingredients were MCC, SPI, and DI water. Other nondairy ingredients in the beverage formulations were 2.5 g of carrageenan (Ticaloid 780 Stabilizer, Tic Gums), 60 g of cellulose gel (Ticaloid Pro HC 988, Tic Gums), 22.5 g of dipotassium phosphate (CAS no. 7758-11-4, ICL Food Specialties) for each 15,000-g batch of beverage. All batches were unsweetened, unflavored model beverage base formulations.

Experiment 1. Four beverage formulations, starting with freshly produced MCC, were made for each replicate. The MCC was diluted to about 6.5% TP (wt/wt) using DI water and analyzed using mid-infrared (**MIR**) analysis (Lactoscope FTA mid-FTIR, Delta Instruments) to verify the protein concentration. The beverages were formulated at a fat content of 0.15% fat at 4 different targeted levels of MDWP removal percentages (95.2%, 91.0%, 83.2%, and 69.3%, respectively). These MDWP removal percentages correspond to actual achieved MCC purities (i.e., CN%TP) of approximately 93.8, 93.2, 92.2, and 90.3%, respectively. After combining all ingredients, formulations were homogenized using a 2-stage homogenizer at 60°C (model NS2006H, GEA Niro Soave) with 20.7 MPa total pressure and 3.4 MPa on the second stage, to ensure homogeneity for all formulations. Beverages were cooled to 4°C after homogenization and stored at 4°C until UP processing later the same day.

Experiment 2. Nine beverage formulations were replicated 3 times, starting with freshly produced MCC from d-1 processing used for each replicate. The MCC was diluted with DI water to a final protein concentration of about 6.5% TP (wt/wt), which was measured and confirmed by MIR analysis (Lactoscope FTA mid-FTIR, Delta Instruments). The beverages were formulated with 3 added fat levels [no added fat (0.15% fat overall), 1% added AMF, and 2% added AMF] and 3 different targeted MCC purity levels (95.2%, 83.3%, and 69.3% of MDWP removed). These targeted percent MDWP reduced values corresponded to the following

mean values for CN%TP purity achieved, respectively: 92.8%, 90.5%, and 87.8%. The AMF (stored at 4°C) was melted on the day of processing using a 70°C water bath. Beverages were formulated at 50°C by slowly mixing in each ingredient using an immersion blender (Waring WSB60, KaTom Inc.) until homogeneous. The liquid AMF (50°C) was then mixed into the beverage formulations, maintained at 50°C. After all the ingredients had been added, the beverage formulations were heated to 60°C and homogenized using a 2-stage homogenizer at 60°C (model NS2006H, GEA, Niro Soave) with 20.7 MPa total pressure and 3.4 MPa on the second stage to ensure homogeneity of each formulation. Beverages were then cooled to 4°C until UP processing (about 2 h later) the same day.

Beverage Processing

For each replicate in both experiments, 15,000 g of each of beverage formulation was well mixed individually, continuously fed to a Microthermics EHVH pasteurization unit (Microthermics) at a flow rate of 1.4 L/min, and then UP processed by direct steam injection (**DSI**): preheated to 90°C, pasteurized at 140°C for 2.3 s under 330-kPa pressure with culinary DSI (model LG-30, Electro-Steam Generator Corp.), cooled via vacuum chamber at −432 mmHg (−57.6 kPa) of vacuum, homogenized using a 2-stage in-line homogenizer (GEA Niro Soavi), and cooled to 10°C. The inlet beverage temperature before steam injection was 90°C; steam injection raised the temperature of the beverage + steam mixture to 142°C. The vacuum applied to the milk + steam mixture reduced the temperature of the milk at the outlet of the vacuum chamber by about 3°C, or back to a temperature of about 87°C. Formulations were homogenized both before and after DSI-UP, to activate hydrocolloids and ensure homogeneity of the samples. The DSI-UP beverages were packaged in 473-mL high-density polyethylene dairy bottles (VWR) under a clean fill hood (model EL422TT-ST, Microthermics, HEPA filter, 0.3-μ filter) and stored at 4°C. Beverages were sampled for testing at 1 d after processing. Sensory and volatile compound analyses were completed within 24 h for each experimental replicate. Samples for chemical composition and SDS-PAGE were frozen in liquid nitrogen and stored at −80°C, and analyses were completed within 120 d.

Analysis Methods

Chemical Composition of Beverages. Milk-based beverages and ingredients were analyzed in triplicate using the following analytical methods: total solids were analyzed by the direct forced-air method (AOACI 2019, method number 990.20), fat by ether extraction (AOACI 2019, method number 989.05), lactose by an enzymatic method (AOACI, 2019, method number 2006.06), TN by AOACI (2019) method number 990.20, NPN by AOACI (2019) method number 990.21), and NCN by AOACI (2019) method number 998.05. True protein was calculated as TN minus NPN multiplied by 6.38; CN was calculated as TN minus NCN multiplied by 6.38; and serum protein content was calculated by subtracting NPN from NCN and multiplying by 6.38.

For monitoring, skim milk, MCC and MF permeate, and SPI and UF permeate composition (i.e., fat, protein, and lactose concentration g/100 g of milk) during the MF processing runs, samples were analyzed using a MIR spectrophotometer (Lactoscope FTA, Delta Instruments). The MIR milk analysis product group was calibrated using modified milk samples produced at Cornell University, as described by Kaylegian et al. (2006). The reference chemistry for the calibration samples was all-laboratory mean reference chemistry for the modified milk calibration samples, as described by Wojciechowski et al. (2016). A milk product testing group was set up on the MIR milk analyzer using traditional virtual filter models for fat A, fat B, TP, and anhydrous lactose measures during the run, to monitor the process and ensure that processing was running properly. The wavelengths, scale factors (i.e., primary slope), and intercorrection factors for each virtual filter model were as described by Kaylegian et al. (2009).

SDS-PAGE Analysis. The polyacrylamide gels used to determine the trace quantity [mm × optical density (**OD**)] of all protein bands (i.e., intact milk casein, casein proteolysis products, and milk serum protein) were made according to the procedure described by Verdi et al. (1987), using the gel unit Protean II and the power unit 3000/300 from Bio-Rad (Bio-Rad Laboratories Inc.) gel electrophoresis system. Samples for SDS-PAGE were prepared by diluting each sample with 0.9 g of a sample buffer that consisted of 10 m*M* Tris-HCl, 1.0% SDS, 20% glycerol, and 0.02% bromophenol blue tracking dye. Samples were prepared with and without 45 m*M* dithiothreitol. Dithiothreitol disrupts the intermolecular disulfide bonds that can be produced as a result of exposing milk serum proteins to high heat in the presence of casein micelles during DSI pasteurization.

