Determining Flavor and Flavor Variability in Commercially Produced Liquid Cheddar Whey

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ABSTRACT

Dried whey and whey protein are important food ingredients. Functionality of whey products has been studied extensively. Flavor inconsistency and flavors which may carry through to the finished product can limit whey ingredient applications in dairy and nondairy foods. The goal of this research was to determine the flavor and flavor variability of commercially produced liquid Cheddar cheese whey. Liquid Cheddar cheese whey from five culture blends from two different stirred-curd Cheddar cheese manufacturing facilities was collected. Whey flavor was characterized using instrumental and sensory methods. Wide variation in whey headspace volatiles was observed between different manufacturing facilities ($P < 0.05$). Hexanal and diacetyl were two key volatiles that varied widely ($P < 0.05$). FFA profiles determined by solid-phase microextraction and degree of proteolysis of the whey samples were also different ($P < 0.05$). Differences in whey flavor profiles were also confirmed by descriptive sensory analysis ($P < 0.05$). Differences in liquid whey flavor were attributed to differences in milk source, processing and handling and starter culture blend. The flavor of liquid Cheddar cheese whey is variable and impacted by milk source and starter culture rotation. Results from this study will aid future studies that address the impact of liquid whey flavor variability on flavor of dried whey ingredients.

(Key words: Whey flavor; Starter culture; Oxidative stability)

Abbreviation key: GC = gas chromatograph, PITC = phenylisothiocyanate, RI = retention index, SPME = solid-phase microextraction.

INTRODUCTION

Whey has become an important ingredient to the dairy and food industries. Since the 1970s, whey production in the U.S. has more than tripled (American Dairy Products Institute, 1999). The functionality of whey has been well studied and documented in recent years. Whey protein concentrate has many functional properties such as inducing gelation and providing viscosity (McDonough et al., 1974; Morr and Foegeding, 1990). Because of the success of this research, many products now contain whey and whey ingredients. However, only 55% of liquid whey produced in the United States is further processed into food or feed ingredients (American Dairy Products Institute, 1999).

In general, whey and dried whey ingredients are expected to have a bland and delicate flavor (Laye et al., 1995). The flavor of whey is complex with many different chemical compounds contributing to the overall flavor. Though whey contains only a small amount of lipid, volatile lipid oxidation products were reported to be the main contributors to off-flavors, both in liquid and dried whey (Swaisgood, 1996). Previous studies have reported a wide variety of volatile compounds in liquid and dried whey including methyl esters, ketones, aldehydes, and FFA (Ferretti and Flanagan, 1971; Hildalgo and Kinsella, 1989; Mills, 1993). Variability in the types and concentrations of free amino acids have also been reported (Mavropoulo and Kosikowski, 1972; Mills, 1993).

Research has shown that there are two reactions responsible for the formation of off-flavors in dairy products (Ramshaw and Dunstone, 1969; Ferretti and Flanagan, 1972; Min et al., 1990; Lee and Morr, 1994). Lipolysis and proteolysis contribute the majority of off-flavors (Lee and Morr, 1994). Lipid oxidation reactions were hypothesized to initiate the deterioration of flavor in whey products through the formation of lipid oxidation products and the promotion of Maillard reactions (Whitfield, 1992). Proteolytic enzymes, including chymosin, carry over into the whey and may also promote off-flavors (Mabbit, 1961; Holmes et al., 1977; Amundson, 1984).

Whey is often implicated as having a stale, undesirable flavor that is unpleasant to consumers (Morr and Ha,

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1991; Branger et al., 1999). Though whey is used in many products, these products tend to be heavily flavored, and off-flavors limit the use of whey in bland or delicately flavored foods such as health beverages or baking mixes. Unfortunately there is a lack of research in whey flavor, especially liquid whey. Flavor variability may be inherent in the liquid whey itself, or may be an outcome of processing techniques. A better understanding of the flavor variability in liquid whey may lead to methods to minimize flavor variability in dried whey ingredients. Research by Tomaino et al. (2001; 2002) studied flavor variability of liquid whey produced in the lab using individual strains of *Lactococcus lactis* subsp. *lactis*. This research demonstrated that there were differences in liquid Cheddar-type whey produced in a noncommercial setting from different starter culture strains. Since wheys are typically produced from mixed starter culture strains and then pooled prior to processing, the next logical step is to determine if there are differences in commercially produced liquid whey from one type of cheese. The objectives of this study were to determine flavor and sources of flavor variability of fresh commercial Cheddar cheese whey utilizing both instrumental and sensory analysis.

