ABSTRACT

WHITSON, MEGAN ELIZABETH. Sources of Flavor in Whey Proteins. (Under the direction of Dr. MaryAnne Drake.)

Whey protein is one of the major players in the growing functional foods market, valued to be >$100 billion by 2012. Mainly utilized in two forms: whey protein concentrate (34-89 % protein; WPC) and whey protein isolate (>90 % protein; WPI), whey protein is both nutritionally and functionally beneficial. These characteristics of whey protein are harnessed in food and beverage applications, but flavor of whey protein continues to be a challenge for its widespread usage. For ingredient applications it is important for spray dried whey products to have a low intensity flavor profile and to be free of off-flavors; however, off-flavors are present and result from many different factors and chemical reactions. Cardboard flavor is one of the most commonly described off-flavors in whey proteins associated with lipid oxidation. It is important to understand which steps in whey protein production impact flavor in order to identify methods to control flavor. Several processing steps are employed to manufacture WPC80 and WPI. During whey protein production, concentrated liquid whey protein (retentate) may be stored for up to 48 hours before spray drying. The objective of this research was to identify volatile components responsible for cardboard flavor in dried whey protein (WPC, WPI) and to determine the impact of liquid retentate storage on flavor and flavor stability of spray dried WPC80 and WPI.

Cardboard and brown paper samples (n=5) soaked in deionized water and whey proteins with and without cardboard flavor were analyzed by gas chromatography mass spectrometry and descriptive sensory analysis to select the potential volatile compound contributors to cardboard flavor. Compounds were evaluated by trained sensory panelists using sniff jars, dose response experiments and whey protein models. Sensory analysis of the aroma of the chemical standards yielded no single compound exhibiting a cardboard aroma, suggesting that cardboard flavor did not result from one compound, but a combination. A combination of compounds (pentanal, heptanal, nonanal, 1-octen-3-one, dimethyl trisulfide) elicited cardboard flavor in whey protein previously deemed free of cardboard flavor. These oxidation products were also found in the brown paper samples and were linked to real cardboard paper production. This study clearly associated the presence of oxidation products with cardboard flavor in whey protein, emphasizing the necessity to control lipid oxidation during processing to reduce cardboard flavor in whey protein ingredients.

Liquid retentate from Cheddar whey protein isolate (WPI) and Mozzarella 80 % whey protein concentrate (WPC80) were evaluated to determine the effect of holding time of liquid retentate on flavor of spray dried whey proteins. Liquid WPC80 and WPI were manufactured and stored at 3C. After 0, 6, 12, 24 and 48 h, product was spray dried (2 kg) and the remaining retentate held until the next timepoint. The design was replicated twice for each product. Powders were stored at 21C and evaluated every 3 mo through 12 mo storage. Flavor profiles of rehydrated proteins were documented by descriptive sensory

analysis. Volatile components were analyzed with solid phase microextraction (SPME) coupled with gas chromatography mass spectrometry (GC-MS) and gas chromatography olfactometry (GC-O). Cardboard flavors increased in both spray dried products with increased retentate storage time and cabbage flavors increased in WPI ($p<0.05$). Concurrent with sensory results, lipid oxidation products (hexanal, heptanal, octanal) and sulfur degradation products (dimethyl disulfide, dimethyl trisulfide) increased in spray dried products with increased liquid retentate storage time, while diacetyl decreased ($P < 0.05$). Shelf stability was decreased in spray dried products from longer retentate storage times. Processors should reduce storage time as they process raw whey into WPC80 or WPI. For maximum quality and shelf life, liquid retentate should be held for less than 12 hours prior to spray drying.

Sources of Flavor in Whey Protein

by
Megan Elizabeth Whitson

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DEDICATION

To my family: the ones who influenced me, loved me; the one who believed in me, coincided with me; and the little one who holds promise of the future.

BIOGRAPHY

A native of North Carolina, Megan Whitson was raised in Burke County. The daughter of a Chemist, Donna Sue Whitson, and an Artist, Monty Joe Whitson, Megan's childhood influences from her parents became her passions as she pursued a degree in the creative side of science: Flavor Chemistry and Sensory Science. Her parents maintain an inviting farm in western NC, where they explore family traditions of brick oven bread baking, quilt making, cheese making, animal husbandry, weaving, and pottery. Her brother, Christopher Whitson, graciously teaches Special Education and coachs basketball for Caldwell Community College. Christopher and his wife, Lauren Boughman Whitson, who teaches Elementary Education, blessed the family with the birth of their first daughter, Bella Grace Whitson in June 2009.

After high school Friday nights of dazzleing the football stadium crowd twirling fire approached an end, Megan traded in her batons to dazzle the college football stadium as a dancer for NC State. During college she was an acitive member of the NCSU Food Science Club, holding offices of Secretary, Awards Banquet co-chair, Wine and Cheese co-chair, and Activitites co-chair. As fate would have it, her Activities co-chair, Drew Watson, soon became her best friend and partner for the future. In 2007, she received two undergraduate degrees in Chemistry and Food Science. Two internships with Kraft Foods

influenced her to persue graduate studies in Sensory and Flavor Chemistry. She then began her Master's degree under Dr. MaryAnne Drake.

Megan's work as a professional seamstress has trained her to design solutions. Each project utilizes a unique artistic perspective, even if employing the same basic building blocks – needle, thread, fabric. In designing food or clothing, one must bring to life something the consumer finds irresistible. Understanding the relationship between a food product's chemical flavor/texture components and the sensory response of consumers is the highest responsibility of the Flavorist / Sensory Scientist, and is where her passion lies; synthesizing these to the benefit of consumers is what she hopes to do as she starts her career.

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and the one who deserves the most thanks….

Drew Watson – my partner on this path, through all the ups and downs; much love to you for always being beside me, making me smile, and always believing in me. Now our journey beings….I can't wait to experience it all with you. Love you, Sweets.

*"WHAT LIES BEHIND US AND WHAT LIES BEFORE US ARE TINY MATTERS COMPARED TO WHAT LIES WITHIN US" – **RALPH WALDO EMERSON***

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CHAPTER 1:

REVIEW OF LITERATURE: WHEY PROTEIN

What is whey?

Maybe little Miss Muffet was onto something when she sat down to drink her curds and whey. Current research on whey is revealing its overwhelming functionality and nutritional qualities. Recorded whey consumption by humans, as a drink and as a porridge additive, dates back to 1825 (Berry, 1923). "Down to the milk-house and drank three glasses of whey" (Pepys' Dairy, 1893). Whey is simply milk without fat and casein (Lampert, 1965), thus it is the by-product of cheese and casein production. In cheese making, addition of rennet or acids naturally coagulate the primary milk protein casein, creating a curd or cheese. The liquid that flows off as the curd precipitates is termed whey (Berry, 1923). Whey is a pale greenish yellow liquid that is slightly opalescent (Berry, 1923). Shakespeare referred to the color in his famed Macbeth, "Go prick thy face and outrid they fear - Thou lily-liver'd boy. What soldiers whey face?" (Shakespeare's *Macbeth*, 1606)

Whey contains lactose, salts, peptides, whey proteins and other non-protein nitrogen substances (Robinson and Wilbey, 1998). The exact composition of whey is determined by the process by which it is produced. The composition of whey is affected by pasteurization of cheese milk, type and amount of bacterial starter cultures, type and concentration of coagulant used, treatment of the cheese curd before whey drainage, and removal of residual lipids from the whey (Johansen et al., 2002). Efficiency of the

cheesemaking process is measured by the amount of casein; thus, large amounts of casein left in the whey indicate a less efficient production and lower quality cheese (Robinson and Wilbey, 1998). In 1923, numbers show a production yield of approximately 90 gallons of whey for 100 gallons of milk (Berry, 1923). Current traditional cheese methods produce whey from approximately 83% of the volume of milk used (Robinson and Wilbey, 1998). In 1970, the average annual production of whey was around 22 billion pounds (Lampert, 1965); while in 2000 the US produced over 1 million metric tons of whey and whey products (1 metric ton = 2204 lbs) (ADPI, 2001). A more recent statistic reports that a large cheese facility produces over 1 million liters of whey daily (Jelen, 2003).

Types of Whey

Whey is divided into two types, rennet or sweet whey and acid whey. These two types of whey are differentiated by the process by which they are made. Rennet whey is obtained when rennet (a proteolytic enzyme) is used to separate the casein and milk serum. Acid whey is produced when acid is used to separate the casein and milk serum. Acid whey is produced from cottage cheese while rennet whey is a byproduct of Cheddar, Swiss, Mozzarella and similar cheeses. Rennet whey contains high concentrations of lactose and lysine rich proteins which lends to its ability to participate in Maillard browning in the presence of moisture (Dattatreya, 2007). Rennet whey is often used for lactose production,

but must be used quickly because after 48 hours it is considered useless for this application (Sienkiewicz and Riedel, 1990). Table 1 outlines the criteria for types of liquid whey.

Table 1.1. Types of Whey

Sweet whey	Titratable acidity 0.1-0.2%, pH value 5.8-6.6 Derived from rennet-coagulated cheese such as Cheddar and from rennet casein manufacture
Medium-acid whey	Titratable acidity 0.2-0.4%, pH value 5.0-5.8 Derived from fresh, acid cheese such as Ricotta and cottage cheese
Acid whey	Titratable acidity 0.4%, pH value <5.0 Derived from fresh, acid cheese and from acid casein manufacture

Adapted from Varnam and Sutherland, 1994

Applications of liquid whey

The value of whey has been quickly rising due to new research revealing practical applications to harness the functional properties. The major use for liquid whey in 1923 was pig feed; however, there were not enough pig farms at that time to keep up with whey production leaving large amounts of whey to be disposed of as waste (Berry, 1923). Bacon curers believed that feeding whey to the pigs actually improved the quality of the bacon

(Berry, 1923). Whey is also valuable for poultry feed due to its high lactose and riboflavin content (Lampert, 1965). Using liquid whey for feeding ruminants is perfect because it ensures a quick turnover with the animals consuming it right away and the flavor quality of the whey is not as much a concern as it would be for human consumption. In the 1970s, liquid whey was proven to increase the yield of corn and hay when sprayed onto the ground of the fields, without increasing groundwater pollution (Watson et al., 1977).

One of the major challenges with utilizing liquid whey is that over 90% of it is water which results in a short shelf life (Berry, 1923). Imbert-Pondaven (1997) proposed that fresh whey stored at temperatures of 5 to 10^{o}C could last for up to 5 days. To combat the issue of shelflife, research in whey preservation was investigated. Formaldehyde and H_2O_2 have both been used as aids in preserving liquid whey; as well as the proposal that propionic acid could also work for this application (Sienkiewicz and Riedel, 1990). Utilization of liquid whey is also problematic due to high transportation costs and deterioration of quality, including emergence of negative sensory characteristics during storage (Jensen and Kroger, 2000; Jelen, 1992). Examples of documented negative sensory attributes include: 'dirty', 'rancid', 'stale', 'cardboardy', 'light-oxidized', 'cabbage/brothy', 'soapy' and 'metallic' (Carunchia Whetstine et al., 2003, 2005; Karagul-Yuceer et al., 2003; Tomaino et al., 2004; Gallardo-Escamilla et al., 2005; Wright et al., 2006, 2008; Javidipour and Qian, 2008).

Manufacture of whey protein concentrates

After liquid whey is skimmed from the cheese curds, it must go through a number of steps to be utilized as a food product or additive. Flow diagrams reported in 1994 designated the heat treatment step to occur before clarification; however, current diagrams illustrate clarification of the liquid whey as the first step (Varnam and Sutherland, 1994; USDEC 2004). Clarification involves the removal of cheese curds still present in the whey. Centrifugation and vibrating screens are the predominant methods for removal of the larger fat particles, while smaller lipids are removed by further centrifugation and microfiltration. Phospholipids present in the whey can lead to membrane fouling and increase lipid oxidation (Varnam and Sutherland, 1994). Clarification helps improve flavor and increase product stability while also creating a more soluble product. By products of clarification include whey cream and cheese fines.

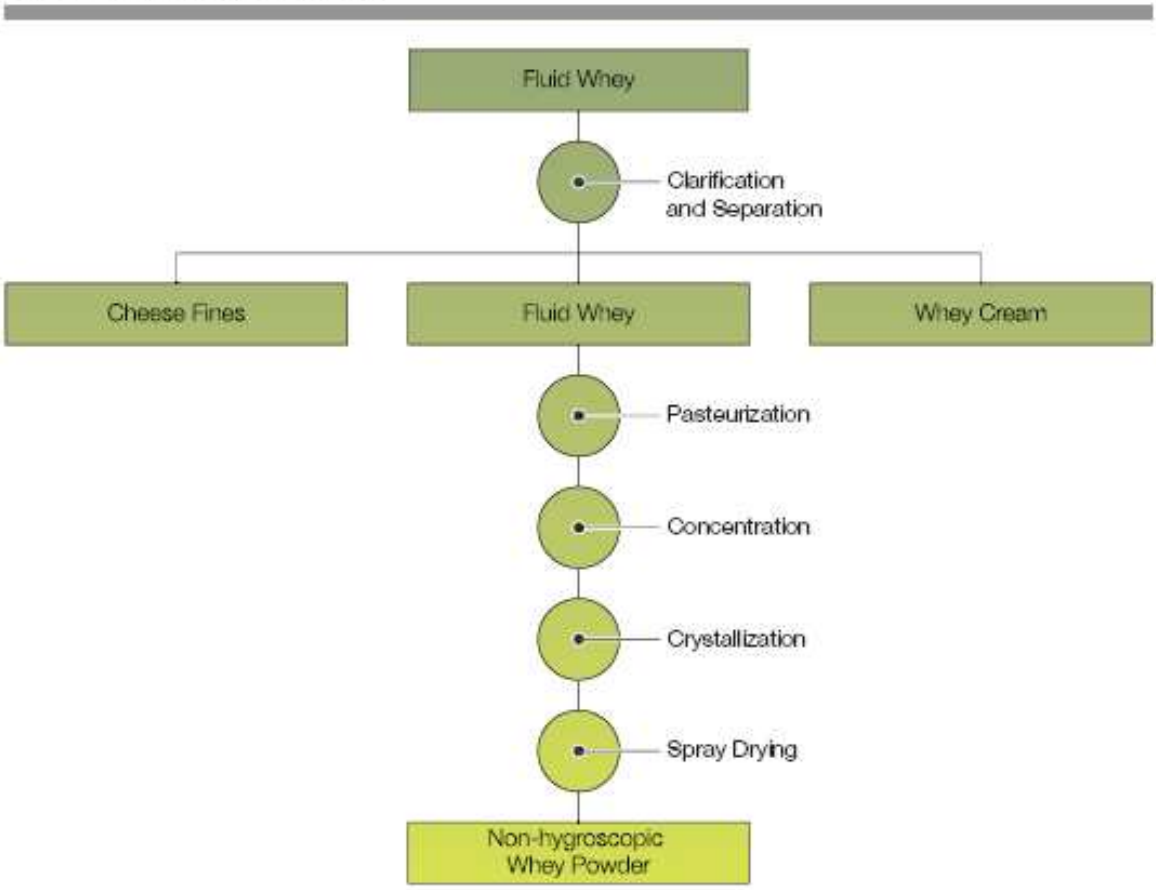

Figure 1.1. Processing of Whey Powder (USDEC, 2004)

Liquid whey, because of its high amounts of starter microorganisms, can support microbial growth and must be kept under refrigeration. A simple heat treatment is the next step and is required to inactivate microorganisms and prevent microbial growth and acid production (Varnam and Sutherland, 1994). Current whey manufacturers employ pasteurization at 71-74^{o}C for 15 seconds as their heat step (Spreer, 1998). If left in the

whey, lactic acid bacteria will produce lactic acid which can alter texture and functionality of the resulting whey powder by making it sticky and hydroscopic. Pasteurization has also been shown to reduce lipid oxidation in fluid whey storage by slowing enzymatic processes (Carunchia Whetstine et al., 2003; Gallardo-Escamilla et al., 2005).

Following pasteurization, whey is then concentrated by ultrafiltration, reverse osmosis, microfiltration, nanofiltration and electrodialysis. Previously, falling film evaporators with mechanical vapor recompression and minimal heat load were used for concentration. Current membrane filtration methods do not involve heat, and therefore do not denature the proteins (Varnam and Sutherland, 1994). Initially whey proteins were also isolated using precipitation techniques; however, current techniques utilize membrane separation and chromatographic processes (Sienkiewicz and Riedel, 1990). These current methods of concentration, ultrafiltration, nanofiltration, reverse osmosis and microfiltration, employ pressure to selectively separate the components of the whey according to molecular weight by pushing them through varying pore sizes. High pressure used in reverse osmosis pushes the whey through a small pore size that allows only water molecules to pass through. Often reverse osmosis is coupled with ultrafiltration or evaporation where the water is then evaporated under vacuum. The advantage of ultrafiltration over reverse osmosis is the lower pressure and temperature required. Ultrafiltration functions by way of a pressure gradient and semipermiable membranes to fractionate individual solutes by structure and size (Sienkiewicz and Riedel, 1990). Pressure

drops over membranes of small pore sizes push water, soluble minerals and lactose through the pores, retaining the larger colloidal molecules (Spreer, 1998). Microfiltration and nanofiltration are similar in theory and method to ultrafiltration, but utilize membranes with smaller pore size to allow for separation of specific components, minerals, microbial flora, and/or lactose from liquid whey (Kelly and Kelly, 1995; Kosikowski and Mistry, 1997b; Tunick, 2008). An alternative to membrane filtration for liquid whey concentration is electrodialysis. Electrodialysis utilizes an electronic current that flows through the liquid whey in a chamber made of ion-permeable walls (Tunick, 2008). Often eletrodialysis is used to demineralize liquid whey (Tunick, 2008).

During these concentration steps, the product that is pushed through the membrane is termed permeate and the product that is retained is called retentate. Permeate from whey processing contains water, lactose, salts, vitamins and amino acids while the retentate contains the proteins. Because of its high lactose content, the use of permeate as a powder is limited, but it has been successfully produced into a lactose syrup (Varnam and Sutherland, 1994). Whey protein retentate is the fraction that remains and contains proteins as well as some water and low molecular matter (Sienkiewicz and Riedel, 1990).

Lactose plays a difficult role in whey processing because it can form a highly hygroscopic anhydrous powder that results in caking and lumping after conversion to the monohydrate form. Major ways of avoiding this problem are lactose crystallization. Both processes ensure that the α-lactose is already converted to the monohydrate form before

drying. Crystallization utilizes controlled cooling and seeding to nucleate lactose crystals that are then centrifuged out of the whey (Varnam and Sutherland, 1994).

Once concentrated, whey can be sold as a concentrated liquid product, or further processed to produce dried whey protein powders. Most common for the production of whey protein, as well as other dried food products, is the method of spray drying (Reineccius, 2004). During spray drying, liquid whey is atomized (sprayed) into a stream of hot air in a heated drying chamber where water in the whey evaporates and is carried away by the hot air. The other components of the whey fall to the bottom of the chamber as powder. Alternative drying processes include a vibrating fluid bed and roller drying. Although still used for production of whey for feed, roller drying isn't common for quality whey powders because the heat required causes the proteins to denature and brown via Maillard browning reactions. After drying, the whey protein is now ready for ingredient applications.

Whey Proteins

The primary proteins in liquid whey are alpha-lactalbumin and beta-lactoglobulin which make up 0.5 to 0.7 % of the soluble protein material. The proteins present in whey are the components that lend to its various applications and high nutritional value as a dried dairy ingredient. Before drying into an ingredient powder, whey proteins are

concentrated to various protein contents, which determine the functional and nutritional properties of the resulting powder. Whey protein can be used to boost the nutritional value of a food product by increasing the protein content (Quach et al., 1999) with the addition of whey protein concentrate powder (WPC) or whey protein isolate powder (WPI). Whey protein concentrate can be prepared from cheese or casein whey and is characterized by 30-89% w/w protein. In 1990, the annual production of WPC in the US was 140 million lbs (Morr and Ha, 1991). In 2005, this figure increased to 170 million kilograms of WPC produced, with 86% of that total utilized for human consumption (Gould, 2006).

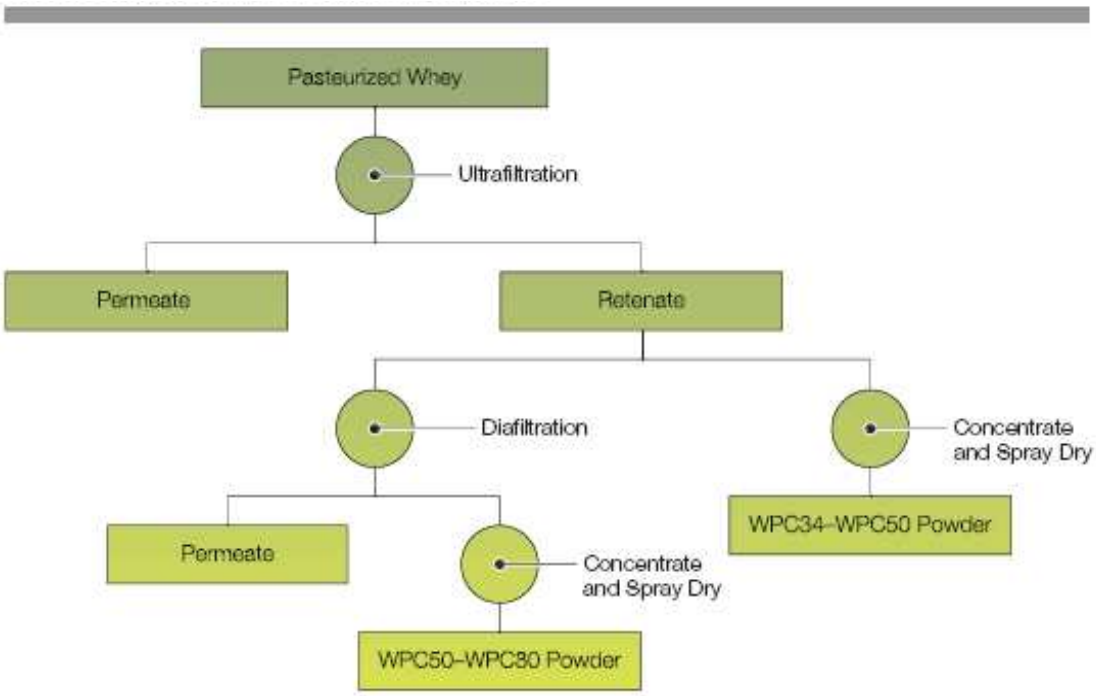

Figure 1.2. Processing of whey protein concentrates (USDEC, 2004)

To be considered whey protein isolate, >90% of the whey powder must be protein. This high protein content is often produced by combining ultrafiltration with diafiltration. Diafiltration is the process of adding water to the retentate and passing it through a second ultrafiltration step (Kelly, 2003; Tunick, 2008). Anion exchange is another method combined with membrane filtration for producing WPI, in which whey components are separated according to ion charges in an ion-exchange tower (Foegeding and Luck, 2003; Tunick, 2008). Recent developments in whey production include the technique of Continuous SEParation (CSEP) chromatographic technology that utilizes column chemistry to separate whey components according to their affinity for the column stationary phase. While the other methods of filtration and anion exchange can only separate whey components according to size and ionic charge, CSEP can separate components according to specific chemical characteristics and interactions allowing for the production of unique WPIs that contain specific components, like quantities of certain proteins, to increase nutritional benefits and applications of the resulting WPI (De Silva et al., 2003). In 2005, production of WPI in the US reached 15.6 million kilograms (Gould, 2006).

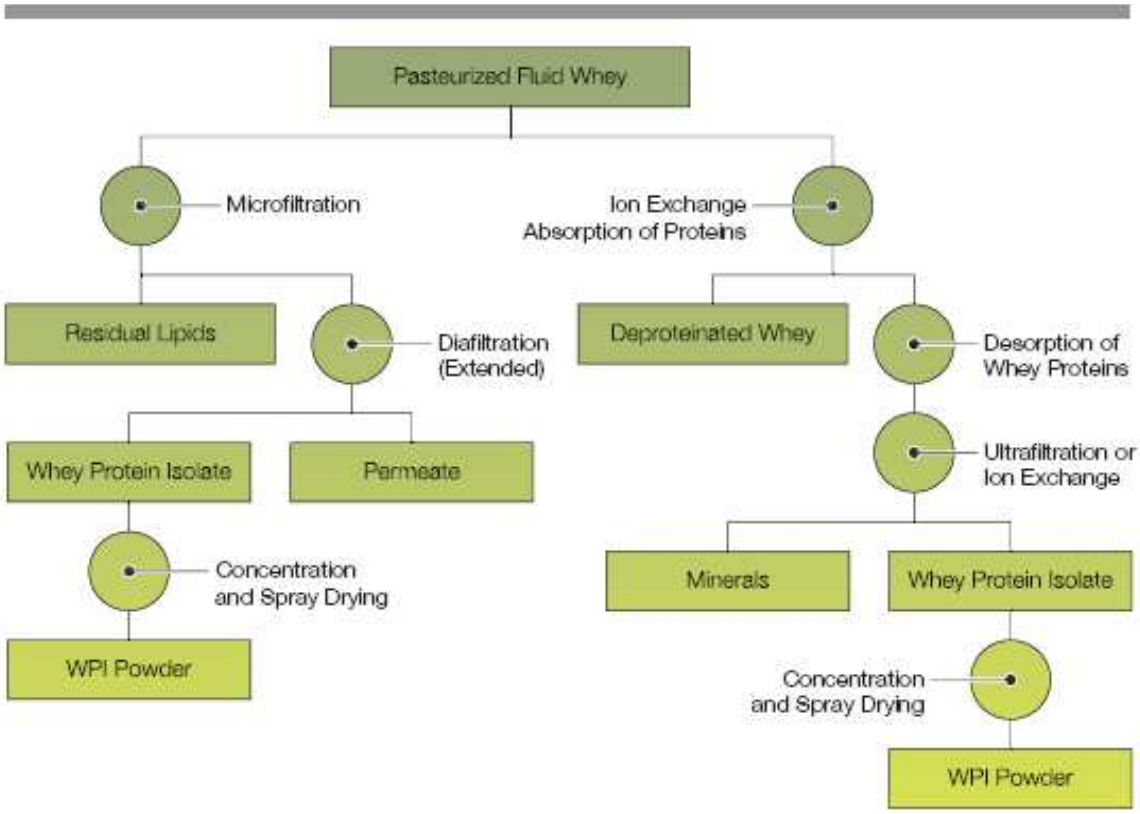

Figure 1.3. Processing of whey protein isolates (USDEC, 2004)

Table 1.2. Average composition of whey protein products

	Protein %	Lactose %	Fat %	Ash %	Moisture %
WPC 34	34.0-36.0	48.0-52.0	3.0-4.5	6.5-8.0	3.0-4.5
WPC 80	80.0-82.0	4.0-8.0	4.0-8.0	3.0-4.0	3.5-4.5
WPI	90.0-92.0	0.5-1.0	0.5-1.0	2.0-3.0	4.5

Adapted from USDEC, 2004

Whey Utilization

Smithers (2008) blamed inconsistency and unreliability of whey proteins in food systems as the number one reason for restricted whey protein utilization over the past 25 years. Utilization of lactose, the main component of whey, was the first step in discovering the functionality of whey. Lactose derivatives have been effectively used in food, pharmaceutical and industrial applications (Smithers, 2008).

Functionality

Protein functionality is defined as a protein's ability to form interfacial films used to stabilize emulsions and foams, interactions to form gel networks and edible films and ability to produce stable sols to be utilized in nutritional drinks (Foegeding et al., 2002). Functional properties include solubility, gelation, aeration, water-binding and emulsification (Kinsella and Whitehead, 1989; Huffman, 1996; Smithers et al., 1996; Korhonen et al., 1998; Foegeding et al., 2002). WPC has many functional properties including gelling, foaming and water binding properties that can be used in food products (Mills, 1992).

Gelling is one of the most important, and widely researched, functional properties of whey. Incorporation of whey protein into gels, after reaching the gel point, determines the whey protein gel microstructure (Verheul and Roefs, 1998a,b; Verheul et al., 1998). This gel microstructure, along with its rheological properties, differentiates whey protein gels into

three classes: fine stranded, mixed or particulate (Clark et al., 1981; Foegeding et al., 1998; Stading et al., 1993). Further heating beyond the gel point decreases gel permeability; permeability decreases as aggregated protein concentration increases (Foegeding et al., 2002). Inversely, elasticity increases, from zero at the gel point, as protein concentration in the gel network increases (Foegeding et al., 2002).

Aggregation kinetics of whey proteins are described through two mechanisms, reaction-limited cluster aggregation (RCLA) where an energy barrier exists, or diffusion-limited cluster aggregation (DLCA) where that energy barrier has already been overcome resulting in protein "sticking" of all collisions (Bijsterbosch et al., 1995). Changes in salt and pH concentration affect the type of aggregation kinetics, RLCA or DLCA (Aymard et al., 1997; Vreeker et al., 1992). WPI gels formed at pH 7 with low salt (NaCl) concentrations followed RCLA kinetics while the same pH and higher salt concentrations eliminated the electrostatic barrier and followed DLCA kinetics (Ikeda et al., 1999). A more elastic, less permeable get network was created with increased pH which increases electrostatic repulsion of the sulfhydryl groups coaxing the proteins to unfold to a greater degree and aggregate rapidly (Foegeding et al., 2002). NaCl alters protein gels by creating a more open, coarse structure that allows for increased permeability and decreased elasticity (McGuffey and Foegeding, 2001; Verheul and Roefs, 1998a).

One advantage to using whey protein to create a gel is its potential to form "cold" gels. Cold gels are produced when the whey proteins are heat-polymerized, at low temperatures,

low ionic strength and high pH. These conditions don't allow a gel to form, so that the product, when the solvent is altered, produces a "cold" gel at 20-37^{o}C (Foegeding et al., 2002). This allows for applications in foods that are sensitive to high heat. Whey proteins are also very beneficial as surfactants, surface active agents that help to form foams and emulsions (Dickinson, 1999). Dressings, ice cream, soufflés and frothed drinks are all examples of food foams and emulsions.

Whey protein hydrolysates (WPH) are formed by enzyme hydrolysis of WPC or WPI to retain nutritional quality and increase functionality (Foegeding et al., 2002). WPHs can be used to improve heat stability, reduce allergenicity, tailor amounts and size of peptides for special diets, produce bioactive peptides and alter functional properties of gelation, foaming and emulsification (Foegeding et al., 2002). Because of these properties, WPH is often utilized in hypoallergenic infant formulae, sports nutrition, and enteral formulas. WPH contains high concentrations of short peptides and a low concentration of free amino acids, which allows for increased efficiency in absorbance because of differences in intestinal adsorption between peptides and free amino acids (Boza et al., 2000; Clemente, 2000).

