ABSTRACT

LIAW, IRIS W. Flavor and Flavor Chemistry of Liquid Mozzarella and Cheddar Cheese Whey. (Under the direction of Dr. MaryAnne Drake.)

Whey protein is widely used in numerous ingredient applications. Cheddar and Mozzarella cheese production are the primary sources for dried whey protein. Flavor of fresh whey may influence the final whey protein flavor. Whey protein flavor is highly variable and off-flavors in dried whey products can carry through into ingredient applications and may negatively affect consumer acceptance. The first objective of this study was to evaluate the impact of antioxidant addition to prevent flavor deterioration of fluid whey over storage and flavor of whey protein. The second objective of this study was to compare the flavor and flavor stability of fresh and stored liquid Cheddar and Mozzarella whey.

For the first objective, Cheddar or Mozzarella liquid whey were manufactured using standard cheese make-procedures. The wheys were pasteurized and subjected to fat separation. Ascorbic acid, whey protein hydrolysate (WPH), or nitrogen flushing were then administered. Wheys with no antioxidant addition and without fat separation were included as controls. Wheys were stored at 3^{o}C and evaluated by descriptive sensory and instrumental analyses after 0, 2, 4, 6, and 8 days. In a subsequent experiment, selected treatments were 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 (SPME-GC-MS). Cardboard flavors increased with

storage in fluid wheys. Liquid wheys with ascorbic acid, WPH or nitrogen flushing had lower cardboard flavor across storage compared to control wheys. Lipid oxidation products, hexanal, heptanal, octanal and nonanal increased in liquid whey during storage, but liquid whey with added ascorbic acid, WPH or nitrogen flushing had lower concentrations of these products compared to untreated controls. Mozzarella liquid whey had lower flavor attribute 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. WPC and liquid whey with added WPH, had a distinct potato flavor which was not present in control products or products with added ascorbic acid.

In the second study, pasteurized, fat-separated Cheddar and Mozzarella wheys were manufactured in duplicate and evaluated immediately or stored for 3 days at 3^{o}C. Sensory properties were documented by descriptive sensory analysis and volatile components were extracted and characterized by solid phase microextraction with gas chromatrography-mass spectrometry (SPME-GC-MS), direct solvent extract (DSE) with solvent assisted flavor evaporation (SAFE) with GC-MS and gas chromatography-olfactometry (GC-O) with aroma extract dilution analysis (AEDA). Cheddar and Mozzarella wheys were distinct by sensory and volatile analyses ($p<0.05$). Fresh Cheddar whey had higher intensities of buttery and sweet aromatic flavors and higher cardboard flavor intensities following storage compared to Mozzarella whey. Fifty aroma-active compounds were identified by GC-O. High aroma impact compounds ($FD_{log3} > 8$) in fresh Cheddar whey included diacetyl, 1-octen-3-one, 2-phenethanol, butyric acid, and (E)-2-nonenal, while those in Mozzarella whey included diacetyl, octanal, (E)-2-nonenal, and 2-phenethanol.

Concurrently, fresh Cheddar whey had increased concentrations of diacetyl, 2/3-methyl butanal, (E)-2-nonenal, 2-phenethanol, and 1-octen-3-one compared to fresh Mozzarella whey. Lipid oxidation products increased in both whey types during storage but increases were more pronounced in Cheddar whey than Mozzarella whey.

Collectively, these studies suggest that lipid oxidation is a primary source of flavor and flavor degradation in fluid whey. Similar aroma-active compounds at different concentrations comprise the flavor of Cheddar and Mozzarella whey and these influence observed differences in lipid oxidation and flavor during subsequent storage. Addition of an antioxidant to liquid whey prior to further processing may be beneficial to flavor of spray dried whey protein.

Flavor and Flavor Chemistry of Liquid Mozzarella and Cheddar Cheese Whey

by
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DEDICATION

This work is dedicated to all students in science graduate programs. It really can be done.

And also to the crazy Liaw family, I did it!

BIOGRAPHY

Iris Wen-Yun Liaw was born and raised in Huntsville, Alabama to Dr. Goang-Shin Liaw, a successful professor, and Barbara Liaw, a faithful library technician. Iris was the baby of the family with two successful older siblings, Judy and Hank, who without their guidance and humor, she would not be the person she is today.

Iris graduated from Virgil I. Grissom High School in May 2003 and began her studies at the University of Georgia in Athens the following summer. Although her childhood aspiration was to become a prima ballerina, Iris was persuaded by Dr. Robert Shewfelt to major in Food Science and Technology in the College of Agriculture. During her stay at UGA, Iris was an active member in the Pamoja Dance Troop and served as the President and Historian of the Alpha Sigma Rho sorority. In the summer of 2006, Iris was fortunate enough to obtain an internship at The Coca-Cola Company. It was here during her internship that sealed Iris's future in continuing her study in the field of Food Science.

In December 2006, Iris graduated from UGA with a Bachelors of Science degree in Food Science. After graduation, she worked at The Coca-Cola Company until beginning graduate school at the North Carolina State University under the direction of Dr. MaryAnne Drake in August 2007. At NC State, while juggling research and classes, Iris became very active in the Food Science Club serving as the Club president for one year. She was extremely proud to be able to represent the IFTSA winners of College Bowl, Product Development, and Chapter of the Year.

Iris is currently on the job prowl and hopes to find time to travel and visit her grandmother before starting a successful career in the food industry. She will remain a faithful Bulldawg, Wolfpack, and Howling Cow ice cream fan.

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CHAPTER 1: LITERATURE REVIEW

Whey Definition

Whey is the liquid by-product of cheese manufacture (Spreer, 1998). This liquid byproduct is mainly comprised of water, lactose, and protein (Morr and Ha, 1993). Whey is categorized into two different types: sweet whey and acid whey. These two whey types differ due to the different methods of cheese curd formation. Sweet whey is derived from the enzyme coagulation of rennet and a starter culture to form cheese curds like Cheddar and Mozzerella (Spreer, 1998). Sweet whey has a pH between 5.8-6.6 and a titratable acidity of 0.10-0.20%. Acid whey is the liquid byproduct of ricotta and cottage cheese where a mineral acid or lactic acid, rather than a starter culture, is used for casein precipitation. Acid whey has a pH between 4.0-5.0 and a titratable acidity of 0.40-0.60% (Kosikowski and Mistry, 1997). Due to the different starter cultures and/or acidulants leading to these two types of whey, sweet and acid whey differ in mineral content, protein concentration, and lactose content, which result in different functional and flavor properties (Gallardo-Escamilla et al., 2005; Bordenave-Juchereau et al., 2005). Whey products differ based on the composition of the original milk, method of curd coagulation, and type of cheese produced (Ji and Haque, 2003). The differences in composition between fluid sweet and acid whey and dried acid and sweet whey are given in Table 1.1.

Table 1.1 Composition of fluid and dried, sweet and acid whey

	Fluid Sweet Whey (%)	Fluid Acid Whey (%)	Dried Sweet Whey (%)	Dried Acid Whey (%)
Total Solids	6.35	6.5	96.5	96
Moisture	93.7	93.5	3.5	4
Fat	0.5	0.04	0.8	0.6
Total Protein	0.8	0.75	13.1	12.5
Lactose	4.85	4.9	75	67.4
Ash	0.5	0.8	7.3	11.8
Lactic Acid	0.05	0.4	0.2	4.2

Fluid sweet whey from Cheddar cheese
Fluid acid whey from Cottage cheese
(Kosikowski and Mistry, 1997)

Because fluid whey has such a high water content, it is beneficial for expense and stability of the whey to lower the moisture content. Plus, the sooner whey is processed, a higher quality and yield of the finished product is achieved (Spreer, 1998; Kosikowski and Mistry, 1997). Typically, acid whey is not widely used in industry because it is difficult to dry and has an objectionable flavor (Kosikowski and Mistry, 1997; Gallardo-Escamilla et al., 2005). Sweet whey is the primary whey source processed and dried.

Whey products

Further manufacturing processes have led to the production of various forms of whey including whey powder, reduced lactose whey, demineralized whey, whey protein concentrates, and whey protein isolate. Fractionation and separation can also further

process whey to concentrate specific glycoproteins such as lactoferrin and lactoperoxidase (Henning et al., 2006).

Whey powder is dried fresh whey containing all the constituents of fresh whey except water. Whey powder has a maximum moisture content of 5% and a minimum lactose content of 70%. Reduced lactose whey is a type of whey powder where at least 40% of the lactose has been removed by enzymatic hydrolysis or physical separation techniques such as precipitation or filtration. Demineralized whey is another type of dried whey where a portion of the minerals or ash content have been removed from pasteurized whey for a maximum of 2.5% mineral content and a maximum of 6% moisture in the final product (USDEC, 2006). Whey protein concentrates (WPC) are produced by removing non-protein constituents, such as lipids, minerals and lactose, from pasteurized whey for a dry product containing 34-80% of protein. For example, WPC34 may not contain less than 34% protein in its finished dry product. Whey protein isolate (WPI) is a dry whey product with no less than 90% protein (USDEC, 2006; Spreer, 1998). To manufacture these products, further processing and advanced technologies are used to take fluid whey to a dried finished product.

Processing whey

Whey can be processed into food ingredients by simple drying or further by removing lipids, minerals, and lactose (Foegeding, 2002). Clarification is the first step in

processing whey. In clarification, all remaining casein or curd particles in the fluid whey are separated out by means of a vibratory sieve or centrifuge (Spreer, 1998). A pretreatment such as fat separation may be performed to remove residual lipids, which could cause oxidation and off flavor development in storage, and/or to remove phospholipoproteins and colloidal calcium phosphate for improved ultrafiltration membrane permeation flux rate and the functionality of the end dry product. Whey is then pasteurized between 71-74^{o}C for at least 15 seconds to prevent starter culture bacteria from growing (Spreer, 1998).

Pressure driven membrane processes have been used to concentrate and separate whey proteins in order to maintain their functional properties and make them suitable for other purposes in the food industry (Suarez et al., 1992). Reverse osmosis, microfiltration, ultrafiltration, diafiltration, and nanofiltration are all separation techniques that utilize a type of membrane with various sized pores for separation based on molecular size and/or chemical composition (Henning et al., 2006; Fenton-May and Hill, 1971). Figure 1.1 below illustrates the different separations of some of the membrane filtration techniques used in the whey industry.

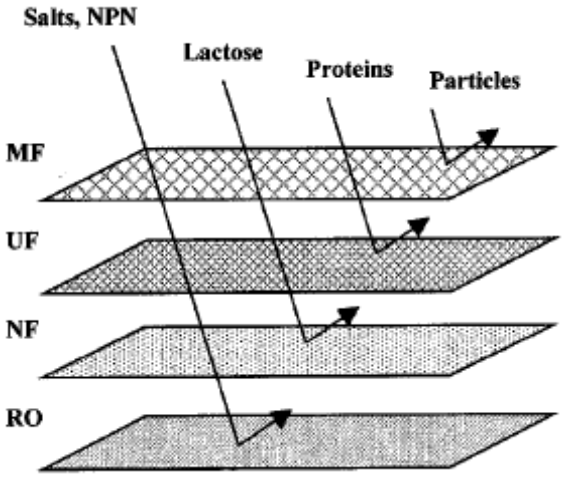

Figure 1.1 -- Illustration of filtration membranes: microfiltration (MF), ultratfiltration (UF), nanofiltration (NF), reverse osmosis (RO) (Saboya and Maubois, 2000)

These membrane separation techniques have the ability to separate specific components from liquid foods into permeate and retentate by means of a semi-permeable membrane. Permeate is the mixture of the liquid, usually containing water, which is able to pass through the membrane. The retentate is then the remaining mixture that does not pass through the membrane and is usually the more valuable product (Kosikowsi and Mistry, 1997).

Reverse osmosis (RO) is a concentration technique that has been used to simply preconcentrate liquid whey before spray drying, for the means of cutting transportation costs, and/or to increase the productive capacity of evaporative equipment (Fenton-May et al., 1971; Suarez et al., 1992). This process utilizes pressure to concentrate the liquid

whey by forcing the water of the liquid whey through a semi-permeable membrane leaving behind concentrated whey (Suarez et al., 1992). Ultrafiltration (UF) is a pressure driven membrane process that not only concentrates whey but also uses a semi-permeable membrane to separate high molecular weight solutes such as whey proteins, fat, and insoluble salts from lower molecular weight solutes such as lactose, water, and soluble minerals. The permeability of the membranes can be controlled during manufacture to specify retaining certain molecular weights (Fenton-May and Hill, 1971; Suarez et al., 1992; Kosikowsi and Mistry, 1997). Today, UF is typically used for fractioning whey components in order to manufacture high protein WPC. After just UF, and subsequent evaporation and spray drying, a whey protein concentrate of approximately 60% protein by weight is produced (Zydney, 1998). Following UF, diafiltration (DF) may be integrated with UF to further reduce lactose, calcium, and water soluble vitamin levels from the retentate. DF reduces these amounts by continuously adding deionized water to the separated retentate as lactose and minerals are simultaneously removed in the filtrate (Morr and Ha, 1993; Zydney, 1998). Kosikowski and Mistry (1997) further explained that DF is merely the act of replacing a certain amount, usually about 50 percent, of permeate removed with clean, salted or acidified water. The process essentially washes the retentate and further purifies and/or concentrates it. After the combined methods of UF and DF, a retentate with approximately 85% protein can be achieved (Zydney, 1998). Microfiltration (MF) separates particles by a membrane based on pore diameters ranging

from 10 µm to 0.05 µm. MF is used for removal of bacteria from milk, defatting whey, the selective separation of somatic cells from whey protein, and removal of serum proteins from fluid milk (Saboya and Maubois, 2000; Kosikowski and Mistry, 1997). The removal of microorganisms by MF provides an alternative for high temperature pasteurization of whey (Maubois and Ollivier, 1997). MF has also been used as an alternative for removing phospholipoproteins in processing whey before beginning UF/DF treatments. The removal of these phospholipoproteins reduces the problem of membrane fouling found on UF membranes which adhere to the membrane and surfaces, decreasing UF permeation flux rates (Morr and Ha, 1993). Microfiltration is required to produce a whey protein concentration of 90% or more (Henning et al., 2006). Nanofiltration utilizes membranes to remove particles with a molecular mass between 300-1000 Daltons. Nanofiltration (NF) has been used to further concentrate UF whey permeate by separating lactose out due to the lower molecular weight of lactose compared to the proteins (Atra et al., 2005). NF has also been utilized to remove salt from whey individually and in combination with MF to remove salt and fat from fluid whey. This separation technique is also an alternative to electrodialysis for demineralizing whey to 32 percent demineralization. Once beyond 32 percent, the combination of nanofiltration and electrodialysis would then be needed (Kosikowski and Mistry, 1997). Nanofiltration is advantageous for demineralization because this method will demineralize and concentrate whey simultaneously (Kosikowski and Mistry, 1997; van der Horst et al., 1995).

Ion exchange adsorption process technology, also known as ion exchange membrane chromatography, has been used as an alternative to MF to produce WPI with ≥ 90% protein and to purify specific proteins from whey such as β –lactoglobulin and α-lactalbumin. The ion exchange process absorbs proteins by protonating the protein molecules in the whey stream by changing the pH of the solution. Then, as the charged particles are passed through the oppositely charged chromatographic membrane (the ion exchanger), these particles will be attracted to the membrane while the rest of the solution continues through. The absorbed proteins are then desorbed by changing the pH again and concentrated once more to produce a product with ≥90% protein or a purified protein (Morr and Ha, 1993; Bhattacharjee et al., 2006). Protein selectivity in ion exchange can be manipulated based on the isoelectric point of the solute to be purified, the type of buffer used, and the temperature (Bhattacharjee et al., 2006; Zydney, 1998).

Over the years, researchers have put together combinations of microfiltration, ultrafiltration, diafiltration, ion exchange, affinity chromatography, nanofiltration, and electrodialysis in order to enhance the functionality of whey and/or fractionate whey protein (Morr and Ha, 1993). Whey protein is best separated and concentrated by means of a combination of these techniques because each technique specializes in a specific separation to control the protein, lactose, and fat ratio in the end product. Finally, the concentrated whey protein is spray dried. The concentrated liquid is transformed into

dried powder by spraying it into a controlled flow of hot air within the drying chamber where liquid from the particles is evaporated (spray-drying) (Henning et al., 2006).

Functions of whey

According to the USDEC (2006), for every ten units of full fat milk used in making cheese, roughly one unit of cheese was produced with nine units of whey left over (10% yield of cheese). The United States alone manufactured approximately 935,000 metric tons of whey in 2006 (USDEC, 2006). Before whey protein functional properties and health benefits became known, cheesemakers had a difficult time disposing whey due to whey having a biological oxygen demand (BOD) value of 32,000 ppm or higher (Kosikowski and Mistry, 1997). Today, whey has gone from being a waste product or animal feed to supplementing baby formula and being a versatile ingredient in the food industry.

Whey has been used for centuries in animal feed for its high value in protein, lactose, calcium, phosphorus, sulfur and water soluble vitamins. Animals including pigs, cattle, poultry, horses, and even dogs can benefit from whey protein as a supplement or a substitute to their normal feed (Sienkiewicz and Riedel, 1990). Nutritionally, whey protein is beneficial for humans for its abundance of minerals, amino acids, and proteins. Demineralized whey has shown great value for creating an infant formula with bovine milk to resemble human milk with the optimal nutritional composition. Bovine milk has

much higher casein and mineral contents in comparison to human milk and thus infant formula is supplemented with demineralized whey or WPC for predominantly whey protein based formulas (de Wit, 1998).

Recent research has revealed specific components of whey that positively impact coronary and colon health. Specifically, peptides derived from whey have been identified to have opiod-like and angiotensin-I-converting enzyme (ACE) inhibiting activity, which may exert an antihypertensive effect and decrease the chance of cardiovascular disease. Opiod activity and ACE inhibiting activity both assist in the regulation of blood pressure. Mullally et al. (1997) found tryptic hydrolysates of β-lactoglobulin, α-lactalbumin, and WPC individually had the ability to inhibit ACE activity and thus possibly promote an antihypertensive effect. Although the ACE inhibitory potencies are lower than commercial drugs used for prevention and treatment of hypertension, it is expected that whey protein-derived ACE inhibitory peptides would have no undesirable side effects (Mullally et al., 1997). A rat model experiment by McIntosh et al. (1998) compared a whey protein diet with a red meat and animal fat diet to find if dietary whey protein would decrease the development of tumors in the colon. Results from this research found an influence of whey protein to have a protective effect against the development of colon cancer in both young and mature rats suggesting the same in human health (McIntosh et al., 1998).

Whey has become very popular in fortified nutritional beverages where even acid whey may be used in conjunction with fruit juices, especially for beverages targeted for diet or athletic protein weight gain (Kosikowski, 1979; Sienkiewicz and Riedel, 1990, Dahm, 2005). Deproteinized cottage cheese whey has been used in combination with grapefruit juice to produce a cheaper grapefruit juice for consumers with additional nutrients. Further, the cottage cheese whey may help reduce the acidity and astringency of grapefruit juice by itself (Branger, et al., 1999). Hydrolyzed and unhydrolyzed mozzarella whey permeate has also been studied as an ingredient in functional beverages. Functional beverages offer some type of health benefit, and is a rapidly growing sector of the beverage market (Beucler et al., 2005). Beucler et al. (2005) has studied consumer acceptance of different whey permeate percentages in sports beverages and found those with lower levels (25% and 50%) of hydrolyzed and unhydrolyzed whey permeate were comparable in visual and flavor properties to commercial products. The whey permeate beverages were also higher in electrolyte (Na, K, Zn, Mg, P) content compared with commercial beverages. Electrolytes are used to deliver rehydration to the human body in sports drinks (Beucler et al., 2005). Whey products such as milk calcium, which is concentrated whey calcium, phosphorus, magnesium and zinc, and WPC35 provide good alternatives for calcium intake (de Wit 1998).

Whey products are used widely by dairies, bakeries, confectionaries, meat processing plants, canned goods and beverage establishments for their various functions in

food quality and stability (Kosikowski, 1979; Morr and Ha, 1993; Foegeding et al., 2002; de Wit, 1998). In the dairy industry, WPC is used as a cheaper alternative to skim milk powder in dairy products like yogurt and ice cream (Henning et al., 2006). Whey butter can be made from separated whey cream (or whey fat), and cheese-like products are also produced from whey (Sienkiewicz and Riedel, 1990). Acid whey is used in dairy products where a tangy flavor is sought, such as in cheese powders and sauces and sherbets (Kosikowski, 1979). In the bakery industry, acid whey is used in bread, biscuits, and crackers for the gold surface color it provides (Kosikowski, 1979). Hydrolyzed whey permeate syrups have been noted as potential replacements for sucrose in white pan breads. Ogunrinola et al. (1988) found varying amounts of demineralized and undemineralized hydrolyzed whey permeate syrups had little significant difference in bread quality compared to the control breads evaluated by a sensory panel. WPC, defatted and normal, can be used for egg substitution to induce and stabilize foams in aerated food products such as meringues and Madeira-type cakes (de Wit, 1998). Partial or full replacement of water by liquid whey can produce better dough kneading, yeast fermentation, greater porosity, and improvement on crust (Sienkiewicz and Riedel, 1990).

