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Effects of milk fat, casein, and serum protein concentrations on sensory properties of milk-based beverages

Ni Cheng,[1] David M. Barbano,[2]* and MaryAnne Drake[1]
[1]Southeast Dairy Foods Research Center, North Carolina State University, Raleigh 27695
[2]Northeast Dairy Foods Research Center, Department of Food Science, Cornell University, Ithaca, NY 14853

ABSTRACT

Our goal was to determine the effect of systematically controlled variation in milk fat, true protein, casein, and serum protein concentrations on the sensory color, flavor and texture properties, instrumental color and viscosity, and milk fat globule size distribution of milk-based beverages. Beverage formulations were based on a complete balanced 3-factor (fat, true protein, and casein as a percentage of true protein) design with 3 fat levels (0.2, 1.0, and 2.0%), 4 true protein (TP) levels (3.00, 3.67, 4.34, and 5.00%) within each fat level, and 5 casein as a percentage of true protein (CN%TP) levels (5, 25, 50, 75, and 80%) within each protein level (for a total of 60 formulations within each of 2 replicates). Instrumental measures of Hunter L and a values and Commission Internationale de l'Éclairage (CIE) b* values, instrumental viscosity, particle size, flavor, sensory texture and sensory appearance evaluations were done on each pasteurized/homogenized beverage formulation. Within each of the 3 fat levels, higher serum protein concentration drove higher aroma intensity, sweet aromatic, cooked/sulfur, cardboard/doughy flavors, and sensory yellowness scores, whereas higher casein concentration drove higher instrumental viscosity in milk protein beverages. Increasing serum protein concentration increased yellowness, sweet aromatic, aroma intensity, cooked/sulfur, and cardboard/doughy flavors across all fat levels and also had the largest effect on L, a, and b* values, sensory whiteness, and opacity within each fat level. Increases in true protein increased throat cling and astringency intensities. Increases in fat concentration were correlated with higher L, a, and b* values, larger particle size, and increased sensory whiteness, mouth coating, cooked/milky, and milkfat flavors. Multiple linear regression of L, a, and b* values produced better predictions of sensory whiteness and yellowness of pasteurized milk protein beverages than simple linear regression of L or b* values, respectively. Formulating milk protein beverages to a higher true protein level increased astringency regardless of fat level. When formulating milk protein beverages, a product developer has a wide range of milk-based protein ingredient choices that differ in price and change price relationship across time. Understanding the expected relative effect of different milk protein ingredients on the textural and flavor characteristics of milk-based beverages could be used to help guide product reformulation decisions and ingredient choices to achieve a specific sensory profile while controlling total beverage ingredient cost.

Key words: casein, serum protein, milk-based beverage

INTRODUCTION

Consumer preference and liking can be influenced by the sensory properties (appearance, flavor, texture) of fluid milk and milk-based protein beverages. Qualitative consumer interviews on fluid milk indicated that better color and whiteness were important reasons that skim milk, 2% fat milk, and whole milk drinkers all preferred milks with higher fat level (McCarthy et al., 2017). Lee et al. (2017) reported that heat treatment [indirect ultra-pasteurization (**UP**), direct UP, and HTST] induced appearance differences (i.e., whiteness) in skim and 2% fat milk that could affect consumer liking. Furthermore, Chung (2009) reported that Korean consumer liking of milks was positively correlated with sweetness, sweet cream flavor, and smooth texture. Grassy odor, raw milk flavor, artificial milk flavor, and rancid flavor were negatively correlated with liking. Consumer acceptance studies of fluid milks by Deane et al. (1967), Chapman and Boor (2001), Gandy et al. (2008), and Lee et al. (2017) indicated that flavors related to heat treatment (cooked/sulfur or eggy flavors) were disliked by American children and adults, and that the flavor of HTST milk was generally accepted in the US market, whereas the flavor of milks produced with higher temperature thermal treatments was not.

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*Corresponding author: dmb37@cornell.edu

Several studies have demonstrated the effects of fat and protein standardization on sensory properties of fluid milk and milk-based protein beverages. Pangborn et al. (1985), Pangborn and Dunkley (1964a,b), Phillips et al. (1995a), Misawa et al. (2016), McCarthy et al. (2017) and Cheng et al. (2019) indicated that the addition of milkfat affected sensory appearance (i.e., whiteness, yellowness, opacity), texture (i.e., mouth coating, viscosity, thickness, and astringency) and flavor (i.e., milkfat) of fluid milk and milk protein beverages. However, visual cues (i.e., whiteness) were the most important factor for differentiation of milks with different fat levels (Phillips et al., 1995a). Phillips and Barbano (1997) reported that suspension of titanium dioxide as a fat substitute in fat-free milk made the milk whiter, which improved perceived sensory appearance, creamy aroma, and texture of fat-free milk.

Protein standardization of fluid milk and milk protein beverages can be divided into 2 approaches: changing true protein (**TP**) concentration by UF and changing casein as a percentage of true protein (**CN%TP**) and true protein concentration by microfiltration and UF. Poulsen (1978) reported that no significant sensory changes were detected for whole milk (3.5% fat), for half-skim milk (1.5 to 1.8% fat), or for skim milk with a range of protein from 1.5 to 6.4%, but a triangle test with 6 panelists was used to make these conclusions. Rattray and Jelen (1996) conducted triangle sensory tests with 15 panelists and reported that skim milk downward standardized in milk protein concentration with addition of UF permeate from either UF of milk or sweet whey to not lower than 2.4% (wt/wt) protein was indistinguishable from normal skim milk, again suggesting that these types of permeates would be suitable for downward standardization of protein concentration of fluid milk. However, appropriate sensory methodology was not applied in either of these studies, which would necessitate additional work to confirm their conclusions. A trained descriptive sensory analysis panel was used to determine the effect of milk protein standardization of skim and 1% fat milk (Quiñones et al., 1997) and 2% and 3% fat milks (Quiñones et al., 1998) on sensory properties of protein standardized fluid milks. Increases in true protein concentration increased appearance scores (center color, edge color, opacity, and visual hang-up) and mouth coating, residual mouth coating, and thickness (Quiñones et al., 1997, 1998). Standardizing the lower fat milk to a higher protein content made the lower fat milks similar to the higher fat milks from a sensory standpoint because of increases in whiteness due to a higher concentration of light-scattering casein micelles (Quiñones et al., 1997, 1998).

Misawa et al. (2016) standardized TP to 4 levels (3.00, 3.67, 4.34, and 5.00%) and CN%TP to 5 levels (5, 25, 50, 75, and 80%) within each TP level for pasteurized 1% and 2% fat milk protein beverages, and Cheng et al. (2019) extended their work to unpasteurized skim, 1%, and 2% fat milk protein beverages. The changes in CN%TP had more effect than TP on the appearance of unpasteurized and pasteurized milk protein beverages and the texture of the pasteurized beverages (Misawa et al., 2016; Cheng et al., 2019). Increases in CN%TP increased the sensory whiteness and opacity but decreased the sensory yellowness of milk protein beverages (Misawa et al., 2016; Cheng et al., 2019). Increases in CN%TP increased throat cling and mouth coating perceived by trained panelists in 2% fat pasteurized beverages even when the visual cues were masked (Misawa et al., 2016).

Principal component analysis (**PCA**) is generally used for sensory or multidimensional data analysis and visualization (Wold et al., 1987). Mean sensory attributes can be used to calculate the principal components (**PC**), and the first 2 PC that contributed to the most of the variability are typically used to generate PCA biplots (Drake et al., 2003, 2010). The correlation coefficient of each sensory attribute (the active variables) to the first 2 PC can be calculated and the sensory attributes can be projected on the biplot. Supplementary variables are the additional objective data measurements (such as compositions and volatile compounds) that can be included or brought into a PCA biplot to better explain the active variables. Pagès and Husson (2001) and Pagès (2005) demonstrated that the introduction of supplementary variables onto the PC-based sensory biplot did not affect the biplot PC, but their correlation coefficient to the PC was calculated and then projected on the biplot in the sensory evaluation of wines and chocolates. This approach puts the active variables and the supplementary variables onto the same data space and can help the interpretation of sensory or multidimensional data. In the current study of standardization of fat, protein, and CN%TP of milk beverages, the idea of bringing milk composition data as the supplementary variables for sensory data (the active variables) may help to better visualize the relative relationships among beverage composition parameters and instrumental and sensory data for milk protein beverages. This novel approach could produce a more clear and concise understanding for milk protein beverages formulation to optimize milk-based beverage sensory properties while controlling ingredient and processing costs.

Our first objective was to determine the effect of systematically controlled variation in milk fat, true protein, casein, and serum protein concentrations on the sensory color, flavor and texture properties, instrumental color and viscosity, and milk fat globule size dis-

tribution of milk-based beverages. Our second objective was to use PCA biplots with supplemental composition variables superimposed on the PCA biplot to provide a visual overview of how the composition of milk protein beverages influences sensory properties as a potential guide for beverage formulation.

MATERIALS AND METHODS

Experimental Design

Beverage formulations were based on a complete balanced 3-factor (fat, TP, and casein as a percentage of true protein) design with 3 fat levels (0.2, 1.0, and 2.0%), 4 TP levels (3.00, 3.67, 4.34, and 5.00%) within each fat level, and 5 CN%TP levels (5, 25, 50, 75, and 80%) within each protein level (for a total of 60 formulations within each replicate) as described by Cheng et al. (2019). The formulation, sensory, and analytical work were conducted in 1 wk for each fat level, and the formulation, processing, and analysis for all the treatments within each fat level was replicated in a second week, for a total of 6 wk of processing (Cheng et al., 2019). Instrumental measures of Hunter lightness (**L**) and red–green color (**a**) values and Commission Internationale de l'Éclairage (CIE) yellow–blue color (**b***) value, instrumental viscosity, particle size, flavor, sensory texture and sensory appearance evaluation were done on each pasteurized/homogenized beverage formulation. The effect of composition, specifically fat, true protein, and CN%TP, on instrumental color and viscosity and sensory properties of pasteurized beverages was determined.

Milk-Based Ingredients, Beverage Formulation, and Processing

The ingredients used for beverage formulation and pasteurization-homogenization processing conditions were as described by Cheng et al. (2018). Each raw milk beverage was well mixed and continuously fed to a Microthermics EHVH pasteurization unit (Microthermics, Raleigh, NC) with a 2-stage homogenizer (GEA Niro Soavi, Parma, Italy) at flow rate of 2 L/min. Each beverage formulation was preheated to 60°C, homogenized (first stage at 17.3 MPa and second stage at 3.4 MPa), HTST pasteurized (73°C for 15 s), cooled to 10°C, and placed in a 4°C cooler. The relative weight percentage of usage of each ingredient in each formulation was as presented by Cheng et al. (2019). Within each fat level (0.2, 1, or 2% fat), TP and CN%TP were targeted at 4 TP levels (3.00, 3.67, 4.34, and 5.00%) and 5 CN%TP (5, 25, 50, 75, and 80%), respectively. Anhydrous lactose concentration was standardized to 4.65% for all formulations.

Analysis Methods

Chemical Composition, Instrumental Color, and Viscosity. Chemical composition analysis, during processing, of ingredients and calculation of formulations based on ingredient composition and instrumental color measurement and viscosity at 4°C and 20°C on pasteurized milk protein beverages were conducted as described by Cheng et al. (2019).

Particle Size. Fat globule particle size of the pasteurized milk protein beverages was measured using a Mastersizer 2000 (Malvern Instrument Ltd., Malvern, UK) as described by Di Marzo et al. (2016), and the particle diameter (in microns) below which 90% of the volume of the particle accounts for [d(0.9)] was reported.

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. Sensory appearance, flavor, and texture/mouthfeel profiling were conducted in separate sessions, and 2 different cohorts of trained panelists were used because of the large numbers of samples and the timeframe. One panel documented appearance attributes (n = 6; 3 men, 3 women, ages 24–56 yr) and one panel documented flavor and texture (n = 8; 3 men, 5 women, ages 23–54 yr). Each panelist had a minimum of 80 h of prior descriptive analysis training on food appearance attributes using the Spectrum method with a 0- to 15-point intensity scale (Meilgaard et al., 2007), and at least 40 h of prior experience with the sensory profiling of fluid milk and dried dairy ingredients using established sensory languages for fluid milk and dairy proteins (Drake et al., 2003; Croissant et al., 2007; Wright et al., 2009; McCarthy et al., 2017). Compusense Cloud (Guelph, ON, Canada) was used for data collection.