Common applications of WPI are to utilize it as a foaming agent similar to egg white proteins. In comparing the two, the interaction of protein with sugar to improve the foam stability was different according to the protein used (Davis and Foegeding, 2006). Further research is being conducted on this application despite the thought that egg white protein

foams require less protein and less whipping time (Pernell et al., 2002). The drive is to understand the physical and chemical interactions of WPI in creating foams to increase the success of this application.

Whey protein can be used to create water binding gels in conjunction with carrageenan. Carrageenan gels with higher whey protein compositions were shown by a sensory panel to be stronger, with higher springiness, firmness, adhesiveness, and water holding capacity (Leskauskaite et al., 2006). These gels can then be added to food products to alter the perceived texture by the consumer.

Table 1.3. Summary of the functionality of whey protein products

Product	**Functional Properties**
Whey powder, permeate	Solids Dispersible Color and flavor development
Sweet whey	Solubility Dispersible Dairy flavor and solids
WPC 34	Solubility Emulsification Protein source Color and protein development
WPC 80	Solubility Emulsification Whipping Fat binding Water binding Heat setting / gelling High protein level
Whey protein isolates, WPI	Solubility High protein level
Demineralized whey products	Low mineral content Whey and lactose Versatile functionality Good source of nutritionally balanced
Bioactive proteins / whey fractions	Reduce cholesterol Enhance iron transport Non-allergenic Antibacterial properties Simulate growth of bifidus bacteria

USDEC, 2004

Nutrition

Nutritional value of whey is not something new. Lactoserum, or whey, has been utilized for medicinal purposes (sepsis, wound healing, stomach disease) throughout the 17th and 18th centuries in Europe where 'whey houses' thrived (Baricelli, 1623; Hoffmann, 1961). A whey house is a location where whey was used for medicinal purposes. Similarly, spas in the 19th century provided whey baths to harness the health benefits of whey (Trelogan, 1970). To gauge the biological value of whey, it is compared against the previous highest benchmark for percentage of nutrient content that can be utilized in the body, egg protein. Whey protein stacks in at 15% higher in biological value than egg protein (Mitchell, 1924; Smithers, 2008).

Whey protein is rich in branched chain amino acids: leucine, isoleucine and valine. These specific amino acids act as metabolic regulators in protein and glucose homoeostasis and lipid metabolism. These characteristic actions could play a role in weight control (Smilowitz et al., 2005; Zemel, 2004). Methionine and cysteine are both sulfur containing amino acids found in whey protein that serve as anti-oxidants, and one-carbon metabolism in the body (Shoveller et al., 2005). Whey proteins increase their bioactivity because not only are the proteins beneficial in promoting good health, but the peptides contained within the proteins often have additional bioactivity benefits than the parent protein (Meisel, 2005).

Lactoferrin, a biological valuable protein found in whey, has been established as an anti-microbial agent applied successfully in increasing the safety of meat (Naidu et al., 2003). Through the stimulation of osteoblast growth and inhibition of osteoclast growth, lactoferrin is being researched as an osteoporosis prevention treatment strategy (Cornish et al., 2004). Anti-microbial effects are also exhibited by lactoperoxidase, an enzyme found in whey. Current products that contain lactoperoxidase help inhibit microorganism growth associated with gingivitis, oral irritation and halitosis (bad breath) in clinical trials (Tenovuo, 2002).

The type of proteins, with established bioactive effects, that are found in highest quantity in whey are immunoglobulins. Whey is not the primary source of immunoglobulin extraction for nutraceutical applications, however, presence of these proteins in whey add to its nutritional quality for human consumption. Immunoglobulins have been shown to improve athletic recovery time and performance, enhance gut health and combat infections (Mero et al., 1997; Bucklety et al., 1998; Playford et al., 1999; Coombes et al., 2000; Mehra et al., 2006). Milk fat globule membrane proteins present in whey include mucin, xanthine oxidase, adipophilin, butyrophilin and others. These proteins may play a role in receptor function, anti-infectivity and anti-microbial (Peterson et al., 2001).

Cell culture and in vivo animal studies present evidence of reducing incidence and burden of certain cancerous tumors with a diet rich in whey proteins compared to diets of casein, meat and soy proteins (McIntosh et al., 1995; Hakkak et al., 2001). Specific

relationships between whey protein and cancer prevention in literature are reported for colon, breast and prostate cancer (Bounous et al., 1991; Gill and Cross, 2000).

Cheese whey has been shown to contain bioactive proteins with multiple growth factors. These growth factors have been extracted from whey protein to create a concentrated extract which is particularly beneficial for wound healing (Regester and Belford, 1999; Rayner et al., 2000; Regester et al., 2003) and mammalian cell growth for fibroblast cell lines (Belford et al., 1995). Human trials are currently underway utilizing this whey growth factor extract in fighting mucositis, painful inflammation of the mucus membranes, in cancer patients (Smithers, 2008).

While the lipids present in whey protein concentrates are deemed an unavoidable processing circumstance, could they be beneficial nutritionally to the product? WPC 80 powders while being ~80% protein, still contain ~4.5% fat. Because this is a dairy product, the fat content is primarily triglycerides. Short chain fatty acids, tetradecanoic (C14), hexadecanoic (C16) and octadecanoic (C18) are in the highest content. Dairy fats also contain small amounts of odd numbered chain and branched fatty acids, small amounts of monounsaturated fatty acids and trace amounts of highly unsaturated fatty acids. Fat content of dairy products varies according to the breed of cow, type of feed and feeding and milking patterns. Dairy lipids are a great source of dietary fat. They also provide natural saturated fatty acids (rather than trans fatty acids). The degree of saturation of the milkfat can be altered by the feed, however, stability can be affected as well.

Dairy lipids contain conjugated linoleic acid (CLA), which is the isomeric fatty acids of 18:2 and 18:1. CLA is derived from linoleic acid in which the double bonds have been conjugated allowing for all cis and trans formations. Dairy products can range from 6.1-3 mg of CLA/ g of fat. Besides diet sources, CLA can be produced from free radical oxidation of linoleic acid in vivo. When compared to linoleic acid, CLA is reported to inhibit melanoma, colorectal and mammary cancer development (MacDonald, 2000). Prevention of proliferation of lung cancer cells may be achieved through cytotoxic lipid oxidation products. This anti-cancer biological property could be explained though CLA enhancing the immune system function. Enhancement from immune system function comes through weight control and possibly by reducing reliance on antibiotics. Because of the fat partitioning quality of CLA, it is proposed to supplement CLA in American diets to control obesity.

Applications

The functional foods market is valued to be >$100 billion by 2012 making it a very valuable market for whey protein (Smithers, 2008). Whey protein is often regarded as a complete source of protein because it contains essential amino acids. These amino acids in conjunction with proteins and minerals help promote lean body mass and limit lipid deposits in adipose tissue (Ha and Zemel, 2003). Because of this nutritional value, most applications of whey are for health benefit.

Whey Cheese

Increase in whey protein knowledge has aided in the production of whey cheese. Whey cheese is simply coagulation of whey protein in fluid whey to form a cheese-like curd. Application of heat and addition of organic acids and/or mineral salts to whey creates a cheese curd at a yield of ~6% (Smithers, 2008). Often milk is added to increase yield and firmness of the curd (Pintado et al., 2001; Smithers, 2008). Ricotta is a type of whey cheese that is produced from Mozzarella or Provolone whey. Flavor is rapidly developed in whey cheese along with microbial growth (indicating a shorter shelf-life) because of its pH and high moisture content (Jelen, 1992; Pintado et al., 2001).

Infant formula

While breast milk is ideal for feeding infants, limitations can require the substitution of human milk with infant formula. Because infant formula is the sole diet item for the infant, it is very important that it be nutritionally complete. Fast growing infants requires high energy intake. Energy sources include fats, carbohydrates and proteins. Carbohydrates and proteins are great sources of energy for adults; however, an infant's intestinal tract can't handle the high solute loads. Therefore, high protein formulas are very easy to produce from whey protein isolate ingredients. In 1999, it was estimated that 30-40 000 tons of whey protein are used annually in infant formulas (Jost et al., 1999). Whey protein concentrates and demineralized whey powder are the primary whey products used for this application. To imitate breast milk it is essential to understand the "mother nature"

world. Human milk contains a protein density of 1.5-1.6 g/100 kcal while regulations require infant formulas to contain 1.8 g/100 kcal protein density (Jost et al., 1999). Infant formulas often contain more protein content in an effort to compensate for the difference in protein between human milk and bovine milk. Infant formulas should contain 50% fat, 9% protein and 41% carbohydrates. Babies also have low levels of amylase, the enzyme responsible for digesting starches, which limits their energy intake from carbohydrates, making fat and protein better sources of energy for the infant.

Often whey protein powders are used in infant formulas to provide the protein source. Whey protein is an ideal protein source, over other sources of protein, because of its essential and semi-essential amino acid profiles (Jost et al., 1999). Reference amino acid profiles from human milk have been developed by regulatory agencies for comparison in infant formula formulations. Added residual lipid content in WPC could benefit the infant by providing trace fat content that is present as short chain essential fatty acids. These fats should be ideal for the infant because they originate from a milk source, and are low molecular weight.

Fat replacers

Whey protein concentrate is used in a food additive, Simplesse 100, which works as a protein-based fat replacer in bakery and dairy products (Mahungu et al., 1999). Microparticulation creates suspended proteins that have fatlike properties. Cheftel and Dumay (1993) utilized the extrusion of whey protein concentrate to produce a semisolid

spread. This nutritional value lies in allowing consumers to replace butter and margarines in their baked goods for whey protein with trace amounts of dietary fat.

Bars and Beverages

While most meal replacement bars contain approximately 300 calories, they must contain 100% of the U.S. Recommended Daily Intake for at least 12 essential vitamins and minerals and 8 to 10 g of protein (Anonymous, 2002). It is expected that the meal replacement industry will reach $8 billion in 2008 (Anonymous, 2004). Research by Childs et al. (2007) compared protein types, soy vs whey, in commercial and prototype meal replacement bars and beverages. Whey protein commercial bars were characterized by higher intensities ($P<0.05$) of cardboard and sweet aromatic flavor and bitter taste, as well as higher textural intensities of surface gloss, adhesiveness, cohesiveness and visual denseness. Similar to the whey bars, commercial beverages that contained whey protein were characterized by bitter taste as well as a metallic flavor; higher intensities ($P<0.05$) of vanillin and sweet aromatic flavors than other beverages were also detected.

Cardboard, metallic and soapy flavors were expected since these are characteristic of rehydrated whey proteins (Russel et al., 2006). However, because the objective of this study was only to compare flavor profiles of fresh soy vs whey protein ingredients (and not fresh vs off-flavor whey ingredients) flavor carry-through into ingredient applications was not addressed. Conclusions from the Childs et al. (2007) study revealed that, despite documented protein-specific flavors in whey protein beverages and bars, consumers

preferred meal replacement bars and beverages utilizing whey protein over those with soy protein when both were presented coded (not labeled) under controlled conditions. However, even though whey protein beverages were rated higher in overall liking than soy protein beverages, they were still scored in the dislike region of the 9 point hedonic scale (<5). Temelli et al. (2004) reported that as the concentration (> 0.5%-1.5%) of whey protein increased in an orange-flavored beverage, greater (undesirable) whey flavors were detected. These results, taken as a whole, suggested that while whey protein to soy protein may be superior in ingredient applications according to consumer liking, flavor still poses hurdles for beverage and bar applications using current formulations. Current research still holds beverage whey protein applications as a sensory challenge, often due to astringency and unpleasant aftertastes. Processing and flavor-masking have been used to address these and other sensory issues with controversial success (Johnson et al., 1996; Beecher et al., 2006; Drake, 2006; Drake et al., 2008).

Another important factor for considering the success of incorporating whey protein into meal replacement bars, is bar hardening. Protein moisture adsorption and/or protein-protein interactions often result in bar hardening after manufacture (Gallo-Torres, 2003; Zhou and Labuza, 2006). Whey protein hydrolysate addition can inhibit bar hardening, and was successfully utilized by Childs et al. (2007). Results showed that whey protein bars decreased in hardness after 14 days, while remaining approximately constant from 48-84 day storage. Bars made with soy protein, on the other hand, were harder at all timepoints

than the whey protein bars. These results represented instrumental testing results, trained panelists did not detect difference in texture across storage.

Other Applications

An interesting application of whey proteins is for encapsulant or co-encapsulant materials. This allows for the protection and delivery of sensitive functional and bioactive components such as omega-3 oils (Augustin et al., 2006), probiotic organisms (Picot and Lacroix, 2004; Crittenden et al., 2006), essential oils and anhydrous milk fat (Rosenberg, 1997).

Increasing popularity and necessity of sustainability has also lapped over into whey production. Whey water is the product that results after all whey solids have been removed and is termed 'cow water' and is potable. While safe to drink, this water has not yet been approved for public drinking, but is an interesting lead for future sustainable strategies (Jelen, 2003; Smithers, 2008).

Edible films, made from WPI, were effective as oxygen barriers at low and intermediate relative humidity levels (McHugh and Krochta, 1994; Mate and Krochta, 1996a). From this, edible coatings have been applied to roasted peanuts to successfully reduce lipid oxidation leading to rancidity by protecting the lipids from oxygen contact (Mate and Krochta 1996b; Mate et al., 1996; Lee et al., 2002). Interestingly, a major concern with whey protein quality is off-flavor production due to lipid oxidation, whereas in this application the whey coating

was utilized to prevent lipid oxidation of the peanuts. In a study by Lee et al. (2002), roasted peanuts without the whey coating were characterized by rancid flavors, and the whey-coated peanuts were characterized by cardboard and burnt attributes after storage. Therefore, since cardboard flavors are often associated with whey lipid oxidation this research could suggest that while lipid oxidation of the peanuts was prohibited and reduced rancid attributes, lipid oxidation still occurred within the whey coating creating the cardboard flavor perceived by the sensory panel. The success of this application should be based not on inhibition of lipid oxidation but rather prevention of off-flavor by consumers. Perhaps the best option would be a shorter peanut shelf life rather than the presence of cardboard flavors. However, if lipid oxidation and production of cardboard flavors from the whey coating were controlled through the whey production process, then the success of this application could be reevaluated.

Whey permeate applications

Whey permeate is the by-product of ultrafiltration of whey to produce WPI and WPC. It is comprised of 65-85% lactose, 8-20% minerals, 1.5% fat and 3-8% protein (USDEC, 2000) Because of its components, whey permeate has limited applications. With fermentation assisted by lactic acid bacteria, whey permeate has been used to produce lactic acid (Talabardon et al., 2000; Fitzpatrick and O'Keeffe, 2001; Fitzpatrick et al., 2001; Macedo et al., 2002). With its high lactose content, application of B-galactosidase to whey permeate can be used to produce oligosaccharides to be used as a functional food ingredient (Rustom

et al., 1998). Permeate syrups have also been developed from hydrolyzed whey permeate and used as a sucrose supplement in canned peaches and pears (Tweedle and Macbean, 1978). No loss in quality was reported from the substitution. Hydrolyzed whey permeate, along with non hydrolyzed permeate has also been used as a brine replacer in canned beans (Chandan et al., 1982).

While these are all successful applications, one of the potential permeate largest applications is in the beverage industry. Functional beverages are a portion of the still beverage market, which is growing annually at twice the rate of carbonated beverages (Williams, 2001). Research by Beucler et al. (2005) examined the design of a fruit-flavored water beverage from Mozzarella whey permeate. Descriptive sensory results characterized the whey permeate beverages as being dairy sour and brothy, which differentiated them from the commercial beverages. Interestingly, consumers in this study indicated water as being the most liked beverage of the samples evaluated; including flavored beverages without whey permeate. Consumers rated the whey permeate beverages as being very high in sweet taste, but low in sweet taste liking, inferring that these beverage formulations were too sweet (Beucler et al., 2005). Results indicated that there was a gap between beverages containing <50% whey permeate and >75% whey permeate, with the lower concentration being liked more and more closely associated to the commercial beverages (Beucler et al., 2005). Conclusions concerning whey permeate hydrolysis were that minimal sensory differences existed when in a beverage application; therefore, it was not necessary

to add this expensive processing step to create a successful whey permeate beverage. Beucler et al. (2005), based on results, reported that a fruit-flavored water beverage was not the best application for securing whey permeate as a ingredient in the beverage market because of the flavors of dairy sour and brothy. They did hypothesize that fruit smoothie or drinkable yoghurt type beverages would be a better platform for integrating whey permeate use at higher levels in the beverage industry (Beucler et al., 2005).

Whey Protein flavor

In order to use liquid whey in applications for human consumption, the flavor must be taken into consideration. Whey flavor is directly affected by the type of cheese produced as well as the quality of milk and the method of whey handling after draining, including the time elapsed (Bodyfelt et al., 1988). Fresh liquid whey is expected to possess a delicate and bland flavor that should relate into powdered whey when reconstituted (Drake et al., 2003). Bodyfelt et al. (1988) described liquid whey as exhibiting a slightly dirty-sweet/ acidic taste and odor and more recent refined descriptive sensory analysis has documented a host of specific flavors in liquid and dried whey ingredients (Karagul-Yuceer et al., 2003; Drake et al., 2003; Carunchia Whetstine et al., 2003, 2005; Tomaino et al., 2004; Gallardo-Escamilla et al., 2005; Russell et al., 2006; Wright et al., 2006, 2008; Drake, 2006; Drake et al., 2008, Table III).

Table 1.4. Sensory descriptive analysis terms

Descriptor	Definition	Reference
Aromatics		
aroma intensity	the overall orthonasal aroma impact	
milky	aromatic associated with milk	room temperature milk
cardboardy	Aromatics associated with wet cardboard and brown paper	2cm x 2cm peiece of brown cardboard boiled in water for 30 min
cooked	aromatic associated with cooked milk	cooked milk
musty	aroma associated with potting soil	
metallic	aroma associated with metal	1-octen-3-one
milkfat	aroma associated with fresh cream	cream, whole milk mozzarella
buttery	aroma assocated with butter	diacetyl
sweet aromatic	sweet aroma associated with dairy products	vanilla cake mix / 20ppm vanillin in milk
pungent	not available	
sulfurous	not available	
rancid	taste associated with sour milk and oxidized fats	oxidized fat
caramelized milk	not available	
natural yoghurt	aroma associated with the fermentation of milk	cultured dairy yoghurt
oaty	aroma associated with oat flakes	oatmeal in milk
cheesy	not available	
heated/boiled milk	aromatic associated with cooked milk	cooked milk
dirty	not available	
chemical	aroma associated with a blend of nonspecific solvents	
acid	taste produced by solution of aqueous acids	citric or lactic acid
stale	not available	
brothy	aromatics associated with broth or boiled potatoes	methional
cabbage	aroma associated with boiled cabbage	cabbage leaf boiled in 500mL in water for 5 min
soapy	aroma associated with soap	lauric acid
doughy/fatty	aroma associated with canned biscuit dough	(Z)-4-heptenal
cucumber	aroma associated with freshly sliced cucumber	(E)-2-nonenal
animal/wet dog	aroma associated with wet dog hair	Knox gelatin
pasta water	aroma associated with water after pasta has been boiled in it	boil pasta in water for 30 min
Basic Tastes		
sweet		
salty		
bitter		
astringency		
sour		
Texture		
mouth coating		
chalky		

Karagul-Yuceer et al., 2003; Tomaino et al., 2004; Carunchia Whetstine et al., 2005; Gallardo-Escamilla et al., 2005; Wright et al., 2006

Volatile compound analysis has also been conducted to pinpoint specific compounds responsible for liquid and dried whey protein flavors. Instrumental analysis of Cheddar cheese liquid whey by Karagul-Yuceer et al. (2003) showed 2,3-butanedione (buttery), 2-butanol (sweet), hexanal (cut grass), 2-acetyl-1-pyrroline (popcorn), methional (boiled potato), (E,E)-2,4-nonadienal (fatty) and (E,E)-2,4-decadienal (fryer oil) to be major players in liquid whey flavor. Mills (1986) identified 2,3-butanedione, 3-methyl butanal, 2-pentanone, pentanal, hexanal, 2-heptanone, heptanal and benzaldehyde from the headspace of WPC from casein whey. Table IV outlines volatile compounds that have been identified in the literature as being present in liquid whey, WPC80 and/or WPI (Karagul-Yuceer et al., 2003; Drake et al., 2003; Carunchia Whetstine et al., 2003, 2005; Mahajan et al., 2004; Tomaino et al., 2004; Gallardo-Escamilla et al., 2005; Russell et al., 2006; Wright et al., 2006; Drake, 2006; Drake et al., 2008; Wright et al., 2008; Javidipour and Qian, 2008)

.

Table 1.5. Volatile Compounds Identified in Liquid Whey

Volatile	Whey type [ab]	Volatile	Whey type [ab]
(E)-2-hexenal	WPC80, agglom. WPC80, WPI, agglom. WPI	butanoic acid	liquid, SWP, WPC80, WPI
(E)-2-nonenal	liquid, SWP, WPC80, WPI	butanol	liquid
(E)-2-octenal	SWP, WPC80	decanal	WPC80, WPI
(E,E)-2,4-decadienal	liquid, SWP, WPC80, WPI	decanoic acid	SWP, WPC, WPI
(E,E)-2,4-nonadienal	SWP, WPI	delta-decalactone	liquid, SWP, WPC80, WPI
(E,E)-2,4-octadienal	SWP	delta-dodecalactone	SWP, WPC80, WPI
(E,Z)-2,4-decadienal	SWP	delta-octalactone	SWP
(E,Z)-2,4-nonadienal	SWP	delta-undecalactone	SWP
(E-Z)-2,6-nonadienal	liquid, SWP, WPC80, WPI	diacetyl	liquid, SWP, WPC80, agglom. WPC80, WPI, agglom. WPI
(Z)-2-nonenal	liquid, SWP, WPI	dimethyl disulfide	SWP, WPC80, inst.WPC80, agglom. WPC80, WPI, agglom. WPI
(Z)-4-heptanal	WPC80	dimethyl sulfide	liquid, WPC80, WPI
(Z)-4-heptenal	SWP	dimethyl trisulfide	liquid, SWP, WPC80, agglom. WCP80, WPI, agglom. WPI
1,2-propadiene; alkenyl	liquid	dimethylamine; allyl	liquid
1,5-octadienone	WPI	dodecanoic acid	SWP
1-dodecane	liquid	ethanol	liquid
1-octen-3-ol	WPC80, agglom. WPC80, WPI, agglom. WPI	ethyl acetate	liquid
1-octen-3-one	liquid, SWP, WPC80, inst. WPC80, WPI	formic acid	SWP
1-propanol	liquid	furfuryl alcohol	SWP
2,3 methyl butanol	liquid	gamma-decalactone	SWP, WPC80
2,3,5-trimethyl pyrazine	SWP	gamma-dodecalactone	SWP
2,3-dimethyl pyrazine	SWP	gamma-hexalactone	SWP
2,3-methylbutanoic acid	liquid	gamma-nonalactone	WPC80
2,5-dimethyl pyrazine	SWP	heptanal	liquid, SWP, WPC80, inst. WPC80, agglom. WPC80, WPI, agglom. WPI
2,5-dimethyl-4-hydroxy-3- (2H) furanone (Furaneol)	SWP, WPC80, WPI	heptanoic acid	SWP, WPC80, WPI
2,6-dimethyl pyrazine	SWP	heptanone	liquid

Table 1.5. continued

Volatile	Whey type [ab]	Volatile	Whey type [ab]
2-acetyl-1-pyrroline	liquid, SWP, WPC80, WPI	hexanal	liquid, SWP, WPC80, inst. WPC80, agglom. WPC80, WPI, agglom. WPI
2-acetylpyrrole	SWP	hexanoic acid	liquid, SWP, WPC80, WPI
2-acetylthiazole	SWP	hydrocarboxyl	liquid
2-butanol	liquid	isobutyric acid	liquid
2-butanone	liquid	maltol	liquid, SWP
2-butanone	liquid	methional	liquid, SWP, WPC80, WPI
2-ethyl pyrazine	liquid, SWP	methyl propanoic acid	liquid
2-ethyl-1-hexanol	WPC80, agglom. WPC80, WPI, agglom. WPI	nonanal	liquid, SWP, WPC80, agglom. WPC80, WPI, agglom. WPI
2-furfural	liquid	nonanoic acid	WPC80, WPI
2-heptanol	WPC80,inst.WPC80	nonanol	WPC80,inst.WPC80
2-heptanone	liquid, WPC80, agglom. WPC80, WPI, agglom. WPI	nonanone	liquid
2-isobutyl-3-methoxypyrazine	liquid, WPC80, WPI	o-aminoacetophenone	WPC80, WPI
2-methoxy phenol (guiacol)	WPC80	octanal	liquid, WPC80, agglom. WPC80, WPI, agglom. WPI
2-methoxy-3-isopropylpyrazine	liquid	octanoic acid	liquid, SWP, WPC80, WPI
2-methyl propanoic acid	SWP	octanol	WPC80,inst.WPC80
2-methyl-3-furanthiol	liquid, WPI	p-Cresol	SWP
2-nonanol	WPC80,inst.WPC80	pentanal	liquid
2-nonanone	liquid, WPC80, agglom. WPC80, WPI, agglom. WPI	pentanoic acid	liquid, SWP, WPC80, WPI
2-octanone	WPC80,inst.WPC80	phenol	liquid
2-pentyl furan	WPC80, agglom. WPC80, WPI, agglom. WPI	phenyl ethyl acetate	WPC80
2-phenethanol	WPC80, WPI	phenylacetaldehyde	SWP, WPI
2-propanol	liquid	propan-1-ol; alkyl	liquid
2-propionyl-1-pyrroline	SWP	propanoic acid	liquid, SWP
2-undecanone	WPC80, agglom. WPC80, WPI, agglom. WPI	skatol	SWP

Table 1.5. continued

Volatile	Whey type [ab]	Volatile	Whey type [ab]
3-hydroxy-4,5-dimethyl-2-(5H)-furanone (Sotolon)	liquid, SWP, WPC80, WPI	toluene	WPC80, agglom. WPC80, WPI, agglom. WPI
3-methoxy-4-hydroxy benzaldehyde (vanillin)	WPC80	***fatty acids***	
3-methyl butanoic acid	SWP	caproic	liquid
3-methyl furan	liquid	caprylic	liquid
4-methyl octanoic acid	WPC80, WPI	capric	liquid
9-decanoic acid	SWP	lauric	liquid
acetaldehyde	liquid	myristic	liquid
acetic acid	liquid, SWP, WPC80, WPI	palmitic	liquid
acetoin	liquid	palmitoleic	liquid
acetone	liquid	stearic	liquid
benzaldehyde	WPC80, agglom. WPC80, WPI, agglom. WPI	oleic	liquid
butadiene	liquid, WPC80, WPI	linoleic	liquid

[a] SWP - Sweet Whey Powder; WPC80 - Whey Protein Concentrate 80% protein; inst. WPC80 - instantized WPC80; agglom. WPC80 - agglomerated WPC80; WPI - Whey Protein Isolate >90% protein; agglom. WPI - agglomerated WPI

[b] Cheese source: *liquid* - Cheddar, Gouda, Mozzeralla, Paneer, Quarg, rennet casein, acid casien, lactic acid casein; *SWP* - Cheddar; *WPC80* - Cheddar, Mozzerella, Monterey Jack; *inst. WPC80* - Cheddar, Monterey Jack; *agglom. WPC80* - Cheddar, Mozzerella; *WPI* - Cheddar; *agglom. WPI* - Cheddar

Karagul-Yuceer et al., 2003; Drake et al., 2003; Mahajan et al., 2004; Tomaino et al., 2004; Carunchia Whetstine et al., 2003, 2005; Gallardo-Escamilla et al., 2005; Russell et al., 2006; Drake, 2006; Wright et al., 2006; Javidipour ang Qian, 2008; Drake et al., 2008; Wright et al., 2008

Flavor variability is widespread among WPC and WPI for several reasons. One reason is that raw liquid whey, possibly from different types of cheese, is pooled together before processing into WPC and WPI (Carunchia Whetstine et al., 2005). Sensory and instrumental analysis by Carunchia Whetstine et al. (2005) demonstrated sensory and volatile compound variability between WPC 80 and WPI as well as flavor variability within both protein products. Walker (1977) identified the non-acidic fraction of volatile compounds from whey as the major contributor to flavor. More recent research by Mills (1992) agreed with Walker (1977) in showing that the basic fraction of volatiles contained the major contributors to WPC flavor. Whey exhibits low sensory flavor intensities, but off flavors may frequently carry-through into ingredient applications (Drake, 2006; Drake et al., 2008). Morr and Ha (1991) hypothesized that flavor could be further reduced during manufacture of WPC through ultrafiltration and diafiltration steps because whey flavor is dialyzable as well as volatilized during drying.

Analyzing for flavor volatiles in whey becomes complicated because whey proteins may bind flavor compounds (Mills and Solms, 1984). Ha et al. (2002) examined dynamic headspace analysis (DHA) as a means of collecting volatiles from Swiss cheese whey protein concentrates. The study involved DHA of powdered WPC compared to DHA of rehydrated WPC. Double the amount of volatiles was identified with the powder WPC compared to the rehydrated WPC solution; however, the DHA system allowed only the powder samples to be heated, releasing more flavor volatiles. Varying the sampling temperature increased

volatile recovery for the powdered WPC samples. Major flavor contributors to Swiss cheese whey proteins in this study were identified as z-propanol, 1-octanol, propanal, 2,3-butanedione and benzothiazole, benzothiazol, dimethyl sulfone and dimethyl disulfide (Ha et al., 2002). These compounds are reported to exhibit gasoline/rubber, sulfur/burnt, and onion/cabbage/putrid odors respectively (Flavornet), but sensory analysis was not conducted to further relate results to flavor perception. Lee et al. (1992) also identified these sulfur compounds in Swiss cheese where they documented increases during aging.

Off-flavors and Their Production in Whey Proteins

For widespread ingredient applications it is important for liquid and powdered whey to exhibit a bland flavor, however, off-flavors are present and result from many different factors and chemical reactions. Such factors/reactions include riboflavin effects, Maillard browning, shelf life storage and lipid oxidation.

Riboflavin Effects

Riboflavin is a water soluble vitamin found in milk (O'Connor and O'Brien, 1994). Decomposition of riboflavin could produce off-flavors in whey protein powders. Decomposition of riboflavin produces a light-oxidized off-flavor in fluid milk, skim milk, whey, and serum protein fractions (Aurand et al., 1966; Sattar and deMan, 1975). Again it is important to consider the pH because pH affects photolysis of riboflavin (Morr and Ha,

1991). Milk contains riboflavin, a water-soluble vitamin and potent photosensitizer, which can induce photooxidation, oxidation due to light (O'Connor and O'Brien, 1994). Combined with fluorescent lighting, riboflavin can also produce superoxide anions and lead to destruction of other milk components (Korycha-Dahl and Richardson, 1979). Further riboflavin interaction with milk proteins in the presence of light can oxidize methionine into methional, a compound with a dirty potato flavor (Sattar et al., 1977b).

