Flavor and stability of milk proteins

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ABSTRACT

A greater understanding of the nature and source of dried milk protein ingredient flavor(s) is required to characterize flavor stability and identify the sources of flavors. The objective of this study was to characterize the flavor and flavor chemistry of milk protein concentrates (MPC 70, 80, 85), isolates (MPI), acid and rennet caseins, and micellar casein concentrate (MCC) and to determine the effect of storage on flavor and functionality of milk protein concentrates using instrumental and sensory techniques. Spray-dried milk protein ingredients (MPC, MPI, caseins, MCC) were collected in duplicate from 5 commercial suppliers or manufactured at North Carolina State University. Powders were rehydrated and evaluated in duplicate by descriptive sensory analysis. Volatile compounds were extracted by solid phase microextraction followed by gas chromatography-mass spectrometry (GC-MS) and gas chromatography-olfactometry. Compounds were identified by comparison of retention indices, odor properties, and mass spectra against reference standards. A subset of samples was selected for further analysis using direct solvent extraction with solvent-assisted flavor extraction, and aroma extract dilution analysis. External standard curves were created to quantify select volatile compounds. Pilot plant manufactured MPC were stored at 3, 25, and 40°C (44% relative humidity). Solubility, furosine, sensory properties, and volatile compound analyses were performed at 0, 1, 3, 6, and 12 mo. Milk proteins and caseins were diverse in flavor and exhibited sweet aromatic and cooked/milky flavors as well as cardboard, brothy, tortilla, soapy, and fatty flavors. Key aroma active compounds in milk proteins and caseins were 2-aminoacetophenone, nonanal, 1-octen-3-one, dimethyl trisulfide, 2-acetyl-1-pyrroline, heptanal, methional, 1-hexen-3-one, hexanal, dimethyl disulfide, butanoic acid, and acetic acid. Stored milk

proteins developed animal and burnt sugar flavors over time. Solubility of MPC decreased and furosine concentration increased with storage time and temperature. Solubility of MPC 80 was reduced more than that of MPC 45, but time and temperature adversely affected solubility of both proteins, with storage temperature having the greatest effect. Flavor and shelf stability of milk proteins provide a foundation of knowledge to improve the flavor and shelf-life of milk proteins.

Key words: milk protein, flavor, shelf life

INTRODUCTION

Milk protein concentrates (MPC) and isolates (MPI) are a relatively new category of dried dairy ingredients. Milk protein concentrate is a complete dairy protein ingredient containing both caseins and whey proteins at a concentration ranging from 40 to 89%, whereas MPI contains a protein concentration of 90% or greater. Milk protein concentrates and isolates are manufactured by concentrating milk proteins (both whey proteins and caseins) from fluid skim milk by membrane filtration followed by spray drying. Globally, MPC production has quadrupled to over 164,000 t from 2000 to 2011 (USDEC, 2012). The United States is both the single largest user in the world and the single largest importer, and the US Dairy Export Council (USDEC) predicts significant growth through the end of the decade to satisfy rising demand. Micellar casein concentrate (MCC) is a product for which commercial production was initiated a few years ago so production data are not yet available. Micellar casein concentrate is produced by microfiltration of skim milk, followed by ultrafiltration to further concentrate proteins before spray drying. In the production of MCC, serum proteins are removed via microfiltration. In production of MPI, filtration is used to remove lactose from milk, but both casein and whey proteins are retained in their original proportions found in milk, without combining separately produced casein (caseinate) and whey proteins (ADPI, 2015). The flavor of these ingredients is an important quality parameter as ingredient flavor can influence consumer liking and acceptability of the finished product.

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Most of the research conducted on MPC, MCC, or caseins has been focused on functionality and very little research has been conducted on flavor or elucidating the compounds responsible for the characteristic flavors of these proteins. Functional characteristics such as solubility during storage (Le et al., 2011a), the effect of NaCl addition during diafiltration on solubility, hydrophobicity, and soluble protein composition (Mao et al., 2012; Sikand et al., 2013), change in protein structure upon oxidation (Dalsgaard et al., 2007), and the influence of the Maillard reaction on functionality (Le et al., 2011a; Thomas et al., 2004) have all been studied in recent years; however, the effects of low (45%) and high (80%) protein content, and low temperature storage (3°C) on solubility and flavor have not been investigated.

Rennet and acid casein have been described as having an animal/wet dog or gluey flavor and characteristic aroma components of rennet casein were 2-aminoacetophenone, hexanoic acid, indole, guaiacol, and *p*-cresol (Karagul-Yuceer et al., 2003). The sensory profiles of higher protein MPC (MPC 77, MPC 80, and MPI) were characterized by tortilla, brothy, cardboard, and animal flavors (Drake et al., 2009). The flavor of MCC has not been reported, and the flavor, flavor chemistry, and flavor stability of milk proteins have not been fully investigated. The objectives of this study were to characterize the flavor and flavor chemistry of milk protein concentrates (MPC 70, 80, 85), isolates (MPI), acid and rennet caseins, and micellar casein concentrate (MCC; experiment 1), and to determine and compare the effects of storage temperature and time on the sensory and functionality (shelf-life) of low (45%) and high (85%) protein MPC over an extended period (1 yr; experiment 2) using instrumental and sensory techniques.

MATERIALS AND METHODS

Experimental Overview

Two experiments (experiments 1 and 2) were included in this study. The purpose of experiment 1 was to determine the sensory profiles and key aroma active volatile compounds in milk proteins. To carry out this objective, 9 commercial milk protein products were collected and one manufactured in the North Carolina State University (NCSU) dairy pilot plant. Products were collected or manufactured in duplicate. Descriptive analysis, headspace extraction and quantification of volatile compounds by GC-MS and gas chromatography-olfactometry (GC-O), and solvent-assisted flavor evaporation (SAFE) followed by aroma extract dilution analysis (AEDA) were performed on the dried milk protein products. The purpose of experiment 2

Table 1. Commercial proteins and manufacturers¹

Manufacturer	Protein type
A	Rennet casein
A	Acid casein
B	MPC 70
B	MPC 85
B	MPI
C	MCC
D	MPC 80
E	MPC 80
E	MPC 85
P	MPC 45
P	MPC 80

¹Two lots of each protein were obtained from each supplier. MPC = milk protein concentrate; MCC = micellar casein concentrate; MPI = milk protein isolate. Letters A–E are commercial suppliers; P means manufactured in the North Carolina State University pilot plant.

was to determine the flavor and functional properties of milk protein concentrates across storage. To conduct this objective, MPC 45 and 80 were produced in the NCSU dairy pilot plant and stored at constant relative humidity (44%) at 3, 25, or 40°C. Samples were measured after 0, 1, 3, 6, and 12 mo. Descriptive analysis, furosine content, solubility, and flavor volatiles by GC-MS were measured on all samples at each time point.

Sample Collection

Commercial Samples and Chemical Standards.

Nine different products were obtained in duplicate lots from 5 different manufacturers across the United States and Canada (Table 1). Upon arrival, samples were stored at –80°C until analysis. Chemical standards were obtained from Sigma-Aldrich (Milwaukee, WI) and ChemStep (Montesquieu, France).

Pilot Plant Production of MPC. Raw skim milk was obtained from the North Carolina State University Dairy Research and Education Unit on 2 different occasions. The milk was HTST pasteurized (720 kg/h; hold time 15 s at 72°C) using a plate heat exchanger (model T4 RGS-16/2, SPX Flow Technology, Greensboro, NC). The milk was ultrafiltered and diafiltered using a laboratory scale ultrafiltration unit (model Lab 46, Filtration Engineering, Champlin, MN) equipped with 4 spiral wound membranes (Snyder Filtration, Vacaville, CA; nominal cutoff: 10,000 Da; surface area 5.0 m²/membrane). The temperature for ultrafiltration was 50°C. For the MPC 45, the skim milk was ultrafiltered and protein monitored by Sprint Rapid Protein Analyzer (CEM, Matthews, NC) and Smart System 5 moisture/solids analyzer (CEM) until 45% protein (wt/wt) was achieved. Total filtration time was approximately 25 min. Total solids before spray drying were approximately 10%. For MPC 80, diafiltration

water was added at a rate of 40% of the original weight of the milk. Total filtration time was approximately 3 h. Once the MPC reached 45 or 80% protein (wt/wt), the MPC retentate was then spray dried (nozzle atomization, model Lab 1, Anhydro Inc., Soeborg, Denmark). Inlet temperature was 200°C and the outlet temperature was 90°C. The MPC 80 was then weighed and separated into lots for flavor analysis or storage. The MPC 80 used in the flavor study were collected and stored in Mylar bags (Uline, Pleasant Prairie, WI) at -80°C following production. The MPC 45 and 80 used in the storage study were weighed, and sealed in laminated poly-nylon vacuum pouches (30NV0608, Elkay Plastics, Austell, GA) at a relative humidity of 44%, boxed (to protect from light exposure), and stored in temperature-controlled incubators at predetermined temperatures (3, 25, or 40°C). Each pouch contained approximately 2 kg of powder such that a new pouch was opened and evaluated at each time point.

Headspace Extraction of Volatile Compounds

GC-MS. Volatile compounds in caseins, MPC/MPI, and MCC powders were extracted by solid-phase microextraction (SPME) and then separated and identified by GC-MS with selective ion monitoring using a modified method of Liaw et al. (2010). Volatile compounds for selective ion monitoring were selected from previous literature (Carunchia Whetstone et al., 2003; Karagul-Yuceer et al., 2003; Drake et al., 2009; Liaw et al., 2011; Campbell et al., 2013), GC-O (described below), and by evaluation of chromatograms collected in scan mode. Dried powders were reconstituted at 10% solids (wt/vol). All samples contained 10% (wt/vol) sodium chloride (Fisher Scientific), and 10 μ L of internal standard solution (2-methyl-3-heptanone in methanol at 81 mg/kg (Sigma-Aldrich) in 20 mL autosampler vials with steel screw tops containing silicone septa faced in Teflon (Microliter Analytical, Suwanee, GA). Samples were injected using a CombiPal autosampler (CTC Analytics, Zwingen, Switzerland) attached to an Agilent 7820A GC with 5975B MSD (Agilent Technologies Inc., Santa Clara, CA). Samples were maintained at 5°C before fiber exposure. Samples were equilibrated at 40°C for 25 min before 30 min fiber exposure of a 1 cm of [divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS); Supelco Inc.] fiber (Supelco, Bellefonte, PA) at 31 mm with 4 s of pulsed agitation at 250 rpm. Fibers were injected for 5 min at a depth of 50 mm. Each sample from each lot was evaluated in triplicate.

The GC method used an initial temperature of 40°C for 3 min with a ramp rate of 10°C/min to 250°C held for 5 min. The SPME fibers were introduced into the

split/splitless injector at 250°C. A ZB-5ms column (ZB-5ms 30 m length $\times$ 0.25 mm i.d. $\times$ 0.25 μ m film thickness; Phenomenex, Torrance, CA) was used for all analyses at a constant flow rate of 1 mL/min. Purge time was set at 1 min. The MS transfer line was maintained at 250°C with the quad at 150°C and source at 250°C. Compounds were identified using the NIST 2005 library of spectra and comparison of spectra of authentic standards injected under identical conditions. Relative abundance for each compound was calculated using the calculated recovery of the internal standard concentration to determine relative concentrations of each compound. Retention indices were calculated using an alkane series (Sigma Aldrich; Vandendool and Kratz, 1963).

GC-O. Solid-phase microextraction followed by GC-O for characterization of aroma active compounds was conducted on all caseins, MPC/MPI (time 0 only), and MCC. Twenty milliliters of each sample (10% solids wt/vol) along with 10% sodium chloride (wt/wt) was dispersed into six 40-mL amber vials (28 $\times$ 98 mm; Supelco Inc.) with a polytetrafluoroethylene/silicone septum (Supelco Inc.) and a stir bar. The vials were heated at 40°C for 20 min with constant stirring. A SPME fiber (DVB/CAR/PDMS, Supelco Inc.) was exposed in each sample at 1 cm for 1 h. The fiber was then injected on an Agilent 6850 gas chromatography-flame-ionization detector (FID) equipped with an olfactometer port (Agilent Technologies Inc.). The GC method used an initial temperature of 40°C for 3 min. The temperature was then increased at a rate of 10°C/min to 150°C, followed by 30°C/min to 200°C, and held for 5 min. Samples were evaluated in duplicate on 2 different columns: polar ZB-WAX (30-m length $\times$ 0.25-mm i.d. $\times$ 0.25- μ m film thickness; Zebron; Phenomenex Inc.) and a nonpolar ZB-5ms (30-m length $\times$ 0.25-mm i.d. $\times$ 0.25- μ m film thickness; Zebron; Phenomenex Inc.). Effluent was split 1:1 between the FID and sniffing port using deactivated fused-silica capillaries (1-m length $\times$ 0.25-mm i.d.; Phenomenex Inc.). The FID sniffing port was held at a temperature of 300°C, with a helium carrier gas flow of 1,018.6 cm/s, and the port was supplied with humidified air at 30 mL/min. Each sample was evaluated on each column by at least 2 highly experienced sniffers (each with >50 h previous experience with GC-O) who recorded aroma character and perceived intensity.

