Comparison of composition and sensory properties of 80% whey protein and milk serum protein concentrates¹

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ABSTRACT

Milk serum protein concentrates (SPC) are proteins found in cheese whey that are removed directly from milk. Because SPC are not exposed to the cheese-making process, enzymatic or chemical reactions that can lead to off-flavors are reduced. The objectives of this study were to identify and compare the composition, flavor, and volatile components of 80% protein SPC and whey protein concentrates (WPC). Each pair of 80% SPC and WPC was manufactured from the same lot of milk and this was replicated 3 times. At each replication, spray-dried product from each protein source was collected. Commercial 80% WPC were also collected from several manufacturers for sensory and volatile analyses. A trained sensory panel documented the sensory profiles of the rehydrated powders. Volatile components were extracted by solid-phase microextraction and solvent extraction followed by solvent-assisted flavor evaporation with gas chromatography-mass spectrometry and gas chromatography-olfactometry. Consumer acceptance testing of acidified 6% protein beverages made with 80% SPC and WPC produced in the pilot plant and with WPC from commercial sources was conducted. The SPC was lower in fat and had a higher pH than the WPC produced in the pilot plant or commercial WPC. Few sensory differences were found between the rehydrated SPC and WPC manufactured in this study, but their flavor profiles were distinct from the flavor of rehydrated commercial WPC. The pilot-plant WPC had higher concentrations of lipid oxidation products compared with SPC, which may be related to the higher fat content of WPC. There was a large difference in appearance between 80% SPC and WPC:

solutions of SPC were clear and those of WPC were opaque. Concentrations of lipid oxidation products in commercial WPC were generally higher than those in pilot-plant SPC or WPC. Sensory profiles of the peach-flavored protein beverage included cereal, free fatty acid, and soapy flavors and bitter taste in beverages made from pilot-plant products, whereas cardboard flavors were detected in those made with commercial WPC. Consumer liking scores for the beverages made with SPC were ranked highest or equally high with beverages made with WPC for aroma, appearance, and mouthfeel, but the beverages made with SPC had lower flavor and overall liking scores compared with beverages made with 3 of the 4 WPC.

Key words: serum protein, whey protein, composition, flavor

INTRODUCTION

Whey proteins are highly functional and nutritious proteins used in a variety of products. The 2 most commonly used value-added forms of whey protein in industry are whey protein concentrate (**WPC**) and whey protein isolate (**WPI**). Whey protein concentrate contains 34 to 89% protein and WPI contains at least 90% protein (USDEC, 2008). Whey proteins have many functional characteristics such as high solubility, dispersibility, water binding, foaming, whipping, emulsification, gelation, and buffering power (Davis and Foegeding, 2007); they are used in an increasing number of food applications.

Whey proteins should ideally have a bland flavor to facilitate application in foods (Drake et al., 2009), but the flavor of these products is highly variable because of the original whey source, processing, and storage (Mahajan et al., 2004; Carunchia Whetstine et al., 2005a; Gallardo-Escamilla et al., 2005; Wright et al., 2006, 2009). Off-flavors may carry through into ingredient applications and thus limit food applications (Drake, 2006; Drake et al., 2009; Wright et al., 2009). Recent research has demonstrated the feasibility of removing whey proteins from skim milk before cheese making

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(Nelson and Barbano, 2005a,b). Before cheese making, these proteins are called serum proteins (**SP**). Because of a lack of familiarity with the term SP, these proteins have also been referred to as “native” whey proteins (Maubois, 2002). Nelson and Barbano (2005a) developed a microfiltration and ultrafiltration method to remove SP from skim milk and subsequently demonstrated that removal of SP from milk before cheese making had no effect on quality of Cheddar cheese (Nelson and Barbano, 2005b). They also reported higher fat recovery and cheese yield in cheeses made from milk with a 65% reduced serum protein content. Because they are not subjected to a cheese make procedure, serum protein concentrates (**SPC**) are not exposed to as many processing conditions that may alter flavor formation, and thus may have the potential to offer a blander flavor profile. Evans et al. (2009) reported few sensory or volatile component differences between 34% SPC and WPC, but distinct compositional differences between these 2 products that might affect functionality were noted, and there were visible appearance differences between the 2 proteins when rehydrated in solution. The 80% WPC are distinct in composition and application from 34% WPC. More obvious differences between whey and serum protein concentrates may be evident. Punidadas and Rizvi (1998) demonstrated that foaming properties of a pH 7.0, dialyzed 5% concentrated serum protein product were comparable to values cited for egg white and better than those cited for products made from cheese whey. The objective of this research was to characterize and compare the composition and sensory properties of 80% WPC and 80% SPC made from the same milk. Sensory and instrumental methods were applied to pilot-plant 80% WPC and SPC manufactured in the pilot plant. A second objective was to compare the composition and sensory properties of the SPC and WPC made in our study with those of 5 commercial 80% WPC.

MATERIALS AND METHODS

Experimental Design

One batch of whole, raw bovine milk (about 1,900 kg) was received from the Cornell University dairy farm, and the milk was divided into 2 portions. One portion of the milk was used for Cheddar cheese manufacture to produce 80% WPC. The other portion was centrifugally separated at 4°C into raw cream and raw skim, and the skim milk was used to produce 80% SPC. This was replicated 3 times with different batches of raw milk. The timeline for the processing was as follows: raw whole milk was received on Monday and split into 2 portions. On Monday, one portion of the

whole raw milk was pasteurized and used for Cheddar cheese making. The Cheddar whey was pasteurized and run through a cream separator and cooled to 4°C. The other portion of raw milk was cold separated to produce raw skim milk. On Tuesday, the raw skim milk from Monday was pasteurized, cooled to 4°C, and stored; the separated pasteurized whey was first UF (stage 1) and then diafiltered (**DF**; stage 2) to produce a liquid 80% WPC that was frozen at -40°C. On Wednesday, the pasteurized skim was microfiltered (**MF**) to produce 65% SP reduced casein concentrate and MF permeate. The MF permeate was cooled to 4°C and held overnight. The MF retentate was further DF twice to produce 95% SP reduced native micellar casein concentrate; its characteristics will be reported in a separate publication. On Thursday, MF permeate was UF (stage 1) and DF (stage 2) to produce 80% liquid SPC that was frozen at -40°C. Frozen 80% liquid SPC and WPC were thawed and then spray dried. Commercial samples of 80% WPC were obtained from 5 companies (2 different lots per company, commercial 25-kg bags) and analyzed under the same conditions for comparison with the samples produced in this study.

WPC Manufacture

The cheesemaking procedure for WPC production was as described in Evans et al. (2009). The average fat and protein contents of the milk for cheese making were $3.73\% \pm 0.05$ and $3.05\% \pm 0.04$, respectively. Whey was drained at pH of 6.35, immediately pasteurized at 72°C for 16 s, continuously cooled to 50°C, and processed with a cream separator. The fat content of the whey before and after separation was $0.24\% \pm 0.03$ and $0.05\% \pm 0.02$, respectively. After separation, the whey was cooled to 4°C and held overnight at $\leq 4^\circ\text{C}$.

Approximately 640 kg of separated whey was heated to 50°C and processed with the UF system in batch recirculation mode using a polyethersulfone spiral-wound UF membrane (model 3838, GEA Niro Inc., Hudson, WI) with a nominal pore size of 10,000 Da. Before processing, the UF membrane was cleaned following the same procedure described in Evans et al. (2009). The water flux was typically about 52 kg/m² per hour. Whey was UF for 2.3 h to achieve a concentration factor of approximately 5.2× and the protein content of the retentate measured by infrared spectrophotometer (Lactoscope FTIR, Delta Instruments, Drachten, the Netherlands) was 47% of protein plus lactose in the retentate. The UF retentate was diluted with pasteurized reverse osmosis water at 50°C to bring the weight back to the original total weight of the starting whey for DF. The membrane was not cleaned before proceeding to the DF stage. The mixture was recirculated through

the membrane for 5 min to ensure complete mixing. Then, the diluted UF retentate was DF to achieve a concentration factor of 6.9 $\times$. Diafiltration was continued until the protein content of the retentate measured by infrared spectrophotometer was 90% of protein plus lactose in the retentate. The total time of DF was 2.4 h. At the end of processing, the retentate drained from the dead volume of the system was mixed with the retentate in the feed vat, mixed, and sampled. The final liquid retentate protein concentrate was weighed, frozen at -40°C in 20-L plastic containers, and stored until spray drying. After producing the 80% WPC liquid concentrate, the UF system was cleaned as described in Evans et al. (2009). The fouled water flux (kg/m^2 per hour) was typically about 32% of the clean water flux (17 vs. 52 kg/m^2 per hour). It was possible to restore the flux after cleaning. The clean water flux after final cleaning was typically about 52 kg/m^2 per hour.

SPC Manufacture

Raw whole milk was processed using the procedures described in Evans et al. (2009) to produce SPC. Skim milk (about 1,100 kg) was heated to 50°C with a DeLaval model A3 plate heat exchanger (DeLaval, Tumba, Sweden) and microfiltered using a pilot-scale, uniform transmembrane pressure MF system (Tetra Alcross MFS-7, TetraPak Filtration Systems, Aarhus, Denmark) equipped with ceramic Membralox membranes (pore diameter: 0.1 μm ; surface area: 1.7 m^2). For a more detailed description of the uniform transmembrane pressure system and process, see Zulewska et al. (2009). The total time of milk processing was about 8.3 h. The MF permeate was cooled to 4°C and held overnight.

On the following day, MF permeate (705 kg) was weighed into a vat, heated to 50°C using a DeLaval model A3 plate heat exchanger, and UF processed. The conditions and parameters of UF processing of MF permeate were the same as for whey processing. The total processing time of first stage was about 3 h at a concentration factor 5.5 $\times$, and the protein content of the retentate measured by infrared spectrophotometer was 41% of protein plus lactose in the retentate. The DF time was 3.3 h and DF (6.9 $\times$) was continued until the protein content of the retentate measured by infrared spectrophotometer was 88% of protein plus lactose in the retentate. The final retentate was frozen at -40°C in 20-L plastic containers and stored until spray drying.