Appearance. Sensory appearance profiling of pasteurized beverages was done at 4 and 20°C in separate panel sessions. Appearance attributes (whiteness, yellowness, and opacity) were consistent with those used in previous research (Phillips et al., 1995b; Misawa et al., 2016; Lee et al., 2017; McCarthy et al., 2017) and the conditions of the appearance evaluation procedure was described by Cheng et al. (2019). The panel was trained for color, opacity, and visual viscosity attributes as described by McCarthy et al. (2017). Briefly, 80 mL of milk was dispensed into 100- × 10-mm clear, plastic Petri dishes (Thermo Scientific, Waltham, MA) and placed onto a white paper background. Paint chips

were used as references for white and yellow color [Behr "Ultra Pure White" PPU18-06 = 0 (L = 97.68, a = −0.84, b = 2.22) and "Glass of Milk" P260-1u = 3.5 (L = 96.21, a = −0.05, b = 11.03); Behr Process Corp., Santa Ana, CA]. For opacity, milks were dispensed into 118-mL black soufflé cups with random 3-digit blinding codes. Water was used as a reference of 0 and full-fat HTST milk was used as reference of 12 for opacity. Visual viscosity was determined by determining the amount of force needed to slurp 1 teaspoon of milk from a spoon. The references for viscosity were water = 0 and heavy cream = 3.2 (Meilgaard et al., 2007). Samples were evaluated in duplicate in a randomized order by each panelist. A 5-min rest was enforced after every 10 samples, and no more than 20 samples were evaluated per session.

Flavor. Sensory flavor profiling was conducted on the pasteurized milk protein beverages at 20°C. Aroma intensity, sweet aromatic, cooked/milky, cooked/sulfur, cardboard, and milkfat flavors were the flavor attributes evaluated. Reference materials were described by Schiano et al. (2017).

Samples (30 mL) were dispensed into translucent lidded 59-mL plastic soufflé cups (stock number P200N, Dart Container Corp., Mason, MI) with random 3-digit blinding codes. Beverages were prepared with overhead lights off to prevent light oxidation. Samples were tempered to room temperature (20°C) before evaluation. Each panelist evaluated each sample in duplicate in a randomized design. A 2-min rest was enforced by a timer after each sample and panelists were instructed to rinse their mouths with spring water. An enforced rest of 10 min followed every 5 samples, with no more than 10 samples evaluated per session.

Texture/Mouthfeel. Sensory texture/mouthfeel profiling of pasteurized beverages was done at 4 and 20°C. The texture attributes sensory in mouth viscosity, mouth coating, throat cling (also called residual smoothness of mouth coating), and astringency of milk protein beverages were adopted from previous work (Foegeding and Drake, 2007; Schiano et al., 2017).

Samples (30 mL) were dispensed into translucent lidded 59-mL plastic soufflé cups (stock number P200N, Dart Container Corp.) with random 3-digit codes. Samples were tempered to room temperature and the temperature confirmed by thermocouple for the 20°C session. Samples were kept in the 4°C walk-in cooler during the 4°C session and a sample was taken out to serve only when needed. Each panelist evaluated each sample in duplicate in a randomized design. A 2-min rest was enforced by a timer after each sample and panelists were instructed to rinse their mouths with spring water. An enforced rest of 15 min was applied following every 5 samples, and no more than 10 samples were evaluated within a session.

Statistical Analysis

The GLM procedure of SAS (version 9.4, SAS Institute Inc., Cary, NC) was used to determine the effect of fat (0.2, 1, and 2%), TP (3.0, 3.67, 4.34, and 5.0%), CN%TP (5, 25, 50, 75, and 80%), measurement temperature of instrumental color (**temp**; 4 and 20°C) of milk protein beverages, and replicate (**rep**) on beverage color and viscosity measured instrumentally. The effect of the same composition parameters temperatures of 4 and 20°C on sensory appearance and sensory texture was determined. All interactions of these parameters were included in the model. Fat, TP, and temperature were treated as categorical variables, whereas CN%TP was handled as a continuous variable. The CN%TP data were transformed (named CNTPT) by mean-centering (Misawa et al., 2016) to avoid collinearity effects on statistical analysis (Glantz and Slinker, 2001). If the F-value for the full model was significant ($P < 0.05$), then the significance of each parameter ($P < 0.05$) and their interactions was determined. The effects of fat, TP (**prot**), heat treatment, temperature of color or viscosity measurement (temp), and replicate (rep) and their interactions were tested for significance using the interaction term of fat × prot × temp × rep as the error term, whereas the effects (linear and quadratic) of the continuous variable of CN%TP and their interactions with the categorical variables were tested for significance using the full model error. A stepwise process was done to remove all nonsignificant terms from the model to produce a final reduced model and type III sum of squares table. To understand the relative amount of total variation explained by each parameter or interaction of parameters on each instrumental color, instrumental viscosity, each sensory appearance metric and each sensory texture metric, the type III sum of squares value for each term was divided by the total type III sum of squares of the significant terms in the model and multiplied by 100. The term "fat" and its related interaction terms in the model were removed from the model when analyzing data to determine the effect of each parameter within each fat level for instrumental color and sensory appearance data. The term "temp" and its interaction terms in the model were removed from the model when analyzing data to determine the effect of each parameter for flavor and particle size data because those measurements were done at only one temperature.

Linear and multiple linear regressions were conducted using Excel (Microsoft Corp., Redmond, WA) data

analysis to generate the prediction models of sensory whiteness and sensory yellowness by using single instrumental parameters (L or b* values) and multiple instrumental parameters (L, a, and b* values).

Principal component analysis was conducted using XLSTAT (Addinsoft, Paris, France) on the active variables—L, a, and b* values, instrumental viscosity, particle size [d(0.9)], sensory whiteness, opacity, yellowness, mouth coating, throat cling, astringency, sweet aromatic, aroma intensity, cooked/milky, cooked/sulfur, cardboard/doughy, and milkfat—for all fat levels to produce PCA vector biplots. The supplementary variables—fat, TP, casein concentration, and serum protein concentration (**SP**)—were projected onto the PCA vector biplots. The supplementary variables have no effect on the PCA vector biplots as their correlation coefficients with the PC are calculated and represented in the PC biplot. The inclusion of these data assist with interpretation of the PCA biplot and may provide a clearer path for beverage formulations, as has been done for other foods (Pagès and Husson, 2001; Pagès, 2005).

RESULTS AND DISCUSSION

Analysis of variance was used to determine the relative effect of variation in fat concentration, true protein concentration, and CN%TP on the sensory properties of HTST pasteurized and homogenized formulated milk-based beverages. The R^2 values for ANOVA models to determine the effect of variation in composition parameters on instrumental color and viscosity and sensory whiteness and opacity were generally higher than those for other sensory color and flavor attributes. The lower the R^2 values for the total models, the lower the power of the ANOVA to correctly quantify the relative contribution of each model factor in explaining variation in sensory parameters, particularly when there were interaction effects.

Instrument and Sensory Color Across All Fat Levels

L Values (Whiteness). The L values of pasteurized and homogenized milk protein beverages increased ($P < 0.05$) with increased fat concentration and increased CN%TP (Table 1 and Figure 1A). Fat, CNTPT, and CNTPT × fat explained 48.75, 39.38, and 3.53%, respectively, of the total variation of L values for the pasteurized milk protein beverages across fat levels, with 91.66% of the total variation in L values explained by these terms in the model (Table 1). Increasing fat level increased the L values more from 0.2% to 1.0% fat than from 1.0% to 2.0% fat and increased CN%TP increased the L value more in the lower fat content pasteurized milk protein beverages ($P < 0.05$, CNTPT × fat interaction in Table 1, Figure 1A). This result confirmed previous studies that light scattering (whiteness) from casein micelles and fat globules was nonadditive and casein micelles were the major source for whiteness of skim beverages, whereas both casein micelles and fat globules increased whiteness of 1% and 2% fat beverages (Quiñones et al., 1997, 1998; Misawa et al., 2016; Cheng et al., 2019). The effect of beverage temperature on instrumental and sensory evaluations of color parameters explained only a very small amount of variation in color (Tables 1, 2, 3, and 4); therefore, data for 4 and 20°C were averaged for presentation in Figures 1 and 2.

Sensory Whiteness. The sensory whiteness of pasteurized and homogenized milk protein beverages increased ($P < 0.05$) with increased fat concentration and increased CN%TP (Table 1 and Figure 1B). Variation in fat, CNTPT, and TP explained 92.89% of the total variation of sensory whiteness, with 51.04, 35.30, and 6.55%, respectively, of the pasteurized beverages across fat levels (Table 1). Increased fat level increased the sensory whiteness more from 0.2% to 1.0% fat than from 1.0% to 2.0% fat (Figure 1B). Increased CN%TP increased the sensory whiteness (Figure 1B), which was consistent with previous studies by Misawa et al. (2016) and Cheng et al. (2019).

Sensory Opacity. Sensory opacity of pasteurized and homogenized milk protein beverages increased ($P < 0.05$) with increased fat concentration and increased CN%TP (Table 1 and Figure 1C). The variation in fat, CNTPT, and CNTPT × fat explained 72.88, 14.93, and 3.06%, respectively, of the total variation of sensory opacity for the pasteurized beverages across fat levels, with 90.87% of the total variation being explained by these 3 parameters in the model (Table 1). Sensory opacity increased with increasing fat content from 0.2% to 2% fat, but the increase was not linear (Figure 1C). Opacity increased much more going from 0.2% to 1% than from 1% to 2% fat. Within each fat level, as CN%TP increased, the sensory opacity of the beverage increased (Figure 1C) but the increase was larger in the skim beverage than in the 1% and 2% fat beverages (i.e., CNTPT×fat interaction, Table 1). Both Misawa et al. (2016) and Cheng et al. (2019) reported that fat and CN%TP increased sensory opacity of milk-based beverages.

a Values (Redness–Greenness). The a values of pasteurized and homogenized milk protein beverages increased ($P < 0.05$) with increased fat concentration but decreased with increased CN%TP (Table 2 and Figure 2A), which confirmed previous work on CN%TP by Misawa et al. (2016) and Cheng et al. (2019). Increased fat concentration levels increased the a values

more from the skim to 1.0% fat beverages than it did from the 1.0% to 2.0% fat beverages (Figure 2A). Increased fat made the milk less green whereas increased CN%TP made the milk more green. Variation in fat and CNTPT explained 80.99 and 9.51%, respectively, of the total variation of a values for pasteurized milk protein beverages across fat levels, with 90.50% of total variation being explained by these 2 terms in the model (Table 2). The effect of increasing concentration of fat on a values was much larger in pasteurized/homogenized beverages than reported for the same unpasteurized beverages (Cheng et al., 2019). The larger effect of fat in pasteurized/homogenized beverages than unpasteurized beverages of the same composition was due to the smaller size, larger number, and increased surface area of the fat globules that was produced by homogenization and caused more light reflection than reported for the unhomogenized fat and casein micelles in the unpasteurized beverages. When more light was reflected by fat, light traveled a shorter distance into the milk before being reflected, which decreased the amount of light absorbed by riboflavin (Misawa et al., 2016; Cheng et al., 2019).

b* Values (Yellowness–Blueness). The b* value of pasteurized and homogenized milk protein beverages increased ($P < 0.05$) with increased fat concentration but decreased with increased CN%TP (Table 2 and Figure 2B). Increased fat made the milk more yellow and increased CN%TP made the milk less yellow. Variation in fat and linear and quadratic effects of CNTPT explained 56.09, 26.38, and 4.98%, respectively, of the total variation in b* values of the pasteurized beverages, with 87.45% of the total variation in b* value explained by these 3 terms in the model (Table 2). In-

Table 1. Pasteurized milk protein beverages: relative percentage of type III sum of squares (for factors with $P < 0.05$) explained by model factors[1] for Hunter L values at a 10° viewer angle (LHA10) using illuminant A, sensory whiteness, and sensory opacity