Solvent Extraction of Volatile Compounds

Direct Solvent Extraction. A representative subset of milk proteins (n = 8; manufacturer A: rennet casein, acid casein, manufacturer B: MPC 85, MPI, manufacturer C: MCC, manufacturer D: MPC 80, manufacturer

E: MPC 80, MPC 85, duplicate lots) was selected based on sensory and SPME results for further analysis via direct solvent extraction with SAFE. Direct solvent extraction was conducted on powders following the methods of Evans et al. (2009). Direct solvent extraction was performed in duplicate on each selected sample. One hundred grams of powder was divided among two 250-mL Teflon bottles with Tefzel closures (Nalgene, Rochester, NY). Thirty milliliters of water was added to each bottle and was allowed to rehydrate for 30 min. Then, 100 mL of diethyl ether (EMD Chemicals Inc., Gibbstown, NJ) was added to the powder/water mixture along with 20 μ L of internal standard at 81 mg/kg each (2-methyl-3-heptanone, 2-methyl-pentanoic acid in ether, Sigma Aldrich). The bottles were agitated using a Roto mix (Tyle 50800, Thermolyne, Dubuque, IA) on high speed for 30 min. The solvent phase was separated from the mixture by low-speed centrifugation ($1,450 \times g$) for 10 min at 10°C. After centrifugation, the ether phase containing the volatile components was collected into an amber glass jar with a Teflon-lined lid. Diethyl ether addition, agitation, and centrifugation were repeated 3 more times each with 50 mL of diethyl ether added to each bottle each time. The extracts were then concentrated under a stream of nitrogen gas to 200 mL. Solvent extracts were stored at -20°C .

SAFE. Solvent extracts were distilled by SAFE (Ace Glassware, Vineland, NJ). The assembly was similar to the methods of Evans et al. (2009) and Whitson et al. (2011). The glass SAFE head, with the 2-L round-bottom flask attached, was connected to 2 glass traps. The primary trap for receiving the final distilled solvent extract and the secondary trap for waste were both submerged in liquid nitrogen via separate Dewar flasks. The round bottom flask was kept at 50°C using a water bath. Vacuum was reached in the glassware using a rough pump/diffusion pump combination. The solvent extract was poured into the top of the SAFE head and introduced into the vacuum system drop-wise until all of the sample was introduced. Distillation was carried out for 10 min under vacuum (1.33 kPa) and kept at 50°C with a circulating water bath. After distillation, the sample was concentrated under a stream of nitrogen gas to 20 mL and transferred to a 50-mL screw-top glass tube for phase separation.

Phase Separation. To separate solution into neutral/basic and acid fractions, the concentrated distillate was first washed twice with 3 mL of sodium bicarbonate (0.5 M; Fisher Scientific) and mixed vigorously. The concentrate was washed a total of 3 times, and after each wash, the bottom layer (water phase) was placed into another glass tube. The resulting concentrate was the neutral/basic fraction and was dried over anhydrous sodium sulfate (VWR International) to remove

any residual water. The neutral/basic fraction was then concentrated to 0.5 mL under a stream of nitrogen gas. The water phase was acidified by adding hydrochloric acid (18% wt/vol; Sigma Aldrich) until a pH of 2 to 2.5 was reached. Acidic volatiles were then extracted by the addition of 5 mL of diethyl ether, mixing vigorously, and removing the top ether phase. This process was repeated 2 more times. The acidic volatiles were then dried over anhydrous sodium sulfate and concentrated to 0.5 mL under a stream of nitrogen gas.

Volatile Analysis of Solvent Extracts

GC-MS. Selected volatile compounds in solvent extraction fractions were detected using both scan and selective ion monitoring simultaneously. Analysis was conducted on an Agilent 7890B with a 5977A mass selector detection (Agilent Technologies Inc.). Separations were performed on a fused silica capillary column Zb-5ms 30 m length $\times$ 0.25 mm i.d. $\times$ 0.25 μ m film thickness; Phenomenex Inc.). Helium was used as the carrier gas at a constant flow of 1.5 mL/min. The GC-MS oven program started with an initial oven temperature of 40°C for 3 min, increasing to 90°C at a rate of 10°C per min, then increasing 5°C per min to 200°C, then 20°C per min to 250°C hold for 5 min and a final hold time of 10 min. The MSD conditions included capillary direct interface temperature, 280°C; source temperature 230°C, quad temperature 150°C. The injector temperature was 250°C.

One microliter of each solvent extract was injected in duplicate in the pulsed splitless (172.4 kPa air for 0.5 min) mode. A 3.75-min solvent delay was programmed into the MS acquisition parameters for solvent injections. Retention indices were calculated using an alkane series (Sigma Aldrich; Vandendool and Kratz, 1963).

GC-O with AEDA. One microliter of solvent extract was injected into the GC injection port, maintained at 250°C, fitted with a direct injection liner (Supelco; Sigma Aldrich). Column conditions and GC-O parameters were identical to those described previously for SPME. Both neutral/basic and acidic fractions from solvent extracts were analyzed in duplicate on both columns. Postpeak intensity of aroma active compounds were evaluated and recorded by 2 experienced sniffers, each with >100 h experience with GC-O of dairy proteins.

The AEDA was conducted on solvent extracts under similar conditions as those used for postpeak intensity GC-O. A step-wise dilution of 1:3 (vol/vol) with diethyl ether was followed until 2 experienced sniffers detected no odorants. The neutral/basic fractions were injected onto the nonpolar Zb-5ms capillary column and acid fractions were injected onto the polar ZB-WAX capil-

lary column. The highest dilution, for which an aroma was still detected, was reported as the flavor dilution factor.

Compound Identification and Quantitation

Mass spectra, retention index, and aroma properties of authentic standards injected under identical conditions on the GC-O and GC-MS were used for aroma active compound identification. Nineteen compounds, 9 of which were fatty acids, were selected for quantification using external standard curves. These compounds were selected due to their high $\log_3$ flavor dilution (FD) values, aroma character, and previous literature (Karagul-Yuceer et al., 2003; Drake et al., 2009). Due to retention times and the mandatory solvent delay on the GCMS after SAFE, 3 of these compounds [hexanal, 1-hexen-3-one, and dimethyl disulfide (DMDS)] were quantified via SPME. The remaining 16 compounds [2-acetylpyroline, 1-octen-3-one, 2-aminoacetophenone, heptanal, nonanal, dimethyl trisulfide (DMTS), methional, and butyric, pentanoic, hexanoic, heptanoic, octanoic, nonanoic, decanoic, dodecanoic, and tetradecanoic acids] were quantified by SAFE. Quantification of selected volatile compounds in SAFE extracts and SPME vials was executed using 5-point external standard curves ranging from 0.50 $\mu\text{g/kg}$ to 2 mg/kg (depending on selected compound) and integrated to an internal standard [81 mg/kg 2-methyl-3-heptanone, 2-methyl-pentanoic acid in ether (SAFE extracts) or water (SPME)]. For SAFE extracts, external standard curves were prepared by adding selected compounds to 30 mL of water, which was then added to 50 g of powder in a 250-mL Teflon bottles with Tefzel closures (Nalgene) and held at 21°C for 30 min. Direct solvent extraction, SAFE, and phase separation proceeded as previously described. Extracts were injected onto the GC-MS using previously described parameters. For SPME, the selected compounds were diluted in water and then added to the milk protein powder, prepared as described previously. Relative abundance of other selected compounds was calculated relative to internal standard recovery.

Descriptive Analysis

Descriptive sensory analysis was conducted on MPC/MPI, MCC, and caseins using a trained descriptive sensory panel and an established dairy flavor language (Drake et al., 2003, 2009). Panelists ($n = 8$) each had more than 50 h of training and 150 h of previous experience with the sensory analysis of dried dairy ingredients using the Spectrum descriptive analysis method (Mei-gaard et al., 2009). All sensory testing was conducted

in accordance with NCSU Institutional Review Board for Human Subjects guidelines.

Powders were rehydrated to 10% solids (wt/vol) with deionized water and dispensed into 3-digit-coded 60-mL lidded cups for evaluation (Solo Cup Company, Champaign, IL). Preparations were conducted with overhead lights off to avoid exposure to light. Samples were evaluated by each panelist in duplicate. Sensory data were collected using Compusense Five, release 4.8 (Compusense, Guelph, Canada).

Solubility Analysis

Solubility analysis was performed on MPC 45 and MPC 85 produced in the NCSU pilot plant at all storage time points. Solutions of 10% protein (wt/vol) were made by rehydrating the MPC to 80% of the total volume desired, for 6 h at room temperature on a stir plate set to 300 rpm (Campbell et al., 2011) and refrigerated overnight at 4°C. The solutions were then equilibrated to 25°C and the pH was adjusted using 1 N NaOH to a pH of 7 and brought to a total volume of 100 mL with deionized water, resulting in 10% (wt/vol) solids solutions. Total protein was measured on the uncentrifuged solutions. Solutions were then centrifuged at $16,500 \times g$ for 10 min at 10°C using a model RC5B centrifuge (Thermo Scientific) and the supernatants were again measured for protein concentration (solubility). Protein concentration was measured by the micro-BCA assay using a kit from Thermo Fisher Scientific/Pierce (Rockford, IL). Protein solutions before and after centrifugation were diluted 1:100 in deionized water. Solutions were added to working reagent in a ratio of 1:8 and pipetted in triplicate into a 96-well plastic plate. Deionized water with working solution was used as a reference blank. The plate was put on a shaker for 30 s and incubated at 37°C for 30 min. The plate was brought to room temperature and read on a Tecan Safire plate reader spectrophotometer at wavelength 562 nm (Tecan, Durham, NC). Analysis was performed in quadruplicate. Solubility was calculated using the following equation (where Abs = absorbance):

$$\text{protein solubility} = 100 - \{[(\text{Abs}_{\text{before}} - \text{Abs}_{\text{after}}) / \text{Abs}_{\text{before}}] \times 100\}.$$

Furosine Analysis

Furosine analysis, adapted from Baptista and Carvalho (2004), was performed on MPC 45 and MPC 85 at each time point. Approximately 30 mg of MPC was placed into a screw-cap (polytetrafluoroethylene-faced septa) glass tube (VWR). Water (HPLC grade, 2.5 mL,

EMD Chemicals) was added and vortexed. Two milliliters of 8 *N* HCl (EMD Chemicals) was added and mixed well. The solution was placed into an oven at 110°C and incubated for 24 h. A 400- μ L aliquot was placed into a glass tube and the HCl was blown down with nitrogen until completely dry. Two milliliters of HPLC water was added and mixed. The solution was then filtered through a 0.45- μ m syringe filter into an HPLC vial (Phenomenex). Furosine was quantified by HPLC (Breeze HPLC, Waters, Milford, MA) using a mobile phase of 0.06 *M* sodium acetate, pH 4.3 with acetic acid at a flow rate 1 mL/min pumped through a binary pump (Waters 1525, Waters). Twenty microliters of sample was injected (Waters 2707 autosampler) onto the column (Lunca 5u C8 100A 250 $\times$ 4.6 mm) at 35°C. Sample was analyzed using a photodiode array detector (Waters 2998) with a maxima of 280 nm. Sample run time was 10 min. All MPC were extracted and evaluated in triplicate. Furosine increase was determined by calculating the percent increase of furosine concentration in the stored MPC to the furosine concentration of the time 0 MPC.

Statistical Analysis

Data in experiment 1 were analyzed by a one-way ANOVA using a general linear model with Fisher's least significant difference for means separation (XLSTAT, version 2015.1.01; Addinsoft Inc., New York, NY). All data in experiment 2 were analyzed by 3-way ANOVA (protein $\times$ temperature $\times$ storage) with Fisher's least significant difference for means separation (XLSTAT, version 2015.1.01; Addinsoft Inc.).

RESULTS AND DISCUSSION

Experiment 1

Instrumental Volatile and Descriptive Analysis. Forty-two odor active events were detected in dried milk proteins by SPME GC-O. Of the 42, 30 were identified by aroma, retention index, and mass spectra, and 12 were tentatively identified by retention index on ZB5 or wax column and odor quality (Table 2). Predominant odorants of the MPC, MCC, and casein samples were also identified by solvent extraction with AEDA. Forty-three odor active events were detected in dried milk proteins by solvent extraction with AEDA. Twenty-two neutral/basic and acid odorants were identified by retention index, aroma, and mass spectra, with 16 tentatively identified by retention index and odor quality, and 5 unknowns (Table 3). All of the odor active compounds listed in Tables 2 and 3 were previously reported as odor-active components of milk,

cheese, casein, skim milk powder (**SMP**), fluid whey, whey powder, whey protein concentrates, or whey protein hydrolysates (Hammond and Hill, 1964; Preininger and Grosch, 1994; Shiratsuchi et al., 1994a,b; Karagul-Yuceer and Cadwallader, 2001; Karagul-Yuceer et al., 2002, 2003; Mahajan et al., 2004; Carunchia Whetstone et al., 2005; Kobayashi et al., 2008; Leksrisompong et al., 2010).

