The Impact of Antioxidant Addition on Flavor of Cheddar and Mozzarella Whey and Cheddar Whey Protein Concentrate

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Abstract: Lipid oxidation products are primary contributors to whey ingredient off-flavors. The objectives of this study were to evaluate the impact of antioxidant addition in prevention of flavor deterioration of fluid whey and spray-dried whey protein. Cheddar and Mozzarella cheeses were manufactured in triplicate. Fresh whey was collected, pasteurized, and defatted by centrifugal separation. Subsequently, 0.05% (w/w) ascorbic acid or 0.5% (w/w) whey protein hydrolysate (WPH) were added to the pasteurized whey. A control with no antioxidant addition was also evaluated. Wheys were stored at 3 °C and evaluated after 0, 2, 4, 6, and 8 d. In a subsequent experiment, selected treatments were then incorporated into liquid Cheddar whey and processed into whey protein concentrate (WPC). Whey and WPC flavors were documented by descriptive sensory analysis, and volatile components were evaluated by solid phase micro-extraction with gas chromatography mass spectrometry. Cardboard flavors increased in fluid wheys with storage. Liquid wheys with ascorbic acid or WPH had lower cardboard flavor across storage compared to control whey. Lipid oxidation products, hexanal, heptanal, octanal, and nonanal increased in liquid whey during storage, but liquid whey with added ascorbic acid or WPH had lower concentrations of these products compared to untreated controls. Mozzarella liquid whey had lower flavor intensities than Cheddar whey initially and after refrigerated storage. WPC with added ascorbic acid or WPH had lower cardboard flavor and lower concentrations of pentanal, heptanal, and nonanal compared to control WPC. These results suggest that addition of an antioxidant to liquid whey prior to further processing may be beneficial to flavor of spray-dried whey protein.

Practical Application: Lipid oxidation products are primary contributors to whey ingredient off-flavors. Flavor plays a critical and limiting role in widespread use of dried whey ingredients, and enhanced understanding of flavor and flavor formation as well as methods to control or minimize flavor formation during processing are industrially relevant. The results from this study suggest that addition of an antioxidant to liquid whey prior to further processing may be beneficial to minimize flavor of spray-dried whey protein.

Introduction

Dried whey ingredients are useful ingredients in food products because of their high solubility, dispersibility, water binding, foaming, whipping, emulsification, gelation, and buffering power (Bryant and McClements 1998; de Wit 1998; Foegeding and others 2002). Whey proteins have gained further popularity for health benefits including increasing colon health (McIntosh and others 1998), cardiovascular health (Mullally and others 1997), and athletic enhancement (Cornish and others 2009).

Flavor is generally recognized as the single most important factor affecting consumer acceptance of food products (Lee and Morr 1994; Drake 2006; Childs and Drake 2009). Dried whey ingre-

dients ideally should have a bland, delicate flavor free from undesirable flavors (Morr and Ha 1993; Wright and others 2009). Off-flavors in whey products can carry through into ingredient applications (Drake and others 2009; Wright and others 2009), and may limit widespread use of these products (Quach and others 1999; Childs and others 2007).

Flavor variability has been identified in liquid whey and dried whey products both between and within manufacturers (Carunchia-Whetstone and others 2003, 2005; Drake and others 2009; Wright and others 2009). Flavor variability has been sourced to the liquid whey itself and is also an outcome of downstream processing and storage techniques (Carunchia-Whetstone and others 2003, 2005; Drake and others 2009). Different starter cultures in the cheese-making process are utilized to produce a variety of cheeses and the resulting liquid wheys have distinct mineral content, protein concentration, and lactose content, as well as distinct flavor properties (Bordenave-Juchereau and others 2005; Gallardo-Escamilla and others 2005).

Many studies have reported that flavor of whey and whey products changes during storage, light exposure, and added heat (Morr

and Ha 1993; Lee and others 1995; Tomaino and others 2004; Wright and others 2009). Aldehydes, including hexanal, have been suggested as the compounds responsible for off-flavors in liquid and dried whey products (Quach and others 1999; Tomaino and others 2004; Wright and others 2009). In 2004, Tomaino and others observed lipid oxidation products in fresh fluid whey and increases in aldehyde concentrations concurrent with increased off-flavors during refrigerated storage of liquid whey. Lipid oxidation products were also prevalent in freshly manufactured whey proteins (Carunchia-Whetstine and others 2005; Evans and others 2009; Wright and others 2009) and concentrations increased with storage time concurrent with increased sensory-perceived off-flavors (Wright and others 2009). Collectively, these studies demonstrate that lipid oxidation products contribute to undesirable flavors in whey proteins.

The 2 main sources of liquid whey in the United States are derived from Cheddar and Mozzarella cheese production and these whey streams were the focus of the current study. The objectives of this study were to evaluate methods to minimize lipid oxidation in liquid whey and whey protein concentrate through the addition of antioxidants to liquid whey. Antioxidants were administered to freshly produced Cheddar and Mozzarella liquid whey to determine if antioxidant addition minimized off-flavor production in liquid whey. Ascorbic acid and whey protein hydrolysate were the antioxidants selected. Previous studies (Jung and others 1998; Lindmark-Månsson and Åkesson 2000; Tong and others 2000; Mortenson and others 2004; Hernandez-Ledesma and others 2005) have confirmed the antioxidative properties of these ingredients. Subsequently, selected treatments were evaluated in finished spray-dried whey protein concentrate (WPC) from Cheddar whey. Sensory and instrumental analyses were applied to document properties of liquid wheys and whey protein concentrates.

Materials and Methods

Fluid whey manufacture

Milk. Raw unhomogenized whole milk (NCSU Creamery, Raleigh, N.C., U.S.A.) for cheese production was high temperature short time (HTST) pasteurized with a plate heat exchanger (APV, APV Co. Ltd., Crawley, West Sussex, U.K.) at 75 °C and a holding time of 28 s. The pasteurized milk was cooled to 3 °C and stored for less than 24 h. The average fat and protein content of the milk were $3.62\% \pm 0.10$ (CEM Smart Trac Rapid Fat Analysis, Matthews, N.C., U.S.A.) and $3.34\% \pm 0.08$ (LactiCheck Milk Analyzer Dual Cow Channel LC-02, P&P Intl. Ltd, Hopkinton, Mass., U.S.A.). During cheese manufacture, the pH of whey and cheese were measured with an electrode (Model IQ150, IQ Scientific Instruments, Inc., Loveland, Colo., U.S.A.) that was standardized at pH, 6.97 and 4.03 at 38 °C and kept immersed in 3M KCl at 38 °C between readings to keep its temperature equal to the temperature of the samples.

Cheddar cheese and Mozzarella wheys. Pasteurized milk (3 °C) was transferred into a cheese vat (Model 4MX, Kusel Equipment Co., Watertown, Wis., U.S.A.). For Cheddar whey, the temperature of the milk was raised to 31 °C with gentle stirring. Once the milk reached 31 °C, calcium chloride [0.0018M] (Dairy Connection Inc., Madison, Wis., U.S.A.) at a rate of 180 mL/454 kg milk and annatto color (double strength, Dairy Connection Inc.) at a rate of 15 mL/454 kg milk were added to the milk along with a freeze-dried starter culture of *Lactococcus lactis* ssp. *lactis* and *Lactococcus lactis* ssp. *cremoris* (Danisco Choozit, Dairy Con-

nection Inc.) at the rate of 50 DCU/454 kg milk. (DCU is a unit of activity.) The milk was continuously stirred and allowed to ripen for 60 min. The ripened milk, at 31 °C, was coagulated with double strength chymosin (40 mL/454 kg milk, Star Ren-net Double Strength, Dairy Connection Inc.) for 30 min with no agitation and no heat. Firm coagulum was cut with 0.95 cm wire knives. Curds and whey were allowed to heal for 5 min and then were gently stirred for 10 min without added heat. The temperature was gradually increased from 31 to 39 °C and continuously stirred over 30 min or until the target whey pH of 6.3 was attained. Cheese whey was immediately drained and pumped into a pasteurizer (Model MPD1050, D&F Equipment Co., McLeansville, N.C., U.S.A.) and pasteurized at 65 °C for 30 min to inactivate the starter culture. Cheddar cheese whey was manufactured in triplicate.