Spray Drying

The conditions for spray drying were the same as described in Evans et al. (2009), except that feed material

(about 55 kg) was kept frozen at -40°C before spray drying. Before drying, the frozen retentate in 20-L plastic containers was thawed at 4°C for 48 h and then the containers were immersed in 50 to 55°C water for 1 to 2 h to allow for complete thawing. The total time of the spray-drying run was approximately 4 h.

Chemical Analyses

Milk for cheese making was analyzed using an infrared spectrophotometer (Lactoscope FTIR, Delta Instruments) for fat content and true protein content (Kaylegian et al., 2006). The fat content of unseparated and separated whey was determined by ether extraction (AOAC, 2000; method 989.05; 33.2.26).

Fresh samples of the final liquid 80% WPC and liquid 80% SPC were analyzed for fat, TS, total N, and NPN content using ether extraction (AOAC, 2000; method 989.05; 33.2.26), forced-air oven drying (AOAC, 2000; method 990.20; 33.2.44), Kjeldahl (AOAC, 2000; method 991.20; 33.2.11), and Kjeldahl (AOAC, 2000; method 991.21; 33.2.12), respectively. The pH of final liquid 80% WPC and 80% SPC was measured with an electrode (model HA 405, Mettler Toledo, Columbus, OH) that was standardized at pH 6.97 and 4.06 at 50°C and kept immersed in 3 *M* KCl at 50°C between readings to keep its temperature equal to the temperature of the buffers and samples.

The commercial 80% WPC and experimental 80% WPC and 80% SPC were reconstituted to 10% solids, and the liquids were analyzed for fat and total N by the methods indicated above. The pH was measured with an electrode (model Electrolyte 9823, Mettler Toledo) that was standardized at pH 7.01 and 4.00 at 22°C . The reconstituted samples (80% WPC, 80% SPC, and five 80% commercial WPC powders) were analyzed for TS content by forced-air oven drying (AOAC, 2000; method 990.20; 33.2.44), and moisture content was calculated. Experimental 80% WPC and 80% SPC were analyzed for NPN using Kjeldahl (AOAC, 2000; method 991.21; 33.2.12). The glycomacropeptide (**GMP**) content (which is soluble in 12% TCA) of WPC was calculated as a difference in NPN content between WPC and SPC powders on a dry basis.

Contents of Ca, P, K, Mg, Na, and S were measured in duplicate by the North Carolina State University Analytical Services Laboratory (Raleigh, NC) using a standard dry ash method with inductively coupled plasma optical emission spectroscopy (**ICP-OES**). For this analysis, 2.5 g of WPC or SPC was ashed overnight at 500°C in a muffle furnace, cooled, and mixed with approximately 1.5 mL of distilled water and 4 mL of 6 *N* HCl (VWR, West Chester, PA). The mixture was heated to dryness on a steam plate to remove silicates,

transferred to a 50-mL volumetric flask with approximately 4 mL of 6 *N* HCl, and brought to volume with distilled water for ICP-OES analysis.

Microbiological Analyses

Samples of pasteurized skim milk for cheese making before and after standardization, pasteurized whey from cheese making, pasteurized skim milk for MF, liquid WPC, liquid SPC, and spray-dried powders were taken for total bacterial and coli counts (Wehr and Frank, 2004; methods 6.020 and 7.020, respectively).

SDS-PAGE

The gels and the milk samples were prepared using the same approach as described in Zulewska et al. (2009). Fresh liquid (after second DF) samples of 80% SPC and 80% WPC were diluted with sample buffer to keep the protein content per sample similar to that of the milk sample. Milk was loaded (8.5 μ L) onto an SDS-PAGE gel, and the samples of 80% SPC and 80% WPC were loaded to achieve a content of proteins per lane similar to that of the milk loading. The gels were scanned and analyzed with USB GS 800 Densitometer using Quantity One 1-D Analysis Software (Bio-Rad Laboratories Inc., Hercules, CA) as described by Evans et al. (2009).

Color Analysis of Liquid 80% WPC and 80% SPC

The Hunter L (lightness), a (red-green), and b (yellow-blue) values for the fresh 80% WPC and 80% WPC liquids (after DF) were determined in duplicate with a MacBeth Color-Eye spectrophotometer (model 2020, Kollmorgen Instruments Corp., Newburgh, NY) with Optiview software from the same company. Hunter values were computed from the diffuse reflectance data in the 360 to 740 nm range, at 20-nm intervals, based on illuminant A. The measurements were done at 23 to 25°C.

Sample Shipping and Storage

Products were shipped on ice by overnight carrier to North Carolina State University (Raleigh). Subsamples (1 kg) were stored in glass jars at -80°C and analyzed within 3 mo of receipt. For all analyses, samples were reconstituted at 10% solids. Sensory analysis samples were reconstituted using deodorized water (prepared by boiling 4 L of distilled water until its volume was decreased by one-third). Instrumental analysis samples were reconstituted using HPLC-grade water (EMD Chemicals Inc., Gibbstown, NJ). Five commercial 80%

WPC (less than 2 mo old) were received on 2 separate occasions and reconstituted using the same methods for comparison with pilot-plant 80% WPC and SPC.

Sensory and Volatile Flavor Compound Analyses

Sensory testing was conducted in compliance with North Carolina State University Institutional Review Board for Human Subjects approval. A trained sensory panel ($n = 10$; 7 females, 3 males, ages 22 to 37 yr) evaluated the flavor attributes of the reconstituted serum and whey proteins and commercial powders using a previously published lexicon for dried dairy ingredients (Drake et al., 2003, 2009; Wright et al., 2009).

Each panelist had more than 150 h of experience with descriptive analysis of dried dairy ingredients and additional training and experience with WPC and SPC aroma and flavor. Consistent with Spectrum descriptive analysis training, panelists were presented with reference solutions of sweet, sour, salty, and bitter tastes to learn to use the universal intensity scale (Meilgaard et al., 1999; Drake and Civille, 2003). Panelists then evaluated and discussed flavor attributes of rehydrated dairy ingredients with a focus on 80% WPC and SPC. Analysis of variance of data collected in preliminary sessions confirmed that the panel and the panelists could consistently identify and scale flavor attributes. Attribute intensities were scaled using the 0 to 15 universal intensity scale characterized by the Spectrum descriptive analysis method (Meilgaard et al., 1999; Drake and Civille, 2003).

Reconstituted products (approximately 30 mL) were dispensed into lidded 58-mL soufflé cups with 3-digit codes and stored at 3°C overnight. Products were tempered to 20°C and served at this temperature with spring water and unsalted crackers for palate cleansing. Panelists evaluated each sample individually in booths in a positive-air-pressure room dedicated to sensory analysis. Each product replication was evaluated by each panelist in duplicate in a randomized balanced block design. Products were scored using paper ballots or computerized ballots using Compusense 5 release 4.8 (Compusense Inc., Guelph, Canada).

Volatile Compound Extraction

Solid-Phase Microextraction GC-MS of WPC and SPC Powders. Volatile compounds were extracted by solid-phase microextraction (SPME) and subsequently separated and identified by GC-MS in triplicate using a modified method of Wright et al. (2006). Products manufactured in this study (pilot-plant 80% SPC and WPC) and commercial products were evaluated by SPME GC-MS. Spray-dried powders

were reconstituted at 10% solids with 10% NaCl (Fisher Scientific, Fairlawn, NJ) and 10 μ L of internal standard solution (2-methyl-3-heptanone in methanol at 81 ppm; Sigma Aldrich, Milwaukee, WI) 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 6890N GC with 5973 inert mass selective detector (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 divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber at 31 mm with 4-s pulsed agitation at 250 rpm. Fibers were injected for 5 min at a depth of 50 mm.

The GC method used an initial temperature of 40°C for 3 min with a ramp rate of 10°C/min to 90°C held for 0 min, followed by a ramp rate of 5°C/min to 200°C held for 10 min, followed by a ramp rate of 20°C/min to 250°C held for 5 min. The SPME fibers were introduced into the split/splitless injector at 250°C. An Rtx-5ms column (Rtx-5ms 30 m length $\times$ 0.25 mm i.d. $\times$ 0.25 μ m film thickness; Restek, Bellefonte, PA) 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 (NIST, 2005). 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; (Van den Dool and Kratz, 1963).

Direct Solvent Extraction of WPC and SPC Powders. Solvent extraction of pilot-plant 80% SPC and WPC was conducted in duplicate using the modified methods of Carunchia Whetstone et al. (2005a). One hundred grams of powder was divided into 4 Teflon bottles (Nalgene, Rochester, NY; capacity of 250 mL) with Tefzel closures (Nalgene). Five milliliters of HPLC-grade water (EMD Chemicals Inc.) was added to each bottle, along with 20 μ L of internal standard at 81 ppm (2-methyl-3-heptanone, 2-methylpentanoic acid in methanol; Sigma Aldrich). Seventy-five milliliters of diethyl ether (EMD Chemicals Inc.) was added to each bottle. The bottles were then shaken for 30 min on a Roto mix (type 5080, Thermolyne, Dubuque, IA) at high speed and centrifuged at $1,459 \times g$ for 10 min to separate the solvent phase from the mixture. After centrifugation, the solvent phase containing the

extracted volatile components was removed by pipette to an amber glass jar. This procedure was repeated twice with the addition of 40 mL of diethyl ether to each bottle each time. After the third round of solvent was removed, the bottles were centrifuged a fourth time and any remaining solvent was removed. The solvent extracted from the bottles was then concentrated to 150 mL using a Vigreux column placed inside a water bath at 40°C.

Solvent-Assisted Flavor Evaporation. Volatile compounds from serum and traditional whey protein extracts were distilled using solvent-assisted flavor evaporation (SAFE; Ace Glassware, Vineland, NJ). The assembly used was similar to that described by Engel et al. (1999). A rough pump/diffusion pump combination was used as the vacuum source. The SAFE apparatus was connected to a separate receiving tube and a waste tube, both submerged in liquid nitrogen. The distillation procedure was carried out over 2 h under vacuum (10^5 Torr). The liquid sample was poured into the SAFE apparatus and introduced dropwise into the vacuum until all of the liquid extract had been placed under vacuum conditions. The SAFE apparatus was kept at 40°C with a circulating water bath.