The weight of MCC beverage used for dilution was calculated based on Kjeldahl TP content of the milk sample, to achieve a loading of approximately 28 μg of true protein loaded per slot. Each MCC plus buffer mixture was placed in a sealed glass vial (Target DP Vials C4000-1W, National Scientific Co.), heated to

100°C using steam, held at 100°C for 3 min, and stored frozen at −80°C until analysis. On the day of SDS-PAGE analysis, diluted MCC were thawed using steam heated to 100°C for 3 min, and then cooled to about 25°C. Each MCC plus buffer mixture was loaded into a different well of the gel. The volume loaded was chosen to achieve a maximum peak OD in the range of 1.0 to 1.4 for the most intense protein band (i.e., α_S-CN) in the sample to avoid overloading and nonlinear response in quantitation. One raw milk was used as a marker in one lane of each gel for evaluation of loading consistency and comparison of resolution of the proteins from gel to gel. Three SDS-PAGE runs were performed with 2 gels at a time. All gels were scanned with a USB GS 800 Densitometer (Bio-Rad Laboratories Inc.) and quantified using Quantity One 1-D Analysis software (version 4.6.7, Bio-Rad Laboratories Inc.). For quantitative analysis, a center line for each lane on each gel was created during scanning of the gel. The lines were adjusted individually to match the middle section of the bands in each lane. Next, each lane background was adjusted using the rolling disk method of subtraction to obtain a straight baseline for each lane. Bands were detected by setting the bracket to cover the width of the bands and then by adjusting each band in each lane by adjusting the height of the brackets to fit each protein band based on visual observation. Trace quantities (mm × OD) of the intact CN (α_S-CN, β-CN, and κ-CN), CN proteolysis products, and serum protein bands, and the sum of all protein bands (i.e., intact CN + CN proteolysis products + serum protein) of the gel for each milk were calculated. The estimation of CN%TP by SDS-PAGE was calculated using the trace quantity (mm × OD) values of [(intact CN bands + CN proteolysis product bands)/all protein bands].

Measurement of CS_2 and H_2S. Hydrogen sulfide (H_2S) and carbon disulfide (CS_2) in MCC beverages were measured using an Agilent 7890B gas chromatograph applied to an Agilent 7000C triple quad mass spectroscopy (GC-MS/MS) and sulfur selective flame photometric detector (Agilent Technologies Inc.) equipped with a ZB-5ms column (30-m length × 0.25-mm internal diameter × 0.25-μm film thickness; Phenomenex) as described by Jo et al. (2019). Samples were introduced using a CombiPal Autosampler (CTC Analytics). Five grams of each formulation was weighed into a 20-mL solid-phase microextraction autosampler vial with a steel screw-top cap containing silicone septa faced in Teflon (Microliter Analytical), along with 20 μL of internal standard (ethyl methyl sulfide in ethyl ether at 1.65 mg/kg; Sigma Aldrich). Analytical conditions and the multiple reaction monitoring transition for selected compounds were as described in Jo et al. (2019). Each formulation was run in triplicate for each experiment replication. MassHunter Qualitative Analysis (B.09.00, Agilent Technologies Inc.) and MassHunter Quantitative Analysis (B.08.00, Agilent Technologies Inc.) software was used for data analysis. The relative concentration of each compound was calculated based on the response ratio of each quantified ion to that of the internal standard.

Orthonasal Best Estimate Threshold

Threshold testing was conducted in accordance with the North Carolina State University Institutional Review Board for the Protection of Human Subjects in Research regulations. H_2S and CS_2 were selected for orthonasal threshold testing based from work done by Jo et al. (2019), who demonstrated that these 2 compounds were the main contributors to sulfur/eggy flavor in thermally processed milk-based products. Orthonasal thresholds for each of these compounds were determined in 95% MDWP-reduced MCC to provide further clarity on concentrations of these 2 compounds that were below sensory detection in this matrix. A modification of the American Society for Testing and Materials procedure E679-9 (ASTM, 2004), an ascending forced-choice method of limits, was used to determine the orthonasal best estimate threshold (**BET**) value for each compound. This method had panelists evaluate the samples in a 7-series 3-ascending forced-choice procedure.

Stock solutions of each compound were prepared in 95% ethanol (Sigma Aldrich). Portions of each stock solution were added to the selected medium [95% MDWP-reduced, ~7.3%-TP (wt/wt) MCC, HTST pasteurized]. The MCC was prepared the day before threshold tests and HTST pasteurized (74°C, 15 s) using a MicroThermics EHVH pasteurization unit (MicroThermics Inc.). The concentrations of H_2S and CS_2 in HTST-pasteurized MCC were below the instrumental limits of detection, and HTST-pasteurized MCC had no discernible sulfur/eggy aroma or flavor (Jo et al., 2019). Blank solutions were prepared using MCC with no added compounds. The orthonasal detection threshold of each compound was determined individually.

Sample solutions were made by geometrically serially diluting the stock solution with MCC using a step factor of 1.5. Thirty milliliters of each serial dilution or blank was poured into clean, 3-digit-coded, 59-mL lidded souffle cups (Dart Container Corp.). The samples for each compound were prepared 2 h before testing so that all solutions could come to equilibrium in the lidded cups (Leksrisompong et al., 2010). Panelists (n = 50) were instructed on the appropriate sniffing tech-

nique and testing procedure before testing, as described by Leksrisompong et al. (2010). Seven series of 3 sample sets were presented to each panelist in ascending order of compound concentration, with sample presentation randomized within each series. Each series had 1 signal cup containing the compound-spiked serial dilution and 2 blank cups containing MCC. Each panelist had to choose a sample cup within each individual series, in which they believed to have detected the signal compound, whether or not such a signal could be detected. A 2-min rest was enforced between each set of 3 samples. The individual BET was taken as the geometric mean of the last concentration with an incorrect response and the first concentration with a correct response, except for the following sequence: if the subject indicated a "not sure" response for the correct choice, that concentration was increased by a factor of 1.41 to adjust for the possibility of a chance correct response (Lawless et al., 2000). The estimate group BET was taken as the geometric mean of the individual BET values. Paper ballots were used for data collection.

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 overall aroma and sulfur/eggy and cooked flavors (Jo et al., 2018, 2019) by 7 panelists (3 men, 4 women, ages 24–50 yr) 1 d after 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 milliliters of each beverage was poured into 59-mL souffle cups (Dart Container Corp.), capped, and labeled with a randomized 3-digit blinding code. Samples were prepared with overhead lights off to prevent light oxidation. Beverages were evaluated at 15°C. Panelists evaluated each treatment in duplicate in a randomized balanced order of presentation. Paper ballots were used for data collection.

Statistical Analysis

For data analysis from experiment 1, the general linear models (GLM) procedure of SAS (version 9.4, SAS Institute Inc.) was used. The ANOVA model included category variables for MDWP removal (n = 4) and replicate (n = 3) for chemical analysis data, and for sensory data, category variables were MDWP removal (n = 4), panelist (n = 7), and replicate (n = 3), with all interaction terms. For data from experiment 2, a 2-way GLM ANOVA model was used (SAS version 9.4). The model included category variables for fat level (n = 3), percent MDWP removal (n = 3 levels), and replicate (n = 3), with all interaction terms for chemical and sensory data, as well as a term for panelist.