Solvent extraction and AEDA of milk proteins and caseins detected many compounds not detected by SPME headspace extraction. These volatile compounds included 2-methyl-1-propanol, heptanal, 1-octen-3-one, E,E 2,4 heptadienal, 2-acetyl thiazole, benzyl alcohol, gamma terpinene, 2-ethyl-3,5-dimethylpyrazine, sotolon, homofuraneol, 2,4-dimethylphenol, 4-ethylphenol, decanal, E,Z-2,4-nonadienal, benzothiazole, acetic acid, β ionone, propionic acid, 5-methyl furfural, butanoic, pentanoic, hexanoic, and decanoic acids, and 1-octadecanol. Similarly, SPME headspace extraction detected many compounds not detected by solvent extraction (SAFE) and AEDA of milk proteins and caseins. No volatile extraction approach is unbiased. Headspace extraction generally recovers more highly volatile compounds, whereas solvent extraction tends to favor higher molecular weight compounds. For these reasons, both extraction approaches were applied in the current study.

The greatest number of odorants (neutral/basic and acid) were identified in acid and rennet caseins. Acidic odorants consisted mainly of short-chain volatile acids (Table 3). On the basis of $\log_3$ FD (>2) factors, the most potent odorants in rennet casein were 1-hexen-3-one, heptanal, methional, 2-acetyl-1-pyrroline, DMTS, 1-octen-3-one, octanal, 2-methoxyphenol, benzothiazole, acetic, propionic, butanoic, and pentanoic acids, and 1-octadecanol. In acid casein, compounds with high $\log_3$ FD factors included hexanal, homofuraneol, methional, 1-octen-3-one, and butanoic acid. The compounds with the highest $\log_3$ FD factors in milk proteins (MPC 70,80, 85, MCC, and MPI) were dimethyl disulfide, hexanal, 1-hexen-3-one, sotolon, homofuraneol, 4-hexen-3-one, heptanal, methional, 1-cyclohexen-3-one, 2-acetyl-1-pyrroline, dimethyl trisulfide, 1-octen-3-one, octanal, guaiacol, nonanal, decanal, benzothiazole, 2-aminoacetophenone, gamma-decalactone, acetic, propionic, butanoic, pentanoic, and hexanoic acids, and delta-dodecalactone.

Among the MPC, MPI, and MCC, the MPC had the greatest number of neutral/basic and acid odorants identified with high (>2) $\log_3$ FD factors. Volatile compounds with the highest $\log_3$ FD factors (>3.5) in MPC were dimethyl disulfide, hexanal, 2-acetyl-pyrroline, 1-octen-3-one, acetic acid, 2-methoxy phenol (guaiacol), nonanal, methional, 2-methyl-1-propanal, and

Table 2. Odor-active compounds detected in caseins, milk protein concentrates (MPC), milk protein isolates (MPI), and micellar casein concentrates (MCC) by gas chromatography-olfactometry using solid-phase microextraction (SPME)

RI (ZB5) ¹	RI (WAX) ²	Method of ID ³	Description	Compound	A: Acid casein	A: Rennet casein	B: MPC 70	B: MPI	B: MPC 85	C: MCC	D: MPC 80	E: MPC 85	E: MPC 80	PP
588	667	RI, O, MS	Pumpkin	Dimethyl sulfide	+	+	+	+	+	+	+			+
627	975	RI, O, MS	Butter	Diacetyl	+	+	+	+	+	+	+	+	+	+
654		RI, O	Metallic/cabbage	Methyl ethyl sulfide							+			+
662	912	RI, O, MS	Cocoa/malty	2-Methyl butanal	+		+			+				+
672		RI, O, MS	Sulfur/cabbage	Dimethyl disulfide	+	+					+			
678		RI, O, MS	Malty/burnt	3-Methyl butanal	+		+	+		+	+	+		
780		RI, O	Fruity	Ethyl 2-methylpropanoate		+					+			
809	1087	RI, O, MS	Burnt	1-Hexen-3-one								+		
832	1079	RI, O, MS	Green	Hexanal	+	+	+	+	+	+	+	+		+
845		RI, O, MS	Mint	1-Hexanol								+		
883	1189	RI, O	Fruity	Ethyl-3-methylbutanoate	+						+			
890	1316	RI, O, MS	Nutty	Furfuryl alcohol		+		+						+
902		RI, O, MS	Cooked	2-Heptanone	+	+	+		+	+	+		+	+
931	1178	RI, O, MS	Fishy	Z-4-Heptenal	+			+					+	
935	1465	RI, O, MS	Potato	Methional	+	+	+	+		+	+	+		
948		RI, O	Brothy	Dimethyl sulfone							+	+		
955	1345	RI, O, MS	Popcorn	2-Acetyl pyrroline	+	+	+	+	+		+	+	+	+
997	1379	RI, O, MS	Sulfur/cabbage	Dimethyl trisulfide	+	+		+	+	+		+		+
1009	1305	RI, O, MS	Mushroom/metallic	1-Octen-3-ol	+	+	+	+	+	+	+	+	+	+
1040	1274	RI, O, MS	Citrus	Octanal	+	+	+	+	+		+	+	+	+
1102		RI, O, MS	Caramel	Maltol							+	+	+	
1109		RI, O, MS	Green/vegetative	3-Nonenal			+			+	+			
1119		RI, O, MS	Burnt/smoky	Guaiacol	+	+	+	+	+	+	+	+	+	+
1126		RI, O, MS	Green/fatty	Nonanal	+						+	+	+	
1139		RI, O	Cardboard/fatty/ carpet	2,6-Dimethylphenol	+	+	+	+	+		+	+		+
1171		RI, O, MS	Cardboard	Z-2-Nonenal	+							+		
1190		RI, O, MS	Green	E,Z-2,6-Nonadienal	+		+				+	+	+	+
1196	1536	RI, O	Cucumber	E-2-Nonenal	+			+		+	+	+		
1204		RI, O, MS	Carpet	2,3-Dimethylphenol		+			+		+		+	
1240		RI, O	Cooked burnt	2,4,6-Trimethylphenol	+		+	+		+	+	+		+
1248		RI, O	Fatty brothy	E,E-2,4-Nonadienal					+			+		
1458		RI, O, MS	Green	E-2-Decenal	+					+	+	+		
1344		RI, O	Licorice	Eugenol		+	+		+				+	
1354	>2000	RI, O, MS	Corn chips	2-Aminoacetophenone	+	+	+		+		+	+	+	+
1358		RI, O, MS	Soapy	E-2-Undecenal	+									
1366		RI, O	Fatty/corn chips	E,Z-2,4-Decadienal	+	+		+	+		+	+	+	
1379		RI, O, MS	Fatty/corn chips	E,E-2,4-Decadienal	+	+		+	+		+	+	+	
1476		RI, O, MS	Green	E-2-dodecenal						+		+		
1578	>2000	RI, O, MS	Sweet	Delta-decalactone		+							+	+
1602		RI, O	Minty	Alpha-curcumene							+			
1663		RI, O	Sweet	Delta-undecalactone				+						
1711		RI, O, MS	Fruity/sweet	Delta-dodecalactone							+			

¹Retention indices (RI) from gas chromatography-olfactometry (GC-O) data on the ZB-5 column.

²RI from GC-O data on the WAX column.

³Method of identification by RI, O (olfactometry), and MS (mass spectrometry) compared with authentic standards.

Table 3. Log₃ FD (flavor dilution) values of odor-active compounds detected in caseins, milk protein concentrates (MPC), milk protein isolates (MPI), and micellar casein concentrates (MCC) in the neutral/basic and acid fractions from solvent extraction with gas chromatography-olfactometry with aroma extract dilution analysis

	RI ¹ WAX	RI ¹ DB5	Fraction ²	Compound	Odor ³	ID ⁴	A: Acid casein	A: Rennet casein	B: MPC 70	B: MPC 85	B: MPI	C: MCC	D: MPC 80	E: MPC 80
1	ND ⁵	718	NB	Dimethyl disulfide	Sulfur	O, RI	2	<1	<1	<1	ND	<1	6	<1
2	ND	802	NB	Hexanal	Green	O, RI, MS	3	2	<1	<1	2	<1	4	<1
3	ND	809	NB	1-Hexene-3-one	Skunky/smoky	O, RI	2	4	<1	<1	<1	ND	3	<1
4	1087	ND	A	2-Methyl-1-propanol	Burning plastic	O, RI	3	2	<1	4	3	ND	3	<1
5	ND	811	NB	Unknown	Mucus	O, RI	<1	<1	2	3	<1	2	ND	<1
6	1025	865	A/NB	Ethyl-3-methylbutanoate	Sweet/citrus	O, RI	2	2	<1	<1	2	<1	<1	2
7	ND	894	NB	Heptanal	Rubber/plastic	O, RI, MS	<1	3	<1	3	<1	<1	<1	2
8	1241	906	A/NB	Z-4-heptenal	Fishy	O, RI	<1	2	<1	<1	2	<1	<1	<1
9	ND	911	NB	Methional	Potato	O, RI	3	3	2	4	3	ND	3	3
10	ND	921	NB	Unknown	Carpet	O, RI	<1	<1	<1	<1	ND	<1	6	<1
11	ND	929	NB	2-Acetyl-1-pyrroline	Popcorn	O, RI, MS	ND	3	4	4	4	2.5	6	5
12	ND	961	NB	Dimethyl trisulfide	Cabbage/garlic	O, RI, MS	2	4	<1	<1	3	2	2	<1
13	1306	984	A/NB	1-Octen-3-one	Mushroom	O, RI, MS	3	3	<1	3	2	2	4.5	4
14	1234	1016	A/NB	Octanal	Citrus	O, RI, MS	<1	3	<1	<1	2	2	3	<1
15	1419	1030	A/NB	(E,E)-2,4 heptadienal	Nutty/grainy	O, RI, MS	<1	<1	ND	<1	ND	2	<1	ND
16	ND	1044	NB	2-Acetyl thiazole	Popcorn	O, RI	ND	<1	<1	<1	ND	ND	ND	<1
17	1846	1052	A/NB	Benzyl alcohol	Fruity/sweet	O, RI, MS	<1	<1	ND	<1	ND	ND	<1	2
18	ND	1067	NB	Gamma terpinene	Pine	O, RI, MS	2	<1	ND	<1	ND	ND	ND	<1
19	1430	1074	A/NB	2-Ethyl-3,5-dimethylpyrazine	Smoky/earthy	O, RI	<1	<1	ND	<1	<1	<1	<1	<1
20	ND	1099	NB	Sotolon	Sweet	O, RI, MS	ND	ND	ND	ND	2	3	3	ND
21	ND	1116	NB	2-Methoxy phenol	Smoky/burnt	O, RI, MS	2	3	<1	<1	2	3	3	4
22	ND	1123	NB	Homofuraneol	Sweet	O, RI, MS	3	2	ND	ND	2	1	1	3
23	1344	1131	A/NB	Nonanal	Fatty/plastic	O, RI, MS	<1	<1	<1	3	2.5	<1	ND	5
24	ND	1157	NB	2,4-Dimethylphenol	Mushroom/smoky	O, RI	2	<1	<1	ND	2	2	<1	<1
25	ND	1165	NB	4-Ethylphenol	Earthy/smoky	O, RI	<1	<1	<1	<1	<1	ND	<1	ND
26	ND	1193	NB	Decanal	Carpet/clay	O, RI, MS	<1	2	2	<1	<1	2	3	<1
27	ND	1212	NB	(E,Z)-2,4-nonadienal	Cucumber	O, RI, MS	<1	<1	<1	<1	<1	<1	ND	<1
28	1968	1233	A/NB	Benzothiazole	Carpet/clay	O, RI	<1	3	3	3	3	3	3	2.5
29	1286	ND	NB	Unknown	Vomit	O	ND	ND	2	ND	ND	ND	ND	ND
30	ND	1350	NB	2-Aminoacetophenone	Tortilla	O, RI, MS	2	ND	<1	2	3	2	<1	<1
31	ND	1362	NB	(E,E)-2,4 decadienal	Fatty	O, RI, MS	<1	2	ND	<1	<1	ND	<1	2
32	1444	ND	A	Acetic acid	Vinegar/sour	O, RI	<1	3	2.5	4	3	<1	ND	3
33	ND	1466	NB	Beta ionone	Sweet/fatty	O, RI, MS	2	<1	<1	2	<1	3	<1	2
34	1514	ND	A	Propionic acid	Garbage	O, RI	2	3	<1	ND	3	<1	2	ND
35	ND	1543	NB	Unknown	Licorice/hay	O	ND	ND	3	ND	<1	ND	<1	3
36	1551	ND	A	5-Methyl furfural	Smoky	O, RI	<1	2	<1	<1	ND	ND	ND	ND
37	ND	1628	NB	Unknown	Stale/dough	O	<1	<1	<1	2	3	3	<1	2.5
38	1684	ND	A	Butanoic acid	Vomit	O, RI, MS	2.5	3	3	4	3	ND	4	5
39	1764	ND	A	Pentanoic acid	Smoky/stinky feet	O, RI, MS	2	3	ND	ND	2	<1	ND	3
40	ND	1784	NB	Delta-dodecalactone	Sweet/coconut	O, RI	<1	<1	<1	<1	3	2	2	<1
41	1809	ND	A	Hexanoic acid	FFA/cheesy	O, RI, MS	<1	<1	3	<1	3	<1	3	ND
42	ND	2092	NB	1-Octadecanol	Soap	O, RI	2	3	2	ND	ND	<1	<1	<1
43	>2,100	ND	A	Decanoic acid	Soapy	O, RI, MS	ND	<1	ND	ND	ND	<1	ND	2

¹Retention indices (RI) from gas chromatography-olfactometry data on the WAX and ZB-5 columns.²A = acid fraction, NB = neutral/basic fraction from solvent extraction with gas chromatography-olfactometry.³Odor description at gas chromatography sniffing port.⁴O = olfactometry, and MS = mass spectrometry.⁵ND = not detected.

butanoic acid. Aroma active compounds with high $\log_3$ FD factors (>2) in MPC that were not high in MPI or MCC were dimethyl disulfide, hexanal, 1-hexen-3-one, heptanal, 1-octen-3-one, octanal, homofuraneol, decanal, and pentanoic acid. Aroma active compounds with high $\log_3$ FD factors in MPI that were not in MPC or MCC were dimethyl trisulfide, 2-aminoacetophenone, propionic acid, and delta-dodecalactone. The MCC had relatively few aroma active compounds with high $\log_3$ FD factors, and only β -ionone had a high $\log_3$ FD factor in MCC and not in MPC or MPI.