Factor	LHA10	Whiteness	Opacity
Fat	48.75	51.04	72.88
Prot	0.31	6.55	2.87
Temp	0.31	0.04	NS[2]
Rep	0.04	NS	0.02[NS]
Fat × prot	1.41	2.84	3.44
Fat × temp	1.35	0.03	0.02
Fat × rep	NS	0.05	0.03
Prot × rep	0.37	NS	NS
Temp × rep	NS	0.01	NS
Fat × prot × temp	NS	NS	0.04
Fat × prot × rep	0.73	0.16	NS
Fat × temp × rep	NS	0.02	NS
Prot × temp × rep	NS	NS	0.06
Fat × prot × temp × rep	0.10[NS]	0.11	0.10
CNTPT	39.38	35.30	14.93
CNTPT × fat	3.53	0.40	3.06
CNTPT × prot	0.48	0.97	0.44
CNTPT × temp	0.63	NS	NS
CNTPT × rep	NS	0.04	NS
CNTPT × fat × prot	0.67	NS	NS
CNTPT × fat × temp	0.20	1.05	0.88
CNTPT × fat × rep	0.23	0.03	0.03
CNTPT × prot × temp	NS	0.05	NS
CNTPT × temp × rep	NS	0.03	0.01
CNTPT × prot × rep	0.34	0.04	NS
CNTPT × CNTPT	0.34	0.31	0.15
CNTPT × CNTPT × fat	NS	0.06	0.79
CNTPT × CNTPT × prot	0.25	0.36	0.04
CNTPT × CNTPT × temp	0.08	NS	NS
CNTPT × CNTPT × rep	NS	0.01	NS
CNTPT × CNTPT × fat × prot	0.33	0.43	0.20
CNTPT × CNTPT × fat × temp	NS	0.03	NS
CNTPT × CNTPT × fat × rep	0.07	NS	NS
CNTPT × CNTPT × prot × rep	0.10	0.02	NS
Sum (%)	100.00	100.00	100.00
R^2	0.98	0.97	0.97
N	480	2,880	2,880

[1]Fat = fat level with 0.2, 1, and 2%; Prot = milk true protein with 3.00, 3.67, 4.34, and 5.00%; Temp = temperature of sensory appearance evaluation with 4°C and 20°C; Rep = replicate; CNTPT = casein as a percentage of true protein with 5, 25, 50, 75, and 80% transformed.

[2]Not significant ($P > 0.05$).

creased fat concentration increased the b* values more from 0.2% to 1.0% fat beverages than it did from the 1.0% to 2.0% fat beverages (Figure 2B). Compounds that absorb light and produce the perception of yellowness (such as carotenoids) are dissolved in milk fat and will increase with increasing fat concentration (Johnson 1974; Misawa et al., 2016; Cheng et al., 2019). Changes in CN%PT caused smaller changes in b* values than changes due to fat concentration of milk protein beverages (Figure 2B).

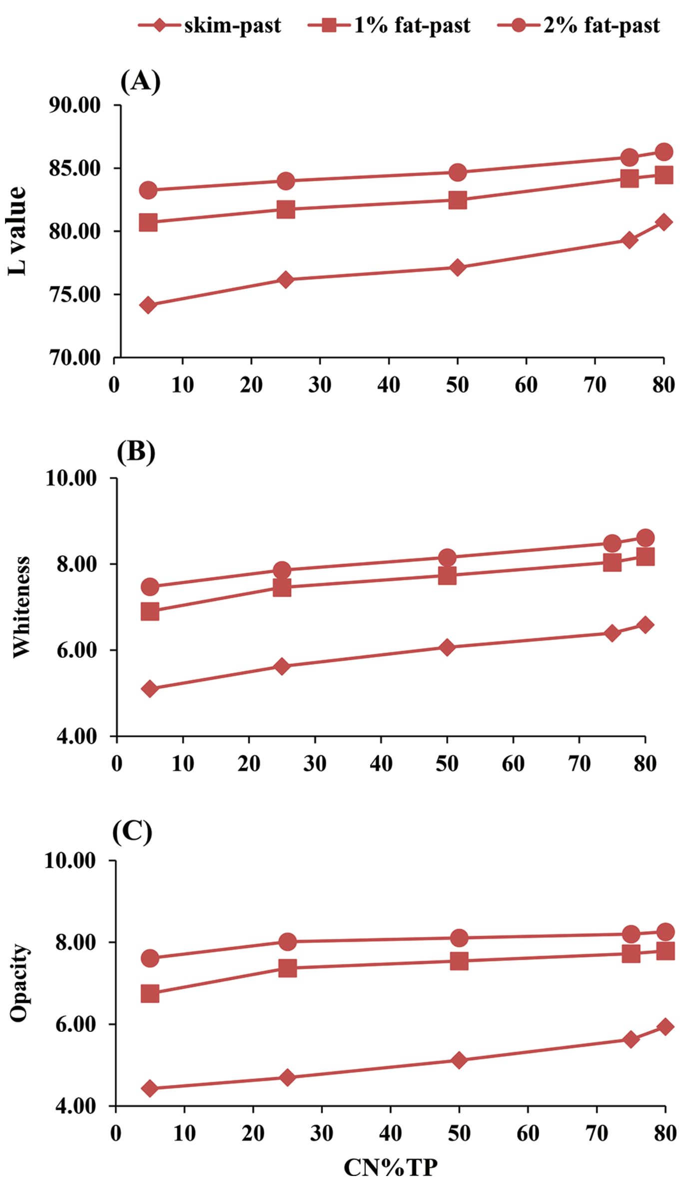

Figure 1. (A) Hunter L values (4°C and 20°C) at 10° viewer angle using illuminant A; (B) sensory whiteness; and (C) sensory opacity for pasteurized (past) milk protein beverages with different fat levels (skim, 1% fat, and 2% fat) as a function of casein as a percentage of true protein (CN%TP).

Sensory Yellowness. The sensory yellowness of pasteurized and homogenized milk protein beverages increased ($P < 0.05$) with increased fat concentration, but sensory yellowness decreased with increased CN%TP for all fat levels (Table 2 and Figure 2C). The effect of CNTPT (72.73%), fat (9.55%), and CNTPT × fat (4.16%) explained 86.44% of the total variation of sensory yellowness for the pasteurized milk protein beverages, but the R^2 value (0.55) was relatively low (Table 2). As CN%TP increased, sensory yellowness scores decreased for beverages of all fat concentrations, but sensory yellowness scores decreased more for skim beverages than beverages with 1% and 2% fat ($P < 0.05$, CNTPT × fat interaction in Table 2, Figure 2C). This is interesting because b* values increased with increasing fat (Figure 2B), whereas sensory yellowness decreased with increasing fat content (Figure 2C). Cheng et al. (2019) reported previously that b* values were not well correlated with yellowness of milk-based beverages and that a combination of L, a, and b* values using multiple linear regression (**MLR**) was a much better predictor of sensory yellowness. Increases in CN%TP also decreased the sensory yellowness scores in unpasteurized beverages (Cheng et al., 2019). When CN%TP is low, casein micelles do not scatter light in the aqueous phase (i.e., liquid phase around the fat globules). If the perceived yellowness is being produced by soluble components in the aqueous phase of the beverage that are absorbing light, then we would expect that removing casein micelles from the skim phase would allow light to travel in the skim (i.e., aqueous) phase and soluble components would absorb wavelengths as a function of their structure. When the casein micelle concentration is high, light would be reflected by the casein micelles and the beverage would appear more white (Figure 1A and B) and less yellow (Figure 2C).

Instrument and Sensory Color Within Fat Levels

Statistical analysis of the instrumental and sensory measures of beverage color within each fat concentration may provide a clearer understanding of the separate effects of protein concentration versus the proportion of true protein that is casein. Microfiltration of skim milk separates milk casein and milk serum proteins, the use of milk micellar casein concentrate and milk serum protein isolate as separate ingredients is a new possibility in formulation of beverages with different sensory characteristics.

L Values (Whiteness). The L values of pasteurized and homogenized milk protein beverages increased ($P < 0.05$) with increased CN%TP for all fat levels (Figure 1A). The linear effect of CNTPT explained 77.36, 96.24, and 94.06% of the total variation of L

values for 0.2%, 1%, and 2% fat beverages, respectively (Table 3 and Figure 1A). The effect of protein and CNTPT × prot explained 4.79 and 3.37% of the total variation of L values for 0.2% fat beverages, respectively (Table 3) and less in the 1% and 2% fat beverages. Thus, within each beverage fat level, the effect of CN%TP on beverage L value was much larger than the effect of protein. This result was consistent with work on unpasteurized milk protein beverages by Cheng et al. (2019). With constant fat concentration, the casein micelle was the major particle that reflected light in milk protein beverages (Misawa et al., 2016; Cheng et al., 2018, 2019). Therefore, to increase beverage whiteness within fat level, increasing casein concentration is more important than increasing protein concentration. Increasing CN%TP may decrease yellowness and greenness because light will be reflected by casein micelles and not be absorbed by soluble components in the aqueous phase of the beverage.

Sensory Whiteness. The sensory whiteness of pasteurized and homogenized milk protein beverages increased ($P < 0.05$) with increased CN%TP for all fat levels (Figure 1B) within each fat level (Table 3). Variation in CNTPT explained 60.98, 83.08, and 87.89% of the total variation (Table 3) in sensory whiteness for 0.2%, 1%, and 2% fat pasteurized products, respectively, and was consistent with the CN%TP on L values. This confirmed previous work that increases in CN%TP increased sensory whiteness of 1% and 2% fat pasteurized, and skim, 1%, and 2% fat unpasteurized milk protein beverages (Misawa et al., 2016; Cheng et al., 2019).

Sensory Opacity. The sensory opacity of pasteurized and homogenized milk protein beverages increased

Table 2. Pasteurized milk protein beverages: relative percentage of type III sum of squares (for factors with $P < 0.05$) explained by model factors[1] for Hunter a (aHA10) and CIE b* (bCA10) values at a 10° viewer angle using illuminant A and sensory yellowness

Factor	aHA10	bCA10	Yellowness
Fat	80.99	56.09	9.55
Prot	2.27	2.10	1.71
Temp	0.33	0.93	0.03[NS2]
Rep	0.07	0.21	0.21[NS]
Fat × prot	1.07	2.06	5.80
Fat × temp	1.19	0.42	NS
Fat × rep	0.25	0.72	0.56
Prot × temp	NS	NS	NS
Prot × rep	0.17	NS	0.30
Temp × rep	NS	NS	NS
Fat × prot × temp	0.43	NS	NS
Fat × prot × rep	NS	0.48	1.04
Prot × temp × rep	NS	NS	NS
Fat × prot × temp × rep	0.12[NS]	0.08[NS]	1.08[NS]
CNTPT	9.51	26.38	72.73
CNTPT × fat	NS	0.38	4.16
CNTPT × prot	0.90	1.15	NS
CNTPT × temp	0.23	NS	0.16
CNTPT × rep	0.09	NS	NS
CNTPT × fat × temp	0.57	1.05	NS
CNTPT × fat × prot	0.36	0.66	0.63
CNTPT × fat × rep	NS	NS	NS
CNTPT × prot × temp	NS	NS	0.34
CNTPT × prot × rep	0.15	0.23	NS
CNTPT × CNTPT	0.71	4.98	0.33
CNTPT × CNTPT × fat	0.32	1.55	NS
CNTPT × CNTPT × prot	0.12	0.18	0.32
CNTPT × CNTPT × temp	NS	NS	0.16
CNTPT × CNTPT × rep	NS	NS	0.18
CNTPT × CNTPT × fat × prot	0.15	0.33	NS
CNTPT × CNTPT × fat × rep	NS	NS	0.73
CNTPT × CNTPT × prot × rep	NS	NS	NS
Sum (%)	100.00	100.00	100.00
R^2	0.99	0.98	0.55
N	480	480	2,880

[1]Fat = fat level with 0.2, 1, and 2%; Prot = milk true protein with 3.00, 3.67, 4.34, and 5.00%; Temp = temperature of sensory appearance evaluation with 4°C and 20°C; Rep = replicate; CNTPT = casein as a percentage of true protein with 5, 25, 50, 75, and 80% transformed.