For Mozzarella whey, the temperature of the pasteurized milk was raised to 35 °C with gentle stirring. Once the milk reached 35 °C, calcium chloride [180 mL/454 kg milk] (Dairy Connection, Inc.) and freeze-dried starter cultures were added. *Streptococcus thermophilus* (50 DCU/454 kg milk, Danisco Choozit, Dairy Connection, Inc.) and *Lactobacillus delbrueckii* ssp. *lactis* and *Lactobacillus helveticus* (20 DCU/454 kg milk, Danisco Choozit, Dairy Connection, Inc.) were added to the warmed milk. The milk was continuously stirred and allowed to ripen for 60 min at 35 °C. The ripened milk, at 35 °C, was coagulated with double strength chymosin (40 mL/454 kg milk, Dairy Connection Inc.) for 30 min with no agitation and no heat. Firm coagulum was cut with 0.95 cm wire knives. Curds and whey were allowed to heal for 5 min and then were gently stirred for 10 min without added heat. The temperature was gradually increased from 35 to 40 °C and continuously stirred over 30 min or until the target whey pH of 6.3 was attained. The whey was then drained and pasteurized at 65 °C for 30 min to inactivate the starter cultures. Mozzarella cheese whey was manufactured in triplicate.

Liquid whey treatments. After pasteurization of Cheddar and Mozzarella liquid whey, 26 L of each liquid whey were collected, cooled in an ice bath to 10 °C, and designated as the fat control (C1). The remaining hot whey (104 L) was run through a separator (Clair Milky, FJ-125 EAP 115V nr 17584-115-3, Warenhandels GmbH, Whitewater, Wis., U.S.A.) for fat separation. The fat content of the whey before and after separation was monitored (CEM Smart Trac Rapid Fat Analysis, Matthews). Separated whey was cooled to 10 °C in an ice bath. This cooled whey was divided into 3 portions. Whey without added treatment was assigned as the separated fat control (C2). Treatment 1 consisted of the addition of 0.05% (w/w) ascorbic acid (VWR Intl., West Chester, Pa., U.S.A.). Treatment 2 consisted of the addition of 0.5% (w/w) whey protein hydrolysate (WPH) (type 8350, Hilmar Ingredients, Hilmar, Calif., U.S.A.) to the liquid whey. Each of these ingredients was incorporated into the cooled whey by gentle agitation with a wire whisk for 5 to 10 min. Preliminary testing with cooled Cheddar whey confirmed that this method of incorporation fully dissolved the ingredients with no visible lumps or sediment. Samples were stored in 950 mL amber glass jars capped with PTFE faced PE-lined screw caps (Fisher Scientific, Hanover Park, Ill., U.S.A.) at 3 °C in the dark. Samples were held at 3 °C for 8 d. Aliquots (1.5 L) were taken from each treatment and tested after 0, 2, 4, 6, and 8 d by descriptive sensory analysis and instrumental volatile analysis. Total solids of whey were evaluated by forced draft oven (AOAC Method 990.19) and protein by Kjeldahl analysis with a conversion factor of 6.38. The pH of wheys was taken at each storage time point to confirm absence of microbial growth.

Descriptive sensory analysis

Sensory testing was conducted in compliance with NCSU Institutional Review Board (IRB) for human subjects approval. A trained sensory panel ($n = 10$, 7 female, 3 male, ages 22 to 37 y) evaluated the flavor attributes of the liquid whey using a previously established lexicon for fluid whey (Carunchia-Whetstine and others 2003; Drake and others 2003; Drake and others 2009). Each panelist had over 150 h of experience with descriptive analysis of dried dairy ingredients, and additional training with liquid whey aroma and flavor. Consistent with SpectrumTM 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 and others 1999; Drake and Civille 2003). Panelists then evaluated and discussed flavor attributes of Cheddar and Mozzarella liquid whey with and without treatment addition. 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-point universal intensity scale characterized by the Spectrum descriptive analysis method (Meilgaard and others 1999; Drake and Civille 2003).

Liquid wheys (approximately 30 mL) were dispensed into lidded 58 mL soufflé cups with 3-digit codes. 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 on separate occasions. Products were scored using paper ballots or computerized ballots using CompusenseTM five version 4.8 (Compusense, Guelph, ON, Canada).

Solid phase microextraction gas chromatography mass spectrometry (SPME GC-MS)

Volatile compounds of wheys were evaluated by SPME GC-MS. SPME GC-MS was conducted using a modified method of Wright and others (2006). Five grams of liquid whey with 10% NaCl (w/w) (VWR Intl.) and 10 μ L internal standard solution (2-methyl-3-heptanone in methanol at 8.1 ppm; Sigma-Aldrich, Milwaukee, Wis., U.S.A.; VWR Intl., West Chester, Pa., U.S.A.) were placed into 20 mL autosampler vials with steel screw tops containing silicone septa faced in teflon (Microliter Analytical, Sawanee, Fla., U.S.A.). Samples were injected using a CombiPal autosampler (CTC Analytics, Zwingen, Switzerland) attached to an Agilent 6890N GC with 5973 inert MSD (Agilent Technologies Inc., Santa Clara, Calif., U.S.A.). Samples were maintained at 5 °C prior to fiber exposure. Samples were equilibrated at 40 °C for 25 min before 30 min fiber exposure of a 1 cm 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, increased at the rate of 5 °C/min to 200 °C, held for 5 min and finally increased at a 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 pressure of 7.06 psi with helium carrier gas, with a purge flow of 1697.7 cm/s. An Rtx-5 ms column (Rtx-5 ms 30 m length $\times$ 0.25 mm inner dia $\times$ 0.25 μ m film thickness; Restek, Bellefonte, Pa., U.S.A.) was used for all analyses at a constant flow rate of 34 cm/s. Purge time was set at 1 min. The MS transfer line was maintained at 250 °C with the Quad at 150 °C and Source at 250 °C. Compounds were identified using

the NIST 2005 library of spectra and comparison of spectra of authentic standards injected under identical conditions. Relative abundance for each compound was calculated using the recovery of the internal standard concentration to determine relative abundance of each compound. Retention indices were calculated using an alkane series (Sigma-Aldrich) (Van den Dool and Kratz 1963). Each sample was injected in triplicate for each treatment replication.

Whey protein concentrate manufacture

Raw milk was obtained from the Cal Poly Dairy (San Luis Obispo, Calif., U.S.A.), pasteurized and used for Cheddar WPC production with the same cheese make procedure as previously described for Cheddar liquid whey production. Fresh liquid whey was collected, pasteurized (71.7 °C for 16 s), fat separated (Alfa-Laval, Richmond, Va., U.S.A.), and cooled. In separate batches, ascorbic acid and WPH (0.05% [w/w] ascorbic acid, 0.5% [w/w] WPH) treatments were incorporated into the cooled liquid whey as previously described. A control of fat separated liquid whey was also produced, receiving no treatments. Whey treatments and control were stored overnight at 4 °C for further processing the next day.