Sensory profiles of the casein, MPC, and MCC samples varied ($P < 0.05$; Table 4). Tortilla/corn chip and cardboard flavors were predominant in all samples, with the exception of acid casein, which was highest in tortilla/corn chip flavor followed by brothy/animal flavor. Only acid and rennet caseins exhibited animal flavor. Tortilla/corn chip flavor has previously been identified as a primary flavor of rennet casein and MPC 80/MPI by descriptive analysis (Karagul-Yuceer et al., 2003; Drake et al., 2009). The flavor of caseins has been characterized by a variety of descriptors including animal/wet dog, cooked, potato/brothy, sulfur, glue, tortilla, and cardboard (Karagul-Yuceer et al., 2003; Drake et al., 2009). Karagul-Yuceer et al. (2003) identified the most potent odorant in rennet casein (based on $\log_3$ FD factors) as 2-aminoacetophenone, which contributed a tortilla and grapelike odor. This compound was previously reported to contribute to a glue/burnt feathers flavor in stored casein (Ramshaw and Dunstone, 1969), stored dry milk (Parks et al., 1964), and sterilized concentrated milk (Arnold et al., 1966). Despite its high aroma activity, Karagul-Yuceer et al. (2003) concluded that the typical odor of rennet casein was primarily caused by hexanoic acid, indole, guaiacol, and p-cresol based upon odor activity values (OAV) and sensory analysis of model mixtures of flavor compounds. In the current study, the significance of 2-aminoacetophenone is consistent with past studies.

Milk proteins (MPC and MPI) have been characterized by descriptors including cooked/milky, sweet aromatic, tortilla, potato/brothy, cardboard, and animal (Drake et al., 2009). Drake et al. (2009) also reported that MPC with lower protein content (56% protein by dry weight) were characterized by fluid milk types of flavor (cooked/milky, sweet aromatic, sweet taste, and cereal, and as protein content increased, the flavor profiles changed and MPC70, MPC 80, and MPI were characterized by tortilla, brothy, cardboard, and animal flavors and higher astringency. The current study characterized milk proteins using the same descriptors, and the MPC 70 and 80 were characterized by low but distinct intensity of tortilla and cardboard flavor, cooked/milky, and sweet aromatic flavors (Table 4).

Table 4. Sensory profiles of caseins, milk protein concentrates (MPC), milk protein isolate (MPI), and micellar casein concentrate (MCC) obtained from various manufacturers¹

Manufacturer	Type	Aroma intensity	Cooked/milky	Sweet aromatic	Sour aromatic	Cardboard	Potato/brothy	Animal	Tortilla/corn chip	Soapy	Fatty	Sour	Astringency
A	Acid casein	4.5 ^a	ND ²	ND	1.8 ^a	2.5 ^a	ND	3.3 ^a	4.6 ^a	ND	ND	1.1 ^a	3.2 ^a
A	Rennet casein	3.8 ^b	ND	ND	ND	2.7 ^a	ND	2.0 ^b	2.6 ^b	ND	ND	ND	2.8 ^b
B	MPC 85	2.6 ^d	1.8 ^a	1.8 ^a	ND	2.3 ^{ab}	1.6 ^a	ND	2.0 ^c	ND	ND	ND	2.6 ^b
B	MPC70	2.3 ^{de}	1.8 ^a	1.5 ^{ab}	1.6 ^a	1.7 ^c	ND	ND	1.1 ^e	ND	1.1 ^b	ND	2.3 ^c
B	MPI	3.2 ^c	ND	ND	ND	2.5 ^a	1.8 ^a	ND	2.6 ^b	ND	1.6 ^a	ND	2.6 ^b
C	MCC	2.5 ^{de}	1.5 ^b	1.3 ^{bc}	ND	2.6 ^a	ND	ND	1.3 ^{de}	ND	ND	ND	2.0 ^d
D	MPC 80	1.1 ^g	1.5 ^b	1.1 ^{cd}	ND	1.0 ^d	ND	ND	2.0 ^c	0.8 ^b	ND	ND	2.3 ^c
E	MPC 80	2.1 ^{ef}	1.0 ^c	0.8 ^c	ND	1.5 ^c	ND	ND	2.1 ^{bc}	1.3 ^a	1.2 ^b	ND	2.3 ^c
E	MPC 85	1.2 ^g	1.0 ^c	1.3 ^{bc}	ND	2.0 ^{bc}	ND	ND	1.0 ^e	1.3 ^a	ND	ND	2.5 ^{bc}
P	MPC 80	1.8 ^f	1.7 ^{ab}	1.0 ^d	ND	1.6 ^c	ND	ND	1.5 ^d	ND	ND	ND	2.5 ^{bc}

^{a-f}Means in a column not sharing a common superscript are different ($P < 0.05$).

¹Attribute intensities were scored on a 0- to 15-point universal intensity scale (Meilgaard et al., 2009). Most dried ingredient flavors fall between 0 and 4 on this scale (Croissant et al., 2009; Wright et al., 2009).

²ND = not detected.

Seven odor active volatile compounds (2-acetylpyrroline, 1-octen-3-one, 2-aminoacetophenone, nonanal, heptanal, DMTS, and methional) from the neutral/basic portion of SAFE extracts were quantified by standard curves. Nine volatile fatty acids (butyric, pentanoic, hexanoic, heptanoic, octanoic, nonanoic, decanoic, dodecanoic, and tetradecanoic) from the acid portion of the SAFE extracts were quantified by standard curves. Three other odor active volatile compounds (hexanal, 1-hexen-3-one, and DMDS) were quantified via SPME. Relative abundance of 15 other volatile compounds was determined by SPME headspace GC-MS (Table 4). Sensory profiles of milk proteins and caseins were generally consistent with volatile compound concentrations and relative abundance (Tables 4 and 5). A correlation between concentration of 2-aminoacetophenone and tortilla/corn chip flavor was observed (Figure 1, $R^2 = 0.87$, $P < 0.05$). The importance of 2-aminoacetophenone to tortilla/corn chip flavor is well established in both corn tortillas and taco shells (Karahadian and Johnson, 1993; Buttery et al., 1994; Buttery and Ling, 1995, 1998) and casein (Badings, 1991; Karagul-Yuceer et al., 2003). The current study confirms the importance of 2-aminoacetophenone to the tortilla flavor of dried milk proteins. However, tortilla flavor in milk proteins may also be caused by several volatile compounds and not just 2-aminoacetophenone based on previous studies (Karagul-Yuceer et al., 2003) and on aroma active compounds detected in the current study. Many of the odor active compounds in corn tortillas, chips, and taco shells have also been identified in the milk protein products of the current study, including E,E and E,Z 2,4 decadienal, acetic acid, 3-methylbutanal, 2-acetyl-1-pyrroline, 2-aminoacetophenone, E-2-nonenal, 2-methylbutanal, E-2-decenal, methional, β -ionone, hexanal, 1-octen-3-ol, and 2-ethyl-3,5-dimethylpyrazine (Tables 2 and 3; Buttery and Ling, 1995, 1998).

Acid casein was characterized by high aroma intensity, sour aromatic, cardboard, animal flavor, and tortilla/corn chip flavors. Acid casein also had high concentrations of 2-aminoacetophenone, E-2-heptenal, and E-2-nonenal. Rennet casein was also characterized by high aroma intensity, cardboard, and animal flavors, but was high in phenylacetaldehyde, sotolon, E-2-heptenal, 2,4 decadienal, 1-hexen-3-one, hexanal, DMDS, DMTS, and 2-pentyl furan. Despite similar sensory profiles, compound concentrations and relative abundance were highly variable likely due to distinct differences in manufacture and processing. It is interesting to note that hexanoic acid was identified as an important flavor contributor to rennet casein in past studies (Karagul-Yuceer et al., 2003), but hexanoic acid was not a factor in the flavor of rennet casein in the current study. This result is consistent with the SAFE GC-O data, which

also lacked a hexanoic acid aroma event. The effects of raw materials, processing and storage all affect flavor and flavor chemistry of dairy ingredients (Smith et al., 2016). It is likely that this discrepancy is due to one or several those factors. Volatile compound differences were also observed among the MPC 80 and MPI from different suppliers.

Many of the volatile compounds related to flavor in milk proteins are either formed by lipid oxidation or Maillard browning. The production of 2-aminoacetophenone in corn tortilla/chip products occurs when corn is heated and exposed to an alkali (Buttery and Ling, 1998), but past studies have also suggested that alkaline conditions catalyzed air oxidation of the amino acid tryptophan to the compound kynurenine, which is then degraded to 2-aminoacetophenone (Tabone et al., 1951). It is possible that in milk protein powders, 2-aminoacetophenone is formed by the oxidation of tryptophan in solution. However, this seems unlikely due to the lack of a strong oxidative step during the manufacture of milk proteins necessary to catalyze the oxidation of tryptophan. It also seems unlikely due to the lack of 2-aminoacetophenone content, and tortilla off-flavor in whey protein concentrates, which often undergo oxidative bleaching during processing (Croissant et al., 2009; Jervis et al., 2012; Fox et al., 2013). The tryptophan content of β -lactoglobulin and α -lactalbumin (whey proteins) is much higher than that of α - and β -caseins (Spies, 1967), which would suggest that if the alkali breakdown of tryptophan was the primary source of 2-aminoacetophenone in milk proteins, it would most certainly also be a major flavor component in whey protein concentrates. Past studies (Spacek, 1954; Parks et al., 1967) have demonstrated that alkaline degradation of kynurenine results in a >90% yield of 2-aminoacetophenone, whereas the yield of 2-aminoacetophenone from tryptophan by alkaline degradation is less than 0.1%. Parks et al. (1964) also determined that kynurenine occurred naturally in bovine milk. It is possible that the 2-aminoacetophenone concentration in milk and milk protein powders is due to the free kynurenine in milk, which may be broken down by nonalkaline means or remains in the cheese during the cheese-make procedure of liquid whey manufacture, preventing its presence in whey protein.