MATERIALS AND METHODS

Sample Collection

Liquid Cheddar cheese whey samples were collected from two different cheese manufacturing facilities. Both facilities manufactured stirred curd Cheddar cheese. One facility was located in California (Plant 1) and one facility was located in Minnesota (Plant 2). Milk from each plant was also collected. Whey (5 L) or milk (1 L) was collected into 500 ml high-density polyethylene bottles (Fisher Scientific, Pittsburg, PA) at the end of the cook procedure or prior to cheesemaking, respectively and was immediately frozen at -20°C . Frozen whey and milk were subsequently shipped overnight on blue ice to North Carolina State University and maintained at -20°C until analysis. Whey and milk were collected on different make days in triplicate across at least two different starter culture rotations at each plant (e.g. whey from each starter culture blend was sampled three times on three different days). Mesophilic lactic starter cultures used in each plant were provided by different starter culture companies. All subsequent analyses on whey and milk, with the exception of descriptive analysis, which was performed in duplicate, were conducted in triplicate. Proximate analyses including pH, titratable acidity, percentage of solids, and percentage of fat were determined to ensure consistency between samples (Table 1) (Marshall, 1993).

Dynamic Headspace-Gas Chromatography Analysis

Wheys or milk were defrosted for 90 min in a 25°C water bath and kept refrigerated (<12 h) until analysis. Sixteen g of whey or milk, $10\ \mu\text{l}$ of an internal standard (0.25mg 1-pentanol (Sigma/Aldrich, St. Louis, MO)/ml distilled water), and a stir bar were added to a 30 ml headspace vial (Supelco, Bellefonte, PA) and crimp sealed with a teflon/butyl septum (National Scientific, Saudi Arabia). Sampling and purge needles were inserted into the vial through the septa and were attached to a Tekmar (Cincinnati, OH) dynamic headspace unit. Samples were placed on a stir plate and agitated during purging with helium (35 ml/min) for 20 min onto a Tenax TA trap (Tekmar, Cincinnati, OH) until desorption. Desorption was for 5 min at 185°C , and the trap was then baked for 20 min at 200°C prior to the next analysis. The desorbed volatiles were transferred to the inlet of a Hewlett Packard 5890 series II gas chromatograph (GC) (Hewlett Packard, Wilmington, DE) equipped with a 30 m DB-1 capillary column (J&W Scientific, Folsom, CA) (0.32-mm I.D., 3- μm film thickness). The temperature was programmed to start at -20°C with a 6 min hold followed by an increase to 60°C at a rate of $8^{\circ}\text{C}/\text{min}$ then an increase to 220°C at $6^{\circ}\text{C}/\text{min}$ with a final 5-min hold. The injector and flame ionization detector were set at 250°C and the carrier gas (He) flow rate was set at 3.15 ml/min. Data collection and integration were performed using ChromPerfect (Justice Laboratories, Denville, NJ). Concentrations of volatiles (relative abundance) were calculated based on the peak area and known concentration of the internal standard and the peak area of the identified volatile. Retention indices (RI) were calculated using an n-alkane series (Van den Dool and Kratz, 1963). A Hewlett Packard MSD 5972 mass spectrometer set at 70eV ionization potential with a scan range of 35 to 350 Da was used along with matching of retention times and retention indices of known compounds to identify volatiles. All the reference compounds were obtained from Sigma-Aldrich (St. Louis, MO).

FFA Analysis

FFA were analyzed using the solid phase microextraction method of Tomaino et al. (2001). After absorption, the fiber was placed into the injector of a Varian 3700 gas chromatogram (Walnut Creek, CA) fitted with a DB-FFAP column (30 m, 0.25 mm I.D., 0.25 μm film thickness; J&W Scientific, Folsom, CA) operating with the split flow off. The temperature program was initially set at 100°C and held for 2 min, then increased at rate of $10^{\circ}\text{C}/\text{min}$ up to 245°C and held for 10 min. The fiber remained in the injector for the entire GC run time at which time the fiber was retracted and removed. Fatty

Table 1. Proximate Composition of Whey and Milk Samples

Sample	pH	Titrateable Acidity ¹	% Solids	% Fat
Whey 1	6.21 ± 0.47	0.20 ± 0.03	6.33 ± 0.76	0.13 ± 0.04
Whey 2	6.25 ± 0.41	0.22 ± 0.05	6.57 ± 0.40	0.16 ± 0.08
Milk 1	6.89 ± 0.07	ND ²	9.57 ± 0.51	3.60 ± 0.25
Whey 3	6.54 ± 0.13	0.18 ± 0.03	6.60 ± 0.72	0.19 ± 0.03
Whey 4	6.60 ± 0.16	0.15 ± 0.03	6.70 ± 0.71	0.17 ± 0.01
Whey 5	6.34 ± 0.29	0.17 ± 0.03	6.77 ± 0.15	0.18 ± 0.01
Milk 2	6.87 ± 0.07	ND	9.73 ± 0.23	3.42 ± 0.08

¹% lactic acid.²ND = not determined.

acids were detected with a flame-ionized detector set at 250°C. Relative abundance of fatty acids was calculated using the peak area and known concentration of the internal standard and the peak area of the identified fatty acid. Milk and whey were tested in triplicate. All chemicals (butanoic acid, hexanoic acid, octanoic acid, decanoic acid, dodecanoic acid, tetradecanoic acid, hexadecanoic acid, heptadecanoic acid, octadecanoic acid, and 9-octadecenoic acid) were obtained from Supelco (Bellafonte, PA).