Phase Separation. Following SAFE, the distillate was concentrated under a nitrogen stream to 20 mL. The concentrated distillate was then washed twice with 3 mL of sodium carbonate (Fisher Scientific) and mixed thoroughly. The bottom (water) phase was removed to a separate test tube. The concentrated solvent was then washed 3 times with 2 mL of saturated sodium chloride solution. Each time, the solution was mixed thoroughly and the water phase removed to the same test tube. The upper layer (ether) containing the neutral/basic (NB) fraction was collected, dried over anhydrous sodium sulfate (VWR International, West Chester, PA), and concentrated to 0.5 mL under a gentle stream of nitrogen gas. Acidic (AC) volatiles were recovered by acidifying the bottom layer (aqueous phase) with hydrochloric acid [18% (wt/vol); Sigma Aldrich] to a pH of 2 to 2.5 and extracting the sample 3 times with 15 mL of ethyl ether. The acidified extract was dried over anhydrous sodium sulfate before concentration to 0.5 mL under a nitrogen gas stream.

GC-MS of SAFE Extracts. An Agilent 6890N GC with 5973 inert mass selective detector (Agilent Technologies Inc.) was used to analyze NB solvent extracts. Separations were performed on a fused-silica capillary column (Rtx-5ms 30 m length $\times$ 0.25 mm i.d. $\times$ 0.25 μ m film thickness; Restek). A carrier gas (helium) at a constant flow rate of 1 mL/min was used. The oven temperature was programmed from 40 to 200°C at a rate of 5°C/min with initial and final hold times of 5

min, respectively. Each extract (2 μ L) was injected in the splitless mode. Duplicate analyses were performed on each sample.

A Varian CP-3380 GC with Saturn 2000 inert mass selective detector (Varian Inc., Palo Alto, CA) was used to analyze AC solvent extracts. Separations were performed on a fused-silica capillary column (Rtx-Wax 30 m length $\times$ 0.25 mm i.d. $\times$ 0.25 μ m film thickness; Restek). A carrier gas (helium) at a constant flow rate of 1 mL/min was used. The oven temperature was programmed from 40 to 200°C at a rate of 5°C/min with initial and final hold times of 5 min, respectively. Each extract (2 μ L) was injected in the splitless mode. Duplicate analyses were performed on each sample.

GC-Olfactometry. Gas chromatography-olfactometry (GC-O) was performed on NB and AC solvent extracts using an HP 5890 series II gas chromatograph (Hewlett-Packard Co., Palo Alto, CA) equipped with a flame-ionization detector (FID), a sniffing port, and a split/splitless injector. Neutral/basic and AC fractions were analyzed from each solvent extraction. Two microliters was injected onto a polar capillary column (Rtx-Wax 30 m length $\times$ 0.25 mm i.d. $\times$ 0.25 μ m film thickness; Restek) and a nonpolar capillary column (Rtx-5ms 30 m length $\times$ 0.25 mm i.d. $\times$ 0.25 μ m film thickness; Restek). Column effluent was split 1:1 between the FID and the sniffing port using deactivated fused-silica capillaries (1 m length $\times$ 0.25 mm i.d.). The GC oven temperature was programmed from 40 to 200°C at a rate of 10°C/min with an initial hold for 3 min and final hold of 20 min. The FID was maintained at a temperature of 300°C, and the sniffing port was maintained at a temperature of 105°C. The sniffing port was supplied with humidified air at 30 mL/min. The post-peak intensity of aroma active compounds was evaluated (Grosch, 1993; van Ruth, 2001). Each extract was sniffed in duplicate by 2 experienced sniffers, each with >50 h training on GC-O of dairy products on both polar and nonpolar columns.

Aroma Extract Dilution Analysis. Aroma extract dilution analysis (AEDA) of solvent extracts was performed under the same conditions as those for post-peak intensity GC-O. Representative samples (2 NB and 2 AC fractions from different batches of SPC and WPC based on examination of post-peak intensity GC-O profiles and mass spectra) of each of the pilot-plant products were analyzed. The NB fractions were injected onto the DB-5ms (Rtx-5ms 30 m length $\times$ 0.25 mm i.d. $\times$ 0.25 μ m film thickness; Restek) capillary column, and AC fractions were injected onto the DB-WAX (Rtx-wax 30 m length $\times$ 0.25 mm i.d. $\times$ 0.25 μ m film thickness; Restek) capillary column. Each sample was diluted stepwise at a ratio of 1:3 (vol/vol) with diethyl ether. Samples were evaluated by 2 experienced

sniffers until no odors were detected. The greatest dilution at which a compound was sniffed was reported as the flavor dilution ($\log_3$ FD) factor (Grosch, 1993).

Volatile Compound Identification

Volatile compounds from SAFE and SPME were identified using the NIST 2005 library of spectra and comparison of spectra of authentic standards injected under identical conditions (NIST, 2005). Relative abundance of compounds 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) (Van den Dool and Kratz, 1963). To verify the identity of aroma-active compounds, retention index and aroma properties were compared with authentic standards injected under identical conditions.

Consumer Testing

Flavor of 80% SPC and WPC in an ingredient application was determined using an acidified, 6% protein, peach-flavored beverage formulation as described by Wright et al. (2009). Both pilot-plant proteins and 3 representative commercial whey proteins were evaluated in beverages. Beverages were prepared by first combining all of the dry ingredients and then slowly blending them into deionized water with agitation from an Oster 2614 hand blender (Sunbeam Products Inc., Boca Raton, FL) until fully blended. Coloring and flavoring were added and the mixture was blended again using the hand blender until fully combined. Descriptive analysis was conducted on each of the peach beverages using the methods described previously. Beverages were evaluated for overall aroma intensity; the flavor attributes sweet aromatic, fruity, cardboard, cereal, free fatty acid, and soapy; and sour, sweet, and bitter tastes.

For consumer testing, approximately 30 mL of each peach beverage was dispensed into 58-mL plastic cups with fitted lids (Sweetheart Cup Co., Owings Mills, MD) labeled with 3-digit codes. Samples were prepared 1 d in advance and stored under refrigeration (5°C). Samples were served at 5°C. Self-reported beverage consumers ($n = 77$) were recruited through university listservs and paper fliers. Consistent with North Carolina State University Institutional Review Board guidelines, consumers were required to sign informed consent forms. Each panelist was first asked a series of demographic questions and then presented samples in a monadic, random, and balanced order. Each beverage was evaluated for overall liking, appearance liking, flavor liking,

and texture/mouthfeel liking using the 9-point hedonic scale. Consumers were provided with deionized water at ambient temperature for palate cleansing between samples and received food treats for their participation. Evaluations were conducted in individual sensory booths using Compusense 5 release 4.8 (Compusense Inc) software for data collection.

Statistical Analyses

To determine if there were significant differences in UF and DF flux, color, composition, or sensory attributes between WPC and SPC, all data were analyzed by ANOVA using the Proc GLM procedures of SAS (SAS version 8.02, 1999–2001; SAS Institute Inc., Cary, NC). The GLM was used to determine if flux changed with the time of processing run after the first 30 min of running UF and during DF between treatments (WPC, SPC). Time was treated as a continuous variable in the split-plot ANOVA models. Distortion of the ANOVA by multicollinearity in the model was minimized by centering the time of running using a mathematical transformation (Glantz and Slinker, 2001). Time was transformed as follows: time transformed = running time – [(last time – first time)/2]. This transformation made the data set orthogonal with respect to time. The model was dependent variable (UF flux, DF flux) = treatment + replicate + treatment × replicate + time + time × replicate + time × treatment + error, with treatment and replicate as category variables and time as a continuous variable. The GLM for the direct comparison of composition from WPC and SPC produced in this study was treatment (WPC, SPC) + replicate + error. The GLM for analysis of sensory data for the direct comparison of composition from WPC and SPC produced in this study was treatment (WPC, SPC) + replicate + panelist + error. The GLM for comparison of composition among samples produced in this study and the commercial powders was dependent variable = treatment (WPC, SPC, commercial powders 1, 2, 3, 4, 5) + error. For descriptive sensory analysis, the GLM was dependent variable = treatment (WPC, SPC, commercial powders 1, 2, 3, 4, 5) + panelist + error. If the *F*-test for the model was significant (i.e., $P < 0.05$), then the least squares means were compared to determine if there were significant differences. Principal component analysis using the Proc Princomp command using the correlation matrix was also applied to sensory and volatile component data of products produced in this study and commercial powders to visualize how products were differentiated across sensory attributes or volatile components (SAS version 9.2, SAS Institute Inc.).

RESULTS AND DISCUSSION

Processing

The largest decrease in UF flux occurred during the initial 30 min of processing for both Cheddar cheese whey and MF permeate. After the initial 15 min of the run, the flux decreased at about 38 and 31% during WPC and SPC processing, respectively, with additional 10 and 23% decreases during the subsequent 15 min for WPC and SPC, respectively (Figure 1). During the first 30 min of DF, the flux improved slightly and then gradually decreased by 8 and 13% at the end of WPC and SPC processing, respectively. Time-dependent differences ($P < 0.05$) in UF and DF flux after 30 min of processing when processing MF permeate from skim milk and whey were detected (Table 1). The mean flux during the time of UF and DF processing was higher for WPC than for SPC (Figure 1); however, flux decreased at a higher rate per hour for WPC than for SPC (i.e., Table 1, significant time × treatment interaction). With longer running times, the flux for processing WPC may have been lower than that for SPC (Figure 1). The flux changed less with time of processing during DF (15.6% of variation in the model explained by time vs. 52.9% for UF) than during UF. All 80% WPC and SPC products conformed to the dry whey grade standards (ADPI, 2002) of <30,000 cfu/g for standard plate count and <10 cfu/g for coliforms. The actual maximum counts for our products were 4,000 cfu/g for standard plate count and <1 cfu/g for coliform.