Before WPC processing, ultrafiltration membranes were cleaned according to a standard washing procedure. First, an alkaline wash with sodium hydroxide (45 min, 50 °C, pH 12) was administered followed by rinsing with deionized water (30 min, 50 °C). Then an acid wash with phosphoric and nitric acids (15 min, 45 °C, pH 2) was administered, followed by a final membrane reconditioning with deionized water. Liquid wheys were ultrafiltrated (UF), followed by diafiltration (DF) and spray drying. Filtrations were carried out continuously (10 °C, 517.107 kPa, 4.456×10^{-3} cu.ft/s) through a Niro R-12 Universal Membrane System (Niro Inc., Hudson, Wis., U.S.A.) equipped with a polymeric spiral wound membrane (Koch Membrane Systems, Mass., U.S.A.) with a nominal separation cutoff of 10000 Daltons. After each production, the membrane was cleaned according to the same standard washing procedure as done before filtration. The retentate stream from the DF process was transferred to the spray-dryer as soon as the protein content reached the target value (total solids, $10 \pm 0.1\%$, and protein content, $6.8 \pm 0.04\%$). Protein was measured by a Rapid-N-Cube nitrogen/protein analyzer unit (Elementar, Germany). Concentrated whey protein was then spray dried (Niro Filterlab, Hudson, Wis., U.S.A.) with an inlet air temperature of 204 °C and an outlet air temperature of 88 °C to obtain whey protein concentrate 65% protein (WPC65). The total filtration production time was approximately 2 h and spray-drying, approximately 45 min. The manufactured WPC65 powder was packaged in Mylar bags (TF-4000 w/Zipper nr 41509, IMPAK Corp., Central City, S.Dak., U.S.A.) and shipped to North Carolina State Univ. by overnight carrier for sensory and instrumental analysis using previously described methods. Each WPC treatment and control was produced in duplicate in a completely randomized design on separate days.

Proximate analysis (fat, protein, moisture, and ash) was conducted in duplicate on each of the WPC using standard methods. Fat content was determined by the Mojonnier method (Mojonnier Bros. Co., Chicago, Ill., U.S.A.) (Atherton and Newlander 1977). Nitrogen content was determined by the Dumas method (Rapid-N-Cube nitrogen/protein analyzer unit) with a 6.38 protein factor (Kirsten and Hesselius 1983). Moisture and ash for WPC samples were analyzed by vacuum oven and ash oven, respectively, and quantified according to AOAC

methods (AOAC 2007) (Isotemp Ash oven Model750, Fisher Scientific).

Statistical analyses

To determine if significant differences existed in sensory and volatile properties between liquid whey controls and treatments, analysis of variance (ANOVA, General Linear Model with repeated measures) was conducted for each whey type (Mozzarella, Cheddar) using XL-STAT (XL-STAT version 2009, Addinsoft, Paris, France). Both main effects (treatment, time) and interactions were investigated. Sensory and instrumental results from WPC were evaluated analogously. Principal component analysis was also applied to the correlation matrix of sensory and volatile component data to visualize how products were differentiated across sensory attributes or volatile components (XL-STAT).

Results

Proximate analysis of liquid whey

Liquid Cheddar and Mozzarella wheys were 6.50 ± 0.10 and 6.45 ± 0.18 percent solids and 1.40 ± 0.20 and 1.30 ± 0.08 percent protein, respectively. Percent fat content of Cheddar and Mozzarella whey prior to fat separation was 0.18 ± 0.04 and 0.21 ± 0.05 , respectively, and after fat separation was 0.07 ± 0.03 for both whey types. The pH of both whey types was 6.38 ± 0.05 and this value did not change with storage time ($P > 0.05$). Addition of ascorbic acid decreased pH (6.13 ± 0.03) ($P < 0.05$). These values were comparable to previous studies (Carunchia-Whetstine and others 2003; Gallardo-Escamilla and others 2005).

Sensory analysis

Liquid whey. Flavor terms documented in liquid wheys included aroma intensity, sweet aromatic, sour aromatic, cardboard, potato/brothy, cheesy/brothy, and cooked/milky (Table 1 and 2). Sour and sweet basic tastes, along with the feeling factor, astringency, were also documented. Previous research has identified these flavors in liquid whey (Carunchia-Whetstine and others 2003; Karagul-Yuceer and others 2003; Tomaino and others 2004; Gallardo-Escamilla and others 2005). Consistent with previous research (Tomaino and others 2004), the flavors of Cheddar and Mozzarella liquid whey changed with storage time ($P < 0.05$). Flavor profiles were also distinct between the controls and treatments ($P < 0.05$). Fresh control wheys were characterized by cooked/milky and sweet aromatic flavors, fresh Mozzarella control whey were also characterized by sour aromatic flavor. Whey with ascorbic acid addition in either cheese type had higher intensities of sour taste, consistent with lower pH values. The WPH treated wheys were characterized by potato/brothy flavor and additionally in Cheddar whey, by cheesy/brothy flavor. These flavors have been previously associated with whey protein hydrolysates (Drake and others 2009).

Over storage time, control Cheddar wheys and Cheddar whey with ascorbic acid increased in cardboard flavor intensity with a simultaneous decrease in cooked/milky flavor. Cardboard flavor was highest in the unseparated fat control whey after 8 d storage. Similar changes were observed with Mozzarella whey (Table 2). As storage time increased, cardboard flavor intensity increased while cooked/milky flavor decreased in control and treated wheys (Table 2). Cooked/milky flavor was lower in WPH-treated whey compared to other wheys initially and this flavor decreased with storage time, similar to other wheys. After 8 d storage, Mozzarella

Table 1—Sensory flavor attributes of Mozzarella liquid whey over storage time.

	Control 1				
	0 d	2 d	4 d	6 d	8 d
Aroma intensity	2.2	2.0	2.1	2.1	2.1
Sweet aromatic	1.1	0.9	0.8	0.8	0.7
Sour aromatic	1.1	0.8	0.5	0.6	0.7
Cardboard	ND	0.8	1.1	1.4	1.6
Potato/brothy	ND	ND	ND	ND	ND
Cooked/milky	3.2	2.5	2.4	2.1	2.1
Sweet taste	1.7	1.6	1.6	1.4	1.6
Sour taste	1.0	0.8	0.8	1.0	0.8
Astringent mouthfeel	1.2	1.3	1.3	1.4	1.3

	Control 2				
	0 d	2 d	4 d	6 d	8 d
Aroma intensity	1.9	1.5	1.7	1.9	2.0
Sweet aromatic	1.1	0.9	0.7	0.6	0.6
Sour aromatic	0.6	0.6	0.6	0.6	0.6
Cardboard	ND	ND	0.9	1.1	1.6
Potato/brothy	ND	ND	ND	ND	ND
Cooked/milky	2.9	2.3	2.1	2.2	2.0
Sweet taste	1.6	1.5	1.4	1.4	1.6
Sour taste	1.0	0.7	0.8	0.8	0.9
Astringent mouthfeel	1.2	1.3	1.2	1.4	1.3

	Ascorbic acid				
	0 d	2 d	4 d	6 d	8 d
Aroma intensity	1.8	1.7	1.8	1.9	1.7
Sweet aromatic	0.7	ND	0.7	0.6	ND
Sour aromatic	0.9	0.6	0.7	0.8	0.9
Cardboard	ND	ND	ND	ND	0.8
Potato/brothy	ND	ND	ND	ND	ND
Cooked/milky	3.0	2.3	2.3	2.3	2.2
Sweet taste	1.6	1.5	1.5	1.4	1.6
Sour taste	1.5	1.2	1.2	1.1	1.1
Astringent mouthfeel	1.2	1.3	1.3	1.4	1.3