Storage Effects

Storage of whey protein is a desirable factor which allows the food industry to better utilize all WPC and WPI. The only problem with the shelflife of whey proteins is the potential for off-flavors produced during storage. The major off-flavor concern associated with dried whey and whey products is a "stale aged" flavor. This is a vague term used prior to mainstream descriptive analysis techniques (Table III). Wright et al. (2008) documented flavor changes during storage of WPC80 and WPI. Flavors documented with storage were variable with protein type (WPC80 vs WPI), facility and whether or not the protein was agglomerated. Predominant storage-related flavors included cardboard, raisin, fatty, and cucumber. Javidipour and Qian published similar research focusing on the storage stability of WPC80 and instantized WPC80. Results indicated an increase of selected volatile compounds over the 15 week storage period. Compounds observed included a range of aldehydes which was suggestive of lipid oxidation in the samples. Lipid oxidation occurred in instantized WPC80 samples faster than the non-instantized WPC80 (Javidipour and Qian,

2008); as also documented by Wright et al. (2008) in agglomerated vs. non-agglomerated whey protein samples. Wright et al. (2008) also observed the presence and increase of lipid oxidation products, particularly aldehydes and ketones, in whey samples over their storage period. During the end of the storage period, Wright et al. (2008) observed further changes in the volatile components suggesting that further breakdown of lipid oxidation compounds was occurring. Both storage studies thus concluded that from 12-18 months storage, degradation of the whey proteins had occurred reguardless of agglomeration or instantization treatment (Javidipour and Qian, 2008; Wright et al., 2008). Wright et al. (2008) also confirmed storage volatile changes in WPC80 and WPI by sensory analysis. Similar to volatile compound results, sensory changes occurred within 12-18 months and these flavor changes were perceived as off flavors by consumers in ingredient applications.

Whey flavor often originates from the milk and cheese making process. In 1992, Whitfield hypothesized that whey flavor compounds were created during the spray drying process that turned liquid whey into powdered whey. During this time Maillard reactions and lipid oxidation initiated flavor development. Mahajan and Qian (2004) studied the aroma compounds present in sweet whey powder to expand the hypothesis outlined by Whitfield (1992). Fresh sweet whey powder samples from two plants were analyzed and up to 45 aroma active compounds identified. Among those compounds identified as being present in the whey powder, some were products formed from autooxidation of lipids, as well as caramelization of sugar and Maillard browning suggesting that spray drying could

impart flavors to sweet whey powder. However, liquid drier feed products were not evaluated, only final spray dried products.

The development of stale off-flavors plays the major role in inhibiting dried whey from being utilized to a greater extent as a food ingredient (Morr and Ha, 1991; Drake, 2006). Maillard browning and lipid oxidation have been demonstrated by numerous research studies as the two factors to control development of these off flavors in dried whey ingredients (Ferretti and Flanagan, 1971a, 1971b; Mills, 1986; Lee and Morr, 1994; Laye et al., 1995; Lee at al., 1996; Stevenson and Chen, 1996; Yang et al., 1998; Mills and Broome, 1998; Quach et al., 1999; Carunchia Whetstine et al., 2005).

Maillard Browning

Temperature of storage has proven to drastically affect the flavor quality of dry dairy products over time (Driscoll et al., 1985; Kieseker and Clarke, 1984). Temperature is often linked to Maillard browning. Dattatreya et al. (2007) examined the effect of temperature and pH on determining shelf life for sweet whey powder (SWP) using accelerated shelf life experiments. Other studies by Sithole et al. (2005) and Javidipour and Qian (2008) also supported the effectiveness of using accelerated shelf life models to predict shelf life of whey. These previous studies evaluated whey powder and WPC80, respectively under normal storage conditions (21^{o}C and 35% relative humidity) while Dattatreya et al. (2007) was concerned with the effects of temperature and pH on the shelf life of sweet whey powder. During handling and storage of liquid SWP, continued fermentation can lead to

higher acidity. Dattatreya used L* values, indicating change in brown pigments of the whey due to Maillard browning reactions, to conclude that storage temperature and initial pH strongly determined shelf life. For sweet whey powder, the higher lactose content was a concern when pH decreased because the acid acted as a catalyst involving the lactose. The results of Dattatreya et al. (2007) supported this concept, showing a higher occurrence of Maillard browning at lower pH. However, the most intriguing aspect of this data was its implications on shelf life with temperature abuse taken into consideration. Temperature abuse can happen during manufacturing, transportation and storage of liquid whey. Heat during spray drying can also initiate browning reactions (Morr and Ha, 1990). Shelf life calculations from this accelerated storage study illustrated that a sample of SWP at high acidity (pH~4.0) held at 60^{o}C will reach its full shelf life (normally up to 2 years) with 0.9 days. If this temperature step was avoided, Dattatreya et al. (2007) hypothesized that the SWP could have up to 262 additional storage days. SWP shelf life was dramatically reduced due to short periods of temperature abuse (Dattatreya et al., 2007)

Another way to combat Maillard browning is to reduce the amount of sugar present. Other manufacture modifications are being explored with WPC to reduce the lactose content to <5% (w/w). Decreasing the lactose content would help improve flavor stability by way of reducing Maillard browning (Morr and Foegeding, 1990). 5-hydroxymethylfurfural (HMF), a compound generated at later stages in Maillard browning, has been used as a quality deterioration marker for whey proteins with results that indicate

that over the shelf life of whey powder at room temperature, not only does moisture content increase, but so does HMF (Dogan and Oral, 2007).

A study by Sithole et al (2005) concluded that while functional properties rapidly decreased as Maillard reactions occurred during 19 month storage of sweet whey powder, sensory aroma and flavors remained constant. Key volatile lipid oxidation components characterized in these studies included aliphatic aldehydes, methyl ketones, 1-pentanol and 1-octen-3-ol. Similar identification of key Maillard browning products of furans, pyrazines, pyrroles and pyrans were also found. Because Maillard reactions involve sugars, whey protein powders with higher lactose contents are more susceptible to this type of deterioration and thus are expected to have a shorter shelflife. Therefore, most of the published research on Maillard off-flavors involve sweet whey powder rather than whey protein concentrates and isolates.

Lipid Oxidation

Lipid oxidation is the production of hydroperoxides from unsaturated fatty acids. Hydroperoxides are tasteless, odorless and unstable and degrade to yield flavorful carbonyls and other compounds (O'Connor and O'Brien, 1994). The chemical reaction mechanism for lipid oxidation starts with an initiation step where free radicals are created by irradiation, metal complexes, enzymes or active oxygen species. Once the free radical is made, it reacts with molecular oxygen to produce a peroxide free radical. The peroxide free radical then reacts with another unsaturated fatty acid to produce a hydroperoxide

(O'Connor and O'Brien, 1994). Oxidative stability is often gauged by the induction period, or lag phase, before hydroperoxides are continually formed rapidly until the maximum is reached (O'Connor and O'Brien, 1994).

Off-flavors in dairy products are often a product of hydroperoxide degradation that can create the following compounds: unsaturated and saturated aldehydes (Frankel et al., 1961), unsaturated and saturated hydrocarbons, unsaturated ketones (Stark and Forss, 1962), saturated and unsaturated alcohols (Hoffman, 1962), and semialdehydes (Frankel et al., 1961). Other compounds have been isolated from milk, rather than the expected carbonyl compounds, indicating that further degradation of the initially produced unsaturated aldehydes, migration of the double bonds or isomerization could all occur during auto-oxidation (Weihrauch, 1988). Lipid oxidation in WPC is believed to result from phospholipoproteins released from the milkfat globule in cheesemaking that are further concentrated during ultrafiltration of whey proteins. As previously addressed, whey proteins, including WPC80 and WPI, still contain residual fat. Researchers have proposed that removal of these phosopholipoproteins prior to ultrafiltration will increase the quality of the resulting WPC (Morr and Foegeding 1990; Rinn et al., 1990). Morr and Foegeding (1990) compared flavor stability of commercial WPI and WPC with results indicating that lipid and lactose concentrations were important factors in flavor stability.

Oxidation of dairy lipids can be affected by many different factors including oxygen, light, metals, anti-oxidants, ascorbic acid, tocopherols, carotenoids, thiols, proteins, enzymes, Maillard browning products, storage temperature, water activity and milk fat globule membrane constituents (O'Connor and O'Brien, 1994). It is this balance of pro-oxidants and anti-oxidants that determines the susceptibility of oxidation in dairy products. Spontaneous oxidation in fluid milk can occur within 48 hours of milking. The category of "spontaneous milk oxidation" describes approximately 12-20% of all raw milk and is determined by heredity, stage of lactation and feeding practices of the cow (Bruhn et al., 1976; Shipe, 1964). While oxygen is a major pro-oxidant, Schroder (1982) showed that removal of dissolved oxygen did not reduce all oxidized flavors in milk. Light-oxidized flavor was reduced, but to prevent copper-oxidized flavors, de-aeration must be conducted to very low oxygen levels.

Water activity has a complex effect on lipid oxidation by promoting oxidation at low and high water activities with the minimum oxidation occurring at intermediate water activity levels (~0.4) (Labuza, 1971). Labuza (1971) hypothesized that a monolayer of water created hydrogen bonding that delayed hydroperoxide degradation and masked pro-oxidants by hydration. At low water activities, this monolayer was not present; however, at higher water levels, pro-oxidants were mobilized and diffused through the food, promoting oxidation. High water activity values can also work to inhibit oxidation because the reactants are diluted and thus have less contact with each other for a reaction to occur.

A study by Gallardo-Escamilla et al. (2005) sought to fill a gap in the literature by understanding flavor characteristics of whey from different cheeses and the relationship of volatile components and sensory profiles. This was the first study that centered on liquid whey flavor, besides those published on Cheddar whey flavor (Carunchia Whetstine et al., 2003; Karagul-Yuceer et al., 2003; Tomaino et al., 2004) since McGugan et al. (1979) researched acid whey. Gallardo-Escamilla et al. (2005) analyzed eleven types of liquid whey ranging from rennet casein whey, medium acid whey, acid whey and cheese whey. The types of cheese whey included Cheddar, Gouda, Mozzarella, paneer or quarg cheeses. Directly after production, samples were frozen for transport and/or frozen at -20^{o}C until time for analysis for which they were thawed overnight. Descriptive analysis of the samples showed statistical differences between all eleven whey samples for all attributes except "caramelized" odor. "Acid", "sweet", "cheesy" and "milky" intensities were the sensory attributes that affected the primary flavor differences in whey. Gallardo-Escamilla et al. (2005) also reported "dirty" odor scores highest in commercially produced Cheddar and acid casein whey. Hypotheses for this finding were the possibility of microbial growth in commercial processing of Cheddar whey and oxidation reactions in acid casein whey (Bodyfelt et al., 1988; Tomaino et al., 2004).

Analytical results of the flavor volatiles were obtained through proton transfer reactions-mass spectrometry (PTR-MS). Gallardo-Escamilla et al. (2005) concluded that this method was adequate in providing volatile results that related to the sensory profiles of the

whey samples, however, principle component analysis of the volatile results only represented 43% of the total variability and partial least squares regression type 1 found only “rancid” and “natural yogurt” attributes to have high validation coefficients. Volatile data characterized “rancid” as being associated with the presence of butadiene or 3-methyl furan and the absence of an allyl radical or dimethylamine and ethanol. Because “rancid” intensities were highest for acid casein whey in this study, Gallardo-Escamilla et al. (2005) hypothesized that the addition of acid during production attributed to this and other “stale” off-flavors. Even though an objective of this study was to understand relationships between sensory and volatile analysis to help control whey flavor, the lack of strong relationships and hypotheses from the data impaired the researchers from outlining future steps that could help control whey flavor.

Perhaps the most enlightening study of lipid oxidation in whey was conducted by Tomaino et al. (2004) in which the objectives were to determine if starter cultures influenced the oxidative stability of liquid whey flavor immediately after clarification and pasteurization and to what affect storage had on both of these parameters. Tomaino et al. (2004) hypothesized that since liquid whey was often transported and stored before drying, reactions that take place during this time that could dramatically affect the flavor of the finished whey powder after concentration and drying. Volatile compounds present in liquid whey were retained during spray drying (Bangs and Reineccius, 1981), which could be attributed to binding of proteins and flavor compounds such as methyl ketones and

aldehydes (lipid oxidation products) (Mills and Solms, 1984). Therefore if oxidation products were produced during storage and transportation, those products could be carried through to the finish whey powder impacting its flavor, functionality and variability (Tomaino et al., 2004).

Tomaino et al. (2004) produced whey at the pilot plant scale using four starter culture strains. Whey was pasteurized at 77^{o}C for 16 seconds, clarified and immediately frozen at -20^{o}C or stored for 14 days at 4^{o}C and then frozen. Volatile analysis indicated 1-propanol, hexanal, nonanal and 2-nonanone as all being present in the liquid whey, but not in the milk used for whey production. Because these compounds were common volatile lipid oxidation products, Tomaino et al. (2004) hypothesized that their presence indicated that oxidation occurred during cheese making and/or whey pasteurization. The liquid whey that had been stored for fourteen days showed a large increase in aldehydes including hexanal, methyl ketones and pentane, verifying that lipid oxidation had occurred. A similar storage study conducted by Lee et al. (1996) also indicated a 127-fold increase of hexanal concentration in WPC, ~75% protein, after six days of accelerated storage. This observation was explained by Tomaino et al. (2004) as being a product of lipid oxidation initiated during processing and the products and continual oxidation being carried over into the dry product due to entrapment in the matrix, hydrophobic interactions or covalent linkages with ε- and α- amino groups of proteins (Kinsella, 1989; Mills and Solms, 1984; Hidalgo and Kinsella, 1989).

Limitations of the study by Tomiano et al. (2004) include using potentially contaminated milk for whey production, not replicating sensory analysis, and projecting conclusions about the impact of potential fermentation compounds on whey ingredients. These fermentation compounds resulted from the starter cultures used to make the cheese from which the whey was produced. This study negates the importance of these fermentation compounds and low molecular weight volatiles. Tomiano et al. (2004) commented that this was a limitation and hypothesized that it was the protein interactions with the higher molecular weight volatile lipid oxidation products that allowed them to be carried through the processing while the others were removed. And so the question still remains as to the importance of these fermentation and low molecular weight volatile compounds on whey flavor. Also, because a significant difference was detected between the 'fresh' liquid whey and 14 day storage whey, the question was raised as to if that time period was too long and when during that time did the changes and reactions occur? Perhaps most importantly, Tomaino et al. (2004) did no remove the fat from fluid whey in their study. This is a standard industrial process step and as such, results from Tomaino et al. (2004) would represent at best the worst case scenario in regards to lipid oxidation.

The importance of these volatile lipid oxidation products lies in their potential effect on flavor. Lipid oxidation products have been associated with cardboard flavor in dairy products, which is a common off-flavor attributed to decreased quality in dried whey products (Hammond, 1989; Drake et al., 2003; Wright et al., 2008). Tomaino et al. (2004)

compared his volatile and sensory data to similar results found by Hall and Anderson (1985) in whole milk powder where significant increases in cardboard aroma, flavor and aftertaste were correlated with hexanal and other volatile lipid oxidation product concentrations. To validate this, Tomaino et al. (2004) examined water-soaked cardboard by dynamic headspace analysis and reported recoveries of hexanal, octanal and heptanal as being the predominate compounds present. An oxidized flavor, described as cardboard, was also observed by Hammond and Seals (1972) when oct-1-en-3-one and octanal were added to milk. This research indicated that many different volatile lipid oxidation products could be responsible for cardboard off-flavors observed in dairy products. However, additional research is needed to further specific responsible compounds.

Because unsaturated fatty acids are susceptible to oxidation, the presence of free fatty acids (FFA) creates a risk for lipid oxidation that could result in off flavors in whey products. Data obtained by Tomaino et al. (2001) and Morr and Foegeding (1990) both indicated a relationship between undesirable flavor and quantity of FFA. Lower protein whey powder, whey protein concentrate (WPC80), was shown by Tomaino et al. (2001) to have a 20-fold greater concentration of FFA than whey protein isolates (~92.6% protein). Similar results by Morr and Foegeding (1990) obtained from sensory analysis indicated that higher “stale”, “old whey” flavors were perceived for WPC than WPI. Increases in cardboard flavor in liquid whey across fourteen days storage were correlated with a decrease in linoleic acid (Tomaino et al., 2004). Oxidation of linoleic acid produced hexanal,

which was also found by Tomaino et al. (2004) to increase over storage time in liquid whey. Tomaino et al. (2004) concluded that more research in understanding the low stability of liquid whey could help outline ways to control oxidation deterioration in order to improve the quality of whey as a food ingredient.

In an effort to extend the results from noncommercial whey provided by Tomaino et al. (2004), Carunchia Whetstine et al. (2003) characterized liquid whey flavor variability in commercial Cheddar cheese whey. Sensory and instrumental volatile analyses were conducted on whey and milk collected in triplicate at the end of the cook procedure or prior to cheesemaking received from two different commercial cheese manufacturing facilities. During these collection days, there were at least two different starter culture rotations.

Volatile instrumental results showed similar compounds present in both milk and whey, including lipid oxidation products: hexanal, pentanal and short-chain aldehydes. Variability of lipid oxidation products was observed between whey from different plants. Plant 2 whey contained higher concentrations of pentanal, 2-butanone and other volatile lipid oxidation products which Carunchia Whetstine et al. (2003) attributed to differences in milk source, processing and handling between plants. Variability was also observed between whey produced within the same plant, with one whey sample having significant differences in 2,4-decadienal concentrations. Within-plant variability was hypothesized to depend on starter culture differences. Carunchia Whetstine et al. (2003) explained the volatile results by dividing them into two distinct classes: aroma active and aliphatic

hydrocarbons. Aroma active compounds have a direct affect on the whey flavor, while aliphatic hydrocarbons do not have any odor, so they were assumed to not have any direct impact on whey flavor. Important aroma active compounds identified were diacetyl (butter aroma), dimethyl sulfide (cabbage/sulfur) and hexanal (grass/fat). Diacetyl concentrations varied from plant to plant, but milk source and whey from the same plant exhibited similar diacetyl concentrations. Milk and whey samples from plant 1, contained 1-octen-3-one which was commonly associated with metallic off-flavors in milk and cheese (Stark and Forss, 1962; Carunchia Whetstine et al., 2003). Carunchia Whetstine et al. (2003) based the presence of aliphatic hydrocarbons on the hypothesis outlined by Laye et al. (1995). They result from the reaction of alkyl free radicals or the decarboxylation of the carbon chains of higher fatty acids. Aliphatic hydrocarbons identified were hexane and octane with octane.

Free fatty acid analysis of the samples also showed differences between plants and within plants. High concentrations of long chain fatty acids were observed in the whey which were attributed to the lipolytic nature of starter culture activity because lipolytic enzymes from the starter cultures cleave the long chain fatty acids off the triglycerides (Jensen et al., 1991; El Soda et al., 1995). Of the long chain fatty acids identified, lauric and mystric acid concentrations were higher in the wheys than in their milk sources (Carunchia Whestine et al., 2003). Some whey samples were observed to have higher concentrations of oleic acid, an unsaturated fatty acid. Carunchia Whestine et al. (2003) hypothesized that

lipid oxidation, which is the breakdown of unsaturated fatty acids, could explain why these whey samples also exhibited an increased perception of cardboard flavors.

In accordance with previous research findings with whey and skim milk powder, pentanal was correlated with cardboard flavors (Tomaino et al, 2001; Karagul-Yuceer et al., 2002). Pentanal along with 2-4 decadienal were both correlated with astringency perception by descriptive sensory analysis. Because these compounds were also produced during lipid oxidation, Carunchia Whetstine et al. (2003) hypothesized a relationship between secondary oxidation products and astringency with a further idea that basic tastes of whey could be affected by interactions between FFA and free amino acids. Descriptive sensory analysis differentiated all wheys, with the unique finding of one whey sample being characterized by musty and sour flavors (Carunchia Whestine et al., 2003). A major conclusion for this study was that whey and milk variability can be explained by differences in volatiles, FFAs, free amino acid content and sensory attributes, which were attributed to differences in starter culture, milk source, feed source and milk processing temperature/time. While this data provides another puzzle piece, the continual prevalence of oxidative-associated flavors and compounds in whey protein justifies the need to understand whey processing factors that could affect oxidative deterioration of whey.

Lipid oxidation in whey protein is well documented, and while off-flavor development is the major concern, in regards to lipid content there is also a loss in nutritional value. Due to lipid oxidation and production of off-flavors, quality is sacrificed

because off-flavors are undesirable to consumers. Nutritional value will be lost in the oxidation of proteins and amino acids as well as any fat soluble vitamins present. Often whey protein concentrates are used in high protein energy shakes marketed to heath conscious individuals. Addition of vitamins in these products is probable, but if they are lipid soluble then they could be lost during oxidation.

There is some concern with the consumption of oxidized products. High consumption of oxidation products can lead to malonaldehyde, diarrhea, poor growth rate, myopathy, cardiopathy, and secondary deficiencies of vitamin A and E. Sterol oxides produced are potentially carcinogenic. For the Eskimo populations, consumption of rancid fat can lead to haemolyic anemia where the red blood cells become more fragile. Off-flavor production from oxidation usually limits consumption of rancid fats, however, WPC is often utilized in bulk packaged protein powder drink mix. These mixes are heavily flavored and usually endure a long shelf-life. Consumers of these products may not dwell on the flavor aspect because of the nutritional value; therefore, the probability of consumption of oxidized WPC is likely.

In summary, previous whey research indicates that lipid oxidation occurs during storage of liquid and powder whey produced in the pilot plant and commercially (Tomiano et al., 2004; Carunchia Whestine et al., 2003). It has also been documented that undesirable cardboard flavors increase in whey protein during storage and are due at least in part to volatile lipid oxidation products (Javidipour and Qian, 2008; Wright et al., 2008).

The question is, when? If the time period of when lipid oxidation products are formed in whey and cardboard and/or differences in flavor are detected is determined, then hypotheses concerning the mechanism and preventative options can be outlined.

As previously stated, during whey production, concentrated liquid whey may be stored in temperature-controlled tanks for up to 48 hours before spray drying. Storage and storage time are variable within and between product facilities. It is hypothesized that during this storage time, reactions occur that produce off-flavors in spray dried whey protein. If off-flavor production increases during storage of liquid whey protein concentrate, then whey processors would be encouraged to spray dry immediately after concentration or methods to minimize oxidation during liquid storage can be identified. While lipid oxidation is hypothesized to be the primary reaction taking place, determining the time period of increased reactions could shed light onto the mechanism and specific catalyst(s) of the reactions occurring. Understanding the reaction mechanism can help to outline specific antioxidants or preventative steps that can be developed to help reach the overall goal of producing a whey protein powder that exhibits a bland flavor for ingredient applications.

The objectives of this research are to determine the effects of storage time of liquid whey concentrate on flavor and flavor stability of spray dried whey proteins: Cheddar WPI and Mozz WPC; and to identify volatile compounds responsible for cardboard flavors in whey proteins.

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CHAPTER 2:

SENSORY CHARACTERIZATION OF CHEMICAL COMPONENTS RESPONSIBLE FOR CARDBOARD FLAVOR IN WHEY PROTEIN

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SENSORY CHARACTERIZATION OF CHEMICAL COMPONENTS RESPONSIBLE FOR CARDBOARD FLAVOR IN WHEY PROTEIN

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ABSTRACT

Cardboard flavor is one of the most commonly described off-flavors in whey proteins. The objective of this research was to identify volatile components responsible for cardboard flavor in dried whey protein concentrate and isolates (WPC, WPI) and characterize them by sensory analysis. Cardboard and brown paper samples (n=5) soaked in deionized water and whey proteins with and without cardboard flavor were analyzed by gas chromatography mass spectrometry and descriptive sensory analysis to select the potential contributors to cardboard flavor. Compounds were evaluated by trained sensory panelists using sniff jars, dose response experiments and whey protein models. Sensory analysis of the aroma of the chemical standards yielded no single compound exhibiting a cardboard aroma, suggesting that cardboard flavor does not result from one compound, but a combination. A combination of compounds (pentanal, heptanal, nonanal, 1-octen-3-one, dimethyl trisulfide) elicited cardboard flavor in whey protein previously deemed free of cardboard flavor.

Key Words: whey protein, cardboard flavor, sensory analysis

PRACTICAL APPLICATIONS

This study established that a combination of pentanal, heptanal, nonanal, 1-octen-3-one, and dimethyl trisulfide elicited cardboard flavor in whey protein and can be utilized as a training reference for identifying cardboard flavor. The direct association of specific lipid oxidation products with cardboard flavor in whey protein emphasize the necessity to control lipid oxidation to reduce this off-flavor in whey protein ingredients. These compounds could potentially be utilized to instrumentally monitor cardboard flavor in whey protein. Hexanal, while a major indicator for lipid oxidation, was not directly indicative of cardboard flavor in whey protein.

INTRODUCTION

Whey protein is one of the major players in the growing functional foods market, valued to be >$100 billion by 2012 (Smithers 2008). The value of whey protein is attributed to its functional and nutritional qualities. Functional properties include solubility, gelation, aeration, water-binding and emulsification (Kinsella and Whitehead 1989; Huffman 1996; Smithers *et al.* 1996; Korhonen *et al.* 1998; Foegeding *et al.* 2002). Nutritionally, whey proteins provide all essential amino acids including branched chain amino acids leucine, isoleucine, and valine which are important for metabolic regulation of protein and glucose

homoestasis and may play a role in weight control through lipid metabolism (Zemel 2004; Smilowitz *et al.* 2005; Smithers 2008).

Despite valuable functional and nutritional properties, for widespread ingredient applications it is important for liquid and powdered whey to exhibit little or no flavor (Drake 2006; Drake *et al.* 2009). Off-flavors are present in dried milk and whey ingredients and potentially result from many different factors and chemical reactions (Karagul-Yuceer *et al.* 2002; Carunchia Whetstine *et al.* 2005; Mahajan *et al.* 2004; Gallardo-Escamilla *et al.* 2005; Wright *et al.* 2006; Wright *et al.* 2009). 'Cardboard' flavors are documented as a common off flavor in whey protein (Hammond 1989; Drake *et al.* 2003; Carunchia Whetstine *et al.* 2005; Russell *et al.* 2006; Drake *et al.* 2009; Wright *et al.* 2009). Earlier dried whey sensory defect references included cardboard off-flavor (Bodyfelt *et al.* 1988), however, it is only recently that this sensory attribute has been assigned a specific reference and definition in a descriptive sensory language (Drake *et al.* 2003). 'Cardboard' flavor is defined as the aroma associated with cardboard and referenced by evaluation of the aroma from cardboard pieces soaked in water (Carunchia Whetstine *et al.* 2005; Russell *et al.* 2006). This is a usable sensory definition and reference, but there are many types of cardboard and the lack of a specific chemical reference is a missing component for training panelists. Also, the actual source(s) of this flavor in whey protein constitute a challenge to development of methods to prevent it.

Volatile lipid oxidation products (VLOP) have been associated with 'cardboard' flavor in many dairy products (Hammond 1989; Carunchia Whetstine *et al.* 2003; Drake *et al.* 2003; Wright *et al.* 2009). Tomaino *et al.* (2004) compared volatile and sensory data from liquid whey to similar results found by Hall and Anderson (1985) in whole milk powder. Both groups observed significant increases in 'cardboard' aroma, flavor and aftertaste following storage. Cardboard aroma, flavor and aftertaste were correlated with hexanal and other volatile lipid oxidation product concentrations. Tomaino *et al.* (2004) examined the volatile compounds in water-soaked cardboard by dynamic headspace analysis and reported recoveries of hexanal, octanal and heptanal as the predominate compounds present.

Several studies have established lipid oxidation products concurrent with loss of desirable flavors and formation of off-flavors in dried dairy products including liquid whey, dried whey and dried whey proteins (WPC and WPI). Tomaino *et al.* (2001) and Morr and Foegeding (1990) both indicated a relationship between undesirable flavor in liquid whey and quantity of free fatty acids. Lower protein whey powder and whey protein concentrate 80 % protein (WPC80), were shown by Tomaino *et al.* (2001) to have a 20-fold greater concentration of FFA than whey protein isolates (~92.6% protein). Similar results by Morr and Foegeding (1990) indicated that higher 'stale' and 'old whey' flavors were perceived for WPC than WPI. Increases in cardboard flavor in liquid whey across fourteen days storage were correlated with a decrease in linoleic acid (Tomaino *et al.* 2004). Oxidation of linoleic

acid produced hexanal, which concurrently increased with storage time (Tomaino *et al.* 2004). Wright et al. (2009) also acknowledged the relationship between FFA and VLOP and attributed 'cardboard' flavor to increased VLOP. These studies established that higher FFA content resulted in higher susceptibility to lipid oxidation; increased lipid oxidation reactions resulted in higher concentrations of VLOP, namely aldehydes. Thus it has been hypothesized that increases in aldehyde concentrations created cardboard flavor. A lack of defined sensory analysis in many of these studies means that a clear relationship between specific lipid oxidation compounds and "cardboard" flavor in whey protein has not been established. There are many by-products of lipid oxidation. Recently, Drake *et al.* (2009) used volatile analysis results in combination with sensory analysis to establish that two specific lipid oxidation products (2, 6-nonadienal and E-2-nonenal) caused cucumber off-flavors in whey protein. Knowledge of the specific compounds that caused this flavor facilitated sensory panelist training and allowed identification of lecithin used in instantizing as a source.

Collectively, previous studies indicate that many different volatile lipid oxidation products could be responsible for 'cardboard' off-flavors observed in whey proteins. Although many different compounds have been correlated with cardboard flavor or hypothesized to be causes of cardboard flavor, there have been no studies to our knowledge that have effectively demonstrated that a specific compound or compound(s) cause cardboard aroma or that when added to fresh whey protein cause cardboard flavor.

Additional research is needed to further identify specific responsible compounds in order to pinpoint fatty acid source(s) or to effectively identity methods to prevent formation or mask this undesirable flavor. The objective of this research was to characterize the sensory properties of chemical compounds associated with 'cardboard' off-flavor in whey protein. A variety of cardboard samples were evaluated by sensory and volatile compound analysis. Analysis of volatile results from cardboards and previous studies with whey proteins were used to select compounds for sensory analysis testing.

MATERIALS AND METHODS

Identification of 'real' cardboard descriptor

A variety of cardboards were collected from different sources including paperboard cardboard, brown corrugated cardboard, white corrugated cardboard, brown paper bag, and white paper bag. Cardboard samples were prepared for evaluation using a modified method from Tomaino *et al.* (2004). Cardboard samples were cut into 1.25 cm squares and 1 g or approximately 5 squares were placed into 59 mL portion cups with three digit codes (Dart, Mason, M.I., U.S.A.). Deodorized water at 100^{o}C was added to each cup to completely cover all cardboard squares. Corrugated cardboard required 20 mL hot water addition while only 10 mL was added to the other cardboard samples. Cups were then

allowed to cool for 30 min, then lidded and allowed to equilibrate for 3 h before sensory evaluation.

