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ARTICLE

Characterization of Flavor of Whey Protein Hydrolysates

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Twenty-two whey protein hydrolysates (WPH) obtained from 8 major global manufacturers were characterized by instrumental analysis and descriptive sensory analysis. Proximate analysis, size exclusion chromatography, and two different degrees of hydrolysis (DH) analytical methods were also conducted. WPH were evaluated by a trained descriptive sensory panel, and volatile compounds were extracted by solid phase microextraction (SPME) followed by gas chromatography−mass spectrometry (GC-MS) and gas chromatography−olfactometry (GC-O). Eleven representative WPH were selected, and 15 aroma active compounds were quantified by GC-MS via the generation of external standard curves. Potato/brothy, malty, and animal flavors and bitter taste were key distinguishing sensory attributes of WPH. Correlations between bitter taste intensity, degree of hydrolysis (using both methods), and concentration of different molecular weight peptides were documented, with high DH samples having high bitter taste intensity and a high concentration of low molecular weight peptides and vice versa. The four aroma-active compounds out of 40 detected by GC-O present at the highest concentration and with consistently high odor activity values in WPH were Strecker derived products, dimethyl sulfide (DMS), 3-methyl butanal, 2-methyl butanal, and methional. Orthonasal thresholds of WPH were lower ($p < 0.05$) than basic taste thresholds suggesting that aromatics and bitter taste are both crucial to control in WPH food applications.

KEYWORDS: Whey protein hydrolysate; enzymatic hydrolysis; degree of hydrolysis; bitter taste; whey protein flavor

INTRODUCTION

Whey protein hydrolysates (WPH) are a good source of bioactive peptides, specific protein fragments that positively influence body functionality and may result in improved human health (*1*). Some bioactivities exerted include anticardiovascular disease activities, ion binding, antioxidant activities, immunomodulatory effects, satiety effects, and antiallergenicity (*1*). To obtain bioactive peptides from whey, enzymatic hydrolysis is usually a method of choice as it can minimize the impact of extreme pH and temperature on protein strands during chemical hydrolysis (via acid or base) (*1*). After hydrolysis to a desired degree of hydrolysis (DH), the fraction of peptide bonds cleaved expressed in percentage, the enzymatic process is inactivated using heat treatment to prevent further breakdown of peptides. Postprocessing such as clarification/filtration to remove insoluble residues or fractionation based on peptide size can also be achieved. Flavor generation steps, where flavor of WPH may differ from that of WPC or WPI, would primarily include the enzymatic treatments, additional thermal treatment for inactivation of enzymes, and final clarification/filtration steps. The peptide fragments generated may also contribute to basic tastes. Reducing peptide chain length and exposing hydrophobic peptides can contribute to bitter taste (*2*), and thermal treatment may elevate thermally generated flavors such as those from the Maillard reaction, lipid oxidation, saponification (*3*, *4*).

Bitter taste has been associated with WPH, and the degree of bitterness may depend upon the enzymes used for hydrolysis, degree of hydrolysis, and specific processing conditions (*2*, *5*). However, to our knowledge, no studies have characterized the flavor and flavor chemistry of WPH. Although bitterness is objectionable, the flavor of WPH can also be objectionable and has been largely overlooked. The objective of this study was to characterize flavor and the volatile sources of WPH flavor by sensory and instrumental analyses. Twenty-two commercial WPH from 8 major global manufacturers were evaluated.

MATERIALS AND METHODS

Chemicals. Compounds (**Table 1**) were obtained from Aldrich (St. Louis, MO) with some exceptions: dimethyl sulfide, *Z*-4-heptenal, phenylacetyldehyde, octanal, and *o*-aminoacetophenone were obtained from Acros Organic (Morris Plains, NJ); δ-decalactone was obtained from Alfa Aesar (Ward Hill, MA).

Whey Protein Hydrolysate Samples. Twenty-two spray dried whey protein hydrolysates (WPH) (approximately 1 kg each) were obtained from 8 global manufacturers, with 2 production codes acquired for each sample. The samples were stored at −20 °C upon receipt and throughout the experiments.

Proximate Analysis. Moisture content was determined on the basis of the ISO 5537:2004 method. Total protein content of WPH was determined using the Kjeldahl method based on ISO 5537:2004 by determining the

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Table 1. Aroma-Active Compounds in WPH by SPME GC-O

description	possible compound	RI (ZB5)[a]	RI (WAX)[b]	method of ID[c]	1[d]	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
sulfur	dimethyl sulfide	<600	667	RI,O,MS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
malty	3-methyl butanal	<600	757	RI,O,MS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
buttery	diacetyl	<600	975	RI,O,MS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
malty/chocolate	2-methyl butanal	637	912	RI,O,MS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
garbage/garlic	unknown	662				+	+		+	+					+			+	+	+	+	+		+	+	+
cheesy	butyric acid[e]	787		RI,O, MS		+			+						+						+					+
grassy	hexanal	801	1079	RI,O,MS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
old whey/vomit	unknown	832	1154		+	+			+		+			+		+						+	+	+	+	
cheesy/dried fruit	2/3-methylbutyric acid	833	1740	RI,O,MS	+		+	+	+	+	+			+	+	+		+		+	+	+		+	+	+
fruity	isopropyl butanoate[e]	850	1189	RI,O		+						+				+										+
cooked nutty	2-methyl-3-furanthiol[e]	859	1316	RI,O	+	+	+	+	+	+	+	+			+	+	+	+	+		+	+	+	+	+	+
fatty/fishy	*Z*-4-heptenal	898	1178	RI,O,MS	+	+	+	+	+	+	+		+	+		+	+	+	+	+	+	+	+	+		
potato	methional	907	1465	RI,O,MS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
sweet popcorn	2-acetyl pyrroline	921	1345	RI,O,MS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
garlic/cabbage	dimethyl trisulfide	960	1379	RI,O,MS		+	+		+			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
mushroom	1-octen-3-one	973	1305	RI,O,MS	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+
citrus/fatty/sweet	octanal	1001	1274	RI,O,MS		+		+		+		+	+	+		+	+	+	+	+	+	+	+	+	+	
rosy	phenylacetyldehyde	1042		RI,O,MS	+	+	+	+	+			+	+	+			+	+		+	+		+	+	+	
phenolic/Band-Aid	*p*-cresol[e]	1074		RI,O			+	+		+		+			+			+	+						+	
burnt/smoky/coffee	guaiacol	1081		RI,O,MS	+	+	+	+	+		+	+		+		+	+	+	+	+	+	+	+	+	+	+
fatty/citrus/sweet	nonanal	1096		RI,O,MS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
curry/maple	sotolone[e]	1095		RI,O		+	+		+			+					+			+	+			+		
rosy/fruity	2-phenethanol[e]	1144		RI,O														+				+				+
fatty/doughy	*Z*-2-nonenal	1146		RI,O,MS	+	+	+	+	+		+		+	+		+				+	+		+		+	+
earthy/soil	unkown	1148											+							+		+		+		
cucumber	(*E*,*Z*)-2,6-nonadienal	1149		RI,O,MS	+	+		+	+	+	+	+		+	+	+				+	+	+	+		+	
cucumber/carpets	*E*-2-nonenal	1160	1536	RI,O,MS	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
bandaid/plastic	unknown	1169				+				+			+		+	+		+		+	+					
cooked nutty	methyl-2-methyl-3-furyl disulfide[e]	1171		RI,O							+	+	+		+		+	+	+	+	+	+	+	+		+
fatty/hay/sweet	decanal	1196		RI,O,MS			+	+		+		+		+	+	+	+	+		+	+	+			+	+
onion/garlic	dimethyl tetra sulfide	1215		RI,O		+			+							+		+	+	+	+	+				+
fatty/hay/sweet	(*E*,*E*)-2,4-nonadienal	1217		RI,O										+						+	+	+	+	+		
minty/hay	2-octyl furan[f]	1237		RI	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+		
hay	*E*-2-decenal[e]	1266	1613	RI,O	+	+	+	+	+		+	+		+		+	+	+		+	+	+				
grapy/tortilla	*o*-aminoacetophenone[e]	1306	>2000	RI,O	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Coconut	Δ-decalactone	1473	>2000	RI,O,MS		+			+	+				+	+		+	+	+	+						
fruity/floral	B-ionone	1499	1975	RI,O,MS	+		+				+	+	+		+	+	+		+	+	+	+	+			+
Peach	gamma-decalactone[e]	1669	>2000	RI,O	+	+											+									+
coconut	unknown	1884			+	+			+			+		+		+	+	+	+							+

