ABSTRACT

PARK, CURTIS WAYNE. The Effect of Acidification, Feed Solids Concentration, and Inlet Temperature on the Flavor of Spray Dried Whey Protein Concentrate. (Under the direction of Dr. MaryAnne Drake).

Flavor is the most important characteristic in determining consumer liking of whey protein ingredient applications. To produce whey protein ingredients, extensive processing must be done which negatively affects the flavor of the resulting powder. The effects of unit operations such as bleaching, starter culture and annatto addition, fat separation, and storage on flavor have been investigated but spray drying parameters have not yet been investigated. Studying the effects of the spray drying parameters of pH, feed solids concentration, and inlet temperature provides further assistance for whey protein manufacturers to be able to minimize off-flavors in whey proteins.

The objective of the first study was to determine the effect of acidification of liquid retentate prior to spray drying on the flavor of whey protein concentrate (WPC). Flavor effects were evaluated with both descriptive sensory analysis and volatile compound analysis using WPC retentate acidified to three different pH values: control (pH 6.5), 5.5, and 3.5. Acidification to pH 3.5 decreased aroma impact and cardboard and sweet aromatic flavors along with concentrations of pentanal, hexanal, heptanal, E-2-heptanal, octanal, E-2-octenal, nonanal, decanal, dimethyl disulfide and dimethyl trisulfide compared to the control and pH 5.5 treatments ($p<0.05$). Acidification to pH 5.5 increased cabbage flavor and DMTS concentration ($p<0.05$). The mechanism for the observed flavor differences was investigated by producing WPC retentate, addition of 150 ppb D_{12}-hexanal, acidification to pH 6.5, 5.5 or 3.5, and spray drying. Recovery of D_{12}-hexanal increased with decreased pH in liquid

retentate and decreased with decreased pH in the rehydrated powder ($p<0.05$). These results demonstrate that acidification prior to spray drying decreases off-flavors in the resulting powder and suggest that the effect is due to decreased interactions between volatile compounds and proteins in liquid retentate and increased interactions in the rehydrated powder.

The objective of the second study was to determine the effect of feed solids concentration and inlet temperature on the flavor of spray dried WPC. WPC was manufactured and spray dried at various solids concentrations (10%, 18%, and 25%) and inlet temperatures (180°C, 200°C, and 220°C). Flavor was analyzed by descriptive sensory and volatile compound analysis. In addition, particle size and surface free fat were also measured. A decrease in feed solids concentration resulted in increased surface free fat, intensities of overall aroma, cardboard and cabbage flavors, and concentrations of pentanal, hexanal, heptanal, decanal, (E)2-decenal, DMTS, DMDS, and 2,4-decadienal ($p<0.05$). A decrease in inlet temperature also resulted in increased surface free fat and cardboard flavor and increased concentrations of pentanal, (Z)4-heptenal, nonanal, decanal, 2,4-nonadienal, 2,4-decadienal, and 2- and 3-methyl butanal ($p<0.05$). Particle size was increased with increasing feed solids concentration ($p<0.05$). These results demonstrate that spray drying WPC at lower temperatures and feed solids concentrations, within the parameters tested, result in increased off-flavors.

The Effect of Acidification, Feed Solids Concentration, and Inlet Temperature on the Flavor of Spray Dried Whey Protein Concentrate

by
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DEDICATION

To Chelsea, you make everything worth it.

BIOGRAPHY

Curtis Wayne Park was born on December 27, 1986 to Wayne and Nancy Park in Urbana-Champaign, Illinois. He has two sisters, Renae and Corinne. In his early years his family moved from Illinois to Utah to Florida and when he was 7 years old they moved to Kennewick, Washington where he lived until graduation from high school. Next, he studied for one semester at Brigham Young University. The following two years were spent serving as a full-time missionary for the Church of Jesus Christ of Latter-Day Saints in the Tuxlta-Gutierrez, Mexico mission. Upon completion of his two year mission he returned to BYU to continue his studies. During his time at BYU he enjoyed being involved in the marching band, pep band, and Synthesis, a jazz band. While at BYU he met the love of his life, Chelsea, and was married in August 2009. During his sophomore year at BYU Curtis found the food science major and decided to take an introductory course. In the spring of 2011 he graduated with a B.S. in Food Science. During his time in the Food Science Department at BYU Curtis worked as a research assistant for Dr. Michael Dunn, a teaching assistant for two different food science classes, and a sensory lab assistant. After graduation, Curtis came to North Carolina State University to study under Dr. MaryAnne Drake with many opportunities to learn and grow as a scientist. He now plans to continue and earn a PhD in Food Science with the post-graduation goal to work in research and development in the food industry.

ACKNOWLEDGEMENTS

I would first like to thank my Mom and Dad for providing me with a home where I could grow up knowing who I am and what I can become. I am also grateful to have had parents who knew the value of an education and were always supportive in everything I wanted to do. I hope that in some way I can repay the kindness and patience you have shown me all these years. I also want to thank my sisters for always being there for me. Who knew that having sisters could be so great?

I want to thank those who have helped me from the NCSU Dairy Processing Plant, I definitely could not have done it without you. I want to thank Gary Cartwright for all your help designing and troubleshooting our processing equipment. I also need to thank Jack Canady for helping me fix everything I broke in the pilot plant.

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To Dr. MaryAnne Drake for giving me this wonderful opportunity and having trust in me to accomplish things I didn't realize I could do. I appreciate the sacrifices you have made for me to be able to earn my degree and better my education.

Lastly, I want to thank my wife, Chelsea, for all of the patience that you have shown me. I love you with all my heart and I am so blessed to have you in my life. You make me want to be a better person every day so that someday I can deserve you.

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Chapter 1: Literature Review

Whey Introduction

Whey was discovered about 3000 years ago when travelers would transport milk in calves' stomachs (Smithers, 2008). It is the liquid that is separated from the cheese curd during the cheese make process (USDA, 2010; Gallardo-Escamilla et al., 2005). Until recently whey was seen as a waste product with little value. It used to be discharged into fields, rivers, lakes, the ocean, sewage systems, or sold as animal feed (Smithers, 2008). None of these methods were economically profitable and as environmental regulations concerning the disposal of whey increased, it was important to find new uses for this byproduct of cheese making. More uses of whey were also necessary because of increased cheese production in the United States. In 2010, 10.4 billion pounds of cheese was made in the United States, a 31% increase from 1999 (USDA, 2010). The increase of cheese production has been accompanied by an increase in whey production since it is a byproduct of cheese making. Within the last 25 years the demand for whey ingredients has steadily increased in the world market as well as the United States (Tunick, 2008).

One major reason why demand for whey has been increased is due to its nutritional properties. Dried whey ingredients can be used to fortify foods with protein to increase the overall nutritional value of the foods (Tunick, 2008). Whey proteins are also used for the functional properties they exhibit. Whey proteins solubilize readily to stabilize emulsions (Burrington, 2005), foams (Renner and Abd El-Salam, 1991), and provide gelation and thermal stability to food products (Foegeding et al., 2002).

Fluid whey is characterized as sweet (pH 6 to 6.3) or acid (pH 4.3 to 4.6) (Morr and Ha, 1993). Sweet whey is derived from rennet coagulation and acid whey is derived from direct acidification. Their compositions can be seen in Table 1.1 below.

Table 1.1 Composition of fluid and acid wheys by percentages (Kosikowski and Mistry, 1997)

Component	Fluid Sweet Whey	Fluid Acid Whey	Dried Sweet Whey	Dried Acid Whey
Total Solids	6.35	6.50	96.5	96.0
Moisture	93.7	93.5	3.5	4.0
Fat	0.5	0.04	0.8	0.6
Total Protein	0.8	0.75	13.1	12.5
Lactose	4.85	4.90	75.0	67.4
Ash	0.5	0.8	7.30	11.8
Lactic Acid	0.05	0.4	0.2	4.2

The protein fraction of whey is made up of β-lactoglobulin, α-lactalbumin, serum albumin, immunoglobulin and protease peptone (Jayaprakasha and Brueckner, 1999). β-lactoglobulin and α-lactalbumin are the major whey proteins making up about 80% of the total protein (Schmidt et al., 1984). Since liquid whey is generally not used as an ingredient itself, it must be further processed. The liquid whey can be dried into a powder or the lactose and minerals can be removed to concentrate the protein content. The concentration of whey proteins increases the value of the product (Onwulata, 2008). With the development of new membrane separation techniques this can now be achieved. The two main concentrated whey

protein products are whey protein concentrate (WPC) and whey protein isolate(WPI). WPC can have a protein content from 34—89% and WPI >90% (USDA, 2010).

Whey Proteins vs. Serum Proteins

After the cheese curd has been formed, the protein in the whey is referred to as whey protein (Varnum and Sutherland, 1994). These proteins are not acid sensitive like casein. When these proteins are in fluid milk they are called serum proteins. If proteins have been removed directly from milk prior to cheese making they are referred to as milk serum proteins because they are not actually in the whey after making cheese (Varnum and Sutherland, 1994). Milk serum proteins play a critical role in ice cream manufacture (Arbukle, 1987). Milk serum proteins can replace up to 25% of the milk solids not fat in ice cream formulation (Arbukle, 1987). Milk serum proteins can provide many useful functional properties such as whipping, body and texture, thermal stability, and storage and melting properties just like whey proteins which are simply these proteins following cheese manufacture. It has been shown that cheese can be made from milk that has had the milk serum proteins removed (Nelson and Barbano, 2005a, 2005b).

Serum proteins can be separated from milk by direct membrane fractionation of fluid milk. Because milk serum proteins are not exposed to the cheese-manufacture process they are free of any decreased functionality or sensory characteristics caused by cheese manufacture and/or whey processing. Evans et al. (2009) reported few sensory differences between serum protein concentrate (SPC) 34% and WPC 34% although flavor intensities of SPC were lower than WPC. When rehydrated to 10% solids SPC34 was clear and WPC34

was cloudy, due to the higher fat content of WPC34. SPC had a lower fat content due to the pore size of the microfiltration membranes used in the initial separation steps to remove serum proteins from skim milk. Increased clarity and lower fat content was also demonstrated with pilot scale manufacture of SPC80 and WPC80 with similar results (Evans et al., 2010). Pilot scale SPC80 had lower cardboard flavors and lower relative abundances of lipid oxidation products than corresponding WPC80. When consumer testing was conducted using a peach-flavored protein beverage fortified with 6% SPC80 or WPC80, consumer liking scores for beverages made with SPC80 were higher or at parity with WPC80 in aroma, appearance, and mouthfeel (Evans et al., 2010). The beverages made with SPC80 had lower consumer liking scores for flavor and overall liking than beverages with commercial WPC80. This was due to cereal and soapy flavors and bitter taste that were present in the SPC80 but not the commercial WPC80. Differences in functionality between native whey proteins (24.1 to 37.3%) and commercial WPC34 have also been observed (Heino et al., 2007). The gel strength, foaming, and emulsification properties of the native whey proteins were higher than WPC. The gel strength was increased in the native whey proteins compared to the WPC because of the absence of glycomacropeptide (Heino et al., 2007). Foam overrun and stability were higher with the native whey proteins due to the significant reduction in fat (Heino et al., 2007).

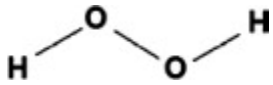
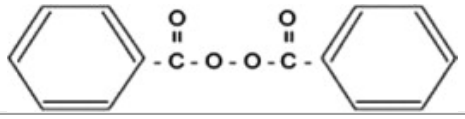
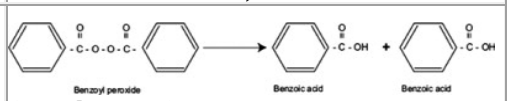
Whey Processing

To obtain whey, cheese must be made. In the cheesemake process the casein micelles are coagulated into a curd either by acid or by an enzymatic process using chymosin or the

industrial enzyme rennet. At this stage the curd expels water along with protein, minerals and lactose. This liquid, whey, is separated from the curd by a draining and filtration process. Once the whey is drained from the cheese curd it must be heat treated as soon as possible or quickly cooled to 5°C to prevent bacterial growth (Anonymous, 2003). This whey is about 93% water and 0.6% protein (Huffman, 2006). Further processing is needed in order to produce whey protein concentrates or isolates. The first step is clarification using centrifugal motion. This is done in order to remove the cheese fines that may be present. After cheese fines have been removed the whey fat must be removed as well using centrifugal motion. Pasteurized whey fat is often used to standardize the fat content in the cheese milk during subsequent cheesemaking. Next, the whey is pasteurized in order to inactivate the starter culture present from cheese making.

An extra processing step must be added to whey that has been produced from Cheddar whey. Because of the desire to have orange Cheddar cheese, in the United States Cheddar cheese milk is colored with the plant pigment annatto. This color mainly stays in the Cheddar curd but it is estimated that 10% of the annatto color is transferred to the liquid whey (Smith, 2012). Because this color is undesirable in products using whey as an ingredient, the liquid whey is bleached (McDonough et al., 1968). The two chemical bleaching agents approved for use in the United States are hydrogen peroxide and benzoyl peroxide. The pros and cons of using either chemical are listed in the table below.

Table 1.2 Summary of hydrogen peroxide and benzoyl peroxide characteristics (Kang et al., 2010)

Item	**Bleaching agent**	
	Hydrogen peroxide	**Benzoyl peroxide**
Structure		
	(National Center for Biotechnology Information, 2008b)	(National Center for Biotechnology Information, 2008a)
Breakdown	$2H_2O_2 \rightarrow O_2 + 2H_2O$ (hydrogen peroxide → oxygen + water) (Smith, 2004)	 (Smith, 2004)
Regulation	21CFR184.1366 (US FDA, 2009a)	21CFR184.1157 (US FDA, 2009b)
Pros	• little to no effect on the nutrients present • more acceptable for usage in other countries	• effective at lower usage levels than hydrogen peroxide • does not require a catalase addition to remove residues • does not pit stainless steel; therefore, is less corrosive to equipment • effective across a wide range of temperatures
Cons	• must be inactivated with catalase • could possibly cause oxidized flavors • corrosive to equipment • less economical to use because it requires much more peroxide for satisfactory bleaching	• possible formation of oxidized flavors • possibility that the carrier used may be considered an allergen • concerns from other countries because it has just been recently approved by Codex

Once the whey has been clarified, fat separated, pasteurized, and bleached the next step in production of whey protein concentrate (WPC) or whey protein isolate (WPI) is to undergo membrane filtration. The most common techniques used for the membrane filtration of dairy products are microfiltration (MF), ultrafiltration (UF), nanofiltration, and reverse osmosis (RO). Membrane filtration concentrates or fractionates one liquid into two liquids of differing composition. This is done through the selective permeability of different constituents in the liquid based on their size (Rosenberg, 1995). In the dairy industry microfiltration is used in protein fractionation, separation of whey fat, removal of bacteria, and fractionation of native milk fat globules (Marcello and Rivisi, 2008; Onwulata, 2008; Fauquant et al., 2005; Michalski et al., 2006).