RESULTS AND DISCUSSION

Beverage Composition

The compositions of the formulated beverages and their MCC purity levels for experiments 1 and 2 are shown in Table 2. The formulated MCC-based beverages in experiments 1 and 2, with varying levels of MCC purity (i.e., percent MDWP removal) had consistent crude and true protein concentration across all formulations of the study design. Additionally, formulation of the beverages achieved the desired range of CN%TP concentrations (i.e., MCC purity levels) among treatments. Measured MCC purity (CN%TP) decreased ($P < 0.05$) as MDWP removal decreased in unheated beverages (Table 2). The mean concentration of fat for the target fat levels of 0.1, 1, and 2% in experiment 2 were 0.14, 0.77, and 1.8% fat, respectively. The measured residual MDWP concentration in the unheated beverages increased as MDWP removal decreased.

Measurement of MCC Beverage Purity Before and After UP Treatment

The MCC purity (CN%TP) was different ($P < 0.05$) among different percent MDWP removal treatments when measured by Kjeldahl analysis before UP processing (Table 3) in both experiments 1 and 2. However, when MCC purity was measured by the Kjeldahl method after UP processing, the absolute purity measures were higher, and no difference in MCC purity among the different percent MDWP treatments was detected in experiments 1 and 2. As expected, the purity of MCC was overestimated after UP processing due to the covalent binding of whey proteins to casein micelles by disulfide bond formation. The apparent increase in purity is larger when the original purity of the MCC is lower (Table 3). To correctly determine MCC purity after thermal processing, a different type of measurement that can differentiate between bonded whey and casein proteins, such as HPLC or SDS-PAGE with the use of a reducing agent, is needed for an accurate assessment of MCC purity.

In contrast to Kjeldahl analysis, measurements of MCC purity before and after UP using SDS-PAGE were not different ($P > 0.05$, Figure 1, Table 4). During

Table 2. Mean (n = 3) micellar casein concentrate (MCC)-based beverage composition (%) with differing target milk-derived whey protein (MDWP) removal percentages before ultrapasteurization for experiments 1 and 2

Experiment 1: MCC-based beverages (~6.5% true protein) at 4 different target MDWP removal percentages and 0.1% fat						
Target MDWP removal %	CP[1]	NPN[2]	TP[3]	CN[4]	MCC purity[5]	Residual MDWP %
95.20	6.57[a]	0.04[a]	6.53[a]	6.15[a]	93.83[a]	0.40[d]
91.00	6.54[a]	0.05[a]	6.49[a]	6.08[b]	93.22[b]	0.44[c]
83.30	6.54[a]	0.04[a]	6.49[a]	6.01[c]	92.18[c]	0.51[b]
69.30	6.49[a]	0.05[a]	6.44[a]	5.84[d]	90.27[d]	0.63[a]
SE	0.03	<0.00	0.03	0.03	0.19	0.01
R^2	0.94	0.55	0.94	0.96	0.97	0.97

Experiment 2: MCC-based beverage composition (~6.5% true protein) at 3 different target MDWP removal percentages and 3 fat contents						
Target MDWP removal %	Target fat content	CP	NPN	CN	MCC purity	Residual MDWP %
95.20	0.1	6.69[a]	0.06[a]	6.16[a]	92.87[a]	0.473[c]
83.30	0.1	6.67[a]	0.07[a]	5.98[b]	90.44[b]	0.631[b]
69.30	0.1	6.74[a]	0.07[a]	5.85[c]	87.68[c]	0.822[a]
95.20	1.0	6.66[a]	0.06[a]	6.12[a]	92.77[a]	0.477[c]
83.30	1.0	6.68[a]	0.06[a]	5.98[b]	90.39[b]	0.635[b]
69.30	1.0	6.68[a]	0.06[a]	5.80[c]	87.58[c]	0.822[a]
95.20	2.0	6.56[a]	0.07[a]	6.03[a]	92.82[a]	0.466[c]
83.30	2.0	6.70[a]	0.07[a]	6.02[a]	90.82[b]	0.609[b]
69.30	2.0	6.71[a]	0.07[a]	5.87[c]	88.30[c]	0.777[a]
SE		0.02	0.0008	0.021	0.091	0.0056
R^2		0.79	0.99	0.927	0.995	0.996

[a–d]Means in the same column followed by a different superscript differ ($P < 0.05$).

[1]CP = total nitrogen (TN) × 6.38.

[2]NPN = nonprotein nitrogen × 6.38.

[3]TP (true protein) = CP − NPN × 6.38.

[4]CN (casein) = (TN − noncasein nitrogen) × 6.38.

[5]MCC purity (casein as a percentage of true protein) = (CN/TP) × 100.

Table 3. Mean (n = 3) micellar casein concentrate (MCC) purity measurement by Kjeldahl analysis before and after ultrapasteurization (UP) processing of differing MCC at about 6.5% total protein for experiments 1 and 2

Experiment 1: MCC-based beverage at 4 different target MDWP[1] removal percentages			
% MDWP removal	MCC purity before UP[2]	MCC purity after UP[2]	Apparent increase in MCC purity
95.20	93.83[b,A]	95.18[a,A]	1.35[D]
91.00	93.22[b,B]	94.76[a,A]	1.54[C]
83.30	92.18[b,C]	94.69[a,A]	2.51[B]
69.30	90.27[b,D]	94.36[a,A]	4.09[A]
SE	0.194		
R^2	0.97		

Experiment 2: MCC-based beverage at 3 different target MDWP removal percentages and 3 fat contents			
95.20	92.80[b,A]	94.03[a,A]	1.75[C]
83.30	90.55[b,B]	93.57[a,A]	3.02[B]
69.30	87.85[b,C]	92.29[a,A]	4.44[A]

[a,b]Means in the same row followed by a different superscript differ ($P < 0.05$).

[A–D]Means in the same column followed by a different superscript differ ($P < 0.05$). SE = 0.096; R^2 = 0.992.

[1]MDWP = milk-derived whey protein.

[2]MCC purity: casein as percentage of true protein (corrected for pasteurization denaturation of MDWP before microfiltration that was retained in MCC) = casein as a percentage of true protein {[(total nitrogen − noncasein nitrogen)/(total nitrogen − nonprotein nitrogen)] × 100}.

sample preparation for SDS-PAGE, samples are heated and dithiothreitol is added to break intermolecular disulfide bonds. As anticipated, whey proteins bonded to casein were disassociated by this process, and residual MDWP was distinguished from casein with or without previous UP processing. The data from this measurement confirmed that SDS-PAGE is a viable method for determining the purity of MCC after a thermal process. Because SDS-PAGE is a slow and difficult method, a more rapid method to determine MCC purity after thermal processing is needed. Similar results were demonstrated in a study conducted by Jovanovic et al. (2007), who evaluated skim milk subjected to varying temperatures (75°C, 80°C, and 90°C for 20 min) followed by SDS-PAGE with and without reducing agent (β mercaptoethanol) to evaluate milk composition after thermal processing. They also demonstrated that SDS-PAGE with a reducing agent was capable of separating bonded whey proteins from casein to measure the 2 types of proteins separately.