[a] Retention indices of the aroma event on the ZB-5 column. [b] Retention indices of the aroma event on the ZB-WAX column [c] Method of identification by RI (retention indices), O (olfactometry), and MS (mass spectrometry) compared with authentic standards. [d] Sample identification. [e] Tentative identification by RI, odor, and the literature. [f] Compound identified by RI and aroma from Greenberg (*34*); (+) indicates the presence of the compound detected by two experienced sniffers, () blank-indicates the absence of compound.

total nitrogen followed by multiplication by a conversion factor of 6.42−6.64 (depending on the degree of protein hydrolysis). Total fat content was determined using the Rose Gottlieb method based on IDF standard 16C:1987 (*6*). Percent ash was determined using a muffle furnace at 550 °C by dividing the initial weight by the residual (ash) weight. All analyses were conducted in duplicate on each sample.

Degree of Hydrolysis. Two methods were applied to evaluate the degree of hydrolysis: the 2,4,6-trinitrobenzene sulfonic acid (TNBS) and *o*-phthaldialdehyde (OPA) methods for comparative purposes.

Measurement of Reactive Amino Groups. The method by which reactive amino groups were measured to determine the extent of protein hydrolysis was adapted from the methods described by Lillard et al. (*7*). The soluble reactive amino groups were measured by the *o*-phthaldialdehyde assay method (OPA) (*8*). Ten percent w/v protein solutions in deionized water were diluted to 1:10 and microcentrifuged at 13,500*g* for 5 min (Beckman Coulter, Fullerton, CA). An aliquot (10−25 μL depending on the free amino acid concentration of the sample) of the sample was reacted with 2 mL of fluoraldehyde *o*-phthalaldehyde reagent solution (Thermo Scientific, Rockford, IL) for 5 min, and the absorbance was evaluated at A_{340} nm. All measurements were performed in duplicate. A microbicinchoninic acid (BCA) assay was used to determine the total soluble protein using BCA Protein Assay Kit (Thermo scientific, Rockford, IL). The results were expressed as μM free amino acid/mg of total soluble protein.

TNBS Method. The percent degree of hydrolysis (% DH) was measured according to the method of Adler-Nissen (*9*). Sample solutions were made by diluting 1 mL of 1% (w/v) whey protein hydrolysate solution with 9 mL of 1% (w/v) sodium dodecyl sulfate (SDS) solution (Sigma Aldrich, St. Louis, MO) to obtain a concentration of 0.1% (w/v protein basis). A 0.25 mL aliquot of protein solution was added to test tubes containing 2 mL of phosphate buffer (pH 8.2) (Sigma Aldrich, St. Louis, MO) Two milliliters of 0.1% (v/v) 2,4,6 trinitrobenzene sulfonic acid solution (TNBS) (Sigma Aldrich, St. Louis, MO) was added to each test tube and vortexed to ensure thorough mixing. The test tubes were incubated at 50 °C for 60 min. The reaction was stopped by the addition of 4 mL of 0.25 N HCL (Chemicals, Gibbstown, NJ) to each tube. These samples were measured at 340 nm against 5 mL of 1% (w/v) SDS blank solution. A leucine standard curve was obtained by diluting 28 mg/L leucine standard (Sigma Aldrich, St. Louis, MO) in 1% SDS to obtain 5.6, 11.2, 16.8, 22.4, and 28 mg/L amino nitrogen leucine standards, and the absorbance values (340 nm) obtained for the leucine standards were plotted.

Gel Permeation High Performance Liquid Chromatography (GP-HPLC). The molecular weight distribution of each whey protein hydrolysate was determined by gel permeation high pressure liquid chromatography (HPLC) using the HPLC system (Varian Inc., Palo Alto, CA) comprising a Prostar 210 binary pump (Varian Inc., Palo Alto, CA), a

Model 410 plus autosampler (Varian Inc., Palo Alto, CA), and a model 310 dual wavelength absorbance detector (Varian Inc., Palo Alto, CA). The column used was a TSK G2000SW separating column (600 × 7.5 mm I.D.) connected to a TSKGEL SW guard column (75 × 7 0.5 mm i.d.) (Tosoh Bioscience, Stuttgart, Germany). The elution was isocratic with a mobile phase composed of 0.1% (v/v) trifluoroacetic acid (T.F.A.) (EMD Chemicals Inc., Gibbstown, NJ) in 30% acetonitrile (v/v) (EMD Chemicals Inc., Gibbstown, NJ), at a flow rate of 0.038 cm s^{-1}. Each hydrolysate sample was diluted to 0.75% (w/v) protein equivalent in water and filtered through a 0.2 μm syringe filter (Millipore, Billerica, MA), and 20 μL was injected on the column. The column was calibrated with protein and peptides standards (0.25% (w/v) in water): bovine serum albumin (67,000 Da), β-lactoglobulin (18362 Da), ribonuclease A (13,700 Da), cytochrome *C* (13,000 Da), aprotinin (6,500 Da), and bacitracin (1,400 Da) obtained from Sigma Aldrich (St. Louis, MO). His-Phe-Arg-Trp (764.8 Da), Leu-Trp-Met-Arg (604 Da), Arg-Pro-Pro (404.4 Da), Leu-Phe (292.8 Da), Asp-Glu (262.2 Da), and Tyr-Gly (240.1 Da) were obtained from Bachem (Torrance, CA). The column void volume (V_0) was estimated from the elution volume of thyroglobulin (600,000 Da), and the total column volume (V_t) was estimated from the elution volume of L-tyrosine HCl (218 Da). The HPLC data was processed to give the percentage of peptides in the molecular weight ranges for 6 different bands: > 10, 5−10, 2−5, 1−2, 0.5−1, and < 0.5 kDa.

Descriptive Sensory Analysis. WPH were reconstituted at 10% solids (w/v) in deionized (DI) water and dispensed into 3-digit coded soufflé cups (Solo Cup, Highland Park, Il) and lidded. Aromatics and basic taste intensities of reconstituted samples were evaluated in triplicate by trained panelists (n = 10) at room temperature (22 °C) using an established sensory language for dried dairy ingredients (*10*). Panelists were between the ages of 23 and 45 years, with each having more than 80 h of experience with descriptive analysis of dried dairy ingredients. Because of the high bitter taste intensity of WPH, no more than 4 samples were evaluated at any session to prevent panelist fatigue, and basic taste profiling was conducted in separate sessions with no more than 3 samples evaluated per session. Panelist expectorated samples and were provided with room temperature deionized water for palate cleaning. A 3 min rest was enforced between samples. Compusense five version 4.8 (Compusense, Guelph, Canada) was used for data collection.

Solid Phase Microextraction: Gas Chromatography Olfactometry (SPME GC-O). All WPH samples were subjected to SPME GC-O to document aroma active compounds and also to capture any occurrence of compounds that may be unique in individual WPH. Ten percent solids WPH (w/v) and 10% sodium chloride (w/v) were reconstituted in HPLC grade water. An aliquot of 20 mL was taken out and placed in two 40 mL amber vials (28 × 98 mm, Supelco, Bellefonte, PA) with a PTFE/Silicone Septum (Supelco, Bellefonte, PA) and a stir bar. The vials were heated, with constant stirring at 40 °C for 30 min before exposure to a SPME fiber (DVB/CAR/PDMS) at 2 cm for 30 min. The fiber was then injected onto an Agilent 6850 gas chromatography-flame ionized detector (GC-FID) equipped with an olfactometer port (Agilent Technologies, Santa Clara, CA) in the front inlet at 3 cm. The GC method used an initial temperature of 40 °C for 3 min. The temperature increased at the rate of 10 °C/min to 150 °C followed by 30 °C/min to 200 °C, and then held for 5 min. Samples were evaluated on two different columns: ZB-5 ms and ZB-WAX plus (30-m length × 0.25-mm i.d. × 0.25-μm df) (Phenomenex Zebron, Torrance, CA). Column effluent was split 1:1 between the FID and the sniffing port using deactivated fused silica capillaries (1-m length × 0.25-mm i.d.) (Phenomenex Zebron). The FID sniffing port was maintained at 300 °C with helium carrier gas flow at 1018.6 cm s^{-1}. Two highly experienced sniffers (each with > 60 h experience with GC-O of dairy products) evaluated each sample in duplicate on each column. The aroma character, retention time, and maximum perceived aroma intensity (0 to 5 point scale) were recorded (*11*).