[2]Not significant ($P > 0.05$).

Table 3. Pasteurized milk protein beverages: relative percentage of type III sum of squares (for factors with $P < 0.05$) explained by model factors[1] for Hunter L values at a 10° viewer angle (LHA10) using illuminant A, sensory whiteness, and sensory opacity at 3 fat levels (0.2, 1, and 2%)

	LHA10			Whiteness			Opacity		
Factor	0.2%	1%	2%	0.2%	1%	2%	0.2%	1%	2%
Prot	4.79	0.02[NS2]	1.49	29.60	11.12	4.10	29.00	0.58	13.61
Temp	2.83	0.07[NS]	0.90	0.08[NS]	0.01[NS]	0.05[NS]	0.01[NS]	NS	0.77
Rep	0.28	0.05[NS]	0.06[NS]	0.12[NS]	0.02[NS]	0.20	0.03[NS]	0.87	0.06[NS]
Prot × temp	NS	0.33	0.28	NS	NS	NS	0.19	1.18	NS
Prot × rep	2.78	NS	0.30	NS	NS	1.12	NS	NS	NS
Temp × rep	0.00	NS	NS	NS	NS	0.07[NS]	NS	NS	0.43
Prot × temp × rep	0.09[NS]	0.15[NS]	0.18	0.68	0.11[NS]	0.07	0.61	0.09[NS]	1.08[NS]
CNTPT	77.36	96.24	94.06	60.98	83.08	87.89	61.66	82.62	70.80
CNTPT × prot	3.37	0.02	NS	5.38	0.70	5.08	6.30	NS	NS
CNTPT × temp	1.62	1.31	1.18	NS	NS	NS	NS	NS	NS
CNTPT × rep	0.58	0.44	NS	0.10	NS	0.49	NS	0.86	NS
CNTPT × prot × temp	NS	NS	NS	0.52	NS	NS	NS	0.78	NS
CNTPT × temp × rep	NS	0.08	0.17	0.34	NS	NS	0.17	NS	NS
CNTPT × prot × rep	2.85	NS	NS	0.32	NS	0.12	NS	NS	NS
CNTPT × CNTPT	0.54	0.86	1.15	0.54	1.74	0.13	1.15	10.78	10.94
CNTPT × CNTPT × prot	1.67	0.09	0.22	1.23	3.22	0.59	0.69	1.48	1.85
CNTPT × CNTPT × temp	0.30[NS]	0.14	NS	0.12	NS	NS	NS	NS	0.47
CNTPT × CNTPT × rep	NS	NS	NS	NS	NS	0.07	NS	0.20	NS
CNTPT × CNTPT × prot × temp	NS	NS	NS	NS	NS	NS	0.20	0.57	NS
CNTPT × CNTPT × prot × rep	0.94	0.20	NS	NS	NS	NS	NS	NS	NS
Sum (%)	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
R^2	0.93	0.99	0.98	0.92	0.90	0.94	0.93	0.75	0.62
N	160	160	160	960	960	960	960	960	960

[1]Prot = milk true protein with 3.00, 3.67, 4.34, and 5.00%; Temp = temperature of sensory appearance evaluation with 4°C and 20°C; Rep = replicate; CNTPT = casein as a percentage of true protein with 5, 25, 50, 75, and 80% transformed.

[2]Not significant ($P > 0.05$).

Table 4. Pasteurized milk protein beverages: relative percentage of type III sum of squares (for factors with $P < 0.05$) explained by model factors[1] for Hunter a (aHA10) and CIE b* (bCA10) values at a 10° viewer angle using illuminant A and sensory yellowness at 3 fat levels (0.2, 1, and 2%)

	aHA10			bCA10			Yellowness		
Factor	0.2%	1%	2%	0.2%	1%	2%	0.2%	1%	2%
Prot	27.78	6.21	3.57	15.58	1.23	0.40	0.51	3.94	26.70
Temp	3.00	11.05	16.89	0.01[NS2]	8.12	5.75	0.02[NS]	0.15[NS]	0.55[NS]
Rep	2.41	0.82	0.23	2.58	0.53	1.72	1.82	0.15[NS]	0.02
Prot × temp	NS	NS	NS	NS	NS	NS	NS	0.54	NS
Prot × rep	5.77	NS	NS	NS	0.12	NS	0.95	NS	NS
Temp × rep	NS	1.01	0.16	NS	0.41	NS	NS	0.26	NS
Prot × temp × rep	0.44[NS]	0.27[NS]	0.19[NS]	1.83[NS]	0.09[NS]	0.23	0.15[NS]	0.26[NS]	6.28[NS]
CNTPT	27.48	74.15	76.44	44.70	79.85	83.60	93.15	88.70	66.46
CNTPT × prot	12.57	2.66	0.67	7.96	1.30	0.19	NS	0.88	NS
CNTPT × temp	5.41	0.44	0.13	1.98[NS]	0.35	1.56	NS	0.35	NS
CNTPT × rep	NS	0.93	0.10	NS	0.33	NS	0.22	NS	NS
CNTPT × prot × temp	NS	NS	NS	NS	NS	NS	NS	0.37	NS
CNTPT × temp × rep	NS	NS	NS	NS	0.05	0.08	NS	0.17	NS
CNTPT × prot × rep	4.16	0.54	NS	2.82	0.49	0.12	NS	0.63	NS
CNTPT × CNTPT	8.69	1.51	1.40	20.63	6.83	6.29	0.76	0.43	NS
CNTPT × CNTPT × prot	2.29	0.41	0.21	1.91	0.16	0.08	0.30[NS]	0.52	NS
CNTPT × CNTPT × temp	NS	NS	NS	NS	NS	NS	NS	0.70	NS
CNTPT × CNTPT × rep	NS	NS	NS	NS	NS	NS	1.81	0.22	NS
CNTPT × CNTPT × prot × temp	NS	NS	NS	NS	NS	NS	NS	0.52	NS
CNTPT × CNTPT × temp × rep	NS	NS	NS	NS	NS	NS	NS	0.36	NS
CNTPT × CNTPT × prot × rep	NS	NS	NS	NS	0.12	NS	0.30[NS]	0.85	NS
Sum (%)	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
R^2	0.85	0.97	0.98	0.88	0.99	0.99	0.74	0.77	0.21
N	160	160	160	160	160	160	950	960	960

[1]Prot = milk true protein with 3.00, 3.67, 4.34, and 5.00%; Temp = temperature of sensory appearance evaluation with 4°C and 20°C; Rep = replicate; CNTPT = casein as a percentage of true protein with 5, 25, 50, 75, and 80% transformed.

[2]Not significant ($P > 0.05$).

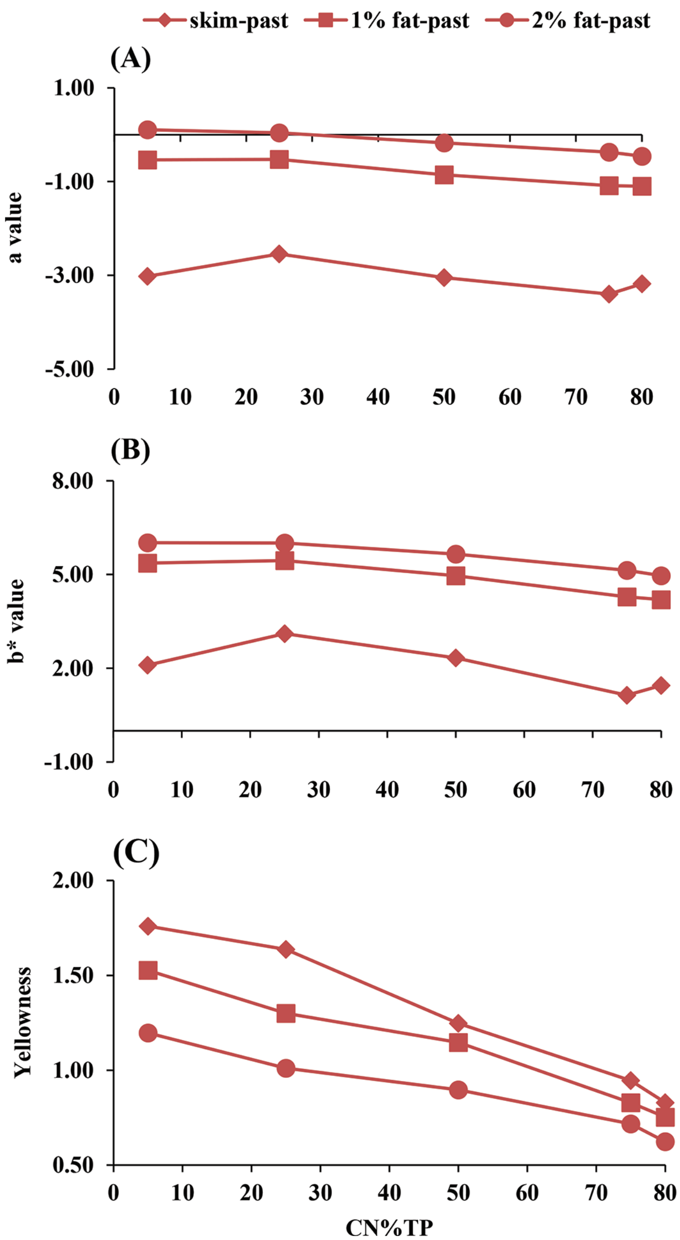

Figure 2. (A) Hunter a values (4°C and 20°C) at 10° viewer angle using illuminant A; (B) CIE b* values (4°C and 20°C) at 10° viewer angle using illuminant A; and (C) sensory yellowness for pasteurized (past) milk protein beverages with different fat levels (skim, 1% fat, and 2% fat) as a function of casein as a percentage of true protein (CN%TP).

($P < 0.05$) with increased CN%TP for all fat levels (Figure 1C) and was consistent with the effect of increasing CN%TP on L values (Figure 1A) and sensory whiteness (Figure 1B), which confirmed results from Misawa et al. (2016). Increased CN%TP also increased sensory opacity of unpasteurized skim, 1%, and 2% fat milk protein beverages (Cheng et al., 2019). The linear plus quadratic effects of CNTPT explained 62.81, 93.40, and 81.74% of the total variation in sensory opacity for the 0.2%, 1.0%, and 2% fat pasteurized beverages, respectively (Table 3).

a and b* Values. Both a and b* values decreased slightly with increased CN%TP but the effect of CN%TP on a and b* values was small relative to the effect of fat (Figure 2A and B). The linear plus quadratic effects for CNTPT explained most of the variation in a and b*values within each fat level (Table 4) but the changes were small (Figure 2A and B) relative to the effect of fat (Table 2) on a and b* values. This result was consistent with the data for the 1% and 2% fat pasteurized beverages in Misawa et al. (2016). Increases in CN%TP decreasing a and b* values within fat levels was also documented in unpasteurized milk protein beverages (Cheng et al., 2019).

Sensory Yellowness. In general, the R^2 values for the ANOVA models for yellowness were lower than for instrumental a and b* values (Table 4), but both sensory parameters decreased with increased CN%TP for 1% and 2% fat beverages (Figure 2C). Misawa et al. (2016) also reported that increases in CN%TP decreased yellowness in 1% and 2% fat pasteurized beverages.

MLR Prediction of Sensory Whiteness and Yellowness. Summary of regression statistics of linear regression and MLR for the whiteness group (L values or combined L, a, b* values) and the yellowness group (b* values or combined L, a, and b* values) of pasteurized milk protein beverages are presented in Tables 5 and 6. Using the L values, the linear regression prediction of sensory whiteness produced an R^2 of 0.75 for the skim, 0.68 for the 1% and 2% fat combined, 0.63 for 1% fat, and 0.67 for 2% fat pasteurized/homogenized beverages (Table 5). The MLR prediction for sensory whiteness including L, a, and b* values, produced a higher R^2 with 0.78 for skim, 0.75 for 1% and 2% fat, 0.87 for 1% fat, and 0.89 for 2% fat pasteurized beverages than the linear regression predictions (Table 5). This result was consistent with the linear and MLR prediction statistics for sensory whiteness of unpasteurized milk protein beverages that MLR using L, a, and b* values together produced a better prediction of sensory whiteness than L values alone (Cheng et al., 2019).