Orthonasal Best Estimate Thresholds

The orthonasal BET values for hydrogen sulfide and carbon disulfide in 95% MDWP reduced MCC were 33.4 ± 5.2 μg/kg and 172.8 ± 51 μg/kg, respectively. The orthonasal BET for these compounds in MCC were higher than those previously reported in skim milk by Jo et al. (2019; 22.5 μg/kg for hydrogen sulfide and 35.2 μg/mg for carbon disulfide), presumably due to the higher protein concentration in MCC, which may bind sulfur compounds and reduce their headspace concentrations. Threshold values for these compounds have not been reported in 95% MDWP-reduced MCC. Sensory thresholds can vary in different mediums, matrices, or methodologies (Leksrisompong et al., 2010). The comparison of an established BET for the compounds of interest with GC-MS/MS relative abundance values and trained panel profiling of beverages is critical to firmly establish the MCC purity range necessary to eliminate sulfur/eggy off-flavors.

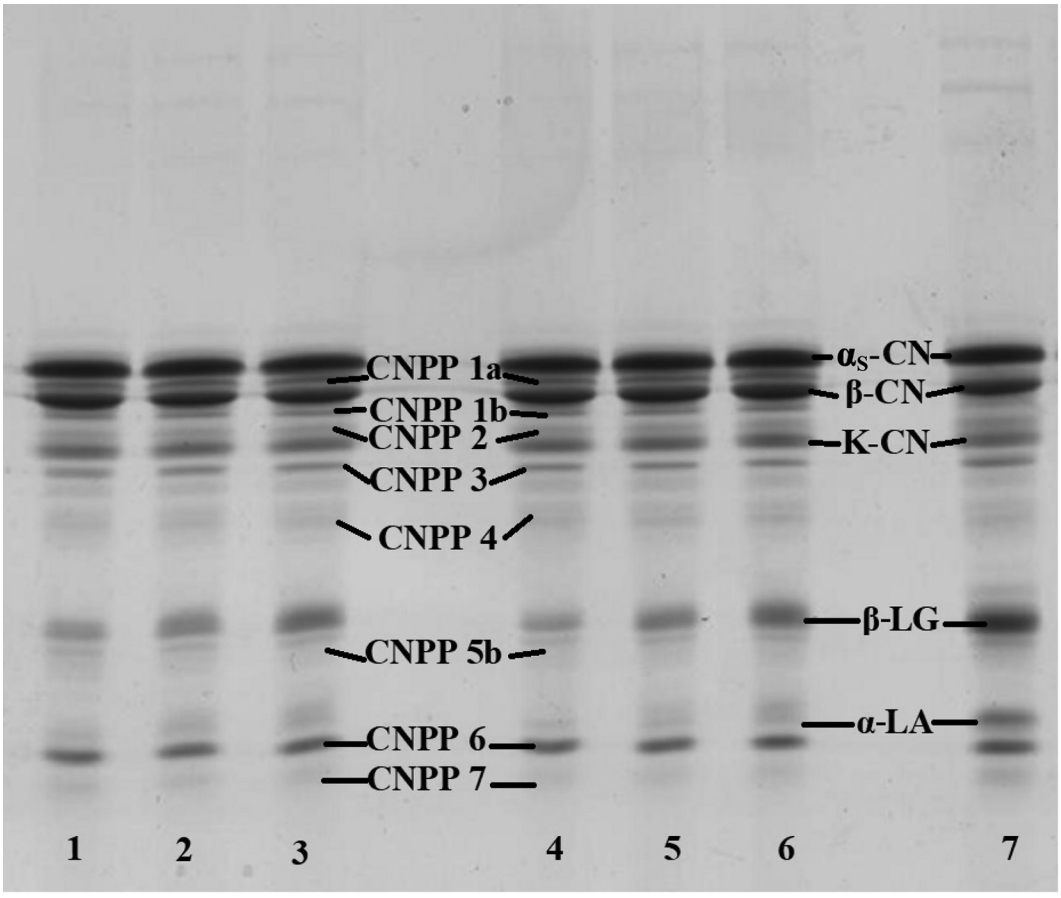

Figure 1. SDS-PAGE electrophoresis gel of micellar casein concentrate (MCC) beverages with varying milk-derived whey protein (MDWP) removal (3 replications). Lanes 1–3: 0.1% fat MCC with MDWP removal targets of 95.2, 83.3, and 69.3% before ultrapasteurization. Lanes 4–6: 0.1% fat MCC with MDWP removal targets of 95.2, 83.3, and 69.3% after ultrapasteurization. Lane 7: pasteurized milk standard. Bands are labeled as α_S-CN, β-CN, κ-CN, β-LG, α-LA, and casein proteolysis products (CNPP) 1a–7.

Table 4. Mean (n = 3) micellar casein concentrate (MCC) purity measurement by SDS-PAGE before and after ultrapasteurization (UP) processing of differing MCC purity percentages at about 6.3% total protein

Target % MDWP[1] removal	MCC purity[2] before UP	MCC purity[2] after UP	Apparent increase in MCC purity
95.20	94.07[a,A]	94.65[a,A]	0.58[A]
83.30	91.89[a,B]	92.47[a,B]	0.58[A]
69.30	89.15[a,C]	89.16[a,C]	0.01[A]

[a]Means in the same row followed by a different superscript differ ($P < 0.05$).

[A–C]Means in the same column followed by a different superscript differ ($P < 0.05$). SE = 0.014; $R^2 = 0.995$.

[1]MDWP = milk-derived whey protein.

[2]MCC purity = casein as percentage of true protein (corrected for pasteurization denaturation of MDWP before microfiltration that was retained in the MCC), calculated using the trace quantity (mm × optical density) values of [(intact CN bands + CN proteolysis product bands)/all protein bands] measured from the SDS-PAGE gel scans.

Trained Panel Profiling and Volatile Sulfur Analysis of Beverages

Experiment 1. Trained panel profiling of the high-protein beverages with varying MCC purity levels demonstrated that overall aroma, cooked/milky flavor, and sulfur/eggy flavor increased ($P < 0.05$) with increasing residual MDWP concentration (Table 5, Figure 2). These results are consistent with results of previous studies. Vogel et al. (2021) evaluated 6.3 and 10.5% protein beverages made with various milk protein blends (SPI, MCC, and milk protein concentrate) and reported that the beverages made with a higher concentration of MDWP as a percentage of TP had higher concentrations of sulfur/eggy flavor by descriptive analysis. Sulfur/eggy flavor is caused by volatile sulfur-containing compounds that decrease in concentration with storage time (Lee et al., 2017; Jo et al., 2018; Vogel et al., 2021). The current study addressed sulfur/eggy flavor at the most conservative time point, within 24 h of heat treatment. In the present study, the only difference among the formulated beverages was the amount of MDWP in the beverages (i.e., the purity of the MCC), and this demonstrated that the amount of MDWP in the MCC is a major contributing factor to sulfur/eggy flavor. The intensity of sulfur/eggy flavor decreased with increasing purity of MCC (Table 5). We found no detectable sulfur/eggy flavor in beverages made with MCC at 95% MDWP removal.