Whey protein hydrolysates (WPH) are another byproduct derived from whey. WPH is WPI that has been broken down into peptides of different sizes and amino acids by means of an enzyme, acids, or alkali. Enzymatic hydrolysis allows the use of milder conditions of pH 6-8 and temperatures around 40-60^{o}C (Sinha et al., 2007). These milder

processing conditions of WPH allow the retention of more nutritional quality and may lead to the development of new products. Interfacial films used to stabilize emulsions and foams have already been designed from whey protein hydrolysates (Foegeding et al., 2002). In confections, condensed whey and whey powder can partially replace evaporated milk and milk powders used to produce caramels, chocolate-flavor coatings, fondants and candy bars. Demineralized whey powder and WPC are also used in the production of butterscotch, caramels, and chocolate (Sienkiewicz and Riedel, 1990). In restructuring new meat products, WPC proteins have been utilized for their abilities to exhibit heat-coagulating and heat gelling properties (Morr and Ha, 1993). Cold-set gelation of whey proteins has also been noted by Bryant and McClements (1998) with the utilization of heat denatured whey proteins and the addition of a salt, such as calcium chloride. Cold-setting ingredients may replace biopolymers which are currently used as thickening or gelling agents in foods such as gelatin in comminuted meat and fish products, or polysaccharides in desserts, sauces and dips (Bryant and McClements, 1998). Hydrolyzed β-lactoglobulin also has the ability to form networks associated with gels like cheese and meat and edible films (Foegeding et al., 2002). Whey protein also has emulsifying properties allowing fat globules to be structural elements in heat induced whey protein gels. These emulsifications have led to the development of new cheese, meat and confectionary products (de Wit, 1998). Canned goods such as canned beans and plums may also utilize whey permeate in substitution for brine and syrup respectively. Hydrolyzed lactose whey

permeate substitution for sucrose in canned fruit is promising (Chandan et al., 1982). In the beverage industry, fermented whey has been used to produce whey champagne, whey beer, and whey wine (Sienkiewicz and Riedel, 1990). Deproteinized acid whey permeate has been used to produce wines with acceptable taste. Low alcohol, effervescent wines, and fortified wines may also be produced with alterations to lactose and sucrose addition (Kosikowski and Wzorek, 1977).

Whey flavor

Flavor is generally recognized as the single most important factor affecting consumer acceptance of food products (Morr and Ha, 1991; Lee and Morr, 1994; Drake, 2006). Although whey is defined as having a bland, delicate flavor free from undesirable flavors, the reality is that flavor and flavor variability of whey have limited its widespread usage in bland and delicately flavored foods (Carunchia-Whetstine et al., 2003; Carunchia-Whetstine et al., 2005; Morr and Ha, 1993; Drake, 2006). Whey may have outstanding functional and nutritional benefits but off-flavors such as cardboard, brothy, diacetyl, sourness and bitterness can prevent use in bland products (Quach et al, 1999; Carunchia Whetstine et al., 2005; Drake, 2006). To identify flavors in whey and other dried dairy ingredients, descriptive sensory analysis and instrumental analysis are performed.

Descriptive sensory and instrumental analysis

Descriptive sensory analysis is an analytical sensory technique that utilizes a group of trained individuals. A vocabulary describing all the flavors in a product is generated and then evaluated by the group of trained individuals. Both attributes and attribute intensities are documented. As panels are trained under the same vocabulary and intensity scale, the panel acts as a uniform instrument (Drake and Civille, 2003). Drake and Civille (2003) recommended that this type of analysis utilize at least seven panelists with several hours of initial training and continued maintenance training. Flavor lexicons are sets of words for describing the sensory properties of a particular product or commodity. The lexicon is then applied or practiced using descriptive sensory analysis techniques. Lexicons are very useful for providing a source of references and definitions for clarification. Plus, communication between other panels and the correlation of descriptive analysis results to instrumental or consumer data can be achieved (Drake and Civille, 2003).

Grosch (1993) suggested that all key flavor compounds could not be identified solely by descriptive analysis and suggested that important odorants could be first located by chromatography. Two types of instrumental analysis were recommended for characterizing flavor; gas chromatograph-olfactometry (GC-O) and gas chromatography-mass spectrometry (GC-MS). Gas chromatography is an analytical instrument that separates volatile compounds in gaseous mixtures. The volatile mixture is injected into an

inert gas stream and carried through a column packed with a solid inert support coated in a resolving liquid phase. The different affinities of the mixture components for the liquid phase on the column result in separation of the components which then flow through a detector cell in order of their separation (McMaster and McMaster, 1998). Several types of detectors can be used to characterize and identify the separated compounds. GC-O is the human sniffing of gas chromatography effluent. In GC-O, after chromatographic separation occurs, the injected sample is split as it exits the column so a portion of it goes to a physical detector, such as a flame ionization detector (FID), where the signal is recorded on a chromatograph and the rest goes to a "sniffing port" where human subjects sniff the effluent and characterize those compounds which display aroma activity (McMaster and McMaster, 1998). GC-O was invented as chemists became interested in determining which volatile components in a given mixture had an odor (Mayol and Acree, 2001). Although the chromatogram of a mixture in question may produce many separated compounds, not all of the compounds will have an odor or play a role in flavor.

Over the years, four different GC-O categories have been established for the collection and processing of GC-O data to estimate the sensory contribution of single odor active compounds (van Ruth, 2001). Charm and aroma extraction dilution analysis (AEDA) are dilution methods which screen the potent, medium and lower volatile odorants of a specific odorant (Grosch, 2001). Charm and AEDA are based on a stepwise dilution to threshold in combination with sensory response measurement (van Ruth,

2001). Detection frequency methods, such as NIF/SNIF (Nasal Impact Frequency/Surface of Nasal Impact Frequency), require a group of assessors to detect specific odors. The frequency of detection is then used to estimate the odor's intensity. Posterior intensity methods record odor descriptions and odor intensities on a scale after a peak has eluted from the column (van Ruth, 2001). Time intensity methods, such as OSME, are based on magnitude estimation of the odor intensity where the subject rates the intensity of the compound odor by using a time-intensity device to produce an odor peak while simultaneously recording verbal descriptions of the peak (Miranda-Lopez et al, 1992).

In 1963, Rothe and Thomas introduced the Odor Activity Value (OAV) which defined the relationship between concentration of an odorant and detection by human threshold. OAV indicates the potency of a specific odorant in a specific sample and the maximum an odorant can contribute to that mixture (Mayol and Acree, 2001). OAVs allow one to rank individual odorants according to their odor contribution based on sensory thresholds and concentration in the food matrix (Schieberle and Steinhaus, 2001; Mayol and Acree, 2001). The odorants detected by GC-O depend on the type of food, amount of food sampled, the extraction technique used, the dilution of the extracted volatiles, and the sample size (Grosch, 1993).

Gas chromatography alone is unable to provide conclusive identification of the detected compounds as they are separated. Many compounds can potentially have the same retention time. In combination with a mass spectrometer (GC-MS), GC is capable

of separating mixtures into their individual components with the ability to identify and then provide quantitative and qualitative information on the amounts and chemical structure of each compound (McMaster and McMaster, 1998). In the mass spectrometer, separated components are first ionized in a high vacuum. The resulting ions and their fragmentation products are then propelled and focused through a magnetic mass analyzer which is then collected and measured in a detector. The fragmentation pattern of a compound is characteristic, similar to the uniqueness of fingerprints, and can be used for conclusive identification of a compound when compared to a chemical reference and/or a chemical reference database (McMaster and McMaster, 1998).

Sources of whey flavor

Ideally, whey and dried whey ingredients should have a bland, delicate flavor free from undesirable flavors (Lee et al., 1995; Morr and Ha, 1990; Tomaino et al., 2004; Drake, 2006). Many studies have focused on dried whey products which initially exhibited a bland flavor immediately after drying, but then displayed off flavors during storage (Morr and Ha, 1993). Some reactions possibly responsible for these off flavors produced during storage include lipid oxidation and Maillard browning (Feretti and Flanagan, 1971; Tomaino et al., 2001; 2004). Flavor variability may be inherent in the whey itself or may be an outcome of downstream processing techniques (Carunchia-Whetstine et al., 2003; 2005). Carunchia-Whetstine et al. (2005) found tremendous

differences in proximate analysis and volatile compounds from various sources of WPC80 and WPI. These variations and off flavors may result naturally from the whey but also from variability in processing techniques and storage (Drake, 2006; Carunchia Whetstine et al., 2005; Karagul-Yuceer et al.,2003).

Lipid oxidation

Lipid oxidation is one of the major causes of deterioration in food. Oxidation reactions during processing and storage lead to the development of off-flavors and off-odors, which can decrease acceptability and also decrease the nutritional quality of foods. Some oxidation products are potentially also toxic (Nawar, 1996; Frankel, 1980; Wong 2003).

Oxidative rancidity is a result of several chemical reactions involving atmospheric oxygen and lipid materials. The reaction causing oxidative rancidity is known as autoxidation (Nawar, 1996; Frankel, 1980; Belitz et al., 1999). Autoxidation is a free radical chain reaction where oxygen reacts with organic substrates to yield hydroperoxides and other oxygenated compounds. This reaction occurs in three steps: initiation, propagation, and termination (Frankel, 1980). The rate at which autoxidation proceeds is affected by the fatty acid composition, degree of unsaturation, the presence of pro- and antioxidants, storage conditions, and the exposure of oxygen to the lipids (Belitz et al., 1999). In order to begin initiation, production of the first few radicals must be catalyzed

by a form of thermodynamic activation energy from hydroperoxide decomposition, metal catalysis, or exposure to light (Nawar, 1996; Frankel, 1980). Propagation reactions continue as hydrogen atoms at positions around unsaturated fatty acid double bonds are abstracted to produce free radical species (Nawar, 1996). Unsaturated fatty acids require less energy for hydrogen abstraction around the fatty acid double bond. This explains the selectivity radicals take to specifically attack unsaturated fatty acids at room temperature while saturated fats remain stable (Belitz et al., 1999). These positions of hydrogen abstraction are then replaced with an oxygen molecule to form peroxy radicals which in turn will abstract hydrogens from other molelcules to produce hydroperoxides. This sequence is repeated until termination where free radicals react and begin to yield nonradical products (Nawar, 1996). Most of the hydroperoxides formed are derivatives from oleic, linoleic, and linolenic acids, the most common long unsaturated fatty acids associated with lipid oxidation (Nawar, 1996; Frankel, 1980; Belitz et al., 1999). These hydroperoxide derivatives are unstable, odorless and tasteless and will further decompose in specific steps to secondary products (Belitz et al., 1999). It is these secondary oxidation products including acids, alcohols, aldehydes, carbonyls, and ketones that can affect flavor, taste, nutritional activity and overall quality of the food product (Wong, 2003).

Secondary oxidation products are also known as volatile lipid oxidation products (VLOP), volatiles not normally present in fresh products (Tomaino et al., 2004). Tomaino

et al. (2004) identified 1-propanol, hexanal, nonanal, and 2-nonanone as VLOPs in pasteurized liquid whey and made the assumption that oxidation had occurred sometime during cheese making and whey pasteurization. This assumption was confirmed when a large increase in aldehydes, especially hexanal, methyl ketones, and pentane, was observed when fluid whey was stored fourteen days in refrigerated storage at 4^{o}C. These findings suggested that all liquid or dry whey ingredients are susceptible to oxidation during storage (Tomaino et al., 2004).

Cardboard flavor in dairy products is a common whey off flavor associated with lipid oxidation products. Actual cardboard soaked in warm water was analyzed to find the principal volatile compounds, which were nonanal followed by hexanal, octanal, and heptanal (Tomaino et al., 2004). Cardboard aroma, flavor, and aftertaste in liquid whey significantly increased during storage over a three week period with significant correlation between the increase of cardboard and hexanal with other VLOP concentrations (Tomaino et al., 2004). Hexanal also increased the most in WPC75 when placed in accelerated storage conditions, 60^{o}C in darkness with a water activity from 0.11-0.79, for six days from 11 ng hexanal/g WPC to 1430 ng hexanal/g WPC (Lee et al., 1996). Additionally, Quach et al. (1999) reported hexanal as the most abundant aldehyde in the headspace of lactic WPC80 when evaluated by headspace solid-phase microextraction (HS-SPME). Both dried whey WPC80 and WPI reconstituted at 10% solids with deodorized, distilled water and liquid whey also exhibited cardboard flavors, most likely due to lipid oxidation

(Russell et al., 2006; Karagul-Yuceer et al., 2003). Hexanal, heptanal, octanal, and nonanal are common lipid oxidation aldehyde products (Morr and Ha, 1991). Increases in these aldehydes strongly support an oxidative mechanism of the decomposition of linoleic and oleic acids (Tomaino et al., 2001).

Light-induced degradation of lipids, proteins, and vitamins can also cause formation of both off flavors and color changes. Light induced protein oxidation in dairy products proceeds very rapidly and is detected after only a short exposure to light. These changes could then lead to loss in nutritional value, product quality and marketability (Mortensen, 2004).

Whey variability

Several recent studies have demonstrated flavor variability within and between fluid whey and whey products collected from different processors (Drake et al., 2003; Russell et al., 2006; Carunchia-Whetstine et al., 2005; Drake, 2006, Karagul-Yuceer et al., 2003). During processing, flavor differences may result from the quality of the milk used for the initial cheese production, the type of cheese manufactured, the method of whey handling after curd separation, and the elapsed time between draining and pasteurization (Bodyfelt et al., 1988). Flavor of fluid whey from different facilities varies, but flavor of whey or whey products within a facility also varies. This variation may come from whey that is typically pooled together from a variety of cheeses before being processed into

WPC and WPI. Starter cultures may also have an impact on fluid whey flavor and subsequent whey products.

Cheese production facilities rotate cheese starter cultures to prevent bacteriophage growth. The type of starter culture applied has an impact on the level of free fatty acids and other volatile compounds in the fluid whey (Tomaino et al., 2001; Karagul-Yuceer et al., 2003, Carunchia-Whetstine et al., 2003). Differences between plants may be caused by milk source, handling, processing and starter culture. Within plant differences may be connected to starter culture rotation, since the milk source and processing should be consistent within the same plant. Gallardo Escamilla et al. (2005) compared fluid whey from a variety of cheeses and starter cultures. The results of this study suggested that starter cultures influenced the flavor of whey to different degrees and roughly reproduced a flavor relative to the original cheese variety. Sweet whey from Cheddar and Gouda exhibited a "cheesy" aroma, and lactic acid casein with no starter culture exhibited a yogurt flavor, while Paneer whey, medium acid whey from a soft heat- and acid-coagulated Indian cheese, also used no starter and lacked any characteristic flavor (Gallardo Escamilla et al., 2005). Research has also shown that WPI may display lower flavor intensities than WPC80 possibly due to lower fat and carbohydrate content and thus fewer sources for volatile flavor components (Russell et al., 2006; Carunchia-Whetstine et al., 2005). In comparing sweet whey powder with previous work on liquid whey

(Carunchia-Whetstine et al., 2003), Sithole et al. (2006) suggested that evaporation and drying of whey did not introduce new flavors but intensified existing flavors.

Milky and sour notes have also been reported in whey products and may be attributed to the production of fermentation products, such as acetaldehyde, ethanol and diacetyl by the starter culture during cheese manufacture (Tomaino et al., 2004; Karagul-Yuceer et al., 2003). Degradation of sulfur containing amino acids can produce off flavors in whey and whey ingredient applications. Wright et al. (2006) identified two sulfur compounds by GC-O and sensory analysis, dimethyl disulfide (DMDS) and dimethyl trisulfide (DMTS), as possible compounds responsible for the cabbage off-flavor of WPI. With the combination of GC-O, GC-MS, sensory analysis, sensory threshold analysis, and model system analysis, dimethyl trisulfide (DTMS) was confirmed as the responsible compound for the WPI cabbage off-flavor. Wright et al. (2006) concluded that DMTS was most likely present in some amount in all WPI but was an off-flavor when levels were above threshold. Benzaldehyde may be responsible for bitter taste in WPC (Quach et al., 1999). Musty/earthy flavor has also been noted in liquid whey and was attributed to pyrazines and an unknown compound (Karagul-Yuceer et al., 2003).

Whey color

Ideally, processed whey should be free from any color for uniformity. Whey manufacturers have encountered problems for whey utilization due to color variability in

whey after processing and during storage. Color is an important characteristic and selection criteria for consumers when making a food choice (Hallagan et al., 1995). Color found in whey may be the result from three general areas: naturally occurring xanthophylls, Maillard reaction products, and annatto addition (Smith, 2004). Discoloration of whey accounts for lower value and is deemed unacceptable by consumers who demand colorless whey products, especially where finished product color is important (Hammond et al., 1975). A premium is paid by the baking industry just for whey powder which is free from color (Chapman et al., 1980).

Xanthophylls are a type of carotenoid naturally found in fruits and vegetables and range in color from yellow to orange, red and brown. Xanthophylls can enter milk through the forage eaten by cows as β-carotene. In the mammary gland, if the β-carotene is not completely converted to vitamin A, a yellow color in the milk is produced, possibly leading to discoloration in whey. Hydrogen peroxide and benzoyl peroxide both have the capability to bleach xanthophyll discoloration in whey but federal regulation prohibits the use of hydrogen peroxide to bleach xanthophylls (Smith, 2004). When heated, Maillard reaction products may be produced and impart tan to dark brown colors in whey and whey products (Smith, 2004).

Annatto is the most commonly used colorant of choice for manufacturers when coloring cheese. In the US, Cheddar cheese commonly has added annatto to provide a characteristic orange color. Whey from cheeses colored with annatto tends to retain some

of this color even after processing (Chang et al., 1977). Annatto is obtained through the extraction of the pigment from the seed of the shrub *bixa orelana*, a tropical plant found in South America, India, East Africa, the Phillipines, and the Carribean (Lauro, 1991; Francis, 2000; Hallagan et al., 1995). The major pigments comprising annatto are bixin and norbixin. Bixin is the major naturally occurring pigment form that comprises 80% of the pigment, and norbixin is the saponified form of bixin. Norbixin is known for its precipitation in the presence of calcium and its fast acting ability to color products while avoiding bleeding into the surrounding medium. Both bixin and norbixin are naturally found in the *cis* form which gives off a redder hue. The *cis* form can be transformed into the more stable *trans* form by means of adding heat to form a more yellow color, thus giving flexibility of a variety of colors from annatto (Lauro, 1991). The figure below illustrates the chemical structures of bixin, *trans* bixin, and norbixin.

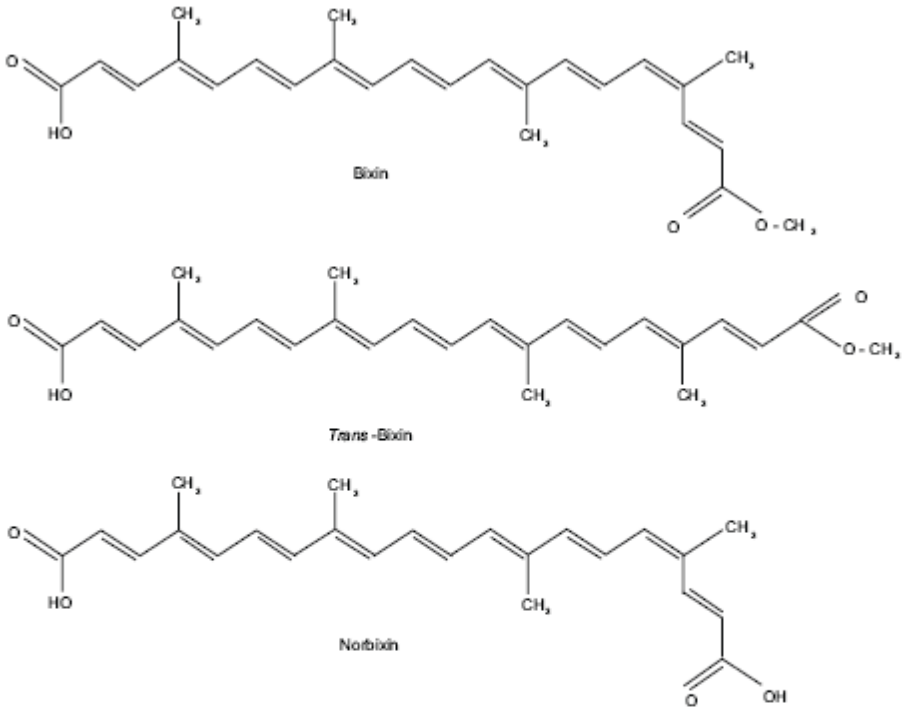

Figure 1.2 Structure of bixin and norbixin (Smith, 2004)

Annatto is able to form even more stable complexes when the carboxyl group of norbixin attaches with divalent metal ions and another carboxyl group. The attachment to other ions and another carboxyl group give additional protection from oxidation and maintain color (Smith, 2004).

Annatto is unstable in increased light and oxygen exposure, high temperatures over 125^{o}C, and in the presence of metal ions (Barnicoat, 1937; Francis, 2000; Smith, 2004). Annatto will exhibit color loss under great light intensity and oxygen availability which both catalyze oxidation of annatto (Smith, 2004). The addition of antioxidants like

ascorbyl palmitate, tocopherols, and polyphenols help protect annatto from color loss by light (Francis, 2000). At low pHs, annatto tends to turn from yellow-orange to pink from isomerization of the pigments (Francis, 2000). The pink color can remain even after bleaching and has been observed when whey powders were used in other products like ice cream (Smith, 2004). Overall, the color of annatto will depend heavily on the plant, growing area, climate, manufacturing and storage conditions (Lauro, 1991; Roundy, 1958).

Annatto is believed to bind with whey proteins, in which norbixin may have some affinity for specific whey components (Hammond et al., 1975). Research has demonstrated that when milk proteins are present, annatto proves to be much more resistant to bleaching, requiring more time or higher bleach concentrations (Chang et al., 1977). Also, once whey is dried, annatto becomes even more highly resistant to bleaching. This resistance may also be the result of annatto's affinity to whey protein and once dried the reaction cannot be reversed to free annatto for bleaching (Smith, 2004).