Reverse osmosis (RO) uses a semi permeable membrane that allows water to pass through but not salt. Because of this, the osmotic pressure on one side of the membrane increases greatly and if pressure is applied to that side pure water can be achieved (Rosenberg, 1995). Nanofiltration is an RO process but differs in that it allows small monovalent ions to pass through the membrane. The fact that this process removes water and minerals makes this process very useful in demineralizing whey, milk, and ultrafiltration of permeate of both milk and whey (Anonymous, 2000). Ultrafiltration (UF) separates macromolecules such as proteins and suspended particles. This is done based on their shapes, sizes, charges, and affinity to the membrane (Bastian et al., 1991). This process is used to concentrate whey proteins while removing smaller molecules such as sugar and salts. Figure 1.1 represents the different membrane separation processes.

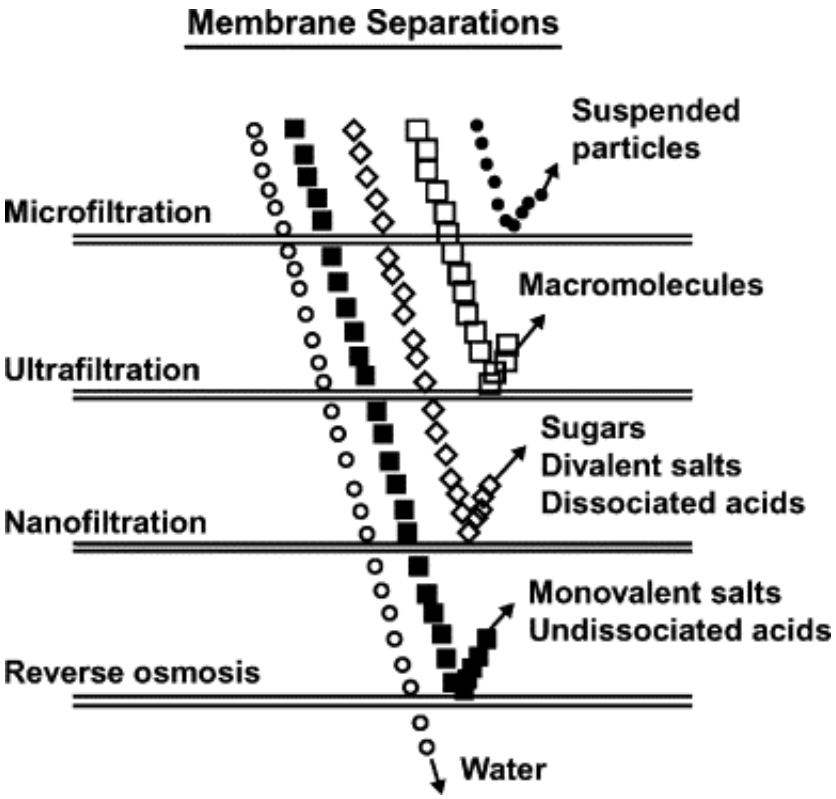


Figure 1.1Membrane separation processes (Henning et al., 2006).

In order to produce whey protein concentrates ultrafiltration is used. UF alone can concentrate the protein in whey to about 50% of the solids. In the case of whey protein concentrates greater than 50% protein, diafiltration (DF) must be used (Kelly, 2003; Onwulata, 2008). DF is a step where water is added back to the ultrafiltered product followed by more ultrafiltration (Johnson and Lucey, 2006; Huffman, 1996; Onwulata, 2008). This step allows for further removal of lactose and minerals, concentrating the whey proteins further. More processing is needed to produce WPI. Because the fat concentrates

during ultrafiltration, fat separation is needed to continue to concentrate the protein. MF along with diafiltration is used to remove the fat from the UF retentate. This will allow for further concentration of the whey proteins > 90%.

Another process that can be used to produce WPI is ion exchange. Ion exchange is used as a pretreatment for the whey before it undergoes ultrafiltration. Ion exchange separates compounds based on their ionic charge rather than their molecular size (Varnum and Sutherland, 1994; Onwulata, 2008). The whey is first acidified to a pH below the isoelectric point of the proteins so that they have a positive charge. The whey is then pumped in a tank with negatively charged resin beads so that the protein is retained and the fat, minerals, and lactose are all removed (Anonymous, 2003; Huffman 1996). The protein is then removed into a different stream by running an alkali solution through the resin which neutralizes the positive charges on the proteins, releasing them from the resin (Anonymous, 2003; Huffman 1996). This whey protein stream can then be concentrated and dried in order to produce WPI. To produce the final dried product, WPC or WPI retentate is spray dried. During spray drying the liquid is atomized and mixed with hot air to evaporate the moisture and the result is a dry powder (Henning et al., 2006).

Lipid Oxidation

Generally, lipids do not contribute significantly to the aroma of foods due to their low volatility, but the decomposition of such can produce off-aromas and off-flavors (McClements and Decker, 2008). The two types of decomposition of lipids are hydrolytic rancidity and autoxidation. Hydrolytic rancidity refers to the liberation of free fatty acids

from the glycerol backbone (McClements and Decker, 2008). The term autoxidation refers to a complex sequence of chemical changes that come from the interaction of lipids with oxygen (Frankel, 1998; McClements and Decker, 2008). Lipid oxidation causes the fatty acids esterified on triacylglycerols and phospholipids to decompose and form volatile compounds. The oxidation of lipids is among the most common causes of deterioration in food. These oxidation reactions can occur during food processing and storage, forming off-flavors which decrease the acceptability and nutritional quality.

Free radicals are the reactive species that cause lipid autoxidation. Free radicals are molecules with unpaired elections, making them very reactive (Frankel, 1998; McClements and Decker, 2008). They oxidize by causing hydrogen abstraction. The free radicals formed on unsaturated lipids form hydroperoxides and other oxygenated species. The rate of autoxidation is determined by temperature, oxygen, degree of fatty acid composition, and the activity of pro and anti-oxidants. Autoxidation can be broken down into 3 steps: initiation, propagation, and termination (Frankel, 1998; McClements and Decker, 2008). In the initiation step an alkyl radical is formed and is stabilized by the delocalization of the radical over the double bonds (McClements and Decker, 2008). These fatty acid radicals are more easily formed with increasing unsaturation. The double bonds in unsaturated fatty acids require less energy for hydrogen abstraction than do saturated fatty acids. This explains the fact that unsaturated fats oxidize at room temperature whereas saturated fats do not (Frankel, 1998; McClements and Decker, 2008). During propagation, oxygen covalently bonds to the fatty acid at the site of the radical because oxygen has 2 radicals. As a result, 1 radical is still left on the oxygen species, resulting in a peroxyl radical (LOO·) (McClements and Decker,

2008). This peroxyl radical can further abstract a hydrogen atom from another molecule forming the hydroperoxide (LOOH), propagating the radical to another molecule. In the final step, termination, two radical species react with each other to form a non-reactive molecule.

Lipid Oxidation of Fat in Whey Products

In whey products, the hydroperoxides formed are mainly from derivatives of oleic, linoleic, and linolenic acids. These are among the most common unsaturated fatty acids to undergo lipid oxidation (Frankel, 1998). The oleic, linoleic, and linolenic acid content (μg/g of liquid whey) in fresh liquid whey is 0.23, 3.33, and 0.86 respectively (Tomaino et al., 2004). In Cheddar whey powder the percentage of oleic, linoleic, and linolenic acids of the total fatty acids present is 32.5%, 2.5%, and 0.6% respectively. The hydroperoxides formed are unstable and will break down to form secondary lipid oxidation products. The hydroperoxides are odorless and flavorless but the secondary lipid oxidation products are not (Frankel, 1998; McClements and Decker, 2008). These secondary lipid oxidation products include acids, alcohols, aldehydes, carbonyls, and ketones and are not normally present in fresh products (Tomaino et al., 2004). In liquid whey a decrease in the free fatty acid linoleic acid was correlated to an increase in volatile lipid oxidation products (Tomaino et al., 2004). In general, lipid oxidation is slowest at high pH and fastest at low pH due to the solubilization of metal catalysts (Frankel, 1998).

The opposite is true in protein emulsions. Decreasing pH can retard lipid oxidation by various mechanisms: metal neutralization by electrostatic repulsion between a positively

charged interface and negatively charged metals, metal binding, physical separation by a barrier, or by chemical reduction of hydroperoxides with sulfhydryl-containing amino acids (Frankel, 1998). It has been shown that the positively charged emulsion droplets in low pH whey protein emulsions slow lipid oxidation by repelling cationic iron (Hu et al., 2003). The relative oxidative stabilities against lipid oxidation at pH 3 in a 0.2% protein oil in water emulsion were in the order of β-lactoglobulin ≥ sweet whey > α-lactalbumin ≥ WPI (Hu et al., 2003).

The compounds responsible for off-flavors in liquid and dried whey products have been shown to be aldehydes (Liaw et al., 2011; Campbell et al., 2011a; Campbell et al., 2011b; Evans et al., 2009; Evans et al., 2010; Whitson et al., 2010; Whitson et al., 2011). Tomaino et al. (2004) observed lipid oxidation products in fresh fluid whey and increases in aldehyde concentrations concurrent with increases in off-flavors during refrigerated storage. Lipid oxidation compounds were also observed in freshly manufactured proteins (Carunchia Whetstine et al., 2005; Whitson et al., 2011). Longer refrigerated storage of liquid WPC retentate correlated to an increase in lipid oxidation compounds (Whitson et al., 2011). Liaw et al. (2011) added antioxidants to liquid whey from both Mozzarella and Cheddar cheese manufacture to determine their effects on the oxidative stability of liquid whey and spray dried WPC. Lipid oxidation compounds were more prevalent in liquid Cheddar whey than in liquid Mozzarella whey. Reduction of fat from liquid whey decreased but did not eliminate lipid oxidation in liquid whey. In both liquid whey and subsequent WPC, addition of whey protein hydrolysate (WPH) or ascorbic acid lower concentrations of the lipid oxidation products hexanal, pentanal, heptanal, octanal, and nonanal were observed compared to

untreated controls. In WPC with added WPH or ascorbic acid lower concentrations of pentanal, heptanal, and nonanal were observed compared to untreated controls. The starter culture used in cheese-manufacture impacts the oxidative stability of both the liquid whey and the resulting WPC (Campbell et al., 2011a; Campbell et al., 2011b).

Phospholipids

Phospholipids are amphiphilic molecules consisting of one or two hydrophobic acyl chains and a hydrophilic head (Rombaut et al., 2006). Phospholipids contain two fatty acids esterified on the glycerol backbone at the sn-1 and sn-2 positions with phosphoric acid on the sn-3 position through a phosphate ester bond (Rombaut and Dewettinck, 2006). In addition to this structure, an organic compound such as choline, ethanolamine, or serine is usually attached to the phosphate group (Rombaut et al., 2006). The structures of common phospholipids in the milk fat globule membrane (MFGM) are shown in Figure 1.2 below.

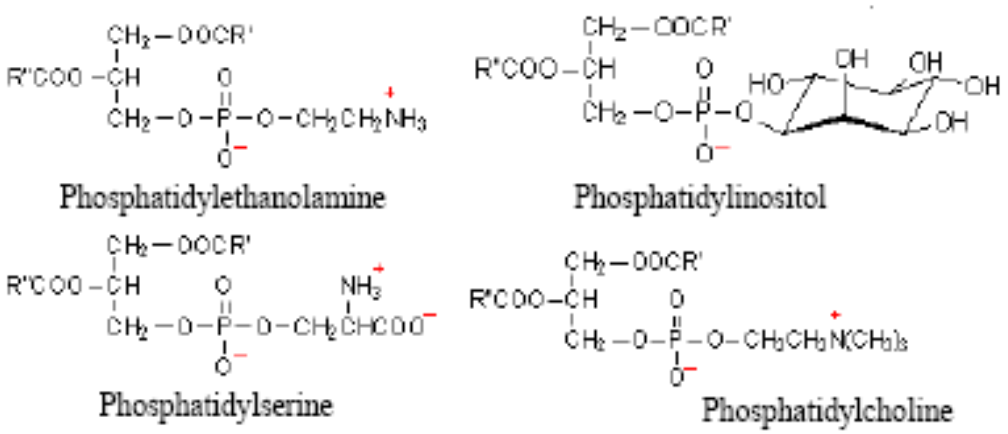


Figure 1.2 Common phospholipids found in the MFGM (Christie, 2012).

Phospholipids account for about 1% of the total bovine milk lipids and about 60% of these come from the MFGM (Gallier et al., 2010). In commercial whey powder phospholipids accounted for 31.4% ± 10.3 of the total lipids (Boyd et al., 1999). Fat rich dairy products have increased amounts of phospholipids due to their high MFGM content (Rombaut et al., 2006). During processing steps (heating, agitation, homogenization and, aeration) the MFGM is ruptured and the phospholipids enter into the aqueous phase (Rombaut and Dewettink, 2006). Because of this phospholipids can be found in products that are not fat rich and in many cases the levels of phospholipids are higher in products with little fat. The polar lipid content (g polar lipid/100 g total lipid) as reported by Rombaut and Dewettink (2006) were 0.35-0.86 for cream, 0.20-0.27 in butter, 19.06 for skim milk, 21.66-33.05 in buttermilk, 0.47 in Cheddar cheese, and 5.32 in Cheddar whey.

Phospholipids have been reported to have pro-oxidant as well as anti-oxidant activity which impacts the flavor of foods (Szuhaj and Sipos, 1989). Phospholipids can negatively affect the flavor of dairy products through oxidation of lipids (Sessa, 1985). For a significant level of auto-oxidation to occur, fatty acids of three or four double bonds must be present in close proximity (O'Connor et al. 1981). Due to the close proximity of polyunsaturated fatty acids to each other on the MFGM and the contact with the aqueous phase there is great potential for oxidation during the high heat treatment of conventional spray drying (Christie et al., 1984). The phospholipid fraction of lipids in milk oxidize more readily than the glyceride fraction (Sattar and deMan, 1975). The whey proteins β-lactoglobulin and α-

lactalbumin have been observed to associate with the MFGM via disulfide bonding with MFGM proteins (Ye et al., 2004). The MFGM material consists of about 65% protein, 7% triglyceride, and 30% phospholipid and accounts for about 22% of the total lipids in WPC (Houlihan and Goddard, 1991). The proteins associated with the MFGM range in molecular weight from about 44 to greater than 155 Kd (Morr and Ha, 1993). Because of the small pore size of UF membranes used to produce WPC the lipids are concentrated as well as the proteins during manufacture. Morr and Foegeding (1990) reported that WPC manufactured by UF membrane processing contained 3.3-7.4% total lipids and 0.8-1.5% phospholipids. The concentrated phospholipids associated with the MFGM increase the potential for lipid oxidation to occur in dried whey ingredients.

Other Sources of Lipid Oxidation

Lipid oxidation can also occur due to the starter culture strain. Cheese producers commonly rotate their starter cultures in an effort to avoid bacteriophage attack on the culture used in cheesemaking. The rotation of starter cultures is a source of flavor variability and lipid oxidation in liquid whey (Carunchia Whetstine et al., 2003; Tomaino et al., 2004). Differences in lipid oxidation have been observed between Cheddar and Mozzarella starter cultures (Campbell et al., 2011a). The different cultures used in producing different types of cheese have also been shown to have an effect on lipid oxidation of liquid whey and WPC80 (Campbell et al. 2011b).