Solid Phase Microextraction: Gas Chromatography−Mass Spectrometry (SPME GC-MS). All WPH samples were subjected to SPME GC-MS for the identification of volatile compounds. The SPME GC-MS method and sample preparation were modified from the method used by Wright et al. (*10*). Ten percent (w/v) of WPH powder and 10% sodium chloride (w/v) were reconstituted in HPLC grade water (EMD Chemicals Inc., Gibbstown, NJ). An aliquot of 5 mL was taken out and placed in three 20 mL SPME vials with steel screw tops containing Teflon-faced silicon septa (Microliter Analytical, Sawanee, FL). Ten microliters of 2-methyl-3-heptanone in methanol (81 ppm) internal standard was added to each vial for relative abundance calculations. Samples were injected using a CTC Analytics CombiPal autosampler (Leap Technologies, Carrboro, NC) attached to an Agilent 6890N GC with 5973 inert MSD (Agilent Technologies, Santa Clara, CA). Samples were maintained at 5 °C prior to fiber exposures. Samples were equilibrated at 40 °C for 25 min before exposure to a 3-phase fiber DVB/CAR/PDMS (Supleco, Bellefonte, PA) at 31 mm for 30 min, with 4 s pulsed agitation at 250 rpm. Fibers were injected for 5 min at a depth of 50 mm. The initial GC temperature was set at 40 °C for 3 min with a ramp rate of 10 °C/min to 90 °C, increased at the rate of 5 °C/min to 200 °C, held for 10 min, and finally increased at the rate of 20 °C/min to 250 °C held for 5 min. SPME fibers were introduced into the split/splitless injector at 250 °C at a pressure of 7.06 psi with helium carrier gas, with a purge flow of 1697.7 cm s^{-1}. A ZB-5 ms column (30-m length × 0.25-mm i.d. × 0.25-μm df) (Phenomenex Zebron) was used for all analyses at a constant flow rate of 34 cm s^{-1}. 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. All samples were analyzed in triplicate using scan mode from 35 to 350 m/z.

Identification of Odorants. Tentative identifications were based on a comparison between the odor properties, retention indices (RI), and/or mass spectra of the unknowns against authentic standards and/or an evaluation of the literature. A compound that was not commercially available, 2-octyl furan, was tentatively identified on the basis of odor properties and a retention index obtained from the literature. An *n*-alkane series (Fluka, Buchs, Switzerland) was used for the calculation of RI (*12*). For positive identification of aroma active compounds, retention indices (RI) on both GC-FID columns, GC-MS column, odor properties, and spectra were compared to those of authentic standard compounds analyzed under identical conditions and the compound match in the NIST Mass Spectral Search Program 2.0 library.

Compound Recovery Utilizing Single Ion Monitoring Mode (SIM). Eleven representative samples were re-evaluated by SPME-GC-MS using the MS set in single ion monitoring (SIM) mode. The SIM mode allowed for improved detection levels by focusing on the unique ion(s) at a certain retention time of a compound of interest, ignoring other ions. The compounds selected were based on GC-O results. Exact retention time on the GC-MS for each compound was determined by injection of authentic standard in scan mode or converted from retention indices (for compounds with no authentic standard commercially available). The unique ions for each compound were selected using spectra acquired from NIST Mass Spectral Search Program 2.0 and the authentic standards. The most abundant ion with m/z greater than 50 was selected. Preliminary work allowed for elimination of some ions that were not detected by the MS (at certain retention time) to provide better sensitivity for the detection of other selected ions. The data were analyzed using MS ChemStation software (Agilent Technologies, Durham, NC).

Quantification of Odorants. Eleven representative samples were selected for the quantification of selected aroma active compounds. Selected compounds were quantified using 5-point external standard curves with internal standard calibration. The standard curves were generated by adding known concentrations of standards to 10% (w/v) reconstituted whey protein isolate (WPI) with composition similar to that of WPH samples. The area of compounds originally present in the protein matrix was served as a baseline prior to the addition of known compound concentration. Response factors (the area response on the GC-MS of a known concentration) relative to the internal standard (81 ppm of 2-methyl-3-heptanone in methanol) of these compounds were obtained and plotted to build a standard curve for each individual compound. The concentrations of the selected compounds in the samples were then quantified using the area ratio of compound to internal standard. Internal standard reproducibility was achieved with a relative standard deviation of 6.2−6.4%.

Odor Activity Values (OAVs). The odor activity values (OAVs) of selected compounds were calculated by dividing the concentration of each compound by the sensory detection threshold in water of each compound obtained from the literature. As threshold values vary tremendously with techniques employed, subjects, and temperature, etc., an attempt was made to keep the source of threshold values from the literature constant (i.e., all of the threshold values were acquired from the same research group except for one compound which was not tested by this group).

Sensory Thresholds. To further demonstrate the high aroma and basic taste activity of WPH, 3 WPH samples with low, medium, and high degree of hydrolysis (and distinct flavor profiles and bitter taste intensities) were selected for orthonasal and basic taste threshold testing. Orthonasal and basic taste threshold tests were conducted separately on different days. Testing solutions were made with an increase in concentration by a factor of 3. Fifteen milliliters of each solution was poured into clean, black (to prevent visual differences) 50 mL plastic soufflé cups with 3 digit codes and lidded (Solo cup, Highland Park, Il). Deionized water was the blank.

Subjects ($n = 25$) were instructed prior to testing and participated in at least three practice orthonasal threshold tests prior to participating in this study. For orthonasal thresholds, subjects were told to open each soufflé cup from the side and to briefly sniff the headspace of each cup in the series without completely removing the cup lid. Subjects rested 1 min between each set of three and were also instructed to sniff their sleeve to assist clearing their nasal passageways between cups. For basic taste thresholds, subjects were blind folded to prevent obvious visual difference in samples and wore nose clips (Mohawk Medical Mall, Utica, NY) in order to prevent the detection of odd samples orthonasally. Subjects were asked to choose the one item from the three that they thought different and to give a certainty judgment (sure/not sure). Panelists were given these concentrations in a series with two appropriate blanks at room temperature.

Seven ascending series were tested for orthonasal thresholds, and 5 ascending series were tested for basic taste thresholds. Series were presented in ascending concentration, and each series was presented in a randomized order and evaluated by panelists using the ASTM ascending forced choice method of limits procedure E679−79 (*13*) with the sure/not sure modification detailed by Lawless et al. (*14*). Threshold testing was conducted in duplicate for each sample on different days. Group thresholds were taken as the geometric mean of the individual best estimate thresholds. Compusense five, version 4.8 (Compusense, Guelph, Canada) was used for data collection.

Statistical Analysis. The estimated group BET values were approximately normally distributed as a result of the Central Limit Theorem. Hence, pairwise comparisons of the group estimates of the BET values were formed using $Z = (BET_2 - BET_1)/((SE_1^2 + SE_2^2)^{1/2})$ as a test statistic that was standard normal under the null hypothesis of common BET values. The pairs computed were basic taste and orthonasal BETs of each WPH, the BETs within the basic taste thresholds, and within the orthonasal thresholds. This pairwise comparison approach was adopted to reduce the type II error rate, which, if too large in an initial study like this, may result in failing to find differences that can be subsequently investigated more thoroughly.

Correlation analysis was conducted using SAS software, version 9.1 (SAS Institute Inc., Cary, NC). Pearson's correlation coefficient was calculated using the Proc Corr procedure. For descriptive sensory analysis, GC-MS quantitation, % DH (TNBS), and (μM/mg) free amino acids in total soluble protein, analysis of variance (ANOVA) with Fisher's least significant difference was used to test significance at $p \leq 0.05$ significance level (XLStat, Addinsoft, New York, NY).