	WPH				
	0 d	2 d	4 d	6 d	8 d
Aroma intensity	2.8	2.7	2.6	2.9	2.6
Sweet aromatic	ND	ND	ND	ND	ND
Sour aromatic	ND	ND	ND	ND	ND
Cardboard	ND	ND	ND	ND	ND
Potato/brothy	2.2	2.0	2.0	2.1	1.6
Cooked/milky	2.1	1.7	1.5	1.5	1.7
Sweet taste	1.7	1.4	1.5	1.4	1.4
Sour taste	1.0	1.0	0.9	0.9	0.9
Astringent mouthfeel	1.2	1.3	1.3	1.4	1.2

	Interactions		
	Trt	Time	Trt * Time
Aroma intensity	<0.0001	0.005	NS
Sweet aromatic	<0.0001	<0.0001	<0.0001
Sour aromatic	<0.0001	<0.0001	<0.0001
Cardboard	<0.0001	<0.0001	<0.0001
Potato/brothy	<0.0001	NS	NS
Cooked/milky	<0.0001	<0.0001	NS
Sweet taste	NS	0.0002	NS
Sour taste	<0.0001	<0.0001	NS
Astringent mouthfeel	NS	NS	NS

ND = not detected. Interactions: Values < 0.05 in trt*time indicate a significant interaction. NS = not significant. Attributes were scored using a 0 to 15-point universal Spectrum intensity scale where 0 = absence of the attribute and 15 = extremely high intensity of attribute. Most whey protein flavors fall between 0 and 4 (Drake and others 2003; Russell and others 2006). Control 1—whey with no fat removal, Control 2—whey with fat removed, ascorbic acid—whey with fat removed and ascorbic acid addition, WPH—whey with fat removed and whey protein hydrolysate added.

Table 2—Sensory flavor attributes of Cheddar liquid whey over storage time.

	Control 1				
	0 d	2 d	4 d	6 d	8 d
Aroma intensity	2.4	2.1	2.1	2.3	2.0
Sweet aromatic	2.0	1.5	0.8	1.1	0.5
Sour aromatic	ND	ND	ND	ND	ND
Cardboard	ND	1.0	1.8	1.9	2.5
Potato/brothy	ND	ND	ND	ND	ND
Cheesy/brothy	ND	ND	ND	ND	ND
Cooked/milky	3.2	3.0	2.4	2.2	1.7
Sweet taste	1.6	1.5	1.4	1.3	1.5
Sour taste	0.5	0.5	0.5	0.5	0.5
Astringent mouthfeel	1.5	1.5	1.5	1.5	1.5
	Control 2				
	0 d	2 d	4 d	6 d	8 d
Aroma intensity	2.4	2.0	2.1	2.0	2.0
Sweet aromatic	2.0	1.3	0.8	1.0	0.5
Sour aromatic	ND	0.1	ND	ND	ND
Cardboard	ND	0.3	1.0	1.5	1.5
Potato/brothy	ND	ND	ND	ND	ND
Cheesy/brothy	ND	ND	ND	ND	ND
Cooked/milky	3.2	3.0	2.6	2.4	2.0
Sweet taste	1.6	1.5	1.4	1.5	1.6
Sour taste	0.5	0.5	0.5	0.5	0.5
Astringent mouthfeel	1.5	1.5	1.5	1.5	1.5
	Ascorbic acid				
	0 d	2 d	4 d	6 d	8 d
Aroma intensity	2.4	2.0	2.1	2.0	2.2
Sweet aromatic	1.7	1.1	1.0	1.3	1.0
Sour aromatic	0.8	0.6	0.6	0.8	1.0
Cardboard	ND	ND	0.6	0.9	0.8
Potato/brothy	ND	ND	ND	ND	ND
Cheesy/brothy	ND	ND	ND	ND	ND
Cooked/milky	3.0	3.1	2.6	2.5	2.3
Sweet taste	1.6	1.5	1.4	1.4	1.5
Sour taste	1.0	1.0	0.9	1.1	1.0
Astringent mouthfeel	1.5	1.5	1.5	1.5	1.5
	WPH				
	0 d	2 d	4 d	6 d	8 d
Aroma intensity	2.9	2.0	3.0	3.0	2.8
Sweet aromatic	1.5	0.7	ND	ND	ND
Sour aromatic	ND	ND	ND	ND	ND
Cardboard	ND	0.3	0.8	0.8	0.5
Potato/brothy	2.7	1.8	2.3	2.5	1.9
Cheesy/brothy	1.3	2.0	1.5	1.6	1.3
Cooked/milky	2.9	2.7	1.8	1.9	1.8
Sweet taste	1.8	1.4	1.6	1.4	1.4
Sour taste	0.5	0.5	0.5	0.5	0.5
Astringent mouthfeel	1.5	1.5	1.5	1.5	1.5
Interactions					
	Trt	Time	Trt*Time		
Aroma intensity	<0.0001	0.002	<0.0001		
Sweet aromatic	<0.0001	<0.0001	0.004		
Sour aromatic	<0.0001	NS	NS		
Cardboard	<0.0001	<0.0001	<0.0001		
Potato/brothy	<0.0001	0.0002	<0.0001		
Cheesy/brothy	<0.0001	NS	0.025		
Cooked/milky	<0.0001	<0.0001	0.017		
Sweet taste	NS	0.0002	NS		
Sour taste	<0.0001	0.002	NS		
Astringent mouthfeel	NS	0.002	NS		

ND = not detected. Interactions: Values < 0.05 in trt*time indicate a significant interaction. NS = not significant. Attributes were scored using a 0 to 15-point universal Spectrum intensity scale where 0 = absence of the attribute and 15 = extremely high intensity of attribute. Most whey protein flavors fall between 0 and 4 (Drake and others 2003; Russell and others 2006). Control 1—whey with no fat removal, Control 2—whey with fat removed, ascorbic acid—whey with fat removed and ascorbic acid addition, WPH—whey with fat removed and whey protein hydrolysate added.

whey controls with and without fat had similar cardboard flavor intensity.

Although flavor changes were similar over time, fresh and stored Mozzarella and Cheddar wheys had distinct flavor profiles ($P < 0.05$) (results not shown). Mozzarella whey had lower intensities of sweet aromatic flavor than Cheddar whey while sour aromatic flavor was only documented in Mozzarella whey. This is in agreement with Gallardo-Escamilla and others (2005) who also reported that Mozzarella liquid whey had higher sour aromatic flavor intensity compared to Cheddar whey. Following storage, Cheddar whey had higher cardboard flavors compared to Mozzarella whey ($P < 0.05$). Differences have also been documented between freshly manufactured Cheddar and Mozzarella WPC80, which also suggests that distinct flavors are present in the respective fresh fluid wheys (Drake and others 2009; Wright and others 2009). Different whey type flavors have been associated with the use of different starter cultures, milk source, and processing techniques to produce different types of cheese (Carunchia-Whetstine and others 2003; Gallardo-Escamilla and others 2005).

Instrumental liquid whey results

Volatiles identified in the liquid wheys included 2-methyl butanal, 3-methyl butanal, hexanal, heptanal, octanal, nonanal, decanal, 2-butanone, 3-hydroxy-2-butanone, 2-pentanone, 2-heptanone, 2-nonanone, and 2,3-butanedione (Table 3 and 4). Previous research has identified most of these volatile compounds in liquid whey (Carunchia-Whetstine and others 2003; Karagul-Yuceer and others 2003; Tomaino and others 2004; Gallardo-Escamilla and others 2005). To our knowledge, decanal, 2-pentanone, and 3-hydroxy-2-butanone have not been documented in fresh liquid whey. Similar to sensory data, the volatile compound concentrations of Cheddar and Mozzarella whey samples changed over time in refrigerated storage. In general, fresh wheys had higher concentrations of 2,3-butanedione and 2-butanone and lower concentrations of all aldehydes compared to wheys stored 8 d. Over storage, volatile lipid oxidation and lipid oxidation decomposition products such as aldehydes, hydrocarbons, alcohols, and olefins were documented.