An increase in storage time of whey prior to processing accelerates lipid oxidation (Campbell et al., 2011a; Liaw et al., 2009; Whitson et al., 2011). The decolorization of whey

using bleaching agents also increases lipid oxidation in WPC (Croissant et al., 2009; Listiyani et al., 2011; Campbell et al., 2012, Kang et al., 2012). The compounds that play a role in storage conditions are lipid oxidation products such as ketones, aldehydes, and free fatty acids, along with protein degradation and Maillard reaction products (Javidipour et al., 2008; Wright et al., 2009).

Maillard Reaction

The Maillard reaction is one of the predominant chemical interactions of food components during processing and storage (Sikorski et al., 2008). Often the Maillard reaction is called nonenzymatic browning because the end products are brown colored pigments. Apart from brown pigments, the Maillard reaction also produces flavor compounds. The reaction requires a reducing sugar and an amine containing compound such as a protein, peptide, or amino acid. In dairy products, lactose is generally an important reducing sugar that participates in the Maillard reaction (Sikorski et al., 2008). Aldehydes and other carbonyls can participate in the Maillard reaction and are predominantly produced from oxidized lipids (Sikorski et al., 2008). The reaction proceeds in three main stages:

1. Reaction of an amine with a reducing sugar to form a glycosyl amine which is then followed by Amadori rearrangement
2. Dehydration of intermediate products, fragmentation of the saccharidic moiety, and Strecker degradation of the products
3. Reactions of intermediate products to produce heterocyclic flavor compounds (Sikorski et al., 2008)

The formation of flavor compounds during the Maillard reaction depends on the type of sugars and amino acids involved, reaction temperature, pH, and water content (Jousse et al., 2002). In general the sugars and amino acids involved determine what flavor compounds are formed and the reaction conditions determine the kinetics (von Boekel, 2006). Increased pH levels increase the reaction rate (Morr, 1991). The figure below shows the different classes of flavor compounds formed from the Maillard reaction.

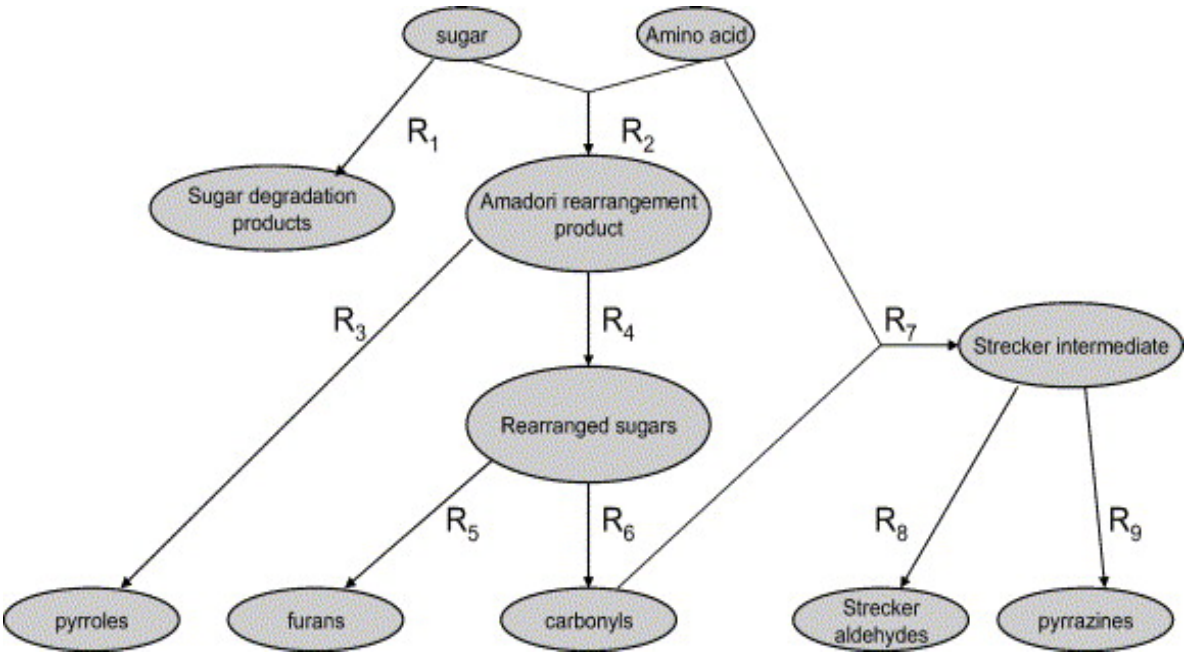


Figure 1.3 Kinetic model of the predominant flavor compounds formed from the Maillard reaction (von Boekel, 2006).

Strecker degradation involves the oxidative degradation of amino acids by dicarbonyl intermediates of the Maillard reaction. This forms unstable Schiff bases that decarboxylate to form enamines. The enamines then undergo hydrolysis to form an aldehyde from the amino acid (Fox, 1992). The Strecker aldehydes play a role in off-flavor formation in dairy products (Fox, 2002).

For the Maillard reaction to be stopped the moisture content must fall below 3%, a value that is not reached in dried dairy ingredients (Sienkiewicz et al., 1990). The Maillard reactions occur faster with increasing temperatures but can occur at refrigerated temperatures as well. Maillard reactions and lipid oxidation reactions are closely related to the flavor of dried whey ingredients (Carunchia Whetstine et al., 2005). The carbonyl compounds that are formed during lipid oxidation are also intermediates of the Maillard reactions (Voziyan et al., 2002). Lipid oxidation products such as methyl ketones, aldehydes, and free fatty acids can also react with the Maillard reaction products to further deteriorate the food quality (Newton et al., 2012). A higher degree of browning and volatile formation was found in model systems containing lipid oxidation products compared to a model system without lipid oxidation products (Adams et al., 2011). Maillard reactions are common in dried whey ingredients due to their high lactose and protein contents (Mahajan et al., 2004; Carunchia Whetstine et al., 2005). In whole milk powder Maillard reactions, lactose crystallization, and radical generation were shown to be closely associated (Thomsen et al., 2005). These radicals have been associated previously with lipid oxidation (Stapelfeldt et al., 1997). Tryptophan was shown to be degraded in β-lactoglobulin due to Maillard reactions (Moreaux and Birlouez-Aragon, 1997). Protein degradation is also associated with off-flavors in dried

whey ingredients (Carunchia Whetstine et al., 2005). As β-lactoglobulin is the major protein in whey, such degradation caused by Maillard reactions can contribute to off-flavors in dried whey ingredients.

Liquid Whey Flavor

Since whey protein products are added as food ingredients for their functionality and nutrition, it is important that they do not contribute flavor to the final product. The most important factor in consumer acceptance of products is flavor (Morr, 1991; Lee and Morr, 1994; Drake, 2006; Childs et al., 2007). Even though dried whey ingredients are defined as having a bland and delicate flavor, the truth is that there are flavors present (Majahan et al., 2004; Carunchia Whetstine et al., 2003; Liaw et al., 2011). These flavors also carry over to the finished product which limit the application of dried whey ingredients in foods (Drake, 2006).

Complex mixtures of volatile compounds in dairy products have been identified dating back to the 1950s thanks to the development of gas chromatography (McGorrin, 2007). The volatile compounds in fluid whey can be placed into one of two different categories: aroma active compounds or aliphatic hydrocarbons (Carunchia Whetstine et al., 2003). Aroma active compounds include alcohols, ketones, and aldehydes. These are compounds that exhibit a perceived aroma (Carunchia Whetstine et al., 2003). These compounds are of interest when analyzing whey flavor because of their aroma activity which means that they exhibit specific aromas. Aliphatic hydrocarbons, however, do not exhibit a perceived aroma at concentrations found in food and thus are not as important when dealing

with flavor (Carunchia Whetstine et al., 2003). Karagul-Yuceer et al. (2003a) identified the aroma active compounds in liquid Cheddar whey. These compounds included: 2,3-butanedione (buttery), hexanal (green), 2-acetyl-1-pyrroline (popcorn), methional (potato), (E,E)-2,4-decadienal (frying oil), and (E,E)-2,4-nonadienal (frying oil) (Karagul-Yuceer et al., 2003a). Most notably, three of these compounds are lipid oxidation compounds.

Flavors in liquid whey can be grouped into one of the following groups: dairy or non-dairy flavors (Carunchia Whetstine et al., 2005; Drake, 2006). Dairy flavors are those that are associated with fresh fluid milk or whey such as sweet aromatic and cooked/milky (Carunchia Whetstine et al., 2005). Non-dairy flavors are those that are not generally associated with fresh fluid milk or whey and include cardboard, animal/wet dog, and cucumber among others (Carunchia Whetstine et al., 2005). The flavor in liquid whey has many different sources. Before whey is processed, it is often pooled together with other whey streams and stored until it is ready to be processed (Tunick, 2008). This creates problems when trying to create a whey product with a consistent bland flavor. The flavor variability can come from milk sources and the processing and handling (Carunchia Whetstine et al., 2003). There is flavor variability between and within manufacturers in both liquid and dried whey products (Drake et al., 2009; Wright et al., 2009; Carunchia-Whetstine et al., 2005).

The use of starter cultures for the production of different cheeses also affects the flavor of the corresponding liquid whey. The mineral, protein, and lactose composition as well as flavor are all affected by different starter cultures (Bordenave-Juchereau et al., 2005;

Gallardo-Escamilla et al., 2005). The type of starter culture was observed more influence on the flavor than the set type (rennet or acid) of the whey (Campbell et al., 2011a). It has been hypothesized that increased concentrations of free fatty acids caused by lipase enzymes in cheese starter cultures in whey were a major contributor to poor flavor in fluid whey (Tomaino et al., 2001; Tomaino et al., 2004).

Often in an industrial setting the whey is produced at one facility and processed in another (Tomaino et al., 2004). This increased storage time of whey has been attributed and demonstrated to be a significant contributor to the flavor of liquid whey (Campbell et al., 2011a; Morr and Ha, 1993; Tomaino et al., 2004; Liaw et al., 2011). Whey that was stored with the addition of the antioxidants ascorbic acid and whey protein hydrolysate had lower intensities of cardboard flavor (Liaw et al., 2010).

Because of the consumer demand for orange Cheddar cheese in the United States, annatto, a plant pigment, is added to Cheddar cheese milk to produce the desired color. This color carries through to the final product. Chemical bleaching is often used to remove this color but with negative consequences on the flavor. There is conflicting published research regarding the flavor and lipid oxidation load of bleached whey. One study done by Mortenson et al. (2008) found that instantizing, ion exchange, and bleaching had no effect on the flavor. The authors noted that the instrumental results had variation but that the variation could not be detected by sensory analysis. However, the products were manufactured in a combination of industrial and pilot plant scale processes. This meant that no products could be compared to each other from one facility. Because of this, the effect of bleaching on the

flavor was confounded. Since the date of the aforementioned publication there have been numerous publications demonstrating the detrimental effects of bleaching fluid whey on the flavor of WPC. Bleaching of fluid whey with hydrogen peroxide resulted in higher intensities of cardboard and fatty flavors and their corresponding volatile lipid oxidation products in WPC70 and WPC80 (Croissant et al., 2009; Jervis et al., 2012). In WPC34 manufacture, bleaching of fluid whey with benzoyl peroxide (50 and 100 mg/kg) resulted in lower intensities of cardboard flavor and volatile lipid oxidation products than bleaching with hydrogen peroxide (500 mg/kg) (Listiyani et al, 2011). Jervis et al. (2012) found similar results when WPC80 was produced.

Ultraviolet radiation, acid-activated bentonite, and ozone were investigated for potential uses as bleaching agents in liquid whey for WPC80 manufacture (Kang et al., 2012). Bleaching with bentonite resulted in WPC80 with lower concentrations of volatile lipid oxidation products and higher bleaching efficacy compared to hydrogen peroxide bleaching. Bleaching with ultraviolet radiation and ozone resulted in distict mushroom/burnt and animal flavors. Relative abundances of volatile lipid oxidation products in the ultraviolet radiation and ozone treatments increased compared to the bentonite and control treatments (Kang et al., 2012). Enzymatic bleaching of liquid whey using the lactoperoxidase system increased intensities of cardboard and cabbage flavors and relative abundances of volatile lipid oxidation compounds compared to an unbleached control (Campbell et al., 2012). However, the bleaching of liquid whey using the lactoperoxidase system was more effective than hydrogen peroxide chemical bleaching and required significantly less hydrogen peroxide. In summary, the removal of the annatto colorant in Cheddar whey through

bleaching increases off-flavor intensities and their corresponding lipid oxidation products. In order to produce a Cheddar WPC with a reduced amount of off-flavor intensity and lipid oxidation alternative methods for removing the colorant such as the use of bentonite must be developed or the use of a different colorant must be investigated.

Spray Dried Dairy Products Flavor

Spray drying is among the most common methods used in the dairy industry to produce dried products. It is used because it can convert a liquid into a powder in a single step and has the ability to monitor the temperature and particle formation process accurately (Moller and Fredsted, 2009). In the 1920s, spray drying was first applied to dairy products to make dry milk powder because refrigerators were not widespread and milk had a very short shelf life (Møller, 2009). Drying a product can greatly decrease the economic costs associated with shipping liquid products by removing the water.

Dry Whey

The two main types of reactions responsible for off-flavor formation in dried whey products are lipid oxidation and proteolysis, both of which could potentially be increased due to heat treatment by spray drying. Swaisgood (1996) stated that the main sources of off-flavors in both liquid and dried whey were volatile lipid oxidation products despite the fact that whey has a little amount of fat. Proteolytic enzymes present in the cheese milk, including chymosin, are also present in the whey and may degrade amino acids, leading to off-flavor formation (Holmes et al., 1993; Amundson, 1984). After the whey is produced from cheese making it can be pooled with other whey streams before processing (Tunick,

2008). This whey can then be further processed into whey powder or into whey protein concentrate or isolate. Sweet liquid whey has been shown to have sweet, bitter, astringent, musty, sweet aromatic, milk fat, and metallic flavors (Carunchia Whetstine et al., 2003). Sweet whey powder was characterized by cooked, oxidized, and barny flavors and salty and sour tastes (Sithole et al., 2006). It has been suggested that new flavors are not developed from the drying of powder, but that existing flavors are intensified (Sithole et al., 2006). Aroma active compounds identified in sweet whey powder included short-chain fatty acids, aldehydes, ketones, lactones, sulfur compounds, phenols, indoles, pyrazines, furans, and pyrroles (Mahajan et al., 2004).

Concentrated Protein Products

Off-flavors develop due to the extensive amount of processing that must be done in order to concentrate and dry milk and whey proteins. There is evidence that proteins bind flavors during processing, contributing to the flavor of the finished product (Stephenson and Chen, 1996). An animal/wet dog flavor has been characterized in WPI (Carunchia Whetstine et al., 2005). This flavor is likely due to protein degradation since it has been identified as a characteristic flavor in caseins and caseinates and both products are high in protein (Karagul-Yuceer et al., 2003b; Drake et al., 2003). The table below shows the references used for descriptive analysis of dried whey proteins.