Alkanals and alkenals, such as decadienal, methylbutanal, nonenal, decenal, and hexanal, are typical products of lipid oxidation. Increased lipid oxidation products over processing, storage, or both is expected and has been demonstrated in pasteurized fluid milk, SMP, whole milk powder, and dried whey protein ingredients (Karagul-Yuceer et al., 2002; Karatapanis et al., 2006; Lloyd et al., 2009; Wright et al., 2009). The autooxidative mechanisms of unsaturated fatty acids in the milk

Table 5. Mean concentration or relative abundance of selected compounds in milk protein and casein powders¹

Item	A: Acid casein	A: Rennet casein	B: MPC 85	B: MPI	C: MCC	D: MPC 80	E: MPC 80	E: MPC 85	P: MPC 80
Concentration ² (μg/kg)									
2-Acetylpyrroline	0.011 ^d	1.88 ^b	0.502 ^d	0.011 ^d	3.73 ^a	1.14 ^c	0.171 ^d	0.523 ^d	1.07 ^c
1-Hexen-3-one*	9.70 ^b	264 ^a	2.35 ^b	0.451 ^b	0.315 ^b	1.20 ^b	1.00 ^b	0.393 ^b	1.11 ^b
1-Octen-3-one	0.238 ^b	104 ^b	528 ^a	20.3 ^b	9.16 ^b	4.98 ^b	20.1 ^b	52.8 ^b	50.7 ^b
2-Aminoacetophenone	12.7 ^a	1.01 ^b	0.053 ^e	0.060 ^e	0.076 ^e	0.630 ^c	0.102 ^{de}	0.055 ^e	0.238 ^d
Hexanal*	52.8 ^b	217 ^a	27.3 ^{bc}	2.31 ^c	1.57 ^c	4.95 ^c	5.29 ^c	7.96 ^c	4.53 ^c
Heptanal	1.16 ^b	210 ^a	190 ^a	3.50 ^b	18.8 ^b	57.9 ^b	203 ^a	30.8 ^b	63.4 ^b
Nonanal	20.5 ^e	16.3 ^e	644 ^a	76.8 ^{cde}	167 ^{bcd}	210 ^{bc}	279 ^b	690 ^a	64.0 ^{de}
DMDS*	6.27 ^b	26.2 ^a	0.500 ^b	0.057 ^b	0.062 ^b	0.093 ^b	0.105 ^b	0.058 ^b	0.460 ^b
DMTS	0.092 ^b	36.7 ^a	2.50 ^b	0.293 ^b	0.593 ^b	0.455 ^b	1.18 ^b	4.48 ^b	3.89 ^b
Methional	0.082 ^d	18.2 ^b	18.9 ^b	0.095 ^d	2.66 ^{cd}	36.1 ^a	8.22 ^c	0.108 ^d	1.99 ^d
Concentration ³ (mg/kg)									
Butyric	6.73 ^b	6.15 ^b	3.05 ^b	11.2 ^b	0.973 ^b	9.10 ^b	109 ^a	ND	2.48 ^b
Pentanoic	0.083 ^c	38.6 ^{ab}	ND	3.03 ^c	4.59 ^{bc}	25.3 ^{abc}	3.43 ^{bc}	2.48 ^c	58.2 ^a
Hexanoic	5.42 ^b	0.015 ^b	5.06 ^b	9.18 ^b	0.012 ^b	0.209 ^b	62.6 ^a	45.5 ^a	10.7 ^b
Heptanoic	0.022 ^c	0.663 ^{bc}	ND	0.710 ^{bc}	1.45 ^{bc}	35.3 ^{ab}	2.34 ^{bc}	2.09 ^{bc}	65.8 ^a
Octanoic	1.89 ^b	0.465 ^b	7.43 ^b	27.6 ^b	10.0 ^b	ND	29.1 ^b	107 ^a	108 ^a
Nonanoic	0.035 ^b	ND	ND	7.25 ^{ab}	6.58 ^{ab}	17.9 ^{ab}	4.18 ^{ab}	9.49 ^{ab}	41.8 ^a
Decanoic	0.044 ^c	0.078 ^c	0.259 ^c	20.5 ^b	0.005 ^c	2.71 ^c	10.0 ^{bc}	43.6 ^a	35.2 ^a
Dodecanoic	2.70 ^c	0.234 ^c	0.020 ^c	19.7 ^{bc}	0.005 ^c	111 ^{ab}	19.1 ^{bc}	100 ^{ab}	139 ^a
Tetradecanoic	4.64 ^b	0.312 ^b	0.023 ^b	2.09 ^b	0.007 ^b	0.038 ^b	1.77 ^b	21.4 ^{ab}	61.7 ^a
Relative abundance (μg/kg)									
Phenylacetaldehyde	2.28 ^b	31.3 ^a	0.089 ^b	4.81 ^b	0.184 ^b	1.56 ^b	43.4 ^a	8.32 ^b	0.167 ^b
Sotolon	0.060 ^b	87.5 ^a	0.199 ^b	0.678 ^b	0.071 ^b	0.150 ^b	71.2 ^a	18.5 ^b	5.06 ^b
Undecanal	11.1 ^{cd}	7.47 ^{cd}	21.3 ^{bc}	111 ^a	4.32 ^d	34.3 ^b	17.5 ^{cd}	5.20 ^d	7.56 ^{cd}
E,Z 2,6 Nonandienal	2.00 ^c	2.50 ^c	0.302 ^c	26.9 ^a	0.143 ^c	1.86 ^c	13.2 ^b	0.561 ^c	0.765 ^c
γ-Terpinene	3.58 ^b	0.109 ^c	0.062 ^c	15.0 ^a	0.564 ^c	0.087 ^c	0.424 ^c	2.38 ^b	0.127 ^c
E-2-Heptenal	13.3 ^{ab}	25.2 ^a	0.170 ^b	3.78 ^b	5.93 ^b	3.70 ^b	3.71 ^b	15.6 ^{ab}	0.611 ^b
γ-Dodecalactone	4.88 ^b	1.08 ^b	1.58 ^b	69.2 ^a	8.75 ^b	2.14 ^b	8.70 ^b	1.86 ^b	2.73 ^b
Guaiacol	10.3 ^c	8.67 ^{cd}	0.142 ^{de}	21.8 ^b	0.079 ^e	0.363 ^{de}	98.0 ^a	4.66 ^{cde}	0.360 ^{de}
2,4 Decadienal	0.158 ^b	40.5 ^a	0.169 ^b	2.28 ^b	2.75 ^b	0.717 ^b	11.7 ^b	18.5 ^b	4.31 ^b
Octanal	0.881 ^d	17.0 ^b	8.89 ^{bcd}	2.75 ^{cd}	6.09 ^{cd}	2.01 ^{cd}	4.67 ^{cd}	10.6 ^{bc}	83.5 ^a
E 2-Nonenal	197 ^a	6.47 ^{bcd}	0.060 ^d	3.26 ^{cd}	0.082 ^d	25.4 ^b	2.89 ^d	5.78 ^{bcd}	25.1 ^{bc}
2-Pentylfuran	2.25 ^b	35.2 ^a	0.202 ^b	24.8 ^a	2.30 ^b	0.562 ^b	36.0 ^a	3.35 ^b	4.08 ^b
E 2-Decenal	6.33 ^e	22.6 ^{cd}	0.341 ^e	1.05 ^e	0.275 ^e	12.8 ^{de}	56.5 ^b	26.8 ^c	75.3 ^a
Decanal	0.536 ^d	10.7 ^c	7.10 ^{cd}	2.48 ^d	5.84 ^{cd}	2.39 ^d	48.3 ^a	7.54 ^{cd}	21.7 ^b
E,E 2,4 Heptadienal	2.36 ^{cde}	5.60 ^c	0.168 ^e	0.749 ^{de}	3.10 ^{cde}	0.589 ^{de}	30.5 ^b	4.70 ^{cd}	39.7 ^a

^{a-c}Means in a row not sharing a common superscript are different ($P < 0.05$).

¹MPC = milk protein concentrate; MPI = milk protein isolate; MCC = micellar casein concentrate. DMDS = dimethyl disulfide; DMTS = dimethyl trisulfide.

²Compounds from neutral/basic portion of solvent-assisted flavor extraction (SAFE) unless otherwise indicated [some compounds were extracted by solid-phase microextraction (SPME) headspace, not SAFE, as denoted by an *].

³Compounds from acid portion of SAFE.

Figure 1. Concentration ($\mu\text{g/kg}$; left y-axis) of 2-aminoacetophenone compared with tortilla/corn chip flavor intensity (right y-axis) in select milk proteins and caseins. Color version available online.

lead to the formation of many aldehydes. They have low flavor thresholds and are commonly characterized by oily, fatty, or tallow odors (Azzara and Campbell, 1992). Whitson et al. (2010) demonstrated that a combination of lipid oxidation products and DMTS caused cardboard flavor in whey proteins. In certain concentrations and combinations with 2-aminoacetophenone, some or all of these compounds may also contribute to tortilla flavor in milk proteins. The alcohol 1-octen-3-ol was important to both the flavor of the milk proteins in this study and corn tortillas and corn chips (Buttery and Ling, 1995, 1998).

The compound 2-acetyl-1-pyrroline is characterized by a popcorn aroma and is a result of the reaction between the AA proline or ornithine and the reactive sugar-degradation product 2-oxopropanal (Schieberle, 1995). The compound 2-acetyl-1-pyrroline is commonly found in many fresh dairy ingredients such as SMP (Karagul-Yuceer and Cadwallader, 2001) and fluid and dried whey powder (Karagul-Yuceer et al., 2003; Mahajan et al., 2004) and contributes a popcorn and cooked flavor. This compound is attributed to sweet aromatic and cooked/milky flavors in milk proteins.

Methional has an aroma similar to boiled potatoes. Methional formation is a result of Strecker degradation of methionine (Tressl et al., 1989) or is a result of methionine breakdown when milk is exposed to light (Badings, 1991). It is also a common flavor-active component of dairy protein powders including liquid whey (Karagul-Yuceer et al., 2003), whey protein concentrate

and whey protein isolate (Carunchia Whetstine et al., 2005), milk powders (Karagul-Yuceer and Cadwallader, 2001), and rennet casein (Karagul-Yuceer et al., 2003). The compound 2-ethyl-3,5-dimethylpyrazine is also a Maillard browning product, specifically produced by Strecker degradation (Manley and Fagerson, 1970) and has a roasted earthy aroma (Grosch and Schieberle, 1997). Products formed from Strecker degradation are heat induced and would likely have higher concentrations in products with high heat treatments.

Experiment 2

Furosine Analysis of MPC During Storage. As expected, furosine levels increased in MPC 45 and 80 over time (Figure 2). Protein level, storage time and storage temperature had an effect on furosine production ($P < 0.05$). The MPC 45 at 3°C demonstrated a 31% furosine increase over 12 mo, whereas the MPC 45 at 25°C and 40°C demonstrated a 650 and 980% increase, respectively (Figure 2). The MPC 80 at 3°C demonstrated an 82% furosine increase over 12 mo, whereas the MPC 45 at 25 and 40°C demonstrated a 510 and 820% increase, respectively (Figure 2). Storage temperature had a larger effect on furosine concentration than protein level.

Furosine is a commonly used measure of protein-bound Amadori products and therefore measures the extent of the Maillard reaction in dairy proteins (Erbersdobler, 1986). Reduction of solubility in milk proteins could

Figure 2. Furosine concentration (mg/100 g of protein) in milk protein concentrate (MPC) 45 and 80 stored at 3, 25, and 40°C for 1, 3, 6, and 12 mo. Color version available online.

be due to covalent cross-linking of the proteins, hydrophobic or hydrogen bonds (Anema et al., 2006; Havea, 2006). The Maillard reaction may not be the primary cause of solubility loss in milk proteins, but Maillard browning products such as glyoxal or methylglyoxal can react with lysine or arginine, causing cross-linking

of proteins and subsequent loss of solubility (Lederer and Klaiber, 1999; Biemel et al., 2001; Le et al., 2011b, 2013). Maillard reactions play an important role in the shelf-life of milk proteins, and furosine is a useful measurement to compare the degree of Maillard browning that has taken place (Le et al., 2011a). The MPC 45

Figure 3. Percent solubility of milk protein concentrate (MPC) 45 and 80 stored at 3, 25, and 40°C for 0, 1, 3, 6, and 12 mo. Color version available online.