Free AA/Protein Hydrolysis Analysis

Free amino acids were analyzed by the high performance liquid chromatography (HPLC) method of Bidlingmeyer et al. (1987). The liquid (thawed) whey or milk was concentrated by freeze drying approximately 150 ml of whey or 100 ml of milk overnight (~20 hours) using a Labconco freeze drier (Kansas City, MO). Upon removal from the freeze drier flask, the whey or milk was ground into a fine powder with a hammer and pestle. Two g of dry powder, along with 1.5 ml of distilled water, were added to a 10-ml test tube in duplicate. For the milk samples, 2 g of dried milk and 2 ml of distilled water were added to 10-ml test tubes in duplicate. The tubes were vortexed until samples were fully hydrated followed by the addition of 5 ml of 0.75 M trichloroacetic acid while vortexing. The samples were allowed to stand for 10 min then they were filtered through Whatman #2 filter paper. 20 µl of filtrate was added to a 6 × 50 mm culture tube and derivatized with phenylisothiocyanate (PITC) as described by Bidlingmeyer et al. (1987). Chromatography was performed with a Hewlett Packard series 1050 HPLC equipped with an autosampler set to inject 20 µl of each replicate. Peak areas were quantitated using a 6-point external calibration curve constructed for each amino acid ($R^2 > 0.991$). All the amino acids were obtained from Sigma-Aldrich.

Descriptive Sensory Analysis

Evaluation of the flavor attributes of whey and milk were conducted by eight experienced descriptive sensory

panelists (6 females, 2 males) using the 15-point universal Spectrum intensity scale (Meilgaard et al., 1991). Panelists were selected based on availability and previous experience with descriptive sensory analysis of dairy products using the universal Spectrum intensity scale. Panelists each had more than 50 h of previous experience in the descriptive sensory analysis of dairy products. Descriptors for whey were developed during four 1-h training sessions. The basic tastes and feeling factors sweet, sour, bitter, salty, astringent, and the volatile flavors cooked/milky, diacetyl, milk fat/lactone, metallic/meat serum, cardboardy, soil/potato-like were included (Table 2). During training sessions, panelists discussed and refined descriptors, definitions and references (Table 2). Discussion and evaluation of wheys was also conducted during training to enable panelists to consistently differentiate and replicate samples. Analysis of data collected from training sessions confirmed that panel results were consistent.

Evaluations were conducted individually in an enclosed room dedicated to sensory analysis and free from external aromas, noise, and distractions. Samples were assigned three digit random codes and evaluated at 21°C in 2-oz plastic soufflé cups with lids during each session. Panelists were instructed to expectorate samples after evaluation. Spring water was available to each panelist for palate cleansing. Panelists evaluated four samples per session with duplicate sessions per day (11 sessions total). Each replicate ($n = 3$) of each starter culture rotation and each milk was evaluated in duplicate by each panelist in a randomized balanced block design.

Statistical Analysis

Data were analyzed using the SAS statistical software (version 8.0, SAS Institute, Cary, NC). Analysis of variance with means separation were performed by PROC GLM and DUNCAN's procedure, respectively, and correlations were evaluated using PROC CORR.

$$\text{Model } Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + e_{ijk}$$

Table 2. Descriptive analysis reference terms.

Term	Definition	Reference	Example
Cooked	Aromatic associated with cooked milk	Cooked milk Heat skim milk to 85°C for 30 min	Mild cheddar
Sweet aromatic/ Diacetyl	Aromatic associated with diacetyl	Diacetyl Drop onto filter paper strips in 125 ml sniff jar	Mild cheddar and cojack shreds
Milk fat/Lactone	Aromatics associated with milk fat	Dodecalactone Drop onto filter paper strips in 125 ml sniff jar	Whole milk mozzarella Fresh coconut Cream
Metallic/Meat serum	Aromatics associated with metal or with juices of a rare steak	1-octen-3-one Drop onto filter paper strips in 125 ml sniff jar	Rare steak tinfoil
Cardboardy	Aroma associated with cardboard	Pentanal Drop onto filter paper strips in 125 ml sniff jar	Cardboard in water
Musty/Soil- like/Potato	Aroma associated with potting soil	Methional Drop onto filter paper strips in 125 ml sniff jar	Potting soil Boiled potatoes
Salty	Basic taste associated with salt	Sodium chloride	0.5% salt in water ¹
Sweet	Basic taste associated with sucrose	Sucrose	5% sugar in water ¹
Sour	Basic taste associated with acid	Citric acid	0.08% citric acid in water ¹
Bitter	Basic taste associated with bitterness	Caffeine	0.08% caffeine in water ¹
Astringency	Drying tongue sensation	Tea	Black tea

¹Universal scale references as described in Meilgaard et al., 1991.