Annatto has historically been derived from plant material and thus considered exempt from certification by the Food and Drug Administration under authority granted by the Federal Food, Drug, and Cosmetic Act of 1938, and the 1960 Color Additive Amendment. According to the Code of Regulations, Title 21, Part 73, Section 30, annatto extract may be safely used for coloring foods, generally in amounts consistent with good manufacturing practices (USDA, 2007). In 1982, the Joint Food and Agriculture

Organization (FAO)/World Health Organization (WHO) Expert Committee on Food Additives (JECFA) established an average daily intake (ADI) of 0.065 mg/kg body weight of bixin (JECFA, 1982). Normal addition rate of annatto colorants with 3% norbixin concentration to cheese is about 42 mL/1000 pounds of milk with a range from 3 mL/1000 pounds to 75 mL/1000 pounds.

Bleaching

Approximately 15-20% of the annatto used to color cheesemilk is retained in the drained liquid whey (Chang, et al., 1977; Smith, 2004; El-Samragy, 2004). It is desirable to bleach whey in order to maintain the same uniform color in the final dried product (Kuramoto and Jezeski, 1954; Chang et al., 1977). Federal food and regulations have permitted the use of benzoyl peroxide and hydrogen peroxide for bleaching whey for colors that have been added to cheese (Smith, 2004). Colored whey is often either bleached or blended with uncolored whey to give an acceptable dry product (Hammond et al., 1975).

Hydrogen peroxide is a colorless liquid used in foods for its bleaching and antimicrobial ability (Cook, 1962). In the United States, hydrogen peroxide is covered by 21CFR 184.1366 and may be used at a rate of <500 ppm and is considered effective at all temperatures and total solids levels. Conditions set by the government also state that hydrogen peroxide residuals must be removed by physical or chemical means during

processing. The addition of catalase is required to help denature residual hydrogen peroxide and cannot exceed 20 ppm catalase based on milk used. Hydrogen peroxide is permitted as a preservative only during electrodialysis where a maximum of 400 ppm may only be used. Finally, hydrogen peroxide must also meet the Food Chemicals Codes (Smith, 2004). The Food Chemicals Codex (FCC) is a collection of internationally recognized scientific standards for the purity and identity of food grade substances (USP, 2008).

Hydrogen peroxide is unique in that it is effective over a wide range of temperatures and solids levels to bleach color while destroying organisms and can then be completely broken down into just water and oxygen (Cooney and Morr, 1972; Roundy 1958; Cook, 1962). Its use has been suggested as a preservative and alternative antimicrobial agent in milk systems, instead of heating processes which greatly denature beneficial proteins (Cook, 1962; Cooney and Morr, 1972).

Although not many enzymes exist in whey, catalase and peroxidase that naturally exist in whey can break down hydrogen peroxide. This denaturation is not beneficial when hydrogen peroxide is used as a preservative but could be beneficial if the hydrogen peroxide is needed to decompose rapidly (Cook, 1962). A study researching hydrogen peroxide in milk containing catalase and peroxidase found a significant increase of hydrogen peroxide decomposition stored at 38^{o}C compared to 57^{o}C. The researcher

believed that at 38^{o}C, catalase and peroxidase were still active while the two enzymes were denatured at 57^{o}C allowing stable hydrogen peroxide concentrations (Roundy, 1958). Amin and Olson (1967) compared raw and pasteurized milk with hydrogen peroxide addition and their results agreed with previous studies. At 54.4 and 57.2^{o}C, the same amount of hydrogen peroxide decomposition occurred in sterilized and raw milk (Amin and Olson, 1967). Because of this problem, many studies have researched and suggested preheating the liquid whey to reduce enzymes before introducing hydrogen peroxide (Cook, 1962). Since fluid whey is subjected to pasteurization or thermalization prior to processing, catalase and peroxidase should not be a concern for hydrogen peroxide use (Smith, 2004).

Hydrogen peroxide used even at low levels causes the denaturation of major and minor whey proteins. A study found proteose peptone was the most susceptible to hydrogen peroxide, then immunogloblulins, bovine serum albumin, B-lactoglobulin, and the least susceptible was alpha lactalbumin (Cooney and Morr, 1972). The addition of heat increased protein denaturation. The minimal whey protein denaturation and aggregation conditions identified were a hydrogen peroxide concentration maintained below 5000 ppm and below 25^{o}C, an amount admittedly higher than the allowed limit of 500 ppm (Cooney and Moor, 1972).

The germicidal affects and limitations of hydrogen peroxide addition in whey have been studied (Cook, 1962; Roundy, 1958; Cooney and Morr, 1971; Amin and Olson,

1967). The amount of peroxide needed depended on the bacteriological quality of the milk, the temperature of the milk at the time the peroxide is added, and the duration of treatment (Roundy, 1958). The bactericidal effectiveness then depended on amount of treatment added and the concentration maintained during treatment (Amin and Olson, 1967). As a sanitizer, hydrogen peroxide was selective on eliminating bacteria. Aerobic spore forming organisms were fairly resistant to the antimicrobial while coliforms were the least resistant (Roundy, 1958). Typical heat treatment caused the destruction of harmful and beneficial organisms. A specific peroxide-catalase treatment can help by selectively destroying undesirable organisms while keeping desirable organisms alive (Roundy, 1958). Due to hydrogen peroxide decomposition by catalase and peroxidase, higher temperatures around 58^{o}C exhibited the best temperature to eliminate pathogens (Amin and Olson, 1967; Roundy, 1958).

Some disadvantages exist when using hydrogen peroxide. Two principle disadvantages to using hydrogen peroxide are the need to add catalase to inactivate residual hydrogen peroxide and the corrosiveness of hydrogen peroxide on equipment. Other issues include the long hold times required and the potential to cause oxidized flavors especially in higher moisture cheeses (Roundy, 1958; Cook, 1962). Hydrogen peroxide also becomes unstable at higher temperatures (>20^{o}C), when in contact with metals such as copper and iron, and certain pH values (> 7.0) (Cook, 1962; Amin and Olson, 1967).

Benzoyl peroxide is a colorless, crystalline solid permitted in the United States under 21CFR 184.1157 for removing color in whey for naturally occurring colors and annatto addition. No dictated rate limits are established for benzoyl peroxide except good manufacturing practices levels in bleaching flour, milk for Italian cheeses and annatto colored whey, concentrated whey and dried whey (GPO, 2001; 2001b; El-Samragy, 2004). Usages of up to 40 ppm have been administered for bleaching flour and cheese milk while Cheddar cheese whey has been successfully bleached with 20 ppm benzoyl peroxide held at 60-63^{o}C for one hour. The dairy industry recommended treatment conditions for bleaching whey with benzoyl peroxide is 20 mg/kg of benzoyl peroxide at 60°C for 15 minutes at pH 6 to 7 (El-Samragy, 2004; Smith, 2004). The peroxide reacts with oxidizable compounds in whey and is converted into water soluble benzoic acid (Smith, 2004). Benzoyl peroxide provides a means of effectively destroying the carotenoid pigments of milk and controlling the color of the cheese (Kuramoto and Jezeski, 1954). When removing color by benzoyl peroxide, the effectiveness depends on the amount used, how it is applied, the whey components present, exposure time and temperature (Smith, 2004).

In bleu cheese manufacture, where milk can be bleached to produce a whiter cheese, Kuramoto and Jezeski (1954) found that optimum carotenoid destruction and flavor preservation occurred at 9 ppm concentration of benzoyl peroxide between 52^{o}C and 63^{o}C held for one to two hours. An increase to 18 ppm of benzoyl peroxide when

held at the same temperatures, caused strong oxidized and tallow notes. They also found that although carotenoids were destroyed, Vitamin A was not significantly affected (Kuramoto and Jezeski, 1954). Washam et al. (1974) also studied the affects of benzoyl peroxide in bleu cheese and found alterations in whey protein under different concentrations of benzoyl peroxide and different accompanying heat treatments. Electrophoresis gel patterns indicated β-lactoglobulin and α-lactoalbumin merged as one wide band instead of two distinct bands with bovine serum albumin, proteose peptone, and immunoglobulin bands were completely invisible. All heat treatments with or without benzoyl peroxide treatment revealed these smeared bands on the gels. However, the researchers determined treatments with 1% or 5% benzoyl peroxide with heat treatments had the most evident changes of smeared gels (Washam et al., 1974). This research also found that as the heat and benzoyl peroxide levels increased, leakage in the bleu cheese increased as well. From this find, Washam et al. (1994) proposed an alteration of the protein matrix had occurred from the treatments, thus creating less water binding. The best bleaching effect on cheese milk occurred with no heat treatment and the addition of 17.8 ppm benzoyl peroxide. This same treatment of 17.8 ppm benzoyl peroxide held for 2 h at 60^{o}C with no heat treatment also did not produce any off flavors. When heat treatment was added, protein denaturation escalated dramatically. Plus, the addition of heating for 2 h at 60^{o}C had no noticeable increased effect on benzoyl peroxide bleaching

and was unable to destroy benzoyl peroxide residues, questioning the necessity of extra heat treatment on whey (Washam et al., 1974).

Chang et al. (1977) researched the use of granular benzoyl peroxide in whey and its decomposition rate dependence on temperature, particle size, stirring rate and pH. Higher temperatures to decompose benzoyl peroxide were not recommended in this study for risk of higher protein denaturation even though increased temperatures could greatly accelerate decomposition. The researchers found that the rate of decomposition of benzoyl peroxide in whey followed first-order kinetics where the speed depended on the size of the benzoyl peroxide particles and the agitation velocity. At 69^{o}C after 2 hours with good stirring and fine mesh peroxide with whey, 91.7% of the radioactive labeled [14C] benzoyl peroxide was decomposed and recovered as benzoic acid. The researchers found that the peroxide particles tended to float and cling to the walls of the tank requiring considerable efforts to eliminate peroxide residue (Chang et al., 1977). Problems with dispersion of benzoyl peroxide were also observed with milk and cream and this could leave a residue that could remain in the cheese curd when used to bleach cheese milk (Washam et al., 1974). A disadvantage of using benzoyl peroxide is its need of a carrier, such as starch, calcium sulfate, magnesium carbonate, etc., which not only may have problems with solubility but could also potentially be an allergen (Smith, 2004).

In comparing the two different bleaching processes, researchers generally believe that benzoyl peroxide is more economical because it requires less peroxide for satisfactory

bleaching, does not need the addition of catalase, and is less corrosive on equipment (Chang et al., 1977; Smith, 2004; Roundy 1958). Unlike hydrogen peroxide, benzoyl peroxide does not reduce microbial populations, inhibit mold growth, or control acid production in whey (Washam et al., 1974). McDonough et al. (1967) compared both hydrogen peroxide and benzoyl peroxide on the decolorization of annatto in whey and found both to be effective bleaching agents, but benzoyl peroxide was better over a wider range of temperatures. Hydrogen peroxide was less effective when curd amounts with natural peroxidases were increased in the whey. Both hydrogen peroxide and benzoyl peroxide bleached whey exhibited oxidized flavors immediately after bleaching, but once spray dried, the off flavors disappeared according to very informal sensory analysis (McDonough et al., 1967; Washam et al., 1974; Roundy, 1958). In the end, the peroxide of choice is up to the manufacturer, the available facilities, time schedules, and the individual manufacturer preference.

Today, whey protein has become an important commodity in the food industry as an ingredient with multiple functional properties, a supplement with numerous health benefits, and simply a nutritious food product. Due to variability in composition and flavor of whey, whey is limited in its widespread usage (Carunchia-Whetstine et al., 2003; Carunchia-Whetstine et al., 2005; Morr and Ha, 1993). Previous studies have found variability in whey due to specific manufacturing processes (Tomaino et al., 2001; Karagul-Yuceer et al., 2003, Carunchia-Whetstine et al., 2003), storage conditions

(Tomaino et al., 2004; Lee et al., 1995), and in the process of bleaching (Washam et al., 1974; Kuramoto and Jezeski, 1954). The objective of this study is to investigate the flavor and flavor stability of liquid whey and additionally with the addition of antioxidants.

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CHAPTER 2: ANTIOXIDANT EFFECTS ON LIQUID AND DRIED WHEY FLAVOR

Interpretive summary (IS) of 100 words or less
Whey protein has become a widely used ingredient in food and health applications. Whey proteins ideally have a bland flavor but flavor variability and off-flavors in whey products limit food applications. This study evaluated the effects of adding antioxidants to liquid whey to decrease off-flavors in dried whey proteins.

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

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ABSTRACT

Whey protein is widely used in numerous ingredient applications. Whey protein flavor is highly variable and off-flavors in whey products can carry through into ingredient applications and negatively affect consumer acceptance. The objectives of this study were to evaluate the impact of antioxidant addition in prevention of flavor deterioration of fluid whey and flavor of whey protein. Cheddar and Mozzarella cheeses were manufactured in triplicate. Fresh whey was collected and pasteurized followed by fat separation. Subsequently, 0.05% w/w ascorbic acid, 0.5% w/w whey protein hydrolysate (WPH), or nitrogen flushing were administered to the pasteurized whey. A control with no antioxidant addition was also evaluated. Wheys were stored at 3^{o}C and evaluated after 0, 2, 4, 6, and 8 days. 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, WPH or nitrogen flushing 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, WPH or nitrogen flushing 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. WPC and liquid whey with added WPH, however, had a distinct potato flavor by sensory analysis which was absent in control or liquid whey or WPC with added ascorbic acid. 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.

Key words: liquid whey, antioxidant, Cheddar, Mozzarella

INTRODUCTION

Liquid whey is formed as a by-product from cheese production and today is a high value raw product stream (USDEC, 2008). Dried whey ingredients are useful ingredients in food products for their high solubility, dispersibility, water binding, foaming, whipping, emulsification, gelation, and buffering power (Morr and Ha, 1993; Bryant and McClements, 1998; de Wit, 1998; Foegeding et al., 2002; Henning et al., 2006). Whey proteins have gained further popularity for health benefits including increasing colon health (McIntosh et al., 1998), cardiovascular health (Mullally et al., 1997), and athletic enhancement (Dahm, 2005).

Flavor is generally recognized as the single most important factor affecting consumer acceptance of food products (Morr and Ha, 1991; Lee and Morr, 1994; Drake, 2006; Childs and Drake, 2009). Dried whey ingredients ideally should have a bland, delicate flavor free from undesirable flavors (Morr and Ha, 1993; Carunchia-Whetstine et al., 2005; Drake, 2006; Wright et al., 2009). Off-flavors in whey products can carry through into ingredient applications (Drake 2006; Childs et al., 2007; Drake et al., 2009; Wright et al., 2009), and may limit widespread use of these products (Quach et al., 1999; Carunchia Whetstine et al., 2005; Drake, 2006; Childs et al., 2007).

Flavor variability has been identified in liquid whey and dried whey products both between and within manufacturers (Carunchia-Whetstine et al., 2005; Drake et al., 2009; Wright et al., 2009). Flavor variability has been sourced to the liquid whey itself and is

also an outcome of downstream processing and storage techniques (Carunchia-Whetstine et al., 2003, 2005; Wright et al., 2009; Drake et al., 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 et al., 2005; Gallardo-Escamilla et al., 2005).

Although liquid whey is typically dried to produce various products, it is often transported and stored prior to drying (Tomaino et al., 2004). Many studies have reported that flavor of whey and whey products also further changes during storage, light exposure and added heat (Morr and Ha, 1993; Lee et al., 1995; Tomaino et al., 2004; Wright et al., 2009). Aldehydes, including hexanal, have been suggested as the compounds responsible for off flavors in liquid and dried whey products (Mills, 1986; Lee et al., 1995; Quach et al., 1999; Tomaino et al., 2004; Wright et al., 2009). In 2004, Tomaino et al. 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 et al., 2005; Evans et al., 2009; Wright et al., 2009) and concentrations increased with storage time concurrent with increased off-flavors (Wright et al., 2009). Collectively, these studies suggest that lipid oxidation products contribute to undesirable flavors in whey proteins.

The two main sources of liquid whey in the United States are derived from Cheddar and Mozzarella cheese production (Smith, 2004), 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, nitrogen blanketing, and whey protein hydrolysate were the antioxidants selected. Previous studies (Frankel, 1998; Jung et al., 1998; Lindmark-Månsson and Åkesson, 2000; Tong et al., 2000; Peña-Ramos and Xiong, 2003; Mortenson et al., 2004; Hernndez-Ledesma et al., 2005) have confirmed the antioxidative properties of these ingredients or treatments. Subsequently, selected treatments were evaluated in finished spray dried whey protein concentrate from Cheddar whey. Sensory and instrumental analyses were applied to document properties of liquid wheys and whey protein concentrates.

MATERIALS AND METHODS

Milk

Raw unhomogenized whole milk (NCSU Creamery, Raleigh, NC) for cheese production was high temperature short time (HTST) pasteurized with a plate heat exchanger (APV, APV Co. Ltd., England) 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 for cheese making were 3.62% ± 0.10 (CEM Smart Trac Rapid Fat Analysis, Matthews, NC) and 3.34% ± 0.08 (LactiCheck Milk Analyzer Dual Cow Channel LC-02, P&P International Ltd, Hopkinton, MA). During cheese manufacture, the pH of whey and cheese were measured with an electrode (Model IQ150, IQ Scientific Instruments, Inc., Loveland, CO) that was standardized at pH 6.97 and 4.03 at 38°C and kept immersed in 3M KCl at 38°C between readings in order to keep its temperature equal to the temperature of the samples.

Cheddar cheese whey

Pasteurized milk (3°C) was transferred into a cheese vat (Model 4MX, Kusel Equipment Company, Watertown, WI). The temperature of the milk was raised to 31°C with gentle stirring. Once the milk reached 31°C, calcium chloride [0.0018M] (Calcium Chloride Lot#0040606-264P, Dairy Connection Inc., Madison, WI) at a rate of 180 ml/454 kg milk and annatto color (Cheese Color DS Double Strength Lot#805129-009P-090607, Dairy Connection Inc., Madison, WI) 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 MA11 LYO 250 DCU Starter Culture Lot #4470 775 828, Dairy Connection Inc., Madison, WI) 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 Rennet Double Strength Lot#0042767-004P, Dairy Connection Inc., Madison, WI) 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, NC) and pasteurized at 65°C for 30 min to inactivate the starter culture. Cheddar cheese whey was manufactured in triplicate.

Mozzarella cheese whey

Pasteurized milk (3°C) was transferred into a cheese vat (Model 4MX, Kusel Equipment Company, Watertown, WI). The temperature of the milk was raised to 35°C with gentle stirring. Once the milk reached 35°C, calcium chloride [0.0018M] (Calcium Chloride Lot#0040606-264P, Dairy Connection Inc., Madison, WI) at a rate of 180 ml/454 kg milk and freeze-dried starter cultures were added. *Streptococcus thermophilus* (50 DCU/454 kg milk, Danisco Choozit TA61 LYO 125 DCU Lot #4470-658-235, Dairy Connection Inc., Madison, WI) and *Lactobacillus delbrueckii* ssp. *lactis* and *Lactobacillus helveticus* (20 DCU/454 kg milk, Danisco Choozit LH100 LYO 20 DCU Lot #4470-735-627, Dairy Connection Inc., Madison, WI) 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, Star Rennet Double Strength Lot#0042767-004P, Dairy Connection Inc., Madison, WI) 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 drained and pumped into a pasteurizer (Model MPD1050, D&F Equipment Co., McLeansville, NC) 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 both Cheddar and Mozzarella liquid whey, 26 L of each liquid whey was collected, cooled to 10^{o}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 #17584-115-3, Warenhandels GmbH, Whitewater, WI) for fat separation. The fat content of the whey before separation was 0.19 $\pm$ 0.04% and after separation was 0.07 $\pm$ 0.02% (CEM Smart Trac Rapid Fat Analysis, Matthews, NC). Separated whey was cooled to 10^{o}C. This cooled whey was divided into four 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 (J.T. Baker Ascorbic Acid, Fine Granular #0938-07, VWR International, West Chester, PA). Treatment 2 consisted of the addition of 0.5% w/w whey protein hydrolysate (WPH) (Whey Protein Hydrolysate 8350, Hilmar Ingredients, Hilmar, CA) to the liquid whey. Each of these ingredients was incorporated into the cooled whey by gentle agitation with a wire whisk for 5-10 min. Preliminary testing with cooled Cheddar whey confirmed that this method of incorporation fully dissolved the ingredients with no visible lumps or sediment. For treatment 3, oxygen was vacuum-pumped from the headspace of the liquid whey and was replaced with a blanket of nitrogen. Treatment 3 was stored in 2 liter round-bottom flasks (Two-Neck Round Bottom Flask, Ace Glass Inc., Vineland, NJ), sealed off with septum (White Septa 24/40, Ace Glass, Inc., Vineland, NJ) and wrapped in foil. All other samples were stored in 950 ml amber glass jars capped with PTFE faced PE-lined screw caps (Fisherbrand Amber Wide-Mouth Packers with PTFE Faced PE-lined Caps, Fisher Scientific, Hanover Park, IL) at 3^{o}C in the dark. Samples were held at 3^{o}C for 8 days. Aliquots (1.5L) were taken from each treatment and tested after 0, 2, 4, 6, and 8 days 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 females, 3 males, ages 22-37 y) evaluated the flavor attributes of the liquid whey using a previously established lexicon for fluid whey (Carunchia-Whetstine et al., 2003; Drake et al., 2003; Drake et al., 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 Spectrum™ descriptive analysis training, panelists were presented with reference solutions of sweet, sour, salty, and bitter tastes to learn to use the universal intensity scale (Meilgaard et al., 1999; Drake and Civille, 2003). Panelists then evaluated and discussed flavor attributes of both 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 ^{TM} descriptive analysis method (Meilgaard et al., 1999; Drake and Civille, 2003).