RESULTS AND DISCUSSION

Proximate Analysis. Proximate composition of WPH was variable: protein content ranged between 66.4 and 88.3%; fat content between 0.00−7.08%; moisture content between 3.37−6.76%, and ash between 1.94−9.09% (**Table 2**). Much of the variability in the composition was due to the initial protein content of the unhydrolyzed protein sources, i.e., hydrolysis derived from WPC 80% protein vs WPI, which is more than 90% protein, as previously shown in other studies (*15*). The ratio of total nitrogen to total protein mass changes depended on the degree of hydrolysis and was corrected in this study by the application of different conversion factors (6.42−6.64). The starter culture, cheese ripening processes, milk composition, processing parameters, and storage time/condition within each manufacturing facility may also impact the proximate composition of WPH since they were obtained from different manufacturers. All values were within expected ranges.

Table 2. Proximate Analysis of WPH

sample ID	% protein	% fat	% moisture	% ash
1	74.6 ± 0.22[a]	4.40 ± 0.40	4.70 ± 0.04	5.32 ± 0.07
2	79.8 ± 0.89	4.72 ± 0.23	5.11 ± 0.42	3.19 ± 0.57
3	74.4 ± 0.19	4.18 ± 0.86	5.97 ± 0.31	7.46 ± 0.36
4	76.3 ± 0.23	0.15 ± 0.02	4.09 ± 0.06	9.09 ± 0.06
5	64.4 ± 13.2	7.08 ± 3.05	6.06 ± 0.23	5.02 ± 0.57
6	85.7 ± 0.13	0.35 ± 0.04	4.74 ± 0.17	2.87 ± 0.05
7	79.2 ± 0.13	6.56 ± 0.04	5.63 ± 0.02	2.93 ± 0.09
8	85.3 ± 1.25	0.69 ± 0.08	3.38 ± 0.57	6.22 ± 0.78
9	77.8 ± 0.25	2.93 ± 0.67	5.21 ± 0.97	4.65 ± 0.11
10	73.3 ± 0.28	5.50 ± 0.75	5.57 ± 0.51	4.51 ± 0.01
11	79.8 ± 1.19	0.30 ± 0.11	6.06 ± 0.33	4.08 ± 0.06
12	80.1 ± 1.31	5.64 ± 1.34	4.88 ± 0.05	3.24 ± 0.23
13	87.0 ± 0.30	0.25 ± 0.02	5.98 ± 0.01	2.72 ± 0.07
14	75.6 ± 3.56	6.19 ± 0.74	5.70 ± 0.01	4.50 ± 1.75
15	85.1 ± 0.81	1.10 ± 1.22	6.76 ± 0.23	2.54 ± 0.14
16	85.6 ± 1.12	2.57 ± 0.12	5.22 ± 0.08	2.89 ± 0.28
17	87.1 ± 0.04	2.97 ± 0.05	4.85 ± 0.11	1.94 ± 0.03
18	86.7 ± 1.30	3.23 ± 0.07	5.00 ± 0.25	2.57 ± 0.12
19	77.3 ± 1.85	5.47 ± 0.61	4.93 ± 0.93	4.36 ± 0.23
20	78.4 ± 0.26	4.36 ± 0.04	5.78 ± 0.13	3.79 ± 0.06
21	77.5 ± 0.69	0.00 ± 0.35	4.60 ± 0.07	7.03 ± 0.02
22	88.3 ± 0.74	0.33 ± 0.04	4.79 ± 0.35	6.22 ± 1.25

[a] Each value is the mean of duplicate analyses of 2 separate lots of each WPH ± standard deviation.

Degree of Hydrolysis. Degree of hydrolysis (%), free amino acids/total soluble proteins values (μM/mg), bitter taste intensities, and molecular weight profiles of WPH are shown in **Table 3**. The DH was determined by the TNBS method which is widely used and reported to be the most suitable for quantification of DH in WPH (*16*). However, another study suggested that the OPA method was more accurate, simple, and more environmentally friendly compared to TNBS (*17*). Thus, the OPA method was also performed. There was a strong positive correlation between the 2 methods ($p < 0.0001$, $r^2 = 0.77$). This implies that the latter method, which is less complicated and less time-consuming, may be sufficient to use when determining the extent of hydrolysis in whey proteins.

The TNBS DH (%) was compared with bitterness and concentration of amino acids of distinct molecular weight cut-offs (**Table 3**). Samples with high degree of hydrolysis were correlated with a lower concentration of large to medium chain peptides (2000−10,000 Da) and high concentration of small peptides (< 500 Da). Using the alternative OPA method of determination of reactive amino acids in total soluble proteins, similar relationships were documented, with minor differences (**Table 3**). The correlations between the degree of hydrolysis determined by both methods and concentrations of peptide molecular weights were expected.

The differences in the concentration of peptides with different molecular weights within a sample contributed to bitter taste intensity (**Table 3**). High bitter taste was correlated with low concentration of larger and medium chain peptides (2,000 to > 10,000 Da) and high concentration of lower molecular weight peptides (< 500−1,000 Da) which agreed with previous studies (*2*, *18*). There was no correlation between bitter taste intensity and concentration of 1,000−2,000 Da peptides. Moreover, the DH using the TNBS method and bitter taste intensity were correlated ($p < 0.001$, $r^2 = 0.32$), with WPH with higher degree of hydrolysis having higher bitter taste intensity and vice versa. However, there was no correlation between the OPA DH results and bitter taste intensity ($p > 0.05$, $r^2 = 0.03$). It is unclear, in spite of a correlation between the TNBS and OPA DH, as to the lack of correlation between OPA DH and bitter taste. However, previous

Table 3. Percent Molecular Weight Distribution of Peptides, Average Molecular Weight of Peptides, % Degree of Hydrolysis, and Bitter Taste Intensities of WPH[a]

	BI Intensity[b]	FAA/TSP (μM/mg)[c]	% DH[d]	>10,000 Da (%)[e*,**,***]	5,000 - 10,000 Da (%)[e*,**,***]	2,000 - 5,000 Da (%)[e*,**,***]	1,000 - 2,000 Da (%)[e]	500 − 1000 Da (%)[e**,***]	<500 Da (%)[e*,**,***]
1	12.4ab	4390b	33.8c	13.2	1.90	4.25	8.03	16.4	56.3
2	9.8cde	1230ghij	6.60ij	55.4	5.10	7.68	6.85	8.12	16.8
3	2.4j	974hij	5.10jk	51.7	4.94	7.91	7.87	9.16	18.5
4	13.5a	5030a	46.7a	0.02	0.07	2.36	9.46	18.5	69.6
5	5.9fg	2060cde	18.6ef	32.4	3.74	9.59	8.30	12.3	33.7
6	11.4abcd	912hij	7.30ij	52.6	6.00	8.03	7.04	9.41	16.9
7	11.1bcd	1760def	12.6gh	22.0	6.99	11.3	10.6	16.6	32.8
8	5.6gh	1620efg	16.6ef	21.0	5.84	13.0	13.0	15.0	32.2
9	3.6ghij	1370fgh	15.2fg	33.9	6.68	11.4	9.49	13.4	25.1
10	9.2de	5360a	39.4b	2.90	0.46	2.01	7.52	17.0	70.6
11	11.7abc	4520b	19.1e	0.00	0.06	4.09	10.8	26.3	58.7
12	4.3ghij	1160hij	5.36jk	12.7	5.25	11.8	19.1	27.9	23.2
13	3.9ghij	804j	2.70kl	61.0	6.49	14.6	4.64	4.54	8.75
14	13.0ab	2000de	18.7e	9.90	2.99	11.9	15.5	20.7	39.1
15	13.4a	2020de	18.5ef	20.9	4.98	14.5	13.9	16.5	29.2
16	3.4hij	1250ghij	9.77hi	36.2	7.04	10.6	10.1	12.4	23.6
17	2.7ij	828ij	1.44 L	49.7	9.01	11.0	8.34	8.44	13.4
18	4.0ghij	995hij	5.66jk	22.2	4.38	11.9	15.3	19.7	26.5
19	8.1ef	2110 cd	18.5ef	16.0	3.45	11.0	13.5	16.5	39.7
20	4.8ghi	1050hij	4.20jkl	23.0	11.55	9.49	15.1	16.0	24.9
21	9.3de	2480c	28.5d	0.00	0.15	3.01	11.2	24.8	60.8
22	8.2e	1280ghi	16.8ef	19.4	7.40	14.8	16.4	19.4	22.5