Activity = Overall mean + (plant)_i + (culture)_j + (plant × culture)_{ij} + (error)_{ijk}, where:

i = 1, 2 (manufacturing plant),
j = 1, 2, 3, 4, 5 (culture), and
k = 1, 2, 3 (replicate).

RESULTS

Volatile Compounds

The volatile products found in the whey and milks are summarized in Table 3. Replicate milk and wheys produced on different make days from each starter culture blend for each plant were not different ($P > 0.05$), so results were averaged. It is important to note that Wheys 1 and 2 were from the same manufacturing facility as Milk 1, and Wheys 3, 4, 5 were from the same manufacturing facility as Milk 2. Aside from being processed at different locations, wheys were made from different starter culture blends. Wheys were not different in composition (Table 1). Most of the compounds found in the whey samples were also found in the milk (Table 3).

Oxidation of lipids produces a wide range of compounds including aldehydes, ketones, alcohols, and alkanes (Frankel et al., 1981). The short-chain aldehydes, pentanal and hexanal, were found in all wheys and milks. Wheys 3, 4, and 5 contained higher amounts of pentanal, and Wheys 3 and 5 contained slightly higher, though not significant, amounts of hexanal as compared to the other whey and milk samples. In general, the

wheys from Plant 2 contained a higher concentration of volatile lipid oxidation products, including pentanal and 2-butanone, than wheys from Plant 1 (Table 3). These differences between plants may be due to differences in milk source, processing, or handling, since these differences are consistent across starter culture rotations within each plant. Some differences may be attributed to starter culture, as wheys produced in the same plants were also distinct from each other. Whey 5 contained higher amounts of 2,4-decadienal ($P < 0.05$) than other wheys. Principal component analysis (Figure 1) of the volatile data indicated that Wheys 3, 4, and 5 were similar to each other, while Wheys 1 and 2 were more similar to each other but distinct from Wheys 3, 4, and 5. There was a distinction among wheys produced at different manufacturing facilities as well as wheys produced from the same manufacturing facility.

FFA

Table 4 summarizes the relative concentrations of FFA. Milks and wheys produced on different make days for each starter culture blend from each plant were not different ($P > 0.05$) so results were averaged. The FFA can be subdivided into two categories: the short chain fatty acids (butyric, caproic, caprylic) and the longer chain fatty acids (capric, lauric, myristic, palmitic, stearic, and oleic). The lower concentrations of short chain fatty acids as compared to concentrations of longer chain fatty acids can be explained because there are more long chain fatty acids found in dairy products (Nawar, 1996;

Table 3. Relative concentration¹ of volatiles in milks and wheys.

Volatile ²	RT ³	RI	W1 ⁴	W2	M1	W3	W4	W5	M2
Acetone	13.2	<500	1193 ^c	1783 ^c	1472 ^c	4746 ^b	3228 ^b	4142 ^b	8901 ^a
Dimethyl sulfide	14.5	<500	8411 ^a	8555 ^a	9512 ^a	ND	ND	1569 ^b	2916 ^b
2-Acetyl-1 pyrroline	14.8	<500	52 ^a	ND	12 ^a	ND	ND	10 ^a	34 ^a
Pentane	15.3	500	ND	ND	12 ^a	ND	ND	ND	ND
Diacetyl	16.5	523	176 ^{a,b}	207 ^{a,b}	244 ^a	52 ^{c,d}	63 ^{c,d}	126 ^{b,c}	17 ^d
2-butanone	16.7	527	ND	142 ^c	55 ^c	437 ^b	259 ^{b,c}	463 ^b	928 ^a
Pentanal	19.6	580	182 ^b	81 ^b	109 ^b	479 ^a	519 ^a	429 ^a	77 ^b
Hexane	20.7	600	85 ^b	324 ^a	185 ^a	ND	ND	ND	ND
Pentenal	21.5	626	ND	ND	4 ^a	ND	ND	3 ^a	ND
1-pentanol (I. S.)	22.1	651	—	—	—	—	—	—	—
Hexanal	23.0	677	433 ^{a,b}	337 ^{a,b}	351 ^{a,b}	590 ^a	549 ^{a,b}	623 ^a	95 ^b
Heptane	23.7	700	31 ^a	134 ^a	245 ^a	ND	128 ^a	31 ^a	6 ^a
Octane	24.2	800	80 ^c	278 ^c	530 ^{b,c}	2009 ^a	3134 ^a	1732 ^{a,b}	1697 ^{a,b}
Nonane	26.7	900	8 ^a	10 ^a	4 ^a	ND	ND	ND	ND
Phenol	28.1	953	ND	168 ^b	4 ^b	207 ^b	14 ^b	297 ^b	889 ^a
1-octen-3-one	28.3	958	9 ^a	8 ^a	16 ^a	ND	ND	ND	ND
Decane	29.5	1000	ND	ND	ND	ND	ND	ND	ND
Octanal	30.4	1036	42 ^{a,b}	15 ^{a,b}	56 ^a	9 ^{a,b}	0.8 ^b	35 ^{a,b}	ND
2-nonanone	31.4	1046	ND	5 ^a	3 ^a	ND	4 ^a	3 ^a	ND
Nonanal	31.7	1085	11 ^a	72 ^a	118 ^a	23 ^a	37 ^a	49 ^a	20 ^a
Undecane	32.1	1100	ND	24 ^a	ND	11 ^a	ND	80 ^a	63 ^a
Dodecane	34.6	1200	2 ^a	8 ^a	8 ^a	9 ^a	10 ^a	16 ^a	15 ^a
2-4-decadienal	36.8	1297	ND	3 ^b	4 ^b	ND	ND	28 ^a	ND
Tridecane	36.9	1300	3 ^a	ND	ND	ND	ND	ND	ND
Tetradecane	39.1	1400	7 ^b	4 ^b	5 ^b	28 ^a	26 ^{a,b}	15 ^{a,b}	23 ^{a,b}
Pentadecane	41.2	1500	ND	ND	ND	ND	ND	ND	ND
Hexadecane	43.1	1600	ND	4 ^a	8 ^a	ND	ND	ND	ND
Heptadecane	45.6	1700	367 ^a	188 ^a	ND	45 ^a	ND	62 ^a	72 ^a