Using the b* values alone, the linear regression prediction of sensory yellowness showed an R^2 of 0.15 for the skim, 0.21 for the 1% and 2% fat, 0.86 for 1% fat, and 0.72 for 2% fat pasteurized beverages (Table 6). Cheng et al. (2019) also previously documented hat instrumental b* values and sensory perception of yellowness were not well correlated for unpasteurized beverages. The MLR prediction for sensory yellowness using L, a, and b* values, produced a much higher R^2 of 0.92 for skim, 0.94 for 1% and 2% fat combined, 0.95 for 1% fat, and 0.92 for 2% fat pasteurized beverages,

Table 5. Linear and multiple linear regression prediction of sensory whiteness (SW) for pasteurized/homogenized skim (0.2%), 1%, and 2% fat milk protein beverages from Hunter L and a (LHA10, aHA10) and CIE b* (bCA10) values at 10° viewer angles

	0.2%		1 and 2%		1%		2%	
Item	LHA10/SW (linear)	LHA10, aHA10 and bCA10/SW (multilinear)	LHA10/SW (linear)	LHA10, aHA10 and bCA10/SW (multilinear)	LHA10/SW (linear)	LHA10, aHA10 and bCA10/SW (multilinear)	LHA10/SW (linear)	LHA10, aHA10 and bCA10/SW (multilinear)
Multiple R	0.8638	0.8810	0.8245	0.8668	0.7913	0.9324	0.8215	0.9468
R^2	0.7461	0.7761	0.6798	0.7513	0.6261	0.8694	0.6748	0.8964
Adjusted R^2	0.7320	0.7341	0.6714	0.7305	0.6053	0.8450	0.6568	0.8770
SE	0.4294	0.4277	0.3292	0.2981	0.3680	0.2306	0.2781	0.1664
Observations	20	20	40	40	20	20	20	20

Table 6. Linear and multiple linear regression prediction of sensory yellowness (SY) for pasteurized/homogenized skim (0.2%), 1%, and 2% fat milk protein beverages from Hunter L and a values (LHA10 and aHA10) and CIE b* (bCA10) values at 10° viewer angles

	0.2%		1 and 2%		1%		2%	
Item	bCA10/SY (linear)	LHA10, aHA10, and bCA10/SY (multilinear)	bCA10/SY (linear)	LHA10, aHA10, and bCA10/SY (multilinear)	bCA10/SY (linear)	LHA10, aHA10, and bCA10/SY (multilinear)	bCA10/SY (linear)	LHA10, aHA10, and bCA10/SY (multilinear)
Multiple R	0.3810	0.9612	0.4601	0.9705	0.9286	0.9769	0.8498	0.9584
R^2	0.1451	0.9239	0.2117	0.9419	0.8622	0.9544	0.7221	0.9185
Adjusted R^2	0.0976	0.9096	0.1910	0.9371	0.8546	0.9458	0.7067	0.9032
SE	0.3659	0.1158	0.2708	0.0755	0.1184	0.0723	0.1366	0.0784
Observations	20	20	40	40	20	20	20	20

respectively (Table 6), than linear regression. Cheng et al. (2019) also reported that using L, a, and b* values together in an MLR produced a better prediction of sensory yellowness for unpasteurized beverages (Cheng et al., 2019). For pasteurized milk protein beverages, MLR (L, a, and b* values) achieved a more robust prediction of sensory whiteness and sensory yellowness than simple linear regression (L or b* values). This approach could be a helpful tool for the use of instrumental color measurements as indicators for sensory color prediction of milk-based beverages.

Across All Fat Levels

Instrumental Viscosity. Variation in fat concentration in the pasteurized milk protein beverages in the current study explained <3% of the observed differences in instrumental viscosity (Table 7). The effect of temperature of instrumental viscosity measurement, variation in true protein concentration, and CNTPT explained 67.33, 14.68, and 8.00% of the total variation of instrumental viscosity for the pasteurized beverages across fat levels, with 90.01% of the total variation explained by the model (Table 7). Viscosity of liquids (including water) increases with decreasing temperature (Figura and Teixeira, 2007). Instrumental viscosity of pasteurized milk-based beverages was higher at 4 than at 20°C (Figure 3), as expected. When solutes and colloidal particles are dispersed in water (as in milk), temperature-induced viscosity changes may be different from those in pure water. Increased concentration of true protein had a larger effect on instrumental viscos-

Table 7. Pasteurized milk protein beverages: relative percentage of type III sum of squares (for factors with $P < 0.05$) explained by model factors[1] for instrumental viscosity; trained sensory panel intensity scores for mouth coating, throat cling, and astringency; and particle size [d(0.9); measured at 40°C]

Factor	Instrumental viscosity	Mouth coating	Throat cling	Astringency	d(0.9)
Fat	2.87	66.56	32.43	4.52	76.07
Prot	14.68	12.94	38.74	42.74	9.26
Temp	67.33	0.02[NS2]	NS	0.55[NS]	—
Rep	0.06	0.39[NS]	2.48	0.35[NS]	0.25
Fat × prot	NS	2.40	2.33	21.19	0.95
Fat × temp	0.77	0.14[NS]	0.19	0.30[NS]	—
Fat × rep	NS	0.83	3.10	1.49	0.25[NS]
Prot × temp	1.67	0.20	0.57	0.39	—
Prot × rep	0.10	NS	0.52	0.50	—
Temp × rep	NS	0.26	NS	1.14	—
Fat × prot × temp	NS	0.63	0.71	1.30	—
Fat × prot × rep	0.41	0.52	1.15	0.93	0.25
Fat × temp × rep	0.06	0.82	1.78	2.83	—
Prot × temp × rep	NS	0.31	NS	NS	—
Fat × prot × temp × rep	0.08[NS]	0.76	0.55	1.51	—
CNTPT	8.00	3.52	3.33	NS	9.24
CNTPT × fat	0.30	2.67	5.77	9.21	1.98
CNTPT × prot	0.72	1.49	0.77	1.48	—
CNTPT × temp	2.59	0.16	0.33	NS	—
CNTPT × rep	NS	NS	0.14	NS	—
CNTPT × fat × prot	NS	0.41	0.43	0.67	0.58
CNTPT × fat × temp	0.06	1.98	1.14	1.13	—
CNTPT × fat × rep	0.00	0.52	0.31	0.56	—
CNTPT × prot × temp	0.16	0.33	0.70	NS	—
CNTPT × temp × rep	NS	NS	0.20	NS	—
CNTPT × prot × rep	NS	NS	0.40	1.08	—
CNTPT × CNTPT	0.14	0.09[NS]	0.03[NS]	0.10[NS]	0.28
CNTPT × CNTPT × fat	NS	0.87	0.78	0.55	0.90
CNTPT × CNTPT × prot	NS	NS	NS	1.84	—
CNTPT × CNTPT × fat × prot	NS	0.62	0.57	2.52	—
CNTPT × CNTPT × fat × temp	NS	NS	0.18	NS	—
CNTPT × CNTPT × fat × rep	NS	0.23	NS	0.77	—
CNTPT × CNTPT × prot × temp	NS	0.25	0.35	NS	—
CNTPT × CNTPT × temp × rep	NS	0.11	NS	0.33	—
Sum (%)	100.00	100.00	100.00	100.00	100.00
R^2	0.98	0.82	0.77	0.65	0.99
N	480	2,880	2,880	2,880	120

[1]Fat = fat level with 0.2, 1, and 2%; Prot = milk true protein with 3.00, 3.67, 4.34, and 5.00%; Temp = temperature of sensory appearance evaluation with 4°C and 20°C; Rep = replicate; CNTPT = casein as a percentage of true protein with 5, 25, 50, 75, and 80% transformed.

[2]Not significant ($P > 0.05$).

ity than increased CN%TP (Figure 3) and the impact of protein concentration on viscosity was greater at 4 than at 20°C.

Sensory Mouth Coating and Sensory Throat Cling. Unlike instrumental viscosity, observed differences in mouth coating and throat cling of the milk protein beverages were not greatly influenced by temperature (4 vs. 20°C) of sensory evaluation (Table 7). Fat, TP, and CN%TP explained 66.56, 12.94, and 3.52% of the total variation in mouth coating across fat levels (Table 7). True protein, fat, and CN%TP contributed to 38.74, 32.43, and 3.33% of the total variation of throat cling across fat levels (Table 7). A higher fat concentration produced more mouth coating and throat cling (Table7, Figure 4A and B). A higher true protein concentration in the beverages also produced more mouth coating and more throat cling (Figure 4A and B), whereas the effect of CN%TP was much smaller (Table 7). Thus, both higher fat and protein contribute to mouth coating and throat cling, but fat explained more of variation in mouth coating, whereas protein explained more of the variation in throat cling. Misawa et al. (2016) also reported that increased CN%TP and TP increased both mouth coating and throat cling ($P < 0.05$) for 1% and 2% fat pasteurized beverages.

Sensory Astringency. The sensory astringency of tartaric acid, tannic acid, and alum produces a mouth-drying sensation (Lee and Lawless, 1991). Sensory astringency values of our HTST beverages ranged from 2 to 2.75 on a 0- to 15-point scale (data not shown). More severe heat treatments (such as UP) increased the astringency of both skim and 2% fat milks from a score of 1.8 to 2.5 on the same 0- to 15-point scale (Lee et al., 2017). Astringency increased from scores of about 2.2 to 2.75 with increased true protein concentration (from 3 to 5% true protein) for beverages at all fat levels (data not shown), with variation in protein concentration explaining about 43% and a fat × prot interaction explaining 21% of the variation in sensory astringency (Table 7) .

Particle Size. Laser light scattering particle volume distribution size analysis results were expressed as d(0.9) (in μm) and reflect the fat globule diameter below which 90% of the volume of fat is contained. Larger fat droplets are likely to contribute more to the mouthfeel of milk. Jackson and Brunner (1960) found that casein was associated with the milk fat globule membrane after milk was homogenized. In the present study, as fat concentration in the milk protein beverages increased, d(0.9) increased (Figure 5). Fat concentration alone explained 76% of the variation in particle size in the beverages (Table 7), but both protein concentration and CN%TP also affected ($P < 0.05$) particle size. Higher TP concentration and higher CN%TP decreased particle size for all fat levels (Figure 5), but the magnitude of the effect of change in TP and CN%TP on d(0.9) increased as fat content decreased ($P < 0.05$, fat × prot and CNTPT × fat interactions in Table 7, Figure 5). Misawa et al. (2016) reported similar results for the effect of fat, protein, and CN%TP on fat globule size in milk. Casein had stronger emulsifying capability than serum protein [i.e., d(0.9) increased

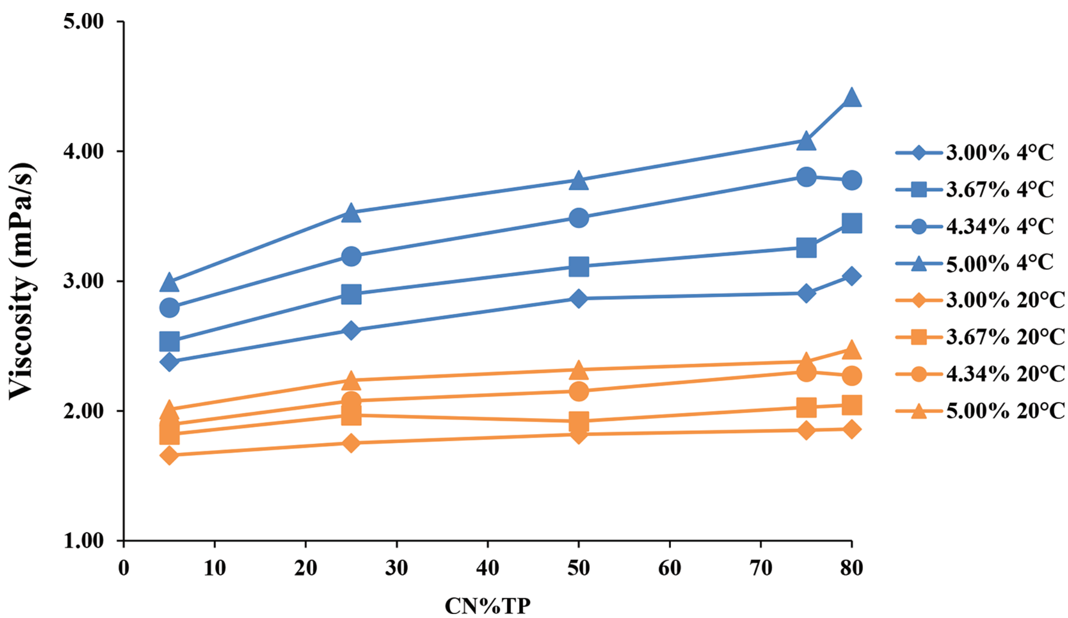

Figure 3. Instrumental viscosity of pasteurized (past) milk protein beverages assessed at different temperatures (4°C and 20°C) at different true protein levels (3.00, 3.67, 4.34, and 5.00%) as a function of casein as a percentage of true protein (CN%TP).

as CN%TP decreased; Figure 5], and homogenization helped to deposit more casein micelles on the interface of milk fat and the serum phase of the beverages, and decreased d(0.9) ($P < 0.05$, fat × prot and CNTPT × fat interactions in Table 7).