Liquid whey products (ca 30 ml) were dispensed into lidded 58 ml soufflé cups with three-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 Compusense ™ version 5.0 (Compusense, Guelph, ON, Canada).

SPME GC-MS

Volatile compounds of wheys were evaluated by SPME GC-MS. SPME GC-MS was conducted using a modified method of Wright et al. (2006). Five grams of liquid whey with 10% NaCl w/w (EMD Chemicals Inc., VWR International, West Chester, PA) and 10 µl internal standard solution (2-methyl-3-heptanone in methanol at 8.1 ppm; Aldrich, Sigma-Aldrich, Milwaukee, WI; VWR International, West Chester, PA) were placed into 20 ml autosampler vials with steel screw tops containing silicone septa faced in Teflon (Microliter Analytical, Sawanee, FL). 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, CA). 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 sec 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 250°C held for 5 min. SPME fibers were introduced into the split/splitless injector at

250°C. An Rtx-5ms column (Rtx-5ms 30 m length × 0.25 mm inner dia × 0.25 um film thickness; Restek, Bellefonte, Pa., U.S.A) was used for all analyses at a constant flow rate of 1 ml/min. Purge time was set at 1 min. The MS transfer line was maintained at 250°C with the Quad at 150°C and Source at 250°C. Compounds were identified using the NIST 2005 library of spectra and comparison of spectra of authentic standards injected under identical conditions. Relative abundance for each compound was calculated using the recovery of the internal standard concentration to determine relative abundance of each compound. Retention indices were calculated using an alkane series (Sigma Aldrich, Milwaukee, WI) (Van den Dool et al., 1963). Each sample was injected in triplicate for each treatment replication.

Whey protein concentrate manufacture

Unhomogenized whole milk from the Calpoly Dairy (San Luis Obispo, CA) was 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 sec), fat separated (Alfa-Laval, Richmond, VA), 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, membranes were cleaned according to a standard washing procedure. First, a phosphoric acid wash (15 min, 45°C, pH 2) was administered, followed by an alkaline wash with sodium hydroxide (20 min, 50°C, pH 12), and a final membrane reconditioning by 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/sec) through a Niro R-12 Universal Membrane System (Niro Inc., Hudson, WI) equipped with a ceramic spiral wound membrane (Koch Membrane Systems, Massachusetts, USA) with a nominal separation cutoff of 10,000 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 ± 0.1%, and protein content, 6.8 ± 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, WI) with an inlet air temperature of 204°C and an outlet air temperature of 88°C to obtain 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 #41509, IMPAK Corp, Central City, SD) and shipped to North Carolina State University by overnight carrier for sensory and instrumental analysis. 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, IL) (Atherton and Newlander, 1977). Nitrogen content was determined by the Dumas method (Rapid-N-Cube nitrogen/protein analyzer unit, Elementar-, Germany) 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, Hanover Park, IL).

Statistical analyses

To determine if significant differences existed in sensory and volatile properties between liquid whey controls and treatments, analysis of variance (ANOVA) was conducted using XL-STAT (XL-STAT version 2009, 1995-2009, Addinsoft, Paris, France). 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 version 2009, 1995-2009, Addinsoft, Paris, France).

RESULTS

Proximate analysis of liquid whey

Liquid Cheddar and Mozzarella wheys were 6.50 $\pm$ 0.10 and 6.45 $\pm$ 0.18 percent solids and 1.40 $\pm$ 0.20 and 1.30 $\pm$ 0.08 percent protein, respectively. Percent fat content of Cheddar and Mozzarella whey prior to fat separation was 0.17 $\pm$ 0.04 and 0.21 $\pm$ 0.05, respectively and after fat separation was 0.07 $\pm$ 0.03 for both whey types. The pH of both whey types was 6.38 $\pm$ 0.05 and this value did not change with storage time. Addition of ascorbic acid decreased pH (6.13 $\pm$ 0.03) ($p<0.05$). These values were comparable to previous studies (Gallardo-Escamilla et al., 2005; Carunchia-Whestine et al., 2003).

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 flavors (Tables 2.1, 2.2). 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 et al., 2003; Karagul-Yuceer et al., 2003; Tomaino et al., 2004; Gallardo-Escamilla, et al., 2005). Consistent with previous research (Tomaino et al., 2004), the flavors of both 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. Initially, Cheddar liquid whey controls both with and without fat separation had similar sensory attributes. Mozzarella fresh whey without fat separation had higher sour aromatic flavor than whey with fat separation. 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 et al., 2009). Initially, nitrogen blanketed Cheddar liquid whey had lower sweet aromatic and cooked/milky flavors than controls. Mozzarella liquid whey blanketed with nitrogen had a similar flavor profile to control whey.

Over storage time, control Cheddar wheys and whey with ascorbic acid and nitrogen treatments 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 2.2). As storage time increased, cardboard flavor intensity increased while cooked/milky flavor decreased in both control and treated wheys (Table 2.2). The whey with WPH was initially characterized by a distinct potato/brothy flavor. 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 whey controls with

and without fat had similar cardboard flavor intensity. Nitrogen blanketed Mozzarella had the highest cardboard intensity after 8 d followed by the two control wheys.

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 et al. (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 (Wright et al., 2009; Drake et al., 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 et al., 2003; Karagul-Yuceer et al., 2003; Gallardo-Escamilla, et al., 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 (Tables 2.3, 2.4). Previous

research has identified most of these volatile compounds in liquid whey (Carunchia-Whetstine et al., 2003; Tomaino et al., 2004; Gallardo-Escamilla et al., 2005; Karagul-Yuceer et al., 2003). To our knowledge, decanal, 2-pentanone, and 3-hydroxy-2-butanone have not been documented in fresh liquid whey. Decanal has been reported in WPC (Carunchia-Whetstine et al., 2005; Stevenson and Chen, 1996), 2-pentanone has been documented in milk as a source of off flavor (Marsili, 1999), and 3-hydroxy-2-butanone (acetoin) has been documented in cheese (Moio and Addeo, 1998) and yogurt (Ott et al., 1997) products. 3-hydroxy-2-butanone has a buttery aroma and in conjunction with 2,3-butanedione is known to contribute to the characteristic sweet and milky aroma in soft immature cheeses such as Mozzarella (Moio and Addeo, 1998).

Similar to sensory data, the volatile compound concentrations of Cheddar and Mozzarella whey samples changed over time in refrigerated storage. In general, fresh whey samples had higher concentrations of 2,3-butanedione and 2-butanone and lower concentrations of all aldehydes compared to wheys after 8 d. Over storage, volatile lipid oxidation decomposition products produced from lipid autoxidation, such as aldehydes, hydrocarbons, alcohols, and olefins (Frankel, 1998) were documented. Short chain aldehydes have also been attributed to cardboard flavor in whey products (Tomaino et al., 2004; Quach et al., 1999; Mills, 1986; Lee et al., 1995).

Cheddar whey. Significant interactions between treatment and time occurred for 2-methyl butanal, 3-methyl butanal, hexanal, and heptanal concentrations in Cheddar whey (Table 2.3). 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 nitrogen treatment was only evaluated through 6 d due to microbial contamination (documented by decrease in pH and off-aromas).

The Cheddar liquid whey instrumental data was 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 whey 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 two aldehydes have been associated with malty and nutty flavors in cheese and milk (Avsar et al., 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 2.4). 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 treatments, while nonanal was not detected in the nitrogen treatment. 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 (Wright et al., 2009; Javidipour and Qian, 2008). For Mozzarella whey, total aldehyde concentration increased up to 4 d and then decreased until 8 d.

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 two whey types. However, unlike Cheddar whey, increases in cardboard flavor over time for Mozzarella

liquid whey samples 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 4-6 d followed by a decrease in concentration at 8 d. This may be due to the further degradation of the aldehydes into other compounds. For example, hexanal has been known to degrade into hexanoic acid (Schieberle and Grosch, 1981). As seen in Table 2.3 and 2.4, aldehyde concentrations were not always lower in Mozzarella liquid whey compared to Cheddar liquid whey. Both Mozzarella control and treatments actually had higher concentrations of specific aldehydes, most commonly heptanal, up until 4 d. After 4 d, Cheddar liquid whey increased in aldehyde concentration while Mozzarella samples decreased. Little research has been conducted on Mozzarella liquid whey. Gallardo-Escamilla et al. (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

Successful treatments from the liquid whey trials were selected for evaluation across the production process into finished spray-dried 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 et al., 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 2.5. Flavors documented in the WPC samples included attributes previously mentioned for liquid whey with the addition of cereal, malty, and serum/oxidized flavors (Table 2.5). Previous research has documented these flavors in WPC (Carunchia-Whetstine et al., 2005; Russell et al., 2006; Wright et al., 2009). Similar to liquid whey, potato/brothy and malty flavors were only detected in the WPH treatment. Only the control WPC was characterized by a serumy/oxidized flavor and the WPC control had the highest intensities of cardboard flavor and aroma intensity ($p<0.05$, Table 2.5).

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 2.7 and Figure 2.1) . Previous research has documented these volatile compounds in Cheddar WPC (Mahajan et al., 2004; Carunchia-Whetstine et al., 2005; Wright et al., 2009; Quach

et al., 1999; Wright et al., 2006; Mills and Broome, 1997; Lee et al., 1995; Stevenson and Chen, 1996). As with fluid whey, the compounds 2-methyl butanal and 3-methyl butanal were only detected in WPC with added WPH. To our knowledge, 2,5-octanedione has not been documented in Cheddar whey products but has been documented in cantaloupe (Beaulieu and Grimm, 2001).

The separated fat control WPC had significantly higher amounts of pentanal, (E,Z)-2,6-nonadienal, 2-heptanone, butanoic acid, and hexanoic acid than the two treated WPC. WPC with ascorbic acid had 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 are further confirmed with sensory results (Carunchia-Whetstine et al., 2005; Wright et al., 2009; Whitfield, 1992).

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 (Tomaino et al., 2004; Karagul-Yuceer et al., 2003; Carunchia-Whetstine et al., 2003), hexanal (Lund and Holmer, 2001; Wright et al., 2009; Quach et al., 1999), nonanal (Tomaino et al., 2004), (E,Z)-2,6-nonadienal (Karagul-Yuceer et al., 2002), 1-octen-3-one (Morr and Ha, 1991; Carunchia-Whetstine et al., 2005) and 2-heptanone (Quach et al., 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 (Wright et al., 2009; Quach et al., 1999). Methional, DMDS, and DMTS have also been documented in commercial WPC and WPI (Wright et al., 2006; Mahajan et al., 2004; Wright et al., 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 et al., 2006; Carunchia-Whetstine et al., 2005). Butanoic and hexanoic acid concentrations in the control WPC were similar to those previously reported in WPC (Carunchia-Whetstine et al., 2005).

DISCUSSION

Lipid autoxidation

Autoxidation of unsaturated fatty acids gives rise to unstable hydroperoxides, which decompose to a wide range of carbonyl products, in particular short chain fatty acids, many of which can contribute to off-flavors in dairy products (Lee and Morr, 1994;

Badings 1991; Carunchia-Whetstine et al., 2005; Drake et al., 2009; 2009; Wright et al., 2009; O'Connor and O'Brien, 2006). Short chain aldehyde detection and increases with storage have been previously documented in Cheddar liquid whey (Tomaino et al., 2004; Karagul-Yuceer et al., 2003; Carunchia-Whetstine et al., 2003; Gallardo-Escamilla et al., 2005). Tomaino et al. (2004) suggested that the actual cheese making process and whey pasteurization initiated the production of these compounds which then increased in concentration over time.

Previous studies have quantified the amounts of free fatty acids in Cheddar liquid whey (Tomaino et al., 2001; 2004; Carunchia-Whetstine et al., 2003; Boyd et al., 1999). In these studies, Cheddar liquid whey had the highest relative concentrations of long chain fatty acids palmitic (C16), oleic (C18:1), and stearic (C18) acids compared to other free fatty acids ranging from butyric (C4) to linoleic (C18:2) (Tomaino et al., 2001; 2004; Carunchia-Whetstine et al., 2003). Over 14 days of liquid whey storage, significant decreases in palmitic (C16), oleic (C18:1), and linoleic (C18:2) free fatty acids (Tomaino et al., 2001; 2004) with subsequent increases in lipid oxidation products were documented (Tomaino et al., 2004). Further, significant correlations were documented between the decrease in linoleic acid concentration and increases in cardboard flavor over storage (Tomaino et al., 2004). Increases in aldehydes support an oxidative mechanism for the decreases in oleic and linoleic acids (Tomaino et al., 2001).

Oxidized oleic acid produces 8-, 9-, 10-, and 11-hydroperoxides which then produce a variety of secondary products including decanal, nonanal, heptanal and octanal (Frankel, 1998). Linoleic acid autoxidation produces 9-, 10-, 12-, and 13-hydroperoxides (Frankel, 1998). From homolytic cleavages of these hydroperoxides, 2,4-decadienal, hexanal, and pentanal are produced among other secondary volatile decomposition products. Hexanal is actually produced from both the 13- and 9-hydroperoxide, as well as from breakdown of some of the unsaturated aldehydes (Frankel, 1998; Schieberle and Grosch, 1981). Ulrich and Grosch (1987) documented hexanal as the major volatile product formed during autoxidation of linoleic acid and methyl linoleate. (E,E)-2,4-decadienal was also identified in this study as a product of linoleic acid autoxidation. Typically, oxidative cleavage at the double bonds causes these reactions but even in the absence of oxygen, 2,4-decadienal and hexanal were obtained from linoleic acid indicating rearrangement of 9- and 13-hydroperoxide isomers as a source of short chain aldehyde production (Schieberle and Grosch, 1981). Additionally, linoleic acid is 40 times more reactive than oleic acid due to its ability to lose a hydrogen atom very readily, and may lead to a greater production of secondary hydroperoxide compounds such as hexanal (Schieberle and Grosch, 1981).

Hexanal was the predominant aldehyde detected in this and other storage studies with Cheddar liquid whey and whey products (Quach et al., 1999; Mills, 1986; Tomaino et al., 2004; Lee et al., 1995; Carunchia-Whetstine et al., 2005). As previously mentioned,

hexanal is formed from two pathways by linoleic acid autoxidation (Frankel, 1998). Further, secondary products including 2,4-decadienal may also continue to degrade. Autoxidative cleavage by oxygen with 2,4-decadienal primarily produces hexanal, serving as another pathway for hexanal formation (Matthews et al., 1971). In reaction mixtures containing both saturated and unsaturated aldehydes, 2,4-decadienal autoxidizes faster than just 2,4-decadienal by itself resulting in more hexanal production which the authors have described as a protective act towards saturated aldehydes, such as hexanal (Schieberle and Grosch, 1981).

Starter cultures may also form lipid oxidation products during fermentation. Lactic acid bacteria, *Lactococcus cremoris*, *Lactococcus lactis*, *Streptococcus thermophilus*, *Lactobacillus helveticus*, and *Lactobacillus bulgaricus*, were found to produce 2,4-decadienal and 2,4-nonadienal (Suriyaphan et al., 2001). This production of 2,4-decadienal serves as yet another pathway for hexanal production. In comparison to equivalent amounts of linoleic acid, 9- and 13- hydroperoxides, 2,4-decadienal autoxidation produces the most hexanal concentration (Schieberle and Grosch, 1981). Additionally, acetic, butanoic, and hexanoic acids were significantly higher in Cheddar whey over 8 d storage compared to Mozzarella whey. Of these three 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 concentration identified in the whey samples.

For both Cheddar and Mozzarella whey, fat separation decreased aldehyde production and lipid oxidation products (Table 2.3, 2.4). Few sensory differences were documented between the two 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 2.2, 2.4). In Cheddar, controls had a similar sensory profile initially, and over time, whey without fat removal had significantly higher cardboard flavor (Table 2.1). Fresh Cheddar controls had similar concentrations of all aldehydes, but after storage, whey without fat removal had higher concentrations of hexanal and total aldehydes than whey with fat removed (Table 2.3).

The increase and subsequent decrease of aldehyde concentrations in Mozzarella liquid whey over 8 d may be explained by the autoxidation of saturated aldehydes (Table 2.4). For example, hexanal and octanal produce hexanoic and octanoic acids, respectively, by autoxidation (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, i.e. 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, especially Cheddar whey, had the highest cardboard flavor intensities and aldehyde concentrations. This is most likely due to an initial higher concentration of free fatty acids than wheys with fat removal. As previously stated, higher free fatty acid concentration leads to higher concentrations of unsaturated aldehydes which not only produce more saturated aldehydes but also decrease saturated aldehyde degradation. Whey with fat, 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 5-7% fat when the whey is concentrated into WPC (Morr and Ha, 1993). These lipids which 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). These lipids are distributed in aqueous cell membranes as thin layers with a large surface area in close contact with pro-oxidants or hydrolytic catalysts in or near the cellular membrane structures (Szuhaj and Sipos, 1989). 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 et al., 1987). The MFGM consists mainly of triaglycerides,

cholesterol, phospholipids, and sphingolipids in varying proportions (Fox and McSweeney, 1998). These polar lipids in dairy products are mainly situated in the MFGM to protect fat droplets from enzymatic attack by lipases (Rombaut et al., 2006). During processing, the MFGM may be disrupted, releasing MFGM and its contents into the skim milk phase and enriching the aqueous phase (Christie et al., 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 (Michalski et al., 2002; Christie et al., 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). The structure of phospholipids results in autoxidation that is far more complicated than that of free PUFA or triglycerides. Phospholipids contain phosphorus, a nitrogen-containing component such as choline, ethanolamine, or serine, unsaturated and saturated fatty acids. The varying structure of the phospholipid may affect the autoxidation rates of both bound and free PUFA constituents (Szuhaj and Sipos, 1989). Phospholipids have many routes to off flavor development. Ester-bonds on

the phospholipid may further oxidize fatty acids, while an amine base can react with hydroperoxides to produce other compounds with off-flavors (Szuhaj and Sipos, 1989).

Autoxidation in liquid milk products usually start with phospholipids due to their highly unsaturated fatty acid residues in contact with the main catalyst, Cu (Walstra and Jenness, 1984). After autoxidation is in progress, milkfat triglycerides are then oxidized. Naturally occurring copper in milk is found in the membrane of fat globules and in aqueous systems, the metal acts as an oxidative catalyst against triglycerides if proteins and phospholipids are present (Walstra and Jenness, 1984). Cheddar and Mozzarella liquid whey actually have very similar concentrations of phospholipid polar species (Rombaut et al., 2007). In WPC75, lipid class composition consisted of in order from greatest concentration, triacylglycerols (neutral lipids), phospholipids (polar lipids), diacylglycerols, free fatty acids, cholesterol ester, cholesterol, and monoacylglycerols (Vaghela and Kilara, 1996; Boyd et al., 1999). Larger polyunsaturated fatty acid proportions, precursors for hydroperoxides and secondary products including aldehydes, were found in the phospholipid fraction as compared to the neutral lipids and total lipid fractions (Boyd et al., 1999). Similar proportions of almost all phospholipid classes were quantified regardless of protein concentration in the whey (Vaghela and Kilara, 1996). During ultrafiltration with a molecular weight cutoff of 8 and 30 kDa, polar lipids, including phospholipids, are retained (Rombaut and Dewettinck, 2006) and may perhaps decrease further phospholipid autoxidation. In addition, these previous studies still

mention the importance of free fatty acids as lipid oxidation products (Vaghela and Kilara, 1996; Boyd et al., 1999).

Overall, lipid oxidation in whey products, and probably in most food products with some lipid concentration, consist of a variety of pathways to produce off-flavors and oxidative products. In this current study, it seems likely that phospholipids play a role in creating oxidative products in both Cheddar and Mozzarella whey. However, different amounts of lipid oxidation products were documented between the two types of whey, despite similar total lipid concentrations in the wheys, 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 et al., 2003; Gallardo-Escamilla et al., 2005), and studies with fermented milks have confirmed a wide variety in type and amount of volatile compound production from *Lactobacillus* strains (Drake et al., 1999) and *Lactococcus* species (Boumerbassi et al., 1995; Seefeldt and Weimer, 2000). The long chain fatty acids precursors to lipid oxidation may be produced by lipolytic enzymes from the specific starter culture (Jensen et al., 1991; Tomaino et al., 2001). Strain differences in Cheddar starter cultures influence the flavor and oxidative stability of liquid Cheddar whey (Carunchia-Whetstine et al., 2003; Tomaino et al., 2004). Thus, it should be no surprise to find vast differences between Cheddar and Mozzarella liquid whey and oxidative stability of these products.