Table 1.3 References for descriptive analysis of whey proteins

Term	Definition	Example/Preparation
Overall aroma intensity	The overall orthonasal aroma impact	Evaluated as the lid is removed from the cupped sample[1]
Sweet Aromatic	Sweet aroma associated with grains, such as oatmeal	Quaker oatmeal (50g soaked in 500g water), vanilla cake mix [4]
Cooked/Milky	Aroma associated with cooked milk	Heated skim milk to 85°C for 30 min [3]
Fatty	Aromatic associated with old fryer oil	(E,E)-2,4-decadienal (2 ppb in skim milk)[4]
Cardboard	Aroma associated with wet cardboard and brown paper	2 × 2 cm piece of brown paper bag boiled in water for 30 min [4]
Cucumber	Aromatic associated with freshly cut cucumbers	One small cucumber diced and placed in a covered bowl[4] or 1 ppm (E)-2-nonenal[1]
Cabbage	Aromatics associated with boiled cabbage	Cabbage leaf boiled in 500 ml water for 5 min[4]
Potato/Brothy	Aromatics associated with broth or boiled potatoes	1 ppm methional in water or boiled potatoes[1]
Animal/Wet dog	Aroma associated with wet dog hair	Dissolve 1 bag of gelatin (28 g) in 2 cups of distilled water[1]
Pasta	Aroma associated with water after pasta has been boiled in it	Boil pasta in water for 30 min[1]
Soapy	Aromatics associated with medium chain fatty acids	White unscented soap bar, 50g soaked in 500 ml water[2]; 1 ppm lauric acid or shaved bar soap[1]
Bitter	Basic taste elicited by various compounds including caffeine and quinine	0.5% caffeine solution[2]
Astringency	Chemical feeling factor characterized by a drying or puckering of the oral tissues	6 tea bags soaked in 500 ml water for 10 min[4]

[1] Carunchia Whetstine et al., 2005; [2] Russell et al., 2006; [3] Karagul-Yuceer et al., 2006; [4] Wright et al., 2009

Mozzarella fluid whey is different in flavor profile from Cheddar fluid whey (Gallardo Escamilla, et al., 2005; Liaw et al., 2011). Dried whey proteins manufactured from Mozzarella and Cheddar whey are also distinct in flavor profile (Wright et al., 2009). Carunchia Whetstine et al. (2005) reported and characterized differences between WPC80 and WPI. The attributes soapy, animal/wet dog, cucumber, and bitter were observed only in WPI and not in WPC80. In another study, however, animal/wet dog was present in WPC at a higher intensity than in skim milk powder (Drake et al., 2003). The flavor profile of spray dried WPC80 was highly variable among different suppliers (Wright et al., 2009; Evans et al., 2010; Carunchia Whestine et al., 2005).

In an effort to try to produce a product similar to WPC or WPI with reduced flavor, research has been done to investigate the sensory and volatile compound quality of SPC. Bleaching is not necessary in SPC because they are not exposed to the annatto color in the cheesemake. Evans et al. (2009, 2010) studied the sensory and volatile compound differences between WPC 34 and SPC34 as well as between WPC80 and SPC80. Spray dried WPC34 had higher concentrations of hexanal, heptanal, pentanal, and nonanal compared to SPC34. SPC80 had lower intensities of sweet aromatic and cereal flavors and aroma intensity. WPC80 had significantly higher concentrations of oxidation products except for decanal. Heptanal, pentanal, and (Z)4-heptenal were not identified in the SPC80. Protein beverages that were made with SPC80 had consumer liking scores that were higher or at parity in aroma, appearance, and mouthfeel as beverages made with WPC80.

Cardboard has been reported to be a major off-flavor in whey proteins (Carunchia Whetstine et al., 2005; Russell et al., 2006). This flavor is defined as the aroma of cardboard and can be referenced by soaking pieces of cardboard in water (Carunchia Whetstine et al., 2005, Russell et al., 2006). Cardboard flavor has been associated with volatile lipid oxidation products (Carunchia Whetstine et al. 2003, Drake et al., 2003, Wright et al., 2009). Tomaino et al. (2004) reported elevated levels of the lipid oxidation products hexanal, octanal, and heptanal in fluid whey with cardboard flavor. In the same study a decrease in linoleic acid was found, giving further proof that the cardboard flavor comes from the oxidation of lipids. Recently, Whitson et al. (2010) demonstrated that the cardboard flavor in whey protein was caused by a combination of pentanal, heptanal, and nonanal in the presence of 1-octen-3-one and dimethyl trisulfide.

Another off-flavor that has been reported in whey proteins is cabbage flavor (Carunchia Whetstine et al., 2005). Some WPI exhibit this off-flavor and others do not (Wright et al., 2006, Carunchia Whetstine et al., 2005). Wright et al. (2006) reported that the compound dimethyl trisulfide was responsible for cabbage off-flavor in WPI. WPI without cabbage off-flavor contained dimethyl trisulfide as well and this was attributed to the fact that the concentration of dimethyl trisulfide was below the orthonasal threshold in those WPI. When methionine degrades it can form dimethyl disulfide and methanethiol which in turn can be further oxidized to dimethyl disulfide and dimethyl trisulfide (Bendall, 2001).

Milk Powder

Dairy powders have developed over the years as a way to extend the shelf-life of milk through various drying techniques. Milk powders can be classified by their composition with skim, whole, and fat-filled milk powders with a fat content of 0%, 26%, and greater than 26% respectively (Vignolles, et al., 2007). The primary dried milk product manufactured in the United States is low-heat skim milk powder (SMP) (Drake et al., 2003). Whole milk powder (WMP) is also manufactured as an ingredient for many applications. The most common method of drying milk is spray drying. Spray drying involves the atomization of the milk into fine particles which are mixed with hot air to remove almost all the water content. Both SMP and WMP are produced by spray drying liquid skim milk and whole milk respectively. Common flavors found in whole milk powders include cooked/sulfurous, milk fat, sweet aromatic, and grassy/hay (Lloyd et al., 2009a). The packaging atmosphere, storage temperature, and storage time all impact the flavor of whole milk powder (Lloyd et al., 2009b). The flavor variability of milk powders can affect the consumer acceptance of ingredient applications (Caudle et al., 2005).

Skim Milk Powder

Skim milk powder (SMP) is used as both an ingredient in food applications as well as for direct consumption. The flavor of SMP is highly variable between suppliers (Drake et al., 2003; Caudle et al., 2005). The flavor of skim milk powder is important because it can carry through into ingredient applications and negatively affect consumer acceptance (Caudle et al., 2005). Differences in processing and storage of SMP between SMP manufactures can account for much of the variability of flavor. The effects of storage on the flavor of SMP

have been reported (Karagul-Yuceer et al., 2002). There is no current research that investigates the influence of process parameters on the flavor of skim milk powder.

Free Fat

The spray drying process not only affects the water content but the structural and physiochemical characteristics as well (Vignolles, et al., 2007). Free fat is one of these important characteristics. Free fat has a loose definition of being fat that is no longer emulsified (Palanuwech et al., 2003). Free fat can be an indicator of damage to the milk fat globule membrane (Kim et al., 2002). A more encompassing definition of free fat in dairy powders is described as fat that is not entirely coated by amphiphilic molecules or protected by a matrix of carbohydrates and proteins during drying (Vignolles et al., 2007). The free fat in milk powders can alter important properties of the dried milk powder such as: oxidative stability, wettability, dispersability, solubility, flowability, and ability to use in chocolate processing applications (Vignolles et al., 2007). Lipid oxidation is increased and oxidative stability is decreased with increasing free fat in dairy powders (Vignolles et al., 2007). Because of the potential for oxidation, control of free fat through changing processing parameters could potentially affect the flavor and flavor stability of SMP.

In milk chocolate manufacture the most expensive ingredient is cocoa butter. Cocoa butter is used to create a flowable product. Whole milk powder with a high free fat content can be used to reduce the amount of cocoa butter needed (Koc et al., 2003). The high free fat content also reduces energy usage during blending (Reimerdes and Mehrens, 1994). Spray-dried whole milk powder contains more than 26% milk fat (Koc et al., 2003). The majority

of this fat is entrapped in a lactose and protein matrix during spray drying. Roller-dried milk has been used because of its high free fat content of greater than 95% compared to about 10% free fat in spray-dried whole milk powder (Reimerdes and Mehrens, 1994).

Processing Affects Free Fat

The pasteurization treatment of milk of 72-74°C for 15 sec has little effect on the composition profile of the milk fat globule membrane (McPherson et al., 1984). Homogenization has two main effects which are: a decrease in fat globule size, and changes in composition of the protective layer of the milk fat globule membrane (Vignolles et al., 2007). The free fat content can be altered depending on the number of passes and pressures during homogenization (Keogh and O'Kennedy, 1999; Tamsma et al., 1959). Crystallization of lactose to α-lactose is used to increase free fat of whole milk powder for chocolate applications (Vignolles et al., 2007). The free fat content of dried milk powders is very dependent on the spray drying parameters. Higher inlet temperatures correspond to a lower free fat content (De Vilder et al., 1976). Free fat content increases with increasing outlet temperatures (Kelly et al., 2002). Larger particle sizes encapsulate more fat during the drying process and thus decrease the amount of free fat (Buma, 1971).

The particle size of the resulting dairy powder can be manipulated through different atomization techniques. Lower inlet temperatures result in a smaller particle size (Nijdam and Lagrish, 2006). Particle size is increased with increasing feed solids content due to its viscosity (Hogan et al., 2001). This is also due to agglomeration of powder particles to each other because of incomplete atomization of high viscosity emulsions (Nijdam and Lagrish,

2006). The current research shows the effects of feed solids, spray dryer temperatures, free fat content, and particle size on functional properties of milk powders but how these relate to flavor and flavor stability is yet to be investigated. There is also a lack of published literature and how these spray drying parameters affect flavor and flavor stability of dried whey ingredients.

Effect of pH on β-lactoglobulin

Protein denaturation is described as a major change in the secondary, tertiary, or quaternary structures without cleavage of backbone peptides (Damadoran, 2008). β-lactoglobulin (β–Lg)is one of the most widely studied proteins. It is hypothesized that the biological role of β–Lg is to transport retinol from the mother to the intestine of the neonatal calf (Kella and Kinsella, 1988). β–Lg exists as a dimer in physiological conditions (Hambling et al., 1992) and the dimer begins to dissociate at pH 3.5 (Townend et al., 1969). The secondary structure of the β–Lg monomers are stabilized by hydrophobic, ionic, and hydrogen bond interactions. There are also two disulfide bridges (de Wit, 2009). There is a free thiol group (Cys^{121}) that, along with the two disulfide bridges, is inaccessible to solvent in native β–Lg (de Wit, 2009).

Upon thermal denaturation of β–Lg, these sulfur containing groups become accessible (Sava et al., 2005). Kella and Kinsella (1988) investigated the thermodynamic stability of β–Lg at low pH. The transition temperature of denaturation increased with decreasing pH, indicating greater protein stability. Boye and Alli (2000) reported an increase in thermal denaturation temperature of β–Lg from 64.9°C at pH 11 to 79.8°C at pH 3. In acid WPC

prepared by UF, the denaturation temperature of β–Lg is highest at pH 3.5 at 88.0°C (Bernal and Jelen, 1985). The thermal denaturation temperatures for WPC at pH 6.5 and 5.5 were 76.9°C and 78.8°C respectively. The presence of milk sugars increases the thermal denaturation temperature. This is due to the increased amount of hydration of the protein molecule, enhancing the water structure around the molecule and thereby increasing its stability (Bull and Breese, 1978). In an isolated β–Lg preparation, the denaturation temperature at pH 3.5 is 81.9°C compared to 88.0°C in WPC (Bernal and Jelen, 1985). The increased protein stability at low pH is due to an increase of hydrogen bonding between carboxy groups and amide groups (Kella and Kinsella, 1988). The reduction of heat stability of β–Lg at pH ≥4 is attributed to the oxidation of free thiol groups and disulfide bonds (Relkin and Mulvihill, 1996). At acidic pH the free thiol group is not reactive (Relkin and Mulvihill, 1996).

Effect of Acidification on Spray Dried WPC Flavor

The typical operating temperatures for the spray drying of dairy products are between 150 and 250°C (Anonymous, 2003). Because of the high temperature there is a great potential for chemical reactions to occur, causing increased off-flavor production. A lower pH prior to spray drying is advantageous for various reasons. As was explained earlier, the rate of lipid oxidation is reduced significantly in a protein rich, low pH aqueous environment (Frankel, 1998). Maillard reactions also are reduced at an acidic pH due to the lower concentrations of unprotonated amine groups on the proteins that are necessary for the reactions (Martins et al., 2000). An increase in the condensation rate increases the rate of

Maillard reactions (Namiki, 1988). At a weakly acidic pH the condensation rate is maximized due to the protonation of carbonyl carbons, making them more subject to nucleophilic attack (Martins et al., 2000). By spray drying at a lower pH the condensation rate is reduced. The stability of β–Lg is also increased at a lower pH which could decrease the amount of protein degradation. The flavor binding behavior of β–Lg is reduced upon heating which is related to conformational changes and aggregation (O'Neill and Kinsella, 1988). The increase of heat stability at low pH could prevent conformational changes and aggregation and prevent the decrease in binding of flavor compounds. As many off-flavor volatile compounds in WPC are aldehydes, an increase in binding affinity could decrease the volatile release and improve the sensory qualities in ingredient applications. Lastly, the higher amount of protonation of the flavor compounds makes them less soluble in water and more volatile. This could significantly reduce the amount of off-flavor volatile compounds in the final spray dried product.

The off-flavors caused by the processing of dried whey protein ingredients are well documented in the literature. How pH, feed solids concentration, and inlet temperature during spray drying affect the flavor of dried WPC80 is yet to be investigated. This would provide dried whey ingredient manufacturers with knowledge to optimize certain processing parameters for minimal off-flavors in the final product. The objective of the first study is to determine the effect of acidification of liquid retentate on the flavor and volatile compounds of spray dried WPC80. The objective of the second study is to determine the effects of feed solids concentration and inlet temperature during the spray drying of WPC80 on the flavor and volatile compounds of the resulting product.