Composition

The liquid WPC before drying had a higher ($P < 0.05$) TS content than the liquid SPC (Table 2), which is explained by differences in fat and NPN contents along with a difference in final UF concentration factor. Fat, CP, and NPN contents of the liquid WPC were significantly higher ($P < 0.05$) than those of SPC (Table 2). The fat remaining in skim milk after cream separation was retained in the MF retentate during MF of skim milk using a ceramic uniform transmembrane pressure membrane with a pore size of 0.1 μm and was not transmitted into the MF permeate with the serum proteins, which will result in a low fat content of the SPC. In contrast, the fat that was not removed from the whey by the cream separator was concentrated by UF and DF in the liquid WPC. Because the high fat content in the WPC obtained from whey after cheese production has a negative effect on flavor (Morr and Ha, 1991), the low fat content in SP products produced directly from skim milk using MF may produce SPC products with different flavor and functional properties

Table 1. Analysis of variance type III sums of squares and *P*-values for UF (after first 30 min of processing run) and diafiltration (DF) flux across the time of processing¹

Item	UF flux		DF flux	
	SS	<i>P</i> -value	SS	<i>P</i> -value
Treatment ¹ (n = 2)	11.82	0.05	64.68	0.07
Replicate (n = 3)	2.33	0.37	4.82	0.67
Treatment × replicate	1.38	0.18	9.67	<0.01
Time	101.24	<0.01	30.47	<0.01
Time × replicate	2.70	0.04	0.30	0.65
Time × treatment	12.33	<0.01	3.89	<0.01
Total	191.24		195.88	
R ²	0.90		0.88	

¹Treatment is the comparison of processing whey versus microfiltration (MF) permeate.

than WPC products. The NPN content of the WPC was higher ($P < 0.05$) than that of SPC (Table 2), which was caused by the presence of GMP in the whey. Glycomacropeptide, a glycoprotein, is unusual for a protein because it is soluble in 12% TCA and will increase the apparent NPN in a sample even though it is not a classical milk NPN compound. Milk NPN compounds pass through a UF membrane into permeate, whereas GMP does not pass through a UF membrane into UF permeate. The pH of the liquid WPC was lower ($P < 0.05$) than that of liquid SPC (Table 2) because of the

Table 2. Mean (n = 3) composition (% by weight) of the 80% serum protein concentrate (SPC) and 80% whey protein concentrate (WPC) liquids after UF/diafiltration and before spray drying

Product	Composition ¹					
	TS	Fat	CP	NPN	TP	pH
SPC	6.81 ^b	0.02 ^b	5.41 ^b	0.10 ^b	5.31 ^b	6.87 ^a
WPC	8.57 ^a	0.79 ^a	6.26 ^a	0.36 ^a	5.90 ^a	6.51 ^b
R ²	0.98	0.82	0.98	0.98	0.98	0.97
SE	0.25	0.10	0.10	0.01	0.10	0.03

^{a,b}Means in the same column not sharing a common superscript are different ($P < 0.05$).

¹CP = total N × 6.38; NPN = 12% trichloroacetic acid N × 6.38; TP = true protein (CP – NPN); pH of the liquid WPC and SPC.

lactic acid from cheese making that is present in the WPC.

The wet- and dry-basis compositions of the spray-dried SPC and WPC powders are presented in Tables 3 and 4, respectively. No significant difference was detected ($P > 0.05$) in moisture content of spray-dried experimental WPC versus SPC (Table 3). There was a difference ($P < 0.05$) in fat content between WPC and SPC (8.12 vs. 0.53, respectively, on a dry basis) (Table 4). Spray-dried SPC had a higher ($P < 0.05$) content of CP than did WPC. Glycomacropeptide, calculated as the difference in NPN content between WPC and SPC, was higher ($P < 0.05$) for WPC than for SPC

Figure 1. Mean (n = 3) ultrafiltration (UF) and diafiltration (DF) flux during the production of 80% whey protein concentrate (WPC) and 80% serum protein concentrate (SPC) using a spiral wound 10-kDa polyethersulfone membrane.

Table 3. Mean ($n = 3$) composition (% by weight) of spray dried 80% serum protein concentrate (SPC) and 80% whey protein concentrate (WPC) calculated on a wet basis

Product	Composition ¹				
	Moisture	Fat	CP	NPN	TP
SPC	7.08 ^a	0.49 ^b	72.39 ^a	2.18 ^b	70.22 ^a
WPC	5.99 ^a	7.63 ^a	67.85 ^b	6.79 ^a	61.06 ^b
R ²	0.53	0.88	0.82	0.99	0.93
SE	0.29	0.73	0.70	0.10	0.71

^{a,b}Means in the same column not sharing a common superscript are different ($P < 0.05$).

¹CP = total N $\times$ 6.38; NPN = 12% trichloroacetic acid N $\times$ 6.38; TP = true protein (CP – NPN); for WPC, this does not include glycomacropeptide.

(Table 4). The calcium and phosphorus contents were slightly higher ($P < 0.05$) in WPC compared with SPC, whereas potassium, sodium, and sulfur contents were slightly lower in WPC compared with SPC (Table 5).

From the functionality point of view, it might be valuable to compare directly the nitrogen fractions of WPC and SPC on a nonfat basis, and this was done in Table 6. There was no significant difference ($P > 0.05$) in CP content for WPC versus SPC (Table 6) on a nonfat basis. However, true protein content (calculated as a difference between total N and NPN; Table 6) was significantly higher ($P < 0.05$) for SPC than for WPC because of the presence of GMP in WPC. Thus, a higher proportion of the CP in the SPC is β -LG and α -LA than in WPC.

The compositions of experimental 80% SPC and WPC were compared with 5 commercial 80% WPC on a wet basis (Table 7) and on a dry basis (Table 8). The moisture content of the experimental SPC was higher ($P < 0.05$) than that of the commercial WPC products (Table 7). There was no difference ($P > 0.05$) in mois-

Table 4. Mean ($n = 3$) composition (% by weight) of spray dried 80% serum protein concentrate (SPC) and 80% whey protein concentrate (WPC) calculated on a dry basis

Product	Composition ¹				
	Fat	CP	NPN	TP	GMP
SPC	0.53 ^b	77.90 ^a	2.34 ^b	75.56 ^a	0.00
WPC	8.12 ^a	72.17 ^b	7.22 ^a	64.95 ^b	4.88
R ²	0.87	0.88	0.99	0.94	NA ²
SE	0.78	0.64	0.10	0.68	NA

^{a,b}Means in the same column not sharing a common superscript are different ($P < 0.05$).

¹CP = total N $\times$ 6.38; NPN = 12% trichloroacetic acid N $\times$ 6.38; TP = true protein (CP – NPN); for WPC, this does not include glycomacropeptide (GMP), calculated as NPN of WPC – NPN of SPC.

²NA = not applicable.

Table 5. Mean ($n = 3$) mineral composition (% by weight) of 80% serum protein concentrate (SPC) and 80% whey protein concentrate (WPC) as calculated on a dry basis

Product	Ca	P	K	Mg	Na	S
SPC	0.40 ^b	0.30 ^b	0.87 ^a	0.06 ^a	0.19 ^a	1.10 ^a
WPC	0.47 ^a	0.35 ^a	0.77 ^b	0.06 ^a	0.17 ^b	0.98 ^b
R ²	0.60	0.74	0.90	0.66	0.93	0.64
SE	0.01	0.007	0.01	0.0009	0.002	0.02

^{a,b}Means in the same column not sharing a common superscript are different ($P < 0.05$).

ture content between 4 commercial WPC (plants 1, 2, 3, and 4) and the experimental 80% WPC, whereas the WPC from plant 5 had lower moisture. The average CP contents on a wet basis of all commercial and experimental powders were $<80\%$ (Table 7), and the experimental WPC was lower in CP ($P < 0.05$) than the commercial WPC powders. The average fat content on a wet basis for the commercial powders was 6.01% (Table 7), and only the powders from plants 2 and 5 were not significantly lower in fat content compared with the experimental 80% WPC (Tables 7 and 8). The SPC produced in this study had a higher pH ($P < 0.05$) than the experimental and commercial WPC (Table 7). The experimental 80% WPC, with a pH of 6.43, was not different from the WPC from plants 1, 2, 3, and 4. The pH of the WPC from plant 5 was lower ($P < 0.05$) than that of all other samples. The CP content, as calculated on a nonfat dry basis, of all commercial WPC powders was significantly higher ($P < 0.05$) than that of the WPC and SPC produced in this study (Table 9). It appears that the DF process used in production of the experimental SPC and WPC did not remove sufficient lactose to achieve a dry-basis CP similar to that of the commercial WPC. Generally, the commercial and experimental WPC and SPC powders varied in mineral content (Table 10) but the differences were small for Ca, P, Mg, Na, and S. The experimental

Table 6. Mean ($n = 3$) composition (% by weight) of spray-dried 80% serum protein concentrate (SPC) and 80% whey protein concentrate (WPC) calculated on a nonfat dry basis

Product	Composition ¹		
	CP	NPN	TP
SPC	78.32 ^a	2.36 ^b	75.97 ^a
WPC	78.66 ^a	7.87 ^a	70.79 ^b
R ²	0.96	0.99	0.96
SE	0.33	0.12	0.41

^{a,b}Means in the same column not sharing a common superscript are different ($P < 0.05$).

¹CP = total N $\times$ 6.38; NPN = 12% trichloroacetic acid N $\times$ 6.38; TP = true protein (CP – NPN).

Table 7. Composition (% by weight) and pH of (n = 3) the experimental 80% serum protein concentrate (SPC) and whey protein concentrate (WPC), and 5 commercial (n = 2) 80% WPC calculated on a wet basis

Source	Product	Composition ¹			
		Moisture	Fat	CP	pH
Commercial WPC	1	5.22 ^{bc}	5.03 ^b	77.14 ^a	6.41 ^b
	2	3.99 ^{bc}	7.72 ^a	77.43 ^a	6.36 ^b
	3	4.24 ^{bc}	4.32 ^b	76.52 ^a	6.36 ^b
	4	4.53 ^{bc}	4.95 ^b	77.30 ^a	6.37 ^b
	5	3.58 ^c	6.76 ^{ab}	75.92 ^a	6.01 ^c
Experimental	SPC	7.08 ^a	0.49 ^c	72.40 ^b	6.77 ^a
Experimental	WPC	5.99 ^{ab}	7.63 ^a	67.85 ^c	6.43 ^b
R ²		0.68	0.74	0.80	0.82
SE: commercial		0.47	0.85	1.01	0.09
SE: experimental		0.39	0.69	0.83	0.08

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

¹CP = total N $\times$ 6.38; pH of the reconstituted (10% wt/vol) commercial and experimental powders.