Table 6. Mean relative abundance ($\mu\text{g/kg}$) of select volatile compounds by protein concentration, storage time, and storage temperature¹

Item	Pentanal	Hexanal	2-Pentylfuran	Octanal	DMS	DMDS	DMTS	Heptanal	Methional	Nonanal	2 MB	3 MB	1-Octen3one	2,4 Decadienal
Protein $\times$ temperature $\times$ time														
45 $\times$ T1 $\times$ 3C	1.26 ^{efghi}	3.47 ^k	0.312 ^c	31.8 ^{kl}	2.30 ^{cdef}	1.52 ^g	0.052 ^{de}	0.323 ^h	0.084 ^{efg}	0.224 ^d	1.53 ^{ghi}	0.432 ^{defg}	1.11 ^{ef}	0.268 ^g
45 $\times$ T1 $\times$ 25C	1.32 ^{efgh}	3.74 ^k	0.297 ^c	35.2 ^{jkl}	2.41 ^{bcd}	4.31 ^g	0.059 ^{cde}	0.396 ^{gh}	0.073 ^{efg}	0.236 ^d	1.56 ^{gh}	0.523 ^{bcd}	1.35 ^{def}	0.554 ^{fg}
45 $\times$ T1 $\times$ 40C	1.38 ^{efg}	5.24 ^{jk}	0.294 ^c	31.0 ^l	2.64 ^{abcde}	15.9 ^{fg}	0.050 ^{de}	0.346 ^h	0.079 ^{efg}	0.196 ^d	1.75 ^{fg}	0.088 ^{kl}	1.29 ^{def}	0.556 ^{fg}
45 $\times$ T3 $\times$ 3C	1.14 ^{fghij}	4.57 ^{jk}	0.309 ^c	48.1 ^{hijkl}	1.83 ^f	3.16 ^g	0.052 ^{de}	0.342 ^h	0.031 ^{fg}	0.110 ^d	1.47 ^{ghi}	0.287 ^{ghij}	0.780 ^f	0.541 ^{fg}
45 $\times$ T3 $\times$ 25C	1.95 ^{de}	6.11 ^{ijk}	0.312 ^c	58.2 ^{fghijkl}	1.81 ^f	7.64 ^g	0.052 ^{de}	0.453 ^{gh}	0.028 ^g	0.173 ^d	2.51 ^{ef}	0.371 ^{efgh}	0.919 ^f	0.632 ^{fg}
45 $\times$ T3 $\times$ 40C	2.29 ^{cd}	10.3 ^{fghijk}	0.412 ^c	69.2 ^{cdefghi}	3.07 ^{ab}	49.6 ^{def}	0.060 ^{cde}	0.578 ^{efgh}	0.035 ^{fg}	0.193 ^d	3.03 ^{de}	0.575 ^{bcd}	0.990 ^{ef}	0.545 ^{fg}
45 $\times$ T6 $\times$ 3C	1.05 ^{fghijk}	5.74 ^{ijk}	0.315 ^c	64.8 ^{defghij}	1.81 ^f	4.23 ^g	0.053 ^{de}	0.369 ^h	0.135 ^{def}	0.214 ^d	1.46 ^{ghi}	0.348 ^{fghi}	1.05 ^{ef}	0.433 ^{fg}
45 $\times$ T6 $\times$ 25C	2.72 ^{bc}	14.1 ^{defgh}	0.353 ^c	85.0 ^{bcd}	2.15 ^{def}	11.4 ^g	0.050 ^{de}	0.546 ^{fgh}	0.088 ^{efg}	0.185 ^d	3.62 ^{cd}	0.486 ^{cdef}	1.11 ^{ef}	0.543 ^{fg}
45 $\times$ T6 $\times$ 40C	2.91 ^{bc}	18.1 ^{bcd}	0.470 ^c	101 ^b	2.96 ^{abc}	80.2 ^{bcd}	0.060 ^{cde}	0.740 ^{cdefg}	0.091 ^{efg}	0.209 ^d	3.95 ^{bc}	0.270 ^{ghij}	1.78 ^{cdef}	1.71 ^{cde}
45 $\times$ T12 $\times$ 3C	0.954 ^{fghijk}	9.52 ^{ghijk}	0.317 ^c	61.7 ^{efghijk}	1.96 ^{ef}	5.79 ^g	0.054 ^{de}	0.392 ^{gh}	0.160 ^{de}	0.195 ^d	1.43 ^{ghij}	0.028 ^l	0.756 ^f	0.721 ^{fg}
45 $\times$ T12 $\times$ 25C	3.38 ^{ab}	19.4 ^{bcd}	0.381 ^c	89.9 ^{bcd}	2.78 ^{abcd}	14.8 ^{fg}	0.045 ^e	0.618 ^{defgh}	0.108 ^{efg}	0.227 ^d	4.60 ^{ab}	0.750 ^a	1.35 ^{def}	1.12 ^{defg}
45 $\times$ T12 $\times$ 40C	3.63 ^a	23.9 ^{bc}	0.543 ^c	136 ^a	3.33 ^a	113 ^b	0.061 ^{cde}	0.927 ^{bcd}	0.092 ^{efg}	0.268 ^d	5.01 ^a	0.363 ^{efgh}	1.99 ^{cde}	1.77 ^{cd}
80 $\times$ T1 $\times$ 3C	0.503 ^{jk}	11.0 ^{fghijk}	0.426 ^c	34.8 ^{ijkl}	0.025 ^g	2.84 ^g	0.050 ^{de}	0.618 ^{defgh}	0.045 ^{fg}	0.626 ^{abc}	0.540 ^{jk}	0.124 ^{ijkl}	0.960 ^{ef}	0.453 ^{fg}
80 $\times$ T1 $\times$ 25C	0.610 ^{ijk}	10.3 ^{fghijk}	0.896 ^c	72.0 ^{bcd}	0.084 ^g	29.2 ^{efg}	0.049 ^{de}	0.849 ^{bcd}	0.110 ^{efg}	0.571 ^{bc}	0.708 ^{hijk}	0.183 ^{ijkl}	1.98 ^{cde}	0.915 ^{defg}
80 $\times$ T1 $\times$ 40C	0.848 ^{fghijk}	16.0 ^{defg}	1.23 ^c	85.1 ^{bcd}	0.098 ^g	95.1 ^{bc}	0.080 ^{cd}	0.883 ^{bcd}	0.129 ^{defg}	0.582 ^{abc}	1.05 ^{ghijk}	0.241 ^{hijk}	2.25 ^{cd}	1.28 ^{def}
80 $\times$ T3 $\times$ 3C	0.369 ^k	7.87 ^{ghijk}	0.428 ^c	29.8 ^l	0.029 ^g	9.64 ^g	0.041 ^e	0.552 ^{fgh}	0.041 ^{fg}	0.458 ^c	0.487 ^k	0.147 ^{ijkl}	1.03 ^{ef}	0.450 ^{fg}
80 $\times$ T3 $\times$ 25C	0.772 ^{ghijk}	16.1 ^{cdefg}	1.03 ^c	82.3 ^{bcd}	0.067 ^g	59.1 ^{de}	0.051 ^{de}	1.02 ^{bc}	0.218 ^{cd}	0.629 ^{abc}	0.790 ^{hijk}	0.189 ^{ijkl}	2.25 ^{cd}	1.14 ^{defg}
80 $\times$ T3 $\times$ 40C	1.00 ^{efghijk}	25.4 ^b	3.88 ^b	94.3 ^{bed}	0.114 ^g	168 ^a	0.091 ^c	1.47 ^a	0.443 ^b	0.640 ^{ab}	1.26 ^{ghijk}	0.470 ^{cdef}	2.83 ^c	3.98 ^b
80 $\times$ T6 $\times$ 3C	0.554 ^{jk}	12.2 ^{efghij}	0.565 ^c	44.3 ^{ijkl}	0.037 ^g	10.3 ^g	0.055 ^{de}	0.733 ^{cdefg}	0.056 ^{efg}	0.600 ^{abc}	0.613 ^{ijk}	0.239 ^{hijk}	1.39 ^{def}	0.763 ^{efg}
80 $\times$ T6 $\times$ 25C	0.931 ^{fghijk}	20.7 ^{bcd}	1.11 ^c	89.1 ^{bcd}	0.064 ^g	80.6 ^{bcd}	0.060 ^{cde}	1.11 ^b	0.273 ^c	0.697 ^{ab}	0.878 ^{ghijk}	0.240 ^{hijk}	2.37 ^{cd}	1.22 ^{defg}
80 $\times$ T6 $\times$ 40C	1.15 ^{fghij}	43.4 ^a	3.70 ^b	98.6 ^{bc}	0.099 ^g	191 ^a	0.158 ^b	1.72 ^a	0.629 ^a	0.737 ^{ab}	1.31 ^{ghijk}	0.611 ^{abc}	5.60 ^b	4.22 ^b
80 $\times$ T12 $\times$ 3C	0.663 ^{hijk}	13.2 ^{defghi}	6.68 ^a	52.4 ^{ghijkl}	0.046 ^g	64.3 ^{cd}	0.061 ^{cde}	0.857 ^{bcd}	0.073 ^{efg}	0.649 ^{ab}	0.707 ^{hijk}	0.341 ^{fghi}	1.62 ^{def}	2.58 ^c
80 $\times$ T12 $\times$ 25C	0.824 ^{fghijk}	17.7 ^{bcd}	0.928 ^c	75.0 ^{bcd}	0.085 ^g	71.0 ^{cd}	0.052 ^{de}	0.952 ^{bcd}	0.297 ^c	0.587 ^{abc}	0.724 ^{hijk}	0.187 ^{ijkl}	1.97 ^{cde}	2.45 ^c
80 $\times$ T12 $\times$ 40C	1.50 ^{ef}	44.5 ^a	6.25 ^a	99.9 ^b	0.088 ^g	197 ^a	0.223 ^a	1.81 ^a	0.720 ^a	0.750 ^a	1.35 ^{ghijk}	0.685 ^{ab}	8.17 ^a	7.86 ^a
Time $\times$ temperature														
T1 $\times$ 3C	0.886 ^f	7.24 ^{cd}	0.369 ^c	33.3 ^f	1.16 ^{bcd}	2.18 ^f	0.051 ^d	0.471 ^f	0.065 ^{de}	0.425 ^{ab}	1.03 ^e	0.278 ^{cde}	1.03 ^{ef}	0.361 ^d
T1 $\times$ 25C	0.969 ^{ef}	7.07 ^{cd}	0.596 ^c	53.6 ^{de}	1.25 ^{abcde}	16.7 ^{ef}	0.054 ^d	0.622 ^{def}	0.091 ^{de}	0.403 ^{ab}	1.13 ^{de}	0.353 ^{bc}	1.67 ^{cde}	0.735 ^d
T1 $\times$ 40C	1.11 ^{ef}	10.6 ^{cd}	0.763 ^c	58.0 ^d	1.37 ^{abcde}	55.5 ^c	0.065 ^{cd}	0.614 ^{def}	0.104 ^d	0.389 ^{bc}	1.40 ^{de}	0.164 ^f	1.77 ^{cd}	0.920 ^d
T3 $\times$ 3C	0.755 ^f	6.22 ^d	0.369 ^c	38.9 ^{ef}	0.930 ^e	6.40 ^f	0.047 ^d	0.447 ^f	0.036 ^e	0.284 ^c	0.982 ^e	0.217 ^{def}	0.907 ^f	0.495 ^d
T3 $\times$ 25C	1.36 ^{de}	11.1 ^{cd}	0.674 ^c	70.2 ^{cd}	0.940 ^e	33.3 ^{de}	0.051 ^d	0.741 ^{de}	0.123 ^{cd}	0.401 ^{ab}	1.65 ^{cd}	0.280 ^{cde}	1.58 ^{cdef}	0.886 ^d
T3 $\times$ 40C	1.64 ^{cd}	17.8 ^b	2.14 ^b	81.7 ^{bc}	1.59 ^{ab}	109 ^b	0.075 ^c	1.02 ^{bc}	0.239 ^b	0.417 ^{ab}	2.14 ^{bc}	0.523 ^a	1.91 ^c	2.265 ^c
T6 $\times$ 3C	0.803 ^f	8.98 ^{cd}	0.440 ^c	54.6 ^{de}	0.925 ^e	7.27 ^f	0.054 ^d	0.551 ^{ef}	0.095 ^{de}	0.407 ^{ab}	1.03 ^e	0.294 ^{cd}	1.22 ^{cdef}	0.598 ^d
T6 $\times$ 25C	1.82 ^{bc}	17.4 ^b	0.731 ^c	87.0 ^{bc}	1.10 ^{cde}	46.0 ^{cd}	0.055 ^{cd}	0.829 ^{cd}	0.181 ^{bc}	0.441 ^{ab}	2.25 ^b	0.363 ^{bc}	1.74 ^{cd}	0.881 ^d
T6 $\times$ 40C	2.03 ^{bc}	30.7 ^a	2.08 ^b	99.8 ^{ab}	1.53 ^{abc}	135 ^a	0.109 ^b	1.23 ^{ab}	0.360 ^a	0.473 ^{ab}	2.63 ^{ab}	0.440 ^{ab}	3.69 ^b	2.97 ^b
T12 $\times$ 3C	0.808 ^f	11.3 ^c	3.49 ^a	57.1 ^{de}	1.00 ^{de}	35.0 ^{cde}	0.058 ^{cd}	0.625 ^{def}	0.117 ^{cd}	0.422 ^{ab}	1.07 ^{de}	0.184 ^{ef}	1.18 ^{def}	1.65 ^c
T12 $\times$ 25C	2.10 ^b	18.5 ^b	0.655 ^c	82.5 ^{bc}	1.43 ^{abcd}	42.9 ^{cd}	0.049 ^d	0.785 ^d	0.203 ^b	0.407 ^{ab}	2.66 ^{ab}	0.468 ^a	1.66 ^{cde}	1.79 ^c
T12 $\times$ 40C	2.56 ^a	34.2 ^a	3.39 ^a	118 ^a	1.71 ^a	155 ^a	0.142 ^a	1.37 ^a	0.406 ^a	0.509 ^a	3.18 ^a	0.524 ^a	5.08 ^a	4.81 ^a
Protein $\times$ temperature														
45 $\times$ 3C	1.10 ^b	5.82 ^d	0.313 ^d	51.6 ^d	1.97 ^c	3.67 ^d	0.053 ^b	0.357 ^e	0.103 ^c	0.186 ^c	1.47 ^c	0.274 ^{bc}	0.927 ^d	0.491 ^d
45 $\times$ 25C	2.34 ^a	10.8 ^c	0.336 ^d	67.1 ^c	2.29 ^b	9.58 ^{cd}	0.051 ^b	0.503 ^d	0.074 ^{cd}	0.205 ^c	3.07 ^b	0.532 ^a	1.18 ^{cd}	0.714 ^{cd}
45 $\times$ 40C	2.55 ^a	14.4 ^b	0.430 ^d	84.5 ^{ab}	3.00 ^a	64.9 ^b	0.057 ^b	0.648 ^c	0.074 ^{cd}	0.217 ^c	3.44 ^a	0.324 ^b	1.51 ^c	1.14 ^b
80 $\times$ 3C	0.522 ^c	11.0 ^c	2.025 ^b	40.3 ^d	0.034 ^d	21.7 ^c	0.052 ^b	0.690 ^c	0.054 ^d	0.583 ^b	0.587 ^d	0.213 ^{cd}	1.25 ^{cd}	1.06 ^{bc}
80 $\times$ 25C	0.784 ^c	16.2 ^b	0.992 ^c	79.6 ^b	0.075 ^d	60.0 ^b	0.053 ^b	0.985 ^b	0.225 ^b	0.621 ^{ab}	0.775 ^d	0.200 ^d	2.14 ^b	1.43 ^b
80 $\times$ 40C	1.12 ^b	32.3 ^a	3.768 ^a	94.5 ^a	0.100 ^d	163 ^a	0.138 ^a	1.47 ^a	0.480 ^a	0.677 ^a	1.24 ^c	0.502 ^a	4.71 ^a	4.33 ^a
Protein $\times$ time														
45 $\times$ T1	1.32 ^d	4.15 ^d	0.301 ^d	32.7 ^e	2.45 ^{ab}	7.24 ^e	0.053 ^c	0.355 ^f	0.079 ^{cd}	0.219 ^d	1.61 ^d	0.348 ^{ab}	1.25 ^{de}	0.459 ^d
45 $\times$ T3	1.79 ^c	7.01 ^d	0.344 ^d	58.5 ^d	2.24 ^b	20.1 ^{de}	0.054 ^c	0.458 ^{ef}	0.032 ^d	0.159 ^d	2.34 ^c	0.411 ^a	0.897 ^e	0.572 ^d
45 $\times$ T6	2.22 ^b	12.6 ^c	0.379 ^{cd}	83.6 ^{ab}	2.31 ^b	31.9 ^{cd}	0.054 ^c	0.552 ^{de}	0.105 ^c	0.203 ^d	3.01 ^b	0.368 ^a	1.31 ^{de}	0.898 ^{cd}
45 $\times$ T12	2.65 ^a	17.6 ^b	0.414 ^{cd}	96.1 ^a	2.69 ^a	44.8 ^c	0.053 ^c	0.646 ^{cd}	0.120 ^c	0.230 ^d	3.68 ^a	0.380 ^a	1.36 ^{de}	1.20 ^c
80 $\times$ T1	0.654 ^f	12.4 ^c	0.851 ^c	64.0 ^{cd}	0.069 ^c	42.4 ^c	0.060 ^c	0.783 ^c	0.094 ^c	0.593 ^{bc}	0.767 ^e	0.183 ^c	1.73 ^{cd}	0.884 ^{cd}
80 $\times$ T3	0.713 ^{ef}	16.4 ^b	1.78 ^b	68.8 ^{cd}	0.070 ^c	79.1 ^b	0.061 ^c	1.01 ^b	0.234 ^b	0.575 ^c	0.848 ^e	0.269 ^b	2.04 ^c	1.85 ^b