Cheddar whey. Significant interactions between treatment and time occurred for 2-methyl butanal, 3-methyl butanal, hexanal, and heptanal concentrations in Cheddar whey (Table 3). As time progressed, concentrations of hexanal, heptanal, octanal, and nonanal increased in the control and treated wheys except for whey with WPH. Whey with WPH decreased in 2-methyl butanal, 3-methyl butanal, and hexanal concentration with a simultaneous increase in decanal concentration. The unseparated fat control (C1) at 8 d had the highest concentration of hexanal overall, while the whey with WPH had the highest concentration of decanal ($P < 0.05$).

The Cheddar liquid whey instrumental data were consistent with descriptive sensory results. Over time, control and treatments except whey with WPH, increased in cardboard flavor and also increased in hexanal, heptanal, octanal, and nonanal concentrations. Cardboard flavor intensities were highest in the unseparated and separated control wheys after 8 d storage and similarly, the control wheys had some of the highest concentrations of hexanal and heptanal. Whey with WPH was characterized by sensory analysis at all times by high intensities of cheesy/brothy and potato/brothy flavors, while instrumentally, this whey had high concentrations of 2-methyl butanal and 3-methyl butanal. These 2 aldehydes have been associated with malty and nutty flavors in cheese and milk (Avsar and others 2004).

Mozzarella whey. Significant interactions in Mozzarella wheys occurred between treatment and time for 2-methyl butanal and 3-methyl butanal, octanal, heptanal, and hexanal, while nonanal concentrations changed only with time. Decanal was not detected in Mozzarella wheys (Table 4). Over 8 d, the concentration of short chain aldehydes decreased ($P < 0.05$). Hexanal concentrations decreased in the separated fat control (Control 2) and all treatments. Hexanal concentration was highest after 2 d in Mozzarella control whey without fat removal and concentrations then decreased through 8 d. Heptanal and octanal concentrations decreased or were not detected in wheys after 8 d. Significant decreases in nonanal were documented in ascorbic acid and WPH

Table 3—Relative abundance of aldehydes (ppb) in Mozzarella liquid whey over storage time.

Control 1					
	0 d	2 d	4 d	6 d	8 d
Nonanal	0.0146	0.0162	0.0210	0.0149	0.00577
Octanal	0.00111	0.000730	0.000786	0.000479	ND
Heptanal	0.0167	0.0134	0.00507	0.00957	0.00394
Hexanal	0.129	0.388	0.143	0.271	0.150
2-Methyl Butanal	ND	ND	ND	ND	ND
3-Methyl Butanal	ND	ND	ND	ND	ND
Control 2					
	0 d	2 d	4 d	6 d	8 d
Nonanal	0.0232	0.0128	0.0218	0.0429	0.0421
Octanal	0.00198	0.000463	0.00141	0.00164	ND
Heptanal	0.0108	0.00827	0.00558	0.00590	0.000754
Hexanal	0.0654	0.152	0.211	0.204	0.0667
2-Methyl Butanal	ND	ND	ND	ND	ND
3-Methyl Butanal	ND	ND	ND	ND	ND
Ascorbic acid					
	0 d	2 d	4 d	6 d	8 d
Nonanal	0.0371	0.0399	0.0532	0.0441	0.00936
Octanal	0.00552	0.00850	0.00986	0.00506	0.00160
Heptanal	0.0112	0.0174	0.0188	0.00623	0.00112
Hexanal	0.0656	0.1025	0.0991	0.0378	0.00443
2-Methyl Butanal	ND	ND	ND	ND	ND
3-Methyl Butanal	ND	ND	ND	ND	ND
WPH					
	0 d	2 d	4 d	6 d	8 d
Nonanal	0.0371	0.0363	0.0308	0.0429	0.0081
Octanal	0.00493	0.00732	0.00784	0.00609	ND
Heptanal	0.0298	0.0323	0.0205	0.0181	ND
Hexanal	0.1817	0.2228	0.1206	0.0988	0.00329
2-Methyl Butanal	0.206	0.428	0.387	0.262	0.0408
3-Methyl Butanal	0.499	0.598	0.327	0.204	0.0441
Interactions					
	Trt	Time	Trt * Time		
Nonanal	NS	0.0008	NS		
Octanal	<0.0001	<0.0001	NS		
Heptanal	<0.0001	<0.0001	NS		
Hexanal	0.0002	0.0002	NS		
2-Methyl Butanal	<0.0008	<0.0001	<0.0001		
3-Methyl Butanal	<0.0001	<0.0001	<0.0001		

ND = not detected. Interactions: Values < 0.05 in trt*time indicate a significant interaction. Attributes where interactions are not significant may be significant within treatment or time if < 0.05. NS = not significant. Control 1—whey with no fat removal, Control 2—whey with fat removed, ascorbic acid—whey with fat removed and ascorbic acid addition, WPH—whey with fat removed and whey protein hydrolysate added. RI values for volatile compounds on a ZB-5 column are listed in Table 7.

treatments. Mozzarella liquid whey controls initially had similar aldehyde concentrations. After storage, whey with fat separation had a higher concentration of nonanal and a lower amount of heptanal compared to the control whey without fat removal. Similar decreases in relative abundance of aldehydes over storage have been documented with WPC and WPI studies (Javidipour and Qian 2008; Wright and others 2009). For Mozzarella whey, total aldehyde concentration increased up to 4 d and then decreased until 8 d.

Table 4—Relative abundance of aldehydes (ppb) in Cheddar liquid whey over storage time.

Control 1					
	0 d	2 d	4 d	6 d	8 d
Nonanal	0.0705	0.0488	0.0552	0.1033	0.1463
Octanal	0.0108	0.00939	0.00834	0.0245	0.0407
Heptanal	0.00979	0.00812	0.0183	0.0583	0.1232
Hexanal	0.110	0.268	1.33	3.33	4.99
2-Methyl Butanal	ND	ND	ND	ND	ND
3-Methyl Butanal	ND	ND	ND	ND	ND
Decanal	0.0122	0.00275	0.00543	0.00971	0.0113
Control 2					
	0 d	2 d	4 d	6 d	8 d
Nonanal	0.0443	0.0233	0.0190	0.0710	0.172
Octanal	0.00726	0.00507	0.00602	0.0165	0.0412
Heptanal	0.00811	0.00477	0.00364	0.0380	0.0903
Hexanal	0.0825	0.1438	0.1967	1.4862	2.9598
2-Methyl Butanal	ND	ND	ND	ND	ND
3-Methyl Butanal	ND	ND	ND	ND	ND
Decanal	0.00537	0.00317	0.00326	0.00554	0.00304
Ascorbic acid					
	0 d	2 d	4 d	6 d	8 d
Nonanal	0.0455	0.0387	0.0282	0.0702	0.1206
Octanal	0.00875	0.00711	0.00696	0.0167	0.0328
Heptanal	0.00832	0.00301	0.00461	0.0203	0.0324
Hexanal	0.0637	0.0153	0.0103	0.281	0.541
2-Methyl Butanal	ND	ND	ND	ND	ND
3-Methyl Butanal	ND	ND	ND	ND	ND
Decanal	0.00867	0.00726	0.00360	0.00584	0.00426
WPH					
	0 d	2 d	4 d	6 d	8 d
Nonanal	0.0752	0.0457	0.0280	0.0688	0.0713
Octanal	0.0130	0.00898	0.0513	0.0405	0.0137
Heptanal	0.0457	0.0270	0.0212	0.0484	0.0242
Hexanal	0.406	0.260	0.223	0.404	0.197
2-Methyl Butanal	0.975	0.955	0.0120	0.449	0.0922
3-Methyl Butanal	0.944	0.775	0.651	0.762	0.157
Decanal	0.0138	0.0115	0.0107	0.0204	0.025
Interactions					
	Trt	Time	Trt * Time		
Nonanal	NS	<0.0001	NS		
Octanal	NS	0.0176	NS		
Heptanal	0.0102	<0.0001	<0.0001		
Hexanal	<0.0001	<0.0001	<0.0001		
2-Methyl Butanal	<0.0001	<0.0001	<0.0001		
3-Methyl Butanal	<0.0001	<0.0001	<0.0001		
Decanal	<0.0001	0.027	NS		