WPC and SPC both had higher K than the commercial powders.

A typical SDS-PAGE gel for 80% WPC and SPC is shown in Figure 2. The relative percentages of casein and serum protein in WPC and SPC are presented in Table 11. The casein content of SPC was higher ($P < 0.05$) than that of WPC (3.76 vs. 1.98%). As was discussed in Evans et al. (2009), band CN2 corresponds to casein proteolysis products and was found in both SPC and WPC. A higher content of CN2 was present in SPC (3.48 vs. 1.98% for SPC and WPC, respectively) and this accounted for most of the difference in casein content between SPC and WPC. The higher level of proteolysis products of casein in SPC versus WPC could be because of a difference in partitioning of these products between cheese and whey in cheesemaking versus partitioning between retentate and permeate during MF. In addition, some casein proteolysis might

have occurred because of native milk protease activity at 50°C during MF processing of the milk that did not occur in the cheese making. The relative β -LG content of WPC was higher ($P < 0.05$) than that of SPC (Table 12). This could be because of a small amount of rejection of β -LG by the MF membrane or because of a minor amount of heat-induced denaturation of β -LG and interaction with casein micelles during heating of the skim milk to 50°C during MF. The mean relative percentages of β -LG and α -LA in the milk used in our study were 79.77 and 20.23, respectively, and were different ($P < 0.05$) compared with those for SPC and WPC (Table 12). The small differences in β -LG and α -LA percentages between SPC and WPC should have only minor, if any, influence on functional properties of the WPC versus SPC compared with the other large differences in composition (e.g., fat and GMP content) reported in earlier tables.

Table 8. Composition (% by weight) of (n = 3) the experimental 80% serum protein concentrate (SPC) and whey protein concentrate (WPC), and 5 commercial (n = 2) 80% WPC calculated on a dry basis

Source	Product	Fat	CP ¹
Commercial WPC	1	5.31 ^b	81.38 ^a
	2	8.02 ^a	80.65 ^a
	3	4.51 ^b	79.91 ^{ab}
	4	5.19 ^b	80.97 ^a
	5	7.02 ^{ab}	78.77 ^{ab}
Experimental	SPC	0.53 ^c	77.90 ^b
Experimental	WPC	8.12 ^a	72.17 ^c
R ²		0.74	0.76
SE: commercial		0.90	1.02
SE: experimental		0.74	0.83

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

¹CP = total N $\times$ 6.38.

Table 9. Composition (% by weight) of (n = 3) the experimental 80% serum protein concentrate (SPC) and whey protein concentrate (WPC), and 5 commercial (n = 2) 80% WPC calculated on a nonfat dry basis

Source	Product	CP ¹
Commercial WPC	1	85.94 ^{ab}
	2	87.70 ^a
	3	83.68 ^b
	4	85.40 ^{ab}
	5	84.73 ^{ab}
Experimental	SPC	78.32 ^c
Experimental	WPC	78.66 ^c
R ²		0.70
SE: commercial		1.33
SE: experimental		1.08

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

¹CP = total N $\times$ 6.38.

Table 10. Mineral composition (% by weight) of ($n = 3$) the experimental 80% serum protein concentrate (SPC) and 80% whey protein concentrate (WPC), and 5 commercial ($n = 2$) 80% WPC as calculated on a dry basis

Source	Product	Ca	P	K	Mg	Na	S
Commercial WPC	1	0.29 ^c	0.31 ^{bc}	0.53 ^{cd}	0.04 ^c	0.32 ^a	1.04 ^{ab}
	2	0.40 ^b	0.47 ^a	0.47 ^d	0.06 ^b	0.22 ^{bc}	1.04 ^{ab}
	3	0.52 ^a	0.37 ^b	0.49 ^{cd}	0.07 ^a	0.12 ^f	1.04 ^{ab}
	4	0.48 ^a	0.36 ^{bc}	0.57 ^c	0.07 ^a	0.24 ^b	1.04 ^{ab}
	5	0.35 ^b	0.33 ^{bc}	0.50 ^{cd}	0.06 ^b	0.14 ^{ef}	0.98 ^b
Experimental	SPC	0.40 ^b	0.30 ^c	0.87 ^a	0.06 ^b	0.19 ^{cd}	1.10 ^a
Experimental	WPC	0.47 ^a	0.35 ^{bc}	0.77 ^b	0.06 ^b	0.17 ^{de}	0.98 ^b
R ²		0.75	0.49	0.88	0.82	0.75	0.35
SE: commercial		0.02	0.03	0.03	0.002	0.02	0.03
SE: experimental		0.01	0.02	0.02	0.001	0.01	0.02

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

Color

There was a very noticeable difference in appearance of the WPC and SPC liquid concentrates (Figure 3). The liquid concentrates obtained after UF and DF processing looked very different; the 80% SPC was clear and 80% WPC was cloudy and opaque. The higher opacity of the 80% WPC was because of the higher fat content and GMP content. Such a difference in clarity may limit the use of WPC for fortification of non-dairy beverages in which clarity of the final beverage is required. The commercial WPC powders were also opaque when reconstituted. These visual observations were confirmed by the Hunter L-value measurement, which can be used as an index of clarity (the higher the L-value, the higher the opacity). Large differences in

L-value between liquid 80% WPC and 80% SPC (52.46 vs. 18.50, respectively) were detected (Table 13). The 80% SPC was also characterized by higher a-value and lower b-value compared with 80% WPC (Table 13). Evans et al. (2009) reported similar results between rehydrated 34% SPC and WPC and hypothesized that differences in fat content contributed to the differences in clarity. Similar distinctions in fat content were observed between 80% SPC and WPC in the present study and may explain the differences in appearance.

Sensory Analysis

SPC and WPC. Pilot-plant 80% SPC and WPC were characterized by low flavor intensities (Table 14), similar to results reported previously for 34% WPC and SPC manufactured in a pilot plant (Evans et al., 2009). The 80% WPC had a higher overall aroma intensity and cereal flavor than SPC, as well as the presence of sweet aromatic flavor that was absent from SPC. Diacetyl was previously documented in 34% WPC and was absent from 34% SPC (Evans et al., 2009), because the diacetyl was produced by the starter cultures during cheese making. Diacetyl flavor was not detected by sensory panelists in 80% WPC or SPC in the current

Figure 2. Sodium dodecyl sulfate-PAGE of proteins in experimental 80% serum protein concentrate (SPC), 80% whey protein concentrate (WPC), and skim milk. Bands are identified on the gel: SP1, SP2, SP3 = high molecular weight serum proteins; Cn1 = α_s -CN (combination of α_{s1} and α_{s2} -CN); Cn2 = casein proteolysis products; Cn3 = proteolysis products of casein. Color version available in the online PDF.

Table 11. Mean ($n = 3$) relative percentage of casein and serum proteins for skim milk and 80% whey protein concentrate (WPC) and 80% serum protein concentrate (SPC) powders by the densitometry analysis of the SDS-PAGE gels

Product	Casein	Serum proteins
Milk	83.23	16.77
SPC	3.76 ^a	96.24 ^b
WPC	1.98 ^b	98.02 ^a
R ²	0.93	0.93
SE: SPC and WPC	0.18	0.18

^{a,b}Means for SPC and WPC in the same column not sharing a common superscript are different ($P < 0.05$).

Table 12. Mean ($n = 3$) relative percentage of β -LG and α -LA for skim milk and 80% whey protein concentrate (WPC) and 80% serum protein concentrate (SPC) powders by the densitometry analysis of the SDS-PAGE gels

Product	β -LG	α -LA
Milk	79.77 ^a	20.23 ^c
SPC	76.05 ^c	23.95 ^a
WPC	77.39 ^b	22.61 ^b
R^2	0.91	0.91
SE: milk	0.33	0.33
SE: SPC, WPC	0.19	0.19

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

study. We speculate that the absence of diacetyl flavor in the 80% WPC was because the water-soluble diacetyl was washed out of the 80% WPC in the DF step, which does not occur in the production of 34% WPC. The passage of water-soluble flavor compounds into UF permeate during the production of 80% WPC would influence the characteristics of lactose and delactosed permeate from whey, but we would expect that these whey-related water-soluble compounds would not be present in permeate from the production of 80% SPC.

Commercial Versus Pilot-Plant Products. Flavor profiles of commercial 80% WPC were similar to those of the pilot-plant products, with the addition of the flavors diacetyl and potato/brothy (Table 15). All of these flavors have been previously reported in 80% WPC and WPI (Drake et al., 2009; Wright et al., 2009). No consistent differences were noted between pilot-plant and commercial products except the presence of cooked/milky flavor in pilot samples, which was absent from commercial samples, and this result was similar to those reported by Evans et al. (2009) with 34% WPC and SPC. Pilot-plant 80% WPC and SPC were clearly distinguished from commercial WPC product using a principal component biplot of sensory attributes (Figure 4). Additionally, the 2 pilot products were similar in flavor profile (Figure 4), whereas there was a large amount of flavor variability among the commercial samples. Previous studies have demonstrated

Table 13. Mean ($n = 3$) Hunter L (lightness), a (red-green), b (yellow-blue) color values of fresh liquid serum protein concentrate (SPC) and whey protein concentrate (WPC)

Product	L	a	b
SPC	18.50 ^b	0.04 ^a	2.01 ^c
WPC	52.46 ^a	-1.47 ^b	4.78 ^b
R^2	0.96	0.78	0.76
SE	1.70	0.30	0.71

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

Figure 3. Liquid 80% whey protein concentrate (WPC; left) and 80% serum protein concentrate (SPC; right) after diafiltration. Color version available in the online PDF.

wide variability among commercial fluid whey, whey powders, and whey proteins (Carunchia Whetstine et al., 2003, 2005a; Karagul-Yuceer et al., 2003; Mahajan et al., 2004; Wright et al., 2009). These differences were attributed to different milk supplies, whey sources, and processing parameters as well as minor but distinct differences in composition, and these distinctions may also represent sources of differences between pilot-plant and commercial products.