Sensory Flavor

Aroma Intensity, Sweet Aromatic, Cooked/Sulfur, and Cardboard/Doughy Flavors. Overall, flavor intensities of beverages were low, with all mean scores <2.2 on a 15-point scale. These flavor intensities are consistent with previous studies on fluid milk and whey proteins (Wright et al., 2009; Oltman et al., 2015; Lee et al., 2017; McCarthy et al., 2017). Dairy flavors generally fall between 0 and 4 on this sensory intensity scale (Drake, 2007; Schiano et al., 2017). All scores for these flavor descriptors decreased ($P < 0.05$) with increased CN%TP (Figure 6A, B, C, and D), whereas differences in fat and protein concentration had little effect on these sensory scores (Table 8 and Figure 6). Variation in CNTPT, CNTPT × fat, and fat explained 63.40, 17.05, and 2.71% of the total variation of aroma intensity for the pasteurized milk protein beverages, respectively, across fat levels with a R^2 of 0.68, whereas variation in CNTPT (71.31%), CNTPT × fat (12.78%), and fat (3.77%) explained most of the variation in sweet aromatic flavor across fat levels, with a R^2 value of 0.61 (Table 8). Higher sweet aromatic flavor may be related to Maillard reactions and lactose degradation compounds, and further work is needed to measure Maillard reaction products in these milk-based beverages. Increases in CN%TP decreased aroma intensity, and sweet aromatic flavor may be due to Maillard reaction–related compounds bonding to casein micelles, resulting in a cleaner, less intense flavor profile for neutral pH milk-based beverages. The linear and quadratic effects of CNTPT explained 83.89 and 7.00% of the total variation of cooked/sulfur flavor for pasteurized milk protein beverages, respectively, across fat levels, with 90.89% of the total variation explained by these terms in the ANOVA model (Table 8). Cooked/sulfur flavors increased as CN%TP decreased (i.e., when SP concentration increased). Serum proteins, particularly β-LG, have been reported as the source of cooked/sulfur flavor in milks due to the presence of sulfur-containing amino acids (such as cysteine), which is the precursor of hydrogen sulfide and carbon disulfide (Hutton and Patton, 1952; Al-Attabi et al., 2009).

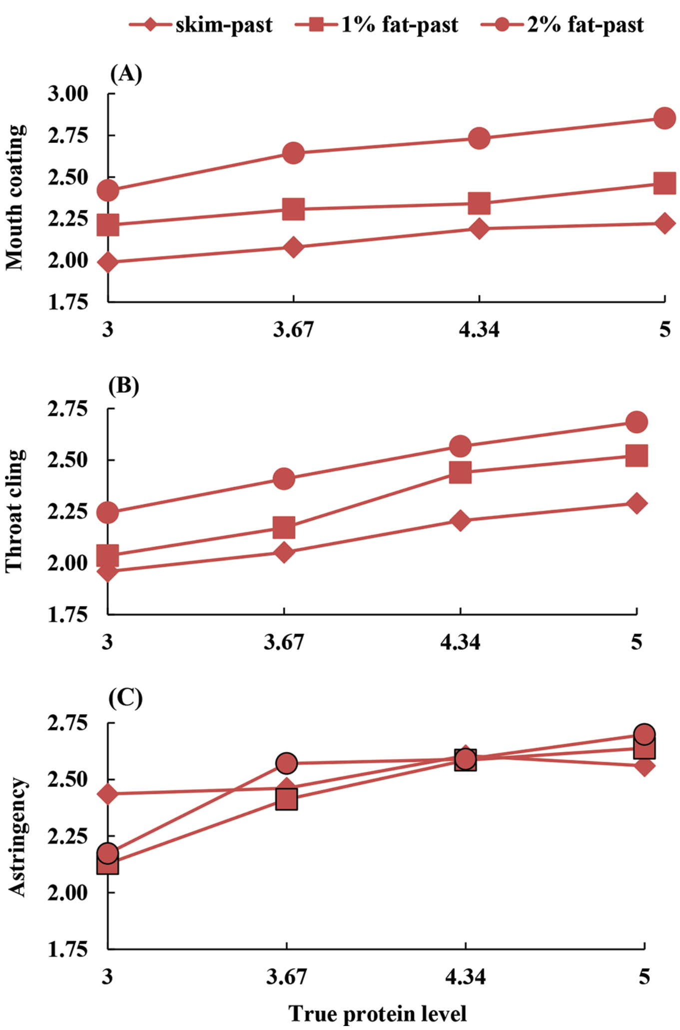

Figure 4. Trained panel sensory intensity scores for pasteurized (past) milk protein beverages: (A) sensory mouth coating, (B) sensory throat cling, and (C) sensory astringency with different fat levels (skim, 1% fat, and 2% fat) as a function of true protein.

Cardboard/doughy flavor increased ($P < 0.05$) when milk SP concentration and proportion of SP increased (i.e., as CN%TP decreased; Figure 6D). Cardboard flavor has been more frequently studied in dried whey protein products and has been sourced to volatile lipid oxidation products (Drake et al., 2003; Wright et al., 2009; Whitson et al., 2010). In the present study, all of the milk protein ingredients were liquid concentrates isolated directly from milk and not cheese whey. Variation in CNTPT (79.17%), prot (4.20%), CNTPT × prot (3.36%) explained about 86.73% of the total variation explained by the model for cardboard/doughy flavor intensity in the pasteurized milk protein beverages across fat levels in the present study (Table 8).

Cooked/Milky. Increasing fat level from 0.2% (skim) to 1% produced a large increase ($P < 0.05$) in cooked/milky flavor intensity but there was little increase from 1% to 2% fat (Figure 7A) in pasteurized milk protein beverages. Increasing CN%TP also caused cooked/milky flavor intensity to increase ($P < 0.05$) by about the same amount within each fat level (Figure 7A). Variation in fat (55.59%), CNTPT (21.89%), and

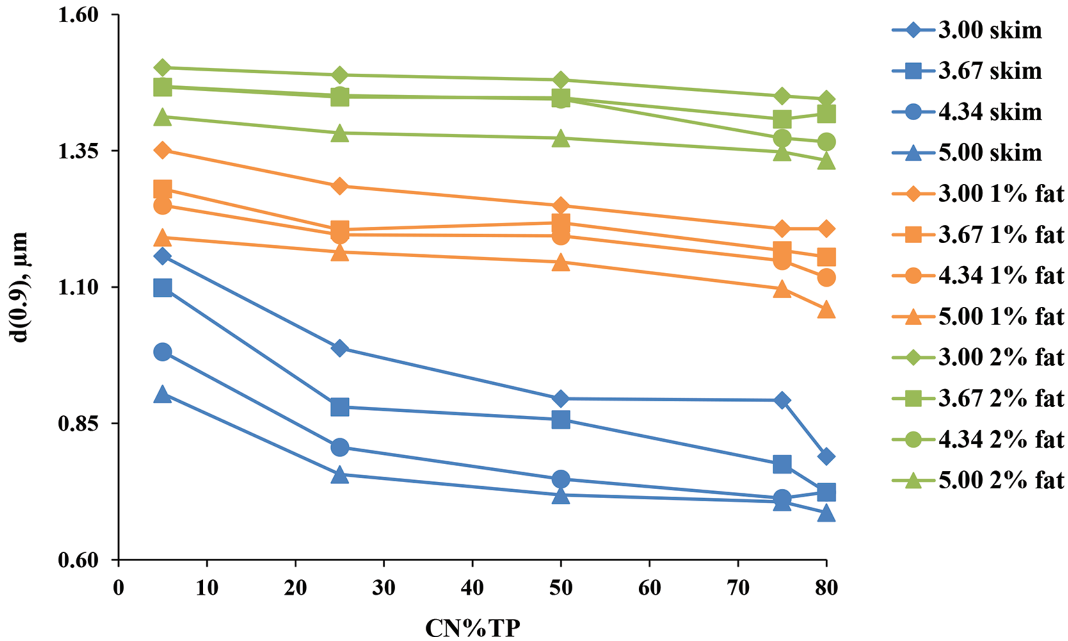

Figure 5. Particle size [d(0.9; µm] for pasteurized milk protein beverages with different true protein levels (3.00, 3.67, 4.34, and 5.00%) as a function of casein as a percentage of true protein (CN%TP) at different fat levels (skim, 1% fat, and 2% fat).

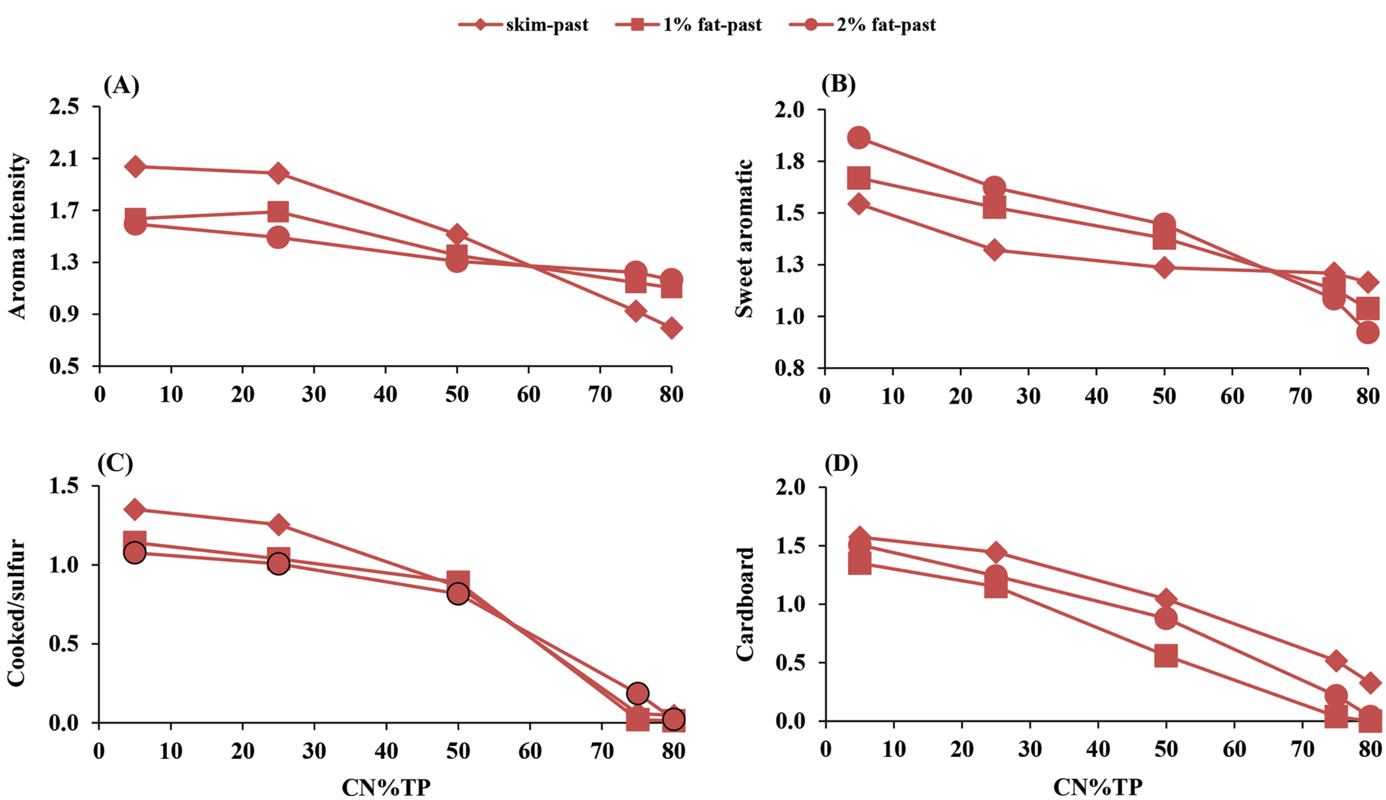

Figure 6. Trained panel sensory intensity scores: (A) aroma intensity, (B) sweet aromatic, (C) cooked/sulfur, and (D) cardboard flavors for pasteurized (past) milk protein beverages with different fat levels (skim, 1% fat, and 2% fat) as a function of casein as a percentage of true protein (CN%TP).