¹ng volatile/ml whey or milk.²All volatiles identified using RT, RI, and MS.³RT = retention time (min) and RI = retention indices were calculated on a DB-1 column; W = whey; M = milk; ND = not detected.⁴Whey 1, Whey 2, and Milk 1 are from the same manufacturing facility; Whey 3, Whey 4, Whey 5, and Milk 2 are from the same manufacturing facility.^{a,b,c,d}Means within rows with different letters are statistically different ($P < 0.05$).

Swaigood, 1996). The long chain fatty acids are cleaved off of the triglycerides by lipolytic enzymes from the starter culture (Jensen et al., 1991; El Soda et al., 1995) and are not typically found as FFA in milk. Therefore, these fatty acids are the ones of most interest in this study since differences in these can be attributed to the lipolytic nature of starter culture activity. Longer chain fatty acids were found in high concentrations in the wheys (Table 4). Lauric and myristic acids increased in wheys compared to their milk sources.

Milk 2 contained significantly ($P < 0.05$) higher concentrations of FFA than did Milk 1. Though differences between milks impacted FFA content of the resulting wheys, there were still observed differences in wheys that were manufactured at the same plant (Table 4). Thus, some differences in FFA observed may be attributed to starter culture. Whey 5 contained higher concentrations of the long chain fatty acids compared to the other wheys ($P < 0.05$).

Protein Hydrolysis

Differences in free amino acids were observed among wheys and milks (Table 5). Replicate milk and wheys

from different make days for each starter culture rotation from each plant were not different ($P > 0.05$) so results were averaged. Milk 2 had a greater concentration ($P < 0.05$) of polar free amino acids than Milk 1. Whey 5 contained significantly higher amounts of the polar amino acids compared to the other wheys ($P < 0.05$). Wheys 1 and 2 were similar to each other in their amino acid content, Wheys 3 and 4 were similar to each other, and 5 was not similar to the other wheys. Whey 5 generally had a higher degree of proteolysis from the other wheys.

Descriptive Analysis

Sensory scores are summarized in Table 6. Some differences, though not significant, were noted in the flavor of the milk from the two facilities. The main difference in the flavor of the milks was that Milk 1 contained feed flavors while Milk 2 did not. These differences in milk flavor may have impacted the flavor of the resulting wheys. Panelists noted that Whey 1 was more sour than the other wheys. Whey 1 was also less sweet and contained higher amounts of musty flavors compared to

Figure 1. Principal component analysis of the five different whey samples within the headspace volatiles.

other wheys. The other wheys did not contain musty flavors at perceivable levels. Wheys 3 and 4 were more cardboardy than the other wheys, and wheys from Plant 2 were more cardboardy in general. Principal component

analysis (Figure 2) of sensory results showed that sensory analysis effectively demonstrated flavor variability in the liquid wheys. Whey 1 was characterized by musty and metallic flavors, while Wheys 3 and 4 were charac-

Table 4. Relative concentrations¹ of free fatty acids in milks and wheys.