[a] Means in columns 1 , 2, and 3 not followed by a common letter are statistically different ($p < 0.05$). [b] Bitter taste intensity determined by descriptive sensory analysis on a 0−15 point scale (Spectrum method) where 0 = absence of bitter taste and 15 = extremely high intensity (*32*) [c] Free amino acid (μM) in mg soluble proteins determined by the OPA method. [d] Percent degree of hydrolysis determined by the TNBS method. [e] Percent of peptides with certain molecular weight >10,000, 5,000-10,000, 2,000-5,000, 1,000-2,000, 500-1,000 and <500 Da, respectively, determined by size exclusion chromatography, * and * correlation between TNBS and peptide with certain molecular weight at $p < 0.001$ and $p < 0.05$, respectively; ** and ** correlation between free amino acid (μM) in mg soluble proteins determined by the OPA method and peptide with certain molecular weight at $p < 0.001$ and $p < 0.05$, respectively; *** and *** correlation between bitter taste intensity and peptide with certain molecular weight at $p < 0.001$ and $p < 0.05$, respectively.

Table 4. Mean Descriptive Sensory Profiles of Selected WPH Rehydrated at 10% (w/v) Solids[a]

sample ID	aroma intensity	cooked/ milky	cooked/ sulfur	malty	cardboard	potato brothy	cheesy brothy	grassy/ herbal	tortilla/ animal	cucumber	scorched	sour	bitter	astringent mouthfeel
1	2.9cde	ND[b]	1.7ab	ND	0.8a	1.1bc	1.3a	1.0a	ND	ND	2.0ab	2.3a	12.4ab	3.0abc
2	2.9 cd	2a	1.0ab	ND	ND	0.5c	ND	0.5a	0.6bc	ND	ND	2.5a	10.1c	3.1bcd
3	2.1ef	1.1ab	1.4ab	ND	ND	1.5bc	0.4a	1.5a	1.1bc	ND	0.9ab	ND	2.4 h	2.5cde
8	1.9f	ND	ND	0.9bc	ND	1.3bc	1a	ND	ND	ND	0.8ab	ND	5.6e	2.1e
9	3.5bc	ND	0.9ab	3.0a	1.0a	2.7ab	1.4a	ND	1.1bc	ND	0.9ab	ND	3.6fg	2.4de
11	2.6def	ND	ND	0.6bc	0.5a	1.6bc	0.9a	ND	0.6bc	ND	3.1a	2.6a	11.7b	2.3de
14	2.5def	ND	ND	0.5bc	1.0a	1.6bc	1.5a	ND	2.6ab	ND	ND	0.8b	13.0a	3.8ab
17	3.7b	ND	2.0ab	ND	ND	2.3abc	ND	ND	2.4ab	0.9b	ND	ND	2.8gh	3.0bcde
18	3.7b	ND	1.2ab	ND	ND	1.5bc	ND	1.3a	2.3b	ND	ND	ND	4.0f	3.4abc
21	4.6a	ND	ND	ND	ND	3.6a	ND	ND	4.4a	ND	2.8a	2.8a	9.2 cd	4.1a
22	3.7b	ND	3.4a	1.8ab	ND	1.5bc	ND	ND	2.0b	2.4a	2.4ab	ND	4.7ef	2.4de

[a] Descriptive sensory profiles of selected WPH using a 0−15 point Spectrum scale (*32*) where 0 = absence of attribute and 15 = extremely high intensity. Most dried ingredient aromatics fall between 0 and 4 on this scale. Means in a column not followed by a common letter are statistically different ($p < 0.05$). [b] ND: not detected.

research reported weak interactions between OPA and cysteine which possibly contributed to an underestimation of DH in WPH (*16*). This may impact the concentration of reactive amino groups obtained from the OPA method in correlation with bitter taste intensity, as bitter scores take into account not only the peptide chain length or the % of small MW peptides but also the physicochemical properties of the peptides.

Peptides containing hydrophobic residues at C terminals were shown to cause bitterness (*2*, *18*). For bitterness to occur, bitter molecules must bind to a bitter taste receptor located in the apical microvilli of taste receptor cells (TRCs). Cheison et al. (*18*) reported that a high hydrophobic WPH containing 71% of peptides with <600 Da and no greater than 4142 Da were extremely bitter. Larger peptides (at least above 3000 to 6000 Da) were able to form hydrophobic interactions among themselves, blocking hydrophobic groups. Peptides between 3 and 6 amino acids contribute to bitterness; however, almost all peptides with L-conformation and hydrophobic side chains elicited bitter taste (*19*). Other factors that contribute to bitter taste include the specific amino acid sequence of the peptide chain (*19*). There were samples in this study acquired from different manufacturers that had low DH but possessed high bitter taste intensities. Commercially and experimentally, processing steps, enzyme cocktails, and protein sources are all influencing factors that contribute to levels of bitterness. Although TNBS and OPA methods were positively correlated, TNBS results were correlated with bitter taste intensity ($p < 0.001$, $r^2 = 0.32$) and may be the method of choice when determining the DH of WPH as it relates to sensory perception of bitterness.

Descriptive Analysis. Since bitter taste for some of the WPH were very high and likely to obscure the contributions of other attributes, PCA biplots of WPH across all sensory attributes and with only aromatics (**Table 4**) were generated (**Figures 1** and **2**). From **Figure 1**, 6 groups of products were observed. Samples with similar attributes were grouped: intensely bitter samples; samples that were characterized by malty, scorched, and potato flavors;