Ascorbic Acid. Lipid oxidation may be inhibited with the addition of antioxidants. Promising artificial antioxidants such as butylated hydroxyanisol (BHA), butylated hydroxytoluene (BHT), and n-propyl gallate exhibit strong antioxidant effect but potential health risks have led to restricted use of these antioxidants (Mahoney and Graf, 1986; Pihlanto, 2006; Peña-Ramos and Xiong, 2003). Due to these risks, recent attempts have been made to find natural food source 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 et al., 1984; Frankel, 1998). In anaerobic, aqueous solutions, ascorbic acid acts as an oxygen scavenger (Frankel, 1998). The addition of ascorbic acid in this study produced an antioxidant effect in both liquid whey and Cheddar WPC. Cardboard flavor in both 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 the finished dried product. When compared to the whey with WPH, 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 2.3, 2.4). This initial ascorbic acid antioxidant activity with subsequent decrease in activity has been observed in previous research (Villota and Karel, 1980; Dave and Shah, 1997; Robertson and Samiengo, 1986; Frankel, 1998). In yogurt prepared with ascorbic acid, the oxygen content was initially lower than the control but after 10-15 d of storage, oxygen levels gradually began to approach the level of the control. After 35 d, yogurt with ascorbic acid addition was not effective. On average, 15-20% of ascorbic acid was lost during fermentation and overnight storage, with 30-35% loss after 5 days, and only 15-20% left at 35 d (Dave and Shah, 1997). Ascorbic acid degradation is independent of initial concentration (Villota and Karel, 1980; 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 began to produce more radicals than it is able to scavenge (Mahoney and Graf, 1986). During storage of milk, ascorbic acid decreases continuously and depletes by consuming dissolved oxygen (Frankel, 1998). In drying, ascorbic acid degradation is independent of its initial concentration but dependent on its moisture content and the drying temperature. Degradation of ascorbic acid in drying follows a first-order reaction. Initially in the drying process, degradation depends on moisture content. As drying proceeds, the degradation rates become dependent on the

increasing temperatures (Villota and Karel, 1980). Regardless of initial ascorbic acid concentration and oxygen level amount, over storage time, ascorbic acid will degrade and antioxidant activity is no longer exhibited (Robertson and Samiengo, 1986; Saguy et al., 1978).

Whey Protein Hydroylsate. Previous studies have demonstrated that food proteins and their hydrolysates have antioxidant activity including milk proteins, casein (Cervato et al., 1999; Wong and Kitts, 2003), casein hydrolysates and low molecular weight casein hydrolysates (Diaz and Decker, 2004), egg yolk (Park et al., 2001), soybean protein hydrolysate (Peña-Ramos and Xiong, 2003; Chen et al., 1996), carnosine, a dipeptide found in skeletal muscle (Decker and Faraji, 1990), and royal jelly protein hydrolysate (Guo et al., 2009). 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 (Pihlanto, 2006; Peña -Ramos et al., 2001). 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.

WPH addition was demonstrated to have an antioxidant effect on cooked pork patties after 8 d of refrigerated storage (Peña-Ramos et al., 2003). Hydrolyzed WPC had greater lipid oxidation inhibition in pork patties compared to hydrolyzed WPI. This

difference was attributed to the production of antioxidant Maillard reaction products (Peña-Ramos et al., 2003). Hydrolyzed β-lactoglobulin and α-lactalbumin, the primary proteins in whey, have also exhibited lipid oxidation inhibition in a model system (Hernández-Ledesma et al., 2005). A particular peptide from β-lactoglobulin had stronger antioxidant activity when compared to BHA (Hernández-Ledesma et al., 2005).

Amino acids may play a very important part in the antioxidant activity of WPH. In 1960, Marcus demonstrated tyrosine, methionine, histidine, lysine, and tryptophan amino acids could chelate metals (Marcuse, 1960). More recently, WPI hydrolysate lipid oxidation delay was attributed to histidine and hydrophobic amino acids (Peña-Ramos et al., 2001). Tryptophan and tyrosine had the highest antioxidant activity in hydrolyzed β-lactoglobulin potentially due to the indolic and phenolic groups of the amino acids acting as hydrogen donors (Hernández-Ledesma et al., 2005). Amino acids may also work together synergistically for a strong antioxidant effect but sometimes the activity of particular combinations of amino acids is lower than the amino acid individually (Hernández-Ledesma et al., 2005).

Protease type for hydrolysis can drastically alter antioxidant activity by forming different amino acid sequences and peptide sizes (Hernández-Ledesma et al., 2005). Pure enzymes for WPI hydrolysis produced peptides and amino acids with little antioxidant activity compared to WPC hydrolysis. This lack of activity has been attributed to a proxidant affect from producing certain amino acids and peptide sequences, especially in

high concentrations (Marcuse, 1960; Peña-Ramos et al., 2001). The same study found crude enzymes with WPI produced an antioxidative effect and further suggested that specific protease type was necessary for lipid oxidation inhibition. Previous research has attributed lipid oxidation inhibition by enzymatic hydrolysis 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; Decker et al., 1992; Chan et al., 1994; Lee et al., 1998; Peña-Ramos et al., 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 et al., 1995) and casein (Suetsuna et al., 2000) attributed the antioxidant success of shorter peptides to specific amino acids. Characteristic amino acid sequences of peptides were required for antioxidative effects typically including valine or leucine at the N-terminus and proline, histidine, or tyrosine somewhere in the sequence (Chen et al., 1995). In hydrolyzed casein, Suetsuna et al. (2000) suggested that the L-glutamic acid and L-leucine (Glu-Leu) sequence was important for the strong free radical scavenging activity of casein derived peptides. In whey, Peña-Ramos et al. (2001) suggested peptides with low molecular weights, <10 kDa, contributed to the antioxidant activity of WPH. Hernández-Ledesma et al. (2005) reported the 3 kDa fraction was mainly responsible for the antioxidant activity in the whole hydrolysate. However, no relationship was found between the degree of hydrolysis

and the antioxidative activity for different whey proteins (Pihlanto, 2006). The activity is inherent to the characteristic amino acid sequences of peptides derived depending on protease specificity (Pihlanto, 2006; Hernández-Ledesma et al., 2005).

Although whey with WPH had lower cardboard flavor in both liquid whey and WPC products, WPH has its own distinct flavor and aroma as well (Drake et al., 2009). As seen in Tables 2.1, 2.2, 2.6, whey with WPH had potato/brothy, cheesy/brothy, and malty flavors that were not detected in the control samples. WPC, after further processing, actually 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 taken to determine if lower concentrations of WPH will minimize WPH-flavor in WPC while maintaining antioxidant effects.

CONCLUSIONS

Both Mozzarella and Cheddar liquid whey had distinct flavor profiles, volatile compound concentrations, and oxidative stability. Lipid oxidation occurred in both 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 differences in dried whey proteins. Fat removal from liquid whey decreased but did not eliminate lipid oxidation. The addition of ascorbic acid and WPH to both liquid whey types 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. In the future, other antioxidants and chelators such as vitamin E, EDTA, alone and in combination with ascorbic acid, should be evaluated for whey oxidation inhibition.

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Figure 2.1 -- Principal component biplot of Cheddar WPC SPME-GC-MS results
Control: Control with fat separation; Ascorbic acid: Whey with ascorbic acid addition; WPH treatment: Whey with WPH addition.

Table 2.1 – Sensory flavor attributes of Mozzarella liquid whey over storage time

	Control 1				
	0d	2d	4d	6d	8d
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				
	0d	2d	4d	6d	8d
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				
	0d	2d	4d	6d	8d
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

Table 2.1 – Continued

	WPH				
	0d	2d	4d	6d	8d
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
	Nitrogen				
	0d	2d	4d	6d	8d
Aroma intensity	2.0	1.8	2.0	2.3	2.3
Sweet aromatic	1.0	ND	ND	ND	ND
Sour aromatic	0.9	0.1	1.0	ND	ND
Cardboard	ND	ND	1.6	2.4	2.3
Potato/Brothy	ND	ND	ND	ND	ND
Cooked/milky	3.1	2.2	1.7	1.4	1.3
Sweet taste	2.0	1.5	1.3	1.2	1.3
Sour taste	1.2	1.0	0.8	0.9	0.9
Astringent mouthfeel	1.3	1.4	1.3	1.0	1.0

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

Table 2.1 – Continued

Means followed by different lowercase letters within a treatment for an attribute signify a difference ($P<0.05$). Uppercase letters in rows following means over the entire table signify differences for an attribute ($P<0.05$). 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 et al., 2003; Russell et al., 2006). LSD=least significant difference.

Table 2.2 – Sensory flavor attributes of Cheddar liquid whey over storage time

	Control 1				
	0d	2d	4d	6d	8d
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				
	0d	2d	4d	6d	8d
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				
	0d	2d	4d	6d	8d
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

Table 2.2- Continued

	WPH				
	0d	2d	4d	6d	8d
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
	Nitrogen				
	0d	2d	4d	6d	8d
Aroma intensity	2.7	3.1	2.0	2.1	2.0
Sweet aromatic	1.3	ND	ND	0.6	1.0
Sour aromatic	ND	ND	0.1	ND	ND
Cardboard	ND	ND	1.8	1.3	1.5
Potato/Brothy	ND	ND	ND	ND	ND
Cheesy/Brothy	ND	ND	ND	ND	ND
Cooked/ milky	2.6	2.0	2.2	2.4	1.8
Sweet taste	2.0	1.4	1.3	1.1	1.2
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

Table 2.2 – Continued

Means followed by different lowercase letters within a treatment for an attribute signify a difference ($P<0.05$). Uppercase letters in rows following means over the entire table signify differences for an attribute ($P<0.05$). 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^{TM} 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 et al., 2003; Russell et al., 2006). LSD=least significant difference.

Table 2.3 – Relative abundance of aldehydes (ppb) in Mozzarella liquid whey over storage time

	Control 1				
	0d	2d	4d	6d	8d
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				
	0d	2d	4d	6d	8d
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				
	0d	2d	4d	6d	8d
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				
	0d	2d	4d	6d	8d
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

Table 2.3 – Continued

	Nitrogen				
	0d	2d	4d	6d	8d
Nonanal	0.0202	0.0123	0.0242	0.00530	ND
Octanal	0.00277	0.00424	0.00362	0.00227	ND
Heptanal	0.00870	0.0137	0.0102	0.00881	ND
Hexanal	0.0880	0.3222	0.3165	0.3392	0.1033
2-methyl Butanal	ND	ND	ND	ND	ND
3-methyl Butanal	ND	ND	ND	ND	ND

	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

Means followed by different lowercase letters within a treatment for a compound signify a difference ($P<0.05$). Uppercase letters in rows following means over the entire table signify differences for a compound ($P<0.05$). 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. LSD=least significant difference.

Table 2.4 – Relative abundance of aldehydes (ppb) in Cheddar liquid whey over storage time

	Control 1				
	0d	2d	4d	6d	8d
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				
	0d	2d	4d	6d	8d
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				
	0d	2d	4d	6d	8d
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				
	0d	2d	4d	6d	8d
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

Table 2.4 – Continued

	Nitrogen			
	0d	2d	4d	6d
Nonanal	0.0448	0.0218	0.0147	0.108
Octanal	0.00722	0.00595	0.00331	0.0225
Heptanal	0.00728	0.00461	0.00361	0.0907
Hexanal	0.134	0.149	0.181	2.89
2-methyl Butanal	ND	ND	ND	ND
3-methyl Butanal	ND	ND	ND	ND
Decanal	0.0033	0.0013	0.0008	0.0032

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

Means followed by different lowercase letters within a treatment for a compound signify a difference ($P<0.05$). Uppercase letters in rows following means over the entire table signify differences for a compound ($P<0.05$). 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. LSD=least significant difference.

Table 2.5 - Gross composition of WPC

WPC Sample	Composition		
	Moisture, %	Fat, %	Protein, %
Control	6.02±0.50	20.5±1.52	64.0±0.67
Ascorbic acid	4.55±0.73	22.2±3.15	66.6±1.31
WPH	3.10±0.33	24.9±2.97	65.0±1.06

Table 2.6 – Sensory flavor attributes of Cheddar WPC

	Mean Scores			
	WPC Control	WPC with Ascorbic acid	WPC with WPH	LSD
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 et al., 2003; Russell et al., 2006). LSD=least significant difference.

Table 2.7 – Relative abundance of volatile compounds (ppb) in Cheddar WPC

Compounds	WPC Control	WPC with Ascorbic acid	WPC with WPH	LSD
Methional	0.155b	0.155b	0.403a	0.125
2-methyl Butanal	ND	ND	3.28a	0.784
3-methyl Butanal	ND	ND	5.00a	0.622
Pentanal	3.22a	1.69b	1.06b	1.20
Hexanal	104a	90.2a	43.8b	37.6
Heptanal	8.20a	6.29a	1.74b	2.27
Octanal	0.795b	1.14a	ND	0.154
Nonanal	1.33ab	3.69a	ND	2.80
(E,Z)-2,6-Nonadienal	1.56a	0.443b	0.295b	0.217
Dimethyl Disulfide	0.0772a	0.0535a	ND	0.0901
Dimethyl Trisulfide	0.656a	0.829a	0.449b	0.196
1-Pentanol	0.747a	0.324a	0.708a	0.544
2,3-Butanedione	3.62b	8.10a	3.49b	3.23
2-Heptanone	2.07a	1.05b	0.288c	0.533
1-Octen-3-one	1.37a	1.02a	1.46a	0.512
2,5-Octanedione	1.57a	1.79a	0.453b	0.779
2-pentyl Furan	3.00a	2.50a	0.474b	1.23
Butanoic acid	1.58a	0.00880b	ND	0.957
Hexanoic acid	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.

CHAPTER 3: FLAVOR AND FLAVOR CHEMISTRY OF MOZZARELLA AND CHEDDAR LIQUID WHEY

Comparison of the Flavor Chemistry and Flavor Stability of Mozzarella and Cheddar whey

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ABSTRACT

Cheddar and Mozzarella whey are the primary sources for whey protein production. Differences in the flavor of fresh whey may influence whey protein flavor. The objectives of this study were to compare the flavor and flavor stability of fresh and stored liquid Cheddar and Mozzarella whey. Pasteurized, fat separated and unseparated Cheddar and Mozzarella wheys were manufactured in duplicate and evaluated immediately or stored for 72 h at 3^{o}C. Flavor profiles were documented by descriptive sensory analysis and volatile components were extracted and characterized by solid phase microextraction with gas chromatrography-mass spectrometry (SPME-GC-MS), direct solvent extract (DSE) with solvent assisted flavor evaporation (SAFE) with GC-MS and gas chromatography-olfactometry (GC-O) with aroma extract dilution analysis (AEDA). Cheddar and Mozzarella wheys were distinct by sensory and volatile analysis ($p<0.05$). Fresh Cheddar whey had higher intensities of buttery and sweet aromatic flavors and higher cardboard flavor intensities following storage compared to Mozzarella whey. Fifty aroma-active compounds were identified by GC-O. High aroma impact compounds ($FD_{log3} > 8$) in fresh Cheddar whey included diacetyl, 1-octen-3-one, 2-phenethanol, butyric acid, and (E)-2-nonenal, while those in Mozzarella whey included diacetyl, octanal, (E)-2-nonenal, and 2-phenethanol. Concurrently, fresh Cheddar whey had increased relative abundances of diacetyl, 2/3-methyl butanal, (E)-2-nonenal, 2-phenethanol, and 1-octen-3-one compared to fresh Mozzarella whey. Lipid oxidation products increased in both whey types during storage but increases were more pronounced in Cheddar whey than

Mozzarella whey. Results suggest that similar compounds in different concentrations comprise the flavor of these two whey sources and that steps should be taken to minimize lipid oxidation during fluid whey processing.

KEYWORDS: Cheddar, Mozzarella, liquid whey, GC-O, flavor chemistry

INTRODUCTION

The United States is the world's largest whey-producing country, manufacturing 1.1 million metric tons of whey and lactose products per year (USDEC, 2004). Whey protein has become an important versatile ingredient in the food industry. The numerous functionalities of whey protein include high solubility, dispersibility, water binding, foaming, whipping, emulsification, gelation, and buffering power and these properties are used widely in the food industry (Morr and Ha, 1993; Foegeding et al., 2002; Bryant and McClements, 1998; Henning et al., 2006). Whey protein has also grown in popularity due to discovered health benefits such as increasing colon health (McIntosh et al., 1998), cardiovascular health (Mullally et al., 1997), and athletic enhancement (Dahm, 2005).

Flavor is generally recognized as the single most important factor affecting consumer acceptance of food products (Drake, 2004). Off-flavors in whey products can carry into ingredient applications and negatively affect consumer acceptance (Drake, 2006; Childs et al., 2007; Wright et al., 2009; Drake et al., 2009). The flavor and flavor variability of whey protein has and continues to limit its widespread usage in bland and delicately flavored foods (Wright et al., 2009; Drake et al., 2009; Carunchia-Whetstine et al., 2003). Flavor variability of liquid whey and whey products have been established between and within manufacturers (Wright et al., 2009; Drake et al., 2009; Carunchia-Whetstine et al., 2003; 2005). Different starter cultures in the cheese-making process are utilized to produce a variety of cheeses and result in liquid wheys with different mineral content, protein concentration, and lactose content, which result in different functional and

flavor properties (Drake et al., 2009; Gallardo-Escamilla et al., 2005; Bordenave-Juchereau et al., 2005).

To our knowledge, little research has been conducted to characterize flavor of liquid whey from different sources. In 2003, Carunchia-Whetstine and others reported distinct volatile compound differences between and within manufacturing facilities when comparing liquid Cheddar whey from different manufacturers. The liquid whey flavor differences were attributed to the milk source, different methods for processing, and starter culture rotation. Karagul-Yuceer and others (2003) identified aroma-active compounds in liquid Cheddar wheys including 2,3-butanedione, 2-butanol, hexanal, 2-acetyl-1-pyrroline, methional, (E,E)-2,4-nonadienal, (E,E)-2,4-decadienal and other short-chain volatile acids. Gallardo-Escamilla and others (2005) evaluated several different types of liquid whey by sensory and headspace analysis but did not identify the distinguishing aroma-active compounds. Previous studies have documented that Mozzarella fluid whey has a different sensory profile from fluid Cheddar whey (Gallardo-Escamilla et al., 2005) and that Mozzarella whey protein concentrate is distinct in flavor from Cheddar whey protein concentrate (Wright et al., 2009; Drake et al., 2009). Instrumental volatile analysis has also suggested that Mozzarella whey proteins have lower concentrations of aldehydes and other volatile compounds over storage time compared to Cheddar whey proteins (Wright et al., 2009), but a direct comparison of the flavor and flavor chemistry of these two primary whey types has not been conducted.

The two main sources of liquid whey in the United States are derived from Cheddar and Mozzarella cheese production (Smith, 2004). Differences in the flavor of

Cheddar and Mozzarella whey protein concentrates may be due to fundamental differences in flavor of the raw product streams: fluid Cheddar and Mozzarella whey. The flavor and flavor components of liquid Cheddar and Mozzarella whey have not been compared. As the two main sources of liquid whey for whey protein manufacture, it is important to understand the flavor chemistry that characterizes the flavors of these products. Understanding the compounds which cause liquid whey flavor may help find methods to improve the flavor variability and stability of liquid whey and ultimately any final whey product. The objectives of this study were to identify and compare the sensory properties and aroma-active compounds of fresh and stored liquid Cheddar and Mozzarella whey.

MATERIALS AND METHODS

Milk

On six separate occasions, raw unhomogenized whole milk (NCSU Creamery, Raleigh, NC) was high temperature short time (HTST) pasteurized with a plate heat exchanger (APV, APV Co. Ltd., England) at 75°C and a holding time of 28 s. The pasteurized milk was cooled to 3^{o}C and stored for no more 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, NC) and 3.34% $\pm$ 0.08 (LactiCheck Milk Analyzer Dual Cow Channel LC-02, P&P International Ltd, Hopkinton, MA), respectively. During cheese manufacture, the pH of whey and cheese were measured with an electrode (Model IQ150, IQ Scientific Instruments, Inc., Loveland, CO) that was standardized at pH 6.97 and 4.03 at 38°C and

kept immersed in 3M KCl at 38°C between readings in order to keep its temperature equal to the temperature of the buffers and samples.

Cheddar cheese whey

Pasteurized milk (3°C) was transferred to a cheese vat (Model 4MX, Kusel Equipment Company, Watertown, WI). The temperature of the cold milk was raised to 31°C with gentle stirring. Once the milk reached 31°C, calcium chloride [0.0018M] (Dairy Connection Inc., Madison, WI) at a rate of 180 ml/454 kg milk and annatto color (cheese color DS double strength, Dairy Connection Inc., Madison, WI) 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 MA11 LYO 250 DCU starter culture, Dairy Connection Inc., Madison, WI) at the rate of 50 DCU/454 kg milk. (DCU is a unit of activity measurement.) The milk was continuously stirred and allowed to ripen for 60 min. The ripened milk, at 31°C, was coagulated with double strength chymosin at a rate of 40 ml/454 kg milk (Star Rennet double strength, Dairy Connection Inc., Madison, WI) 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 vat pasteurizer (Model MPD1050, D&F Equipment Co.,

McLeansville, NC) and pasteurized at 65°C for 30 min to inactivate the starter culture. Cheddar cheese whey was manufactured in duplicate.

Mozzarella cheese whey

Pasteurized milk (3°C) was transferred into a cheese vat (Model 4MX, Kusel Equipment Company, Watertown, WI). The temperature of the cold milk was raised to 35°C with gentle stirring. Once the milk reached 35°C, calcium chloride [0.0018M] (Dairy Connection Inc.) at a rate of 180 ml/454 kg milk and freeze-dried starter cultures were added. The starter culture included *Streptococcus thermophilus* at the rate of 50 DCU/454 kg of milk (Danisco Choozit TA61 LYO 125, Dairy Connection Inc., Madison, WI) and *Lactobacillus delbrueckii* ssp. *lactis* and *Lactobacillus helveticus* (20 DCU/454 kg milk, Danisco Choozit LH100 LYO 20 DCU, Dairy Connection Inc.). 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 drain pH of 6.3 was attained. The whey was drained, pumped into a vat pasteurizer and pasteurized at 65°C for 30 min to inactivate the starter cultures. Mozzarella cheese whey was manufactured in duplicate.