Figure 4. Principal component biplot of sensory attributes of 80% whey (WPC) and serum protein concentrates (SPC) manufactured in this study and 5 commercial 80% WPC. WPC80 = experimental 80% WPC; SPC80 = experimental 80% SPC; commercial whey proteins are designated by numbers 1 to 5; PC1 and PC2 = principal components 1 and 2.

Table 14. Mean sensory attributes¹ of 80% serum protein concentrate (SPC) and whey protein concentrate (WPC) produced in this study (n = 3)

Product	Aroma intensity	Sweet aromatic	Cardboard	Cereal	Cooked/milky	Astringent
SPC	1.2 ^b	ND ²	0.8 ^a	1.2 ^b	1.1 ^a	1.6 ^a
WPC	1.6 ^a	0.8	1.3 ^a	1.9 ^a	1.1 ^a	1.9 ^a
R ²	0.74	NA ³	0.65	0.81	0.09	0.67
SE	0.11	NA	0.11	0.07	0.14	0.12

^{a,b}Means in the same column not sharing a common superscript are different ($P < 0.05$).

¹Intensities were scored on a 0 to 15 universal scale where 0 = none and 15 = very high intensity (Meilgaard et al., 1999). Dried whey ingredient intensities usually fall between 0 and 4 on this scale (Drake et al., 2003; Wright et al., 2009). Attributes not listed were not detected.

²ND = not detected.

³NA = not applicable.

Instrumental Volatile Analysis

SPME GC-MS: SPC Versus WPC. Differences were also noted in the relative abundance of selected compounds (Table 16). The 80% WPC had higher aldehyde concentrations compared with SPC except for decanal. Pentanal, heptanal, and Z-4-heptenal were not detected in SPC. The WPC had significantly higher fat content (Table 4) than the SPC, which may have contributed to higher concentrations of lipid oxidation products in WPC. These results are consistent with relative abundance results reported for 34% WPC and SPC (Evans et al., 2009), with a lower overall number of aldehydes in 80% SPC compared with 80% WPC. Diacetyl was not detected in 80% SPC, which was also expected because this compound is produced during cheesemaking; this difference between SPC and WPC was also observed for 34% protein products. Gas chromatography-olfactometry results (FD factors from AEDA; Table 17) suggested that hexanal played

a larger role in SPC flavor compared with WPC flavor, whereas relative abundance calculations (Table 16) showed lower concentrations of hexanal in SPC compared with WPC. Gas chromatography-olfactometry results identify aroma-active compounds and suggest which compounds play the largest role in flavor. Because sensory thresholds for compounds vary, aroma activity is not related to concentration when different compounds or products are compared.

Pilot-Plant Versus Commercial Products. Differences were noted between pilot-plant and commercial 80% protein products (Table 18; Figure 5). Pilot-plant samples were generally lower in diacetyl, 2-methyl butanal, hexanal, heptanal, and pentanal compared with most of the commercial WPC (Table 18). Commercial sample 3, a Mozzarella WPC, was the exception. This WPC was distinct in volatile profile from the other WPC, which were sourced from Cheddar whey. Carunchia Whetstine et al. (2005a) and Wright et al. (2009) demonstrated a range of flavor (sensory and instrumen-

Table 15. Mean sensory attributes¹ of the experimental 80% serum protein concentrate (SPC) and whey protein concentrate (WPC) produced in this study (n = 3) and 5 commercial 80% WPC (n = 2)

Source	Product	Aroma intensity	Sweet aromatic	Diacetyl	Cardboard	Cereal	Cooked/milky	Potato	Astringent
Commercial WPC	1	1.8 ^{bc}	ND ²	ND	1.6 ^b	1.1 ^b	ND	ND	2.3 ^{ab}
	2	1.2 ^d	0.8 ^b	ND	1.1 ^{cd}	ND	ND	ND	2.1 ^a
	3	1.4 ^{cd}	1.5 ^a	ND	1.0 ^{cd}	1.1 ^b	ND	ND	2.3 ^{ab}
	4	2.3 ^a	ND	ND	2.2 ^a	ND	ND	1.4	2.2 ^{ab}
	5	1.0 ^d	0.8 ^b	1.1	1.3 ^{bcd}	0.8 ^c	ND	ND	2.5 ^a
Experimental	SPC	1.2 ^d	ND	ND	0.8 ^d	1.2 ^b	1.1 ^a	ND	1.6 ^d
Experimental	WPC	1.6 ^{bc}	0.7 ^b	ND	1.3 ^{bcd}	1.9 ^a	1.1 ^a	ND	1.9 ^c
R ²		0.74	0.65	NA ³	0.70	0.91	0.77	NA	0.56
SE: commercial		0.14	0.16	NA	0.15	0.09	0.17	NA	0.14
SE: experimental		0.11	0.13	NA	0.11	0.07	0.14	NA	0.12

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

¹Intensities were scored on a 0 to 15 universal scale where 0 = none and 15 = very high intensity (Meilgaard et al., 1999). Dried whey ingredient intensities usually fall between 0 and 4 on this scale (Drake et al., 2003; Wright et al., 2009). Attributes not listed were not detected.

²ND = not detected.

³NA = not applicable.

Table 16. Mean ($n = 3$) concentrations of selected aroma-active compounds ($\mu\text{g}/\text{kg}$) of spray-dried 80% serum protein concentrate (SPC) and 80% whey protein concentrate (WPC) produced in this study isolated using solid-phase microextraction

Product	Hexanal	Heptanal	Diacetyl	Pentanal	Octanal	Nonanal	Decanal	2-Methyl butanal	Z-4-Heptenal
SPC	1.06 ^b	ND ¹	ND	ND	1.04 ^a	0.5 ^b	2.32 ^a	0.26 ^b	ND
WPC	6.58 ^a	0.43	0.50	0.66	0.78 ^a	1.14 ^a	0.74 ^b	0.77 ^a	2.3
R ²	0.72	NA ²	NA	NA	0.24	0.79	0.92	0.80	NA
SE	0.74	NA	NA	NA	0.10	0.07	0.08	0.05	NA

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

¹ND = not detected.

²NA = not applicable.

tal) among commercial 80% WPC and WPI and noted distinctions between Cheddar- and Mozzarella-sourced whey protein products. A previous study comparing 34% SPC and WPC flavors also demonstrated distinct volatile compound differences between pilot-plant and commercial samples (Evans et al., 2009). Processing and handling differences, time required for processing, different raw product streams (e.g., Mozzarella vs. white Cheddar vs. colored Cheddar), and composition differences are all possible sources of these differences.

GC-O

Compounds Detected. Forty-six aroma-active compounds were detected in the pilot plant 80% WPC and SPC by GC-O (Table 17). The compounds identified were aldehydes, ketones, esters, sulfur-containing compounds, and free fatty acids. Of these compounds, 13 were positively identified by mass spectra, retention index (**RI**), and odor properties of authentic standards; 26 compounds were tentatively identified by comparing RI and odor with standards; 3 were tentatively identified by RI and previously published standards; and 4 remained unknown (Table 17). These compounds are primarily products of lipid or protein oxidation. Most of the compounds identified have been previously identified in whey, WPC, or WPI (Karagul-Yuceer et al., 2003; Mahajan et al., 2004; Carunchia Whetstine et al., 2005a; Wright et al., 2006, 2009; Mortenson et al., 2008; Evans et al., 2009).

Saturated and unsaturated aldehydes were detected in all samples (Table 17). These compounds are lipid oxidation products and have previously been documented in dried whey products (Mahajan et al., 2004; Carunchia Whetstine et al., 2005a; Gallardo-Escamilla et al., 2005; Wright et al., 2006; Javidipour and Qian, 2008; Wright et al., 2009). Five acids (acetic, butanoic, pentanoic, decanoic, and 4-methyl octanoic acids) were identified in the pilot-plant products. Of these 5 fatty acids, butanoic, 4-methyl octanoic acid, and decanoic

acid showed high aroma activity ($\text{FD}_3 \geq 3$; Table 17). These 3 acids have been reported as aroma-active compounds in whey products (Mahajan et al., 2004; Carunchia Whetstine et al., 2005a; Gallardo-Escamilla et al., 2005; Wright et al., 2006; Mortenson et al., 2008). Free fatty acids can be formed from the action of milk or bacterial lipase reacting with triglycerides and phospholipids found in the milk (Ferretti and Flanagan, 1971; Kim et al., 2003). Five ketones [diacetyl (buttery), 1-hexen-3-one (rubber), 1-octen-3-one (mushroom), (Z)-1,5-octadien-3-one (geranium), and 1-nonen-3-one (mushroom)] were identified in both SPC and WPC.

Four sulfur-containing compounds (dimethyl disulfide, dimethyl trisulfide, methional, and thienylthiol) were identified in the pilot-plant whey products. Each

Figure 5. Principal component biplot of solid-phase microextraction volatile compound results for 80% whey (WPC) and serum protein concentrates (WPC) manufactured in this study and 5 commercial 80% WPC. WPC80 = experimental 80% WPC; SPC80 = experimental 80% SPC; commercial whey proteins are designated by numbers 1 to 5; PC1 and PC2 = principal components 1 and 2.