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Chapter 2: The Effect of Acidification of Retentate on the Flavor of Spray Dried Whey Protein Concentrate

The Effect of Acidification of Retentate on the Flavor of Spray Dried Whey Protein Concentrate

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Abstract

Off-flavors in whey protein negatively influence consumer acceptance of whey ingredient applications. Clear acidic beverages are a common application of whey protein and recent studies have demonstrated that beverage processing steps, including acidification, enhance off flavor production from whey protein. The objective of this study was to determine the effect of pre-acidification of whey protein retentate prior to spray drying on flavor of dried whey protein concentrate (WPC). Two experiments were performed to achieve the objective. In both experiments Cheddar cheese whey was manufactured, fat-separated, pasteurized, bleached (250 ppm hydrogen peroxide), and ultrafiltered (UF) to obtain WPC80 retentate that was 13% solids (wt/wt). In experiment I, the liquid retentate was then acidified using a blend of phosphoric and citric acids to the following pH values: no acidification (pH 6.5), pH 5.5, or pH 3.5. UF permeate was used to normalize the protein concentration of each treatment. The retentates were then spray dried. In experiment II, 150 ppb D_{12}-hexanal was added to each treatment, followed by acidification and spray drying. Both experiments were replicated 3 times. Flavor properties of the spray dried WPC were evaluated by sensory and instrumental analyses. Pre-acidification to pH 3.5 resulted in decreased cardboard and sweet aromatic flavor intensities and aroma intensity and an increase in soapy flavor ($p<0.05$) with decreased concentrations of pentanal, hexanal, heptanal, E-2-heptanal, octanal, E-2-octenal, nonanal, decanal, dimethyl disulfide and dimethyl trisulfide ($p<0.05$). Adjustment to pH 5.5 prior to spray drying increased cabbage flavor ($p<0.05$), and concentrations of nonanal, and dimethyl trisulfide ($p<0.05$). The flavor effects of pre-acidification were consistent regardless of the pH the solutions were adjusted

to after spray drying. Pre-acidification to pH 3.5 increased D_{12}-hexanal recovery in liquid retentate and decreased D_{12}-hexanal recovery in the resulting powder ($p<0.05$). These results demonstrate that acidification of WPC80 retentate to pH 3.5 prior to spray drying decreases off flavors in spray dried WPC. Results suggest that the mechanism for off flavor reduction is due to decreased protein interactions with volatile compounds at low pH in liquid retentate and increased interactions in the resulting powder.

Introduction

Advances in processing technology have increased the production of whey protein concentrate. In 2010, approximately 194 million kilograms of whey protein concentrate (WPC) (25 to 89.9 percent protein) and approximately 27 million kilograms of whey protein isolate (WPI), (>90 percent protein) were produced in the United States (USDA, 2011). Whey protein has high nutritional value and unique functional properties and is incorporated into sports drinks, meal replacement beverages, and infant formula. Protein beverages can be formulated at different acidity levels including neutral (pH 6-7), mildly acidic (pH 4-5), or highly acidic (pH<3.5) (Bastian, 2004). Whey protein is least soluble at its isoelectric point (pH 5.2) and solubility increases with decreasing pH (Pelagrine and Gasparetto, 2005) making whey protein ideal for acidic beverages. Low pH whey protein beverages are not only clear but also require a more mild thermal pasteurization process and are regarded as shelf stable.

Astringency and off-flavors in acidic whey protein beverages reduce consumer acceptance (Beecher et al., 2008; Childs and Drake, 2010). When whey protein is used as an

ingredient it is expected to be bland in flavor and colorless. Off-flavors in whey protein (cardboard, cabbage, fatty, and potato) are generated from multiple sources including, cheese starter culture, processing, and storage conditions (Whitson et al., 2011; Liaw et al., 2010; Campbell et al., 2011a; Carunchia Whetstine et al., 2005; Wright et al., 2009). These off-flavors can carry through into beverages and negatively influence consumer acceptance (Evans et al., 2009; Evans et al., 2010; Wright et al., 2009). Volatile lipid oxidation products are primarily responsible for these off-flavors (Carunchia Wheystine et al., 2005; Wright et al., 2009; Whitson et al., 2010; Kang et al., 2012). Bleaching of liquid whey is one of the primary sources of off-flavors in dried whey proteins (Croissant et al., 2009; Kang et al., 2012; Listiyani et al., 2011; Jervis et al., 2012). Other unit operations including liquid storage time, dry storage, and instantization also can negatively influence whey protein flavor (Wright et al., 2009; Whitson et al., 2011).

Recent studies have demonstrated that beverage processing steps (acidification and heat treatment) may also have detrimental effects on whey protein flavor contributions (White et al., 2013). Commercially, there are acidified dried WPI ready-made for beverages. These proteins are acidified prior to spray-drying and provide a value-added application that are purportedly lower in aromatic flavor intensities. To our knowledge, no published studies have investigated the effect of acidification prior to spray drying on the flavor of WPC. The objective of this study was to investigate whether pre-acidification (prior to spray drying) impacted the flavor of the subsequent powder. This objective was achieved by application of sensory and instrumental analyses of WPC made from bleached Cheddar whey that was

spray dried at different pH values (6.5, 5.5, and 3.5). A subsequent study investigated the mechanism of flavor reduction using a stable isotope.

Materials and Methods

Experimental Design

Two experiments (experiment I and II) were included in this study. The purpose of experiment I was to determine the effect of acidification of retentate on the flavor of spray dried WPC. To make the WPC, liquid whey was pasteurized, fat separated, bleached, ultrafiltered, and diafiltered to 13% solids (wt/wt) with 80% (wt/wt) of the solids as protein. Each batch of liquid WPC80 retentate that was made was adjusted to either pH 6.5, 5.5, or 3.5. Fresh liquid permeate was added to the pH 6.5 and 5.5 treatments to dilute the protein to the same level as the pH 3.5 treatment. The resulting retentates were 74% protein on a dry weight basis and were subsequently spray dried. The entire experiment was replicated three times.

The purpose of experiment II was to investigate volatile compound association with WPC at different pH values (6.5, 5.5 and 3.5) before and after spray drying. One batch of liquid Cheddar whey was manufactured, pasteurized, fat separated, bleached, ultrafiltered, and diafiltered to produce liquid WPC80 retentate similar in composition as in experiment I. The retentate was then split into three equal portions and spiked with D_{12}-hexanal (150 µg/kg) (CDN Isotopes, Quebec, Canada). The pH of each portion of WPC80 retentate was then adjusted to 6.5, 5.5, or 3.5 with liquid permeate added to the pH 6.5 and 5.5 treatments

as in experiment I. The retentates (WPC74) were then spray dried. The entire experiment was replicated three times.

Liquid Whey and WPC80 Production

Raw whole milk was obtained from the North Carolina State University Dairy Research and Education Unit (Raleigh, NC). The milk was vat pasteurized (Model MPD1050, Micro Process Design, D & F Equipment Co, McLeansville, NC) at 63°C for 30 min. 195 kg were pasteurized for use on day 1 and 117 kg were pasteurized, cooled, and stored overnight at 4°C for use on day 2. The milk was then cooled to 31°C and transferred to a cheese vat (Model 4MX, Kusel Equipment Company, Watertown, WI). Mesophilic starter culture containing *Lactococcus lactis ssp. lactis* and *Lactococcus lactis ssp. cremoris* (Danisco Choozit MA11 LYO, Dairy Connection Inc., Madison, WI) was added at the rate of 50 DCU/454 Kg of milk. A calcium chloride solution (50% w/v, Dairy Connection Inc.) was also added at the rate of 0.39 ml/Kg of milk. The milk was then allowed to ripen for 60 min. Double strength annatto color (Cheese Color DS Double Strength, Dairy Connection Inc.) was added after 30 min of ripening at the rate of 15 ml/454 kg of milk, diluted 20 times in deionized water. Then, the milk was coagulated with double strength recombinant rennet (Dairy Connection Inc.) for 30 min at a rate of 0.09 mL/kg of milk diluted 80 times in deionized water. The coagulum was then cut and the curd was allowed to rest for 5 min. The curd and whey were stirred for 30 min while the temperature was increased gradually to 39°C. The pH was monitored and once a pH of 6.35 was achieved the whey was drained through a sieve to remove cheese fines. The drained whey was fat separated using a hot bowl

centrifugal separator (Model SI600E, Agri-Lac, Miami, FL). After fat separation the whey was pasteurized at 63°C for 30 min. Following pasteurization, the whey from day 1 was split into 2 batches of about 68 kg each in 60 L stainless steel kettles (model 601BP Polar Ware Company, Kiel, WI). All of the whey from day 2 was used as one batch. Treatment assignment to each batch was randomized and balanced across the three replications. Each batch was bleached with 250 µg/kg hydrogen peroxide (35% w/v, VWR International, Westchester, PA) for 1 h at 60°C. Catalase (20 mg/kg, FoodPro CAT, Danisco, New Century, NJ) was added at 20 µg/kg to remove the hydrogen peroxide upon completion of bleaching. The inactivation of the hydrogen peroxide was confirmed by using EM Quant peroxide test strips (EMD Chemicals Inc., Gibbstown, NJ).

After bleaching, the whey was subjected to ultrafiltration (UF) to concentrate the protein to 80% on a dry weight basis (13% solids). Prior to UF, the membrane cartridges were cleaned with a 0.1N sodium hydroxide solution (VWR International) followed by a rinsing of deionized water. After the rinse step, each batch of whey was concentrated using a UF system (model Pellicon 2, Millipore Inc., Billerica, MA) using 5 cartridges of polyethersulfone membrane filters (model P2B010V05, nominal separation cutoff = 10,000 kDa, surface area = 0.5 m^2). The pump used to circulate the product was a variable speed peristaltic pump (model 77410–10, Cole Palmer, Vernon Hills, Il) equipped with model 77601–00 pumpheads with silicone tubing (model 96440–73, Cole Palmer).

The flux during UF was calculated every 15 min. Diafiltration was used to aid the removal of lactose and minerals to further concentrate the protein. The whey was

concentrated by a factor of 2 and 33% of the original weight of the liquid whey was added back as deionized water. The whey was concentrated again by a factor of 2 and 17% of the original weight of the liquid whey was added as deionized water. Total deionized water addition was equal to 50% of the original weight of the liquid whey. The protein concentration was confirmed with a Sprint™ Rapid Protein Analyzer (CEM, Matthews, N.C.). Percent solids were analyzed using a Smart System 5 moisture/solids analyzer (CEM). The total time for UF was approximately 2.5 h.

Once the WPC was made, the 3 batches were subjected to one of the following treatments: Control pH (6.5), pH 5.5, and pH 3.5. For experiment II, D_{12}-hexanal (CDN Isotopes) was added (150 µg/kg) to each treatment prior to acidification. The acidified samples were acidified using a blend of phosphoric (Mallinckrodt Baker, Inc., Phillipsburg, NJ) and citric (VWR International) acids. Because acidification to pH 3.5 diluted the protein concentration, UF permeate was added to the control and pH 5.5 retentates to equalize the protein concentration across all samples. The retentates were dried in a spray dryer (model Lab 1, Anhydro Inc., Soeberg, Denmark) with an inlet temperature of 200°C and outlet temperature of 90°C. The dried powdered samples were collected in mylar bags (TF-4000, Impak Corp., Central City, SD) and stored at -80°C until subsequent analyses. Both experiments were replicated three times.

Proximate Analysis

The percent moisture of the dried whey proteins was measured using a vacuum oven (AOAC, 2000; method number 990.20: 33.2.44). The fat content was measured using a

Modified Mojonnier ether extraction (AOAC, 2000; method number 932.06; 33.5.08). Total protein was measured using the Kjeldahl method (AOAC, 2000; method number 991.20; 33.2.11) by multiplying total nitrogen by a factor of 6.38.

Descriptive Analysis

All sensory testing was performed in compliance with the North Carolina State University Institutional Review Board for Human Subjects guidelines. Spray dried powders were collected and rehydrated to 10% solids (wt/vol) in deionized (DI) water. There were three pH values from the three treatments: 6.5, 5.5, and 3.5. Each of these treatments was rehydrated to 10% solids (wt/vol) in DI water and either acidified or neutralized to each of the following pH values: 6.5, 5.5, and 3.5. In total, every experimental replication had 9 samples for evaluation. The acid used to acidify the samples was a 10% solids (wt/vol) solution of a mixture of phosphoric (Mallinkrodt Chemical, Phillipsburg, NJ) and citric (J.T. Baker, Mallinkrodt Baker, Phillipsburg, NJ) acids, the base used to neutralize the samples was a 10% solids (wt/vol) solution of sodium hydroxide (VWR International, Westchester, PA). All samples were evaluated at 10% solids (wt/vol).

The rehydrated powders were dispensed into 3-digit coded soufflé cups (Solo Cup, Highland Park, Il), lidded, and tempered to 21°C. Aromatics and basic tastes intensities were evaluated by trained panelists (n = 8) using an established sensory language for dried dairy ingredients and a 0 to 15 point Spectrum^{TM} intensity scale (Wright et al., 2009). Panelists were between the ages of 23 and 45 y and each had over 150 h of experience with descriptive analysis of dried dairy ingredients. Panelists expectorated samples and were provided with

room temperature DI water for palate cleansing. Each sample was evaluated in duplicate by each panelist. Paper ballots or Compusense Five version 4.8 (Compusense, Guelph, Canada) was used for data collection.

Instrumental Volatile Compound Analysis

Spray dried powders were collected and rehydrated to 10% solids (wt/vol) in HPLC grade water (Campbell et al., 2011b). Powders were pH adjusted as described for descriptive analysis. Volatile compounds were extracted by head space-solid phase microextraction-gas chromatography-mass spectrometry (SPME-GC-MS). A CombiPal Autosampler (CTC Analytics, Zwingen, Switzerland) was used for injections. All injections were made on an Agilent 6890N GC with 5973 inert MSD with a ZB-5 ms (30 m × 0.25 mm ID × 0.25 μm) column (Phenomenex, Torrance, Calif., U.S.A.). After neutralization or acidification, 5 ml of each sample was added to 20 ml SPME vials (MicroLiter Analytical Supplies Inc., Suwanee, Ga., U.S.A). An internal standard compound (10ul of 81ppm 2-methyl-3-heptanone in ether; Sigma Aldrich) was added to each vial along with 0.5g of sodium chloride (VWR International). The GC method was adapted from Campbell et al. (2011b). Each sample was prepared in triplicate. Equilibration took place over 25 min at 40°C while the samples were agitated at 250 rpm. A DVB/Carboxen/PDMS 1 cm fiber (Supelco, Bellefonte, PA) was used. Compounds were identified using the National Institute of Standards and Technology (2002) mass spectral database and authentic standards injected under identical conditions. Compounds of interest (White et al., 2013; Campbell et al., 2011b; Jervis et al., 2012) were either quantified by relative abundance using the internal

standard or by constructing 5 point standard curves with D_{12}-hexanal, D_{18}-Octanal, and D_6-DMDS (CDN Isotopes) into samples that were pH adjusted as described previously.

For experiment II D_{12}-hexanal was monitored using selective ion monitoring (SIM) mode with the ion mass of 64 used for quantification. Both liquid retentate and rehydrated powders were analyzed at 10% solids (wt/vol). Five point standard curves were constructed using D_{12}-hexanal in treated samples that were not originally spiked with D_{12}-hexanal.

Statistical Analysis

The data was analyzed with two-way analysis of variance (ANOVA) with means separation (SAS, version 9.3, SAS, Cary, NC). Fisher's least significant difference (LSD) test was used to analyze differences among sample means.

Results

Proximate Analysis

The fat and moisture of the spray dried WPC produced in experiments I and II were consistent ($p>0.05$). The fat content averaged 3.94% ± 0.47 and the moisture content averaged 4.85% ± 0.70. The protein content was 76.3% ± 0.40, 74.8% ± 1.53, and 73.8% ± 0.65 for the control (pH 6.5), pH 5.5, and pH 3.5 treatments respectively. The spray dried WPC from experiment II were not different ($p>0.05$) in fat, moisture, and protein composition from the powders in experiment I.