Whey Processing

After the pasteurization of both Cheddar and Mozzarella liquid whey, a portion (30 L) of the liquid whey was collected and cooled to 10^{o}C as the control without fat separation (C1). The remaining whey was then run through a separator (Clair Milky, FJ-125 EAP 115V #17584-115-3, Warenhandels GmbH, Whitewater, WI) for fat separation. The fat content of the whey before separation was 0.20 $\pm$ 0.04% and after separation was 0.07 $\pm$ 0.04% (CEM Smart Trac Rapid Fat Analysis, Matthews, NC). Separated whey was cooled to 10^{o}C. The fat-separated whey was collected and designated as the fat-separated control (C2). Samples were stored in 950 ml amber glass jars capped with PTFE faced PE-lined screw caps (Fisherbrand Amber Wide-Mouth Packers with PTFE Faced PE-lined Caps, Fisher Scientific, Hanover Park, IL) at 3^{o}C in the dark for 3 days. Aliquots (1.5L) of each whey were tested at 0 and 3 days for descriptive sensory analysis and volatile compound extraction and separation. Total solids and protein content of collected wheys were evaluated by forced draft oven (AOAC, 2007).

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 females, 3 males, ages 22-37 y) evaluated the flavor attributes of the liquid whey using a previously established lexicon for fluid whey (Carunchia-Whetstine et al., 2003, Drake et al., 2008; Drake et al., 2003). 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 Spectrum™ descriptive analysis training, panelists were presented with reference solutions of sweet, sour, salty, and bitter tastes to learn to use the universal intensity scale (Drake et al., 2003; Meilgaard et al., 1999). Panelists then evaluated and discussed flavor attributes of both 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 et al., 1999; Drake and Civille, 2003).

Liquid whey products (ca 30 ml) were dispensed into lidded 58 ml soufflé cups with three-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 on separate occasions. Products were scored using paper ballots or computerized ballots using Compusense ™ version 5.0 (Compusense, Guelph, Canada).

Volatile compound extraction

SPME GC-MS

Volatile compounds of wheys were evaluated by SPME GC-MS. SPME GC-MS was conducted using a modified method of Wright et al. (2006). Five grams of liquid whey with 10% NaCl w/w (EMD Chemicals Inc. Sodium Chloride Crystals #SX0420-1,

VWR International, West Chester, PA) and 10 µl internal standard solution (2-methyl-3-heptanone in methanol at 8.1 ppm; Aldrich, Sigma-Aldrich, Milwaukee, WI; VWR International, West Chester, PA) were placed into 20 ml autosampler vials with steel screw tops containing silicone septa faced in Teflon (Microliter Analytical, Sawanee, FL). 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, CA). 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 sec 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 250°C held for 5 min. SPME fibers were introduced into the split/splitless injector at 250°C. An Rtx-5ms column (Rtx-5ms 30 m length × 0.25 mm inner dia × 0.25 um film thickness; Restek, Bellefonte, Pa., U.S.A) was used for all analyses at a constant flow rate of 1 ml/min. Purge time was set at 1 min. The MS transfer line was maintained at 250°C with the Quad at 150°C and Source at 250°C. Each sample was injected in triplicate for each treatment replication.

Direct solvent extraction of liquid whey

Solvent extraction was conducted using the modified methods of Carunchia-Whetstine et al. (2003). One liter of liquid whey was divided into six Teflon bottles (Nalgene, Rochester, NY, capacity of 250 ml) with Tefzel closures (Nalgene, Rochester, NY). Forty-five ml of diethyl ether (EMD Chemicals In., Gibbstown, NJ), 50 g sodium

chloride (EMD Chemicals Inc. Sodium Chloride Crystals #SX0420-1, VWR International, West Chester, PA) and 20 µl of internal standard at 81 ppm (2-methyl-3-heptanone, 2-methyl-pentanoic acid in methanol, Sigma Aldrich, Milwaukee, WI) were added to each bottle. The bottles were then shaken for 30 min on a Roto mix (Type 50800: Thermolyne Dubuque, IA) at high speed and centrifuged at 1459 x g for 10 min in order to separate the solvent phase from the mixture. After centrifugation, the solvent phase containing the extracted volatile components was removed by pipette from each centrifuge bottle to the same amber glass jar. This extraction was repeated twice with the addition of 45 ml of diethyl ether to each bottle, each time. After the solvent was removed the third time, the bottles were centrifuged a fourth time without ether addition and remaining solvent was collected into the same amber glass jar. The extracted solvent was then concentrated to 150 ml under a gentle stream of nitrogen gas.

Solvent assisted flavor evaporation (SAFE)

Volatile compounds from the liquid whey extracts were distilled using SAFE (Ace Glassware, Vineland, NJ). The assembly used was similar to that described by Engel et al. (1999). A diffusion pump combined with a rotary vane pump was used as the vacuum system. The SAFE apparatus was connected to a primary trap and a secondary trap submerged in liquid nitrogen. The distillation procedure was carried out over 1 h under vacuum (10^5Torr). The liquid sample was poured into the SAFE apparatus and introduced drop-wise into the vacuum until all of the liquid extract had been placed under vacuum conditions. The SAFE apparatus was kept at 40°C with a circulating water bath.

Phase Separation

Following SAFE, the distillate was concentrated under a gentle nitrogen stream to 20 ml. The concentrated distillate was washed twice with 3 ml of sodium bicarbonate (Fischer Scientific, Fairlawn, NJ) and mixed thoroughly. After each wash, the bottom layer, the water phase of the distillate, was removed and collected into a separate test tube. The concentrated solvent was then washed with 2 ml of saturated sodium chloride solution three times. Each time, the solution was mixed thoroughly and the water phase removed and collected into the same test tube. The remaining layer was collected as the neutral/basic fraction. Acidic volatiles were recovered by acidifying the water layer collected from the concentrated distillate with hydrochloric acid (18 w/v %) (Sigma Aldrich, Milwaukee, WI) to a pH of 2-2.5. The acidified sample was then extracted three times with 5 ml ethyl ether. All extracts were dried over anhydrous sodium sulfate (VWR International, West Chester, PA) before concentration to 0.5 ml under a nitrogen gas stream.

Gas chromatography-mass spectrometry (GC-MS) *of SAFE extracts*

An Agilent 6890N GC with 5973 inert mass selector detection (MSD) (Agilent Technologies Inc., Santa Clara, CA) was used to analyze solvent extracts. Separations were performed on a fused silica capillary column (Rtx-5ms 30 m length × 0.25 mm inner dia × 0.25 um df; Restek, Bellefonte, PA). Helium at a constant flow rate of 1ml/min was used. The oven temperature was programmed with an initial temperature of 40^{o}C for 3

min, a ramp of 10°C/min up to 90°C, followed by a ramp of 5°C/min up to 200°C and 10 min holding time, followed by another ramp of 20°C/min to a final temperature of 250°C for a final hold time of 10 min. The MSD held a solvent delay for 3 min. MSD scan parameters were set from 35 to 300 m/z. Each extract (2 µl) was injected in the splitless mode. Duplicate analyses were performed on each sample replication.

Gas chromatography-olfactometry (GC-O)

GC-O analysis was performed using an HP 5890 series II gas chromatograph (Hewlett-Packard Co., Palo Alto, CA) equipped with a flame ionization detector (FID), a sniffing port, and a split/splitless injector. Neutral/basic and acidic fractions were analyzed from each solvent extraction. Two µl were injected onto a polar capillary column (Rtx-Wax 30 m length × 0.25 mm inner dia × 0.25 um film thickness; Restek, Bellefonte, PA) and a nonpolar capillary column (Rtx-5ms 30 m length × 0.25 mm inner dia × 0.25 um film thickness; Restek, Bellefonte, PA). Column effluent was split 1:1 between the FID and the sniffing port using deactivated fused silica capillaries (1-m length × 0.25-mm inner dia). The GC oven temperature was programmed from 40°C to 200°C at a rate of 10°C/min with an initial hold for 3 min and final hold of 20 min. The FID was maintained at a temperature of 300°C. The sniffing port was maintained at 105°C. The sniffing port was supplied with humidified air at 30 ml/min. The post-peak intensity of aroma active compounds was evaluated (Grosch, 1993; van Ruth, 2001).

Each extract was sniffed in duplicate by two experienced sniffers, each with >50 h experience on GC-O of dairy products on both polar and nonpolar columns.

Aroma Extract Dilution Analysis (AEDA)

AEDA was performed under the same conditions as those for post peak intensity GC-O. Two representative samples of both Cheddar and Mozzarella liquid wheys were analyzed. NB fractions were injected onto the DB-5ms (Rtx-5ms 30 m length × 0.25 mm inner dia × 0.25 um df; Restek, Bellefonte, Pa., U.S.A.) capillary column and acid fractions were injected onto the DB-WAX (Rtx-Wax 30 m length × 0.25 mm inner dia × 0.25 um film thickness; Restek, Bellefonte, Pa., U.S.A.) capillary column. Each sample was diluted stepwise at a ratio of 1:3 (vol/vol) with diethyl ether. Samples were evaluated by two experienced sniffers until no odors were detected. The greatest dilution in which a compound was sniffed was reported as the flavor dilution (log_3FD) factor (Grosch, 1993).

Volatile Compound Identification

Volatile compounds from SAFE and SPME were identified using the NIST 2005 library of spectra and comparison of spectra of authentic standards injected under identical conditions. Relative abundance of compounds was calculated using the calculated recovery of the internal standard concentration to determine relative concentrations of each compound. Retention indices were calculated (van den Dool and Kratz, 1963) using an alkane series (Sigma Aldrich). For aroma active compound identity verification, mass

spectra, retention index, and aroma properties were also compared with authentic standards injected under identical conditions.

Statistical analyses

Analysis of variance (ANOVA) was conducted to determine if differences existed in sensory properties or volatile compound relative abundances using XL-STAT (XL-STAT version 2009, 1995-2009, Addinsoft, Paris, France). 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 $\pm$ 0.10 and 6.45 $\pm$ 0.18 percent solids and 1.40 $\pm$ 0.20 and 1.30 $\pm$ 0.08 percent protein, respectively. Percent fat content of Cheddar and Mozzarella whey prior to fat separation was 0.18 $\pm$ 0.04 and 0.21 $\pm$ 0.05, respectively and after fat separation was 0.07 $\pm$ 0.03 for both whey types. The pH of both whey types was 6.38 $\pm$ 0.05 and this value did not change with storage time. These values were comparable to previous studies (Gallardo-Escamilla et al., 2005; Carunchia-Whestine et al., 2003).

Sensory Analysis

Liquid whey. Flavor terms documented in liquid wheys included aroma intensity, sweet aromatic, sour aromatic, cardboard, and cooked/milky flavors (Table 3.1). Sweet taste, along with the feeling factor, astringency were also documented. Previous research has identified these flavors in liquid whey (Carunchia-Whetstine et al., 2003; Karagul-Yuceer et al., 2003; Tomaino et al., 2004; Gallardo-Escamilla, et al., 2005).

Consistent with previous research on Cheddar liquid whey (Tomaino et al., 2004), the flavors of both Cheddar and Mozzarella liquid whey changed with storage time ($p<0.05$) (Table 3.1). Sour aromatic was not detected in Cheddar liquid whey (Table 3.1). Initially, Cheddar liquid whey with and without fat separation were similar and were characterized by sweet aromatic and cooked/milky flavors. After storage, both Cheddar samples acquired a cardboard flavor while cooked/milky and sweet aromatic flavors decreased in intensity. Cardboard intensity was higher in Cheddar whey without fat separation ($p<0.05$). Cooked/milky and sweet aromatic attributes decreased with time, regardless of fat separation (Table 3.1).

Aroma intensity and sour aromatic attributes were lower in Mozzarella whey with fat separation than the whey without fat separation, however, fresh Mozzarella liquid whey with and without fat separation were similar in flavor (Table 3.1). After storage, similar to Cheddar whey, both Mozzarella samples decreased in cooked/milky flavor while cardboard flavor was detected. As with Cheddar whey, cardboard flavor intensity was higher in whey without fat separation ($p<0.05$). The inclusion of fat separation in liquid whey processing, a standard unit operation in industrial whey processing, affected

the flavor stability of liquid whey. In Mozzarella, fat separation lowered cardboard flavor intensity after storage and decreased sour aromatic flavor before storage. In Cheddar whey, fat separation decreased cardboard flavor intensity after storage.

Cheddar and Mozzarella liquid whey were similar in how they changed over storage: cardboard flavor increased (Table 3.1, Figure 3.1). The two whey types had similar sensory attributes but were distinct in flavor, especially in intensity. Cardboard flavor was highest in the unseparated fat Cheddar whey after storage. Both fresh Cheddar liquid wheys had higher sweet aromatic intensities than both Mozzarella wheys. As such, fresh and stored Mozzarella liquid wheys were blander in overall flavor compared to Cheddar liquid whey. This is in agreement with previous studies which also reported differences in Cheddar and Mozzarella fluid whey flavor and between Cheddar and Mozzarella whey protein products (Gallardo-Escamilla et al., 2005; Wright et al., 2009; Drake et al., 2008). Whey type differences have been associated with the use of different starter cultures and processing techniques used to produce different types of cheese (Carunchia-Whetstine et al., 2003; Karagul-Yuceer et al., 2003; Gallardo-Escamilla, et al., 2005).

Volatile analysis

Aldehyde relative abundance. The relative abundance of selected volatile aldehydes in the wheys, hexanal, heptanal, octanal, nonanal, and decanal, were calculated. These aldehydes are well known as volatile lipid oxidation products in liquid whey (Carunchia-Whetstine et al., 2003; Tomaino et al., 2004; Gallardo-Escamilla et al., 2005;

Karagul-Yuceer et al., 2003). To our knowledge, decanal has not been documented in fresh liquid whey but has been reported in WPC (Carunchia-Whetstine et al., 2005; Stevenson and Chen, 1996; Evans et al., 2009).

Two different extraction methods were used for determining volatiles in the liquid wheys (Tables 3.2, 3.3). In order to extract representative volatiles from the wheys, SPME was used to detect lower molecular weight volatiles while SAFE was applied to recover higher molecular weight volatiles. Aldehydes, except for decanal in Mozzarella, were detected by both extraction methods. Decanal was not detected by the SPME method but was detected by SAFE. Relative abundance of the compounds by each extraction method also differed, as expected, due to extraction differences between the methods. The SAFE method purportedly has greater compound recovery than SPME in milk products (Havemose et al., 2007). According to the theoretical principle behind SPME, the absorption coating of the SPME fiber does not have a large enough partition coefficient between the coating and sample matrix to exhaustively extract most of the analytes (volatile compounds) in the matrix (Zhang et al., 1994). Higher sensitivity allows easier identification of compounds with low concentration and potentially high impact on the food matrix by GC-MS (Havemose et al., 2007). Consistent with these differences in extraction capacity, total aldehyde concentrations from Mozzarella whey recovered by SPME displayed no significant changes throughout storage or with the removal of fat (Table 3.2). In contrast, recovery by SAFE indicated significant differences due to storage and fat removal (Table 3.3). SPME, although not as sensitive as SAFE, is a useful technique for fast and simple applications (Havemose et al., 2007).

Unlike the multiple steps involved in SAFE, SPME integrates sampling, extraction, concentration, and sample introduction into a single step without the use of solvent (Zhang et al., 1994).

Short chain aldehydes have been attributed to cardboard flavor in whey products (Tomaino et al., 2004; Quach et al., 1999; Mills, 1986; Lee et al., 1995; 1996). Similar to sensory data, the volatile compound concentrations of Cheddar and Mozzarella whey samples changed over time in refrigerated storage. Cheddar whey without fat separation had higher amounts of hexanal after storage than Cheddar whey with fat separation by both extraction methods (Tables 3.2, 3.3). Overall, hexanal was most abundant in both Cheddar and Mozzarella whey by the SPME method. However, based on the SAFE method results, hexanal was the least abundant aldehyde detected. Nonanal, instead, was the most abundant aldehyde detected by the SAFE method in both Cheddar and Mozzarella whey. In agreement with SPME results, hexanal did increase during storage in Cheddar whey without fat separation. Total aldehyde concentration was highest in Cheddar liquid whey without fat removal by both SAFE and SPME methods. These results are consistent with sensory changes with storage and fat separation (Figure 3.1).

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 two whey types (Figure 3.1). However, increases in cardboard flavor over time for Mozzarella liquid whey samples were not concurrent with aldehyde concentrations after storage. Instead of an increase in short chain aldehyde concentration during storage, there was no change or a

decrease. This may be due to the further degradation of the aldehydes into other compounds, for example hexanal has been known to degrade into hexanoic acid (Schieberle and Grosch, 1981).

GC-O

Post peak time intensity and AEDA aroma analysis were performed on neutral/basic and acidic fractions for each whey before and after storage to characterize flavor-contributing volatile compounds (Table 3.4). A variety of aromas were detected including aldehydes, ketones, esters, sulfur compounds, pyrazines, and free fatty acids. Fifty aroma active compounds were found in whey samples. Of these compounds, 29 were tentatively identified by comparing RI, odor, and MS with authentic standards, 8 were tentatively identified by comparing RI and odor with authentic standards, 3 were tentatively identified by RI and previously published standards and 10 remained unknown. The majority of these compounds were reported previously in liquid or dried whey protein products (Karagul-Yuceer et al., 2003; Morr and Ha, 1991; Evans et al., 2009a; Evans, 2009; Carunchia-Whetstine et al., 2005; Wright et al., 2006; Mahajan et al., 2004; Quach et al., 1999).

AEDA is a semiquantitative technique used to understand the most potent aroma and flavor contributing compounds in a sample. Compounds with high FD factors are considered more crucial to a product's flavor than those with lower FD factors. AEDA does not represent actual concentrations of the compounds in the sample (Audouin et al., 2001). Instead, $\log_3$ FD factors for the same compound differing by $\geq$2 $\log_3$ factors are

considered suggestive of concentration differences (Audouin et al., 2001). To our knowledge, aroma active volatiles have only previously been reported with liquid Cheddar wheys (Karagul-Yuceer et al., 2003).

Most of the 50 aroma-active compounds identified in the liquid whey samples are produced by lipid oxidation or protein degradation by proteolysis and non-enzymatic browning. These are the two main flavor reactions in dairy products (Ferretti and Flanagan, 1971; Min et al., 1990). First, lipid oxidation in whey processing initiates during cheese manufacture (Tomaino et al., 2004). Lipid oxidation begins with the autoxidation of unsaturated fatty acids producing unstable hydroperoxides, which decompose to a wide range of carbonyl products, including short chain fatty acids, many of which can contribute to off-flavors in dairy products (Lee and Morr, 1994; Badings 1991; Carunchia-Whetstine et al., 2005; Drake et al., 2008; 2009; Wright et al., 2009; O'Connor and O'Brien, 2006).

Free fatty acids in whey are known to cause oxidation by hydrolysis and become precursors for lipid oxidation products. These free fatty acids are originally bound to triglycerides and phospholipids found in the milk but become detached during processing. In milk, free fatty acids only represent <0.5% of total fat and are not typically classified as a separate lipid class of milk (Cadwallader et al., 2007). Short chain free fatty acids ($\leq$8 carbons) can impart desirable and undesirable flavors depending on the product and the concentration of the compound (Cadwallader et al., 2007). Short chain free fatty acids, acetic (vinegar) and butyric (cheesy) acids, were identified in this study and in previous

studies with liquid whey (Karagul-Yuceer et al., 2003; Tomaino et al., 2001; 2004). Starter cultures may also impact the free fatty acid level in whey products especially in Cheddar cheese by releasing these free fatty acids from triglycerides and phospholipids found in the milk (Ferretti and Flanagan, 1971; Kim et al., 2003; Tomaino et al., 2001; Carunchia-Whetstine et al., 2003) and at high concentrations, can impart rancid flavors.

Unsaturated fatty acids of longer lengths have also been found in Cheddar liquid whey (Tomaino et al., 2001; 2004; Carunchia-Whetstine et al., 2003). Some of these acids include oleic, linoleic, and linolenic acids which are known to oxidize and produce aromatic aldehydes, ketones, and alcohols (Frankel, 1998). Oxidized oleic acid produces 8-, 9-, 10-, and 11-hydroperoxides which then produce secondary products including decanal, nonanal, heptanal octanal, decenal, and 2-nonenal (Frankel, 1998). Linoleic acid autoxidation produces 9-, 10-, 12-, and 13-hydroperoxides, and further degrade into 2,4-decadienal, hexanal, and 2,4-nonadienal among other secondary volatile decomposition products. Linolenic acid autoxidation produces 9-, 12-, 13-, and 16- hydroperoxides and degrades to produce propanal and butanal (Frankel, 1998).

As previously mentioned, lipid oxidation is not completely responsible for the formation of the identified aroma-active compounds. The second flavor reaction in dairy products results from protein degradation consisting of proteolysis and non-enzymatic browning (Ferretti and Flanagan, 1971; Min et al., 1990). These processes produce aromatic volatile compounds by amino acid catabolism (Yvon and Rijnen, 2001; Urbach, 1995). The degradation of proteins creates free amino acids which can then produce amines, aldehydes, alcohols, acids, phenols, sulfur compounds and indoles by means of

decarboxylation, transamination, oxidation, and reduction (Yvon and Rijnen, 2001; Urbach, 1995). The addition of enzymes and bacterial starter culture used to produce cheeses may still exist in liquid whey even after heat treatment of collected whey. The existence of these enzymes may lead to more amino acid degradation and subsequent catabolism to form aromatic compounds during the storage of liquid whey (Eriksson, 1970).