Table 17. Aroma-active compounds detected in experimental 80% serum protein concentrate (SPC) and whey protein concentrate (WPC) by solvent-assisted flavor evaporation with gas chromatography-olfactometry with aroma extract dilution analysis

No.	Compound	Fraction ¹	Odor ²	Log ₃ flavor dilution ³ (aroma intensity ⁴)		RI ⁵		Methods of identification ⁶
				WPC	SPC	DB-5	DB-Wax	
1	Diacetyl	NB	Buttery	3 (1)	ND ⁷	604		RI, odor
2	Acetic acid	AC	Vinegar	<1 (1.5)	ND	643	1365	RI, odor
3	2/3-Methylbutanal	NB	Malty	<1 (2.4)	<1 (1)	647		RI, odor
4	Ethyl propionate	NB	Fruity	1 (1.5)	<1 (1)	708	945	RI, odor
5	Dimethyl disulfide (DMDS)	NB	Garlic	<1 (1.2)	<1 (1.5)	744	1101	RI, odor, MS
6	1-Hexen-3-one	NB	Rubber	2 (1.3)	ND	759		RI, odor
7	Hexanal	NB	Green/earthy	<1 (1.8)	4 (1)	791	922	RI, odor, MS
8	Butyric acid	AC	Butyric/cheesy/sweaty/malty	3 (2)	1 (2.5)	807	1630	RI, odor
9	2-Methyl-3-furanthiol	NB	Vitamin	<1 (3)	<1 (4)	853		RI, odor, MS
10	2-Methylbutyl acetate	NB	Dried fruit	<1 (3.5)	ND	888	1061	RI, odor
11	Z-4-Heptenal	NB	Fishy/fatty	1 (1)	1 (1)	891	1138	RI, odor, MS
12	Methional	NB	Potato	4 (2)	3 (1.5)	899	1358	RI, odor, MS
13	Pentanoic acid	AC	Sour/sweetish/Swiss cheese	<1 (3.2)	<1 (2)	906		RI, odor
14	2-Acetyl-1-pyrroline ⁷	NB	Popcorn	4 (2.5)	3 (2)	914	1290	RI, odor
15	4, 5-Dimethyl thiazole	NB	Burnt	ND	3 (1)	939		RI, odor, MS
16	Dimethyl trisulfide (DMTS)	NB	Garlic	2 (3.4)	3 (3.25)	945	1379	RI, odor
17	1-Octen-3-one	NB	Mushroom	4 (1.5)	3 (2)	972	1225	RI, odor, MS
18	(Z)-1,5-octadien-3-one ⁶	NB	Geranium	2 (3)	<1 (1.75)	978		RI
19	Octanal	NB	Citrus	2 (3)	<1 (2.5)	995	1157	RI, odor, MS
20	2-Acetylpyridine	NB	Popcorn	<1 (2)	<1 (2)	1019		RI, odor
21	Phenylacetaldehyde	NB	Honey/floral	<1 (1.5)	ND	1041		RI, odor
22	Thienylthiol	NB	Weeds/sulfur	ND	<1 (2)	1050	1504	RI, odor
23	2-Methoxy phenol	NB	Fatty/smoky	3 (2.3)	9 (2.5)	1054		RI, odor, MS
24	1-Nonen-3-one ⁶	NB	Mushroom	<1 (1.8)	ND	1072		RI
25	E,Z-2-6-Nonadienal	NB	Burnt/cucumber	3 (2)	2 (2)	1080	1499	RI, odor, MS
26	Nonanal	NB	Fatty	3 (2.7)	3 (3)	1096	1406	RI, odor, MS
27	3-Hydroxy-4,5-dimethyl-2(5H)-furanone (sotolon)	AC	Maple	<1 (3)	<1 (3)	1116	2186	RI, odor
28	2-Phenethanol	NB	Rose-y	4 (3.5)	4 (2)	1142	1880	RI, odor
29	Unknown	NB	Stale/garbage	<1 (2)	ND	1143		
30	E-2-Nonenal	NB	Cucumbers	4 (3)	6 (2)	1149	1583	RI, odor, MS
31	2-Isobutyl-3-methoxypyrazine	NB	Bell pepper	3 (3.5)	2 (2)	1162	1403	RI, odor
32	Ethyl octanoate	NB	Sweet/fatty/citrus	<1 (1.8)	<1 (2.75)	1192	1436	RI, odor
33	Decanal	NB	Fatty	6 (1.9)	2 (2)	1214	1484	RI, odor, MS
34	Unknown	NB	Cilantro	<1 (2.7)	ND	1261		
35	Unknown	NB	Oatmeal	6 (3)	2 (1)	1266		
36	γ-Octalactone	NB	Coconut	<1 (2.5)	ND	1285	1918	RI, odor
37	Decanoic acid	AC	Fatty/stale	6 (1.5)	2 (1.75)	1310	2361	RI, odor
38	γ-Nonalactone	NB	Cilantro/waxy	9 (3)	9 (2.25)	1358	2042	RI, odor
39	4-Methyl octanoic acid	NB	Waxy	4 (3)	<1 (2)	1412	2176	RI, odor
40	Skatole	NB	Mothball	<1 (2)	<1 (1.5)	1459	2486	RI, odor
41	γ-Decalactone	NB	Floral/peach	1 (2)	<1 (1.5)	1483	2228	RI, odor
42	δ-Decalactone	NB	Waxy/coconut	3 (1.5)	2 (1.5)	1532	2103	RI, odor
43	Unknown	NB	Sweet/soap	<1 (2.7)	ND	1572	1972	
44	γ-Dodecalactone	NB	Peach	1 (3)	<1 (1.25)	1661	2389	RI, odor
45	(E,E)-2,4-Nonadienal	NB	Green	<1 (3.25)	<1 (1.5)		1796	RI, odor
46	Tetradecanol	AC	Bread/coconut	2 (1.5)	ND		2159	RI, odor

Continued

Table 17 (Continued). Aroma-active compounds detected in experimental 80% serum protein concentrate (SPC) and whey protein concentrate (WPC) by solvent-assisted flavor evaporation with gas chromatography-olfactometry with aroma extract dilution analysis

¹NB = neutral/basic compounds; AC = acidic compounds.

²Odor description at the GC sniffing port.

³Flavor dilution factors were determined on a DB-5MS column for NB compounds and on a DB-WAX column for AC compounds.

⁴Aroma intensity at the GC sniffing port, on a scale of 1 to 5.

⁵Retention indices were calculated from GC-olfactory data.

⁶Compounds were identified by comparison with authentic standards on the following criteria: retention index (RI) on DB-WAX and DB-5MS columns, odor property at the GC sniffing port, and mass spectra in the electron impact mode. Positive identification indicates that mass spectral data compared with authentic standards.

⁷ND = not detected.

of these compounds has been documented in whey products (Mahajan et al., 2004; Carunchia Whetstine et al., 2005a; Wright et al., 2006, 2009; Mortenson et al., 2008; Evans et al., 2009). These sulfur-containing compounds have aromas associated with garlic, cabbage, and potato. Thermally generated compounds were also identified in both products. 2-Acetyl-1-pyrroline was tentatively identified by RI and odor and has been documented in published work on fluid whey (Karagul-Yuceer et al., 2003) and dried whey proteins (Carunchia Whetstine et al., 2005a; Wright et al., 2006), as has 2-methyl-3-furanthiol. 2-Isobutyl-3-methoxypyrazine, 2-acetylpyridine, 2-acetyl-1-pyrroline, 2-methyl-3-furanthiol, and sotolon are thermally generated compounds from Maillard and caramelization reactions (Blank et al., 1996; Friedman, 1996; Mahajan et al., 2004). Three esters were identified in the products: ethyl propionate, 2-methylbutyl acetate, and ethyl octanoate. These compounds have sweet fruity aromas and are formed by an enzymatic or chemical pathway resulting in the esterification of fatty acids with primary alcohols (Dumont and Adda, 1978). 2-Methylbutyl acetate has been reported in 34% SPC (Evans et al., 2009). Ethyl propionate and ethyl octanoate have not been previously identified in whey or whey proteins; however, they have been reported in various cheeses (Qian and Reineccius, 2003, in Parmesan; Larrayoz et al., 2001, in ewe's milk cheese; Moio et al., 2000, in Gorgonzola).

2-Phenethanol, 2-methoxyphenol, and skatole were identified in the products and these compounds have been previously identified in dried whey and whey proteins (Mahajan et al., 2004; Carunchia Whetstine et al., 2005a). Lactones, with fruity, dairy, and tropical aromas, were identified in both products. Lactones are believed to be formed from triglycerides containing a hydroxyl acid moiety (Parliment et al., 1966) during the pasteurization of milk (Dimick et al., 1969). Lactones have been reported in liquid Cheddar whey (Karagul-Yuceer et al., 2003), WPC and WPI (Carunchia Whetstine et al., 2005a; Wright et al., 2006), and Cheddar cheese (Carunchia Whetstine et al., 2005b).

SPC Versus WPC. Five compounds were more prevalent in 80% SPC than in WPC (higher FD₃ factors) or were not detected in WPC (Table 17). Hexanal (green), 2-methoxy phenol (smoky), and E-2-nonenal (cucumber) had higher FD factors in SPC compared with WPC, and thenylthiol (garlic) and 4,5-dimethyl thiazole (burnt) were not detected in WPC but were present in SPC. These compounds most likely result from the alternative processing parameters, especially the multiple filtration steps at 50°C associated with separating serum proteins from casein. The SPC had a significantly lower fat content compared with the pilot-plant WPC. The lower fat content means the lipid

Table 18. Mean concentrations of selected volatile compounds ($\mu\text{g/L}$) of spray dried ($n = 3$) experimental 80% serum protein concentrate (SPC) and spray-dried ($n = 3$) whey protein concentrate (WPC) produced in this study and 5 commercial ($n = 2$) 80% WPC isolated using solid-phase microextraction

Source	Product	Hexanal	Heptanal	Diacetyl	Pentanal	Octanal	Nonanal	Decanal	2-Methylbutanal	Z-4-Heptenal
Commercial WPC	1	61.2 ^a	4.25 ^a	1.16 ^{bc}	6.11 ^a	1.18 ^a	2.25 ^a	0.09 ^c	2.78 ^{ab}	2.66 ^a
	2	26.5 ^b	2.15 ^b	2.92 ^a	3.9 ^b	0.70 ^{bc}	1.92 ^a	0.27 ^c	3.15 ^a	2.23 ^a
	3	1.4 ^c	0.63 ^c	0.57 ^{bcd}	1.03 ^c	0.42 ^c	0.73 ^c	0.78 ^b	1.17 ^{cd}	2.39 ^a
	4	44.6 ^{ab}	2.57 ^b	1.37 ^b	3.36 ^b	1.1 ^{ab}	1.63 ^{ab}	0.08 ^c	1.99 ^{abc}	2.31 ^a
	5	40.01 ^b	2.04 ^b	2.86 ^a	3.66 ^b	0.53 ^c	1.83 ^a	0.15 ^c	1.72 ^{abc}	2.50 ^a
Experimental	SPC	1.06 ^c	ND ¹	ND	ND	1.04 ^{ab}	0.5 ^c	2.32 ^a	0.26 ^c	ND
Experimental	WPC	6.58 ^c	0.43 ^c	0.50 ^{cd}	0.66 ^c	0.78 ^{abc}	1.14 ^{bc}	0.74 ^b	0.77 ^{cd}	2.32 ^a
R^2		0.65	0.73	0.67	0.63	0.33	0.54	0.86	0.43	0.02
SE: commercial		7.1	0.35	0.31	0.68	0.16	0.24	0.14	0.50	0.41
SE: experimental		5.8	0.29	0.25	0.55	0.13	0.20	0.11	0.40	0.34

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

¹ND = not detected.

oxidation products that do form play a more significant role in the overall flavor, as do compounds not formed by lipid oxidation. By GC-O analysis, 18 compounds were more prevalent in 80% WPC or absent altogether from SPC (Table 17). Most of these products are associated with the cheesemaking process and starter culture fermentation (Singh et al., 2003), along with lipid oxidation products. The increased number of lipid oxidation products in WPC might also be associated with the higher fat content in WPC compared with SPC (Table 4) or because of starter culture fermentation (Tomaino et al., 2004).