Sensory and Volatile Compound Analysis

Experiment I

The primary source of off-flavors in whey protein ingredients is lipid oxidation (Croissant et al., 2009; Evans et al., 2010; Whitson et al., 2010). Many of the volatile lipid oxidation products in dried whey protein ingredients are above sensory threshold values and thus contribute to the flavor of the whey proteins (Whitson et al., 2010). Starter culture, light exposure, storage, heat treatment, and bleaching all can increase volatile lipid oxidation products in whey proteins (Campbell et al., 2011a; Liaw et al., 2011; Tomaino et al., 2004; Croissant et al., 2009; Jervis et al., 2012). Spray drying liquid WPC retentate at pH 5.5 and 3.5 had significant effects on the flavor of the subsequent powder compared to the control (pH 6.5) (Tables 2.1, Figure 2.1). Interactions between spray drying pH and evaluation pH were significant for aroma, sweet aromatic, cardboard, and soapy flavor intensities ($p<0.05$). In general, samples spray dried at pH 6.5 or pH 5.5 were characterized by increased aroma and increased sweet aromatic and cardboard flavors ($p<0.05$) (Table 2.1). Spray drying at pH 5.5 increased cabbage flavor, decreased sweet aromatic flavor and did not affect aroma or cardboard flavor intensities compared to pH 6.5 ($p<0.05$). WPC acidified to pH 3.5 prior to spray drying was characterized by increased soapy flavor and decreased aroma impact and cardboard and sweet aromatic flavors (Figure 2.1). When evaluated at pH 3.5, all samples had decreased aroma and cardboard flavor intensity, but WPC spray dried at pH 3.5 had the lowest intensities regardless of the evaluation pH compared to WPC spray dried at pH 6.5 or 5.5 ($p<0.05$) (Table 2.1 and Figure 2.1). Evaluation of WPC at pH 5.5 increased cabbage

flavor intensity for WPC spray dried at pH 6.5 or 5.5 but had no effect on WPC spray dried at pH 3.5 ($p<0.05$).

Consistent with sensory results, interactions between spray drying pH and evaluation pH were significant for all volatile compounds except decanal and 2,4-nonadienal ($p<0.05$) (Figure 2.1, Table 2.2). Samples that were spray dried at pH 6.5 and 5.5 were characterized by increased concentrations of the aldehydes hexanal, pentanal, decanal, nonanal, octanal and the protein degradation product DMDS (Figure 2.1). Acidification to pH 3.5 prior to spray drying decreased concentrations of pentanal, hexanal, heptanal, nonanal, and DMTS at each of the evaluation pH values compared to WPC spray dried at pH 6.5 or 5.5 ($p<0.05$) (Table 2.2). Consistent with sensory results for cabbage flavor, an evaluation pH of 5.5 increased DMTS concentration in all samples ($p<0.05$). The evaluation pH of 3.5 decreased concentrations of pentanal, hexanal, heptanal, nonanal, and DMTS for all treatments but WPC spray dried at pH 3.5 were always lower than those spray dried at pH 6.5 or 5.5 ($p<0.05$). These results demonstrate that acidification of WPC retentate prior to spray drying reduces off-flavor intensities and their corresponding volatile compounds regardless of the pH at which the samples are rehydrated and evaluated.

Experiment II

The results from Experiment I demonstrated that acidification of WPC retentate to pH 3.5 reduced cardboard and cabbage flavor intensities along with lipid and protein volatile oxidation compounds. Experiment II was devised to further explain the mechanism for the reduction of oxidation volatile compounds. The decrease in volatile lipid oxidation

compounds (VLOC) in WPC spray dried at pH 3.5 observed in Experiment I may be attributed to a decrease in lipid oxidation, increased volatilization during spray drying, or an increase in association between the VLOC and whey protein. No differences in VLOC were observed when WPC34 was spray dried versus freeze-dried, suggesting that lipid oxidation during spray drying is minimal (Evans et al., 2009). Additionally, due to the short residence time and the fact that spray drying droplets usually do not reach temperatures above 60°C, it is unlikely that a reduction of lipid oxidation is the mechanism (Schuck, 2013). A decrease in lipid oxidation or increased volatility during spray drying would reduce the amount of VLOC present and an increase in association with the whey protein would reduce the amount of VLOC in the headspace and thus reduce off-flavor intensity.

Hexanal was chosen for Experiment II because it was one of the aldehydes from Experiment I that showed a dramatic decrease in concentration in samples spray dried at pH 3.5 and is also a product of lipid oxidation (Frankel et al., 1981). Deuterated hexanal (D_{12}-hexanal) is not produced naturally during lipid oxidation of WPC but displays the chemical properties of hexanal. Increased recovery of D_{12}-hexanal in the liquid retentate was observed at pH 3.5 compared to pH 5.5 or 6.5 ($p<0.05$) (40.8 vs. 20.0 vs. 11.6 ppb, respectively). Interactions were significant for spray dry pH and evaluation pH in the WPC powders ($p<0.05$) (Table 2.3). WPC spray dried at pH 3.5 decreased the recovery of D_{12}-hexanal compared to WPC spray dried at pH 6.5 or 5.5 when evaluated at pH 6.5 (24.7 vs. 59.7 vs. 47.2 ppb, respectively) and pH 5.5 (18.2 vs. 35.5 vs. 27.5 ppb, respectively) ($p<0.05$). When evaluated at pH 3.5, D_{12}-hexanal recovery decreased in each treatment and was not different between treatments ($p>0.05$) (Table 2.3). This result is consistent with the volatile

compound analysis in experiment I where hexanal concentration decreased in all treatments when evaluated at pH 3.5 (Table 2.2).

Discussion

Cardboard flavor in whey proteins has been sourced to pentanal, heptanal, and nonanal in the presence of DMTS and 1-octen-3-one (Whitson et al., 2010). In general, WPC spray dried at pH 3.5 had lower concentrations of pentanal, heptanal, nonanal, and DMTS. This result was consistent with decreased cardboard flavor intensity in these samples. WPC spray dried at pH 3.5 exhibited soapy flavor at all evaluation pH values whereas WPC spray dried at pH 5.5 and 6.5 only exhibited soapy flavor when evaluated at pH 3.5. Soapy flavor has also been documented in rehydrated instantized and non-instantized WPI, WPC80, and SPC at neutral pH suggesting that this flavor is not solely due to pH (Wright et al., 2009; Evans et al., 2010; Drake et al., 2009) although White et al. (2013) reported that acidification increased soapy flavor in WPI solutions. At this time, the source of soapy flavor remains unknown. Cabbage flavor in whey proteins is due to increased concentrations of DMTS (Wright et al., 2006). The WPC in this study with concentrations of DMTS >0.268 µg/kg all exhibited cabbage flavor whereas cabbage flavor was absent in WPC with DMTS concentrations below that level.

Whey proteins have been reported to bind to flavor compounds and reduce flavor intensity (Hansen and Heinis, 1991). In WPI, it was found that β–lactoglobulin (β–Lg) was the main protein responsible for the binding of flavor compounds (Kuhn et al., 2007). At low pH, the thermal denaturation temperature of the major protein in WPC, β–Lg, is increased

which could decrease the amount of protein denaturation during spray drying (Bernal and Jelen, 1985). At pH 5.5, the thermal denaturation temperature is only slightly elevated (Bernal and Jelen, 1985). Both decreases and increases in flavor binding affinity have been reported in denatured whey proteins compared to native whey proteins (O'Neill and Kinsella, 1988; Hansen and Booker, 1996). Conformational changes could result in increased binding of VLOC at low pH compared to pH 6.5 or 5.5. Below pH 3.5, β–Lg changes from existing as a dimer to a monomer (Townend, 1969). When at low pH, the monomeric form of β–Lg allows for more solvent accessibility to the hydrophobic region in the interior of the protein (Uhrinova et al., 2000). As many off-flavor volatile compounds in WPC are hydrophobic, an increase in accessibility to the hydrophobic region of β–Lg could decrease volatile compound release and reduce off-flavor intensities in ingredient applications. In addition, the higher amount of protonation of the off-flavor compounds at low pH makes them less soluble in water and more volatile (van Ruth and Villenueve, 2002). As such, greater volatility due to increased partitioning in headspace may significantly reduce concentrations of off-flavor volatile compounds in the final spray dried product, consistent with the results observed in this study. White et al. (2013) reported increases in cardboard and potato/brothy flavor intensities (along with soapy flavor) in non-instantized WPI that was acidified. The WPI used in that study was commercial and other studies have found significant differences between commercial and experimentally made whey proteins (Evans et al., 2010). Consistent with our results, White et al. (2013) did observe a decrease in volatile lipid oxidation compounds in WPI that was acidified compared to a control that was not acidified. In experiment II, a reduction in D_{12}-hexanal recovery was observed when the WPC was

evaluated at pH 3.5 for the WPC spray dried at pH 6.5 or 5.5 indicating an increase in association with the protein. The WPC spray dried at pH 3.5 was unchanged regardless of the evaluation pH and always had decreased recovery of D_{12}-hexanal compared to WPC spray dried at pH 6.5 or 5.5. This result leads us to propose that the decreased protein interactions with aldehydes at low pH resulted in more unbound (volatile) hexanal available. Spray drying whey protein retentate at pH 3.5 volatilized more D_{12}-hexanal and there was less in the subsequent powder. In experiment I, the WPC spray dried at pH 3.5 was always lowest in concentration in hexanal regardless of evaluation pH. A combination of increased volatility in liquid retentate and decreased recovery of D_{12}-hexanal in the powders spray dried at pH 3.5 was observed in Experiment II and lead to the conclusion that VLOC are decreased during spray drying at pH 3.5 due to increased volatility due to decreased protein interactions and/or decreased solubility followed by increased association of VLOC with whey protein upon rehydration.

Conclusions

Whey proteins used for acidified beverage applications that are acidified to pH 3.5 prior to spray drying have decreased intensities of off-flavors and decreased concentrations of many lipid oxidation compounds compared to whey proteins spray dried at higher pH. D_{12}-hexanal was more volatile in acidified liquid WPC retentate and less was recovered in the subsequent powder rehydrated to pH 3.5. These results suggest that the mechanism for decreased off-flavor intensities is due to increased volatility during the spray drying process and increased flavor-protein interactions at pH 3.5 compared to pH 6.5. Producing a pre-

acidified whey protein ingredient for acidified beverage applications is advantageous due to decreased off-flavor intensities compared to direct acidification of the rehydrated whey protein powder.

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Table 2.1 Descriptive analysis means for rehydrated (10% solids) WPC spray dried at different pH values and evaluated at different pH values

Spray Dry pH	6.5	5.5	3.5	6.5	5.5	3.5	6.5	5.5	3.5
Evaluation pH	6.5			5.5			3.5		
Aroma Intensity	2.6^{bc}	2.4^{c}	1.4^{e}	2.9^{bc}	3.1^{a}	1.3^{e}	2.0^{d}	2.0^{d}	1.5^{e}
Sweet Aromatic	1.3^{a}	0.9^{b}	ND[1]	ND	ND	ND	ND	ND	ND
Cardboard	2.7^{b}	2.6^{b}	1.1^{d}	2.9^{ab}	3.2^{a}	1.4^{cd}	1.7^{c}	1.6^{c}	1.1^{d}
Fatty	1.0^{a}	ND	ND	ND	ND	ND	ND	ND	ND
FFA	ND	ND	ND	ND	ND	ND	0.6^{a}	ND	ND
Cabbage/Brothy	ND	0.8^{ab}	ND	0.8^{abc}	1.2^{a}	ND	ND	1.1^{a}	ND
Soapy	ND	ND	1.6^{cd}	ND	ND	2.2^{a}	1.9^{bc}	1.4^{d}	2.0^{ab}

Statistical analysis with means separations using Fisher's (LSD) with 95% confidence interval. Means in a row followed by a different letter are different ($p \leq 0.05$). [1]ND: not detected.

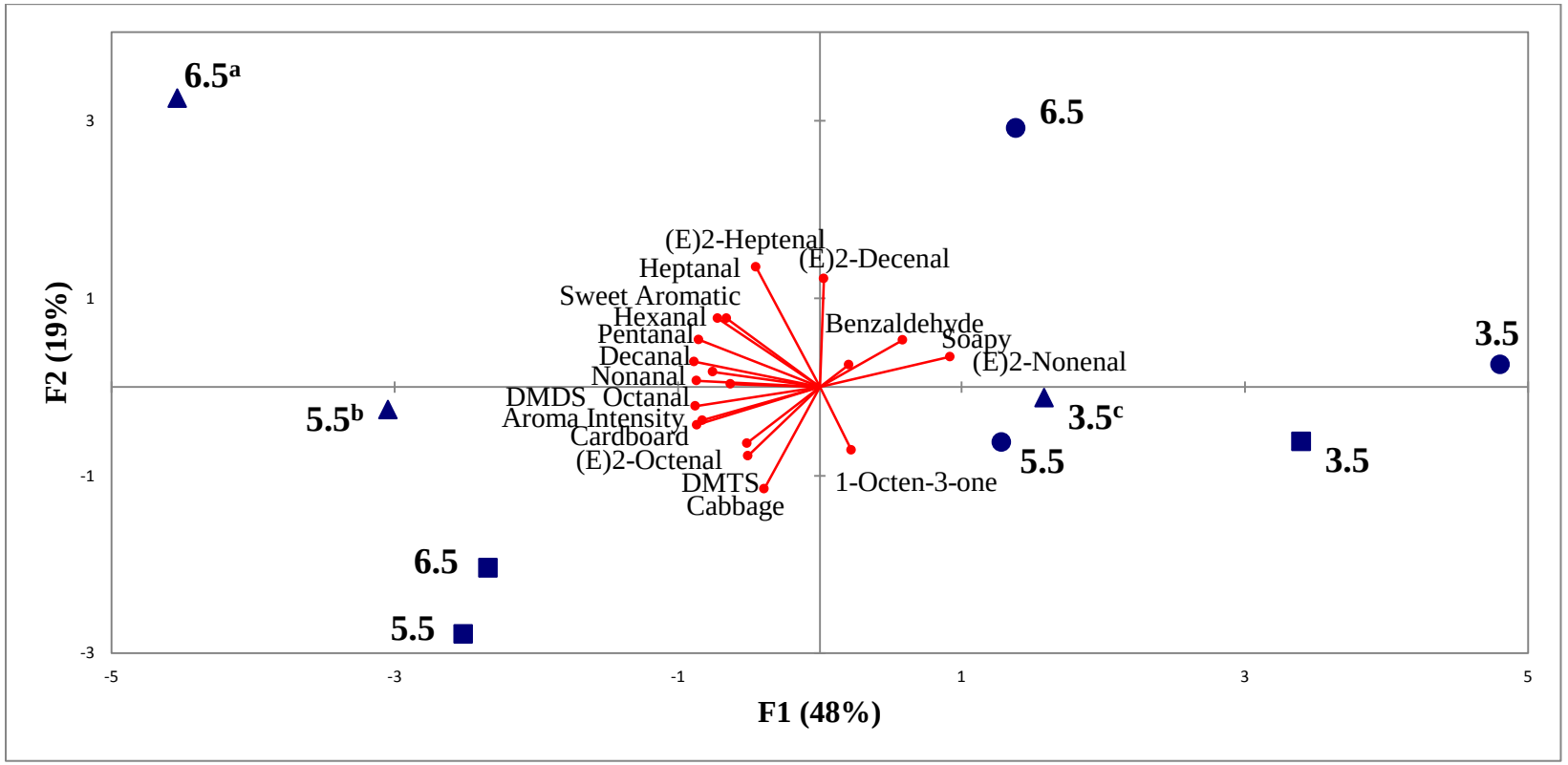

Figure 2.1 Principal component biplot of sensory and volatile compound analyses of rehydrated WPC spray dried at different pH values