Free Fatty Acid ²	RT ³	W1	W2	M1	W3	W4	W5	M2
Butyric	7.3	0.03 ^c	0.05 ^c	0.27 ^{b,c}	0.25 ^{b,c}	0.37 ^b	0.56 ^b	1.18 ^a
Caproic	9.9	0.10 ^c	0.37 ^{b,c}	1.08 ^{a,b}	0.48 ^{b,c}	1.28 ^a	1.33 ^a	1.78 ^a
Caprylic	12.4	0.47 ^e	1.24 ^{c,d}	0.87 ^{d,e}	1.44 ^c	1.52 ^c	4.21 ^a	2.17 ^b
Capric	14.5	2.21 ^d	3.69 ^c	2.20 ^d	5.06 ^b	5.15 ^b	13.66 ^a	4.62 ^{b,c}
Lauric	16.5	2.85 ^e	4.28 ^d	1.73 ^e	6.07 ^{b,c}	6.31 ^b	15.11 ^a	4.91 ^{c,d}
Myristic	18.7	6.05 ^c	8.03 ^c	2.56 ^d	12.26 ^b	14.13 ^b	27.88 ^a	8.39 ^c
Palmitic	21.5	11.75 ^d	12.14 ^d	6.15 ^e	14.41 ^{c,d}	17.24 ^{b,c}	31.31 ^a	18.99 ^b
Heptadecanoic	23.3	—	—	—	—	—	—	—
Stearic	25.6	3.79 ^c	3.67 ^{b,c}	2.65 ^c	3.17 ^{b,c}	4.10 ^b	6.55 ^a	6.27 ^a
Oleic	26.4	3.36 ^{d,e}	4.37 ^{d,e}	2.91 ^e	5.37 ^{c,d}	7.09 ^{b,c}	12.05 ^a	8.05 ^b
Total FFA		30.61	37.84	20.41	48.50	57.19	112.64	56.36

¹ng free fatty acid/ml whey or milk.

²All volatiles identified using RT.

³RT = retention time (min); W = whey; M = milk.

a,b,c,d,e Means within rows with different letters are statistically different ($P < 0.05$).

Table 5. Concentrations¹ of free amino acids in milks and wheys.

Free Amino Acid ²	R ^{2,3}	W1	W2	M1	W3	W4	W5	M2
Aspartic Acid	0.972	38 ^c	27 ^c	4 ^c	18 ^c	5 ^c	245 ^a	113 ^b
Glutamic Acid	0.993	157 ^{b,c}	179 ^{b,c}	89 ^c	138 ^{b,c}	133 ^c	479 ^a	260 ^b
Serine	0.999	8 ^{b,c}	3 ^c	1 ^c	64 ^a	15 ^{a,b}	66 ^a	56 ^{a,b}
Asparagine	0.999	9 ^c	13 ^{b,c}	17 ^{b,c}	36 ^{b,c}	40 ^{b,c}	124 ^a	51 ^b
Glycine	0.979	20 ^c	22 ^c	14 ^c	21 ^c	7 ^c	160 ^a	81 ^b
Glutamine	0.972	0.8 ^d	32 ^{c,d}	0.8 ^{c,d}	15 ^{c,d}	172 ^b	264 ^a	74 ^c
Histadine	0.978	71 ^b	87 ^b	42 ^b	201 ^a	242 ^a	243 ^a	50 ^b
Threonine	0.959	79 ^{a,b}	136 ^a	47 ^b	140 ^a	113 ^{a,b}	28 ^b	16 ^b
Alanine	0.999	76 ^{a,b}	113 ^a	24 ^b	10 ^b	10 ^{a,b}	10 ^b	10 ^b
Arginine	0.988	15 ^a	10 ^a	4 ^b	8 ^{a,b}	8 ^{a,b}	4 ^b	ND
Proline	0.997	1 ^d	14 ^{b,c}	0.8 ^d	3 ^{c,d}	2 ^{c,d}	21 ^a	15 ^{a,b}
Cystine	0.999	11 ^{a,b}	8 ^{a,b}	8 ^b	6 ^b	8 ^{a,b}	18 ^a	11 ^{a,b}
Tyrosine	0.999	8 ^a	4 ^a	0.8 ^c	0.8 ^{b,c}	3 ^{b,c}	5 ^{a,b}	ND
Valine	0.999	ND	ND	ND	ND	ND	371 ^a	156 ^b
Methionine	0.996	10 ^c	293 ^{b,c}	64 ^{b,c}	375 ^a	245 ^{a,b}	10 ^c	10 ^c
Cysteine	0.991	138 ^b	24 ^b	10 ^b	156 ^b	10 ^b	171 ^a	ND
Isoleucine	0.988	86 ^{b,c,d}	160 ^{a,b,c}	8 ^d	190 ^{a,b}	235 ^a	38 ^{c,d}	31 ^{c,d}
Leucine	0.996	116 ^b	68 ^{b,c}	179 ^a	ND	ND	ND	ND
Phenylalanine	0.999	13 ^a	11 ^a	18 ^a	14 ^a	10 ^a	11 ^a	8 ^a
Tryptophan	0.998	20 ^{c,d}	30 ^{b,c}	0.8 ^d	10 ^{c,d}	5 ^{c,d}	54 ^{a,b}	63 ^a
Lysine	0.999	9 ^a	ND	6 ^a	ND	ND	13 ^a	14 ^a
Total free amino acid		886	1234	538	1406	1263	2335	1019