Saturated and unsaturated aldehydes were detected in all wheys in the present study. Aldehydes are among the most potent flavor compounds with flavor thresholds less than 1 ppm in milk (Mills et al., 1986; Karagul-Yuceer et al., 2003b). Additionally, lower molecular weight aldehydes have even lower aroma thresholds (Kinsella et al., 1967). Short chain aldehyde detection and increases with storage were identified in this study and have been previously documented in Cheddar liquid whey (Tomaino et al., 2004; Karagul-Yuceer et al., 2003; Carunchia-Whetstine et al., 2003; Gallardo-Escamilla et al., 2005). Unsaturated aldehydes, (Z)-4-heptenal, (E)-2-nonenal, (E,E)-2,4-nonadienal, (E,Z)-2,6-nonadienal, and (E)-4,5-epoxy-2(E)-decenal, were identified in all samples and all have been identified previously in liquid whey except (E)-4,5-epoxy-2(E)-decenal (Karagul-Yuceer et al., 2003). (E)-4,5-epoxy-2(E)-decenal has been detected as a potent odorant in heated butter (Gassenmeier and Schieberle, 1994) and rennet casein (Karagul-Yuceer et al., 2003c. These unsaturated aldehydes are formed from the degradation of unsaturated fatty acids and further degrade to produce secondary products including saturated aldehydes (Frankel, 1998). Unsaturated aldehydes have been associated with stale, cardboard off flavors in dairy products (Karagul-Yuceer et al., 2002; 2003; Morr

and Ha, 1991). The saturated aldehydes, 2/3-methyl butanal, hexanal, heptanal, octanal, nonanal, and decanal have also been identified in dried and liquid whey products (Evans et al., 2009; Carunchia-Whetstine et al., 2003a; 2005; Karagul-Yuceer et al., 2003a; Gallardo-Escamilla et al., 2005) and have also been associated with cardboard, fatty and unclean flavors in dairy products (Lloyd and Drake, 2009; Wright et al., 2009; Tomaino et al., 2004).

In this study, after storage, (E,E)-2,4-nonadienal, (E,Z)-2,6-nonadienal, (E)-4,5-epoxy-2(E)-decenal, hexanal and nonanal increased (FD factor increases of 2 or greater) in Cheddar whey regardless of fat removal. In Mozzarella whey, only octanal increased after storage in whey with fat removal (Table 3.4). Both (E,Z)-2,6-nonadienal and decanal had higher FD factors in Mozzarella whey without fat removal than whey with fat removal regardless of storage. (E)-2-nonenal decreased in Cheddar whey with fat separation over storage while the FD factor for this compound increased in Mozzarella whey with fat separation. All of these aldehydes, including octanal had higher FD factors in Cheddar whey without fat removal than Cheddar whey with fat removal.

Phenylacetaldehyde and 2-phenethanol (floral/rosy aroma) have been identified in whey products (Carunchia-Whetstine et al., 2005; Mahajan et al., 2004). These compounds are derived from the Strecker degradation of aromatic amino acids, especially phenylalanine (Singh et al., 2003; Carunchia-Whetstine et al., 2005). Phenacetaldehyde did not change significantly in either whey in this study. However, 2-phenethanol decreased in Cheddar samples after storage while Mozzarella samples increased after

storage. Cheddar whey without fat removal had higher intensities than whey with fat removal.

Six ketones were detected in the wheys and have all been reported in whey protein products (Evans et al., 2009; Carunchia-Whetstine et al., 2005; Karagul-Yuceer et al., 2003). Diacetyl (2,3-butanedione) and 2,3-pentanedione have a buttery aroma. Diacetyl is commonly found in dairy products, however its formation mechanism is still being debated (McSweeney and Sousa, 2000). Diacetyl may be formed as a thermally generated aromatic compound produced from non-enzymatic browning during milk pasteurization (Scanlan et al., 1968), from lactic fermentation by the oxidative decarboxylation of alpha-acetolactic acid (Cronin and Rispin, 1996), or through the use of specific starter cultures used for cheese production (Cronin and Rispin, 1996; McSweeney and Sousa, 2000). In model systems, researchers have also noted that the lack of an amino acid or amine will not form diacetyl or 2,3-pentanedione. The mechanism responsible for ketone formation only occurs with the Maillard reaction and not base-catalyzed cleavage (Weenen and Apeldorn, 1996). Diacetyl concentration also increases as temperature increases (Scanlan et al., 1968). Both diacetyl and 2,3-pentanedione are also important aroma contributors in yogurt fermented by mesophilic and thermophilic mixed lactic starters (Imhof et al., 1994; 1995; Gallardo-Escamilla et al., 2005b; Ott et al., 2000). 2,3-pentanedione has a higher odor impact in *Streptococcus thermophilus* fermented milk than 2,3-butanedione (Imhof et al.,1994;1995). In agreement with this current study, Mozzarella liquid whey, produced from a thermophilic starter culture blend, had higher FD log factors of 2,3-pentanedione

and lower FD log factors of diacetyl compared to Cheddar liquid whey samples (Table 3.4).

(Z)-1,5-octadien-3-one, 1-hexen-3-one, 1-octen-3-one, 1-nonen-3-one, and o-aminoacetophenone have been identified as compounds responsible for stale, cardboard, or metallic flavors in dairy products (Morr and Ha, 1991; Carunchia-Whetstine et al., 2005; Parks et al., 1964; Karagul-Yuceer et al., 2002). (Z)-1,5-octadien-3-one has a geranium odor and has recently been identified in whey and serum protein concentrates (Evans et al., 2009). This ketone is a degradation product of linolenic acid (Ulrich and Grosch, 1988). 1-hexen-3-one has a sweet/rubbery aroma, while 1-octen-3-one and 1-nonen-3-one have a mushroom aroma. 1-hexen-3-one has been found in nonfat dry milk (Karagul-Yuceer et al., 2002), butter (Mallia et al., 2008); and fresh goat cheese (Carunchia-Whetstine et al., 2003b). 1-octen-3-one is a lipid oxidation product formed by the oxidation of linoleic acid (Frankel et al., 1998) and has been associated with cardboard flavor in Cheddar liquid whey (Karagul-Yuceer et al., 2003). (Z)-1,5-octadien-3-one and 1-octen-3-one contribute to the metallic off-flavor in butter and in combination with (E)-2-nonenal, may cause cardboard or oxidized off-flavor in skim milk (Carunchia-Whetstine and Drake, 2007), and are contributors to Cheddar cheese flavor (Zehentbauer and Reineccius, 2002). 1-nonen-3-one has been detected in yogurt (Ott et al., 1997; 2000) and dried whey protein (Evans et al., 2009). Ott et al. (1997) hypothesized this ketone may originate from unsaturated fatty acid oxidation. Both Cheddar and Mozzarella whey increased in 1-hexen-3-one and 1-octen-3-one after storage in whey with fat removal (Table 3.4). (Z)-1,5-octadien-3-one was not detected in Mozzarella without fat removal

and 1-nonen-3-one was not detected in any Mozzarella samples nor in Cheddar whey with fat removal after storage.

o-Aminoacetophenone (stale/grape/tortilla aroma) has been reported in Cheddar WPC and nonfat dry milk powder (Carunchia-Whetstine et al., 2005; Karagul-Yuceer et al., 2002; 2004b). o-Aminoacetophenone may be formed from the degradation of free amino acids such as tryptophan during the presence of the Maillard reaction (Parks et al., 1964). This compound has been associated with stale flavor in stored milk powders and rennet casein (Parks et al., 1964; Karagul Yuceer et al., 2003c). In this study, o-aminoacetophenone FD factors increased after storage in all samples except Cheddar liquid whey with fat removal.

Methional, dimethyl disulfide (DMDS), and dimethyl trisulfide (DMTS) have all been documented in whey products (Yvon and Rijnen, 2001; Wright et al., 2006; Mahajan et al., 2004; Wright et al., 2009; Evans et al., 2009). Methional (cooked potato aroma) is a Strecker degradation product formed from the degradation of methionine. From methionine, DMDS and DMTS with onion/cabbage odors are further degradation products from intermediate products methanethiol and dimethyl sulfide (Yvon and Rijnen, 2001; Wright et al., 2006; Carunchia-Whetstine et al., 2005). Sulfur compounds may also originate from the release of activated sulfhydryl groups from β-lactoglobulin during heat denaturation of whey proteins (Calvo and De la Hoz, 1992). Sulfur compounds have particularly low sensory thresholds and may contribute considerably to the flavor of a food even at low concentrations (Morr and Ha, 1993; Wright et al., 2006). Methional decreased in both Cheddar and Mozzarella samples after storage, although Cheddar

samples decreased more drastically than Mozzarella. DMDS was not detected in Mozzarella samples after storage (Table 3.4).

Delta-decalactone, delta-dodecalactone, gamma-nonalactone and gamma-octalactone were detected in wheys and have previously been reported in whey products (Karagul-Yuceer et al., 2003; Evans, 2009; Carunchia-Whetstine et al., 2003). In the current study, Cheddar whey samples increased in delta-decalactone and gamma-nonalactone after storage while only Cheddar whey without fat removal increased in gamma-octalactone after storage. In Mozzarella samples, delta-decalactone and delta-dodecalactone had high FD factors in fresh whey with fat separation, while gamma-nonalactone increased after storage in Mozzarella whey without fat removal. Lactones are thermally generated aroma compounds produced during heat processing (Dimick et al., 1969; Parliment et al., 1966). In fresh milk fat, hydroxy acids, naturally formed in the mammary gland of ruminants from saturated fatty acids, are esterified into triglycerides which form saturated lactones from hydrolysis during heating or storage (Dimick et al., 1969). Lipid concentration of the whey or milk determines the amount of lactones produced (Carunchia Whetstine et al., 2005). Fresh Mozzarella whey with fat removal had higher intensities of delta-decalactone and delta-dodecalactone compared to whey without fat removal while stored Cheddar whey without fat removal had higher intensities of gamma-nonalactone and gamma-octalactone compared to whey with fat removal. Fat removal in Cheddar liquid whey seemed to decrease lactone intensity while fat removal in fresh Mozzarella liquid whey increased intensity. Since lactones are heat induced,

Mozzarella liquid whey may have higher lactone intensity due to the higher temperatures utilized during Mozzarella cheese production and whey pasteurization.

Other thermally generated compounds, pyrrolines, pyrazines, and furanones were also detected in wheys. 2-Isopropyl-3-methoxypyrazine, 2-isobutyl-3-methoxypyrazine, sotolon, 2-methyl-3-furanthiol and 2-acetylpyridine are products of the Maillard reaction which most likely form during heat processing of lysine with glucose or fructose (Friedman, 1996; Mahajan et al., 2004; Arnoldi and Corain, 1996). All have been previously reported in liquid Cheddar whey (Karagul-Yuceer et al., 2003). 2-acetyl-1-pyrroline was tentatively identified by retention index and aroma. This compound has a low flavor threshold and contributes to cooked popcorn-like notes in fresh liquid whey (Karagul-Yuceer et al., 2003). 2-acetyl-1-pyrroline is believed to be formed by the interaction of pyruvaldehyde with 1-pyrroline which is formed from proline (Bendall, 2001; Karagul-Yuceer et al., 2003). 2-methyl-3-furanthiol may be formed from the degradation of sulfur-containing amino acids (Lee et al., 1996) or more specifically, from the retro-Aldol reaction of mercapto-2-propanone and hydroxylacetaldehyde during the Maillard reaction (Hofmann and Schieberle, 1996). 2-methyl-3-furanthiol and 2-acetylpyridine both decreased in intensity after storage in Cheddar samples while 2-methyl-3-furanthiol increased in Mozzarella whey with fat but decreased with fat removal after storage. The exact opposite was observed for 2-acetylpyridine in Mozzarella wheys. Both Cheddar and Mozzarella increased in 2-isopropyl-3-methoxypyrazine after storage in whey with fat separation. 2-isobutyl-3-methoxypyrazine was detected only in Cheddar whey.

Sotolon (3-hydroxy-4,5-dimethyl-2(5H)-furanone) is a sugar degradation product generated by heat (Takahashi et al., 1976) by means of the 4-hydroxyisoleucine biopathway (Hofmann and Schieberle, 1996). Another sotolon production pathway may originate from the reaction of hydroxyacetaldehyde and 2,3-butanedione (Hofmann and Schieberle, 1996). Sotolon has been reported in skim and whole milk powder, whey protein concentrate, and cheese (Karagul-Yuceer et al., 2002; Carunchia-Whetstine et al., 2003b; Evans, 2009). This compound may increase in stored products due to Maillard reactions (Karagul-Yuceer et al., 2002) and has a sweet, sugar-like aroma, which may contribute to sweet aromatic flavor (Carunchia-Whetstine et al., 2003b). In agreement with previous studies, all samples increased in sotolon after storage. Sweet aromatic flavor decreased after storage in Cheddar wheys, but this may be due to increases in aroma-active lipid oxidation products (with stale, fatty aromas) which may have overpowered sensory contributors of sotolon.

Esters, ethyl propionate and ethyl hexanoate, both with fruity aromas, are formed by the esterification of alcohols by bactericidal enzymes (Dumont and Adda, 1979). Ethyl propionate has been documented in whey and serum protein (Evans, 2009) and ethyl hexanoate has been documented in milk (Friedrich and Acree, 1998). Both esters have been documented in cheese (Yang and Min, 2006). Ethyl propionate decreased in Cheddar without fat removal while this compound increased in Mozzarella whey with fat removal over storage. Ethyl hexanoate decreased in Cheddar with fat removal and was not detected in Cheddar without fat removal after storage.

p-Cresol (4-methylphenol), a phenolic compound with a phenol/bandaid-like aroma, and skatole (3-methylindole), a nitrogen-containing heterocyclic compound with a fecal/mothball aroma have been identified in this study and have been identified in milk (Bendall, 2001), nonfat dry milk (Karagul-Yuceer et al., 2002), whole and skim milk powders (Carunchia-Whetstine and Drake, 2007), rennet casein (Karagul-Yuceer et al., 2003c) and dried whey protein products (Evans et al., 2009). p-Cresol and skatole contribute to the flavor of cheeses and farmhouse Cheddar cheese at low levels (Suriyaphan et al., 2001). Guaiacol (2-methoxy-phenol), also a phenolic compound, has a smoky/sweet odor and has been detected in butter (Mallia et al., 2008), aged cheese (Wallace and Fox, 1997) and whey products (Evans et al., 2009; Carunchia-Whetstine et al., 2005). The formation of these compounds in milk and dairy products may result from a multiple of reasons including pasture-derived, amino acid catabolism, direct addition (for specific cheese production), processing, or contamination from the environment. In pasture, certain types of weeds (Crucifera), lucerne, or *Brassica* sp. may increase the concentration of indole, skatole, mercaptans, sulfides, nitriles, and thiocyanates during the cow's metabolism (Forss, 1979). The loss of the alanine moiety from tyrosine and tryptophan causes the formation of phenol and indole, respectively (Dumont and Adda, 1978). Heat treatment may release free phenolic compounds (O'Connell and Fox, 2001) and result in Maillard reaction-derived cresol and phenol compounds in whey powder (Ferretti and Flanagan, 1971). Inadequately filtered rennet may also produce p-cresol from salt-tolerant lactobacilli in Gouda cheese (Badings et al., 1967). p-Cresol and skatole during storage of nonfat dry milk (Karagul-Yuceer et al., 2002). Cheddar whey

samples increased in p-cresol with storage and increased in skatole only in Cheddar whey without fat removal. Although Mozzarella samples did not increase or decrease significantly, skatole had higher intensity in whey with fat removal than whey without fat removal.

DISCUSSION

To our knowledge, aroma active volatiles have only previously been reported with liquid Cheddar wheys (Karagul-Yuceer et al., 2003). Similar results from the current study were reported in the previous work. Of 34 aroma active volatiles identified by Karagul-Yuceer et al. (2003), 18 were also identified in the 50 aroma active volatiles of the present study with liquid whey. Although the sensory and instrumental methods used in both studies were similar, Cheddar whey samples were not from the same location. Karagul-Yuceer et al. (2003) received commercial liquid whey samples from two different Cheddar-cheese producers, who also rotated culture blends, resulting in four different starter culture blends used for Cheddar cheese production. The different manufacturers also probably utilized milk from different sources and possibly different regions of the country. These whey samples were produced, frozen, shipped overnight, and then thawed upon receipt. In the present study, only one starter culture blend was used for each cheese type and milk used for the study was received from the same dairy during the same season. Further, unlike the previous study, in the current study, fresh whey was not frozen before volatile compound extraction at 0 d. Freezing liquid whey at -20^{o}C does not inhibit the progression of lipid oxidation (Tomaino et al., 2004) and this may have influenced aroma-active compound recoveries. Previous work has also reported variability in volatile

and sensory results between and within whey manufacturers (Carunchia-Whetstine et al., 2003). These differences may explain why only 18 of the 34 previously documented aroma active volatile compounds were detected in the current study. Although there was less variability in whey production in the current study, more aroma active volatiles were identified.

Cheddar and Mozzarella dried whey protein products have distinct flavor differences which have been evaluated and identified by instrumental and sensory analysis (Carunchia-Whetstine et al., 2005; Wright et al., 2009). In the current study, Cheddar and Mozzarella liquid wheys were evaluated before further whey processing to understand initial flavor differences in liquid wheys.

Hexanal and nonanal were the predominant aldehydes detected by SPME and SAFE, respectively (Tables 3.2, 3.3). Both aldehydes have been detected in other storage studies with Cheddar liquid whey and whey products (Quach et al., 1999; Mills, 1986; Tomaino et al., 2004; Lee et al., 1995; Carunchia-Whetstine et al., 2005) and have been attributed to cardboard off-flavor. Hexanal is formed from linoleic acid oxidation while nonanal is formed from oleic acid oxidation (Frankel, 1998). Both aldehydes increase due to the addition of a lactic acid starter culture (Drake et al., 1999). Hexanal is also produced by the degradation of secondary lipid oxidation products and degradation of products from lactic acid bacteria starter cultures (Suriyaphan et al., 2001), such as 2,4-decadienal (Matthews et al., 1971). Although cardboard flavor was detected in Mozzarella liquid whey after storage (Table 3.1), liquid wheys without fat removal,

especially Cheddar whey, had the highest cardboard flavor intensities and aldehyde concentrations.

Of the 50 aroma compounds detected by GC-O, guaiacol was the only compound not detected in Cheddar liquid wheys after storage regardless of fat separation. 1-nonen-3-one, 2-isobutyl-3-methoxy pyrazine, and (Z)-1,5-octadien-3-one were not detected in any Mozzarella whey samples and DMDS was not detected in Mozzarella whey without fat removal. Thus, most compounds detected by GC-O were identified in both Cheddar and Mozzarella samples. Based on differences of $\log_3$ FD factors $\geq$2 of the same compound signifying a concentration difference (Audouin et al., 2001), only 3 compounds, 2,3-pentanedione, 1-hexen-3-one, and 2-isopropyl-3-methoxy pyrazine increased or decreased after storage similarly amongst all the samples. This small similarity between Mozzarella and Cheddar whey indicates the differences in the development of the aroma compounds due to different starter cultures and perhaps heat treatment during the cheesemake procedure and how these initial differences may influence changes that occur with the removal of fat and time in storage. The main differences between the two whey types were the intensity of the compounds, i.e. based on the FD log factor, and how the compounds increased or decreased after storage due to fat separation.

Focusing on each whey individually, hexanal, (E,E)-2,4-nonadienal, (E)-4,5-epoxy-2(E)-decenal, nonanal, (E,Z)-2,6-nonadienal, gamma-nonalactone, and delta-decalactone all increased in Cheddar whey after storage regardless of fat removal. (E,E)-2,4-nonadienal, (E)-4,5-epoxy-2(E)-decenal, and nonalactone were affected by fat

removal as these compounds were higher in Cheddar whey without fat separation. 2-acetylpyridine, methional, 2-phenethanol decreased over time regardless of fat content. 2-phenethanol decreased but intensities were higher in whey without fat separation. Octanal and phenacetaldehyde remained the same after storage but both were higher in whey without fat separation. The abundance of aroma-active lipid oxidation compounds suggest, similar to Tomaino et al. (2004), that lipid oxidation initiates during the cheese make-procedure. Assuming lipid oxidation does initiate during the cheesemaking process, then this autocatalytic reaction will continue to oxidize and form degradation products throughout time in storage. Also, lipid oxidation requires lipids as a substrate to initiate oxidation. Lipid oxidation still occurred in Cheddar liquid whey with fat removal however, the decrease in fat content also decreased aldehyde content and FD factors compared to whey without any fat removal (Tables 3.2, 3.3, 3.4). Fat separation resulted in decreased concentrations of lipid oxidation products by GC-MS, GC-O, and sensory analysis in Cheddar whey. 2-acetylpyridine, guaiacol, methional, 2-phenethanol are all products of free amino acid degradation. These degradation products may have decreased during storage due to further degradation by a variety of chemical reactions.

In fresh Cheddar liquid whey, the volatile compounds with the highest FD factors ($\geq$8) were diacetyl, 1-octen-3-one, 2-phenethanol, butyric acid, and (E)-2-nonenal. These compounds represent aromatic volatiles from starter culture fermentation, lipid oxidation, and protein degradation. In stored Cheddar liquid whey, diacetyl, 1-octen-3-one, butyric acid, nonanal, delta-decalactone and (E)-4,5-epoxy-2(E)-decenal were highest ($\geq$8 FD factors). After storage, lipid oxidation products 1-octen-3-one, butyric acid, nonanal, and

(E)-4,5-epoxy-2(E)-decenal had high FD factors indicating their strong presence in Cheddar liquid whey. In agreement with sensory analysis, diacetyl, associated with sweet aromatic sensory attribute, did remain in Cheddar liquid whey after storage time although at a lower intensity. Also, the increase in aldehydes agreed with the increase of cardboard flavor noted by sensory evaluation of Cheddar liquid whey (Table 3.1). Nonanal also increased in both Cheddar wheys after storage by SAFE-GC-MS data and GC-O. Although delta-decalactone typically is produced during heat processing, it also increased in refrigerated storage.