Descriptive sensory analysis. Descriptive sensory analysis was performed on real cardboard samples in water to identify which of the five cardboard types created a cardboard aroma reminiscent of the cardboard aroma and flavor in whey products. Panelists (n = 10) each had more than 300 h of previous experience with the Spectrum™ descriptive analysis method (Meilgaard et al. 1999) determining the presence and intensities of the cardboard flavor attribute in liquid and dried whey products using an established sensory language (Drake *et al.* 2009; Wright *et al.* 2009). Aroma was evaluated by cracking the lid of the sample cup and using short sniffs. A reference sample of 10 % solids (w/v) rehydrated Cheddar WPI (18 mo old) in deionized water was provided as an example of cardboard flavor in whey protein. This WPI sample was documented as having a high cardboard intensity and thus was deemed suitable for determining the similarity of cardboard aroma in whey protein to the aroma of the real cardboard samples. Samples were evaluated at room temperature and each sample was evaluated in duplicate by each panelist. Aroma characteristics of the real cardboard samples were described as well as their similarity to the reference whey protein sample.

Solid phase micro extraction. Solid phase micro extraction (SPME) followed by gas chromatography – mass spectrometry (GC-MS) was conducted using a modified method from Wright *et al.* (2006), on all real cardboard samples and the reference WPI. Cardboard

samples were cut into 1.25 cm squares and 1 g or approximately 5 squares were placed into 20 ml autosampler vials with steel screw tops containing silicone septa faced in Teflon (Microliter Analytical, Sawanee, F.L., U.S.A.). Five mL HPLC grade water at 100^{o}C was added to each vial and allowed to equilibrate for 3 h before analysis. WPI reference sample was rehydrated to 10 % solids (w/v) with HPLC grade water (EMD Chemicals Inc., Gibbstown, N.J., U.S.A.) at room temperature and 5 mL of the rehydrated WPI reference sample was added to a vial to serve as a reference sample. Each vial was spiked with 10 uL internal standard solution (2-methyl-3-heptanone in methanol at 8.1 ppm) and 0.5 g sodium chloride (Sigma Aldrich, Milwaukee, W.I., U.S.A.) was added. A CombiPal autosampler (CTC Analytics, Zwingen, Switzerland) was utilized for injection into an attached Agilent 6890N GC with a 5973 inert MSD (Agilent Technologies Inc., Santa Clara, C.A., U.S.A.). Vials were placed in a randomized balanced order in the autosampler to account for the time delay during sample analysis (analysis time for 1 sample was 47 min). Vial temperature was maintained at 5^{o}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 (source) 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 followed by a final hold time of 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. Real cardboard samples and the reference WPI were analyzed in triplicate.

Identification and evaluation of potential cardboard chemical components

Chemical compounds were selected for evaluation based on their presence and relative concentrations in whey protein with cardboard flavor and real cardboard from SPME GC-MS analysis in the current study and from previously published literature on liquid whey (Carunchia Whetstine *et al.* 2003; Tomaino *et al.* 2004) and dried whey ingredients (Carunchia Whetstine *et al.* 2005; Wright *et al.* 2006; Wright *et al.* 2009; Evans *et al.* 2009). Potential cardboard chemical compounds selected included: pentanal, hexanal, heptanal, octanal, nonanal, decanal, benzaldehyde, 1-octen-3-ol, 1-octen-3-one, 1-pentanol, methional, dimethyl disulfide, dimethyl trisulfide (Sigma Aldrich, Milwaukee, W.I., U.S.A.).

Sniff jars. Sniff jars were prepared to evaluate the aroma of each chemical compound external to any food matrix. Five 1 cm squares of filter paper (Fisher Scienctific, Fairlawn, N.J., U.S.A.) were placed in the bottom of individually labeled 120 mL lidded amber glass jars (VWR International, West Chester, P.A., U.S.A.). Approximately 3 uL of each compound was dropped onto the filter paper and the jar quickly lidded. Combination sniff jars were also prepared for: heptanal/octanal, heptanal/nonanal, heptanal/benzaldhyde, heptanal/hexanal/octanal/nonanal/benzaldehyde, pentanal/heptanal, heptanal/hexanal,

pentanal/heptanal/hexanal, pentanal/heptanal/nonanal, 1-octen-3-ol/heptanal, 1-pentanol/heptanal, in which approximately 3 uL of each chemical was added to the jar. Sniff jars with three-digit codes were evaluated in duplicate by the same descriptive analysis panel (n=10) and aroma characteristics described.

Dose response. After sniff jar evaluation, chemicals with potential cardboard aroma were then serially diluted to determine their aroma across a wide concentration range (Vara-Ubol *et al.* 2004). It is well known that compounds can elicit distinct aromas across a concentration range (Drake and Civille 2003; Avsar *et al.* 2004). Each chemical was diluted in propylene glycol (Sigma Aldrich, Milwaukee, W.I., U.S.A.) to concentrations of 0.001, 0.01, 0.1, 1, 10, 100, and 1,000 ppm. Fragrance testing strips (Sephora, Raleigh, N.C., U.S.A.) were used to evaluate the aroma of each diluted chemical by dipping them 1.25 cm into the chemical solution and storing the strip in a coded 20 mL screw top vial. Aromas were allowed to equilibrate in the tube for 1 day before aroma evaluation.

Each chemical was evaluated in duplicate by the same descriptive panel (n=10) during multiple sessions on one day; three chemicals per session. Panelists evaluated the chemicals from lowest to highest concentration and were instructed to take quick sniffs of the fragrance testing strip. The odor of each dilution was then described as "cardboard-like" or not. Additional odor characteristics and their intensities were recorded for each dilution. To reduce carryover between compounds, a 10 min rest period was implemented

between compounds. Nasal passages were cleared between evaluations by sniffing fresh air or each panelist's own sleeve.

Model systems

WPI models. Whey protein model systems were prepared using a freshly manufactured commercial WPI (Midwest supplier, USA) that was absent of cardboard aroma and flavor (as determined by descriptive sensory analysis) with the addition of selected chemicals to determine if we could create cardboard flavor in whey protein. Aldehydes were prepared in 95% ethanol (Everclear, Luxco, Inc., St. Louis, MO) (for aroma and flavor evaluation) at concentrations representing their average relative abundance identified in commercial whey proteins (including those with and without cardboard flavor by descriptive sensory analysis). Sulfur compounds and 1-octen-3-one were prepared in 95% ethanol at near threshold concentrations because preliminary studies indicated that above threshold, their aroma characteristics were not "cardboard-like". Ethanol was used as the diluent for evaluation because it is safe for human consumption.

WPI was prepared as previously described, 10% solids (w/v) in deionized water. Chemical solutions were introduced by clean, disposable micropipettes. After chemical addition, the WPI was completely mixed. Appropriate blanks with no ethanol added, and only ethanol added were prepared. Additionally, a cardboard reference WPI sample (a commercial WPI with a high intensity of cardboard flavor) was prepared for comparison.

WPI and WPI models were dispensed into lidded soufflé cups with 3-digit codes. WPI models were evaluated for aroma and flavor characteristics in duplicate by descriptive sensory analysis. In addition to evaluating the individual chemicals in WPI model systems, to account for perceptual interactions of odorants, such as inhibition or suppression, combinations of the chemicals in WPI were also evaluated.

n-1 / Omission. Odor characteristics from WPI model systems indicated that cardboard flavor was potentially caused by a combination of chemical compounds rather than one single compound. To determine which chemical(s) in the combination had the largest contribution to cardboard aroma, n-1 or omission experiments were conducted (Karagul-Yuceer *et al.* 2004). Model systems were prepared as previously described. All chemicals except one (n-1) were introduced into each whey model. Appropriate blanks and cardboard reference WPI were all provided along with one whey model sample containing all compounds (n). Descriptive sensory panelists evaluated the samples by determining if the odor was more or less similar to n with the omission of that chemical compound compared to a reference WPI that was predetermined to have high intensities of cardboard flavor. Similarity to the reference WPI was scored on a 10 point scale where 1 = not similar at all and 10 = highly similar. Models in which cardboard aroma was not detected were assigned a low similarity score which indicated that the chemical compound omitted made a large contribution to cardboard flavor (Drake and Civille 2003; Karagul-Yuceer et al. 2004). Each model was evaluated in duplicate by each panelist.

Statistical analysis

Principal component analysis was conducted on selected volatile compounds identified in cardboards and whey proteins. Sensory panel similarity scores were subjected to analysis of variance with means separation (Fisher's least significant difference). Analyses were conducted using SAS (version 9.1, Cary, NC).

RESULTS & DISCUSSION

Identification of 'real' cardboard descriptor

Sensory panelists evaluated real cardboard samples to determine which type of cardboard exhibited an aroma similar to cardboard flavor observed in whey protein. All types of cardboard were differentiated by their aroma profiles (Table 1). Panelists determined that paperboard cardboard, similar to that found on the back of paper pads, was most similar (9/10 panelists consistently selected it as the most similar to cardboard flavor in WPI) to the reference WPI with a high intensity of cardboard aroma (Table 1).

SPME GC-MS analysis of three of the cardboards identified 39 volatile compounds (results not shown) several of which were aldehydes (VLOP) (Figure 1). Paperboard cardboard was characterized by the presence of aldehydes. Aldehydes are VLOP created from the degradation of unsaturated fatty acids (Tomaino *et al.* 2004) and are commonly reported in whey proteins (Hammond 1989; Drake *et al.* 2003; Carunchia Whetstine *et al.*

2005; Russell *et al.* 2006; Drake *et al.* 2009; Wright *et al*. 2009; Evans *et al.* 2009). Relative abundances of hexanal, octanal and nonanal were the highest identified volatile components of paperboard cardboard. Hexanal and octanal, along with heptanal, were also recovered in the headspace of cardboard analyzed by Tomaino *et al.* (2004).

It may seem odd to compare the flavor of an animal-based product (whey protein) to the aroma of cardboard, an inanimate everyday object; cardboard is principally composed of plants and trees. It is important to consider how cardboard is manufactured. Cardboard or paperboard is defined as paper that is over 0.3mm in thickness and is manufactured from virgin paper pulp and additives to aid in processing (Smook 1982). Concentrating on lipid oxidation reactions, there are two possible sources of lipids in cardboard: the paper pulp itself, and as an additive during processing. Pulp from trees contains cellulose, hemicelluloses, lignin (the outer layer of fiber), and extractives (Smook 1982). Extractives, also known as tall oil, vary depending on the tree source, but they include resin acids, fatty acids, terpenoid compounds, alcohols, and phenols (Smook 1982). Extractives make up a very low percentage of pulp but Southern pines, which are most widely utilized for cardboard production, contain the highest percentage of extractives. Southern pine extractives contain approximately 40% fatty acids (Smook 1982). During processing of paper pulp to cardboard, these fatty acids are saponified during cooling and evaporation steps and removed. Thus the objective in cardboard processing is to remove these lipids, a similar objective to liquid whey processing, but the opportunity for residual

lipids is present. Heat from cardboard processing could promote oxidation of these residual lipids or of the tall oil before it is removed and produce VLOP in the finished cardboard.

The second possible source of lipids in cardboard is as an additive to the paper pulp. Manufacture of cardboard requires the addition of a minimal amount of starches, gums, and/or rosin. These additives increase the internal strength of the paper product as well as provide other advantages such as improving 'feel' by reducing 'fuzz' and decreasing rate of water penetration (Smook 1982). While starches and gums are the most popular additive, rosin is specifically added as a sizing agent because it is responsible for increasing resistance to water penetration (Smook 1982). Rosin is an amber colored natural resin tapped from southern pines or processed from tall oil, thus it is an amphipathic material having lipid characteristics. Rosin can be added on the wet end of cardboard processing where it is added into the pulp or on the dry end where a thin layer is sprayed on the cardboard surface (Smook 1982). During processing, the cardboard pulp is often drum dried, for which heat is applied. Therefore, the residual lipids present in the rosin could be oxidizing during processing and result in VLOP in cardboard.

Identification and evaluation of potential cardboard chemical components

Sniff jars. Aroma characteristics of each volatile compound were described (Table 2). One single chemical did not exhibit a cardboard aroma alone. Pentanal and heptanal were both

described as having cardboard-like aromas, however, fatty and oxidized aromas were also detected and at higher intensities than the cardboard aroma. Aromas of benzaldhyde, 1-pentanol, 1-octen-3-ol and dimethyl disulfide were distinct from the targeted cardboard aroma and thus were discontinued from subsequent analyses. After sniff jar evaluation, it was concluded that a combination of chemical components was likely responsible for cardboard flavor in whey proteins. A review of published gas chromatography olfactometry (GC-O) data from whey protein and dairy products also does not reveal any single odor detected or chemical compound identified that by itself exhibits a cardboard aroma which also suggests that a combination of compounds are responsible for creating cardboard flavor (Karagul Yuceer *et al.* 2002, 2003; Mahajan *et al.* 2004; Carunchia Whetstine *et al.* 2005; Mortenson *et al.* 2008; Evans *et al.* 2009; Liaw 2009).

Dose response. Sniff jar evaluation indicated that no single chemical exhibited cardboard aroma, while some had cardboard-like qualities (Table 2). Therefore, dose response analysis was used to determine if any of these chemicals posed a cardboard-like aroma at a specific concentration. Aroma characteristics over the concentration range for each chemical were described (Table 3). For all compounds the concentration range included their threshold value, indicated by the blank aroma detected; except dimethy trisulfide, whose threshold value was below the concentration range evaluated (Wright *et al.* 2006). The highest concentration evaluated (1000 ppm) was above the relative concentrations of each compound previously reported in whey or whey protein (Tomaino *et al.* 2004;

Carunchia Whetstine *et al.* 2005; Drake *et al.* 2009; Wright *et al.* 2009; Evans *et al.* 2009). All compound aromas were described as being more harsh at the highest concentration. While the aromas of the aldehydes did not change drastically over the concentration range, aroma characteristics of the two sulfur compounds and ketone were altered by the concentration at evaluation. Cardboard-like aromas were detected twice: 1000 ppm pentanal and 0.1 ppm 1-octen-3-one. However, in agreement with sniff jar results, when cardboard aromas were detected, additional aromas were also described. These results also suggested that a single compound was not responsible for cardboard flavor in whey or whey protein. Because of its characteristic citrus aroma at all concentrations, octanal was discarded and not evaluated in model systems.

Model systems

WPI models. While results from both sniff jars and dose response analysis suggested that cardboard flavor depended on a combination of chemical components, it was necessary to evaluate the impact of individual chemicals and combinations of chemicals in a whey protein model system. Concentrations of the aldehydes for model systems were determined by selecting a concentration previously documented in WPC34, WPC80, and WPI (Carunchia Whetstine *et al.* 2005; Wright *et al.* 2009; Evans *et al.* 2009) (Table 4). Due to the overwhelming odors created by 1-octen-3-one and DMTS, the concentration of these two compounds for application in model systems was close to their threshold values in

water and WPI respectively (van Gemert 2003; Wright *et al.* 2006). Odor Activity Values (OAV = concentration / threshold) were calculated to determine the effect of each chemical at these relative concentrations on the overall aroma of the sample (Table 4). According to OAV theory, compounds with a higher OAV value have a greater contribution to the overall aroma or flavor of a product than compounds with smaller OAV values (Qian and Reineccius 2003). Based on OAV values, nonanal and hexanal contributed the most to the aroma of whey protein, however, sensory results did not indicate that these compounds contributed cardboard aromas (Table 2, 5). Results from sensory evaluation of individual compounds in whey protein indicated that aromas of pentanal and heptanal were closest to the reference cardboard WPI (Table 2); however the aroma of both were still significantly different from the reference cardboard WPI. Combinations of aldehydes, because they are primary VLOP, were evaluated to find a better match to the reference cardboard WPI flavor. Initial evaluations pointed toward a combination of pentanal, heptanal, and nonanal; however, sensory panelists were not satisfied with this model as a good representation of cardboard flavor in WPI when directly compared to the reference high cardboard flavor WPI. Therefore, there was a need to explore other compounds in conjunction with the current aldehydes.

Sulfur compounds were the next target because of the large amount of sulfur gases produced from cardboard production (Smook 1982). While sulfur dioxide is the principle gas emitted, it doesn't pose an odor problem because its threshold is a thousand times

higher than reduced sulfur gases. Hydrogen sulfide, methyl mercaptan, dimethyl sulfide, and dimethyl disulfide are the four reduced sulfur gases that are emitted from a cardboard mill that produce odor pollution at levels that require action (Smook 1982). Because reduced sulfur gases are so prevalent in cardboard production, and were found in the SPME analysis of 'real' cardboard samples, it was hypothesized that a sulfur component added to the current aldehyde model would better simulate cardboard flavor. Volatile sulfur compounds are also present in fluid whey and dried whey proteins due to heat degradation and oxidation of sulfur containing amino acids during whey protein processing or by free radicals initiated during lipid oxidation (Karagul Yuceer *et al.* 2003; Carunchia Whetstine *et al.* 2005; Wright *et al.* 2006; Evans *et al.* 2009). Sulfur compounds chosen for analysis were based on the relative concentrations of sulfur compounds present in whey protein with and without cardboard flavor: methional, dimethyl disulfide (DMDS), dimethyl trisulfide (DMTS). Wright *et al.* (2006) demonstrated that when present at high concentrations ($\geq 0.80 \pm 0.45$ ppb in WPI), DMTS was responsible for cabbage off flavor in WPI. They also demonstrated that DMTS was likely present at some concentration in most WPI which further indicates that DMTS at low concentrations could impart aromas affecting the overall flavor of whey protein. Additionally, 1-octen-3-one was added to the compounds evaluated because it has been documented in whey proteins, milk powders and cardboard samples (Carunchia Whetstine *et al.* 2003; Wright *et al.* 2009; Evans *et al.* 2009). 1-octen-3-one exhibits an earthy/mushroom aroma that could express the earthy note of cardboard.

Evaluation of WPI models with methional, dimethyl disulfide, and DMTS individually demonstrated that the addition of DMTS to the WPI base created cabbage and cardboard aromas. Addition of 1-octen-3-one to the WPI base created cardboard and metallic aromas. While the latter two compounds both exhibited some cardboard aroma, alone they were not a match to the reference cardboard WPI. Subsequent models were evaluated with combinations of the aldehydes, 1-octen-3-one and DMTS until an appropriate model was created. The final model contained pentanal (10 ppb), heptanal (70 ppb), nonanal (500 ppb), 1-octen-3-one (1 ppb), and DMTS (0.8 ppb). This model was labeled n. When compared to the reference WPI with high intensities of cardboard flavor, the model 'n' scored 8.7 ± 0.25 on a 1-10 degree of similarity scale where 10 = identical and 1 = not similar at all.

Pentanal, heptanal, and nonanal are all aldehydes that increase in concentration as lipid oxidation reactions occur. 1-octen-3-one is a ketone that can also be produced via lipid oxidation. Lipid oxidation has already been associated with cardboard flavor in many dairy products (Hammond 1989; Carunchia Whetstine *et al.* 2003; Drake *et al.* 2003; Tomaino *et al.* 2004; Wright *et al.* 2009) without identification of specific oxidation compounds. Previous studies have hypothesized hexanal as a source of cardboard flavor and proposed hexanal concentrations as a benchmark. The results in the current study indicate that while hexanal is a major VLOP, it is not responsible for cardboard flavor in whey. An additional model with added hexanal at concentrations found in whey protein (n + hexanal) was

evaluated to ensure that the model with hexanal was not more similar to the reference WPI than the n model. When the model with added hexanal was compared to reference WPI with cardboard flavor, panelists scored this model 4.5 $\pm$ 0.3 in similarity, indicating that it was not similar to WPI with cardboard flavor. The overall similarity of the model with added hexanal was also changed and distinct in aroma from the n model (Table 5). Lloyd et al. (2009) evaluated storage changes of whole milk powder (WMP) by sensory and instrumental analysis. They conducted multiple linear regressions to determine volatile compounds that were most predictive of storage off flavors in WMP (grassy and painty flavors). Similar to fluid whey and whey protein, hexanal was the VLOP recovered at the highest concentrations. However, hexanal, even though it has an aroma similar to one of the WMP off flavors (grassy) was not a sole predictor of WMP off-flavors. This study also suggested that while hexanal was a good indicator of lipid oxidation, it was not necessarily the primary source of off-flavor or a good predictor of off-flavors. Similar studies with warmed over flavor (WOF) in meat (often described as cardboard-like) which is attributed to lipid oxidation have also demonstrated that this off-flavor is not caused by hexanal (St Angelo *et al.* 1987; Konopka *et al.* 1995; Kerler and Grosch 1996).

VLOPs are not formed from the same pathway, nor do they appear at the same rate (Marsili 1999; Lee and Min 2009). Chemical pathways of lipid oxidation are determined by the free fatty acid that is being oxidized. Tomaino *et al.* (2004) correlated the decrease of linoleic acid (18:2) with increased cardboard flavor during 14 day storage of liquid whey.

Oleic acid (18:1) also significantly decreased during this storage period (Tomaino *et al.* 2004). Milkfat is composed of approximately 25% oleic acid and only 2% linoleic acid; however, the phospholipid component of the milkfat globule membrane is 6% linoleic acid (Frankel 2005) and this fraction is enriched in whey proteins. When autooxidized, oleate forms 8-, 9-, 10-, and 11- hydroperoxides which are further cleaved on the ester side to produce heptanal, octanal, nonanal, and decanal aldehydes. Autooxidized linoleate forms 9- and 13- hydroperoxides that are cleaved to produce 2,4 decadienal, pentanal, and hexanal. Additional hexanal is produced through the cleavage of the 12-hydroperoxide formed from photooxidized linoleic acid (Frankel 2005). The reaction rate of linoleic acid is 40 times faster than that of oleic acid (Frankel 2005). Further oxidation and breakdown of unsaturated aldehydes can also yield hexanal (Schieberle and Grosch 1981). These multiple pathways resulting in hexanal production and the reaction kinetics of lipid oxidation explain why hexanal is the primary aldehyde observed at highest concentration in oxidized food products. However, when comparing results from instrumental analysis, sensory thresholds and sensory analysis of model systems should be applied to clearly associate volatile compounds with sensory responses. Nonanal exhibits a lower threshold value than hexanal; other VLOPs, such as 1-octen-3-one, exhibit an even lower threshold (Table 4). As such, some VLOPs, such as hexanal, may be indicators of lipid oxidation and possible cardboard flavor, but not causative of cardboard flavor. Our model system results indicate that hexanal does not cause cardboard flavor (Table 5).

N-1 models. To validate the model and determine what components were most important in creating cardboard flavor, n-1 models were evaluated. N-1 models were evaluated on a degree of similarity scale (Table 5), comparing the n-1 model to the n model containing all chemical components. For n-1 analysis, when model similarity decreased, it indicated that the chemical compound omitted made a large contribution to cardboard flavor in whey protein (Drake and Civille 2003; Karagul-Yuceer et al. 2004). When 1-octen-3-one and DMTS were removed from the model system, the overall similarity score decreased and was not described as cardboard-like. These results indicated that even though these two compounds were at very low concentrations, they were necessary to impart cardboard flavor in WPI. Out of the aldehydes, pentanal, was the least important aldehyde for creating cardboard flavor in the model.

CONCLUSION

Cardboard flavor was not observed from one single chemical compound, but the aroma / flavor in whey protein was produced by multiple volatile chemicals. A combination of pentanal, heptanal, and nonanal in the presence of 1-octen-3-one and DMTS produced cardboard flavor in whey protein. This study further clearly associated the presence of VLOP with cardboard flavor in whey protein, emphasizing the necessity to control lipid oxidation during processing to reduce cardboard flavor in whey protein ingredients. Future work should also explore the relative concentrations of these compounds at which

cardboard flavor is not detected to utilize as a volatile benchmark for occurrence of lipid oxidation reactions and presence/absence of off-flavors rather than hexanal concentrations alone.

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TABLES AND FIGURES

TABLE 2.1
AROMA CHARACTERISTICS OF REAL CARDBOARD SAMPLES SOAKED IN DI WATER[1]

Cardboard type	Aroma characteristics[2]
Paperboard (PC)	sweet / cardboard / clean
brown corrugated (CC)	harsh / cardboard / glue / pungent
white corrugated	harsh / glue
brown paper bag (WFB)	low intensity / cardboard / not sweet
white paper bag	fatty / fryer oil / cardboard

[1] 1g of each sample was soaked in 100°C DI water for 3.5h before evaluation
[2] Aroma was evaluated in duplicate by a trained sensory panel; n=10. Descriptors provided represent words used consistently by 6 or more panelists.

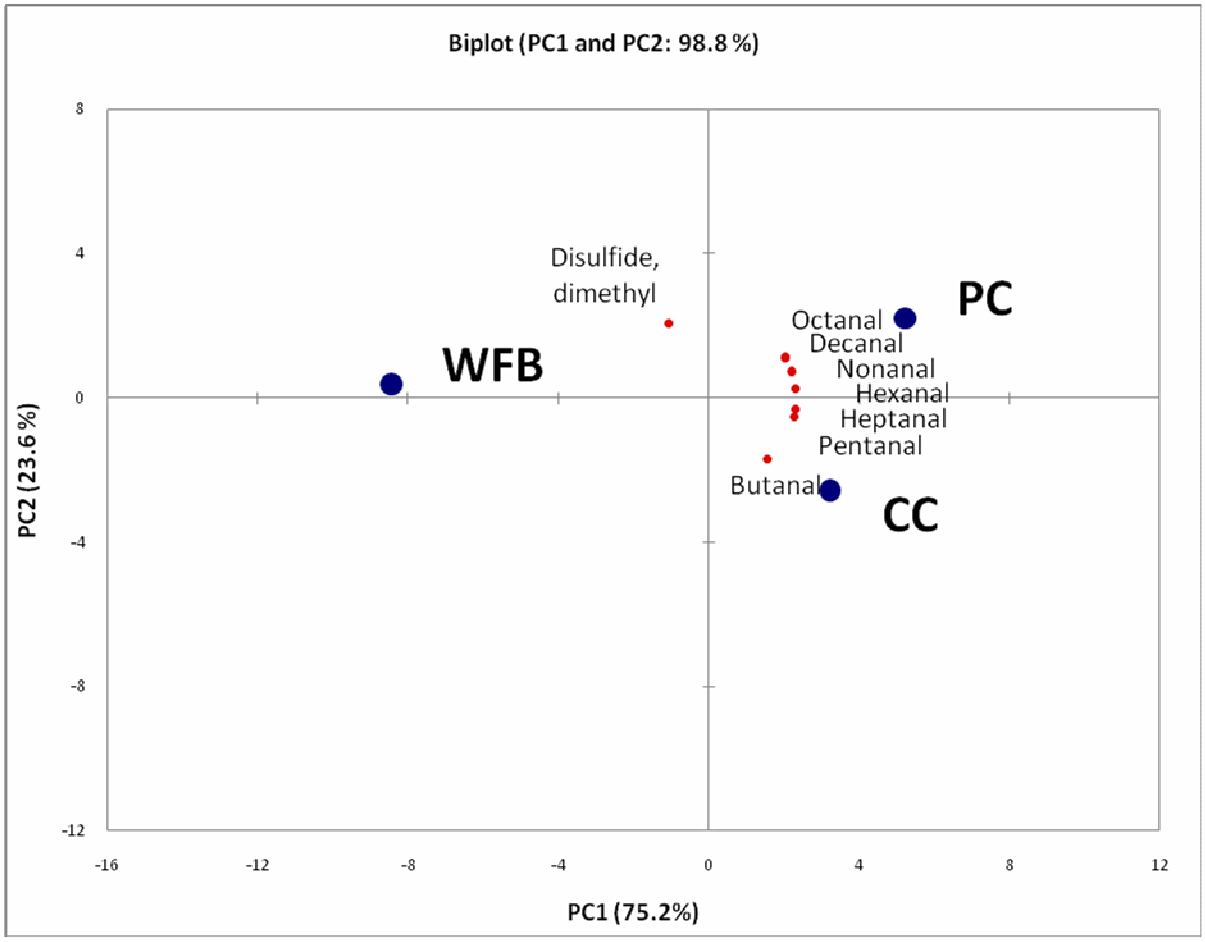

FIGURE 2.1

PRINCIPLE COMPONENT BIPLOT OF 'REAL' CARDBOARD SAMPLES WITHIN VOLATILES IDENTIFIED BY SPME GC-MS (CC= CORRUGATED CARDBOARD, PC= PAPERBOARD CARDBOARD, WFB= BROWN PAPER BAG)

TABLE 2.2
AROMA CHARACTERISTICS OF SINGLE COMPOUNDS IN SNIFF JARS

Compound	**Aroma Characteristics**[1]
benzaldehyde	cherry / almond
Pentanal	fatty / oxidized / cardboard
Hexanal	fatty / grassy
Heptanal	fatty / cardboard
Octanal	citrus
Nonanal	fatty
1-pentanol	balsamic
1-octen-3-ol	mushroom / earthy / green
1-octen-3-one	mushroom / earthy / green
methional	potato / earthy
dimethyl disulfide	brothy
dimethyl trisulfide	cabbage / garlic

[1] Aroma of coded sniff jars was evaluated in duplicate by a trained sensory panel; n=10 Descriptors provided represent words used consistently by 6 or more panelists.

TABLE 2.3
DOSE RESPONSE OF SELECTED CHEMICALS PROPOSED TO CONTRIBUTE TO CARDBOARD FLAVOR

	Odor characteristics at different concentration levels (ppm)[1]						
Compound	**0.001**	**0.01**	**0.1**	**1**	**10**	**100**	**1000**
pentanal	no odor					malty / fatty	sweet / cardboard / malty
hexanal	no odor				grassy / sweet		
heptanal	no odor		oily / fatty	green / fatty			grassy / fatty
octanal	no odor				sweet / citrus	citrus / waxy	citrus / waxy / fatty / honey
nonanal	no odor				fatty	fatty / waxy / green	waxy / floor polish
1-octen-3-one	no odor	mushroom	mushroom / metallic / cardboard	mushroom	mushroom / soil	mushroom / green / fruit	mushroom / fruit / soil
methional	no odor		potato		earthy / potato / fatty	potato / potato flesh and eyes	
dimethyl trisulfide	garlic		harsh / garlic / cabbage			burnt garlic	rotten cabbage

[1] Aroma of coded compounds was evaluated in duplicate by a trained sensory panel; n=10
Descriptors provided represent words used consistently by 6 or more panelists.