(▲) Samples evaluated at pH 6.5. (■) Samples evaluated at pH 5.5. (●) Samples evaluated at pH 3.5.
[a]WPC spray dried at pH 6.5 [b]WPC spray dried at pH 5.5 [c]WPC spray dried at pH 3.5
Table 2.2 Concentration (µg/kg) of selected volatile compounds in WPC spray dried and rehydrated to different pH values

Spray Dry pH	6.5	5.5	3.5	6.5	5.5	3.5	6.5	5.5	3.5
Evaluation pH	6.5			5.5			3.5		
[1]Pentanal	13.0^{a}	9.99^{b}	4.94^{d}	7.29^{c}	8.82^{b}	4.80^{d}	4.89^{d}	3.55^{e}	3.21^{e}
[1]Hexanal	260^{a}	151^{c}	106^{e}	190^{b}	129^{d}	77.6^{f}	144^{c}	102^{e}	27.9^{g}
[1]Heptanal	23.6^{a}	15.2^{c}	12.4^{d}	15.6^{c}	15.6^{c}	12.5^{d}	19.4^{b}	14.5^{cd}	6.38^{e}
[1](E)2-Heptenal	12.2^{a}	6.08^{c}	3.85^{de}	2.38^{ef}	2.01^{f}	1.55^{f}	9.12^{b}	4.62^{cd}	2.29^{f}
[2]Octanal	14.8^{a}	11.1^{b}	13.9^{a}	11.8^{b}	9.84^{c}	8.58^{d}	6.9^{e}	11.9^{b}	5.09^{f}
[2](E)2-Octenal	5.96^{bc}	3.48^{d}	6.30^{bc}	10.5^{a}	6.70^{b}	2.00^{e}	3.16^{d}	5.34^{c}	3.34^{d}
[2]Nonanal	109^{a}	113^{a}	77.2^{cd}	76.1^{d}	97.3^{b}	60.1^{e}	70.2^{d}	84.8^{c}	41.1^{f}
[2](E)2-Nonenal	1.20^{c}	0.310^{e}	0.450^{e}	3.49^{a}	0.683^{de}	0.832^{cd}	3.49^{a}	2.76^{b}	2.39^{b}
[2]Decanal	7.05^{ab}	6.99^{ab}	4.27^{c}	7.56^{a}	6.60^{b}	4.01^{c}	7.45^{a}	6.54^{b}	3.14^{d}
[2](E)2-Decenal	4.07^{b}	2.41^{d}	2.42^{d}	2.18^{de}	2.33^{d}	1.42^{e}	5.59^{a}	3.31^{c}	3.56^{bc}
[4]1-Octen-3-one	0.356^{de}	0.283^{e}	0.347^{de}	0.651^{a}	0.524^{b}	0.665^{a}	0.465^{bc}	0.680^{a}	0.395^{cd}
[3]DMDS	1.05^{b}	0.944^{c}	0.505^{e}	1.24^{a}	0.786^{d}	0.283^{g}	0.347^{f}	0.254^{g}	0.152^{h}
[3]DMTS	0.104^{e}	0.727^{a}	0.032^{fg}	0.268^{c}	0.773^{a}	0.074^{ef}	0.182^{d}	0.500^{b}	0.016^{g}
[4]Benzaldehyde	0.671^{e}	0.097^{g}	0.975^{d}	0.465^{f}	0.094^{g}	1.04^{d}	2.39^{b}	3.13^{a}	1.77^{c}
[4](E,E) 2,4 Nonadienal	ND[5]	ND	ND	ND	ND	ND	0.433^{a}	0.495^{a}	0.357^{a}

Statistical analysis with means separations using Fisher's (LSD) with 95% confidence interval. Means in a row followed by a different letter are different ($p \leq 0.05$). [1]Compounds quantified with D_{12}-hexanal. [2]Compounds quantified with D_{18}-octanal. [3]Compounds quantified with D_6-DMTS. [4]Compounds quantified with relative abundance. [5]ND: not detected.

Table 2.2 Recovery (µg/kg) of D_{12}-hexanal in rehydrated spray dried powders at different pH values

pH at Spray Drying	Evaluation pH	D_{12}-hexanal
6.5	6.5	59.7^{a}
6.5	5.5	35.5^{c}
6.5	3.5	27.5^{d}
5.5	6.5	47.2^{b}
5.5	5.5	27.5^{cd}
5.5	3.5	23.5^{de}
3.5	6.5	24.7^{de}
3.5	5.5	18.2^{e}
3.5	3.5	22.3^{de}

Statistical analysis with means separations using Fisher's (LSD) with 95% confidence interval. Means in a column followed by a different letter are different ($p \leq 0.05$). All quantification was with 5 point standard curves with $R^2>0.97$.

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Chapter 3: The Effect of Feed Solids Concentration and Inlet Temperature on the Flavor of Whey Protein Concentrate

The Effect of Feed Solids Concentration and Inlet Temperature on the Flavor of Spray Dried Whey Protein Concentrate

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* Use of names, names of ingredients, and identification of specific models of equipment is for scientific clarity and does not constitute any endorsement of product by authors, North Carolina State University, or the Southeast Dairy Foods Research Center.

Abstract

Off flavors in whey protein negatively influence consumer acceptance of whey ingredient applications. Previous research has demonstrated that unit operations in whey protein manufacture, such as liquid storage and bleaching, promote off-flavor production in whey protein. The objective of this study was to determine the effects of feed solids concentration in liquid retentate and spray drier inlet temperature on the flavor of dried whey protein concentrate (WPC). Cheddar cheese whey was manufactured, fat-separated, pasteurized, bleached (250 ppm hydrogen peroxide), and ultrafiltered (UF) to obtain WPC80 retentate that was 25% solids (wt/wt). The liquid retentate was then diluted with deionized water to the following solids concentrations: 25%, 18%, and 10%. Each of the treatments was then spray dried at the following temperatures: 180°C, 200°C, and 220°C. The experiment was replicated 3 times. Flavor of the WPC80 was evaluated by sensory and instrumental analyses. Particle size and surface free fat were also analyzed. Both main effects (solids concentration and inlet temperature) and interactions were investigated. A decrease in feed solids concentration resulted in increased surface free fat, increased intensities of overall aroma, cabbage and cardboard flavors and increased concentrations of pentanal, hexanal, heptanal, decanal, (E)2-decenal, DMTS, DMDS, and 2,4-decadienal ($p<0.05$). A decrease in inlet temperature also resulted in increased surface free fat and intensity of cardboard flavor and increased concentrations of pentanal, (Z)4-heptenal, nonanal, decanal, 2,4-nonadienal, 2,4-decadienal, and 2- and 3-methyl butanal ($p<0.05$). Particle size was higher for powders from increased feed solids concentration and increased inlet temperature ($p<0.05$). These results demonstrate that an increase in feed solids

concentration in the liquid retentate and inlet temperature within the parameters tested decreases off-flavor intensity in the resulting WPC80.

Introduction

One of the most important factors in consumer acceptance of products with whey protein is flavor (Childs et al., 2007). Whey protein is commonly used as a food ingredient due to its unique functional and nutritional properties. It is important that whey protein does not contribute flavor in the final application (Drake et al., 2009). Dried whey ingredients are reported as having a bland and delicate flavor but off-flavors are present (Majahan et al., 2004; Wright et al., 2009; Liaw et al., 2011; White et al., 2013). These flavors also carry over to the finished product and can limit the application of dried whey ingredients in foods (Evans et al., 2010; White et al., 2013). The most common off-flavors in whey proteins include cardboard, cabbage, soapy, fatty, and potato (Evans et al., 2010; White et al., 2013; Whitson et al., 2011; Wright et al., 2009). Aldehydes are the primary volatile compounds associated with these off flavors (Whitson et al., 2011; Wright et al., 2009).

Off-flavors in whey proteins are often associated with different processing steps. Campbell et al. (2011a) demonstrated that the use of starter culture increased lipid oxidation products in WPC compared to WPC made from rennet-set whey. Different starter cultures impact lipid oxidation to varying degrees: mesophilic starters have a larger effect than thermophilic (Liaw et al., 2011). Bleaching of Cheddar whey increases off-flavors and corresponding volatile lipid and protein oxidation products (Croissant et al., 2009; Kang et al., 2012; Listiyani et al., 2011; Jervis et al., 2012). Storage of liquid whey or liquid retentate

also increases off-flavors and volatile compounds (Whitson et al., 2011; Campbell et al., 2011b). Whey protein may be agglomerated to increase wettability and solubility for use in beverage applications. Both steam agglomeration and instantization increase off-flavor intensity in whey proteins (Wright et al., 2009; White et al., 2013). The role of spray dry parameters on whey protein flavor has not been investigated.

In order to achieve a dry powder, whey proteins are spray dried. The parameters used during spray drying can greatly affect the physical characteristics of the powder. The following parameters are among those that can be altered: feed solids concentration, inlet temperature, outlet temperature, and nozzle air pressure. One important powder characteristic to measure is free fat. Free fat can be defined as the fat that is not surrounded by amphiphilic molecules (Vignolles et al., 2007). Higher free fat has been associated with increased lipid oxidation and decreased oxidative stability in whole milk powder (Vignolles et al., 2007). Increased inlet temperatures and decreased outlet temperatures correspond to a lower free fat content (De Vilder et al., 1976; Kelly et al., 2002). The use of nozzle atomization rather than rotary atomization decreased the free fat along with increased nozzle pressure during nozzle atomization (Kelly et al., 2002). Larger particle sizes encapsulate more fat during the drying process and thus decrease the amount of free fat (Buma, 1971). Increased inlet temperatures result in larger particle size (Nijdam and Lagrish, 2006). Increasing the feed solids concentration will also result in a larger particle size (Hogan et al., 2001).

Previous research has demonstrated effects of feed solids concentration and inlet temperature on physical characteristics of milk powder such (free fat and particle size). To our knowledge, how these parameters relate to flavor in dried whey ingredients has yet to be investigated and no studies have evaluated these parameters in whey proteins. The objective of this study was to determine the effect that feed solids concentration and inlet temperature had on the flavor of spray dried WPC. This objective was achieved by means of sensory and instrumental analyses of WPC80 made from bleached Cheddar whey that was spray dried at 10%, 18%, or 25% solids (wt/wt) and at an inlet temperature of 180°C, 200°C, or 220°C.

Materials and Methods

Experimental Design

The experiment was conducted across two days. On day one, WPC80 retentate was manufactured, cooled, and held overnight at 4°C. Liquid Cheddar whey was manufactured, pasteurized, fat separated, bleached, ultrafiltered, and diafiltered to 25% solids (wt/wt) with 80% of the solids as protein. On day two, WPC80 retentate was split into three treatments and deionized water was added to two treatments to achieve solids concentrations of 10%, 18% and 25% (wt/wt). Each solids concentration was then spray dried at 180°C, 200°C, or 220°C. The experiment was replicated three times.

Liquid Whey and WPC80 Production

Whole, raw bovine milk (815 Kg) was obtained from the North Carolina State University Dairy Research and Education Unit. The milk was HTST pasteurized (720 kg/hr) with a plate heat exchanger (model T4 RGS-16/2, SPX Flow Technology, Greensboro, NC)

at 72°C with a holding time of 16 sec. The milk was then cooled to 31°C and transferred to a cheese vat (Kusel Equipment, Watertown, WI). Mesophilic starter culture containing *Lactococcus lactis ssp. lactis* and *Lactococcus lactis ssp. cremoris* (Danisco Choozit MA11 LYO, Dairy Connection Inc., Madison, WI) was added at the rate of 50 DCU/454 Kg of milk. A calcium chloride solution (50% w/v, Dairy Connection Inc.) was also added at the rate of 0.39 ml/Kg of milk. The milk was then allowed to ripen for 60 min. Double strength annatto color (Cheese Color DS Double Strength, Dairy Connection Inc.) was added after 30 min of ripening at the rate of 15 ml/454 kg of milk, diluted 20 times in deionized water. Next, the milk was coagulated with double strength recombinant rennet (Dairy Connection Inc.) for 30 min at a rate of 0.09 mL/kg of milk diluted 80 times in deionized water. The coagulum was then cut and the curd was allowed to rest for 5 min. The curd was stirred for 30 min while the temperature was increased gradually to 39°C. The pH was monitored and once a pH of 6.35 was achieved the whey was drained through a sieve to remove cheese fines. The drained whey was fat separated using a hot bowl centrifugal separator (Model SI600E, Agri-Lac, Miami, FL). After fat separation the whey was HTST pasteurized (750 kg/h) at 72°C for 15 sec. The whey was then subjected to bleaching using 250 ppm hydrogen peroxide (35% w/v, VWR International, Westchester, PA) at 50°C for 1 h. The hydrogen peroxide was then removed using 20 ppm catalase (FoodPro CAT, Danisco, New Century, NJ).

The whey was ultrafiltered using a pilot scale ultrafiltration unit (Model Lab 46, Filtration Engineering, Champlin, MN). Four spiral wound UF membranes were used (Synder Filtration, Vacaville, CA; nominal cutoff: 10,000 Da, surface area 5.0 m^2). The

temperature for UF was 50°C. The flux was recorded every 15 min. Diafiltration water was added as 40% of the original weight of whey. The run time was approximately 2.5 h.

Immediately before processing, the soak solution (0.54% vol/vol, Hydrisoak, Hydrite Chemical, Brookfield, WI) was flushed out of the UF membranes until neutral pH was attained. Next, the membranes were washed for 15 min with a combination of liquid alkaline cleaner (0.24% vol/vol, Hydriflux, Hydrite Chemical) and sanitizer with 150-180 ppm chlorine (Multi-Chlor, Diversy Inc., Sturtevant, WI) diluted in water at a pH of 10.5-11.0. The membranes were then flushed with water until a neutral pH was attained. The clean water flux was measured at 54 kg/m^2 per hour. The UF system was then drained of the remaining water to prepare for UF of whey.

Approximately 660 kg of whey was heated to 50°C in a cheese vat (Kusel Equipment) and transferred to the UF system to be processed. The operating inlet pressure was 210 kPa and the outlet retentate pressure was 140 kPa. The starting flux of the whey was approximately 45 kg/ m^2 per hour. The whey was ultrafiltered until a concentration factor of about 3x was achieved. Deionized water was then added as 40% of the original weight of whey. The whey was then ultrafiltered until a concentration factor of about 15x was achieved. The protein concentration was confirmed with a Sprint™ Rapid Protein Analyzer (CEM, Matthews, NC). Percent solids were analyzed using the Smart System 5 moisture/solids analyzer (CEM). The percent solids in the liquid retentate was 24.28% ± 0.60%. The liquid retentate was held overnight at 4°C.