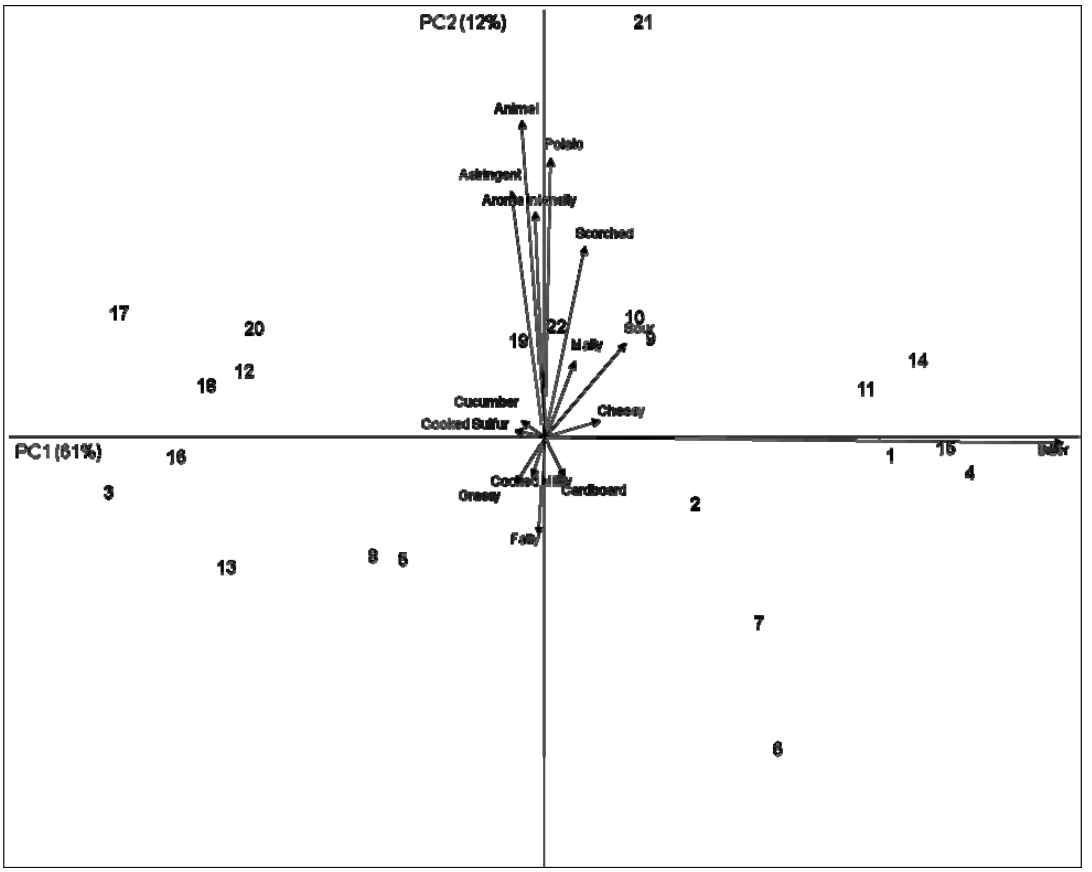

Figure 1. PCA biplot of sensory properties of WPH with all sensory attributes. Numbers 1−22 represent WPH samples, and vectors represent sensory attributes. This plot contains 6 groups total (group numbers were arbitrarily assigned): group 1 (samples 1, 4, 15, 11, and 14); group 2 (samples 19, 22, 10, and 9); group 3 (samples 2, 7, and 6); group 4 (samples 5 and 8); group 5 (samples 3, 16, and 13); group 6 (samples 12, 17, 18, and 20).

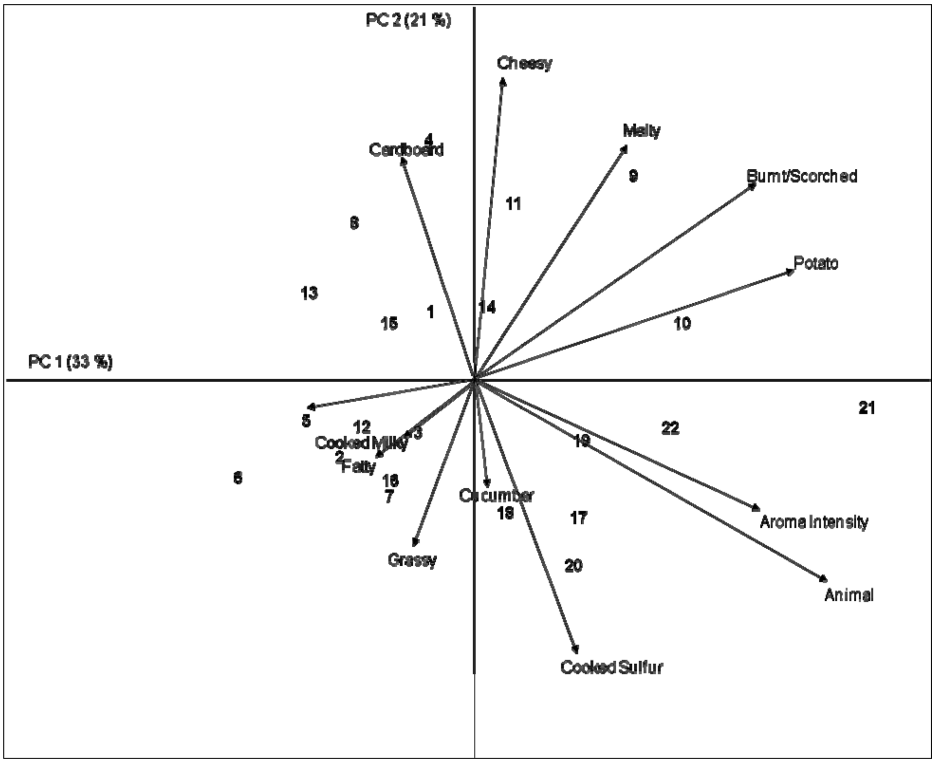

Figure 2. PCA biplot of sensory properties of WPH without basic tastes (bitter and sour) and astringency. Numbers 1−22 represent WPH samples, and vectors represent sensory attributes. This plot contains 5 groups total (group numbers were arbitrarily assigned): group 1 (samples 9 and 10); group 2 (samples 19, 22, 18, 17, and 20); group 3 (samples 2, 3, 5, 6, 7, 12, and 16); group 4 (samples 1, 4, 8, 13, and 15); group 5 (samples 11 and 14).

samples that lacked those flavor characteristic; samples with medium bitterness, low aroma intensity, and low animal flavor; and samples with medium bitterness and high animal flavor. Product number 21 was distinct in sensory profile and was characterized by high aroma intensity, potato/brothy and animal flavors, and bitterness. **Figure 2** shows the PCA biplot of sensory flavors excluding basic tastes and astringency to investigate the groupings of samples due to flavors alone. Five groupings were observed: samples that were characterized by potato/brothy, malty, and burnt/scorched flavors; samples that were characterized by cooked/sulfur and animal flavors and high aroma intensity; samples that were characterized by grassy flavor and lack of burnt/scorched notes; samples that were discriminated on the basis of cardboard flavor intensity; and samples that were characterized by the presence of cheesy, malty, and potato/brothy flavors and lack of cooked milky, fatty, and grassy flavors. Sample 21 was once again distinct from other samples, mainly by high aroma intensity.

Similar groupings were observed between the two biplots. More variability was explained on the first two principle components in **Figure 1** compared to that in **Figure 2**. This confirms that basic taste, especially bitterness, impacts the groupings of samples and differentiates the WPH but that aromatics also differentiate the WPH. There were no correlations between bitter intensity scores and individual sensory aromatic (flavor) intensities ($p > 0.05$), meaning that low bitter taste did not equate to low flavor in other sensory categories or vice versa. However, there was a positive correlation between bitter intensity scores and sour taste ($p < 0.05$; $r^2 = 0.79$). Sour taste was perceived in the samples that were highly bitter. Specificity of the peptides and extent of hydrolysis may play roles in sour and bitter tastes perceived. Acidic amino acid dipeptides (Val-Glu and Val-Asp) were reported to exhibit sour and slight bitter tastes (*20*).

From examination of sensory biplots and DH results, 11 representative WPH were chosen for instrumental volatile quantitation by GC-MS (samples: 1, 2, 3, 8, 9, 11, 14, 17, 18, 21, and 22) (**Table 5**). Some of the major flavors of these samples were cooked/sulfur, potato/brothy, cheesy, tortilla/animal, and malty. These descriptors are unique to WPH; they are not commonly reported in unhydrolyzed whey proteins (*10*, *15*, *21*). Flavors such as cooked/milky, cardboard, cucumber, cabbage/brothy, and fatty, which are commonly found in WPC and WPI, were also present in WPH (*10*, *15*). Flavors present in many WPH, animal and potato/brothy, have been documented as major attributes in rennet caseins (*5*, *22*). Liberated amino acids/peptides due to protease and thermal treatment generate flavor compounds in casein that are similar to those of WPH, probably derived from the same amino acid precursors.

Gas Chromatography−Olfactometry (GC-O). Forty aroma-active compounds were detected by solid phase microextraction (SPME) GC-O of WPH (**Table 1**), of which, 21 compounds were positively identified (detected by MS, RI, and odor as compared to the authentic standards), 14 compounds were tentatively identified (detected by RI and odor compared to authentic standards or RI and odor compared with published literature), and 5 compounds were unknown. Most of these compounds have previously been documented in dairy products and are generated by lipid oxidation, lipolysis, protein proteolysis, and glycolysis of sugars (*15*, *21*, *23*, *24*), except for methyl-2-methyl-3-furyl disulfide, 2-octyl furan, and *E*-2-decenal. Eight out of the 40 aroma active compounds detected by SPME GC-O were present in all WPH. The presence or absence of these compounds in different concentrations WPH (**Table 1**) likely contributes to the differences in sensory profile of each product (**Table 4**). All of the compounds quantified except guaiacol were present at concentrations