¹μg free amino acid/ml whey or milk.²All free amino acids identified using RT (retention time in min).³R² calculated on an 8-point standard curve; W = whey; M = milk.^{a,b,c,d}Means within rows with different letters are statistically different ($P < 0.05$).

terized by cardboady flavors. Wheys 2 and 5 had more fresh dairy flavors (cooked/milky and sweet aromatic/diacetyl) compared with other wheys.

DISCUSSION

There were two distinctions of whey flavor observed. There were differences in flavor between plants, and

differences within plants. Differences between plants may be caused by milk source, handling, and processing, and starter culture. Differences found within plants may be attributed to starter culture rotation, since the milk source, handling, and processing would be the same for wheys produced in the same plant. It is important to stress that wheys from each plant for each starter culture rotation were sampled across three different usage days

Table 6. Sensory profiles of wheys and milks.

Descriptor	Mean Scores ^{1,2}						
	W1 ³	W2	M1	W3	W4	W5	M2
Salty taste	1.13 ^a	1.38 ^a	0.52 ^b	1.12 ^a	1.09 ^a	1.23 ^a	0.54 ^b
Sour taste	2.25 ^a	2.11 ^{a,b}	0.15 ^c	1.64 ^b	1.63 ^b	1.63 ^b	0.18 ^c
Sweet taste	1.29 ^c	1.90 ^b	2.19 ^{a,b}	1.67 ^{b,c}	2.29 ^{a,b}	1.83 ^{a,b}	2.55 ^a
Cooked	0.75 ^b	1.04 ^b	2.52 ^a	0.96 ^b	1.00 ^b	1.06 ^b	2.21 ^a
Sweet aromatic/ Diacetyl	0.45 ^{b,c}	1.03 ^a	0.00 ^c	0.67 ^b	0.46 ^{a,b}	0.73 ^a	0.00 ^c
Milk fat/Lactone	0.23 ^b	0.23 ^b	2.75 ^a	0.19 ^b	0.41 ^b	0.48 ^b	2.82 ^a
Metallic/Meat							
Serum	1.54 ^a	1.32 ^a	0.00 ^b	1.48 ^a	1.23 ^a	1.50 ^a	0.00 ^b
Cardboady	0.39 ^{b,c}	0.32 ^c	0.13 ^{c,d}	0.75 ^a	1.00 ^a	0.54 ^{a,b}	0.00 ^d
Musty/Soil- like/Potato	1.33 ^a	0.17 ^b	0.00 ^b	0.13 ^b	0.19 ^b	0.10 ^b	0.00 ^b
Astringency	1.42 ^{a,b}	1.36 ^{a,b}	1.38 ^{a,b}	1.23 ^{a,b}	1.39 ^{a,b}	1.63 ^a	1.17 ^b
Feed	0.00 ^b	0.00 ^b	0.82 ^a	0.00 ^b	0.00 ^b	0.00 ^b	0.00 ^b

¹Scores based on a universal 15-point intensity scale (Meilgaard et al., 1991).²Duplicate analyses of each replicate by each panelist (2 analyses × 3 replicates × 8 panelists).³W = whey, M = milk.^{a,b,c,d}Means within rows with different letters are statistically different ($P < 0.05$).

Figure 2. Principal component analysis of the five different whey samples and their sensory characteristics.

to minimize the possibility of sanitation or process deviation as a primary source of variability. In all cases the three replications were consistent in instrumental and sensory properties ($P > 0.05$).

There were two different classes of volatile compounds in wheys. First, there were aroma-active compounds (alcohols, ketones, aldehydes), such as diacetyl, dimethyl sulfide and hexanal. Aroma-active compounds have a discernable aroma. These compounds are obviously important because they may contribute to whey flavor. Secondly, there were aliphatic hydrocarbons. These are commonly found in whey and do not contribute directly to flavor since they do not have an odor. Laye et al. (1995) hypothesized that formation of these aliphatic hydrocarbons may result from the decarboxylation of the carbon chains of higher fatty acids or from the reaction of alkyl free radicals. Frankel et al. (1981) also noted alkanes as products of lipid oxidation. Hexane was found in Wheys 1 and 2, as well as Milk 1. However, only Whey 2 contained significantly higher concentrations of hexane compared

with the other wheys. The presence of hexane in wheys from Plant 1 may be due to milk source. Octane was detected in all samples, both wheys and milks, but the samples from Plant 2 (Wheys 3, 4, 5 and Milk 2) contained higher concentrations of this compound ($P < 0.05$). Diacetyl is a common flavor constituent of fermented dairy products, including young Cheddar cheese. Diacetyl was detected in higher concentrations in Milk 1 compared to Milk 2 and Wheys 1 and 2 had higher concentrations of diacetyl compared to Wheys 3 or 4. Milk and wheys from Plant 1 contained trace amounts of 1-octen-3-one. This is a common ketone found in milk and cheese, and can impart a metallic off-flavor (Stark and Forss, 1962).