ND = not detected. Interactions: Values < 0.05 in trt*time indicate a significant interaction. Attributes where interactions are not significant may be significant within treatment or time if < 0.05. NS = not significant. Control 1—whey with no fat removal, Control 2—whey with fat removed, ascorbic acid—whey with fat removed and ascorbic acid addition, WPH—whey with fat removed and whey protein hydrolysate added. RI values for volatile compounds on a ZB-5 column are listed in Table 7.

Mozzarella liquid whey instrumental results were consistent with descriptive sensory data in that the volatile composition of Mozzarella liquid whey was very different from Cheddar liquid whey as were the sensory profiles of these 2 whey types. However, unlike Cheddar whey, increases in cardboard flavor over time for Mozzarella liquid wheys were not concurrent with increased aldehyde concentrations after 8 d. Instead of an increase in short chain aldehyde concentration over 8 d, there was an increase up to 4 to 6 d, followed by a decrease in concentration at 8 d. This may be due to the further degradation of the aldehydes into other compounds or reaction with sulfur compounds. For example, hexanal can degrade into hexanoic acid (Schieberle and Grosch 1981) and both saturated and unsaturated aldehydes can react with hydrogen sulfide and thiol compounds (Boelens and others 1974). Alternatively, aldehydes may bind to proteins and thus appear to decrease in concentration. However, given that the protein concentration of Cheddar and Mozzarella wheys are similar, if protein binding occurred, we would have expected to observe similar changes with Cheddar whey. As observed in Tables 3 and 4, aldehyde concentrations were not always lower in Mozzarella liquid whey compared to Cheddar liquid whey. Mozzarella control whey and treatments actually had higher concentrations of specific aldehydes, most commonly heptanal, up until 4 d. After 4 d, Cheddar liquid wheys increased in aldehyde concentration while Mozzarella samples decreased. Little research has been conducted on Mozzarella liquid whey. Gallardo-Escamilla and others (2005) compared liquid wheys of various cheese types and similar to our results, concluded that Mozzarella fluid whey was different in flavor and volatile profile from Cheddar liquid whey.

Whey protein concentrate

Cheddar whey was selected for WPC manufacture. Results from liquid whey trials indicated that Mozzarella liquid whey did not change as drastically as Cheddar liquid whey over time by sensory or instrumental analysis. Previous studies have also documented that Mozzarella WPC80 has a distinct and mild flavor and generally lower concentrations of all volatile compounds over storage time than Cheddar WPC (Wright and others 2009), suggesting that further antioxidant research would be more beneficial for Cheddar whey than Mozzarella whey. The separated fat control and the treatments ascorbic acid and WPH were selected for WPC production.

Analysis of whey protein concentrate. Gross composition of Cheddar WPC are listed in Table 5. Flavors documented in the WPC samples included attributes previously noted in liquid whey with the addition of cereal, malty, and serum/oxidized flavors (Table 6). Previous research has documented these flavors in WPC (Carunchia-Whetstine and others 2005; Russell and others 2006; Evans and others 2009; Wright and others 2009). Similar to liquid

Table 5—Gross composition of WPC (mean $\pm$ standard deviation).

WPC sample	Composition		
	Moisture, %	Fat, %	Protein, %
Control	6.02 $\pm$ 0.50a	20.5 $\pm$ 1.52a	64.0 $\pm$ 0.67a
Ascorbic acid	4.55 $\pm$ 0.73ab	22.2 $\pm$ 3.15a	66.6 $\pm$ 1.31a
WPH	3.10 $\pm$ 0.33b	24.9 $\pm$ 2.97a	65.0 $\pm$ 1.06a

Means are from duplicate analyses from 2 experimental replications. Means in a column followed by different letters are different ($P < 0.05$). Control—no treatment, ascorbic acid—added ascorbic acid, WPH—added whey protein hydrolysate.

whey, potato/brothy and malty flavors were only detected in the WPH treatment. Only the control WPC was characterized by a serummy/oxidized flavor and the WPC control had the highest intensities of cardboard flavor and aroma intensity ($P < 0.05$, Table 5).

Volatiles identified in Cheddar WPC included 2-methyl butanal, 3-methyl butanal, pentanal, hexanal, heptanal, octanal, nonanal, dimethyl disulfide (DMDs), 2-heptanone, 1-pentanol, 2,5-octanedione, 2-pentyl furan, butanoic acid, and hexanoic acid (Table 7 and Figure 1). Previous research has documented these volatile compounds in Cheddar WPC (Stevenson and Chen 1996; Mills and Broome 1997; Quach and others 1999; Mahajan and others 2004; Carunchia-Whetstine and others 2005; Wright and others 2009). As with fluid whey, the compounds 2-methyl butanal and 3-methyl butanal were only detected in WPC with added WPH.

The control WPC had significantly higher amounts of pentanal, (E,Z)-2,6-nonadienal, 2-heptanone, butanoic acid, and hexanoic acid than the 2 treated WPC. WPC with ascorbic acid had

Table 6—Sensory flavor attributes of Cheddar WPC.

	Mean scores			LSD
	WPC control	WPC with ascorbic acid	WPC with WPH	
Aroma intensity	2.5a	2.1b	1.5c	0.28
Sweet aromatic	1.1a	1.1a	ND	0.10
Cardboard	2.0a	1.5b	1.1b	0.41
Potato/brothy	ND	ND	0.90a	0.23
Cereal	1.8a	1.5a	1.3a	0.63
Malty	ND	ND	1.1a	0.13
Serum/oxidized	1.1a	ND	ND	0.09
Cooked/milky	1.1b	1.5a	ND	0.34
Astringent	1.6a	1.6a	1.8a	0.42
Sweet	1.3a	1.3a	1.4a	0.18

Means followed by different lowercase letters within a treatment for an attribute signify a difference ($P < 0.05$). Attributes were scored using a 0 to 15-point universal Spectrum intensity scale where 0 = absence of the attribute and 15 = extremely high intensity of attribute. Most whey protein flavors fall between 0 and 4 (Drake and others 2003; Russell and others 2006). LSD = least significant difference.

Table 7—Relative abundance of volatile compounds (ppb) in Cheddar WPC.