Table 5. Concentration in Parts Per Billion (ppb) of Selected Compounds in WPH by SPME GC-MS[a]

samples	dimethyl sulfide $R^2 = 0.94$	3-methyl butanal $R^2 = 0.94$	diacetyl $R^2 = 0.99$	2-methyl butanal $R^2 = 0.93$	hexanal $R^2 = 0.97$	*Z*-4-heptenal $R^2 = 0.98$	methional $R^2 = 0.91$	dimethyl trisulfide $R^2 = 0.98$	octanal $R^2 = 0.98$	phenylacetylde-hyde $R^2 = 0.98$	guaiacol $R^2 = 0.98$	nonanal $R^2 = 1.00$	*E*-2-nonenal $R^2 = 0.96$	*E*,*Z*-2,6-nonadienal $R^2 = 0.88$	1-octen-3-one $R^2 = 0.92$
1	29.8def	217cde	7.26c	33.8def	9.61efg	1.21de	56.4 cd	0.04d	0.29 g	3.98d	0.05c	3.19def	1.22d	0.34cde	1.36f
2	47.1cde	116de	60.5b	13.6ef	17.1e	1.73bcd	26.0d	0.04d	0.51f	1.63d	0.05c	5.78c	0.76d	0.30de	2.31ef
3	56.6bcd	27.2e	14.1c	38.9def	11.2efg	0.57efgh	74.1 cd	0.05 cd	0.30 g	2.66d	0.19bc	2.87def	2.03 cd	0.91bcd	1.76ef
8	50.0 cd	39.5e	3.99c	20.0def	17.0e	0.171gh	22.4d	0.06 cd	0.27 g	1.50d	0.59a	1.73ef	4.21a	1.674a	5.90bc
9	82.7ab	1350a	29.5c	365a	55.6b	1.03e	525a	0.28b	0.75e	33.1b	0.18bc	2.91def	0.90d	0.19e	12.4a
11	51.7 cd	346c	24.5c	55.0 cd	6.74fgh	0.58efg	4.45d	0.06 cd	0.83e	4.08d	0.10c	10.9b	2.19 cd	1.16ab	2.31ef
14	15.8fg	149cde	91.5a	39.7def	65.9a	1.40cde	33.5d	0.23bc	1.02de	2.73d	0.09c	2.67def	2.60bcd	1.07ab	5.14 cd
17	2.08 g	1131a	13.9c	203b	39.7c	2.73a	131bc	0.14c	1.32c	81.6a	0.35b	3.93 cd	1.35d	1.44ab	12.3a
18	1.26 g	23.1e	19.6c	10.6f	29.4d	2.14b	2.88d	0.09 cd	1.11d	1.06d	0.12c	3.21def	1.42d	1.06ab	3.62de
21	61.1bc	713b	124a	91.4c	14.9e	0.43fgh	215b	3.24a	2.28b	30.3b	0.18bc	22.0a	2.97bc	1.19ab	2.40ef
22	23.1efg	2.67e	0.381c	7.41f	4.38gh	0.00 h	0.00d	0.04d	0.15 g	1.37d	0.19bc	1.28f	0.856d	1.16ab	3.58de

[a] Mean concentration of selected compounds in WPH by SPME GC-MS using the SIM mode quantified using five-point external standard curves; R^2 = correlation coefficient of regression line; Means in a column not followed by a common letter are statistically different ($p < 0.05$).

greater than reported sensory detection thresholds, which suggest that these compounds play roles in WPH flavor. Furthermore, there were no correlations between the quantified compounds and bitter taste intensity ($p > 0.05$).

Major Flavor Compounds

Reaction of Proteins. 3-Methyl butanal and methional were key volatile compounds present in all WPH samples and had high odor impact (high OAVs; **Table 6**). These compounds are Strecker degradation products and may be derived from further degradation of amino acids from the initial enzyme mixture used to produce the WPH or may be formed during the heating step to inactivate the enzymatic protein hydrolysis process. This heat treatment can stimulate Maillard reaction-generating intermediates that react with α-amino acids to form specific aldehydes including 3-methyl butanal and methional. However, the concentration of these compounds was not related to the degree of hydrolysis of the samples ($p > 0.05$).

2- and 3-Methyl butanal were present at high concentration in many WPH samples (**Table 5**) and were derived from the degradation of branched chain amino acids: isoleucine and leucine, respectively (*3*). This is expected because whey protein itself contains high concentrations of branched chain amino acids. These compounds were previously documented in 34% serum protein concentrate and 34% protein WPC (*21*) and cheese (*3*), but not in liquid or dried whey (*23*, *25*, *26*). 2- and 3-methyl butanal are likely the main contributors to malty flavor in WPH. These compounds were not potent aroma impact compounds in WPC 34 (*21*). Multiplication of these compounds can be viewed to be primarily generated from the hydrolysis of WPC or WPI and thermal treatment. Sample 9 contained the highest concentrations of 2- and 3-methyl butanal ($p < 0.05$, **Table 5**) and also had the highest intensity of malty flavor ($p < 0.05$, **Table 4**) documented by descriptive panelists. However, there were other samples that had 2- and 3-methyl butanal concentrations but did not have malty flavor by descriptive analysis. The malty flavor may have been overpowered by other potent aromatics such as cooked/sulfur or potato/brothy (samples 1, 2, 3, 17, 18, and 21; **Table 4**).

Methional was detected by SPME GC-O and GC-MS in all selected WPH (except for sample 22 on GC-MS; **Table 5**) and was a key contributor to the flavor of WPH as indicated by its high OAV (**Table 6**). The other sulfur containing aroma-active compounds present in WPH samples were DMS, 2-methyl-3-furanthiol, methyl 2-methyl-3-furyl disulfide, DMTS, and dimethyl tetrasulfide. All of these compounds except for methyl 2-methyl-3-furyl disulfide have been documented in sweet whey powder (*23*), liquid cheddar whey (*25*, *26*), WPC 80, 34, and WPI (*15*, *21*), serum protein (*21*), and different types of cheese, i.e., camembert, munster, and cheddar (*3*). Methyl 2-methyl-3-furyl disulfide was previously reported in ultrahigh temperature (UHT) skim milk powder (*27*). The sources of sulfur compounds may be derived from the major protein fractions in whey protein, β-Lg and α-La, which are sulfur-containing molecules. Samples with higher methional concentration (9, 17, and 21; **Table 6**) had high potato/brothy flavor (**Table 4**), indicating that methional is a major contributor to potato/brothy flavor. Cooked/sulfur flavor may be associated with many sulfur containing compounds, and DMS may be one of the major contributors to this flavor since it had a relatively high OAV in many WPHs.

Other Strecker degradation derived compounds detected in some WPH were phenylacetaldehyde, 2-phenethanol, *p*-cresol, guaiacol, 2-acetyl pyrroline, and sotolon. These compounds did not impart their own specific flavor characteristics but may have enhanced some of the cooked, scorched, or unclean flavors in WPH. Phenylacetyldehyde and 2-phenethanol contribute to rosy unclean off-flavors in cheddar cheese (*3*) and in sweet whey

Table 6. Odor Activity Values (OAV)[a] Calculated for Selected Compounds in WPH

samples	DMS	3-methyl butanal	diacetyl	2-methyl butanal	hexanal	*Z*-4-heptenal	methio-nal	dimethyl trisulfide	octanal	phenylacetylde-hyde	guiacol	nonanal	*E*-2-nonenal	*E,Z*2,6-	1-octen-3-one
thresholds (ppb) (refs)	0.3 (*33*)	0.2 (*34*)	3.0 (*34*)	3.0 (*34*)	4.5 (*34*)	0.2 (*35*)	0.2 (*34*)	0.01 (*34*)	0.7 (*34*)	4.0 (*34*)	3.0 (*34*)	1.0 (*34*)	0.08 (*34*)	0.01 (*33*)	0.05 (*34*)
1	99.3	1090	2.42	11.3	2.14	6.05	282	4.00	0.41	1.00	0.02	3.19	15.3	34.0	27.2
2	157	580	20.2	4.53	3.80	8.65	130	4.00	0.73	0.41	0.02	5.78	9.50	30.0	46.2
3	189	136	4.70	13.0	2.49	2.85	371	5.00	0.43	0.67	0.06	2.87	25.4	91.0	35.2
8	167	198	1.33	6.67	3.78	0.86	112	6.00	0.39	0.38	0.20	1.73	52.6	167	118
9	276	6750	9.83	122	12.4	5.15	2630	28.0	1.07	8.28	0.06	2.91	11.3	19.0	248
11	172	1730	8.17	18.3	1.50	2.90	22.3	6.00	1.19	1.02	0.03	10.9	27.4	116	46.2
14	52.7	745	30.5	13.2	14.7	7.00	168	23.0	1.46	0.68	0.03	2.67	32.5	107	103
17	6.93	5660	4.63	67.7	8.82	13.7	655	14.0	1.89	20.4	0.12	3.93	16.9	144	246
18	4.20	116	6.53	3.53	6.53	10.7	14.4	9.00	1.59	0.27	0.04	3.21	17.8	106	72.4
21	204	3570	41.3	30.5	3.31	2.15	1080	324	3.26	7.58	0.06	22.0	37.1	119	48.0
22	77.0	13.4	0.13	2.47	0.97	0.00	0.00	4.00	0.21	0.34	0.06	1.28	10.7	116	71.6