Research has shown that starter cultures can form lipid oxidation products (including hexanal) during fermentation (Forss, 1979; Suriyaphan et al., 2001). Lipid oxidation products formed during cheesemaking would be expected in liquid whey because there is some binding of lipid oxidation products by whey proteins (Kinsella,

1989). Lipid oxidation and the subsequent production of short chain aldehydes are main contributors to off-flavors in dairy products (Lee and Morr, 1994). Badings (1991) concluded that oxidation flavors were related to the breakdown of unsaturated fatty acids. Wheys 3, 4, and 5 had higher amounts of free oleic acid than Wheys 1 and 2. This high amount of unsaturated FFA and subsequent oxidation may have caused the increased perception of cardboary flavors in Wheys 3 and 4. Pentanal was correlated to cardboary flavors ($R = 0.894$, $P = 0.04$) and has been previously associated with this flavor in whey and skim milk powder (Tomaino et al., 2001; Karagul-Yuceer et al., 2002).

El Soda et al. (1995) reported that milk lipase was destroyed during pasteurization leaving the starter culture as the only source of lipolytic enzymes in whey and cheese. Milk from Plant 2 had higher concentrations of FFA compared to milk from Plant 1 and this may explain the greater concentrations of FFA in wheys from Plant 2 compared to wheys from Plant 1 (Table 6). However, Whey 5 contained greater concentrations of all FFA except butyric acid than Wheys 3 and 4 and Milk 2. Because the differences in Whey 5 were consistently observed across three sampling days, plant sanitation or process deviation seems unlikely. These differences in Whey 5, distinct from milk source and other wheys from the same plant, indicate higher lipolytic activity from this starter culture compared to the other starter culture blends used.

Astringency was found in all whey and milks and was correlated to pentenal and 2–4 decadienal ($R = 0.870$, $P = 0.05$) for both compounds. These compounds are both formed during lipid oxidation, and there may be a relationship in that secondary oxidation products contribute to astringency. Previous studies with milk have attributed astringency to proteins (Harwalker et al., 1993). The lack of other significant correlations between sensory and instrumental results may be due to the small number of samples (5) analyzed and/or that the joint effects of several chemicals may generate certain flavors. Volatile data did not show differences in the wheys as clearly as descriptive sensory analysis (Figure 1; Figure 2). Perception and detection of other compounds (FFA, proteolysis) were not accounted for in Figure 1. Additionally, sensory analysis included the basic tastes as well as volatile flavors, and the interaction of the FFA and free amino acids may play a role in these basic tastes.

Several factors contributed to the differences among the wheys in the present study. Milks from the two plants contained varying amounts of volatiles, FFA, and free amino acids. Milk source played a role in the variability of whey flavor since compounds found in milks were also found in wheys. Feed source and milk processing temperature/time will impact flavor of milk and

the resulting wheys (Scanlan et al., 1968; Badings and Neeter, 1980; Calvo de la Hoz, 1992; USDA AMS, 2000). Dimethyl sulfide is often found in milk that has been pasteurized at higher temperatures (Lindsay, 1996). Milk 1 contained high amounts of this compound, and dimethyl sulfide was found in higher concentrations in the wheys from Plant 1 ($P < 0.05$) compared with the wheys from Plant 2.

Another contributing factor in whey flavor variability was the starter culture blends used to produce Cheddar cheese and subsequently Cheddar whey. *Lactococcus* species and strains contain differences in lipase activity (Meyers et al., 1996), proteolytic activity (Broadbent et al., 1998) and differences in type and amount of volatile compound production (Boumerbassi et al., 1995; Seefeldt and Weimer, 2000). Wheys from the same manufacturing facility were differentiated indicating that starter culture rotation for the same cheese type can also impact whey flavor. In particular, Whey 5 was different from all other wheys, containing higher concentrations of FFA and free amino acids. The prevalence of oxidative compounds in all wheys and oxidative-associated flavors by sensory analysis also emphasizes the need to identify steps in subsequent whey processing to minimize oxidative deterioration.

If flavor variability in liquid whey can be diminished, then dried whey ingredients could be produced to a higher quality standard. The utilization of whey and whey ingredients then could be increased. Implicitly, wheys made from different types of cheese have different flavor (i.e., Mozzarella vs. Cheddar). Results of the current study indicate that flavor variability also exists within wheys from one cheese type. More research in this area needs to be conducted to fully understand whey flavor variability and the impact of liquid whey flavor variability on dried whey ingredients.

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