Table 8. Pasteurized milk protein beverages: relative percentage of type III sum of squares (for factors with $P < 0.05$) explained by model factors[1] for trained panel flavor intensity sensory scores

Factor	Aroma intensity	Sweet aromatic	Cooked/ milky	Cooked/ sulfur	Cardboard/ doughy	Milkfat
Fat	2.71	3.77	55.59	0.13	2.94	94.68
Prot	0.92	0.56[NS2]	7.13	0.70	4.20	0.15[NS]
Rep	1.04	0.16[NS]	0.44[NS]	NS	NS	0.85
Fat × prot	2.58	2.26	4.51	1.01	2.53	NS
Fat × rep	0.92	0.53	NS	0.24	0.04	1.92
Prot × rep	NS	0.42	0.72	NS	0.10	NS
Fat × prot × rep	0.45	1.09	1.37	0.11[NS]	0.18	0.43
CNTPT	63.40	71.31	21.89	83.89	79.17	0.01[NS]
CNTPT × fat	17.05	12.78	0.42	0.92	0.26	0.22
CNTPT × prot	1.56	0.88	1.92	NS	3.36	0.46
CNTPT × rep	0.88	0.19	0.49	NS	NS	0.17
CNTPT × fat × prot	0.97	0.78	1.56	5.38	2.95	NS
CNTPT × fat × rep	0.67	0.23	1.28	0.15	NS	0.20
CNTPT × prot × rep	0.75	NS	0.54	NS	0.10	0.10
CNTPT × CNTPT	1.68	0.07	0.07[NS]	7.00	1.49	NS
CNTPT × CNTPT × fat	1.63	1.97	NS	NS	0.20	0.53
CNTPT × CNTPT × prot	0.89	NS	0.40	0.16	0.09	NS
CNTPT × CNTPT × rep	NS	NS	0.45	NS	NS	0.09
CNTPT × CNTPT × fat × prot	1.90	2.27[NS]	NS	0.15	2.40	NS
CNTPT × CNTPT × fat × rep	NS	0.25[NS]	0.44	0.15	NS	NS
CNTPT × CNTPT × prot × rep	NS	0.49[NS]	0.77	NS	NS	0.18
Sum (%)	100.00	100.00	100.00	100.00	100.00	100.00
R^2	0.68	0.61	0.80	0.83	0.86	0.93
N	1,920	1,920	1,920	1,920	1,920	1,920

[1]Fat = fat level with 0.2, 1, and 2%; Prot = milk true protein with 3.00, 3.67, 4.34, and 5.00%; Rep = replicate; CNTPT = casein as a percentage of true protein with 5, 25, 50, 75, and 80% transformed.

[2]Not significant ($P > 0.05$).

prot (7.13%) explained, in total, 84.61% of the total variation of cooked/milky flavor for pasteurized milk protein beverages across fat levels (Table 8).

Milkfat. The variation in fat concentration explained 94.68% of the total variation of milkfat flavor for the pasteurized milk protein beverages (Table 8), whereas no effect of variation in CN%TP on milkfat flavor (Figure 7B) was detected ($P > 0.05$). Increasing fat increased milkfat flavor (Figure 7B). Higher milkfat flavor intensity could be due to higher fat content and associated higher concentration of lactones, which contribute to milkfat flavor (Drake et al., 2010).

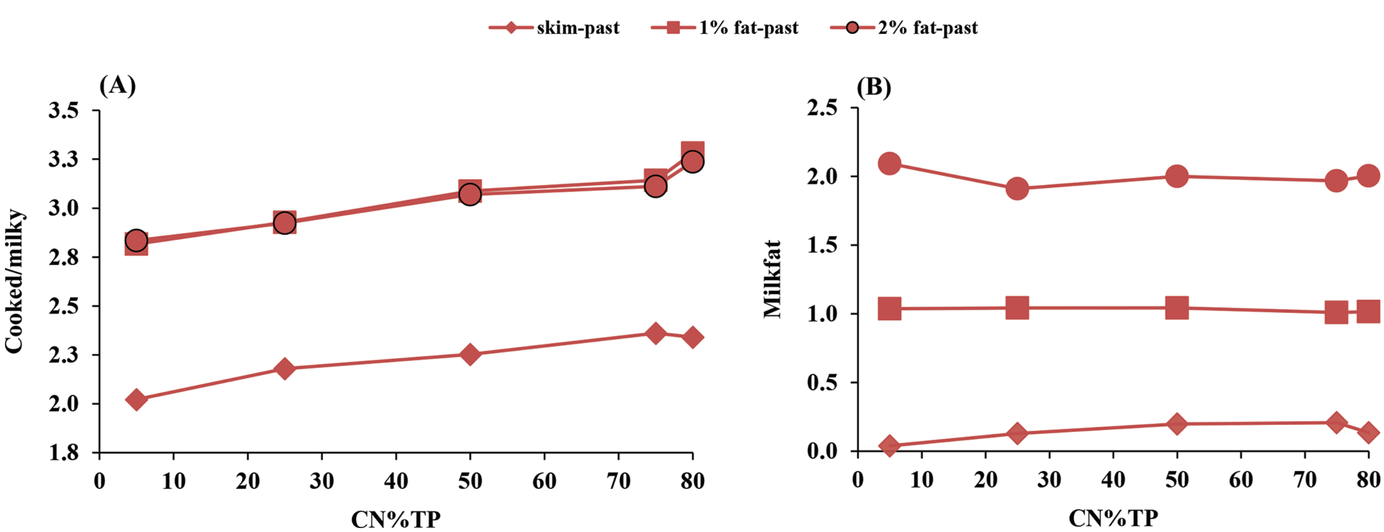

Figure 7. Trained panel sensory intensity scores: (A) cooked/milky and (B) milkfat flavors for pasteurized (past) milk protein beverages with different fat levels (skim, 1% fat, and 2% fat) as a function of casein as a percentage of true protein (CN%TP).

Biplot Analysis

Before the biplot analysis, the instrumental and sensory measures for color, texture, and flavor were reviewed and sorted. First, all the instrument and sensory metrics from Tables 1, 2, 5, and 6 for the ANOVA across all fat levels that had an R^2 >0.6 were identified and split into 2 groupings. One group included the parameters where fat concentration explained the highest proportion of variation in the data and the other group was where CN%TP or TP concentration explained the highest proportion of variation in the data. The same sorting of measures by composition parameters that explained the percentage of total variation was done using a criterion of R^2 >0.50% for the ANOVA data for the analysis within each fat concentration (i.e., 0.2, 1.0, and 2.0%). Thus, the results of the ANOVA were used as a sorting tool to form the basic grouping of parameters in the biplot analysis of the data.

Biplots were used in the current study to provide a novel approach to visualize relationships among beverage composition variables and sensory outcomes. Vector biplots were used to overlay the vectors for the milk component (e.g., fat, TP, casein, and SP) concentrations as supplemental variables on the vector space for instrumental and sensory data vectors for the population of milk beverages for all fat levels. This allows a visualization of relationships of the variation in beverage composition parameters and the dependent sensory parameters. For example, if 2 composition parameters (e.g., fat and casein) both produce increased beverage whiteness, then a product developer has the opportunity to achieve the same visual whiteness perception with different product compositions and different ingredient choices or concentrations. Thus, ingredients that may minimize cost or maximize a particular nutritional attribute can be prioritized, while achieving the same perceived whiteness.

Vector Biplots: Within Fat Levels. Separate vector biplots are presented for skim (Figure 8), 1% (Figure 9), and 2% fat (Figure 10) beverages. Within each of the 3 fat levels, a higher concentration of SP drove higher aroma intensity, sweet aromatic, cooked/sulfur, and cardboard/doughy flavors, and sensory yellowness

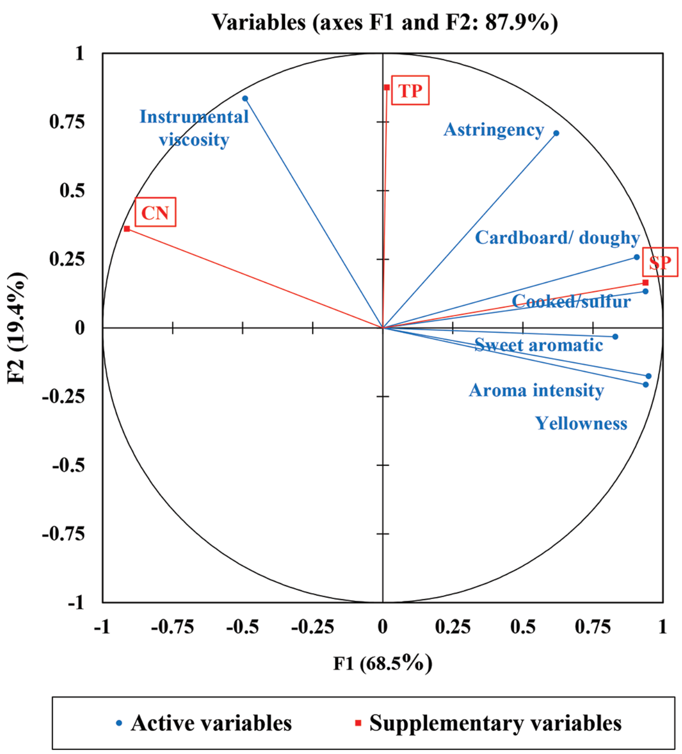

Figure 8. Principal component analysis biplot of skim milk pasteurized beverages for parameters where casein as a percentage of true protein contributed the most to the total variation of each parameter: instrumental viscosity, trained panel sensory yellowness (yellowness), astringency, sweet aromatic, aroma intensity, cooked/sulfur and cardboard/doughy with overlaying true protein (TP), casein protein (CN) and serum protein (SP) as supplementary variables.

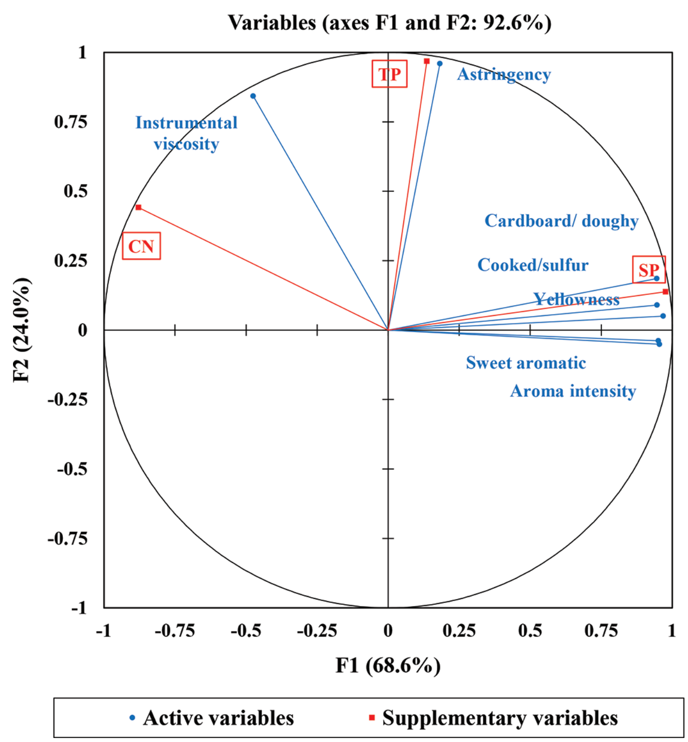

Figure 9. Principal component analysis biplot of 1% fat pasteurized milk protein beverages for parameters where casein as a percentage of true protein contributed the most to the total variation of each parameter: instrumental viscosity, trained panel sensory yellowness (yellowness), astringency, sweet aromatic, aroma intensity, cooked/sulfur and cardboard/doughy with overlaying true protein (TP), casein protein (CN) and serum protein (SP) as supplementary variables.