Compounds	RI (ZB5)	WPC Control	WPC with ascorbic acid	WPC with WPH	LSD
Methional	925	0.155b	0.155b	0.403a	0.125
2-Methyl Butanal	647	ND	ND	3.28a	0.784
3-Methyl Butanal	637	ND	ND	5.00a	0.622
Pentanal	695	3.22a	1.69b	1.06b	1.20
Hexanal	801	104a	90.2a	43.8b	37.6
Heptanal	882	8.20a	6.29a	1.74b	2.27
Octanal	995	0.795b	1.14a	ND	0.154
Nonanal	1096	1.33ab	3.69a	ND	2.80
(E,Z)-2,6-Nonadienal	1149	1.56a	0.443b	0.295b	0.217
Dimethyl Disulfide	744	0.0772a	0.0535a	ND	0.0901
Dimethyl Trisulfide	960	0.656a	0.829a	0.449b	0.196
1-Pentanol	773	0.747a	0.324a	0.708a	0.544
2,3-Butanedione	<600	3.62b	8.10a	3.49b	3.23
2-Heptanone	894	2.07a	1.05b	0.288c	0.533
1-Octen-3-one	973	1.37a	1.02a	1.46a	0.512
2,5-Octanedione	985	1.57a	1.79a	0.453b	0.779
2-Pentyl Furan	988	3.00a	2.50a	0.474b	1.23
Butanoic acid	835	1.58a	0.00880b	ND	0.957
Hexanoic acid	1024	1.68a	0.103b	0.00743b	0.876

Means followed by different lowercase letters within a treatment for a compound signify a difference ($P < 0.05$). ND = not detected. LSD = least significant difference. RI—retention indices of the volatile compound on the ZB-5 column.

higher concentrations of octanal than the WPC control and higher concentrations of hexanal, heptanal, DMTS, 2,3-butanedione, 2-heptanone, 2-pentyl furan, and 2,5-octanedione than WPC with WPH. Octanal, nonanal, butanoic acid, and DMDS were not detected in WPC with WPH while 2-methyl butanal and 3-methyl butanal were only detected in WPC with WPH. Methional concentration was significantly higher in WPC with WPH compared to control and ascorbic acid treated WPC. The volatile compounds detected represent a range of Maillard browning, lipid oxidation, or fermentation compounds. Many of the compounds found in WPC, especially the aldehydes and ketones, confirm the evolution of lipid oxidation during storage and were further confirmed with sensory results (Carunchia-Whetstine and others 2005; Wright and others 2009).

Propanal, pentanal, hexanal, heptanal, octanal, nonanal, decanal, nonadienals, 1-pentanol, 2-pentyl furan, and 2-heptanone are products of free fatty acid decomposition (Frankel 1998). Pentanal (Carunchia-Whetstine and others 2003; Tomaino and others 2004), hexanal (Quach and others 1999; Wright and others 2009), nonanal (Tomaino and others 2004), (E,Z)-2,6-nonadienal (Karagul-Yuceer and others 2003), 1-octen-3-one (Carunchia-Whetstine and others 2005), and 2-heptanone (Quach and others

1999) have been associated with stale, cardboard flavors in whey and dairy powders. In agreement with previous studies, hexanal was the most abundant compound quantified in WPC (Quach and others 1999; Wright and others 2009). Methional, DMDS, and DMTS have also been documented in commercial WPC and WPI (Mahajan and others 2004; Wright and others 2009). Methional is a Strecker degradation product formed from the degradation of methionine. From methionine, DMDS and DMTS are further degradation products from intermediate products methanethiol and dimethyl sulfide (Wright and others 2006). Butanoic and hexanoic acid concentrations in the control WPC were similar to those previously reported in WPC (Carunchia-Whetstine and others 2005).

Discussion

Lipid autooxidation in fluid whey and WPC

Autooxidation of unsaturated fatty acids gives rise to unstable hydroperoxides, which decompose to a wide range of carbonyl products, many of which can contribute to off-flavors in dairy products (Lee and Morr 1994; O'Connor and O'Brien 2006; Drake and others 2009; Wright and others 2009). Short chain

Figure 1—Principal component biplot of Cheddar WPC volatile compounds and WPC treatments. Control: Control with fat separation; ascorbic acid: whey with ascorbic acid addition; WPH treatment: whey with WPH addition.

aldehyde detection and increases with storage have been previously documented in Cheddar liquid whey (Carunchia-Whetstine and others 2003; Tomaino and others 2004; Gallardo-Escamilla and others 2005). Tomaino and others (2004) suggested that the actual cheese making process initiated the production of these compounds, which then increased in concentration over storage time and/or processing, an observation confirmed in the current study with Cheddar and Mozzarella wheys.

Previous studies have quantified the amounts of free fatty acids in Cheddar liquid whey (Tomaino and others 2001, 2004; Carunchia-Whetstine and others 2003). Over 14 d of liquid whey storage, significant decreases in palmitic (C : 16), oleic (C18 : 1 ω 9), and linoleic (C18 : 2 ω 6) free fatty acids with subsequent increases in lipid oxidation products were documented (Tomaino and others 2004). Further, significant correlations were documented between the decrease in linoleic acid concentration and increases in cardboard flavor over storage (Tomaino and others 2004). Hexanal was the predominant aldehyde detected in this and other storage studies with Cheddar liquid whey and whey products (Quach and others 1999; Tomaino and others 2004; Carunchia-Whetstine and others 2005).

Starter cultures may also form lipid oxidation products during fermentation. Lactic acid bacteria, *Lactococcus cremoris*, *Lactococcus lactis*, *Streptococcus thermophilus*, *Lactobacillus helveticus*, and *Lactobacillus bulgaricus*, produced 2,4-decadienal and 2,4-nonadienal in milk (Suriyaphan and others 2001). Degradation of 2,4-decadienal serves as yet another pathway for hexanal production (Schieberle and Grosch 1981). In the current study, volatile acetic, butanoic, and hexanoic acids were significantly higher in Cheddar whey over 8 d storage compared to Mozzarella whey. Of these 3 acids, only acetic acid was detected in Mozzarella whey with fat. In the current study, Cheddar liquid whey had more lipid oxidation than Mozzarella liquid whey based on the total aldehyde concentrations identified in the whey samples.

For Cheddar and Mozzarella whey, fat separation decreased aldehyde production and lipid oxidation products (Table 3 and 4), confirming that efficient fat separation is a critical parameter in industrial whey processing. Few sensory differences were documented between the 2 Mozzarella control wheys. However, control Mozzarella wheys had similar aldehyde concentrations initially, but over time, the fat separated control had a lower concentration of total aldehydes than the control whey without fat separation (Table 2 and 4). In Cheddar wheys, controls had a similar sensory and volatile profiles initially, and over time, whey without fat removal had significantly higher cardboard flavor and higher concentrations of hexanal and total aldehydes than whey with fat removed (Table 3).

The increase and subsequent decrease of aldehyde concentrations in Mozzarella liquid whey over 8 d may be explained by the autooxidation of saturated aldehydes (Table 4). For example, hexanal and octanal produce hexanoic and octanoic acids, respectively, by autooxidation (Schieberle and Grosch 1981) and nonanal produces octanal, octanol, hexanal, heptanal (Forney 1974), and nonanoic acid (Lillard and Day 1964). Saturated aldehydes degrade significantly slower than unsaturated aldehydes and in the presence of unsaturated aldehydes, oxidation of saturated aldehydes is decreased (Schieberle and Grosch 1981). Thus, the initial concentration of free fatty acids in whey may first determine the concentration of aldehydes, that is, lipid oxidation products, followed by the degradation of unsaturated aldehydes and then finally degradation of the saturated aldehydes into carboxylic acids. In relation to the current study, liquid wheys without fat removal, es-

pecially Cheddar whey, had the highest cardboard flavor intensities and aldehyde concentrations. This is most likely due to an initial higher concentration of fat and free fatty acids than wheys with fat removal. Whey without fat removal, especially Cheddar whey, may have higher lipid oxidation products than Mozzarella whey due to its higher initial free fatty acid and unsaturated aldehyde concentration.