TABLE 2.4
MODEL SYSTEM CONCENTRATIONS AND ODOR ACTIVITY VALUES

Compound	Concentration for whey protein model system (ppb)	Orthonasal threshold in water (ppb)	OAV (concentration / threshold)	Concentration in whey protein reference	threshold reference
pentanal	10	8	1.25	EVANS ET AL. 2009	VAN GEMERT 2003
hexanal	1000	7.5	133.33	CARUNCHIA WHETSTINE ET AL. 2005	VAN GEMERT 2003
heptanal	70	10	7.00	WRIGHT ET AL. 2009	VAN GEMERT 2003
nonanal	500	3.5	142.86	CARUNCHIA WHETSTINE ET AL. 2005	VAN GEMERT 2003
1-octen-3-one[1]	1	0.6	1.67		VAN GEMERT 2003
methional[1]	0.4	0.31	1.29		VAN GEMERT 2003
dimethyl trisulfide[1]	0.8	0.8	1.00		WRIGHT ET AL. 2006

[1] threshold concentrations were used in model systems, not mean concentration found in whey protein. Mean concentration found in whey protein for 1-octen-3-one, methional, and dimethyl trisulfide are 7ppb, 0.16ppm, and 0.8ppb respectively (WRIGHT ET AL. 2006; EVANS ET AL. 2009).

TABLE 2.5

SENSORY EVALUATION OF MODEL SYSTEMS: SIMILARITY TO REFERENCE CARDBOARD WPI MODEL N, N = PENTANAL, HEPTANAL, NONANAL, 1-OCTEN-3-ONE, DIMETHYL TRISULFIDE[1,2]

chemical omitted or added	degree of similarity to cardboard WPI model with all compounds (N[3,4,5])	Comments
N - pentanal	8.5[a]	cardboard / mushroom / green / waxy
N - heptanal	6.8[b]	cardboard
N - nonanal	7.0[b]	cardboard
N - 1-octen-3-one	5.5[c]	green
N - dimethyl trisulfide	6.0[b,c]	green / soapy
N + hexanal	5.0[c]	Sweet, grassy, white papery

[1] N = cardboard free WPI with 10 ppb pentanal, 1000 ppb heptanal, 500 ppb nonanal, 1 ppb 1-octen-3-one, 0.8 ppb dimethyl trisulfide

[2] N (total model) scored a 8.75 when compared to the reference cardboard WPI using the same 1-10 scale

[3] degree of similarity scale: 1=not similar at all, 10=identical

[4] Aroma was evaluated by a trained sensory panel; n=10

[5] values with different letters are significantly different ($p<0.05$); all compounds in N, except pentanal, were critical to eliciting cardboard flavor. 1-octen-3-one and dimethyl trisulfide were most important.

CHAPTER 3:

EFFECT OF LIQUID RETENTATE STORAGE ON FLAVOR OF SPRAY DRIED WHEY PROTEIN CONCENTRATE AND ISOLATE

EFFECT OF LIQUID RETENTATE STORAGE

ON FLAVOR OF SPRAY DRIED WHEY PROTEIN CONCENTRATE AND ISOLATE

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ABSTRACT

Flavor continues to be an issue for wide spread usage of whey proteins. During whey protein production, concentrated liquid whey protein (retentate) may be stored for up to 48 hours before spray drying. The objective of this research was to determine the effects of holding time of liquid retentate on flavor of spray dried whey proteins: Cheddar whey protein isolate (WPI) and Mozzarella 80 % whey protein concentrate (WPC80). Liquid WPC80 and WPI were manufactured and stored at 3C. After 0, 6, 12, 24 and 48 h, product was spray dried (2 kg) and the remaining retentate held until the next timepoint. The design was replicated twice for each product. Powders were stored at 21C and evaluated every 3 mo through 12 mo storage. Flavor profiles of rehydrated proteins were documented by descriptive sensory analysis. Volatile components were analyzed with solid phase microextraction (SPME) coupled with gas chromatography mass spectrometry (GC-MS) and gas chromatography olfactometry (GC-O). Cardboard flavors increased in both spray dried products with increased retentate storage time and cabbage flavors increased in WPI ($p<0.05$). Concurrent with sensory results, lipid oxidation products (hexanal, heptanal, octanal) and sulfur degradation products (dimethyl disulfide, dimethyl trisulfide) increased in spray dried products with increased liquid retentate storage time, while diacetyl decreased ($P < 0.05$). Shelf stability was decreased in spray dried products from longer retentate storage times. Processors should reduce storage time as they process raw whey into WPC80 or WPI. For maximum quality and shelf life, liquid retentate should be held for less than 12 hours prior to spray drying.

INTRODUCTION

Whey protein is one of the major players in the growing functional foods market, valued to be >$100 billion by 2012 (Smithers, 2008). Whey protein is mainly utilized in two forms: whey protein concentrate (34-89 % protein; WPC) and whey protein isolate (>90 % protein; WPI). In 2007, 357.4 million pounds of WPC was produced (IDFA, 2008). While production and applications are increasing, Smithers (2008) blamed inconsistency and unreliability of whey proteins in food systems as the number one reason for restricted whey protein utilization over the past 25 y. Both nutritional and functional characteristics of whey protein are utilized in food applications. Whey proteins exhibit low sensory flavor intensities, but off flavors may frequently carry-through into ingredient applications, limiting product success (Drake, 2006; Childs et al., 2007; Drake et al., 2009; Wright et al., 2009).

For widespread ingredient applications, it is important for spray dried whey products to exhibit a bland flavor; however, off-flavors are present and result from many different factors and chemical reactions. Such factors/reactions may include riboflavin effects, Maillard browning and lipid oxidation (Aurand et al., 1966; Sattar and deMan, 1975; Dattatreya et al., 2007; Morr and Foegeding, 1990; Rinn et al., 1990; Carunchia Whetstine et al., 2003; Tomaino et al., 2004; Gallardo-Escamilla et al., 2005). Volatile lipid oxidation products (VLOP) have been associated with cardboard flavor in dairy products, which is a

common off-flavor in dried whey products (Hammond, 1989; Drake et al., 2009; Wright et al., 2009).

Tomaino et al. (2004) hypothesized that transportation and storage of liquid whey could dramatically affect the flavor of the finished whey powder after concentration and drying. Volatile compounds present in liquid whey are retained during spray drying (Bangs and Reineccius, 1981). Therefore if oxidation products were produced during storage and transportation of liquid whey, those products could be carried through to the final whey powder impacting its flavor and functionality (Tomaino et al., 2004). Tomaino et al. (2004) documented an increase of VLOP over a 14 day liquid whey storage period. Tomaino et al. (2004) did not remove the fat from the pilot plant- produced fluid whey in their study nor did they process the liquid whey to a final spray dried product. Fat removal is a standard industrial process step and as such, results from Tomaino et al. (2004) would represent the worst case scenario in regards to lipid oxidation. However, Carunchia Whetstine et al. (2003) also documented 'cardboard' off flavors and VLOP in commercial fluid whey samples indicating that lipid oxidation was a potential concern even when the lipid content was minimal according to industrial practice. Undesirable flavors also increased in dried whey protein during storage and were due at least in part to increases in volatile lipid oxidation products (Wright et al., 2009).

During whey production, concentrated liquid whey may be stored in temperature-controlled tanks for up to 48 h before spray drying. Storage and storage time are variable

within and between facilities. During this storage time, chemical reactions may occur that produce off-flavors in spray dried whey protein. If off-flavor production increases during storage of liquid whey protein retentate, then whey processors would be encouraged to spray dry immediately after concentration or methods to minimize oxidation during liquid storage should be identified. The objective of this research was to determine the effects of storage time of liquid whey protein retentate on flavor and flavor stability of spray dried whey proteins: Cheddar WPI and Mozzarella WPC80.

MATERIALS AND METHODS

Experimental design

Two industrial manufacturers (Cheddar WPI manufacturer, West Coast, USA and Mozzarella WPC80 manufacturer, Midwest, USA) of whey protein were contacted and agreed to participate in the study. These two manufacturers were selected based on their interest and willingness to participate, representation of Mozzarella and Cheddar whey products, as well as their pilot plant storage and drier facilities which were required to enable them to participate in the study. Each facility, on two separate occasions, collected 2271 L of liquid whey protein retentate (Mozzarella WPC80 retentate 28.27 ± 0.62 % solids, 23.18 ± 0.68 % protein; Cheddar WPI retentate 28.39 ± 0.36 % solids, 25.87 ± 0.43 % protein). For each facility, the liquid retentate was subsequently immediately spray dried at

the manufacturer's pilot plant facility (Mozzarella WPC80 or Cheddar WPI) or held at 3C in a stainless steel temperature controlled holding tank for 6, 12, 24 or 48 h prior to spray drying. At each time point, a portion of the liquid retentate was collected and frozen while another portion was spray dried and the powder collected (figure 1). Approximately 2.4 L of frozen liquid whey retentate and 2 kg of powdered whey protein were received for each time point. Liquid samples were shipped on dry ice and spray dried samples were shipped on ice gel packs by overnight carrier to NCSU (Raleigh, NC).

Whey Protein Production

Mozzarella WPC80 and Cheddar WPI Process Overview

Mozzarella WPC 80 retentate (approx. 189 liters) was collected from the commercial ultrafiltration/diafiltration filtration system and immediately transported via truck to the industrial pilot plant facility in a 189 L High Density Polyethylene drum. Temperature of the retentate was approximately 3 ^{o}C. It took approximately 2 h to transport the drum from the manufacturing plant to the pilot plant. Retentate temperature did not change during the transport. Upon arrival, a liquid sample was taken for shipment to NCSU and approx 38 liters were immediately spray-dried. The remaining 151 L of retentate was placed in a jacketed stainless steel tank. Chilled water was circulated through the jacket of the tank to maintain a 3 ^{o}C retentate temperature. The agitator of the tank was turned off, except for a

few min prior to each sampling. The liquid samples were collected in 4 liter low density polyethylene blend amber cube containers (Hedwin, VWR International, West Chester, P.A., U.S.A.) and immediately frozen at -40 ^{o}C. Sampling occurred at the prescribed intervals. The retentate was spray dried at each timepoint on a pilot scale drier. Inlet drier temperature was 182 ^{o}C and the outlet temperature was 91 ^{o}C. Pressure atomization was used on a single nozzle. Dry powder samples were collected and immediately sealed in mylar bags (Impack Corp., Los Angeles, C.A., U.S.A.). The drier was not cleaned between intervals, but all liquid delivery equipment, such as the drier tanks, pumps, and piping were cleaned after each sample was dried. This process was replicated on two different occasions. The process for Cheddar WPI production was similar except that pilot plant facilities were adjacent to the manufacturing facility and no transportation or time delay occurred between collection of retentate and spray drying of the first timepoint.

Sample Preparation

Frozen liquid WPC 80 and WPI retentate were quickly thawed by placing the containers in a room temperature water bath. The water was continually replenished to accelerate thawing. Overall thawing time was 1 h 45 min. All time points (0, 6, 12, 24 and 48 h) were thawed at the same time. After thawing, the liquid samples were then diluted to 10 % solids by adding 300 ml deodorized water to 150 ml retentate. This diluted whey was then portioned into random 3 digit coded lidded sample cups for descriptive sensory

analysis. Another set of sample vials were filled with a small portion of thawed whey to be used for volatile analysis. Excess liquid WPC 80 retentate was portioned into one liter fluorinated ethylene propylene containers (Nalgene, Rochester, N.Y., U.S.A.) and frozen at -15^{o}C for future analysis. Careful attention was taken to ensure all steps with the thawed frozen liquid retentate were carried out quickly. These steps, including descriptive analysis were completed within 4 h. Spray dried samples (300 g) were stored in mylar bags (Impak Corp.) at -80C until sensory and instrumental analysis (less than 120 days). Additional spray dried WPC80 and WPI (300g) were stored in mylar bags at room temperature (20 +/- 1^{o}C, 50% relative humidity) for up to one y. During that time, products were analyzed every four mo by sensory and volatile compound analysis to document storage time effects.

Proximate Analysis

Proximate analyses were conducted in duplicate on each sample. Total solids in retentate or moisture in WPC/WPI was conducted by forced draft oven. Total nitrogen was determined by Kjehdahl analysis and converted to protein using a conversion factor of 6.38 (Wehr and Frank, 2004). Fat content of WPC/WPI was determined by the Mojonnier method (Mojonnier Bros. Co., Chicago, I.L., U.S.A.) (Wehr and Frank, 2004). Color measurements were taken with a handheld Minolta Chromameter CR-300 (Minolta Camera Co., Japan) for all powder WPC80/WPI hold times at each 4 mo analysis timepoint during powder storage. WPC80/WPI powder was filled into 60 x 15 mm round polystyrene petri

dish (Becton Dickinson Labware, Franklin Lakes, NJ). L*a*b* measurements were taken at four random places on each petri dish.

Descriptive Analysis

Descriptive sensory analysis was performed on liquid retentate (adjusted to 10 % solids) and rehydrated spray dried WPC/WPI. Panelists (n = 10) each had more than 100 h of previous experience with the sensory analysis of dairy products with the Spectrum™ descriptive analysis method (Meilgaard et al., 1999) and an established sensory language for liquid and dried whey products (Drake et al., 2009; Wright et al., 2009). Liquid and spray dried samples were evaluated at 10% solids (w/v). A warm-up sample and deionized water were both provided. Aroma was first evaluated by cracking the lid of the sample cup and using short sniffs, followed by flavor analysis in the mouth. Attribute intensities were scored using a universal 0 to 15 point universal intensity scale consistent with the Spectrum™ method (Meilgaard et al., 1999). Both liquid and powder samples were evaluated at room temperature and each sample was evaluated in duplicate by each panelist. Data were collected using paper ballots or Compusense™ 5.0 (Compusense, Guelph, Canada).

Headspace extraction of volatile compounds

Solid Phase Micro Extraction (SPME)

Solid phase micro extraction (SPME) followed by Gas Chromatography – Mass Spectrometry (GC-MS) was conducted using a modified method from Wright et al. (2006), on both liquid and powders at time 0 and following 4, 8 and 12 mo storage for WPC80 and WPI powders. For GC-MS analysis preparation, WPC 80 and WPI liquid retentate for each time point was diluted to 10 % solids using HPLC grade water (EMD Chemicals Inc., Gibbstown, N.J., U.S.A.). Five mL diluted liquid whey retentate and 0.5 g sodium chloride (Sigma Aldrich, Milwaukee, W.I., U.S.A.) was added to 20 ml autosampler vials with steel screw tops containing silicone septa faced in Teflon (Microliter Analytical, Sawanee, FL). Powdered WPC80 and WPI were rehydrated to 10 % solids in HPLC grade water. Sodium chloride was also added at 10% w/v. Five mL whey / salt solution was added to 20 ml autosampler vials with steel screw tops containing silicone septa faced in Teflon (Microliter Analytical) and each vial spiked with 10 uL internal standard solution (2-methyl-3-heptanone in methanol at 8.1 ppm). A CombiPal autosampler (CTC Analytics, Zwingen, Switzerland) was utilized for injection into an attached Agilent 6890N GC with a 5973 inert MSD (Agilent Technologies Inc., Santa Clara, C.A., U.S.A.). Vials were placed in a randomized balanced order in the autosampler to account for the time delay during sample analysis (analysis time for 1 sample was 47 min). Vial temperature was maintained at 5^{o}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 (Supelco, Bellefonte, P.A., U.S.A.) 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. WPC 80 and WPI liquid and powder retentate from each time point were analyzed in triplicate.

Solvent extraction of volatile compounds

Direct Solvent Extraction

Direct solvent extraction (DSE) was conducted on spray dried samples following the methods of Evans et al. (2009). DSE was performed in duplicate with 0 and 48 h timepoint samples for all spray dried WPC80 and WPI replications at 0 mo. Whey powder (100 g) was divided among four 250 mL Teflon bottles with Tefzel™ closures (Nalgene®, Rochester, N.Y., U.S.A.). Seventy-five mL of diethyl ether (EMD Chemicals In., Gibbstown, NJ) was added to each bottle along with five mL of HPLC water (Gibbstown, NJ). Each bottle was then spiked with 20 uL of internal standard at 81 ppm (2-methyl-3-heptanone, 2-methyl-pentanoic acid

in methanol, Sigma Aldrich, Milwaukee, WI), and bottles were agitated using a Roto mix (Type 50800; Thermolyne, Dubuque, Iowa, U.S.A.) on high speed for 30 min. Solvent phase was separated from the mixture by centrifugation at 1459 x g for 10 min. After centrifugation, the ether phase, containing extracted volatile components, was collected into an amber glass jar with a Teflon^{TM}-lined lid. Diethyl ether addition, agitation and centrifugation steps were repeated twice with 40 mL diethyl ether added to each bottle each time. Centrifugation was repeated a fourth time after the ether phase was removed from the third round. Any remaining solvent was then removed. Solvent extracts were stored at -20^{o}C.

Solvent assisted flavor evaporation

Solvent extracts were distilled by solvent-assisted flavor evaporation (SAFE) (Ace Glassware, Vineland, N.J., U.S.A.). The assembly utilized was similar to the methods of Engel et al. (1999) as reported by Evans et al. (2009). The glass SAFE head, with the 2 L round bottom flask attached, was connected to two glass traps. The primary trap for receiving the final distilled solvent extract and secondary trap for waste were both submerged in liquid nitrogen via separate dewar flasks. The round bottom flask was kept at 40^{o}C using a water bath. Vacuum was reached in the glassware using a rough pump/diffusion pump combination. The solvent extract was poured into the top of the SAFE head and introduced into the vacuum system drop-wise until all sample was introduced. Distillation was carried out for 10 min under vacuum (10 Torr) and kept at 40^{o}C with a circulating water bath.

Phase separation

After distillation, the sample was concentrated under a stream of nitrogen gas to 20 mL and transferred to a 50 mL screw top glass tube for phase separation into acid and neutral/basic fractions. The concentrated distillate was washed twice with 3 mL of sodium bicarbonate (0.5M)(Fisher Scienctific, Fairlawn, N.J., U.S.A.) and mixed vigorously. After each wash, the bottom layer, or water phase, was pipette into another glass tube. The concentrate was then washed three times with 2 mL of saturated sodium chloride solution, mixed vigorously and the bottom water phase removed into the same glass tube. The resulting concentrate was the neutral/basic fraction and was dried over anhydrous sodium sulfate (VWR International, West Chester, P.A., U.S.A.) to remove any residual water. The neutral/basic fraction was then concentrated to 0.5 mL under a stream of nitrogen gas. The water phase was acidified by adding hydrochloric acid (18 w/v %)(Sigma Aldrich, Milwaukee, W.I., U.S.A.) dropwise until a pH of 2-2.5 was reached. Acidic volatiles were then extracted by the addition of 15 mL diethyl ether over three steps of adding 5 mL diethyl ether, vigorously mixing and removing the top ether phase. The acidic volatiles were dried over anhydrous sodium sulfate (VWR International, West Chester, P.A., U.S.A.) and concentrated to 0.5 mL under a stream of nitrogen gas.

Solvent-Assisted Flavor Evaporation Optimization

Prior to solvent extraction, preliminary studies were conducted to optimize vacuum distillation holding time during SAFE methodology. A commercial Cheddar WPI sample was

extracted by DSE with diethyl ether on six different occassions to evaluate three distillation holding times in duplicate. SAFE and phase separation were carried out as previously described in duplicate with these extracts for 10 min, 1 h or 2 h hold times after all sample was introduced into the vacuum system. Extracts were evaluated by GC-MS and GC-O to determine if SAFE hold times impacted volatile compound recovery.

Volatile Analysis of solvent extracts

Gas Chromatography – Mass Spectrometry (GC-MS)

GC-MS analysis was conducted on an Agilent 6890N GC with a 5973 inert mass selector detection (MSD) (Agilent Technologies Inc., Santa Clara, Ca., U.S.A.). 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., U.S.A.). Helium was utilized as the carrier gas at a constant flow of 1 mL/min. The GC-MS oven program started with an initial oven temperature of 40^{o}C for 5 min, increasing to 200^{o}C at a rate of 5^{o}C per min and a final hold time of 45 min. MSD conditions included: capillary direct interface temperature, 280^{o}C; ionization energy, 70eV; mass range 35 to 300 amu; EM voltage (Atune+200V); scan rate, 5 scans/s.

Two micro liters of each solvent extract, neutral/basic and acidic fractions, were injected in the splitless mode. A 5 min solvent delay was programmed into the MS

acquisition parameters for solvent injections. Each solvent extract fraction was analyzed in duplicate. For SPME samples, fibers were injected in triplicate into the same GC-MS and followed the aforementioned GC method (from the SPME section) with the same MS parameters as for solvent volatile analysis. A solvent delay was not needed for headspace SPME analysis.

Gas Chromatography – Olfactometry (GC-O)

GC-O of solvent extracts was conducted using two HP 5890 series gas chromatographs (Hewlett-Pakard Co., Palo Alto, Calif., U.S.A.) equipped with flame ionization detectors (FID), a sniffing port, and a splitless injector. One GC-O was fitted with a polar capillary column (Rtx-Wax 30 m length × 0.25 mm inner dia × 0.25 um film thickness; Restek, Bellefonte, Pa., U.S.A.) and another with a nonpolar capillary column (Rtx-5ms 30 m length × 0.25 mm inner dia × 0.25 um df; Restek). Both neutral/basic and acidic fractions from solvent extracts were analyzed in duplicate on both columns. Two uL of solvent extract were injected into the GC injection port, maintained at 250^{o}C, fitted with a direct injection liner (Supelco). The GC oven temperature was initially held at 40^{o}C for 5 min and then increased to 200^{o}C at a rate of 10^{o}C/min with a final hold time of 20 min. Deactivated fused silica capillaries (1-m length × 0.25-mm inner dia) were used to split the column effluent 1:1 between the FID and the sniffing port. The FID and sniffing port were maintained at 300^{o}C and 105^{o}C respectively. Humidified air was supplied to the sniffing port at 30mL/min. Post peak intensity of aroma active compounds was evaluated (Grosch

1993; van Ruth, 2001) and recorded by two experienced sniffers, each with > 150 h experience with GC-O of dairy products.

GC-O analysis of SPME headspace volatiles was conducted on a nonpolar capillary column (Rtx-5ms 30 m length × 0.25 mm inner dia × 0.25 um df; Restek). Samples were prepared as described for SPME GC-MS. The fiber was desorbed for 5 min at a depth of 3 cm in the injection port, held at 250^{o}C, fitted with a SPME inlet liner (Supelco). The GC oven temperature was initially held at 40^{o}C for 5 min and then increased to 200^{o}C at a rate of 10^{o}C/min with a final hold time of 5 min. All other GC-O parameters were similar to solvent GC-O analysis. Each sample was prepped in duplicate and sniffed in duplicate. Post peak intensity of aroma active compounds was evaluated (Grosch 1993; van Ruth, 2001) and recorded by two experienced sniffers, each with > 150 h training on GC-O of dairy products.

Aroma Extract Dilution Analysis (AEDA)

AEDA was conducted on solvent extracts under similar conditions as those used for post peak intensity GC-O. Neutral basic fractions were injected onto the non-polar 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 polar 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. A step wise dilution of 1:3 (v/v) with diethyl ether was followed until two experienced sniffers detected no odorants. The highest dilution, for which an aroma was still detected, was reported as the flavor dilution factor (Grosch, 1993).

Compound Identification

For positive identification of volatile compounds extracted by SAFE and SPME, ion spectras of compounds were compared to the NIST 2005 library of spectra and spectra of authentic standards injected under identical conditions. Recovery of the internal standard concentration was used to calculate the relative abundance of each compound identified. Retention indices were calculated using an alkane series (Sigma Aldrich, Milwauke, WI, U.S.A.) (Van den Dool and Kratz 1963). Mass spectra, retention index, and aroma properties of authentic standards injected under identical GC-O conditions were used for aroma active compound identification.

Statistical Analysis

Statistical differences in sensory and instrumental data were determined by analysis of variance (ANOVA) using Fisher's Least Significant Difference (LSD) as a post hoc test (XLStat). Principle component analysis was utilized to visualize the impact of hold times (0, 6, 12, 24 and 48 h) on the sensory attributes and volatile compound profiles of spray dried products.

RESULTS AND DISCUSSION

WPC80 and WPI Composition

There were no compositional differences between 0-48 h time points for Mozzarella liquid retentate ($P > 0.05$). Ash was 0.82 ± 0.05; total solids were 28.2 ± 0.62; protein was 23.2 ± 0.68; fat was 1.35 ± 0.02. Proximate analysis of Mozzarella WPC80 from each storage point were also similar (carbohydrate 10.09 ± 0.49; ash 2.75 ± 0.09; moisture 6.28 ± 1.21; protein 76.44 ± 1.06; fat 4.45 ± 0.11).

Proximate analysis of Cheddar WPI retentate was variable ($p<0.05$) for protein and total solids between 0 and 48 h time point samples although the range was small (<1%). Ash content was 3.08 ± 0.05; total solids was 28.39 ± 0.24; protein was 25.9 + 0.3, fat was 0.06 ± 0.01. Small, but statistically significant differences ($p<0.05$) were also noted between moisture and protein for spray dried WPI (Table 1). All products (Mozzarella and Cheddar; powder and liquid retentate) were within expected ranges for their proximate compositions (Jelen, 2000).

Sensory Descriptive Analysis

Aroma and flavor attributes detected in all samples can be split into two groups: dairy flavors which are flavors associated with fresh fluid milk or whey (cooked/milky, diacetyl, cereal), and non-dairy flavors which are flavors not associated with fresh fluid milk or whey (cardboard, cabbage, serumy, fatty) (Drake et al., 2003, Carunchia Whetstine et al., 2005, Drake et al., 2009, 2010). For ingredient applications, whey proteins should have as little flavor as possible. Milder fresh dairy flavors in whey proteins are easier to incorporate into other food systems (ingredient applications) compared to non-dairy flavors in whey proteins. Cardboard, cabbage, and fatty flavors in whey proteins readily carry-through into beverage applications (Caudle et al., 2005; Wright et al., 2009; Drake et al., 2009).

Liquid Mozzarella retentate. Aroma intensity increased with retentate hold time. Aroma intensity was highest for the 48 hour time point, while 0, 6, 12, and 24 had lower aroma intensities (Table 2). Cardboard, astringency and serumy flavors also increased with increased retentate hold time. In contrast, cooked/milky flavor decreased as retentate hold time increased. Cereal flavor did not change. Overall, for liquid mozzarella retentate samples, there was an increasing trend for non dairy flavors and a decreasing trend for dairy flavors as retentate hold time increased from 0 to 48 h.

Mozzarella WPC 80 - 0 month. Similar to the liquid retentate results, Mozzarella WPC also increased in aroma intensity, cardboard, and serumy flavors as the retentate hold time increased from 0 to 48 h ($p < 0.05$) (Table 3, Figure 2). Fatty flavor also increased with

retentate hold time ($p < 0.05$). No statistical difference was detected for astringency. Flavor attributes were more intense for Mozzarella WPC80 than the liquid Mozzarella retentate even initially, which might suggest that spray drying pronounces flavor perception and differences.

Liquid Cheddar retentate. There were flavor differences between Cheddar and Mozzarella retentates (Table 3, 4). Mozzarella retentate had cooked/milky and serumy flavors which were not documented in Cheddar retentates while Cheddar retentate displayed a fatty flavor that increased with storage time. Other flavor attributes were similar for both types of retentates. Aroma intensity, cardboard, and fatty attributes all increased in Cheddar retentate as hold time increased (Table 4).

Cheddar WPI – 0 month. Cabbage/brothy, potato, and chlorine flavors were descriptive sensory attributes that have been associated with Cheddar whey protein and not Mozzarella whey protein (Wright et al., 2009). Aroma intensity, cabbage/brothy, and cardboard attributes increased in Cheddar WPI 0 to 48 h of retentate storage (Table 5, Figure 3). Cardboard flavor showed the greatest increase, starting at intensities of 0.88 for 0 h and increasing to 2.38 in WPI from retentate stored for 48 h at 3C. There was no discernable trend for chlorine flavor among the time points. No statistical differences were detected for potato and astringency attributes.

SPME GC MS of liquid Mozzarella and Cheddar retentate

Volatile compounds differentiating retentate storage times were first analyzed with SPME GC-MS, a dynamic headspace technique which targeted the recovery of low molecular weight compounds. Relative abundance of selected VLOPs, namely aldehydes, for Mozzarella WPC80 liquid retentate storage times are presented in Table 6. Hexanal and heptanal concentrations both increased with increased retentate holdtime. Other aldehydes: butanal, octanal, and decanal showed no significant differences. A statistical increase in overall aldehyde concentration was observed by 48h of retentate storage for Mozzarella WPC80 liquid retentate. While aldehydes increased, diacetyl concentrations significantly decreased. These volatile results are consistent with sensory results, both indicating that while retentate storage time increased, fresh flavors associated with diacetyl decreased and off-flavors associated with aldehydes increased. VLOPs were also detected by SPME GC-MS for Cheddar WPI liquid retentate (Table 7). Unlike Mozzarella relative abundances, relative concentrations of compounds examined in Cheddar WPI retentate were not significantly different over 48h storage of liquid retentate at 3C ($P < 0.05$). Beyond aldehydes, sulfur compounds: dimethyl disulfide, and dimethyl trisulfide were also detected in the Cheddar WPI retentate, consistent with sensory detection of sulfur flavors (cabbage and potato flavors in the spray dried Cheddar WPI.

Tomaino et al. (2004) also reported VLOP compounds in liquid Cheddar whey and hypothesized that because these compounds were common volatile lipid oxidation products, their presence indicated that oxidation occurred during cheese making and/or whey pasteurization. Two storage studies reported similar increases of hexanal concentration in liquid whey and WPC after six and fourteen days of storage (Tomaino et al., 2004; Lee et al., 1996). This observation was explained by Tomaino et al. (2004) as being a product of lipid oxidation initiated during processing. The oxidative products and continued oxidation were carried over into the dry product due to entrapment in the matrix, hydrophobic interactions or covalent linkages with ε- and α- amino groups of proteins (Kinsella, 1989; Mills and Solms, 1984; Hidalgo and Kinsella, 1989). However, few studies have actually documented sensory and volatile compound analysis in both liquid and spray dried whey products and fewer still, to our knowledge, have followed volatile compounds and sensory profiles from fluid whey through processing and into spray dried products in a controlled pilot plant study. Croissant et al. (2009) evaluated the impact of bleaching on flavor of liquid whey and WPC66 manufactured from the same liquid whey. They documented volatile compounds and sensory changes in fluid whey that were consistent with the volatile compounds and sensory profile of the spray dried WPC66, suggesting that observations from fluid whey are indicative of the quality of the final spray dried product.

The importance of these volatile lipid oxidation product observations lies in their effect on flavor. Lipid oxidation products have been associated with cardboard flavor in dairy products, which is the off-flavor most commonly attributed to decreased quality in whey (Hammond, 1989; Drake et al., 2003). Tomaino et al. (2004) compared his volatile and sensory data from fluid whey to similar results found by Hall and Anderson (1985) in whole milk powder where a significant increase of cardboard aroma, flavor and aftertaste were significantly correlated with hexanal and other volatile lipid oxidation product concentrations. To validate this, Tomaino et al. (2004) examined water soaked cardboard by dynamic headspace analysis and reported hexanal, octanal and heptanal as the predominate compounds present. Current results from this research coincide with the previous findings of volatile compounds as they relate to lipid oxidation and sensory quality in whey.