Continued

Table 6 (Continued). Mean relative abundance ($\mu\text{g/kg}$) of select volatile compounds by protein concentration, storage time, and storage temperature¹

Item	Pentanal	Hexanal	2-Pentylfuran	Octanal	DMS	DMDS	DMTS	Heptanal	Methional	Nonanal	2 MB	3 MB	1-Octen-3-one	2,4-Decadienal
80×T6	0.879 ^{df}	25.4 ^a	1.79 ^b	77.3 ^{bc}	0.067 ^c	94.1 ^b	0.091 ^b	1.19 ^a	0.319 ^a	0.678 ^a	0.935 ^c	0.363 ^a	3.12 ^b	2.06 ^b
80×T12	0.996 ^{cd}	25.1 ^a	4.62 ^a	75.8 ^{bc}	0.073 ^c	110 ^a	0.112 ^a	1.20 ^a	0.364 ^a	0.662 ^{ab}	0.928 ^c	0.404 ^a	3.92 ^a	4.29 ^a
Temperature														
3C	0.813 ^c	8.45 ^c	1.16 ^b	46.0 ^c	1.00 ^c	12.7 ^c	0.052 ^b	0.523 ^c	0.078 ^c	0.385 ^b	1.03 ^c	0.243 ^c	1.09 ^c	0.776 ^c
25C	1.56 ^b	13.5 ^b	0.664 ^c	73.3 ^b	1.18 ^b	34.7 ^b	0.052 ^b	0.744 ^b	0.150 ^b	0.413 ^{ab}	1.92 ^b	0.366 ^b	1.66 ^b	1.07 ^b
40C	1.84 ^a	23.3 ^a	2.09 ^a	89.5 ^a	1.55 ^a	114 ^a	0.098 ^a	1.06 ^a	0.277 ^a	0.447 ^a	2.34 ^a	0.413 ^a	3.11 ^a	2.74 ^a
Time														
T1	0.991 ^d	8.32 ^d	0.576 ^c	48.3 ^c	1.26 ^{ab}	24.8 ^d	0.057 ^c	0.569 ^c	0.087 ^c	0.406 ^{ab}	1.19 ^d	0.265 ^c	1.49 ^c	0.672 ^c
T3	1.25 ^c	11.7 ^c	1.06 ^b	63.6 ^b	1.15 ^b	49.6 ^c	0.058 ^c	0.737 ^b	0.133 ^b	0.340 ^b	1.59 ^c	0.340 ^b	1.46 ^c	1.21 ^b
T6	1.55 ^b	19.0 ^b	1.08 ^b	80.5 ^b	1.18 ^{ab}	63.0 ^b	0.072 ^b	0.871 ^a	0.212 ^a	0.367 ^{ab}	1.97 ^b	0.366 ^{ab}	2.22 ^b	1.48 ^b
T12	1.82 ^a	21.4 ^a	2.51 ^a	86.0 ^b	1.38 ^a	77.8 ^a	0.083 ^a	0.927 ^a	0.242 ^a	0.446 ^a	2.30 ^a	0.392 ^a	2.64 ^a	2.75 ^a
Protein														
45	2.00 ^a	10.3 ^b	0.360 ^b	67.7 ^a	2.42 ^a	26.0 ^b	0.054 ^b	0.502 ^b	0.084 ^b	0.203 ^b	2.66 ^a	0.377 ^a	1.20 ^b	0.784 ^b
80	0.810 ^b	19.9 ^a	2.26 ^a	71.5 ^a	0.070 ^b	81.6 ^a	0.081 ^a	1.05 ^a	0.253 ^a	0.627 ^a	0.870 ^b	0.305 ^b	2.70 ^a	2.27 ^a

^{a-f}Means in a column not sharing a common superscript are different ($P < 0.05$).

¹DMS = dimethylsulfide; DMDS = dimethyltrisulfide; DMTS = dimethyltrisulfide; 2MB = 2 methylbutanal; 3MB = 3 methylbutanal; T1 = 1 mo storage, T3 = 3 mo storage, T6 = 6 mo storage, T12 = 12 mo storage. 3C = stored at 3°C, 25C = stored at 25°C, 40C = stored at 40°C.

and 80 stored at 40°C became noticeably browner at 3 mo, whereas it was 6 mo before both MPC 45 and 80 stored at 25°C became noticeably brown. This visual color change corresponded to an increase in furosine content of approximately 400 to 500% (Figure 2).

Solubility of MPC During Storage. Temperature, storage time, and protein level had an effect on solubility of MPC powders ($P < 0.05$) (Figure 3). The solubility of MPC 45 stored at 3 and 25°C was reduced by 9 and 15%, respectively, over 12 mo whereas the solubility of MPC 45 stored at 40°C decreased by approximately 28% (Figure 3). Temperature had a more pronounced effect on the solubility of MPC 80 than time. The solubility of MPC 80 stored at 3°C was reduced by approximately 15% after 12 mo, whereas the solubility of MPC 80 stored at 25°C was reduced by 51% and at 40°C, solubility was reduced by 88%.

The solubility of MPC has been extensively studied (Schuck et al., 1994; Anema et al., 2006; Havea 2006; Baldwin and Truong, 2007; Le et al., 2011a,b) Interestingly, whereas past studies agree that solubility of stored MPC is an issue, the solubility of MPC during storage is highly variable and dependent on many factors. For instance, Havea (2006) produced 6 MPC 85 powders, one of which had a solubility of 53% after 2 d, whereas another, which had had 30% of its calcium replaced by sodium ions, had a solubility of 98% after 7 wk of storage at 20°C. Anema et al. (2006) tested the solubility of MPC 85 at temperatures ranging from 20° to 50°C of which, after 60 d of storage, the 20°C treatment retained 100% solubility, whereas the 50°C treatment dropped to approximately 20% solubility after 10 d. Le et al. (2011a) studied the effect of relative humidity (RH) and temperature on MPC 80 solubility and demonstrated a 50% drop in solubility of MPC 85 stored at 25°C with a RH of 44% (the RH used in this study) after 12 wk. The solubility of MPC 80 stored at 40°C with a RH of 44% decreased to approximately 20% after 4 wk. Whereas the variables in these previous studies vary, the current study solubility results are consistent with past studies, especially Le et al. (2011a), whose storage conditions matched those of the current study (except for the lack of a 3°C storage treatment). Ultimately, the purpose of experiment 2 was to determine the shelf life of MPC 80 stored under specific conditions, and although flavor is of utmost importance to the shelf life of any food product, usability, specifically solubility in the case of MPC 80 must also be taken into account. These results confirm that temperature abuse during storage for a short period renders MPC virtually unusable in liquid suspensions. Solubility and furosine content were inversely correlated ($R^2 = 0.84\text{--}0.97$, $P < 0.05$), which suggests that Maillard browning products

Table 7. Sensory profiles of milk protein concentrates (MPC45 and 80) stored for 0, 1, 3, 6, and 12 mo at 3, 25, and 40°C¹

Item ²	Aroma intensity	Sweet aromatic	Cooked milky	Tortilla/corn chip	Cardboard	Cabbage	Burnt sugar	Animal	Astringency
45 T0	2.5 ^c	3.4 ^a	2.7 ^{bc}	0.5 ^h	1.0 ^k	ND ³	ND	ND	1.8 ⁱ
45 3C T1	2.1 ^{defg}	2.2 ^{bc}	3.0 ^{abc}	ND	1.1 ^{jk}	ND	ND	ND	2.2 ^{ghi}
45 3C T3	2.3 ^{cdef}	1.7 ^{cd}	2.5 ^{cde}	ND	1.5 ^{hijk}	ND	ND	ND	2.3 ^{fgh}
45 3C T6	2.0 ^{defgh}	1.6 ^{de}	2.3 ^{def}	ND	1.7 ^{fghi}	ND	ND	ND	2.2 ^{ghi}
45 3C T12	2.0 ^{defgh}	1.1 ^{ef}	2.0 ^{efgh}	0.9 ^{fgh}	2.1 ^{efg}	ND	ND	ND	2.3 ^{fgh}
45 25C T1	2.0 ^{defgh}	2.5 ^b	3.3 ^a	ND	1.0 ^k	ND	ND	ND	2.3 ^{fgh}
45 25C T3	2.3 ^{cdef}	2.2 ^{bc}	3.1 ^{ab}	ND	1.8 ^{fghi}	ND	ND	ND	2.3 ^{fgh}
45 25C T6	2.1 ^{defg}	1.4 ^{de}	2.7 ^{bc}	ND	2.1 ^{efg}	ND	1.3 ^{cd}	ND	2.2 ^{ghi}
45 25C T12	2.3 ^{cdef}	0.8 ^f	2.0 ^{efgh}	0.9 ^{fgh}	2.9 ^{cd}	ND	1.8 ^{bc}	0.5 ^c	2.0 ^{hi}
45 40C T1	2.3 ^{cdef}	0.8 ^f	2.5 ^{cde}	ND	1.3 ^{ijk}	ND	0.8 ^d	ND	2.5 ^{efgh}
45 40C T3	2.0 ^{defgh}	1.2 ^{def}	1.9 ^{fgh}	ND	1.7 ^{fghi}	ND	1.3 ^{cd}	ND	2.6 ^{defg}
45 40C T6	3.2 ^b	1.1 ^{ef}	1.8 ^{fghi}	0.6 ^{gh}	3.0 ^{cd}	ND	2.1 ^{ab}	ND	3.0 ^{bcde}
45 40C T12	2.8 ^{bc}	ND	0.6 ^l	1.3 ^{ef}	3.8 ^{ab}	ND	2.5 ^a	1.8 ^b	2.8 ^{cdef}
80 T0	1.8 ^{fghi}	1.4 ^{de}	2.0 ^{efgh}	1.3 ^{ef}	1.8 ^{fghi}	ND	ND	ND	2.5 ^{efg}
80 3C T1	1.5 ^{hi}	ND	1.9 ^{fgh}	1.6 ^e	1.3 ^{ijk}	ND	ND	ND	3.1 ^{bcd}
80 3C T3	1.6 ^{ghi}	ND	2.1 ^{efg}	1.6 ^e	1.6 ^{ghij}	ND	ND	ND	3.1 ^{bcd}
80 3C T6	1.3 ⁱ	ND	1.8 ^{fghi}	1.4 ^{ef}	2.2 ^{ef}	ND	ND	ND	3.1 ^{bcd}
80 3C T12	1.5 ^{hi}	ND	1.3 ^{ijk}	1.6 ^e	2.5 ^{de}	ND	ND	0.8 ^c	3.1 ^{bcd}
80 25C T1	1.6 ^{ghi}	ND	1.5 ^{hij}	1.5 ^e	1.3 ^{ijk}	ND	ND	ND	3.3 ^{bc}
80 25C T3	1.9 ^{defgh}	ND	1.5 ^{hij}	1.7 ^e	2.1 ^{efg}	ND	ND	ND	3.1 ^{bcd}
80 25C T6	2.8 ^{bc}	ND	1.6 ^{ghi}	1.8 ^{de}	2.8 ^{cd}	1.0 ^{cd}	ND	0.7 ^c	2.9 ^{cde}
80 25C T12	2.4 ^{cde}	ND	1.3 ^{ijk}	2.3 ^{cd}	3.1 ^c	1.5 ^{bc}	ND	1.6 ^b	3.5 ^b
80 40C T1	1.5 ^{hi}	ND	1.1 ^{kl}	2.4 ^c	2.0 ^{efgh}	ND	1.2 ^d	0.7 ^c	3.5 ^b
80 40C T3	2.5 ^c	ND	0.8 ^{kl}	2.8 ^{bc}	2.8 ^{cd}	0.6 ^d	2.2 ^{ab}	1.8 ^b	4.2 ^a
80 40C T6	3.8 ^a	ND	ND	3.3 ^b	3.3 ^{bc}	1.8 ^b	ND	2.8 ^a	4.4 ^a
80 40C T12	4.0 ^a	ND	ND	4.4 ^a	4.3 ^a	2.8 ^a	ND	3.1 ^a	4.5 ^a