Approximately 0.2% residual lipids are left in liquid whey, which amount to 5% to 7% fat when the whey is concentrated into WPC (Morr and Ha 1993). These lipids that remain in the whey even after fat separation are believed to be released from the milkfat globule membrane into the whey during cheesemaking (Morr and Ha 1991). Phospholipids have also been considered as a possible source of off-flavors in whey products (Morr and Ha 1993). Phospholipids of milk and dairy products are concentrated in either the milk fat globule membrane (MFGM) or in other membraneaceous material that are probably derived from the MFGM (Christie and others 1987). The MFGM consists mainly of triacylglycerides, cholesterol, phospholipids, and sphingolipids in varying proportions (Fox and McSweeney 1998). During processing, the MFGM may be disrupted, releasing MFGM and its contents into the skim milk phase and enriching the aqueous phase (Christie and others 1987; Rombaut and Dewettinck 2006). Before processing, fat rich products are high in polar lipids as they are situated on the MFGM, but after processing and subsequent MFGM rupturing, low fat products such as skim milk, buttermilk, butterserum (aqueous phase of butter), and whey also become enriched in polar lipids (Christie and others 1987; Rombaut and Dewettinck 2006). In cream, butter and cheese, polar lipids are less than 1% (w/w) of total lipids, but the concentration is much higher in skimmed products like whey (Rombaut and Dewettinck 2006). Phospholipids are more unsaturated than the triglyceride fraction of milk (Rombaut and Dewettinck 2006). Cheddar and Mozzarella liquid whey, when manufactured from whole milk as was the case in the current study, have very similar concentrations of phospholipid polar species (Rombaut and others 2007).

Overall, lipid oxidation in whey products consists of a variety of pathways to produce off-flavors and oxidative products. It seems likely that phospholipids play a role in the oxidative products in Cheddar and Mozzarella whey. However, different amounts of lipid oxidation products were documented between the 2 types of whey, despite similar total lipid concentrations in the wheys and in the initial cheesemilks, suggesting the role of other influencing factors such as the type of starter culture.

Whey made from different types of cheese have different flavor profiles (Carunchia-Whetstine and others 2003; Gallardo-Escamilla and others 2005), and studies with fermented milks have confirmed a wide variety in type and amount of volatile compound production from *Lactobacillus* strains and *Lactococcus* species (Boumerbassi and others 1995). Strain differences in Cheddar starter cultures can also influence the flavor and oxidative stability of liquid Cheddar whey (Carunchia-Whetstine and others 2003; Tomaino and others 2004). Thus, it should be no surprise to find vast differences between Cheddar and Mozzarella liquid whey and oxidative stability of these products. Lipid oxidation products, prevalent in liquid whey, were also observed in WPC. Lipid oxidation products are primary contributors to the flavor of WPC34, WPC80, and WPI (Carunchia Whetstine and others 2005; Evans and others 2009). Previous studies have similarly demonstrated that flavors and flavor compounds documented in fluid whey are indicative of flavors in spray-dried WPC (Croissant and others 2009).

Ascorbic acid. Lipid oxidation may be inhibited with the addition of antioxidants. Ascorbic acid is a natural and promising antioxidant for food systems. Ascorbic acid can be described as an antioxidant, prooxidant, metal chelator, reducing agent, and oxygen scavenger (Frankel 1998). As an antioxidant, ascorbic acid acts as a radical scavenger by reacting with radicals to form less reactive radicals. Ascorbic acid can inhibit or retard hydroperoxide decomposition and ultimately aldehyde formation by interfering with propagation or initiation by hydrogen donation to the radicals (Niki and others 1984; Frankel 1998). The addition of ascorbic acid in this study produced an antioxidant effect in liquid whey and Cheddar WPC. Cardboard flavor in liquid and WPC were significantly lower in whey with ascorbic acid addition compared to control whey. Ascorbic acid addition also resulted in lower total aldehyde concentration in liquid whey and WPC compared to control whey and WPC. However, addition of ascorbic acid to liquid whey seemed to exhibit a greater antioxidant effect compared to its effect in the finished dried product WPC. In liquid whey, both treatments had similar reduced concentrations of aldehydes over storage time. Conversely, in WPC, ascorbic acid did not maintain the same aldehyde inhibiting effects as WPC with WPH (Table 3 and 4). This initial ascorbic acid antioxidant activity with subsequent decrease in activity has been observed in previous research with other foods (Villota and Karel 1980; Robertson and Samaniego 1986; Dave and Shah 1997). As ascorbic acid is consumed as an antioxidant, the concentration of ascorbic acid will eventually fall in the prooxidant range and begin to produce more radicals than it is able to scavenge (Mahoney and Graf 1986). Over storage time, ascorbic acid will degrade and antioxidant activity is no longer exhibited (Saguy and others 1978; Robertson and Samaniego 1986).

Whey protein hydrolysate. Previous studies have demonstrated that food proteins and their hydrolysates have antioxidant activity including milk proteins, casein (Cervato and others 1999; Wong and Kitts 2003), casein hydrolysates and low molecular weight casein hydrolysates (Diaz and Decker 2004), whey protein hydrolysates (Peña-Ramos and Xiong 2003), egg yolk (Park and others 2001), and soybean protein hydrolysate (Chen and others 1996; Peña-Ramos and Xiong 2003). Model systems have demonstrated that the antioxidative properties of whey proteins can be increased by fractionation or hydrolysis with certain enzymes, as some peptides and fractions possess stronger antioxidative effects than others (Peña-Ramos and Xiong 2001; Hernandez-Ledesma and others 2005; Pihlanto 2006). In this study, WPH addition to liquid whey and further production to spray-dried WPC successfully led to a decrease in cardboard flavor and short chain aldehyde concentration compared to the control liquid whey and WPC.

Previous research has attributed lipid oxidation inhibition by enzymatic hydrolysis of proteins to metal chelation and termination of radicals due to specific amino acid residue side chains or through the specific peptide structure (Yamashoji and Kajimoto 1980; Peña-Ramos and Xiong 2001). The accessibility to the peptides may also alter the antioxidant effect. Smaller peptides and amino acids have greater accessibility compared to larger peptides and proteins (Moosman and Behl 2002). Previous research with soy protein (Chen and others 1995) and casein (Suetsana and others 2000) attributed the antioxidant success of shorter peptides to specific amino acids. In whey, Peña-Ramos and Xiong (2001) suggested peptides with low molecular weights, < 10 kDa, contributed to the antioxidant activity of WPH.

Although whey with WPH had lower cardboard flavor in liquid whey and WPC products, WPH has its own distinct flavor

and aroma as well (Drake and others 2009). Whey with WPH had potato/brothy, cheesy/brothy, and malty flavors that were not detected in the control samples. WPC, after further processing, had lower intensities of these flavors than the liquid whey with the same added WPH concentration, but WPH-specific flavors were still evident. Further research should be conducted to determine if lower concentrations of WPH or different types of WPH will minimize WPH-flavor in WPC while maintaining or enhancing antioxidant effects.

Conclusions

Mozzarella and Cheddar liquid whey had distinct flavor profiles, volatile compound concentrations, and oxidative stability. Lipid oxidation occurred in Mozzarella and Cheddar liquid whey over storage time. However, lipid oxidation products were more prevalent in Cheddar whey than Mozzarella whey, which may contribute to flavor differences in dried whey proteins. Fat removal from liquid whey decreased but did not eliminate lipid oxidation. The addition of ascorbic acid or WPH to either liquid whey type decreased cardboard off-flavor and aldehyde concentration over time, and WPH was most effective as an antioxidant in finished Cheddar WPC. Ascorbic acid antioxidant activity may become exhausted through the production of the dried whey product, thus exhibiting less antioxidant properties than WPH. Hexanal was the most abundant volatile compound found in liquid whey and WPC. These results suggest that lipid oxidation is evident in fresh liquid whey and steps should be taken to further minimize lipid oxidation during whey processing. The addition of antioxidants may enhance quality of WPC.

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