Evans et al. (2009) evaluated aroma-active compounds in pilot-plant 34% SPC and WPC. Eighteen of the 29 compounds identified in the pilot-plant 34% WPC and SPC were identified in the pilot-plant 80% WPC and SPC (diacetyl, acetic acid, 2/3-methylbutanal, dimethyl disulfide, hexanal, butyric acid, 2-methylbutyl acetate, Z-4-heptenal, methional, 2-acetyl-1-pyrroline, dimethyl trisulfide, 1-octen-3-one, octanal, thenylthiol, 2-methoxy phenol, E,Z-2-6-nonadienal, 2-phenethanol, and

E-2-nonenal) in the current study. More aroma-active compounds were identified in the 80% WPC and SPC (46) than in the 34% SPC and WPC (29). The additional compounds identified in the 80% protein samples were primarily aldehydes (5), lactones (5), and ketones (4). Similar to 34% WPC and SPC, the 80% WPC had higher FD factors for diacetyl, acetic acid, and butyric acid compared with 80% SPC. These compounds are associated with starter culture fermentation (Singh et al., 2003). The differences observed between 80% WPC and SPC were larger than those observed between 34% WPC and SPC. These more pronounced differences can be attributed to further processing and concentration of the whey and serum proteins, resulting in larger differences in composition.

Consumer Testing

An acidified 6% protein peach-flavored beverage was selected as an ingredient application. Previous studies have demonstrated that this application was gener-

Table 19. Mean sensory attributes¹ of 6% protein peach-flavored beverages manufactured ($n = 2$) from the experimental 80% serum protein concentrate (SPC) and 80% whey protein concentrate (WPC) in this study and 3 commercial 80% WPC

Source	Product	Aroma intensity	Fruity	Sweet aromatic	Cardboard	Cereal	FFA	Soapy	Bitter	Sweet taste	Sour taste	Astringent
Commercial WPC	2	2.8 ^{ab}	2.8 ^a	2.1 ^a	1.9 ^a	ND ²	ND	ND	ND	5.5 ^a	3.5 ^a	3.5 ^a
	3	3.0 ^a	2.5 ^{ab}	2.2 ^a	0.8 ^b	ND	ND	ND	ND	6.0 ^a	3.5 ^a	3.2 ^a
	4	1.5 ^b	2.0 ^{abc}	2.0 ^a	2.4 ^a	ND	ND	ND	ND	5.8 ^a	3.5 ^a	3.7 ^a
Experimental	SPC	2.3 ^{ab}	1.5 ^c	2.0 ^a	ND	1.5 ^a	1.3	2.3 ^a	1.8 ^a	5.5 ^a	3.3 ^{ab}	3.6 ^a
Experimental	WPC	3.0 ^a	1.8 ^{bc}	2.3 ^a	ND	2.0 ^a	ND	1.3 ^b	0.8 ^b	5.8 ^a	3.0 ^b	3.6 ^a
R^2		0.75	0.78	0.90	0.98	0.95	NA ³	0.98	0.96	0.75	0.81	0.90
SE		0.20	0.15	0.07	0.09	0.12	NA	0.08	0.09	0.07	0.08	0.28

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

¹Intensities were scored on a 0 to 15 universal scale where 0 = none and 15 = very high intensity (Meilgaard et al., 1999). Dried whey ingredient intensities usually fall between 0 and 4 on this scale (Drake et al., 2003; Wright et al., 2009). Attributes not listed were not detected.

²ND = not detected.

³NA = not applicable.

Table 20. Consumer ($n = 77$) liking scores¹ of 6% protein peach-flavored beverages manufactured from the experimental 80% serum protein concentrate (SPC) and 80% whey protein concentrate (WPC) in this study and 3 commercial 80% WPC

Source	Product	Aroma liking	Overall liking	Appearance liking	Flavor liking	Mouthfeel/thickness liking
Commercial WPC	2	4.58 ^b	5.1 ^b	5.8 ^{ab}	5.2 ^a	5.8 ^a
	3	5.55 ^a	5.8 ^a	5.6 ^b	5.6 ^a	5.9 ^a
	4	3.58 ^c	3.7 ^d	5.4 ^b	3.6 ^c	5.2 ^b
Experimental	SPC	5.50 ^a	4.4 ^c	6.2 ^a	4.3 ^b	5.6 ^{ab}
Experimental	WPC	5.54 ^a	5.2 ^b	6.2 ^a	5.2 ^a	5.8 ^a
R ²		0.19	0.14	0.05	0.13	0.26
SE: commercial		0.12	0.13	0.11	0.13	0.11
SE: experimental		0.15	0.15	0.12	0.16	0.13

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

¹Consumers scored attributes using a 9-point hedonic scale where 1 = dislike extremely and 9 = like extremely.

ally liked by consumers compared with other protein beverages and that it was sensitive to ingredient flavor carry-through (Childs et al., 2007; Drake et al., 2009; Wright et al., 2009). Representative commercial 80% WPC numbers 2, 3, and 4, selected based on descriptive sensory profiles, were also incorporated into beverages and compared with beverages made with pilot-plant 80% protein products.

SPC Versus WPC. Descriptive sensory analysis of 6% protein peach beverages revealed differences between beverages made from pilot-plant SPC and WPC, as well as differences between beverages made from pilot-plant products and those made from commercial WPC (Table 19). One of the main differences between pilot-plant SPC and WPC beverages was the presence of FFA flavor in beverages made from SPC and the absence of FFA flavor in beverages made from WPC. Reconstituted SPC at neutral pH had no detectable levels of FFA flavor (Table 14). However, a shift to a lower pH (beverage pH was 3.83 to 3.9) may increase the volatility of FFA with pKa values of 4.75, 4.82, 4.86, and 4.84 for acetic, butyric, propionic, and hexanoic acids, respectively. The SPC also had lower fat content compared with WPC (Table 8), which may have provided a reservoir for protonated FFA, allowing them to be undetected by sensory analysis of the products made with 80% WPC. Volatile compound analyses were not conducted on acidified solutions, meaning that the concentrations of the volatile FFA in these products may have been overlooked. The peach-flavored beverages made with pilot-plant SPC and WPC also contained soapy flavor and bitter taste intensities (Table 19). Soapy flavor has been noted in WPI rehydrated under neutral conditions and has been associated with medium-chain fatty acids (Carunchia Whetstine et al., 2005a). Free fatty acids (short and medium chain lengths) may also be the source of bitter taste (Carunchia Whetstine et al., 2005a). The peach-flavored beverage made with SPC had higher intensities of soapy flavor and bitter taste

compared with that made with WPC. Consistent with trained panel descriptive profiles, overall liking scores of the (higher fat) beverages made from pilot WPC (no FFA flavor) were higher compared with those for the (lower fat) beverages made from pilot SPC (FFA flavor; Table 20).

Pilot-Plant Versus Commercial Beverages.

Trained panel profiles of beverages made from commercial and pilot-scale proteins were also distinct (Table 19). Cereal and soapy flavors and bitter taste were documented in the beverages made using pilot-plant 80% SPC and WPC but were not present in the beverages made with commercial 80% WPC. In contrast, cardboard flavor was present in all 3 beverages made from commercial 80% WPC but was not detected in beverages made with pilot-plant SPC or WPC. Cardboard and brothy flavors have been previously documented in beverages with added whey protein (Childs et al., 2007; Drake et al., 2009; Wright et al., 2009).

Consumer liking scores were highest for beverages made with one commercial protein (product 3 originating from Mozzarella instead of Cheddar whey; Table 20). Beverages made with pilot-plant WPC and commercial protein sample 2 were liked second best. Both beverages made with pilot-scale protein products scored significantly higher in appearance liking than beverages made with the commercial protein products. Beverages made with commercial sample 4 scored the lowest in overall liking and liking attributes. This protein had the highest cardboard flavor intensity when rehydrated in water (Table 15). Previous studies have also demonstrated that off-flavor intensities (such as cardboard flavor) in whey proteins rehydrated in water are indicative of flavor carry-through potential in beverages (Drake et al., 2009; Wright et al., 2009).

CONCLUSIONS

The 80% SPC had lower fat content and higher pH compared with pilot-plant and commercial WPC. Pilot-

plant rehydrated 80% SPC and WPC were similar in flavor profile. Volatile compound differences were noted; pilot-plant WPC generally had higher concentrations as well as a higher number of lipid oxidation products than those of pilot SPC. Similarly, lipid oxidation products were generally higher in commercial 80% WPC samples compared with the SPC and WPC produced in this study. These results suggest that additional commercial processing parameters contribute to flavor and flavor variability in WPC. Acidified peach-flavored protein beverages made with pilot-plant or commercial proteins differed in sensory profiles and consumer liking and revealed differences that were not identified in rehydrated neutral pH protein solutions, emphasizing the need for ingredients to be evaluated in representative ingredient applications. Differences between pilot-plant products and commercial samples as well as the variability just among the commercial samples suggest that other processing factors have an influence on the flavor of WPC.

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