Jo et al. (2019) demonstrated that hydrogen sulfide and carbon disulfide were the main contributors to sulfur/eggy flavor and were derived from MDWP denaturation by heat. Higher residual MDWP percentage resulted in higher ($P < 0.05$) relative abundance of hydrogen sulfide and carbon disulfide (Table 5, Figure 3) in the MCC-based beverages, and this result was consistent with the higher sulfur/eggy sensory scores. Jo et al. (2019) reported that hydrogen sulfide was the primary contributor to sulfur/eggy flavor, which is also consistent with the instrumental and lower BET values for hydrogen sulfide than carbon disulfide in the current study. Vogel et al. (2021) also reported that milk-based high-protein beverages with higher MDWP concentration contained higher concentrations of hy-

Table 5. Mean (n = 3) sensory intensity scores, volatile compound relative abundance, and residual milk-derived whey protein (MDWP) concentrations (%) for micellar casein concentrate-based beverages at about 6.5% true protein, at varying MDWP removal percentages and 0.1% fat, 1 d after processing

MDWP removal %	Sensory intensity scores[1]			Volatile sulfur compound[2] (μg/kg)		Residual MDWP %
	Overall aroma	Cooked/milky	Sulfur/eggy	Hydrogen sulfide	Carbon disulfide	
95.20	1.8[d]	3.7[d]	ND[3]	9.27[d]	33.28[d]	0.40[d]
91.00	2.2[c]	4.0[c]	0.7[c]	21.88[c]	58.52[c]	0.44[c]
83.30	2.6[b]	4.2[b]	1.3[b]	35.37[b]	100.74[b]	0.51[b]
69.30	4.4[a]	4.4[a]	2.2[a]	58.65[a]	148.81[a]	0.63[a]
SE	0.04	0.02	0.03	1.43	7.35	0.01
R^2	0.89	0.90	0.97	0.94	0.78	0.97

[a–d]Means in the same column followed by a different superscript differ ($P < 0.05$).

[1]Attribute intensities were scored on a 0- to 15-point universal intensity scale (Meilgaard et al., 2007).

[2]Relative mean concentration in triplicate (μg/kg).

[3]ND = not detected.

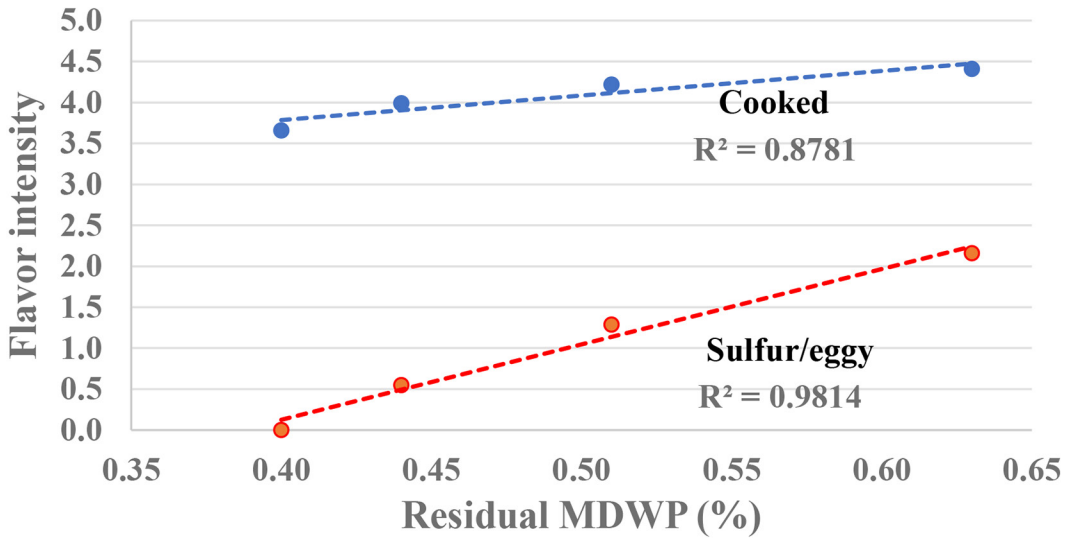

Figure 2. Mean (n = 3) sensory descriptive analysis flavor intensity scores for cooked/milky and sulfur/eggy flavors in micellar casein concentrate (MCC)-based beverages based on residual milk-derived whey protein (MDWP) percentage at about 6.5% protein, formulated to 15 g of protein per 240-mL serving.

drogen sulfide and carbon disulfide following UP than those with lower MDWP. The results of the current study establish that a 95% MDWP removal, which is equivalent to about 93.2% purity, results in no detectable sulfur/eggy flavor in a thermally processed MCC-based beverage.

Experiment 2. Overall aroma intensity was higher ($P < 0.05$) at lower MDWP removal (i.e., lower MCC purity) within the same fat content level (Table 6). Cooked/milky flavor was not different among beverages ($P > 0.05$), and perceived milk fat flavor increased ($P < 0.05$) as expected with increasing percent milk fat. Both percent MDWP removal and fat content affected sensory perception of sulfur/eggy flavor ($P < 0.05$). Sulfur/eggy flavor was not detected in beverages with 95% MDWP removal at any fat content level. At MDWP reductions of 83.3% and 69.3% MDWP, sulfur/eggy flavor increased ($P < 0.05$), but the increase was less in the beverages with higher fat content (Table 6). Beverages with 2% added milk fat at 69.3% MDWP removal had lower sulfur/eggy flavor than 1% milk fat 69.3% MDWP removal, and sulfur/eggy flavor in these beverages was lower than that of 0.1% milk fat 69.3% MDWP removal. The presence of fat had a masking effect on the sensory detection of sulfur/eggy flavor in UP-processed MCC-based high-protein beverages. Fat dissolves sulfur volatile compounds and decreases their concentration in the container headspace, which may lead to lower headspace detection of volatile sulfur compounds and decreased sulfur/eggy flavor by sensory analysis. In contrast, in the absence of fat, vapor pressure of volatile sulfur compounds in container headspace can increase (Hatchwell, 1996), leading to a higher intensity and higher instrumental relative abundance and higher scores by a trained sensory panel, as reported in the current study. Previous studies with dairy products have also documented the influence of fat on sensory perception. Drake et al. (2010) demonstrated that fat and its effects on sensory thresholds and flavor perception were the primary sources of flavor differences between full-fat and reduced-fat Cheddar cheese rather than a difference in specific volatile compounds.