In the current study, volatile compound changes were observed in Mozzarella retentate, but not in Cheddar retentate, which could be attributed to processing and/or compositional differences. Special attention was taken to control cooling/handling storage differences and process time variability, however, it has been proposed that lipid oxidation can occur at -20C (Liaw, 2009). Additionally, the lipid concentration of the Mozzarella retentate was higher than the Cheddar retentate (1.35 vs 0.06) and this difference could certainly impact total aldehyde concentrations. Because lipid oxidation is the primary reaction that produces aldehydes then initial lipid content as well as lipid quality (oleic vs

linoleic content) plays a role in the final types of aldeydes and concentrations produced. Once produced, some aldehydes are much more unstable than others, and readily degrade into other aldehydes (Frankel et al., 2005). For example, nonanal is produced from the 9- and 10- hydroperoxide of oleate. In a circumstance like whey protein where 8-, 9-, 10-, and 11- oxo esters of oleate are present in a mixture, different lipid oxidation mechanisms determine the final aldehyde ratio. Photosensitized lipid oxidation, as compared to autoxidation, favors cleavage of the 9- and 10- hydroperoxides, and thus increases the nonanal concentration (Frankel et al., 2005). A subsequent decrease in nonanal concentration could be due to oxidation of the aldehyde. As lipid oxidation advances and saturated aldehydes accumulate, unsaturated aldehydes are flavored to oxidize into lower aldehydes and dialdehydes (Frankel et al., 2005). A big spike in the hexanal concentration of Mozzarella liquid retentate from 24h to 48h could be attributed to the multiple lipid oxidation pathways that produce hexanal, both direct hydroxide cleavage and aldehyde degradation (Frankel et al., 2005). These various lipid oxidation pathways could also explain why hexanal and heptanal were observed at higher initial concentrations in Cheddar retentate than Mozzarella retentate. Fatty acid composition, starter cultures, and processing parameters are all additional points of variability between Cheddar and Mozzarella retentate that could translate into VLOP variability (Liaw, 2009).

SPME GC-MS of spray dried WPC80 and WPI

Relative abundances of select VLOPs for spray dried Mozzarella WPC80 and Cheddar WPI (at 0 month shelf life storage) are reported in Tables 8 and 9 respectively. Spray dried Mozzarella WPC80 followed similar volatile trends as the liquid retentates. Hexanal, heptanal, and total aldehyde relative abundances increased as retentate holdtime increased. No discernable trend was observed for the other aldehydes at 0 month. Differences present in liquid Mozzarella retentate translated into differences in the final spray dried WPC80. Unlike liquid retentate, spray dried Cheddar WPI headspace volatiles changed with increased retentate storage time, consistent with sensory changes. Changes were documented for dimethyl disulfide, dimethyl trisulfide, octanal and nonanal (Table 9). Octanal and nonanal and dimethyl trisulfide increased in WPI with increased retentate storage time. Dimethyl disulfide increased and then decreased with increased retentate storage time.

Tomaino et al. (2004) hypothesized that since liquid whey was often transported and stored before drying, reactions that take place during this time could dramatically affect the flavor of the finished whey powder after concentration and drying. Volatile compounds present in liquid whey have been shown to be retained during spray drying (Bangs and Reineccius, 1981), which could be attributed to binding of proteins and flavor compounds such as methyl ketones and aldehydes (lipid oxidation products) (Mills and Solms, 1984). Our results are consistent with these hypotheses. Oxidation products are produced during

storage, and these products are carried through to the finished whey powder impacting its flavor.

VLOPs are not formed from the same pathway, nor do they appear at the same rate (Marsili 1999; Lee and Min 2009). The free fatty acid that is being oxidized determined the chemical pathway that is followed and thus the VLOPs produced. Tomaino et al. (2004) stored liquid whey at refrigeration temp for 14 days and monitored the free fatty acid content. A correlateion between the decrease of linoleic acid (18:2) with increased cardboard flavor was documented. Oleic acid (18:1) also significantly decreased during this storage period (Tomaino *et al.* 2004). Lipid content in whey proteins is primarly composed of the phospholipid component of the milkfat globule membrane, which is 6% linoleic acid (Frankel 2005). While linoleic acid is at a higher content in the milkfat globule membrane, milkfat, as a whole, is 25% oleic acid and only 2% linoleic acid (Frankel 2005). During autooxidation, 8-, 9-, 10-, and 11- hydroperoxides are formed from oleate. These specific hydroperoxides further cleave on the ester side to produce heptanal, octanal, nonanal, and decanal aldehydes. 9- and 13- hydroperoxides are formed from autooxidized linoleate and cleaved to produce 2,4 decadienal, pentanal, and hexanal. These initial pathways are followed during autooxidation; however, different pathways are followed when free fatty acids are photooxidized. Photooxidation of linoleic acid forms the 12-hydroperoxide which produces hexanal when cleaved (Frankel 2005). Linoleic acid has a reaction rate that is 40 times faster than that of oleic acid (Frankel 2005). If oxidation is allowed to continue, the

intial unsaturated aldehydes can breakdown to also produce hexanal (Schieberle and Grosch 1981). High concentrations of hexanal in oxidized food products can be attributed to the multiple oxidation and breakdown pathways that form hexanal and the reaction kinetics of lipid oxidation that favor these specific pathways.

Direct comparison of sensory perception of cardboard flavor and the relative abundance of total aldehydes clearly indicated an increasing trend for both, flavor and aldehyde concentration as retentate holdtime increased for both whey types (Figures 4 and 5). Sensory and volatile analysis substantiates the hypothesis that as whey retentate is held for extended times before spray drying, lipid oxidation reactions may lead to increased production of off-flavors that alters the clean flavor profile expected of whey protein powders.

GC-O of spray dried WPC80 and WPI

For GC-O analysis, flavor compounds were extracted using DSE SAFE, a solvent based extraction method which targets the recovery of medium and high molecular weight compounds. Post peak intensity aroma analysis was conducted for all timepoints. Aroma extract dilution analysis (AEDA), a serial dilution sniffing technique, was also conducted on all samples to generate semi-quantitative values for aroma-active compounds (Tables 10, 11). Flavor dilution factors (FD $\log_3$) are presented to illustrate the potency of each aroma

active compound. Compounds with high FD factors indicate a more potent compound than those with lower FD factors. FD factor differences within a compound are considered suggestive of concentration differences when FD factors differ by ≥2 $\log_3$ (Audouin et al., 2001).

Ninety four aroma active compounds were detected in Mozzarella WPC80 (Table 10) and one hundred and seven aroma-active compounds were detected in Cheddar WPI (Table 11). Sixty one of the identified aroma active compounds were present in both Mozzarella WPC80 and Cheddar WPI. Identified aroma active compounds included a wide range of aldehydes, ketones, esters, pyrazines, free fatty acids, and sulfurs. Many of these compounds have been previously identified in whey and dairy ingredients (Stevenson and Chen, 1996; Lee et al., 1996; Karagul-Yuceer et al., 2003; Carunchia Whetstine et al., 2003; Wright et al., 2009; Evans et al., 2009). Differences between 0h and 48h retentate holdtimes were apparent for both types of whey protein. Nineteen compounds increased between 0 and 48h in Mozzarella WPC80; of these, 9 were VLOPs. Additionally, 12 compounds were only present in WPC80 from retentate stored for 48h but not 0h and 1 compound was present at 0h but not 48h. For Cheddar WPI, 29 compounds increased between 0 and 48h; of these, 10 were VLOPs and 5 were sulfur degradation products. Fifteen compounds were only present in 48h but not 0h and 11 compounds were present at 0h but not 48h. In many cases, aroma compounds were more potent, indicated by a higher log dilution factor in the 48h products than at 0h. Identification of key aroma active

volatiles and their relative intensities pinpoints the major flavor reactions occurring within the 48h retentate holdtime.

Off-flavors in dairy products are often a product of hydroperoxide degradation that can create the following compounds: unsaturated and saturated aldehydes (Frankel et al., 1961), unsaturated and saturated hydrocarbons, unsaturated ketones (Stark and Forss, 1962), saturated and unsaturated alcohols (Hoffman, 1962), and semialdehydes (Frankel et al., 1961). Other compounds have been isolated from milk, rather than the expected carbonyl compounds, indicating that further degradation of the initially produced unsaturated aldehydes, migration of the double bonds or isomerization could all occur during auto-oxidation (Weihrauch, 1988). VLOPs are associated with cardboard flavor in whey proteins. Research by Hammond and Seals (1972) indicated that many different volatile lipid oxidation products could be responsible for cardboard off-flavors observed in dairy products.

Hexanal is one of the most widely identified VLOPs in whey protein. Hexanal, along with aldehydes: pentanal, hexanal, heptanal, nonanal, and decanal were identified in both whey types. These aldehydes exhibited higher FD factors in Mozzarella WPC80 than Cheddar WPI. Aldehyde FD factors didn't follow any trends with increasing liquid retentate holdtimes for either whey type; however, differences between FD factors for samples were detected. This lack of correlation could be attributed to the formation of aldehydes from different lipid oxidation pathways and the concurrent breakdown of aldehydes into other

aldehydes. Additionaly, the entrapment of small molecular weight aldehyes in the protein matrix would account for decreased perception (Carunchia Whetstine et al., 2005). Another important VLOP is the ketone, 1-octen-3-one, which elicits a mushroom aroma. 1-octen-3-one has been previously documented in whey power (Carunchia Whetstine et al., 2005; Wright et al., 2009; Evans et al., 2009; Liaw, 2009). FD factors for 1-octen-3-one were higher for Mozzarella WPC80 than Cheddar WPI. As liquid retentate holdtime increased for Mozzarella WPC80, 1-octen-3-one FD factors also increased. Cheddar WPI indicated an opposite trend with a much smaller range between the two holdtimes. Another ketone, 1-hexen-3-one which is characterized by a plastic rubber aroma, increased in FD factor for both whey types as liquid retentate holdtime increased. VLOPs are attributed as being the major source of off flavors in whey products, however, it is important to consider the other aroma active compounds identified.

Nine sulfur compounds were identified in Cheddar WPI and only four in Mozzarella WPC80. Methional, dimethyl disulfide, and dimethyl trisulfide have been previously identified in whey (Carunchia Wheststine et al., 2005; Wright et al., 2006; Wright et al., 2009; Evans et al., 2009; Liaw, 2009). Methional is a documented Strecker degradation product formed from methionine. Methionine also degrades into dimethyl sulfide and methanthiol which further breakdown to form dimethyl disulfide and dimethyl trisulfide (Bendall, 2001; Carunchia Whestine et al., 2005; Wright et al., 2006). The intermediate product, dimethyl sulfide was also identified. Relative abundance values of DMDS and

DMTS for Cheddar WPI indicate increases and decreases in DMDS and DMTS concentrations due to the production of these two compounds from various intermediate products from different Strecker degradation pathways (Table 9). Thiophene, methylthiophene, thenythiol, 2-methyl-3-furanthiol, and 2-methyl-3-methyldithiofurane were all identified in Cheddar WPI but not previously identified in whey. Three of these compounds have been reported in skim and whole milk powder (Carunchia Whetstine et al., 2003). Cheddar WPI was more characterized by sulfur compounds not only because more sulfur compounds were detected, but they were also detected at higher FD factors and post peak intensities than for Mozzarella WPC80. The major observation for identified sulfur compounds was that for both whey types, Mozzarella WPC80 and Cheddar WPI, FD factors and post peak intensities increased as retentate hold time increased from 0h to 48h. This increase is consistent with the increase of cabbage/brothy, and potato sensory attributes as retentate hold time increased. Due to their low threshold values, sulfur compounds play a major role in the flavor of whey protein (Wright et al., 2006).

Strecker degradation refers to the degradation of amino acids to form volatile flavor compounds. As previously discussed, methionine degrades to produce sulfur compounds. Phenylalanine degrades to produce 2-phenethanol, phenyl ethyl acetate, and phenylacetaldehyde. These compounds have been previously identified in sweet whey powder, cheddar cheese, WPC, WPI, liquid Cheddar whey, and liquid Mozzarella whey (Singh et al., 2003; Mahajan et al., 2004; Carunchia Whetstine et al., 2005; Evans et al.,

2009; Liaw, 2009) 2-Phenethanol and phenylacetaldehyde both elicit a rosy aroma. In this study for both whey types, phenylacetaldehyde increased with increased retentate holdtime while 2-phenethanol decreases with increased retentate holdtime. Degradation of aromatic amino acids can also produce guaiacol which elicits a smoky aroma (Singh et al., 2003; Carunchia Whetstine et al., 2005; Evans et al., 2009). Guaiacol was identified with FD factors of ≥ $\log_3$ 6 in Mozzarella WPC80 and increased to $\log_3$ 9 after 48h retentate holdtime. Cheddar WPI FD factors for guaiacol were lower than those for Mozzarella WPC80, but they also increased with increased retentate holdtime. Strecker degradation has a slower reaction rate than lipid oxidation (Wallace and Fox, 1997); thus, while it may be a major source of flavor in aged products like cheese, it is less likely the source of off flavors in whey protein (Swaisgood, 1996; Carunchia Whetstine et al., 2005).

Lactones are formed during the pasteurization of milk when hydroxyl fatty acids from milkfat are esterifed (Dimick et al., 1969). Coconut and peach aromas are created by lactones (Singh et al., 2003). Because lactones are produced from milkfat it has been documented that WPC80 contains more lactones that WPI (Carunchia Whetstine et al., 2005). Eight lactones were identified for both Mozzarella WPC80 and Cheddar WPI. There were no discernable trends observed correlating lactone concentrations with retentate storage time. Nor were lactones detected at routinely higher FD factors in one whey type than the other. The major difference was the identification of vanillin in Mozzarella WPC80 and not in Cheddar WPI. Vanillin is also formed during pasteurization and originates from

plant lignin in the cow rumen (Cobb et al., 1963). Vanillin has been identified in skim milk powder and WPC80 (Karagul-Yuceer et al., 2001, 2002; Carunchia Whetstine et al., 2005). Vanillin was only identified in the 0h Mozzarella WPC80 sample. Lactones and vanillin are associated with fresh dairy flavors and are desirous; therefore, their decrease after 48h of retentate storage might result in a whey product that was categorized as less fresh (Drake et al., 2009).

Aside from Strecker degradation products and lactones, other thermally generated compounds were identified. Maillard browning reactions of residual lactose initiated in thermal processing (pasteurization, spray drying) in whey production can produce pyrollines, pyrazines, and furanones (Morr and Ha, 1990; Freidman, !996; Mahajan et al., 2004). Morr and Foegeding (1990) reported that decreasing lactose content would reduce Maillard browning and might improve flavor stability. Dogan and Oral (2007) identified 5-hydroxymethylfurfural as a volatile marker for Maillard browning in whey proteins, however, that compound was not identified in the current study. As expected, Mozzarella WPC80 had a higher lactose composition than Cheddar WPI. Sotolon and furaneol were both initially identified in sweet whey powder (Mahajan et al., 2004), but were further confirmed in WPC80 and WPI (Carunchia Whetstine et al., 2005; Evans et al., 2009; Liaw, 2009). While sotolon was detected in both types of whey, furaneol was only detected in Cheddar WPI. Higher FD factors for sotolon were reported for Mozzarella WPC80 than Cheddar WPI as expected due to the higher lactose content. FD Factors for sotolon furaneol

increased in Mozzarella WPC80 as retentate holdtime increased. In addition to these compounds, pyrazines and pyrollines, compounds exhibiting cooked/nutty and popcorn aromas were detected in both Mozzarella WPC80 and Cheddar WPI. Flavors elicited by these compounds are often associated with fresh whey flavors. 2-Acetyl-1-pyrolline and 2-acetylpyidine FD factors increased for both whey types as retentate hold time increased. This supports the sensory results indicating that fresh flavor attributes decreased with increased retentate holdtime. Carunchia Whetstine et al. (2005) concluded that Maillard browning products were likely not the source of off-flavors in fresh dried whey products.

Some volatile compound differences between Mozzarella WPC80 and Cheddar WPI can be attributed to their composition. Lipid oxidation was pinpointed as a major off flavor producing reaction in liquid and spray dried whey in the current results. Thus, we would expect that Mozzarella WPC80 would contain a higher concentration of volatile lipid oxidation products because both the initial retentate lipid content and the lipid content in the final spray dried product was higher for WPC80 than WPI (Morr and Ha, 1990). Relative abundance results support this hypothesis because the total aldehyde concentration for Mozzarella WPC80 was much higher than the total aldehyde concentration for Cheddar WPI. Additionally, GC-O flavor dilution factors for hexanal and nonanal were higher for Mozzarella WPC80 than Cheddar WPI. While higher lipids may increase the concentration of VLOPs, the detection of VLOPs is also determined by the matrix. Increased detection of VLOPs in Mozzarella WPC80 could also be attributed to protein binding of volatile flavor

compounds (Mills and Solms, 1984; Quach et al, 1999). WPI by definition contains a higher protein concentration. Specifics of the flavor binding qualities of proteins are still controversial as literature results are not always consistent. Plug and Haring (1993) suggested that aldehydes bind to proteins through Van der Waals forces, hydrogen bonds, and covalent bonds. However, in all circumstances, the detection of aldehydes is decreased when higher protein concentrations are present (Ng et al., 1989; Hansen and Heinis, 1992).

VLOPs concentrations are not only dependant upon the initial lipid concentration, but also the presence of pro-oxidants. Metal ions are considered the main catalyst for non-enzymatic lipid oxidation (St. Angelo, 1992; Frankel, 2005). In this case, Cheddar WPI was had higher ash content than Mozzarella WPC80 which could correspond to higher mineral content. Cardboard flavor, the off flavor associated with lipid oxidation was higher in Cheddar WPI than Mozzarella WPC80 even though total aldehyde concentrations were higher for Mozzarella WPC80. This suggests that while aldehydes are the primary VLOPs produced, they are not solely responsible for eliciting cardboard flavor in whey protein. Therefore predicting the quality (absence of off flavors) of a whey product whether WPC80 or WPI, Mozzarella or Cheddar is not dependant upon one factor (starter culture, composition, pH, temperature, storage) but on all factors as they interact together during cheesemaking, whey concentration, spray drying, and shelf life storage. Whitson et al. (2009) recently demonstrated that cardboard flavor in whey proteins was due to a combination of specific VLOPs and dimethyl trisulfide, a sulfur degradation compound.

WPC80 / WPI shelf life

When whey is utilized in the industry, rarely is it as fresh from the manufacturer as the 0 month powder samples analyzed in this study. Thus, the powders were evaluated every 4 months for up to 1 year of shelf life. Zero, four, eight and twelve month descriptive sensory data for Mozzarella WPC80 and Cheddar WPI were evaluated by principle component analysis to provide a visual illustration of the effects of retentate hold time on the storage stability of whey proteins for each flavor attribute (WPC80: Figure 6; WPI: Figure 7).

Mozzarella WPC80 with retentate hold time of 48 h still had higher intensities of cardboard, serumy, fatty, and aroma intensity attributes compared to WPC80 with retentate hold times of 12 h or less across 12 mo of storage. A decrease in intensity from 0 to 48 h retentate hold time was still observed for cereal flavor after 12 months storage. Cheddar WPI from retentate held 48 h also still indicated increased intensities of cardboard, cabbage, potato, chlorine, and aroma flavor attributes even after 12 months of WPI storage compared to Cheddar WPI retentate held for 0 h. This indicates that while the 0 h sample was the most bland sample for both whey types at 0 months, it was still the most bland sample (according to off-flavor development) after a 12 month shelf life; further indicating that initial retentate hold time during whey production has an impact on initial quality and storage stability of the resulting spray dried whey protein.

Volatile analysis of spray dried Mozzarella WPC80 and Cheddar WPI showed differences between retentate holdtimes throughout the 12 m shelf life (Tables 8 and 9). Total aldehyde relative abundance was highest at 48 h retentate storage for each timepoint for Mozzarella WPC80. Sulfur degradation compounds were highest at 12, 24, and 48 h retentate storage for each timepoint for Cheddar WPI.

Alterations in the flavor profiles of whey proteins are very important factors for ingredient applications. Previously off-flavors in milk powder were shown to carry through into ingredient applications and have a negative impact on consumers (Caudle et al., 2005). Similar results have been reported for WPC80 and WPI in beverage applications (Drake, 2006; Drake et al., 2009; Wright et al., 2009). Childs et al. (2007) evaluated the consumer acceptance of meal replacement bars and beverages containing soy and whey protein. Consumers preferred whey protein applications over soy applications, however, they disliked both. Furthermore, Wright et al. (2009) evaluated consumer acceptance of peach flavored beverages with a 6% added whey protein. Acceptance of WPC80 peach beverages decreased when WPC80 was stored prior to incorporation into beverages. Similar results were documented with WPI beverages when WPI was stored for as little as 9 mo. Stronger flavors in whey proteins means more potential for flavor carry-through into ingredient applications.

CONCLUSION

During liquid storage of whey protein retentate at 3C, chemical reactions continue to occur that produce off-flavors in the retentates and spray dried whey protein. Concurrent with sensory results, lipid oxidation products (octanal) and sulfur degradation products (dimethyl disulfide, dimethyl trisulfide) increased in spray dried Cheddar products with increased liquid retentate storage time. Total aldehyde relative abundances (butanal, hexanal, heptanal, octanal, nonanal) were higher in spray dried Mozzarella WPC80 and increased with increasing liquid retentate storage time. Major volatile aroma compounds that characterized Mozzarella WPC80 and Cheddar WPI flavor were aldehydes and Strecker degradation products. Shelf stability, as evaluated by sensory and volatile compound analyses, was decreased in spray dried products from longer retentate storage times. It is critical for processors to reduce intermediate storage time in liquid state as they process raw whey into WPC80 or WPI. By so doing, they can dramatically improve flavor of whey protein products. Based on this study, for maximum quality and shelf life, liquid retentate should be held for less than 12 hours prior to spray drying.

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TABLES AND FIGURES

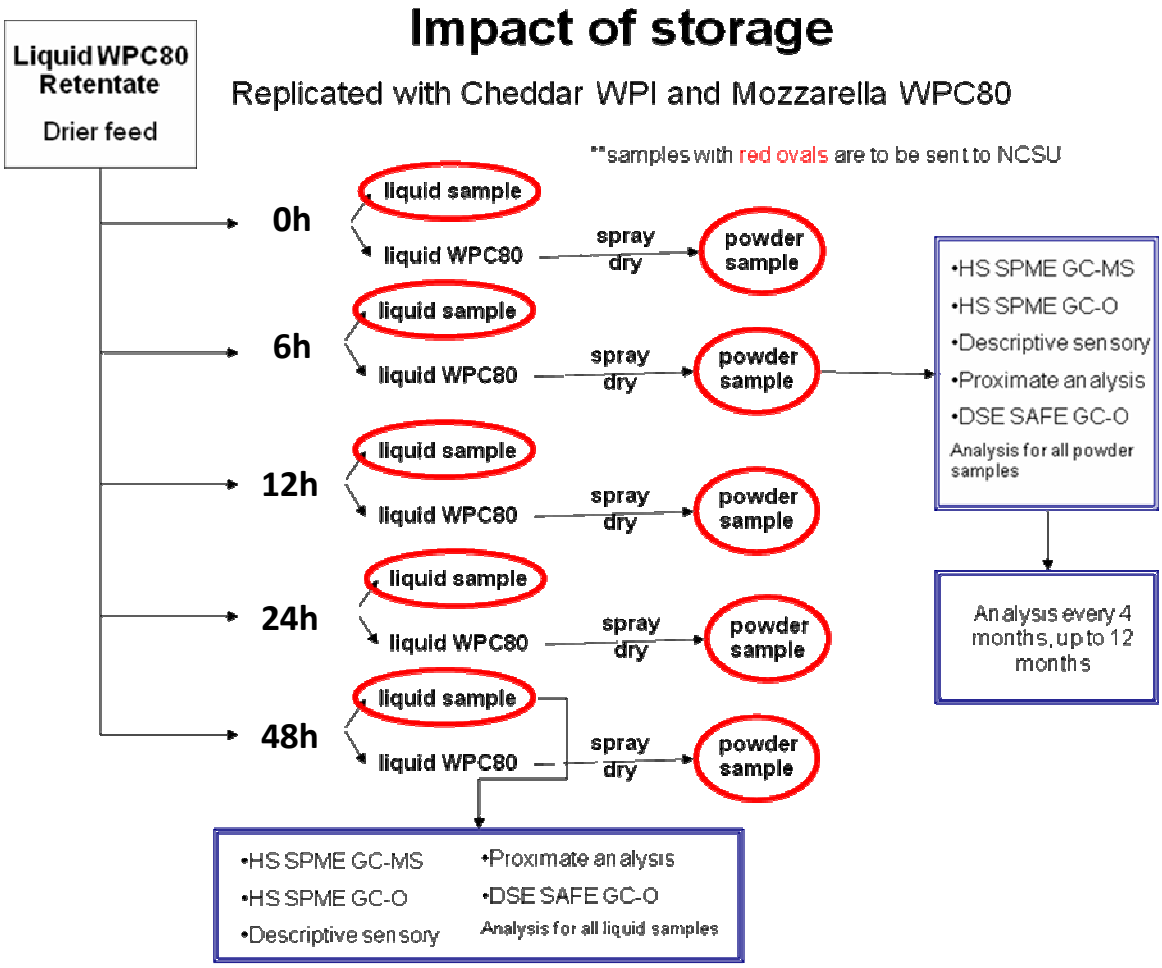

Figure 3.1. Experimental Design

Table 3.1. Proximate analysis of Cheddar WPI

	Retentate Holdtime (h)[1]				
Attribute	**0**	**6**	**12**	**24**	**48**
ash (% dry weight basis)	3.10[a]	3.05[a]	3.02[a]	3.13[a]	3.13[a]
moisture (%)	4.69[b,c]	4.40[c,d]	4.26[d]	5.11[a]	4.81[a,b]
protein (% dry weight basis)	88.70[a,b]	89.50[a]	89.11[a,b]	88.53[b]	88.36[b]
fat (% dry weight basis)	0.38[a]	0.42[a]	0.45[a]	0.38[a]	0.38[a]

[1] Means in a row followed by different letters are significantly different ($P < 0.05$)

Table 3.2. Descriptive sensory analysis of liquid Mozzarella retentate

	Retentate Holdtime (h)[1]				
Attribute	**0**	**6**	**12**	**24**	**48**
aroma intensity	1.18[b]	1.28[b]	1.33[b]	1.42[b]	1.75[a]
cooked milky	1.33[a]	1.00[a,b,c]	1.05[a,b]	0.67[c]	0.75[b,c]
cardboard	ND	ND	ND	1.12[a]	1.38[a]
cereal	1.25[a]	1.13[a]	1.22[a]	1.12[a]	1.00[a]
astringency	1.75[b]	2.22[a,b]	2.08[b]	2.10[b]	2.68[a]
serumy	0.88[c]	1.57[a,b]	1.45[b]	1.43[b]	1.95[a]

[1] Means in row followed by different letters are significantly different ($P < 0.05$).
Intensities are score on a 0 to 15-point universal scale; 0 = none and 15 = very high (Mielgaard et al., 1999). Most dried ingredient flavor intensities fall between 0 and 4 on this scale (Drake et al., 2003, 2009; Wright et al., 2009).
ND = not detected consistently by all panelists; mean intensity score < 0.50.

Table 3.3. Descriptive sensory analysis of Mozzarella WPC80 (0 month) across varying retentate storage times

	Retentate Holdtime (h)[1]				
Attribute	**0**	**6**	**12**	**24**	**48**
aroma intensity	1.33[b]	1.58[b]	1.45[b]	1.90[a,b]	2.45[a]
cardboard	1.00[b]	1.23[b]	1.05[b]	1.75[a]	1.95[a]
cereal	1.50[a]	1.30[a,b]	1.38[a,b]	1.33[a,b]	1.15[b]
astringency	2.05[a]	2.00[a]	2.15[a]	2.05[a]	2.05[a]
serumy	ND	ND	ND	0.75[b]	1.13[a]
fatty	ND	ND	ND	0.83[b]	1.78[a]

[1] Means in row followed by different letters are significantly different ($P < 0.05$).
Intensities are score on a 0 to 15-point universal scale; 0 = none and 15 = very high (Meilgaard et al., 1999). Most dried ingredient flavor intensities fall between 0 and 4 on this scale (Drake et al., 2003, 2009; Wright et al., 2009).
ND = not detected consistently by all panelists; mean intensity score < 0.50.

Table 3.4. Descriptive sensory analysis of liquid Cheddar retentate across holdtime at 3C

	Retentate Holdtime (h)[1]				
Attribute	**0**	**6**	**12**	**24**	**48**
aroma intensity	1.13[b]	1.25[a,b]	1.25[a,b]	1.25[a,b]	1.53[a]
cereal	0.50[a]	0.50[a]	0.50[a]	0.60[a]	0.73[a]
cardboard	1.25[b]	1.40[b]	1.53[a,b]	1.65[a,b]	1.93[a]
fatty	ND	ND	0.50[b]	0.50[b]	1.08[a]
astringency	2.50[a]	2.58[a]	2.58[a]	2.58[a]	2.65[a]

[1] Means in row followed by different letters are significantly different ($P < 0.05$).
Intensities are score on a 0 to 15-point universal scale; 0 = none and 15 = very high (Mielgaard et al., 1999). Most dried ingredient flavor intensities fall between 0 and 4 on this scale (Drake et al., 2003, 2009; Wright et al., 2009).
ND = not detected consistently by all panelists; mean intensity score < 0.50.

Table 3.5. Descriptive sensory analysis of Cheddar WPI (0 month)

	Retentate Holdtime (h)[1]				
Attribute	**0**	**6**	**12**	**24**	**48**
aroma intensity	1.55[b]	1.90[a,b]	2.18[a,b]	2.53[a]	2.20[a,b]
cabbage brothy	ND	0.88[b]	1.18[b]	1.18[b]	1.60[a]
potato	0.50[a]	ND	ND	0.90[a]	0.50[a]
cardboard	0.88[c]	1.53[b,c]	1.83[a,b]	2.13[a,b]	2.38[a]
chlorine	0.63[a]	ND	ND	ND	0.70[a]
astringency	1.83[a]	1.88[a]	1.93[a]	1.95[a]	2.00[a]

[1] Means in row followed by different letters are significantly different ($P < 0.05$).
Intensities are score on a 15-point universal scale; 0 = none and 15 = very high (Meilgaard et al., 1999). Most dried ingredient flavor intensities fall between 0 and 4 on this scale (Drake et al., 2003, 2009; Wright et al., 2009).
ND = not detected consistently by all panelists; mean intensity score < 0.50.