In Mozzarella whey, only 2-phenethanol and o-aminoacetophenone increased after storage. Acetic acid decreased after storage in the samples but remained higher in wheys with fat. As seen in previous studies with dried dairy products, o-aminoacetophenone is an indication of storage and typically increases over storage time (Karagul-Yuceer et al., 2002; Carunchia-Whetstine et al., 2005; Parks et al., 1964). As a short chain free fatty acid, acetic acid is considered a type of lipid in dairy products, thus it is no surprise that concentrations of acetic acid were lower in whey with fat removal. Decreases of acetic acid over time may be due to degradation of the acid during storage. The compound with the highest FD factor in fresh Mozzarella was diacetyl ($\geq$8 FD factor) followed by butyric acid and 2-acetyl-1-pyrroline ($\geq$ 6 FD factors). In agreement with sensory analysis, fresh Mozzarella whey had high intensities of cooked/milky and sweet aromatic attributes before storage (Table 3.1). After storage, 2-phenethanol, octanal and (E)-2-nonenal had FD factors $\geq$8 in Mozzarella whey. These compounds indicate an increase in lipid oxidation after storage and an increase in compound formation due to degradation of free

amino acids. Once again, sensory results indicated an increase in cardboard flavor in Mozzarella whey after storage consistent with GC-O results.

GC-O results indicate more changes occurred in Cheddar whey due to storage time and fat removal than Mozzarella whey. Sensory flavor intensities were also generally higher in Cheddar whey compared to Mozzarella whey at each time point. Regardless of fat removal, Cheddar liquid wheys had more aldehyde increases with storage time than Mozzarella wheys. Aldehydes are known lipid oxidation products and are frequently responsible for off flavors in dairy products (Tomaino et al., 2004; Morr and Ha, 1991; Wright et al., 2009; Karagul-Yuceer et al., 2002). By GC-O, Cheddar and Mozzarella liquid wheys have a similar aroma-active compound makeup but as previously mentioned, the intensity and change due to fat removal and storage of the compounds differed between the whey types. Aromas of compounds can change with concentration (Drake and Civille, 2003; MacGibbon and Taylor, 2006) and thus affect overall sensory perception. Sensory results indicated the flavor profile of the two types of whey were distinct from each other and GC-MS volatile and GC-O results documented differences in selected volatile compounds in each whey type. These results suggest that Cheddar whey may be more prone to off-flavor development during processing into whey protein compared to Mozzarella whey, independent of downstream processing differences that may occur. Studies with commercial whey protein concentrates have also suggested that whey proteins from Mozzarella and Cheddar whey are distinct in flavor (Wright et al., 2009; Drake et al., 2009).

Mozzarella and Cheddar cheese whey in the current study differed due to the starter culture and the temperature profile used to produce the specific type of cheese whey. The starter culture produces different volatile compounds and enzymes which influence initial flavor and can impact storage-induced changes (Tomaino et al., 2004). Even different strains of the same starter cultures can produce distinct volatile compound profiles (Tomaino et al., 2004; Carunchia-Whetstine et al., 2003). Besides starter culture differences, the degree of protein denaturation, enzyme addition, lipase, milk source, and other components can influence the flavor development and flavor stability of whey and ultimately, the finished product of dried whey protein (Drake et al., 2009). Liquid whey flavor varies with cheese type (Tomaino et al., 2004; Gallardo-Escamilla et al., 2005). The flavor, sensory and volatile components of fresh liquid whey from thermophilic starter cultures will differ from whey of mesophilic starter culture. The starter cultures which produce characteristic cheese also produce whey which is characteristic to that cheese. Mozzarella and Cheddar whey are as different as their cheeses.

CONCLUSIONS

Differences in liquid whey may explain the flavor and flavor variability of dried whey protein (Drake et al., 2009). This study confirms that Mozzarella and Cheddar whey are distinct in flavor and volatile compounds initially. This study also indicates lipid oxidation continues to occur in fluid whey regardless of pasteurization, fat separation, or cold storage. Results also suggest that Cheddar whey may be more prone to lipid oxidation than Mozzarella whey. Finally, all compounds identified by GC-O data have

been identified in dried whey products (Carunchia-Whetstine et al., 2005; Mahajan et al., 2004; Wright et al., 2006; Wright et al., 2009; Evans et al., 2009a; Evans, 2009; Karagul-Yuceer et al., 2003; Mortenson et al., 2008; Stevenson and Chen, 1996). Thus, the volatiles that contribute to flavor in whey protein are already present in fluid whey. Whey manufacturers need to identify methods to process whey quickly to decrease lipid oxidation and off-flavor development. In the future, further research should be conducted to further clarify the role that additives and processes used in cheese making play in fluid whey and whey protein flavor.

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Table 3.1 -- Sensory flavor attributes of Cheddar and Mozzarella liquid whey initially and after 3 d at 3°C

Sensory Attribute	Cheddar				Mozzarella			
	No Fat Separation		Fat Separation		No Fat Separation		Fat Separation	
	0 d	3 d	0 d	3 d	0 d	3 d	0 d	3 d
Aroma intensity	2.4a	2.1b	2.4a	2.0b	2.2ab	2.0b	2.0bc	1.6c
Sweet aromatic	2.0a	1.1b	2.0a	1.0b	1.1b	0.9b	1.1b	0.8b
Sour aromatic	ND	ND	ND	ND	1.1a	0.7b	0.6b	0.6b
Cardboard	ND	1.4a	ND	0.6c	ND	1.0b	ND	0.5c
Cooked/ milky	3.2ab	2.7cd	3.2a	2.8bcd	3.2ab	2.4de	2.9abc	2.2e
Sweet	2.0a	2.0a	2.0a	2.0a	1.7b	1.6b	1.6b	1.5b
Astringent	1.5a	1.5a	1.5a	1.5a	1.5a	1.5a	1.5a	1.5a

Means in a row followed by different lowercase letters signify differences ($P<0.05$). ND=not detected. 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 et al., 2003; Russell et al., 2006). LSD=least significant difference.

Table 3.2 -- Relative abundance of aldehydes (ppb) in Cheddar and Mozzarella liquid whey initially and after 3 d at 3°C by SPME

Cheddar Liquid Whey					
	No Fat Separation		Fat Separation		LSD
	0 d	3 d	0 d	3 d	
Decanal	0.0122aA	0.00409aBC	0.00537aB	0.00321aBC	0.0104
Nonanal	0.0705aA	0.0520abAB	0.0443abB	0.0212bCD	0.0382
Octanal	0.0108aA	0.00887abAB	0.00726abBC	0.00554bC	0.00440
Heptanal	0.00979aAB	0.0133aAB	0.00811abAB	0.00420bB	0.00581
Hexanal	0.110bB	0.798aA	0.0825bB	0.170bB	0.378
Total Aldehydes	0.213bB	0.877aA	0.148bB	0.204bB	0.404
Mozzarella Liquid Whey					
	No Fat Separation		Fat Separation		LSD
	0 d	3 d	0 d	3 d	
Decanal	ND	ND	ND	ND	--
Nonanal	0.0146aD	0.0186aD	0.0232aCD	0.0173aD	0.0175
Octanal	0.00111aD	0.000758aD	0.00198aD	0.00938aD	0.00223
Heptanal	0.0167aA	0.00927aAB	0.0109aAB	0.00693aAB	0.0115
Hexanal	0.1289aB	0.2655aB	0.0654aB	0.1816aB	0.202
Total Aldehydes	0.161aB	0.295aB	0.101aB	0.207aB	0.218

Means followed by different lowercase letters within a whey type for a compound signify a difference for that whey
sample over time ($P<0.05$). Uppercase letters in rows following means signify differences amongst all samples of the whey type for a compound ($P<0.05$). ND=not detected. LSD=least significant difference.

Table 3.3 -- Relative abundance of aldehydes (ppb) in Cheddar and Mozzarella liquid whey initially and after 3 d at 3°C by SAFE

Cheddar Liquid Whey					
	No Fat Separation		Fat Separation		LSD
	0 d	3 d	0 d	3 d	
Decanal	1.45aABC	1.91aA	1.25aBC	1.49aAB	0.730
Nonanal	14.5bBC	24.4aA	8.83bBC	8.65bBC	8.54
Octanal	2.02bB	2.35bB	6.37aA	2.77bB	3.13
Heptanal	0.926bBC	1.48abAB	0.764bBC	2.36aA	1.30
Hexanal	0.577bB	0.951aA	0.502bBC	0.591bB	0.241
Total Aldehydes	19.5abBC	31.1aA	17.7bBC	15.9bBC	11.7
Mozzarella Liquid Whey					
	No Fat Separation		Fat Separation		LSD
	0 d	3 d	0 d	3 d	
Decanal	1.57aAB	1.23abBC	1.21abBC	0.823bC	0.601
Nonanal	15.4aB	13.4abBC	12.4abBC	7.67bC	6.04
Octanal	1.47abB	0.911bB	1.71aB	1.18abB	0.622
Heptanal	1.25aBC	1.11aBC	0.975abBC	0.503bC	0.513
Hexanal	0.424abBC	0.389abBC	0.575aB	0.309bC	0.230
Total Aldehydes	20.1aB	17.0abBC	16.9abBC	10.5bC	7.47

Means followed by different lowercase letters within a whey type for a compound signify a difference for that whey sample over time ($P<0.05$). Uppercase letters in rows following means signify differences amongst all samples of the whey type for a compound ($P<0.05$). ND=not detected. LSD=least significant difference.

Table 3.4 -- Aroma active compounds in Cheddar and Mozzarella liquid wheys by gas chromatography olfactometry with aroma extract dilution analysis

							Log_3 Flavor Dilution Factor[5]							
							Cheddar				Mozzarella			
	Retention Index[1]						Unseparated Fat		Separated Fat		Unseparated Fat		Separated Fat	
Obs.	DB5	DB WAX	Compound	Methods of Identification[2]	Fraction[3]	Odor[4]	0 d	3 d	0 d	3 d	0 d	3 d	0 d	3 d
1	605	860	2,3-Butanedione	RI, Odor, MS	NB	Buttery	7	8	10	8	5	7	8	5
2	646	693	2/3-Methylbutanal	RI, Odor, MS	NB	Malty/Chocolate	7	5	6	6	5	3	<1	4
3	648	1392	Acetic Acid	RI, Odor, MS	AC	Vinegar	7	5	3	6	6	4	5	2
4	684	1000	2,3 Pentanedione	RI, Odor	NB	Buttery	1	1	1	1	2	2	2	3
5	715		ethyl Propionate	RI, Odor	NB	Sweet/Dried fruit	5	<1	2	2	<1	<1	<1	4
6	718	1039	Dimethyl Disulfide	RI, Odor	NB	Vegetable brothy/Sulfur	<1	<1	<1	2	<1	ND	<1	ND
7	763	1045	1-Hexen-3-one	RI, Odor	NB	Sweet/Rubbery	2	3	4	7	4	4	2	4
8	791	1024	Hexanal	RI, Odor, MS	NB	Grassy	<1	2	<1	2	2	<1	<1	2
9	794	1111	Unknown		NB	Fatty/Vomit	1	<1	4	<1	1	<1	1	3
10	813	1785	Butyric acid	RI, Odor, MS	AC	Cheesy	9	8	7	7	7	7	7	5
11	864	1315	2-methyl-3-Furanthiol	RI, Odor, MS	NB	Cooked/Nutty	1	<1	4	<1	3	5	3	<1
12	895	1194	Z-4 Heptenal	RI, Odor, MS	NB	Fishy/Fatty	2	1	3	2	2	1	<1	1
13	909	1402	Methional	RI, Odor, MS	NB	Potato	3	1	6	<1	4	3	5	4
14	917	1303	2-acetyl-1-Pyrroline	RI, Odor[6]	NB	Popcorn	7	6	7	5	7	7	6	7
15	936	1131	Heptanal	RI, Odor, MS	NB	Citrus	<1	<1	<1	<1	ND	<1	<1	1
16	937	1126	Unknown		NB	Minty	3	3	4	7	3	3	1	3
17	973	1340	Dimethyl Trisulfide	RI, Odor, MS	NB	Garlic	1	ND	1	<1	<1	<1	2	1
18	977	1260	1-Octen-3-one	RI, Odor, MS	NB	Mushroom	11	6	5	10	5	6	4	7
19	983		(Z)-1,5-octadien-3-one	RI, Odor[6]	NB	Geranium	2	1	1	1	ND	ND	1	<1
20	1005	1256	Octanal	RI, Odor, MS	NB	Fruity/Citrus	3	3	1	<1	2	1	1	9
21	1025	1656	2-Acetylpyridine	RI, Odor, MS	NB	Popcorn	3	1	3	1	5	3	1	3

Table 3.4 -- Continued

22	1032	1244	ethyl Hexanoate	RI, Odor	NB	Fruity	1	ND	2	<1	1	<1	<1	<1
23	1050		Phenylacetaldehyde	RI, Odor, MS	NB	Rosy	3	3	1	2	1	<1	<1	2
24	1078		1-Nonen-3-one	RI, Odor[6]	NB	Mushroom	5	<1	<1	ND	ND	ND	ND	ND
25	1080		Unknown		NB	Cooked/Nutty	5	7	7	1	7	9	8	8
26	1089	1395	2-isopropyl-3-Methoxypyrazine	RI, Odor, MS	NB	Bell pepper	5	5	2	5	5	6	4	7
27	1095	1308	Nonanal	RI, Odor, MS	NB	Citrus/Fatty	4	7	5	8	6	7	5	6
28	1096		Guaiacol	RI, Odor, MS	NB	Smokey/Sweet	<1	ND	<1	ND	4	1	<1	<1
29	1114		p-Cresol	RI, Odor, MS	NB	Phenol/Bandaid	1	3	2	3	3	2	2	3
30	1129	2156	Sotolon	RI, Odor, MS	NB	Curry/Maple	1	4	1	2	<1	1	<1	2
31	1149	1859	2-Phenethanol	RI, Odor, MS	NB	Rosy	10	7	8	3	6	9	6	10
32	1156	1602	(E,Z)-2,6-Nonadienal	RI, Odor, MS	NB	Cucumbers	1	3	<1	2	2	3	1	1
33	1162	1682	(E)-2-Nonenal	RI, Odor, MS	NB	Carpet/Lipstick	8	7	8	4	4	3	2	8
34	1168	1700	2-isobutyl-3-Methoxypyrazine	RI, Odor	NB	Bell pepper	<1	ND	<1	<1	ND	ND	ND	ND
35	1176		Unknown		NB	Cooked/Brothy/Nutty	3	5	6	6	5	3	4	6
36	1197	1489	Decanal	RI, Odor, MS	NB	Sweet/Fatty	ND	1	1	<1	2	1	ND	<1
37	1222		(E,E)-2,4-Nonadienal	RI, Odor	NB	Hay/Fatty	<1	6	1	3	<1	5	3	<1
38	1244		Unknown		NB	Minty/Stale/Hay	2	2	<1	<1	3	3	3	<1
39	1265		gamma-Octalactone	RI, Odor, MS	NB	Coconut	4	6	1	ND	4	<1	3	3
40	1290	1625	Unknown		NB	Oatmeal	3	5	5	6	2	5	3	6
41	1323	2223	o-Aminoacetophenone	RI, Odor, MS	NB	Grape	<1	4	2	ND	<1	4	ND	5
42	1327		Unknown		NB	Tortilla	3	7	4	<1	<1	6	<1	5
43	1370		gamma-Nonalactone	RI, Odor, MS	NB	Cilantro	<1	7	1	3	<1	3	<1	<1
44	1392		(E)-4,5-epoxy-2(E)-Decenal	RI, Odor	NB	Metallic/Grainy	2	8	4	6	3	4	4	5
45	1410		Unknown		NB	Soap/Waxy	<1	3	3	5	<1	<1	<1	<1
46	1422		Skatole	RI, Odor, MS	NB	Mothball	3	5	4	<1	3	2	4	4
47	1448	1687	Unknown		NB	Cooked/Nutty	2	5	4	3	4	3	5	5
48	1525		delta-Decalactone	RI, Odor, MS	NB	Coconut	5	8	4	7	4	4	6	5
49	1595		Unknown		NB	Soapy	<1	<1	1	2	<1	3	2	<1

Table 3.4 -- Continued

50	1689	delta-Dodecalactone	RI, Odor, MS	NB	Peach	<1	1	2	1	<1	2	5	3

[1]Retention indices were calculated from gas chromatography-olfactometry data

[2]NB=neutral/basic compounds; AC=acidic compounds

[3]Compounds were identified by comparison with authentic standards based on retention index (RI) on DB-Wax and DB-5MS columns, odor detection at the sniffer port, and mass spectra in the electron impact mode. Positive identifications indicate that mass spectral data was compared with authentic standards.

[4]Odor description detected by gas chromatography-olfactometry sniffing port

[5]Flavor dilution factors were determined on a DB-5MS column for neutral/basic (NB) compounds and on a DB-Wax for acidic (AC) compounds.

[6]Compound identified by comparing RI and aroma with literature (Avsar et al., 2004; Ott et al., 1997)

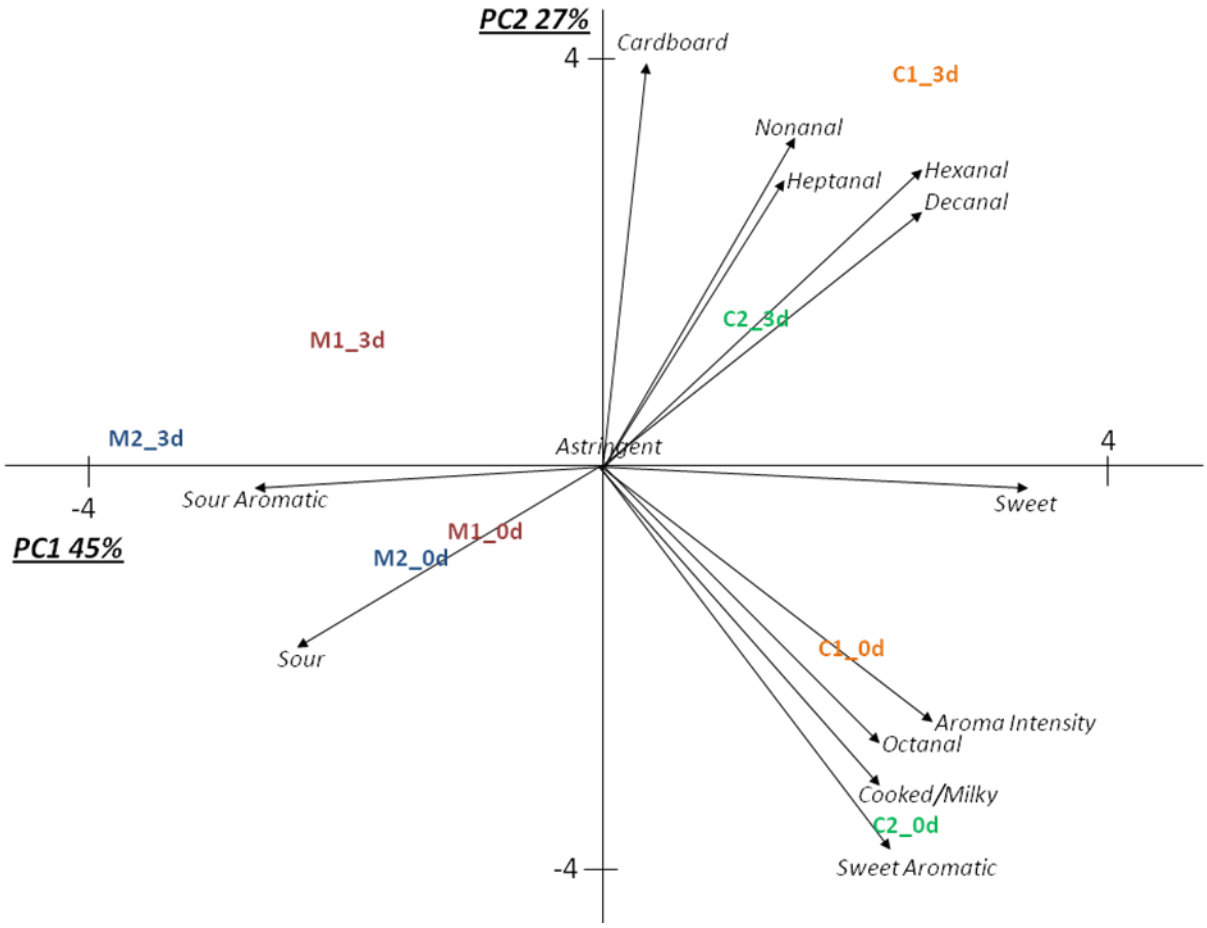

Figure 3.1 -- Principal component biplot of Cheddar and Mozzarella liquid whey sensory and SAFE-GC-MS results. M1: Mozzarella whey with no fat removal, M2: Mozzarella whey with fat removal, C1: Cheddar whey with no fat removal, C2: Cheddar whey with fat removal. 0d: Fresh liquid whey, 3d: Liquid whey after storage