Consistent with sensory results, both percentage MDWP removal and fat content affected relative abundances of hydrogen sulfide and carbon disulfide (Figures 4 and 5). Percent MDWP removal had a greater effect (i.e., higher type III sum of squares and more variation explained) than fat content on both hydrogen sulfide and carbon disulfide relative abundance (Figures 4 and 5), consistent with sensory perceived sulfur/eggy flavor. The relative abundance of both volatile sulfur compounds increased ($P < 0.05$) with decreased ($P < 0.05$) MDWP percent removal and lower MCC purity, with an interaction of MDWP removal and fat level ($P < 0.05$). In the presence of fat, both sulfur compounds decreased ($P < 0.05$), although the decrease due to increasing fat was not as large as the effect of higher percent MDWP removal. Lowering the concentrations of sulfur volatiles in the headspace by increasing the fat content will lead to a decreased intensity of perceived sulfur/eggy flavor (Table 6). Keršiene et al. (2008) made model custards from skim or full-fat milk pow-

Table 6. Mean (n = 3) sensory intensity scores[1] for overall aroma, cooked/milky, sulfur/eggy, and milk fat flavor of ultrapasteurized micellar casein concentrate-based beverages with differing target milk-derived whey protein (MDWP) removal percentages and fat content, containing about 6.5% true protein

Target MDWP removal %	Target fat content %	Overall aroma	Cooked/milky	Sulfur/eggy	Milk fat	Residual MDWP %
95.20	0.1	1.8^{c}	4.0^{a}	ND[2]	ND	0.473^{c}
83.30	0.1	2.7^{b}	4.2^{a}	2.1^{b}	ND	0.631^{b}
69.30	0.1	3.6^{a}	4.1^{a}	3.0^{a}	ND	0.822^{a}
95.20	1.0	2.0^{c}	4.2^{a}	ND	1.3^{b}	0.477^{c}
83.30	1.0	2.3^{b}	4.4^{a}	1.1^{d}	1.9^{a}	0.635^{b}
69.30	1.0	2.9^{a}	4.4^{a}	1.9^{b}	1.2^{b}	0.822^{a}
95.20	2.0	2.0^{c}	4.4^{a}	ND	2.0^{a}	0.466^{c}
83.30	2.0	2.2^{b}	4.4^{a}	0.4^{e}	1.9^{a}	0.609^{b}
69.30	2.0	2.8^{a}	4.4^{a}	1.6^{c}	1.9^{a}	0.777^{a}
SE		0.04	0.45	0.04	0.02	0.006
R^2		0.92	0.38	0.97	0.98	0.996

$^{a–e}$Means in the same column followed by a different superscript differ ($P < 0.05$).

[1]Attribute intensities were scored on a 0- to 15-point universal intensity scale (Meilgaard et al., 2007).

[2]ND = not detected.

der in an effort to determine the effect of milk fat on the flavor and rheological properties of model desserts. Keršiene et al. (2008) reported similar findings, in that an increased amount of fat resulted in a significant decrease in the headspace concentration of volatile flavor compounds through hydrophobic interactions.

Both higher purity of MCC (i.e., less residual MDWP in the beverage) and increasing milk fat content will reduce the sensory detection of sulfur/eggy off-flavors in UP milk-based beverages. Increasing MCC ingredient purity for high-protein beverages will have the largest influence on reduction in sulfur/eggy off-flavor. A MDWP removal of 95% (or MCC purity of 93.5 to 94% or higher) will eliminate the sensory detection of sulfur/eggy off-flavor in UP milk-based protein beverages. To achieve the high-level purity of MCC required to eliminate sulfur/eggy off-flavor, it is likely that ceramic membrane MF will be required (Zulewska et al., 2009).

CONCLUSIONS

The purity of MCC measured by the Kjeldahl method (CN%TP) was higher after thermal treatment than before, due to covalent binding of whey protein to

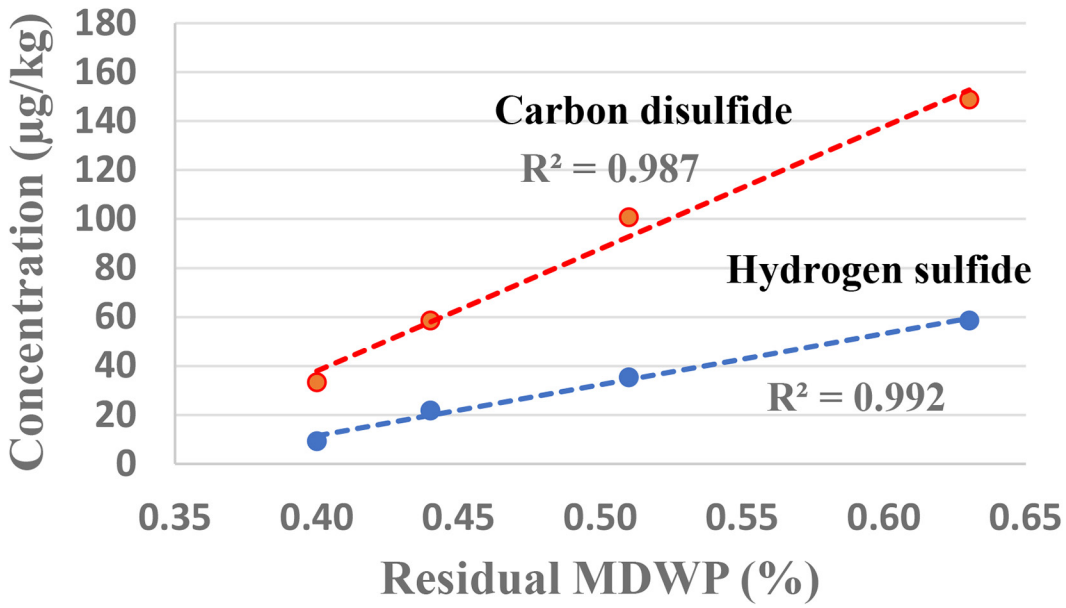

Figure 3. Mean (n = 3) relative abundance (µg/kg) of hydrogen sulfide and carbon disulfide in micellar casein concentrate (MCC)-based beverages based on residual milk-derived whey protein (MDWP) percentage at 6.5% total protein, formulated to 15 g of protein per 240-mL serving.