Table 3.6. Relative abundance of selected volatiles in liquid Mozzarella retentate stored for 0, 6, 12, 24, and 48h

	Concentration (ppb)[1]				
Compound	**0 h**	**6 h**	**12 h**	**24 h**	**48 h**
Diacetyl	0.49[a]	0.25[b]	0.20[b]	0.20[b]	0.07[b]
Butanal	1.22[a]	1.87[a]	1.91[a]	2.01[a]	1.61[a]
Hexanal	0.15[b]	0.20[b]	0.99[b]	1.26[b]	11.79[a]
Heptanal	0.03[b]	0.08[b]	0.05[b]	0.16[b]	1.07[a]
Octanal	0.12[a]	0.11[a]	0.11[a]	0.13[a]	0.12[a]
Decanal	ND	0.01[a]	0.01[a]	ND	0.01[a]
Total Aldehydes	1.52[b]	2.26[b]	3.06[b]	3.57[b]	14.61[a]

[1] means within rows followed by different letters are significantly different ($P < 0.05$)

ND = not detected

Table 3.7. Relative abundance of selected volatiles in liquid Cheddar retentate stored at 0, 6, 12, 24, and 48h

	Concentration (ppb)[1]				
Compound	**0 h**	**6 h**	**12 h**	**24 h**	**48 h**
Diacetyl	0.22	0.26	0.26	0.23	0.19
Dimethyl Disulfide	2.84	4.36	4.98	9.34	2.34
Hexanal	7.69	6.58	7.98	6.79	5.51
Heptanal	0.30	0.38	0.42	0.37	0.27
Dimethyl Trisulfide	0.47	0.81	0.95	1.34	0.56
Octanal	0.07	0.07	0.07	0.07	0.05
Nonanal	0.12	0.32	0.35	0.20	0.15
Total Aldehydes	8.18	7.36	8.83	7.43	5.97

[1] all means within rows were not significantly different ($P > 0.05$)

A peak for butanal could not be integrated and as such was considered not detected.

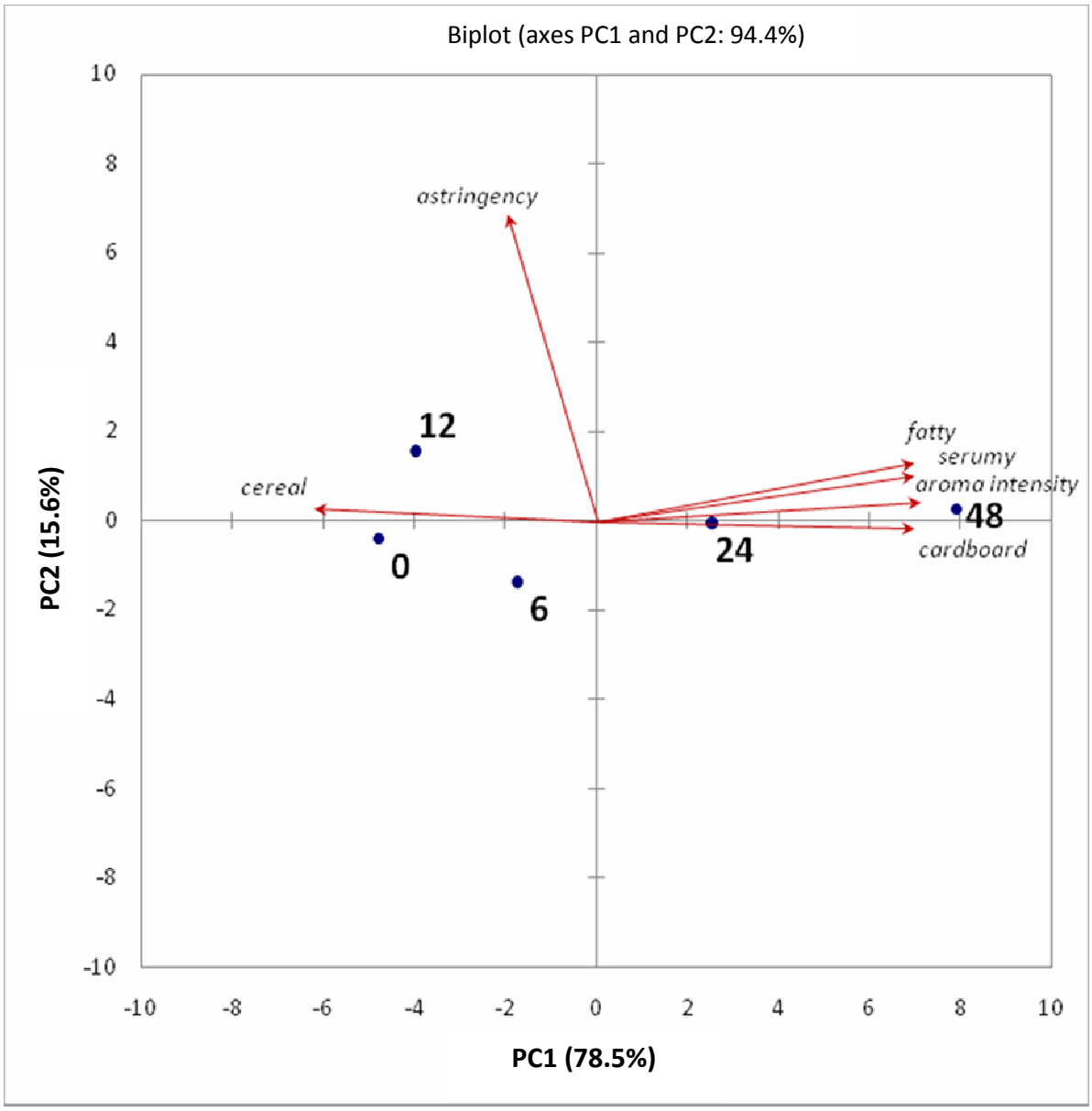

Figure 3.2. Descriptive sensory analysis principle component biplot of Mozzarella WPC80 from liquid retentate stored for 0, 6, 12, 24, 48 h

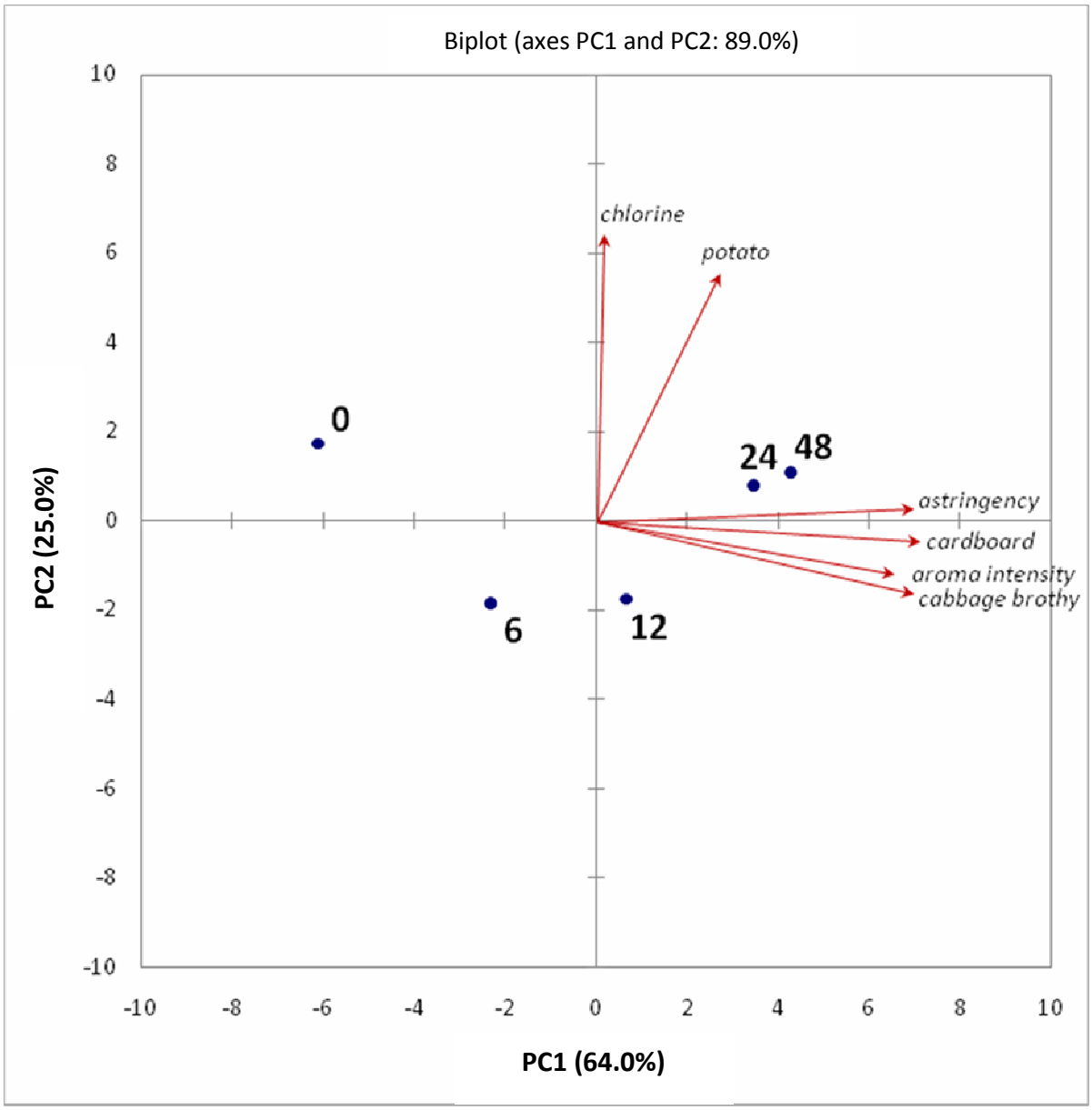

Figure 3.3. Descriptive sensory analysis principle component biplot of Cheddar WPI from liquid retentate stored for 0, 6, 12, 24, 48 h

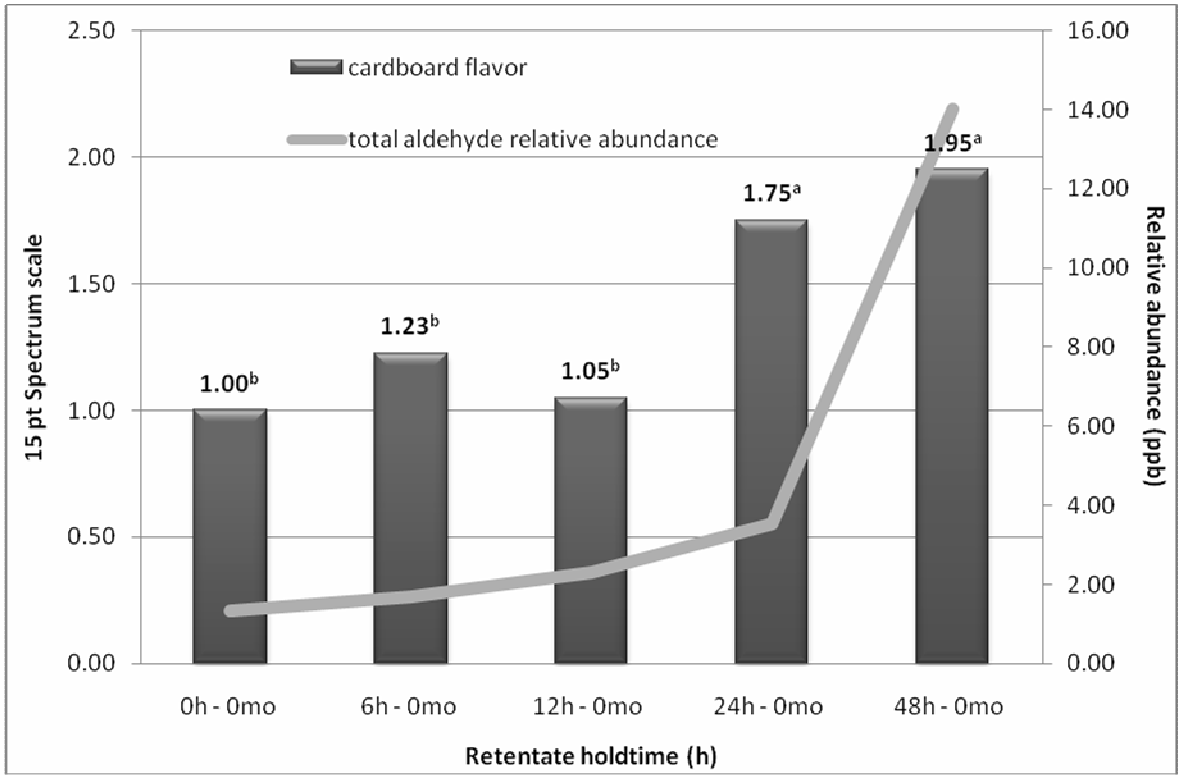

Figure 3.4. Comparison of descriptive analysis cardboard flavor intensity to SPME total aldehyde relative abundance (ppb) in Mozzarella WPC80 from liquid retentate stored for 0, 6, 12, 24 or 48 h

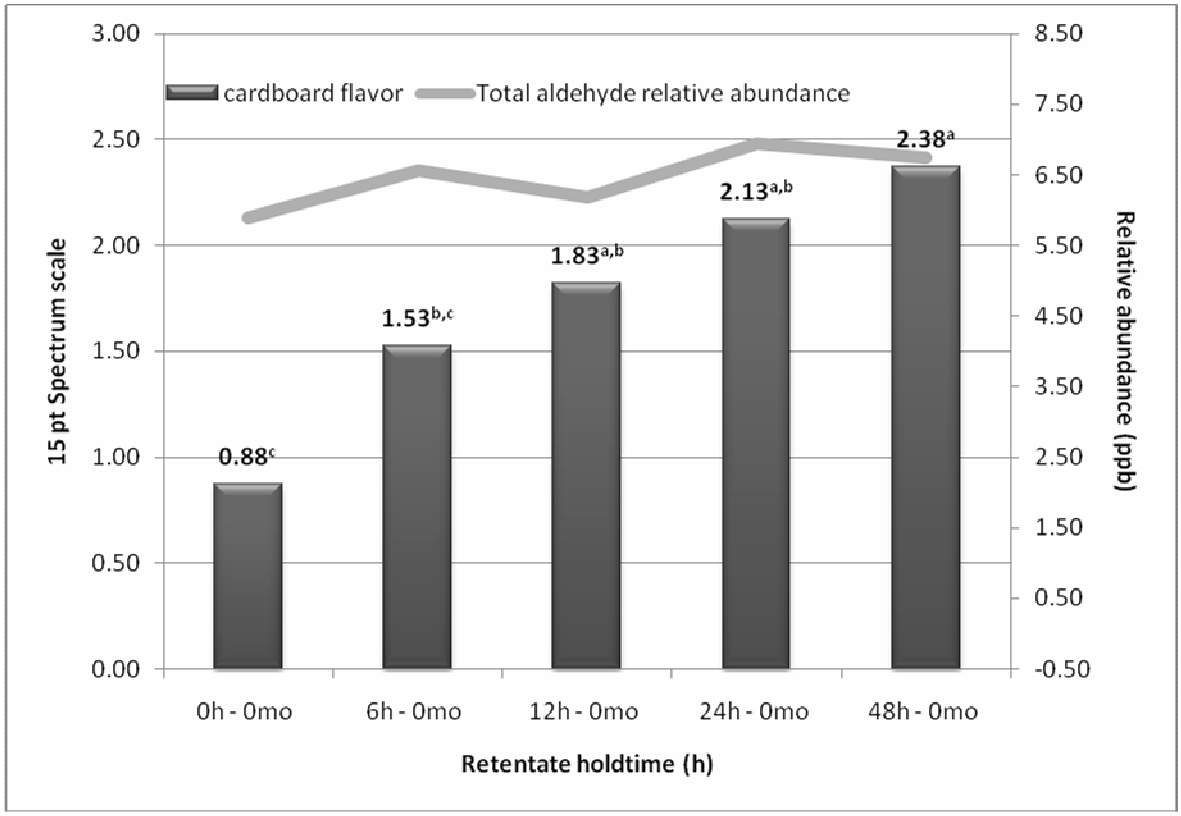

Figure 3.5. Comparison of descriptive analysis cardboard flavor intensity to SPME total aldehyde relative abundance (ppb) in Cheddar WPI from liquid retentate stored for 0, 6, 12, 24 or 48 h

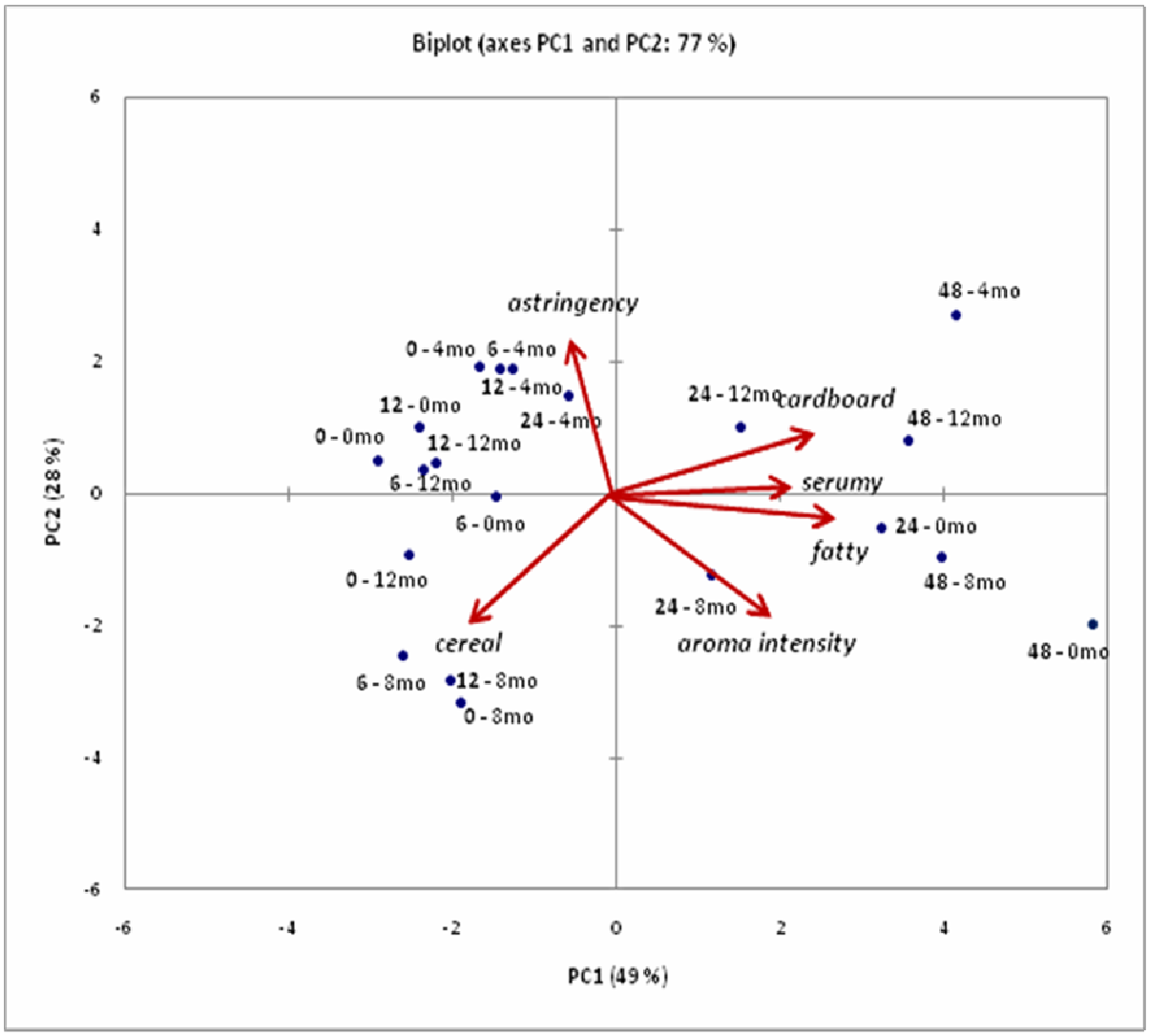

Figure 3.6. Descriptive sensory analysis principal component biplot of Mozzarella WPC80 from liquid retentate stored for 0, 6, 12, 24 or 48 h across 12 months storage at 21C (0, 4, 8, 0r 12 months). For data point labels, the first number is the retentate storage time and the second number is WPC80 storage time.

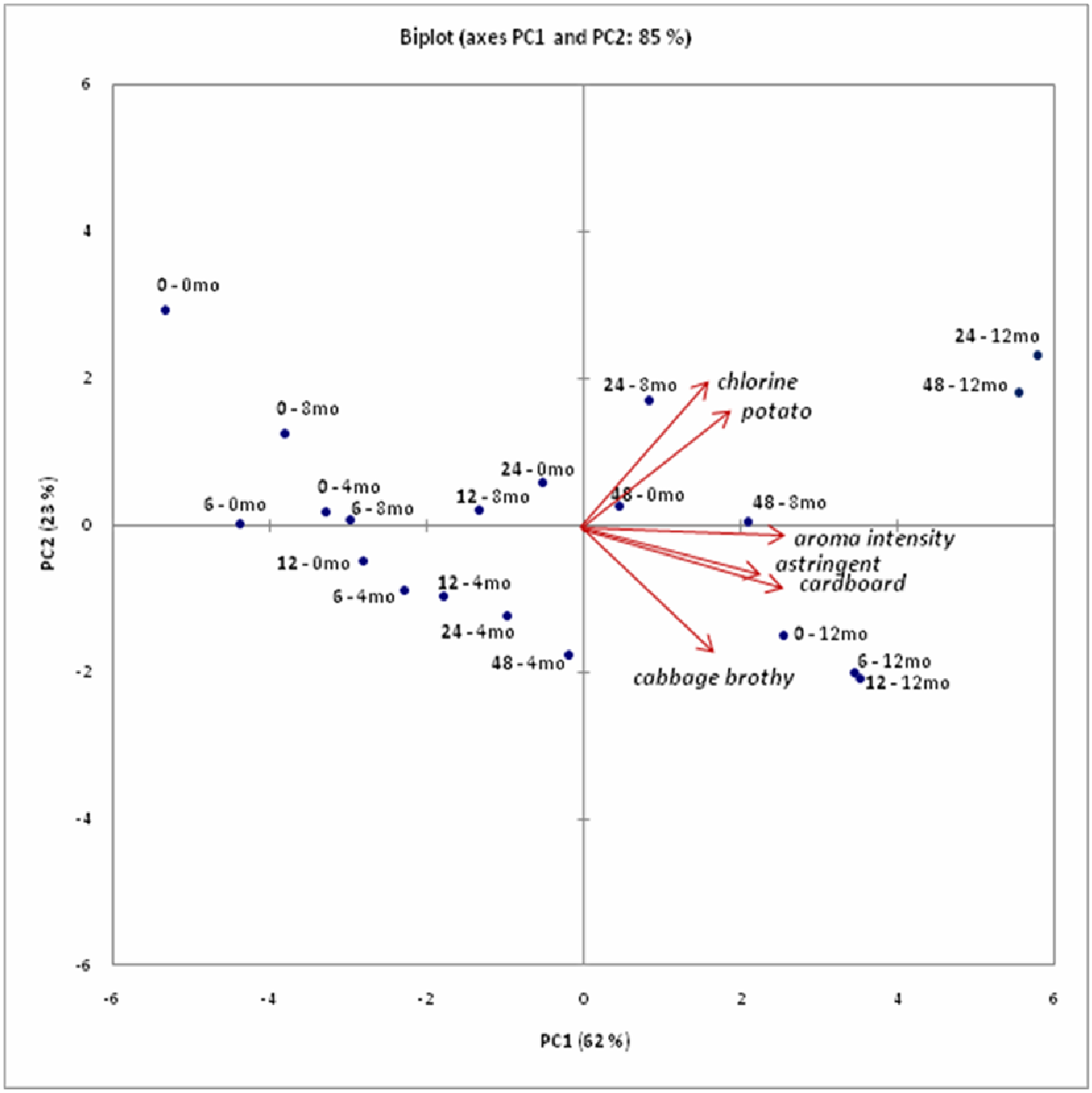

Figure 3.7. Descriptive sensory analysis principal component biplot of Cheddar WPI from liquid retentate stored for 0, 6, 12, 24 or 48 h across 12 months storage at 21C (0, 4, 8, 0r 12 months). For data point labels, the first number is the retentate storage time and the second number is WPI storage time.

Table 3.8. SPME relative abundance of select VLOPs in Mozzarella WPC80 from liquid retentate stored for 0, 6, 12, 24 or 48 h across 12 months storage at 21C (0, 4, 8, or 12 months)

	Concentration (ppb)[1]									
	0 month					4 month				
Compound	**0 hr**	**6 hr**	**12 hr**	**24 hr**	**48 hr**	**0 hr**	**6 hr**	**12 hr**	**24 hr**	**48 hr**
Diacetyl	$0.12^{a,b,A,B,C,D}$	$0.28^{a,A,B,C}$	$0.21^{a,b,A,B,C,D}$	$0.21^{a,b,A,B,C,D}$	$0.10^{b,D}$	$0.15^{a,B,C,D}$	$0.14^{a,C,D}$	$0.17^{a,B,C,D}$	$0.18^{a,B,C,D}$	$0.21^{a,A,B,C,D}$
Butanal	$1.06^{a,A,B,C}$	$1.33^{a,A}$	$1.17^{a,A,B,C}$	$1.33^{a,A}$	$1.31^{a,A}$	$0.62^{a,C}$	$0.68^{a,B,C}$	$0.74^{a,B,C}$	$0.87^{a,A,B,C}$	$0.99^{a,A,B,C}$
Hexanal	$0.15^{b,J}$	$0.17^{b,J}$	$0.97^{b,J}$	$1.94^{b,J}$	$11.69^{a,I,J}$	$18.11^{c,H,I}$	$21.31^{c,G,H,I}$	$23.17^{c,F,G,H,I}$	$40.52^{b,B,C,D}$	$52.10^{a,A,B}$
Heptanal	$0.03^{b,G}$	$0.08^{b,G}$	$0.05^{b,G}$	$0.17^{b,G}$	$0.91^{a,F,G}$	$2.14^{b,E,F}$	$2.53^{b,D,E,F}$	$2.38^{b,D,E,F}$	$3.55^{a,b,B,C,D,E}$	$5.06^{a,A,B,C}$
Octanal	$0.10^{a,F}$	$0.10^{a,F}$	$0.10^{a,F}$	$0.11^{a,F}$	$0.11^{a,F}$	$0.26^{b,D,E,F}$	$0.30^{b,C,D,E,F}$	$0.29^{b,C,D,E,F}$	$0.47^{a,b,B,C,D,E}$	$0.67^{a,A,B}$
Nonanal	ND	ND	ND	ND	ND	$0.08^{b,C,D,E,F,G}$	$0.08^{b,C,D,E,F,G}$	$0.09^{b,B,C,D,E,F,G}$	$0.14^{a,b,A,B,C}$	$0.19^{a,A}$
Decanal	ND	$0.01^{a,E,F}$	$0.01^{a,E,F}$	ND	$0.01^{a,E,F}$	$0.05^{a,A}$	$0.05^{a,A,B,C}$	$0.05^{a,A,B}$	$0.05^{a,A}$	$0.05^{a,A}$
Dodecanal	ND	ND	ND	ND	ND	$0.04^{a,B,C}$	$0.05^{a,A,B,C}$	$0.06^{a,A,B}$	$0.08^{a,A}$	$0.08^{a,A,B}$
total aldehyde	$1.34^{b,I}$	$1.69^{b,I}$	$2.29^{b,I}$	$3.54^{b,I}$	$14.03^{a,H,I}$	$21.31^{c,G,H}$	$25.00^{c,F,G,H}$	$26.78^{c,F,G,H}$	$45.69^{b,B,C,D}$	$59.14^{a,A,B}$

	Concentration (ppb)[1]									
	8 month					12 month				
Compound	**0 hr**	**6 hr**	**12 hr**	**24 hr**	**48 hr**	**0 hr**	**6 hr**	**12 hr**	**24 hr**	**48 hr**
Diacetyl	$0.28^{a,A,B}$	$0.26^{a,A,B,C}$	$0.26^{a,A,B,C}$	$0.20^{a,A,B,C,D}$	$0.33^{a,A}$	$0.16^{a,b,B,C,D}$	$0.16^{a,b,B,C,D}$	$0.14^{b,C,D}$	$0.19^{a,A,B,C,D}$	$0.16^{a,b,B,C,D}$
Butanal	$1.17^{a,b,A,B,C}$	$0.95^{a,b,A,B,C}$	$0.99^{a,b,A,B,C}$	$0.84^{b,A,B,C}$	$1.39^{a,A}$	$0.87^{a,A,B,C}$	$1.30^{a,A,B}$	$0.87^{a,A,B,C}$	$1.17^{a,A,B,C}$	$0.67^{a,B,C}$
Hexanal	$21.71^{b,F,G,H,I}$	$29.74^{b,D,E,F,G}$	$25.37^{b,E,F,G,H}$	$31.06^{b,D,E,F,G}$	$57.90^{a,A}$	$32.82^{a,C,D,E,F,G}$	$40.27^{a,B,C,D}$	$34.57^{a,C,D,E,F}$	$37.29^{a,C,D,E}$	$44.68^{a,B,C}$
Heptanal	$2.30^{b,D,E,F}$	$3.28^{b,C,D,E}$	$2.58^{b,D,E,F}$	$3.05^{b,D,E}$	$5.97^{a,A}$	$4.15^{a,A,B,C,D,E}$	$5.46^{a,A,B}$	$4.35^{a,A,B,C,D}$	$4.24^{a,A,B,C,D}$	$5.23^{a,A,B,C}$
Octanal	$0.25^{b,E,F}$	$0.36^{b,C,D,E,F}$	$00.27^{b,D,E,F}$	$0.33^{b,C,D,E,F}$	$0.72^{a,A,B}$	$0.57^{a,A,B,C,D}$	$0.85^{a,A}$	$0.61^{a,A,B,C}$	$0.70^{a,A,B}$	$0.88^{a,A}$
Nonanal	$0.06^{a,b,E,F,G,H}$	$0.07^{a,b,D,E,F,G}$	$0.03^{b,G,H}$	$0.05^{a,b,F,G,H}$	$0.11^{a,B,C,D,E}$	$0.09^{a,B,C,D,E,F,G}$	$0.13^{a,A,B,C,D}$	$0.01^{a,B,C,D,E,F,G}$	$0.11^{a,B,C,D,E,F}$	$0.15^{a,A,B}$
Decanal	$0.01^{b,E,F}$	$0.06^{a,C,D}$	$0.02^{a,b,D,E}$	$0.01^{b,E,F}$	$0.04^{a,B,C,D}$	$0.05^{a,A,B,C}$	$0.06^{a,A}$	$0.04^{a,A,B,C,D}$	$0.05^{a,A}$	$0.05^{a,A}$
Dodecanal	ND	ND	$0.01^{a,C,D}$	ND	$0.01^{a,D}$	$0.06^{a,A,B}$	$0.08^{a,A,B}$	$0.06^{a,A,B}$	$0.06^{a,A,B}$	$0.06^{a,A,B}$
total aldehyde	$25.50^{b,F,G,H}$	$34.43^{b,D,E,F,G}$	$29.26^{b,E,F,G}$	$35.34^{b,D,E,F}$	$66.14^{a,A}$	$38.62^{a,C,D,E,F}$	$48.15^{a,B,C,D}$	$40.60^{a,C,D,E,F}$	$43.62^{a,C,D,E}$	$51.72^{a,A,B,C}$

[1] Means in rows followed by different lowercase letters are significantly different within storage month time-point ($P < 0.05$); Means in rows followed by different uppercase letters are significantly different within overall 12 month storage period.