^{a–l}Means in a column not sharing a common superscript are different ($P < 0.05$).

¹Attribute intensities were scored on a 0- to 15-point universal intensity scale (Meilgaard et al., 2009). Most dried ingredient flavors fall between 0 and 4 (Croissant et al., 2009; Wright et al., 2009).

²T1 = 1 mo storage, T3 = 3 mo storage, T6 = 6 mo storage, T12 = 12 mo storage. 3C = stored at 3°C, 25C = stored at 25°C, 40C = stored at 40°C.

³ND = not detected.

are reacting with AA, causing cross-linking of proteins and subsequent solubility loss.

Instrumental Volatile and Descriptive Analysis of MPC During Storage. Storage time, temperature, and protein content also affected volatile compounds and flavor of MPC powders. Aldehydes (hexanal, heptanal, octanal, nonanal, methional, 2 and 3-methylbutanal, E,E 2,4 decadienal) and other lipid oxidation products such as 2-pentylfuran and 1-octen-3-one and sulfur-containing volatile compounds (dimethyl sulfide, dimethyl disulfide, dimethyl trisulfide) increased with time and as storage temperature increased in both MPC 45 and MPC 80 (Table 6).

Generally, MPC 45 stored at lower temperatures for less time were associated with sweet aromatic and cooked milky flavors and a sweet taste. As the powders aged, aroma intensity, cardboard, animal, and tortilla/corn chip flavors increased. The MPC 45 stored at 25°C exhibited a burnt sugar flavor after 6 mo, whereas MPC 45 stored at 40°C exhibited this after only 1 mo of storage. No burnt sugar flavor was detected in MPC 45 stored at 3°C. The MPC 45 stored at 25 and 40°C exhibited an animal flavor after 12 mo, whereas this

flavor was not detected in product stored at 3°C (Table 7). Because burnt sugar flavor followed the trend of Maillard reactions (furosine), it seems likely that this flavor can be attributed to Maillard reaction products such as sotolon or furaneol. These compounds are formed by thermal treatment of sugars and have distinct burnt sugary aromas, and have been previously reported in stored SMP (Karagul-Yuceer et al., 2002), sweet whey powder (Mahajan et al., 2004), and whey protein (Carunchia Whetstine et al., 2005) extracted by solvent extraction. These compounds were also odor active in fresh MPC 80 in experiment 1 by solvent extraction, but not by SPME, so they likely were not well extracted by SPME. Additional work would be needed to confirm this hypothesis. Cardboard flavor in whey protein concentrates has been sourced to pentanal, heptanal, and nonanal in the presence of DMTS and 1-octen-3-one, and lipid oxidation compound increases are the likely source of cardboard flavor in the stored milk proteins (Whitson et al., 2010).

Similar to MPC 45, MPC 80 stored at lower temperatures for less time were associated with sweet aromatic and cooked milky flavors. As the powders aged, overall

aroma intensity, cardboard, animal, cabbage, and tortilla/corn chip flavors increased. This result is consistent with an increase in the lipid oxidation and protein degradation volatile compounds that cause these off-flavors. Cabbage flavor in whey protein isolate has been sourced to DMTS (Wright et al., 2006). Compounds attributed to cardboard and cabbage flavor increased during storage (pentanal, heptanal, nonanal, 1-octen-3-one, and DMTS, Table 6). The compounds, E,E 2,4 decadienal, 3-methylbutanal, methional, and hexanal,

possible sources of corn chip, fatty, and potato flavors, increased during storage in MPC 80 and 45. The MPC 80 stored at 3°C exhibited an animal flavor after 12 mo, whereas MPC 80 stored at 25 and 40°C exhibited this flavor after 6 and 1 mo, respectively. The MPC 80 stored at 25°C exhibited a cabbage flavor after 6 mo, but burnt sugar flavor was not detected. The MPC 80 stored at 40°C exhibited cabbage flavor after 3 mo and burnt sugar flavor after 1 mo. These 2 flavors were not detected in MPC 80 stored at 3°C. Unlike the MPC

Figure 4. Principal component biplot of sensory attributes of milk protein concentrate (MPC) 45 stored at 3, 25, and 40°C for 0, 1, 3, 6, and 12 mo. T0 = no storage, T1 = 1 mo storage, T3 = 3 mo storage, T6 = 6 mo storage, T12 = 12 mo storage. 3C = stored at 3°C, 25C = stored at 25°C, 40C = stored at 40°C. Color version available online.

Figure 5. Principal component biplot of sensory attributes of milk protein concentrate (MPC) 80 stored at 3, 25, and 40°C for 0, 1, 3, 6, and 12 mo. T0 = no storage, T1 = 1 mo storage, T3 = 3 mo storage, T6 = 6 mo storage, T12 = 12 mo storage. 3C = stored at 3°C, 25C = stored at 25°C, 40C = stored at 40°C. Color version available online.

45, MPC 80 stored at all 3 temperatures exhibited the tortilla/corn chip flavor immediately after production rather than following storage.

The principal component biplots generated from sensory analysis and relative abundance means can be used to approximate similarities in the stage of shelf-life of the MPC 45 and 80 powders by the increase in animal, tortilla/corn chip, cardboard, cabbage, and aroma intensity and increase in corresponding lipid oxidation and heat-induced volatile compound concentration over time (Figures 4, 5, 6, and 7). For instance, MPC 45 stored at 3°C for 6 mo was similar in flavor to MPC 45 stored at 25°C for 3 mo and MPC 45 stored at 40°C for 1 mo, whereas MPC 45 stored at 25°C stored for 6 mo had a similar flavor profile to MPC 45 stored at 40°C

for 3 mo. It is important to note that MPC 45 stored at 3°C for 12 mo never developed the off-flavor profile of MPC 45 stored at 25°C by 6 mo and MPC 45 stored at 40°C by 3 mo. Milk protein concentrate (45%) stored at 40°C for 6 mo was more highly associated with tortilla, cardboard, animal, and burnt sugar flavors than MPC 45 stored at 25°C for 12 mo.

The effect of storage temperature on the flavor of MPC 80 was more pronounced than its effect on MPC 45. The MPC 80 stored at 3°C for 12 mo was similar in flavor to MPC 80 stored at 25°C for 6 mo and MPC 80 stored at 40°C for 1 mo, whereas MPC 80 stored at 25°C for 12 mo had a similar flavor profile to MPC 45 stored at 40°C for 3 mo. The effect of temperature is especially important to consider during transport over-

Previous studies on MPC have focused on functionality, primarily solubility, rather than instrumental volatile and sensory properties. Whereas a few studies have been published on sensory properties of milk proteins, to the authors' knowledge, the effect of storage on milk protein flavor has not been previously addressed. Compounds associated with lipid oxidation and Maillard browning are the primary source of flavor in milk proteins and storage increased the concentration of many of these compounds. Although lipid content in the powders is relatively low, lipid oxidation products have a low odor threshold and are well established as

contributors to off flavor in dried dairy ingredients (Carunchia Whetstine et al., 2005; Drake et al., 2009). Aldehydes (hexanal, heptanal, octanal, nonanal, methional, 2 and 3-methylbutanal, E,E 2,4 decadienal) and other lipid oxidation products such as 2-pentylfuran and 1-octen-3-one and sulfur-containing volatile compounds increased over time and as storage temperature increased in both MPC 45 and MPC 80. Drake et al. (2006) compared flavor stability of SMP stored at 21 or 35°C, and Wright et al. (2009) studied the flavor stability of whey protein concentrates (80%) and isolates across 18 mo storage at 21°C. Wright et al. (2009) documented that lipid oxidation increased during storage of whey proteins, confirming the involvement of lipid oxidation in dried dairy protein ingredients during storage. Drake et al. (2006) also documented lipid oxidation compounds in SMP stored at 21°C as well as increased Maillard reaction production in SMP stored at 35°C. They documented that different flavors as well as different volatile compounds formed in milk powders

stored at different temperatures. These results collectively are consistent with the results from the current study with MPC.

CONCLUSIONS

Flavor is of utmost importance to the success of milk protein products. The flavor of different milk protein products varied depending on the composition, manufacturing process, and storage. Milk proteins were characterized by sweet aromatic, sour aromatic, cardboard, potato/brothy, brothy/animal, tortilla/corn chip, soapy, and fatty flavors. Tortilla flavor is a defining flavor of milk protein ingredients and many of the odor active volatile compounds found in corn tortillas, corn chips, and taco shells were also odor active compounds in milk proteins. Key flavor active compounds in milk proteins were 1-hexen-3-one, 2-acetyl-1-pyrroline, benzothiazole, 1-octadecanol, homofuraneol, 1-octen-3-one, 1-hexen-3-one, sotolon, homofuraneol, 4-hexen-3-one, 1-cyclohexen-3-one, dimethyl disulfide,

dimethyl trisulfide, guaiacol, methional, hexanal, heptanal, octanal, nonanal, decanal, 2-aminoacetophenone, acetic, propionic, butanoic, pentanoic, and hexanoic acids, gamma-decalactone, and delta-dodecalactone. The MPC 45 and 80 stored at lower temperatures for less time were associated with sweet aromatic and cooked milky flavors and a sweet taste. As the powders aged, aroma intensity, cardboard, animal, burnt sugar, and tortilla/corn chip flavors increased. Storage temperature played a large role in the shelf life of MPC. At high temperature (40°C), 1 mo storage was sufficient to reduce solubility of MPC 45 and 80 below the solubility of 3 and 25°C stored MPC 45 and 80. Storage at high temperatures (40°C) also reduced the flavor related shelf life of MPC 45 by 50%, and by 75% in MPC 80 compared with MPC 45 and 80 stored at 25°C. Based on sensory and functional changes, shelf life of MPC at temperatures greater than 40°C is less than 3 mo, up to 6 mo at 25°C, and more than 12 mo at 3°C. Special care must be taken during shipping and storage or the effective shelf-life of MPC is drastically reduced.

Figure 7. Principal component biplot of relative abundance of selected volatile compounds of milk protein concentrate (MPC) 80 stored at 3, 25, and 40°C for 0, 1, 3, 6, and 12 mo. T0 = no storage, T1 = 1 mo storage, T3 = 3 mo storage, T6 = 6 mo storage, T12 = 12 mo storage. 3C = stored at 3°C, 25C = stored at 25°C, 40C = stored at 40°C. DMS = dimethyl sulfide; DMDS = dimethyl disulfide; DMTS = dimethyl trisulfide; 2MB = 2 methylbutanal; 3MB = 3 methylbutanal. Color version available online.

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