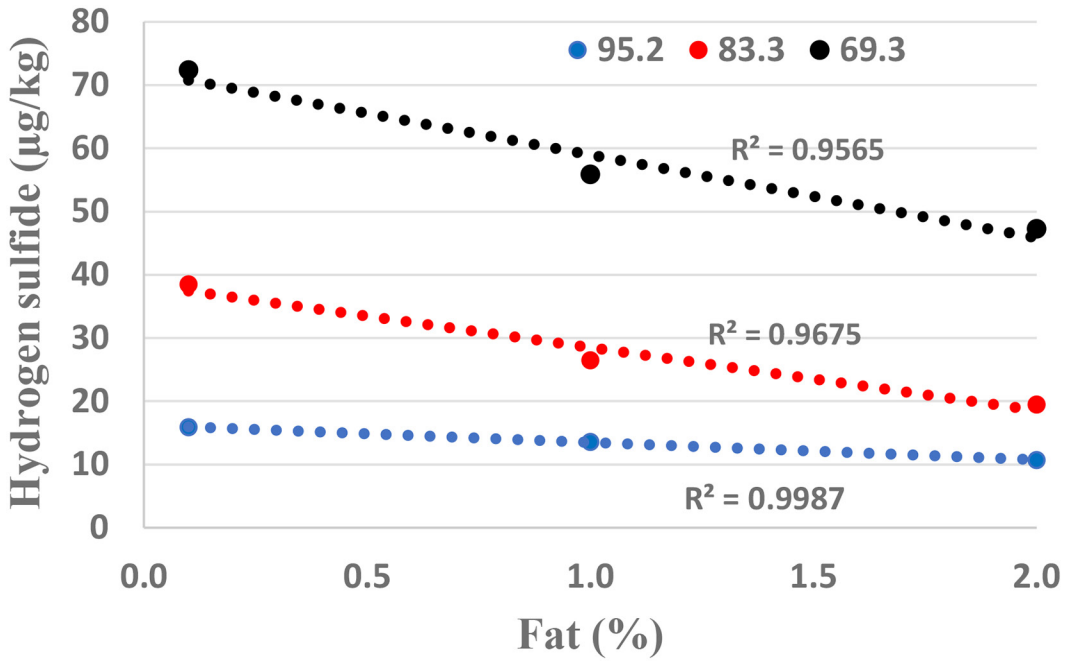

Figure 4. Hydrogen sulfide concentration (µg/kg) in ultrapasteurized milk protein-based beverages with differing target milk-derived whey protein removal percentages (95.2, 83.3, and 69.3%) at about 6.5% protein with 0.1, 1.0, or 2.0% fat formulated to 15 g of protein per 240-mL serving.

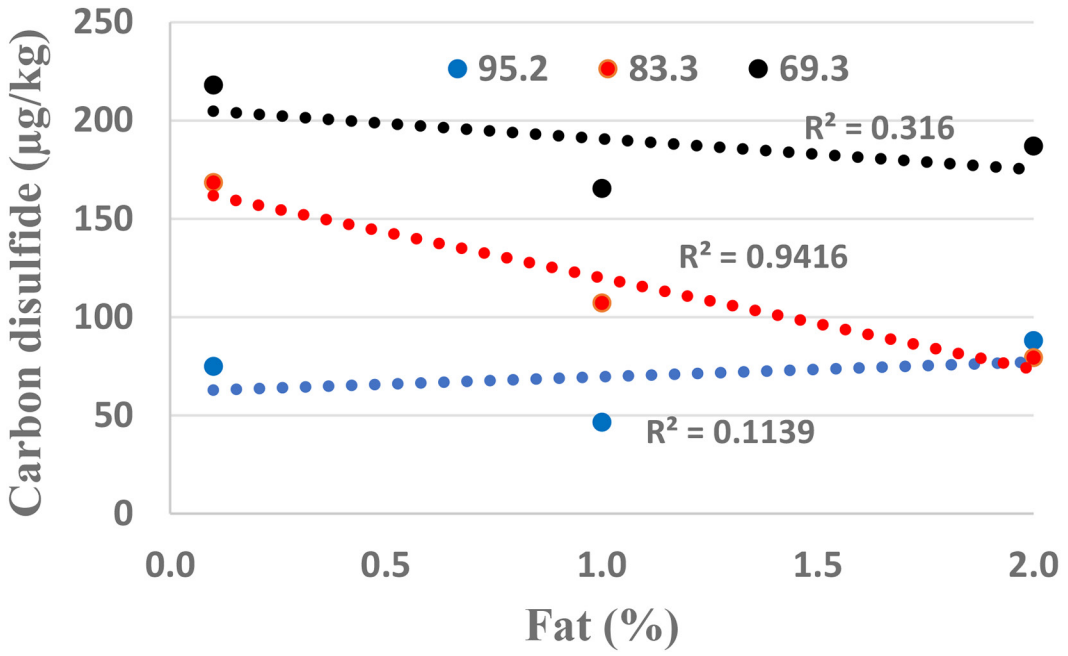

Figure 5. Carbon disulfide concentration (µg/kg) in ultrapasteurized milk protein beverages with differing target milk-derived whey protein removal percentages (95.2, 83.3, and 69.3%) at about 6.5% true protein with 0.1, 1.0, or 2.0% fat formulated to 15 g of protein per 240-mL serving after ultrapasteurization.

casein micelles, and therefore purity measurement by Kjeldahl analysis must be performed before UP heat treatment to avoid overestimation of MCC purity. We found that SDS-PAGE was a viable method to determine the purity of an MCC-based product before and after heat treatment. The purity (or completeness of removal of whey protein by microfiltration) of micellar casein concentrate had a significant effect on the flavor profile of thermally processed beverages at 6.5% protein, made with fresh liquid MCC as the milk protein ingredient. No sulfur/eggy flavor was detected in MCC beverages when 95% of the MDWP was removed (MCC purity about 93.5 to 94%) from skim milk by microfiltration at 0.1, 1, and 2% fat. As the fat content of a high-protein beverage produced with MCC increased, sulfur/eggy flavor and relative abundances of hydrogen sulfide and carbon disulfide decreased. However, the effect of increasing milk fat on reducing sulfur/eggy flavor in MCC-based beverages at 6.5% protein was less than that of increasing MDWP removal from MCC.

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REFERENCES

ADPI. 2017. ADPI releases new industry standards for whey proteins obtained directly from milk. Cheese Reporter, 12-08-2017, page 9. Accessed Sep. 12, 2021. https://npaper-wehaa.com/cheese-reporter/2017/12/?page=9.

AOACI. 2019. Official Methods of Analysis. 21st ed. Association of Official Analytical Chemists.

ASTM (American Society for Testing and Materials). 2004. Standard practice for determination of odor and taste thresholds by a forced-choice ascending concentration series method of limits. E 679-04. ASTM.

Carter, B. G., N. Cheng, R. Kapoor, H. G. Meletharayil, and M. A. Drake. 2021. Microfiltration derived casein and whey proteins from milk. J. Dairy Sci. 104:2465–2479. https://doi.org/10.3168/jds.2020-18811.

Cheng, N., D. M. Barbano, and M. A. Drake. 2018. Hunter versus CIE color measurement systems for analysis of milk-based beverages. J. Dairy Sci. 101:4891–4905. https://doi.org/10.3168/jds.2017-14197.