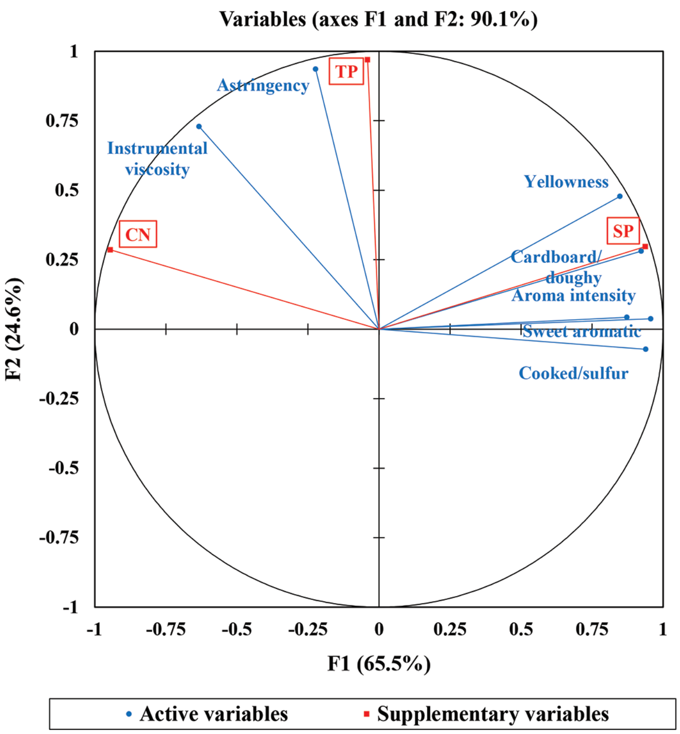

Figure 10. Principal component analysis biplot of 2% fat pasteurized milk protein beverages for parameters where casein as a percentage of true protein contributed the most to the total variation of each parameter: instrumental viscosity, trained panel intensity scores for sensory yellowness (yellowness), astringency, sweet aromatic, aroma intensity, cooked/sulfur and cardboard/doughy with overlaying true protein (TP), casein protein (CN) and serum protein (SP) as supplementary variables.

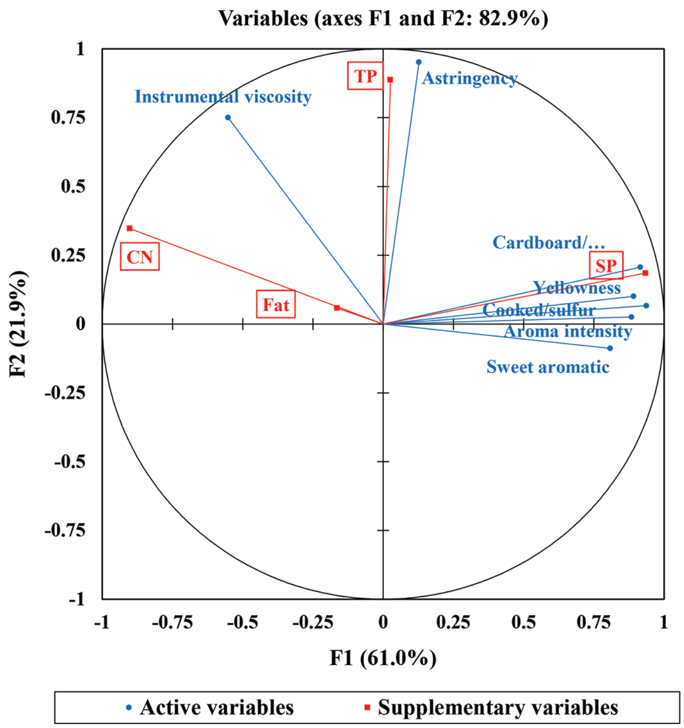

Figure 11. Principal component analysis biplot of pasteurized milk protein beverages at all fat levels for parameters where casein as a percentage of true protein contributed the most to the total variation of each parameter: instrumental viscosity, trained panel sensory yellowness (yellowness), astringency, sweet aromatic, aroma intensity, cooked/sulfur and cardboard/doughy with overlaying fat, true protein (TP), casein protein (CN) and serum protein (SP) as supplementary variables.

intensity scores, whereas a higher casein concentration drove higher instrumental viscosity. Formulating the beverages to higher TP levels, regardless of type of milk protein, increased the astringency within each fat level.

Vector Biplots: Among Fat Levels. Separate vector biplots are presented for the sensory parameters from all fat levels combined that were influenced the most based on the ANOVA (Tables 1, 2, 5, and 6) by casein and protein (Figure 11) and by fat (Figure 12). As expected, the effects of casein and SP concentrations on sensory parameters shown within fat levels (Figure 8, 9, 10) were similar and clear when data from all fat levels were combined (Figures 11, 12, 13). The parameters that were influenced the most (Tables 1, 2, 5, and 6) by protein type were instrumental viscosity, astringency, aroma intensity, cardboard/doughy, cooked/sulfur, and sweet aromatic flavors and yellowness (Figure 11). Instrumental viscosity was increased by increased casein and fat concentrations, whereas astringency was increased by TP concentration. All other parameters increased with increased milk SP concentration. The sensory parameters that were influenced the most (Tables 1, 2, 5 and 6) by difference in fat concentration (i.e., milkfat and cooked/milky flavors, throat cling, mouth coating, instrumental color L, a, and b* values, opacity, and whiteness) followed the direction of the fat supplemental factor in the vector biplot (Figure 12). In Figure 13, all sensory measures with an R^2 >0.6 are shown for all formulations to provide a complete view of the matrix of 60 formulated milk based beverages in the current study. Increasing fat concentration drove increased intensity scores for whiteness/color and mouthfeel parameters, whereas increasing SP concentration drove higher yellowness and heat-derived flavor intensity scores in the beverages. Higher casein concentration produced higher sensory whiteness, L values, and cooked/milky flavors and higher instrumental measures of viscosity.

CONCLUSIONS

Within each of the 3 fat levels, higher SP concentration produced higher aroma intensity, sweet aromatic, cooked/sulfur, and cardboard/doughy flavors,

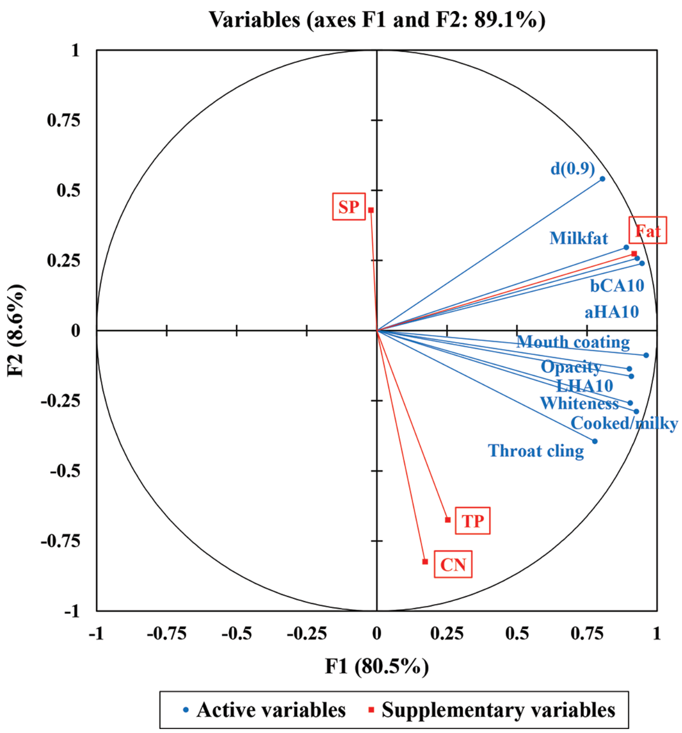

Figure 12. Principal component analysis biplot of pasteurized milk protein beverages at all fat levels for parameters where fat contributed the most to the total variation of each parameter: Hunter L (LHA10), a (aHA10) and CIE b* (bCA10) at 10° viewer angles using illuminate A, particle size [d(0.9)], trained panel sensory whiteness (whiteness), opacity, mouth coating, throat cling, cooked/milky and milkfat with overlaying fat, true protein (TP), casein protein (CN) and serum protein (SP) as supplementary variables.

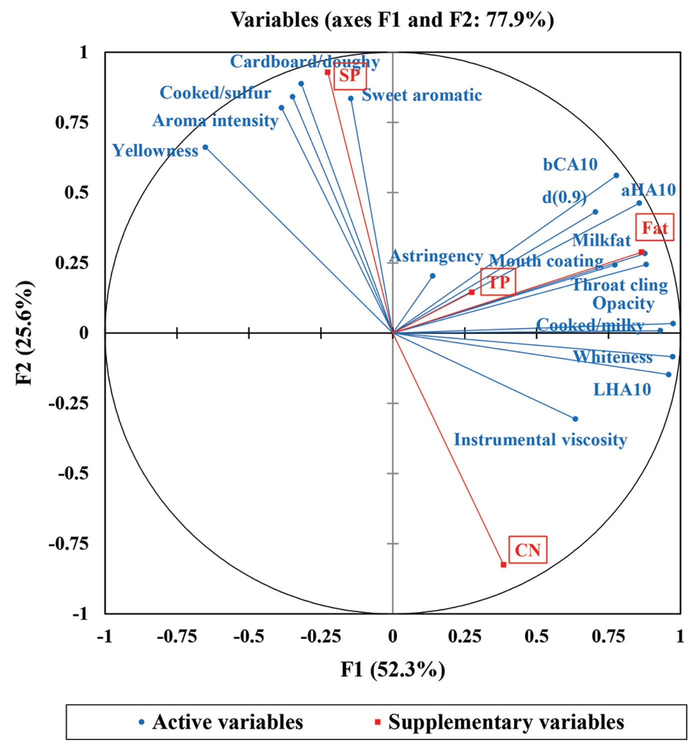

Figure 13. Principal component analysis biplot of pasteurized milk protein beverages at all fat levels and all sensory parameters for Hunter L (LHA10), a (aHA10), and CIE b* (bCA10) at 10° viewer angles using illuminate A, instrumental viscosity, particle size [d(0.9)], trained panel sensory whiteness (whiteness), opacity, yellowness, mouth coating, throat cling, astringency, sweet aromatic, aroma intensity, cooked/sulfur, cooked/milky, cardboard/doughy and milkfat with overlaying fat, true protein (TP), casein protein (CN) and serum protein (SP) as supplementary variables.

and sensory yellowness scores, whereas higher casein concentration produced higher instrumental viscosity in milk protein beverages. Increasing SP concentration increased yellowness, aroma intensity, sweet aromatic, cooked/sulfur, and cardboard/doughy flavors across all fat levels and also had the largest effect on L, a, and b* values, sensory whiteness, and opacity within each fat level. Increases in TP increased sensory intensity scores for throat cling and astringency. Increases in fat concentration were correlated with higher L, a, and b* values, larger particle size, increased sensory whiteness, mouth coating, and cooked/milky and milkfat flavors. Multiple linear regression of L, a, and b* values produced better predictions of sensory whiteness and yellowness of pasteurized milk protein beverages than simple linear regression of L or b* values, respectively. Formulating milk protein beverages to a higher TP level increased astringency regardless of fat level. When formulating milk protein beverages, a product developer has a wide range of milk-based protein ingredient choices that differ in price and change price relationship across time. Understanding the expected relative effect of different milk protein ingredients on the textural and flavor characteristics of milk-based beverages could be used to help guide product reformulation decisions and ingredient choices to achieve a specific sensory profile while controlling total beverage ingredient cost.

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