ND = not detected

Table 3.9. SPME Relative abundance of select VLOPs in Cheddar WPI from liquid retentate stored for 0, 6, 12, 24 or 48 h across 12 months storage at 21C (0, 4, 8, or 12 months)

	Concentration (ppb)[1]									
	0 month					4 month				
Compound	**0 hr**	**6 hr**	**12 hr**	**24 hr**	**48 hr**	**0 hr**	**6 hr**	**12 hr**	**24 hr**	**48 hr**
Diacetyl	$0.23^{a,D}$	$0.30^{a,C,D}$	$0.33^{a,B,C,D}$	$0.30^{a,C,D}$	$0.26^{a,D}$	$0.45^{a,A,B,C,D}$	$0.53^{a,A,B}$	$0.59^{a,A}$	$0.56^{a,A,B}$	$0.59^{a,A}$
DMDS[2]	$3.41^{b,H}$	$6.47^{b,H}$	$7.78^{a,b,H}$	$11.97^{a,G,H}$	$6.36^{b,H}$	$14.24^{b,F,G,H}$	$18.91^{a,b,E,F,G,H}$	$20.52^{a,b,D,E,F,G,H}$	$28.64^{a,C,D,E,F,G}$	$20.36^{a,b,D,E,F,G,H}$
Hexanal	$5.42^{a,A,B,C}$	$5.71^{a,A,B,C}$	$5.30^{a,A,B,C}$	$6.24^{a,A,B,C}$	$5.73^{a,A,B,C}$	$6.97^{a,A,B}$	$9.95^{a,A}$	$6.32^{a,A,B,C}$	$6.16^{a,A,B,C}$	$6.48^{a,A,B,C}$
Heptanal	$0.26^{a,C}$	$0.39^{a,B,C}$	$0.38^{a,B,C}$	$0.37^{a,B,C}$	$0.42^{a,B,C}$	$0.52^{a,A,B,C}$	$0.82^{a,A}$	$0.61^{a,A,B}$	$0.53^{a,A,B,C}$	$0.54^{a,A,B,C}$
DMTS[3]	$0.51^{b,F}$	$0.98^{a,b,D,E,F}$	$1.15^{a,b,D,E,F}$	$1.54^{a,C,D,E,F}$	$0.91^{a,b,E,F}$	$1.76^{a,B,C,D,E}$	$2.56^{a,A,B,C}$	$2.61^{a,A,B,C}$	$3.33^{a,A}$	$2.62^{a,A,B,C}$
Octanal	$0.07^{b,C}$	$0.09^{a,b,C}$	$0.09^{a,b,C}$	$0.08^{a,b,C}$	$0.13^{a,B,C}$	$0.15^{a,A,B,C}$	$0.23^{a,A}$	$0.19^{a,A,B}$	$0.16^{a,A,B,C}$	$0.11^{a,B,C}$
Nonanal	$0.14^{b,F}$	$0.38^{a,b,B,C,D,E,F}$	$0.42^{a,B,C,D,E}$	$0.27^{a,b,D,E,F}$	$0.47^{a,B,C,D,E}$	$0.57^{a,b,A,B,C}$	$0.62^{a,b,A,B}$	$0.82^{a,A}$	$0.51^{a,b,B,C,D}$	$0.36^{b,B,C,D,E,F}$
total aldehyde	$5.89^{a,A,B,C,D}$	$6.57^{a,A,B,C,D}$	$6.19^{a,A,B,C,D}$	$6.95^{a,A,B,C,D}$	$6.75^{a,A,B,C,D}$	$8.21^{a,A,B}$	$11.62^{a,A}$	$7.94^{a,A,B,C}$	$7.36^{a,A,B,C,D}$	$7.49^{a,A,B,C,D}$

	Concentration (ppb)[1]									
	8 month					12 month				
Compound	**0 hr**	**6 hr**	**12 hr**	**24 hr**	**48 hr**	**0 hr**	**6 hr**	**12 hr**	**24 hr**	**48 hr**
Diacetyl	$0.57^{a,A}$	$0.56^{a,A,B}$	$0.46^{a,A,B,C,D}$	$0.48^{a,A,B,C,D}$	$0.50^{a,A,B,C}$	$0.35^{a,A,B,C,D}$	$0.49^{a,A,B,C,D}$	$0.36^{a,A,B,C,D}$	$0.21^{a,D}$	$0.47^{a,A,B,C,D}$
DMDS[2]	$30.63^{a,C,D,E,F}$	$33.49^{a,B,C,D,E}$	$36.68^{a,A,B,C,D,E}$	$47.25^{a,A,B,C}$	$29.14^{a,C,D,E,F,G}$	$39.79^{b,A,B,C,D,E}$	$56.49^{a,b,A,B}$	$58.43^{a,A}$	$58.40^{a,A}$	$41.21^{b,A,B,C,D}$
Hexanal	$6.71^{a,A,B,C}$	$4.84^{a,A,B,C}$	$4.32^{a,B,C}$	$5.23^{a,A,B,C}$	$4.99^{a,A,B,C}$	$0.31^{a,b,C}$	$1.75^{a,B,C}$	$0.71^{a,b,B,C}$	$0.26^{b,C}$	$0.30^{a,b,C}$
Heptanal	$0.59^{a,A,B}$	$0.54^{a,A,B,C}$	$0.52^{a,A,B,C}$	$0.50^{a,A,B,C}$	$0.47^{a,B,C}$	$0.29^{a,B,C}$	$0.28^{a,B,C}$	$0.32^{a,B,C}$	$0.27^{a,B,C}$	$0.24^{a,C}$
DMTS[3]	$1.72^{b,B,C,D,E}$	$2.62^{a,b,A,B,C}$	$2.77^{a,A,B}$	$3.11^{a,A}$	$2.54^{a,b,A,B,C}$	$2.28^{a,A,B,C,D,E}$	$3.55^{a,A}$	$3.28^{a,A}$	$3.54^{a,A}$	$2.40^{a,A,B,C,D}$
Octanal	$0.19^{a,A,B}$	$0.14^{a,b,B,C}$	$0.12^{a,b,B,C}$	$0.12^{a,b,B,C}$	$0.10^{b,B,C}$	$0.07^{a,C}$	$0.06^{b,c,C}$	$0.07^{a,b,C}$	$0.06^{a,b,c,C}$	$0.05^{c,C}$
Nonanal	$0.24^{a,D,E,F}$	$0.32^{a,C,D,E,F}$	$0.41^{a,B,C,D,E,F}$	$0.19^{a,E,F}$	$0.23^{a,D,E,F}$	$0.16^{a,b,E,F}$	$0.22^{a,D,E,F}$	$0.21^{a,D,E,F}$	$0.15^{b,c,E,F}$	$0.10^{c,F}$
total aldehyde	$7.23^{a,A,B,C,D}$	$5.83^{a,A,B,C,D}$	$5.37^{a,B,C,D}$	$6.04^{a,A,B,C,D}$	$5.79^{a,A,B,C,D}$	$0.82^{b,C,D}$	$2.31^{a,B,C,D}$	$1.32^{a,b,B,C,D}$	$0.74^{b,D}$	$0.70^{b,D}$

[1] Means in rows followed by different lowercase letters are significantly different within storage month time-point ($P < 0.05$); Means in rows followed by different uppercase letters are significantly different within overall 12 month storage period.

[2] DMDS = Dimethyl Disulfide

[3] DMTS = Dimethyl Trisulfide

ND = not detected

Table 3.10. Aroma extract dilution analysis of Mozzarella WPC80 from liquid retentate stored for 0 and 48h

No.	Compound	Fraction	Odor[1]	Log_3FD^2	(Aroma intensity)[3]	Retention Index		Method of identification
				0h	48h	DB-5	DB-WAX	
1	dimethyl sulfide	NB	glue	ND	1 (0.5)	571		RI, Odor, MS
2	unknown	NB	old powder whey	ND	<1 (2.0)	609		Odor
3	diacetyl	NB	buttery	3.5 (1.3)	1.5 (1.0)	614	936	RI, Odor, MS
4	2/3-methylbutanal	NB	malty buttery sweet	ND	<1 (1.8)	637	828	RI, Odor, MS
5	acetic acid	AC	vinegar	9 (0.5)	7 (0.5)	661	1360	RI, Odor, MS
6	3-hydroxy-2-butanone	NB	malty musty	2.5 (1.0)	1 (1.0)	688		RI, Odor
7	pentanol	NB	sweet fruity	3 (3.0)	2 (1.0)	712	1240	RI, Odor
8	unknown	NB	onion	1 (1.0)	<1 (1.5)	720		Odor
9	ethyl isobutyrate	NB	sweet rubbery	1 (2.0)	<1 (1.8)	726	943	RI, Odor
10	1-hexen-3-one	NB	plastic rubber	ND	1 (1.5)	732	1040	RI, Odor
11	pentanal	NB	sweet rubbery	1.5 (1.8)	2 (1.5)	763	1133	RI, Odor
12	dimethyl disulfide	NB	earthy	ND	<1 (2.0)	779	1191	RI, Odor, MS
13	hexanal	NB	grassy lingers	5 (0.9)	4 (1.0)	786	1075	RI, Odor, MS
14	unknown	NB	fatty	1.5 (0.8)	ND	800		Odor
15	unknown	NB	sickening brothy	2.5 (1.3)	1 (2.0)	803	1053	Odor
16	butanoic acid	AC	cheesy	8 (0.5)	6 (0.5)	868	1510	RI, Odor, MS
17	o-xylene	NB	geranium rubbery	2 (1.0)	3.5 (0.8)	883	1272	RI, Odor
18	heptanal	NB	mushroom fatty	ND	<1 (2.8)	896	1234	RI, Odor, MS
19	Z-4-heptenal	NB	fishy fatty	3.5 (2.3)	4.5 (1.0)	899	1147	RI, Odor, MS
20	methional	NB	citrus potato	3 (1.5)	<1 (2.2)	907	1371	RI, Odor, MS
21	unknown	NB	garlic	ND	2 (1.5)	919		Odor
22	2-acetyl-1-pyrroline	NB	popcorn	5 (0.9)	1 (1.0)	927	1264	RI, Odor
23	methyl thiazoline	NB	brothy onion	2.5 (1.0)	3 (2.0)	953	1420	RI, Odor
24	unknown	NB	fatty	1 (2.0)	<1 (2.3)	964		Odor

Table 3.10. continued

No.	Compound	Fraction	Odor[1]	Log_3FD^2 0h	(Aroma intensity)[3] 48h	Retention Index DB-5	Retention Index DB-WAX	Method of identification
25	dimethyl trisulfide (DMTS)	NB	garlic	3.5 (1.1)	4.5 (0.5)	971	1321	RI, Odor, MS
26	1-octen-3-one	NB	mushroom	7.5 (0.5)	8.5 (0.8)	981	1234	RI, Odor, MS
27	(Z)-1,5-octadien-3-one	NB	geranium fatty	2 (2.5)	2 (2.0)	987	1353	RI, Odor
28	unknown	NB	citrus fatty	ND	2 (1.0)	992		Odor
29	unknown	NB	licorice	ND	<1 (2.0)	996		Odor
30	unknown	NB	citrus oapy lingers	3.5 (1.3)	1 (1.0)	1002		Odor
31	octanal	NB	citrus	ND	4 (1.3)	1007	1234	RI, Odor, MS
32	(E,E)-2,4-heptadienal?	NB	fatty	1 (2.5)	1 (1.5)	1012	1346	RI, Odor
33	2-acetyl thiazole	NB	coconut mothball fatty	ND	1 (1.5)	1017		RI, Odor
34	2-acetylpyridine	NB	popcorn	3 (1.1)	1 (1.5)	1028		RI, Odor
35	unknown	NB	coconut soap	1 (2.0)	2 (1.5)	1042		Odor
36	phenylacetaldehyde	NB	rosy sweet	1 (1.3)	6 (1.0)	1052	1417	RI, Odor, MS
37	(E)-2-octenal	NB	fatty citrus	4 (1.3)	4.5 (0.8)	1062		RI, Odor
38	tetramethylpyrazine	NB	nutty mushroom	2 (1.8)	8 (0.5)	1081		RI, Odor
39	unknown	NB	bell pepper	1 (2.8)	2 (1.8)	1088		Odor
40	2-methoxy phenol (guiacol)	NB	smoky	6 (1.5)	9 (0.8)	1090		RI, Odor, MS
41	nonanal	NB	fatty lingers citrus	6 (2.8)	6.5 (1.3)	1102	1333	RI, Odor, MS
42	p-cresol	NB	phenolic	2.5 (1.5)	<1 (2.0)	1112		RI, Odor, MS
43	3-hydroxy-4,5-dimethyl-2(5H)-furanone (sotolon)	NB	curry lingers	2.5 (1.5)	6 (1.5)	1117		RI, Odor, MS
44	unknown	NB	phenolic foral citrus	ND	5 (1.0)	1119		Odor
45	unknown	NB	diff phenolic	ND	5 (1.0)	1131		Odor
46	3-Hydroxy-4,5-dimethyl-2(5H)-furanone (sotolon)	NB	maple curry	ND	4 (0.8)	1143		RI, Odor, MS
47	unknown	NB	garlic earthy	1.5 (1.5)	ND	1151		Odor

Table 3.10. continued

No.	Compound	Fraction	Odor[1]	Log_3FD^2 0h	(Aroma intensity)[3] 48h	Retention Index DB-5	Retention Index DB-WAX	Method of identification
48	2-phenethanol	NB	rosy lingers	6 (1.5)	4.5 (1.8)	1149		RI, Odor, MS
49	(E,Z)-2,6-nondienal	NB	cukes lingers	5.5 (1.7)	6.5 (1.3)	1158	1423	RI, Odor, MS
50	(E,Z)-2,6-nondienal / (E)-2-nonenal	NB	carpets lingers	9 (0.9)	8 (1.0)	1166	1460	RI, Odor, MS
51	(E)-2-nonenal	NB	sweet cukes	ND	<1 (3.5)	1174	1502	RI, Odor, MS
52	2-isobutyl-3-methoxy pyrazine	NB	brothy beef	3 (1.8)	3 (1.5)	1178		RI, Odor, MS
53	o-cresol	NB	phenolic	ND	3 (1.3)	1189		RI, Odor
54	pheylethylacetate	NB	sweet cilantro	3.5 (2.4)	<1 (2.7)	1197		RI, Odor, MS
55	(E,E)-2,4-nonadienal	NB	peachy fruity fatty	ND	4.5 (1.0)	1202	1574	RI, Odor
56	unknown	NB	grain sweet curry maple	4 (1.5)	2 (1.8)	1209		Odor
57	decanal	NB	oxidized lingers	5.5 (1.3)	4 (1.8)	1222		RI, Odor, MS
58	unknown	NB	oatmeal	ND	2.5 (1.5)	1234		Odor
59	beta-cyclocitral	NB	minty licorice	4.5 (1.5)	2 (1.5)	1249		RI, Odor, MS
60	phenylethyl acetate	NB	rosy cukes	6 (1.0)	1 (1.5)	1256		RI, Odor
61	gamma-octalactone	NB	oatmeal hay	3 (2.0)	2.5 (1.3)	1266	1850	RI, Odor, MS
62	delta-octalactone	NB	cilantro stale tortilla	1 (2.0)	3.5 (1.3)	1274		RI, Odor
63	unknown	NB	sweet oatmeal waxy	7 (0.8)	7 (0.9)	1282	1665	RI, Odor
64	unknown	NB	metallic sweet	ND	2.5 (2.0)	1294		Odor
65	unknown	NB	cinamon soapy waxy	4 (2.0)	5 (1.3)	1304		Odor
66	unknown	NB	waxy cilantro metallic	2 (2.0)	3 (2.5)	1314		Odor
67	decanoic acid	AC	stale	6 (1.5)	2 (2.0)	1328	1923	RI, Odor
68	o-aminoacetophenone	NB	tortilla lingers	5.5 (1.9)	4.5 (2.0)	1332		RI, Odor
69	(E,E)-2,4-decadienal	NB	fatty tortilla bandaid	6.5 (1.0)	6 (1.3)	1344	1717	RI, Odor
70	(E)-2-undecenal	NB	coconut soap	3 (1.8)	2.5 (1.5)	1357		RI, Odor
71	gamma-nonalactone	NB	waxy coconut cilantro	3 (1.3)	6 (0.5)	1367		RI, Odor

Table 3.10. continued

No.	Compound	Fraction	Odor[1]	Log_3FD^2 0h	(Aroma intensity)[3] 48h	Retention Index DB-5	Retention Index DB-WAX	Method of identification
72	(E)-4,5-epoxy-2(E)-decenal	NB	grainy metallic	8 (0.8)	7 (0.9)	1394		RI, Odor
73	4-ethyl-octanoic acid	NB	soap	3 (1.5)	3.5 (1.3)	1427		RI, Odor
74	skatole	NB	mothaball	5.5 (1.3)	1.5 (1.8)	1439		RI, Odor, MS
75	unknown	NB	vanilla	<1 (3.3)	ND	1451		Odor
76	unknown	NB	waxy coconut lingers	ND	4 (0.8)	1453		Odor
77	unknown	NB	mothball	2 (2.0)	<1 (1.5)	1465		Odor
78	gamma-decalactone	NB	peach floral lingers	4 (1.5)	3.5 (1.8)	1504		RI, Odor
79	delta-decalactone	NB	coconut peach	4 (2.0)	6 (0.8)	1532		RI, Odor, MS
80	6-(Z)-dodecen-gamma-lactone	NB	soapy lingers dirty	ND	2 (1.5)	1621		RI, Odor
81	gamma-dodecalactone	NB	peach lingers	3.5 (1.9)	2 (1.5)	1718		RI, Odor, MS
82	delta-dodecalactone	NB	peach	3 (1.8)	2.5 (1.8)	1762		RI, Odor, MS

[1] Odor description at the gas chromatograph (BC) sniffing port

[2] Flavor dilution factors were determined on a DB-5MS column for neutral/basic (N/B) compounds

[3] Aroma intensity at the GC sniffing port, on a scale from 1-5

Table 3.11. Aroma extract dilution analysis of Cheddar WPI from liquid retentate stored for 0 and 48h

No.	Compound	Fraction	Odor[1]	Log_3FD^2 (Aroma intensity)[3] 0h	Log_3FD^2 (Aroma intensity)[3] 48h	Retention Index DB-5	Retention Index DB-WAX	Method of identification
1	dimethyl sulfide	NB	sulfur	<1 (1.5)	<1 (1.5)	536	792	RI, Odor, MS
2	unknown	NB	old stale whey sickening	1 (2.0)	4 (1.0)	543	820	Odor
3	diacetyl	NB	buttery	3.5 (1.2)	3 (1.0)	587	926	RI, Odor, MS
4	3-methyl propanal	NB	lingering burnt	ND	<1 (2.0)	592		RI, Odor
5	acetic acid	AC	vinegar	8 (0.8)	7 (0.5)	630	1347	RI, Odor, MS
6	2/3-methylbutanal	NB	chocolate malty	ND	5 (1.0)	632	887	RI, Odor, MS
7	acetoin	NB	buttery	<1 (1.8)	2.5 (1.0)	659	1232	RI, Odor
8	3-hydroxy-2-butanone	NB	buttery malty	ND	5 (1.0)	668		RI, Odor
9	ethyl isobutyrate	NB	rubbery	1 (1.3)	ND	668	991	RI, Odor
10	ethyl propionate	NB	fruity	1 (1.5)	2.5 (1.3)	690		RI, Odor
11	methylthiophene	NB	weeds catty	1 (1.4)	<1 (1.8)	699	1108	RI, Odor
12	pentanol	NB	fruity burnt tar	ND	1 (1.3)	704		RI, Odor
13	1-hexen-3-one	NB	rubberu solvent	2 (1.3)	6 (1.0)	752	1080	RI, Odor
14	pentanal	NB	chocolate malty	2 (1.3)	ND	773	1122	RI, Odor
15	dimethyl disulfide	NB	earthy onion sweet	ND	3 (1.5)	776	1095	RI, Odor, MS
16	hexanal	NB	grassy	3 (1.3)	3.5 (1.0)	786	1060	RI, Odor, MS
17	2-methyl thiophene	NB	rubbery burnt brothy	1 (2.0)	1.5 (1.8)	792	1031	RI, Odor
18	unknown	NB	sickening fatty	5.5 (1.0)	6.5 (1.3)	805	1144	Odor
19	unknown	NB	skunky sulfur sour stinky	1 (1.8)	3 (1.5)	812		Odor
20	butanoic acid	AC	cheesy	6 (0.8)	4 (1.0)	832	1613	RI, Odor, MS
21	2-methyl-3-furanthiol	NB	cooked nutty	3 (1.0)	3 (0.8)	860	1328	RI, Odor, MS
22	unknown	NB	fatty old stale whey	ND	1.5 (1.0)	878		Odor
23	o-xylene	NB	metallic geranium	2.5 (1.0)	ND	880	1179	RI, Odor
24	propionic acid	AC	skunky fatty fishy earthy	<1 (1.5)	<1 (2.3)	887	1482	RI, Odor

Table 3.11. continued

No.	Compound	Fraction	Odor[1]	Log_3FD^2 (Aroma intensity)[3] 0h	Log_3FD^2 (Aroma intensity)[3] 48h	Retention Index DB-5	Retention Index DB-WAX	Method of identification
25	2-methyl pyrazine	NB	popcorn	ND	5 (1.3)	893	1223	RI, Odor
26	Z-4-heptenal	NB	fishy fatty	2.5 (1.5)	2.5 (1.5)	896	1236	RI, Odor, MS
27	heptanal	NB	citrus fatty	ND	<1 (2.0)	902	1254	RI, Odor, MS
28	methional	NB	potato	4 (0.8)	5 (0.5)	905	1458	RI, Odor, MS
29	pentanoic acid	AC	skunk	2 (1.0)	2 (1.0)	909	1046	RI, Odor
30	2-acetyl-1-pyrroline	NB	popcorn	7 (0.8)	6 (1.5)	918	1339	RI, Odor
31	methyl hexanoate	NB	fruity	ND	2 (1.0)	924	1162	RI, Odor
32	3-octanone	NB	minty	ND	5 (1.0)	936	1191	RI, Odor
33	methyl thiazoline	NB	garlic	2.5 (0.8)	ND	951	1472	RI, Odor
34	4, 5-dimethyl thiazole	NB	bell pepper earthy	2 (3.0)	2 (2.0)	956	1287	RI, Odor
35	dimethyl trisulfide (DMTS)	NB	garlic cabbage	5.5 (1.5)	7 (1.0)	971	1398	RI, Odor, MS
36	1-octen-3-one	NB	mushroom	4 (1.5)	3.5 (1.5)	980	1299	RI, Odor, MS
37	(Z)-1,5-octadien-3-one	NB	geranium	3.5 (1.3)	2.5 (2.0)	987	1307	RI, Odor
38	octanal	NB	citrus	3 (1.5)	2 (2.0)	1005	1294	RI, Odor, MS
39	(E,E)-2,4-heptadienal	NB	fatty	ND	1 (2.0)	1014		RI, Odor
40	unknown	NB	cooked nutty	1 (2.0)	ND	1015		Odor
41	unknown	NB	earthy	ND	1 (1.3)	1019		Odor
42	2-acetylpyridine	NB	popcorn	2.5 (1.5)	1.5 (1.5)	1028	1654	RI, Odor
43	2-acetyl thiazole	NB	brothy	<1 (1.8)	<1 (1.8)	1039	1678	RI, Odor
44	phenylacetaldehyde	NB	rosy perfume	3 (1.0)	3.5 (1.0)	1052	1623	RI, Odor, MS
45	thenythiol	NB	sulfur weeds	3.5 (1.0)	4.5 (1.5)	1061		RI, Odor
46	(E)-2-octenal	NB	cukes fatty	ND	2.5 (1.8)	1066		RI, Odor
47	1-nonen-3-one	NB	medicinal vitamin	4 (0.5)	ND	1070		RI, Odor
48	furaneol™ (2,5-dimethyl-4-hydroxy-3-(2H) furanone)	NB	burnt sugar	ND	<1 (2.0)	1079		RI, Odor

Table 3.11. continued

No.	Compound	Fraction	Odor[1]	Log_3FD^2 (Aroma intensity)[3] 0h	Log_3FD^2 (Aroma intensity)[3] 48h	Retention Index DB-5	Retention Index DB-WAX	Method of identification
49	tetramethylpyrazine	NB	cooked nutty	3.5 (0.8)	3 (1.8)	1084		RI, Odor
50	unknown	NB	fatty	2 (2.0)	<1 (2.0)	1087		Odor
51	2-methoxy phenol (guiacol)	NB	smoky	4.5 (1.2)	6.5 (0.8)	1092		RI, Odor, MS
52	nonanal	NB	fatty	2.5 (1.5)	4 (1.3)	1106	1303	RI, Odor, MS
53	p-cresol	NB	phenol	2 (2.0)	1 (3.3)	1112		RI, Odor, MS
54	unknown	NB	fatty phenol	2 (1.5)	1.5 (1.8)	1120		Odor
55	3-hydroxy-4,5-dimethyl-2(5H)-furanone (sotolon)	NB	curry cooked scorched	2.5 (1.0)	2.5 (1.0)	1129	2006	RI, Odor, MS
56	2-phenethanol	NB	rosy	4.5 (2.0)	5.5 (1.3)	1153		RI, Odor, MS
57	(E,Z)-2,6-nondienal	NB	cukes	1.5 (1.8)	4 (1.3)	1162	1606	RI, Odor, MS
58	(E,Z)-2,6-nondienal / (E)-2-nonenal	NB	carpets	5.5 (1.0)	6.5 (0.5)	1169	1593	RI, Odor, MS
59	(E)-2-nonenal	NB	cukes	2 (2.0)	ND	1174	1593	RI, Odor, MS
60	2-methyl-3-methyldithiofurane	NB	cooked	3.5 (1.3)	4.5 (1.3)	1183	1529	RI, Odor
61	2-isobutyl-3-methoxy pyrazine	NB	bell pepper	<1 (3.0)	ND	1185	1490	RI, Odor, MS
62	Unknown	NB	sweet burnt curry	ND	1 (1.2)	1193		Odor
63	o-cresol	NB	bandaid	<1 (2.4)	ND	1195	2021	RI, Odor, MS
64	ethyl octanoate	NB	cukes mint hay fatty citrus	2 (1.0)	3 (0.5)	1205	1521	RI, Odor
65	phenylethyl acetate	NB	yeasty rosy	2 (1.5)	ND	1212		RI, Odor
66	(E,E)-2,4-nonadienal	NB	yeasty hay	1 (1.0)	1 (1.0)	1226		RI, Odor, MS
67	decanal	NB	fatty	2 (3.0)	3.5 (1.3)	1228	1538	RI, Odor
68	beta-cyclocitral	NB	licorice	1 (2.0)	3 (0.5)	1254	1593	RI, Odor, MS
69	unknown	NB	cukes	2.5 (0.8)	3 (1.5)	1262		Odor
70	gamma-octalactone	NB	sweet cooked soapy	ND	2.5 (1.0)	1265	1834	RI, Odor, MS
71	unknown	NB	rubbery sweet	ND	2 (1.0)	1276		Odor
72	delta-octalactone	NB	cilantro coconut	2 (0.5)	1 (2.0)	1278	1961	RI, Odor

Table 3.11. continued

No.	Compound	Fraction	Odor[1]	Log_3FD^2 (Aroma intensity)[3] 0h	Log_3FD^2 (Aroma intensity)[3] 48h	Retention Index DB-5	Retention Index DB-WAX	Method of identification
73	unknown	NB	oatmeal	3 (1.5)	3.5 (1.0)	1289	2245	Odor
74	unknown	NB	cilantro waxy soapy	3 (2.0)	1.5 (2.5)	1310		Odor
75	decanoic acid	AC	stale	2 (1.0)	2.5 (2.0)	1321		RI, Odor
76	unknown	NB	mint grapy stale	2 (2.0)	<1 (3.0)	1327		Odor
77	o-aminoacetophenone	NB	grape stale	1.5 (2.3)	2 (2.3)	1332		RI, Odor
78	unknown	NB	tortilla	5.5 (0.8)	4 (1.0)	1339		Odor
79	(E,E)-2,4-decadienal	NB	tortilla stale mothball	4 (1.5)	5 (1.5)	1347	1733	RI, Odor
80	(E)-2-undecenal	NB	latex	3 (1.0)	<1 (2.5)	1357		RI, Odor
81	unknown	NB	mothball	ND	2 (2.0)	1362		Odor
82	4-methyl octanoic acid	NB	stale oatmeal fatty	4.5 (0.8)	5.5 (0.8)	1375	212	RI, Odor
83	gamma-nonalactone	NB	cilantro	1 (1.5)	1.5 (2.5)	1381	2021	RI, Odor
84	(E)-4,5-epoxy-2(E)-decenal	NB	metallic grainy	4.5 (1.5)	2.5 (1.0)	1397		RI, Odor
85	5-ethyl-(3H)-furan-2-one	NB	oatmeal	ND	3 (1.5)	1416	1699	RI, Odor
86	4-ethyl-octanoic acid	NB	waxy soapy	<1 (2.3)	2.5 (1.0)	1427		RI, Odor
87	skatole	NB	mothball	4 (1.0)	2 (2.3)	1437		RI, Odor, MS
88	2-dodecenal	NB	cooked sweet fatty	4 (0.5)	2 (1.0)	1476	1690	RI, Odor
89	gamma-decalactone	NB	peach lingers	4.5 (0.8)	1.5 (2.9)	1505		RI, Odor
90	delta-decalactone	NB	coconut lingers	2 (1.0)	2 (1.5)	1543	2124	RI, Odor, MS
91	6-(Z)-dodecen-gamma-lactone	NB	soap lingers	3 (1.0)	3 (1.5)	1603	1961	RI, Odor
92	gamma-dodecalactone	NB	peach	3 (1.1)	3 (1.0)	1710	2386	RI, Odor, MS
93	delta-dodecalactone	NB	peach lingers	2 (0.8)	2 (1.8)	1756		RI, Odor, MS

[1] Odor description at the gas chromatograph (BC) sniffing port

[2] Flavor dilution factors were determined on a DB-5MS column for neutral/basic (N/B) compounds

[3] Aroma intensity at the GC sniffing port, on a scale from 1-5