Cheng, N., D. M. Barbano, and M. A. Drake. 2019. Effects of milk fat, casein and serum protein concentrations on sensory properties of milk-based beverages. J. Dairy Sci. 102:8670–8690. https://doi.org/10.3168/jds.2018-16179.

Christensen, K. R., and G. A. Reineccius. 1992. Gas chromatographic analysis of volatile sulfur compounds from heat milk used static headspace sampling. J. Dairy Sci. 75:2098–2104. https://doi.org/10.3168/jds.S0022-0302(92)77968-5.

Dalgleish, D. D. 1990. Denaturation and aggregation of serum proteins and caseins in heated milk. J. Agric. Food Chem. 38:1995. https://doi.org/10.1021/jf00101a001.

Drake, M. A., R. E. Miracle, and D. J. McMahon. 2010. Impact of fat reduction on flavor and flavor chemistry of cheddar cheeses. J. Dairy Sci. 93:5069–5081. https://doi.org/10.3168/jds.2010-3346.

Guichard, E. 2002. Interactions between flavor compounds and food ingredients and their influence on flavor perception. Food Rev. Int. 18:49–70. https://doi.org/10.1081/FRI-120003417.

Hatchwell, L. C. 1996. Implications for fat on flavor. Pages 14–23. Flavor-food interactions. R. J. McGorrin and J. V. Leland, ed. American Chemical Society.

Hutton, J. T., and S. Patton. 1952. The origin of sulfhydryl groups in milk proteins and their contributions to "cooked" flavor. J. Dairy Sci. 35:699–705. https://doi.org/10.3168/jds.S0022-0302(52)91658-5.

Jang, H. D., and H. E. Swaisgood. 1990. Disulfide bond formation between thermally denatured β-lactoglobulin and κ-casein in casein micelles. J. Dairy Sci. 73:900–904. https://doi.org/10.3168/jds.S0022-0302(90)78746-2.

Jo, Y., D. M. Benoist, D. M. Barbano, and M. A. Drake. 2018. Flavor and flavor chemistry differences among milks processed by high-temperature, short-time pasteurization or ultra-pasteurization. J. Dairy Sci. 101:3812–3828. https://doi.org/10.3168/jds.2017-14071.

Jo, Y., B. G. Carter, D. M. Barbano, and M. A. Drake. 2019. Identification of the source volatile sulfur compounds produced in milk during thermal processing. J. Dairy Sci. 102:8658–8669. https://doi.org/10.3168/jds.2019-16607.

Jovanovic, S., M. Barac, O. Macej, T. Vucic, and C. Lacnjevac. 2007. SDS-PAGE analysis of soluble proteins in reconstituted milk exposed to different heat treatments. Sensors (Basel) 7:371–383. https://doi.org/10.3390/s7030371.

Kaylegian, K. E., G. E. Houghton, J. M. Lynch, J. R. Fleming, and D. M. Barbano. 2006. Calibration of infrared milk analyzers: Modified milk versus producer milk. J. Dairy Sci. 89:2817–2832. https://doi.org/10.3168/jds.S0022-0302(06)72555-3.

Kaylegian, K. E., J. M. Lynch, J. R. Fleming, and D. M. Barbano. 2009. Influence of fatty acid chain length and unsaturation on mid-infrared milk analysis. J. Dairy Sci. 92:2485–2501. https://doi.org/10.3168/jds.2008-1910.

Keršiene, M., A. Adams, A. Dubra, N. De Kimpe, and D. Leskauskaite. 2008. Interactions between flavour release rheological properties in model custard desserts: Effect of starch concentration and milk fat. Food Chem. 108:1183–1191. https://doi.org/10.1016/j.foodchem.2007.11.011.

Lawless, H., C. Hartono, and S. Hernandez. 2000. Thresholds and supra-threshold intensity functions for capsaicin in oil and aqueous based carriers. J. Sens. Stud. 15:437–447. https://doi.org/10.1111/j.1745-459X.2000.tb00281.x.

Lee, A. P., D. M. Barbano, and M. A. Drake. 2017. The influence of ultra-pasteurization by indirect heating versus direct steam injection on skim and 2% fat milks. J. Dairy Sci. 100:1688–1701. https://doi.org/10.3168/jds.2016-11899.

Leksrisompong, P., D. M. Barbano, A. E. Foegeding, P. Gerald, and M. A. Drake. 2010. The roles of fat and pH on the detection thresholds and partition coefficients of three compounds: Diacetyl, δ-dacalactone, and furaneol. J. Sens. Stud. 25:347–370. https://doi.org/10.1111/j.1745-459X.2009.00264.x.

Li, Z., R. Marshall, H. Heymann, and L. Fernando. 1997. Effect of milk fat content on flavor perception of vanilla ice cream. J. Dairy Sci. 80:3133–3141. https://doi.org/10.3168/jds.S0022-0302(97)76284-2.

Lynch, J. M., D. M. Barbano, and J. R. Fleming. 1998. Indirect and direct determination of the casein content of milk by Kjeldahl nitrogen analysis: Collaborative study. J. AOAC Int. 81:763–774. https://doi.org/10.1093/jaoac/81.4.763.

Meilgaard, M., G. V. Civille, and B. T. Carr. 2007. Descriptive analysis techniques. Chapter 10 in Sensory Evaluation Techniques, 4th ed. CRC Press.

Misawa, N., D. M. Barbano, and M. A. Drake. 2016. Influence of casein as a percentage of true protein and protein level on color and texture of milks containing 1 and 2% fat. J. Dairy Sci. 99:5284–5304. https://doi.org/10.3168/jds.2016-10846.

Verdi, R. J., D. M. Barbano, M. E. Dellavalle, and G. F. Senyk. 1987. Variability in true protein, casein, nonprotein nitrogen, and proteolysis in high and low somatic cell count milks. J. Dairy Sci. 70:230–242. https://doi.org/10.3168/jds.S0022-0302(87)80002-4.

Vogel, K. G. III, B. G. Carter, N. Cheng, D. M. Barbano, and M. A. Drake. 2021. Ready-to-drink protein beverages: Effects of milk protein concentration and type on flavor. J. Dairy Sci. 104:10640–10653. https://doi.org/10.3168/jds.2021-20522.

Wojciechowski, K. L., C. Melilli, and D. M. Barbano. 2016. A proficiency test system to improve performance of milk analysis methods and produce reference values for component calibration samples for infrared milk analysis. J. Dairy Sci. 99:6808–6827. https://doi.org/10.3168/jds.2016-10936.

Zulewska, J., and D. M. Barbano. 2014. The impact of linear velocity and flux on performance of ceramic graded permeability membranes when processing skim milk at 50°C. J. Dairy Sci. 97:2619–2632. https://doi.org/10.3168/jds.2013-7635.

Zulewska, J., M. Newbold, and D. M. Barbano. 2009. Efficiency of serum protein removal from skim milk with ceramic and polymeric membranes at 50°C. J. Dairy Sci. 92:1361–1377. https://doi.org/10.3168/jds.2008-1757.

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