[a] OAV is the concentration (ppb) quantified in this Table for specific compounds divided by threshold concentrations (ppb) reported in the literature.

powder (*23*). *p*-Cresol has been documented in many dairy products including sweet whey powder (*23*), fresh and stored milk powder (*28*), and cheddar cheese (*3*). Guaiacol has been found in WPC (*15*) and rennet casein (*22*). 2-Acetyl pyrroline was documented in sweet whey powder (*23*), liquid cheddar whey (*24*), and stored nonfat dry milk (NFDM) where it has been purported to contribute sweet/cooked/popcorn-like flavors (*28*). Sotolon has been associated with stored skim milk powder (*28*) and sweet whey products (*23*, *15*). The 2 branched chain free fatty acids (FFA) present in the WPH, 2-methylbutyric and isovaleric acid, were derived from microbial metabolism of branched-chain amino acids L-isoleucine and L-leucine, respectively (*29*). These compounds were reported in cheese ripening (*3*), nonfat dried milk (*28*), casein (*22*), and liquid cheddar whey (*24*). They were not previously reported in whey powder.

Lipolysis/Hydrolysis and Oxidation of Fats. Lipid oxidation products were flavor-contributing compounds found in both WPC and WPI (*15*, *23*). In this study, fat content was related to the abundance of lipid oxidation products. Samples that had lower fat content (samples 8, 11, 21, and 22; 0.00−0.69% fat) had lower concentrations of hexanal compared to samples containing higher fat (sample 14; 6.19%), although, not significantly lower than other moderate fat content samples (2.93−4.72% fat). Sample 22, which had the highest protein content (88%), had the lowest lipid oxidation products quantified: hexanal, *Z*-4-heptenal, octanal, nonanal, *E*-2-nonenal, and 1-octen-3-one. There were no relationships between fat content with other lipid oxidation compounds (aldehydes and ketones).

Lipid oxidation had been shown to initiate during whey production, as an impact of the starter culture fermentation (*26*). In samples, 2-nonenal, *E*,*Z*-2,6-nonadienal, *E*,*Z*-2,4 decadienal, *Z*-4-heptenal, *Z*-2-nonenal, (*E*,*Z*)-2,6-nonadienal, *E*-2-nonenal, (*E*,*E*)-2,4-nonadienal, *E*-2-decenal, hexanal, octanal, nonanal, and decanal were detected by SPME GC-MS and GC-O (**Tables 1** and **5**). These compounds, except for *E*-2-decenal, have been detected in whey proteins, and elevated levels of these compounds have been documented during storage and agglomeration of whey protein (*10*). 1-Octen-3-one, *Z*- and *E*-2-nonenal, *E*,*Z*-2,6-nonadienal, and *E*,*E*-2,4-decadienal were associated with cardboard flavor in liquid and dried whey (*15*, *24*). *E*-2-decenal was reported in fresh chevre-style goat cheese (*30*). Only samples 1, 9, 11, and 14 were noted as cardboard by descriptive analysis (**Table 4**). However, there was not one single individual lipid oxidation product (aldehyde or ketone) that gave rise to this off flavor, and this flavor may be derived from a combination of lipid oxidation products. Moreover, it is possible that other attributes such as cooked/sulfur, cheesy/brothy, potato/brothy, or animal may be more potent and may overpower the sensory perception of cardboard flavor.

E-2-Nonenal, *E*,*Z*-2,6-nonadienal, *Z*-4-heptenal, and hexanal display an array of grassy, fatty, cucumber, stale, and fishy aromas (**Table 1**). Hexanal was present at the highest concentration out of all aldehydes quantified in this study, although it was not one of the high aroma impact compounds by odor activity values (**Table 6**). *E*,*Z*-2,6-Nonadienal had a high OAV relative to that of other aldehydes in many WPH, suggesting that this compound may play a major role in contributing to lipid oxidation related flavors (**Table 6**). A furan compound, 2-*n*-octyl furan, with a minty/hay aroma, was tentatively identified by comparison of RI and odor description. This compound has been documented in meat and bone meal flavor (*31*) but has not previously been identified in whey proteins or other dairy products.

Ketones that were detected in WPH samples were 1-octen-3-one, *o*-aminoacetophenone, and β-ionone. 1-Octen-3-one was previously associated with cardboard off-flavor in liquid whey (*24*). *o*-Aminoacetophenone has been identified in WPC and WPI (*15*), skim milk powder (*28*), and rennet casein (*22*), and was present in all WPH samples (**Table 1**). It has a grapy/stale/tortilla characteristic and was associated with stale flavor in stored milk powders and casein (*22*, *28*). β-Ionone was documented in dried milk ingredients (*28*), and to our knowledge, has not been identified in whey products. 2,3-Butanedione (diacetyl) contributes to buttery flavors in various dairy products and was reportedly formed by lactic acid fermentation by oxidative decarboxylation of α-acetolactic acid (*30*). It can be generated as an intermediate during high temperature treatment nonenzymatic Maillard browning and can later participate in Strecker degradation with other free amino acids (*31*).

Compounds that occur via the reaction of lipolysis in this study were butyric acid, δ-decalactone, and γ-decalactone. Butyric acid has a cheesy aroma and has previously been documented in dried WPC and WPI (*15*) and contributes to distinctive aroma in cheddar cheese (*3*). δ-Decalactone and γ-decalactone are lactone compounds that have been reported in WPC, WPI (*15*, *23*), and rennet casein (*22*). These compounds may contribute, in part, to cooked/milky flavor in WPH.

Orthonasal and Basic Taste Thresholds of WPH. Bitterness is usually an objectionable characteristic of WPH, but the flavor of WPH should receive the same attention. There were no significant differences in the orthonasal thresholds between the 3 selected WPH with different amino acid profiles and bitter taste intensity, nor were there differences in the basic taste thresholds between these products ($p > 0.05$), illustrating a nonvariability in the detection limits of different samples across the degree of hydrolysis range tested and bitter taste intensity (**Table 7**). However, there were differences between the basic taste thresholds and orthonasal thresholds of all 3 WPH tested, with the basic taste thresholds being approximately 10 times greater than orthonasal thresholds ($p < 0.001$). This implies that, the flavor of WPH is noticeable at a lower WPH concentration than the detection of bitterness. Many approaches exist for masking or minimizing bitter taste in food; when the source of the bitter taste is known, however, masking malty, animal, and potato flavors in nonsavory food applications such as a beverage may be more challenging (Bastian, E., Glanbia Nutritionals, personal communication).

WPH displayed an array of aromatic flavors. The unique flavor profile of WPH was mainly composed of protein degradation compounds. There were lower concentrations of lipid oxidation products present in WPH, and these may not be as important, depending on the fat content. Flavor contributions of WPH are undoubtedly a challenge and cannot be overlooked. Altering processing steps, enzymes, or the use of complementary ingredients could be applied to lessen if not overcome the strong objectionable flavor of WPH.

Table 7. Orthonasal and Basic Taste Best Estimate Thresholds (BET) of Rehydrated WPH in Parts Per Million (ppm) and Their Standard Errors[a]

	orthonasal		basic taste	
WPH	BET (ppm)	SE	BET (ppm)	SE
1	246b	99	3600a	746
3	191b	65	3900a	827
8	519b	150	4910a	1200

[a] BET values within a row not followed by a common letter are statistically different ($p < 0.05$).

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