Spray Drying

The liquid retentate was split into three equal portions. Deionized water was added to two of the portions to achieve solids levels of 25%, 18%, and 10% (wt/vol). Next, the treatments were dried in a spray dryer (model Lab 1, Anhydro Inc., Soeberg, Denmark) with an inlet temperature of 180°C, 200°C, or 220°C and an outlet temperature of 95°C. A total of nine treatments were spray dried. The dried WPC80 powders were collected in mylar bags (TF-4000, Impak Corp., Central City, SD) and stored at -80°C until subsequent analyses. The order of the treatments was structured so that a balanced experiment was conducted.

Proximate Analysis

Percent moisture of powders was measured using a vacuum oven (AOAC, 2000; method number 990.20: 33.2.44). The total fat content of powders was measured using a Modified Mojonnier ether extraction (AOAC, 2000; method number 932.06; 33.5.08). Total protein of powders was measured using the Kjeldahl method (AOAC, 2000; method number 991.20; 33.2.11) by multiplying total nitrogen by a factor of 6.38.

Descriptive Analysis

All sensory testing was performed in compliance with the North Carolina State University Institutional Review Board for Human Subjects. Spray dried WPC80 powders were rehydrated to 10% solids (wt/vol) in deionized (DI) water. The rehydrated WPC80 were dispensed into 3-digit coded soufflé cups (Solo Cup, Highland Park, Il), lidded, and tempered to 21°C. Aromatics and basic taste intensities were evaluated in duplicate by trained panelists (n = 8) using an established sensory language for dried dairy ingredients and

a 0 to 15 point universal Spectrum^{TM} intensity scale (Wright et al., 2009). Panelists were between the ages of 23 and 45 y and each had over 150 h of experience with descriptive analysis of dried dairy ingredients. Panelists expectorated samples and were provided with room temperature DI water for palate cleansing. Paper ballots or Compusense Five version 4.8 (Compusense, Guelph, Canada) were used for data collection.

Volatile Compound Analysis

Spray dried powders were collected and rehydrated to 10% solids (wt/vol) in HPLC grade water (Campbell et al., 2011a). Volatile compounds were extracted by head space-solid phase microextraction-gas chromatography-mass spectrometry (SPME-GC-MS) as described by Park et al. (2013). Volatile compounds evaluated were selected based on previously published works establishing them as important to the flavor of whey proteins (White et al., 2013; Jervis et al., 2012; Whitson et al., 2010; Wright et al., 2006). Each sample was prepared in triplicate. Compounds were identified using the National Institute of Standards and Technology (2002) mass spectral database and with authentic standards injected under identical conditions. Relative abundance was calculated for each compound using the calculated recovery of the internal standard.

Surface Free Fat and Particle Size

Surface free fat was measured by ether extraction (GEA Niro Method No.A 10a) (GEA, 2005). Particle size distribution was determined by laser diffraction (Bluewave S3500, Microtrac, Montgomeryville, PA). Measurements were made in duplicate for each WPC powder.

Statistical Analysis

Two-way analysis of variance (ANOVA) with means separation was used to analyze the data (SAS, version 9.3, SAS, Cary, NC). Differences between the sample means were analyzed by Fisher's least significant difference (LSD).

Results

Proximate Analysis

The fat, moisture, and protein contents of the spray dried WPC80 powders were consistent between replications ($p>0.05$). The fat, moisture, and protein contents for the WPC80 powders averaged 4.31% ± 0.52, 3.21% ± 0.41, and 82.7% ± 0.59 respectively on a wet weight basis (WWB).

Sensory and Volatile Compound Analyses

Volatile lipid oxidation products are the main source of off-flavors in whey proteins. Different unit operations in whey protein manufacture can affect the flavor of spray dried whey proteins such as starter culture, heat treatment, bleaching, acidification, and storage (Tomaino et al., 2004; Campbell et al., 2011b; Whitson et al., 2011; Jervis et al., 2012; Park et al., 2013). The spray drying parameters of solids concentration and inlet temperature had significant effects on the flavor of WPC80 (Tables 3.1 and 3.2) (Figure 3.1). Interaction effects between solids concentration and inlet temperature were not significant for sensory results ($p>0.05$). Overall, an inlet temperature of 180°C increased cardboard flavor intensity compared to 200°C ($p<0.05$) but not 220°C ($p>0.05$) (2.4 vs. 2.1 vs. 2.3). Inlet temperature had no effect on aroma intensity, sweet aromatic or cabbage flavors, or astringency ($p>0.05$).

A solids concentration of 10% increased aroma intensity compared to 18% and 25% ($p<0.05$) (2.3 vs. 2.1 vs. 2.0). Increasing solids concentration increased sweet aromatic flavor intensity (Not detected vs. 0.77 vs. 1.2) and decreased cardboard flavor intensity (2.8 vs. 2.1 vs. 1.9) ($p<0.05$). Cabbage flavor was only detected in WPC spray dried at 10% solids.

In contrast to sensory results, interactions between solids concentration and inlet temperature were observed for many volatile compounds. Interaction effects were significant for the following volatile compounds: hexanal, heptanal, decanal, DMTS, 1-octen-3-one, and 2,4-decadienal ($p<0.05$) (Table 3.2, Figure 3.1). In congruence with sensory results, increased inlet temperatures decreased volatile compound relative abundances in general. An inlet temperature of 220°C, compared to 180°C or 200°C, decreased concentrations of pentanal (2.75 µg/kg vs. 3.73 µg/kg vs. 3.47 µg/kg) and (Z)4-heptenal (0.827 µg/kg vs. 0.934 µg/kg vs. 1.01 µg/kg) ($p<0.05$). Increased inlet temperatures (200°C or 220°C) compared to 180°C decreased the concentration of nonanal (9.10 µg/kg vs. 7.73 µg/kg vs. 7.52 µg/kg). When spray dried at 18% or 25% solids, concentrations of hexanal and heptanal decreased with increasing inlet temperature ($p<0.05$).

WPC80 spray dried at 10% solids were very distinct from those spray dried at 18% or 25%. WPC80 spray dried at 10% solids were characterized by increased concentrations of hexanal, pentanal, octanal, nonanal, heptanal, (E)2-decenal, DMDS, and DMTS (Figure 3.1). In general, increasing solids concentration (10% vs. 18% vs. 25%) decreased concentrations of pentanal (4.57 µg/kg vs. 3.27 µg/kg vs. 2.11 µg/kg), nonanal (8.98 µg/kg vs. 8.05 µg/kg vs. 7.32 µg/kg), (E)2-decenal (0.044 µg/kg vs. 0.035 µg/kg vs. 0.023 µg/kg), DMDS (6.91

µg/kg vs. 3.61 µg/kg vs. 1.55 µg/kg), and 2,4-nonadienal (0.034 µg/kg vs. 0.031 µg/kg vs. 0.028 µg/kg). WPC80 spray dried at 25% solids had decreased relative abundances of hexanal and heptanal at each of the inlet temperatures compared to 10% or 18% ($p<0.05$). Relative abundances of decanal and DMTS decreased in WPC80 spray dried with increasing solids concentration at each of the inlet temperatures ($p<0.05$).

In general, lipid oxidation products were higher in WPC80 spray dried at 180°C and 10% solids compared to WPC80 spray dried at higher inlet temperatures and solids. These results are consistent with sensory results as the parameters 180°C and 10% solids increased cardboard and cabbage flavor intensities. There was no general differences between spray drying at an inlet temperature of 200 or 220°C in flavor intensities or volatile compounds. Spray drying at higher percent solids concentration reduced off-flavor intensities and volatile lipid oxidation products. These results demonstrate that within the parameters tested, higher temperatures and feed solids concentration should be used to decrease off-flavor intensities in WPC80. While both inlet temperature and solids concentration affected the flavor of spray dried WPC80 these results suggest that the feed solids concentration has more of an effect than that of inlet temperature.

Surface Free Fat and Particle Size

The particle sizes of the spray dried powders were distinct. Interaction effects between inlet temperature and solids concentration were not significant ($p>0.05$). An inlet temperature of 180°C decreased the average particle size compared to 200 or 220°C (36.8 µm vs. 41.0 µm vs 41.7 µm) ($p<0.05$). Average particle size increased with increasing feed

solids concentration (25.1 µm vs. 40.0 µm vs. 54.3 µm) ($p<0.05$). Both inlet temperature and feed solids concentration affected the percent surface free fat, but interactions between solids concentration and inlet temperature were not significant ($p>0.05$). An inlet temperature of 200°C increased the surface free fat compared to 220°C ($p<0.05$) (3.20% vs. 2.65%) but was not different from 180°C (2.87%) ($p>0.05$). Decreasing feed solids concentration increased the surface free fat ($p<0.05$) (1.73% vs. 2.32 % vs. 4.67%). Overall, increasing inlet temperature and feed solids concentration increased particle size and decreased surface free fat.

Discussion

Increases in cardboard and cabbage flavor intensities in whey proteins has been attributed to lipid oxidation (Croissant et al., 2009; Whitson et al., 2010; Wright et al., 2006; Jervis et al., 2012.; Kang et al., 2012). The primary volatile compounds responsible for off-flavors in whey proteins are aldehydes (Whitson et al., 2010). Antioxidant addition to WPC was reported to decrease aldehyde concentration in WPC (Liaw et al., 2010). The use of bleaching agents increased secondary lipid oxidation products in whey proteins (Jervis et al., 2012; Kang et al., 2012; Listiyani et al., 2011). Sithole et al (2006) suggested that spray drying does not create new flavors but that it intensifies flavors already present. Evans et al (2009) reported that WPC that was spray dried had decreased intensities of aroma and sweet aromatic flavor and no significant differences in volatile compounds compared to WPC that was freeze dried. The effect of spray drying parameters on solubility, denaturation, and surface composition has been investigated. MPC particles that were spray dried at lower

inlet temperatures in the range of 77°C to 178°C were found to be more spherical, had increased solubility, and lower amounts of denaturation (Fang et al., 2012). In that study, the MPC was spray dried at different inlet temperatures and outlet temperatures confounding the effect of inlet temperature alone on the MPC functionality. Kim et al. (2009) reported that both whole milk and skim milk powders spray dried at increased feed solids concentration and inlet temperatures had decreased concentrations of fat and protein on the powder surface. The results presented in this study are in congruence with those observations.

It may be intuitive to hypothesize that increased inlet temperatures increase the heat load on WPC during spray drying and therefore increase lipid oxidation. However, in the current study elevated inlet temperatures during spray drying decreased the intensity of off-flavors and many lipid oxidation volatile compounds (pentanal, hexanal, heptanal, nonanal, and (Z)4-heptenal) in WPC80. Increased inlet temperatures do not necessarily mean that the temperature of the particles is increased. During spray drying the temperature of the particle generally does not reach higher than 60°C if the parameters are chosen correctly due to the evaporative cooling of water (Schuck, 2013). Increased inlet temperatures (150-180°C) and outlet temperatures (60-80°C) during spray drying of yogurt increased the sensory acceptability (Bielecka and Majkowska, 2000; Koc et al., 2010). Bernard et al. (2011) reported that an increase of inlet temperature during the spray drying of WPC80 from 170°C to 237°C did not increase the amount of whey protein denaturation. During spray drying there are two main stages of drying that occur in the liquid droplets. First, water is evaporated at a constant rate because there is enough water to keep the droplet saturated. As the water evaporates, the size of the droplet decreases. Second, once the moisture content in

the droplet is too low, a particle is formed with an outer layer containing a crust or skin and water evaporation is decreased (Kim et al., 2009). Because elevated inlet temperatures increase the moisture removal rate the crust is formed more quickly and the particle has less time to shrink which increases the particle size (Birchal et al., 2005; Nijdam and Langrish, 2006). As such, decreased oxidation and off-flavors are not surprising with increased inlet temperatures because the heat load on each individual particle is not significantly higher and the rapid formation of a rigid crust on the exterior of the particle decreases the migration of fat and protein to the surface.

The increase in average particle size of WPC80 due to increased inlet temperature and feed solids concentration is in congruence with previously published literature with whole milk powders (Buma, 1971; Nijdam and Langrish, 2006). The decrease in surface free fat in powders with larger particles is due to a lower surface area to volume ratio of the powder particle, allowing it to encapsulate more fat (Beristain et al., 2001). The higher surface free fat in whole milk powders spray dried at lower inlet temperatures and lower solids concentrations can be more susceptible to lipid oxidation due to increased interactions with oxygen (Keogh and O'Kennedy, 1999). Although previous studies have applied these observations to whole milk powders, it seems logical that they may apply to other powders with lower fat contents and differences in surface free fat. Keogh and O'Kennedy (1999) observed an increase in lipid oxidation in whey protein stabilized emulsions with higher concentrations of surface free fat. Treatments that resulted in higher percentages of surface free fat (lower inlet temperatures and lower % solids) were those that were observed with

increased secondary lipid oxidation products along with cardboard and cabbage flavor intensities.

Conclusions

Spray drying of WPC80 at increased temperatures and solids concentrations within the parameters tested resulted in decreased off-flavor intensities. Spray drying at 10% solids increased cardboard and cabbage flavor intensities along with volatile lipid oxidation products. Spray drying with an inlet temperature of 180°C increased cardboard flavor intensity compared to 220°C. These sensory and instrumental results were also consistent with particle size and surface free fat results. Decreasing the inlet temperature decreased the particle size and increased the surface free fat. These results demonstrate that an increase in feed solids concentration and inlet temperature, within the parameters tested, decrease off-flavors in WPC80 by increasing the particle size which in turn decreases the surface free fat.

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Table 3.1 Descriptive analysis results of rehydrated WPC80 spray dried at different solids concentration and inlet temperatures

Treatment	Aroma Intensity	Sweet Aromatic	Cardboard	Cabbage	Astringency
10% 180°C	2.3[a]	[1]ND	3.1[a]	0.8[a]	2.0[a]
10% 200°C	2.2[ab]	ND	2.7[b]	0.8[a]	1.9[a]
10% 220°C	2.2[abc]	ND	2.8[b]	1.0[a]	2.0[a]
18% 180°C	2.0[bc]	1.0[ab]	2.2[c]	ND	2.0[a]
18% 200°C	2.0[c]	0.8[bc]	2.1[cd]	ND	2.0[a]
18% 220°C	2.1[bc]	0.7[c]	2.1[cd]	ND	2.0[a]
25% 180°C	2.0[bc]	1.2[a]	1.9[de]	ND	2.0[a]
25% 200°C	2.1[bc]	1.2[a]	1.8[e]	ND	1.9[a]
25% 220°C	2.1[bc]	1.2[a]	1.9[de]	ND	2.0[a]

Statistical analysis with means separations using Fisher's (LSD) with 95% confidence interval. Means in a column followed by a different letter are different ($p \leq 0.05$). [1]ND: not detected.

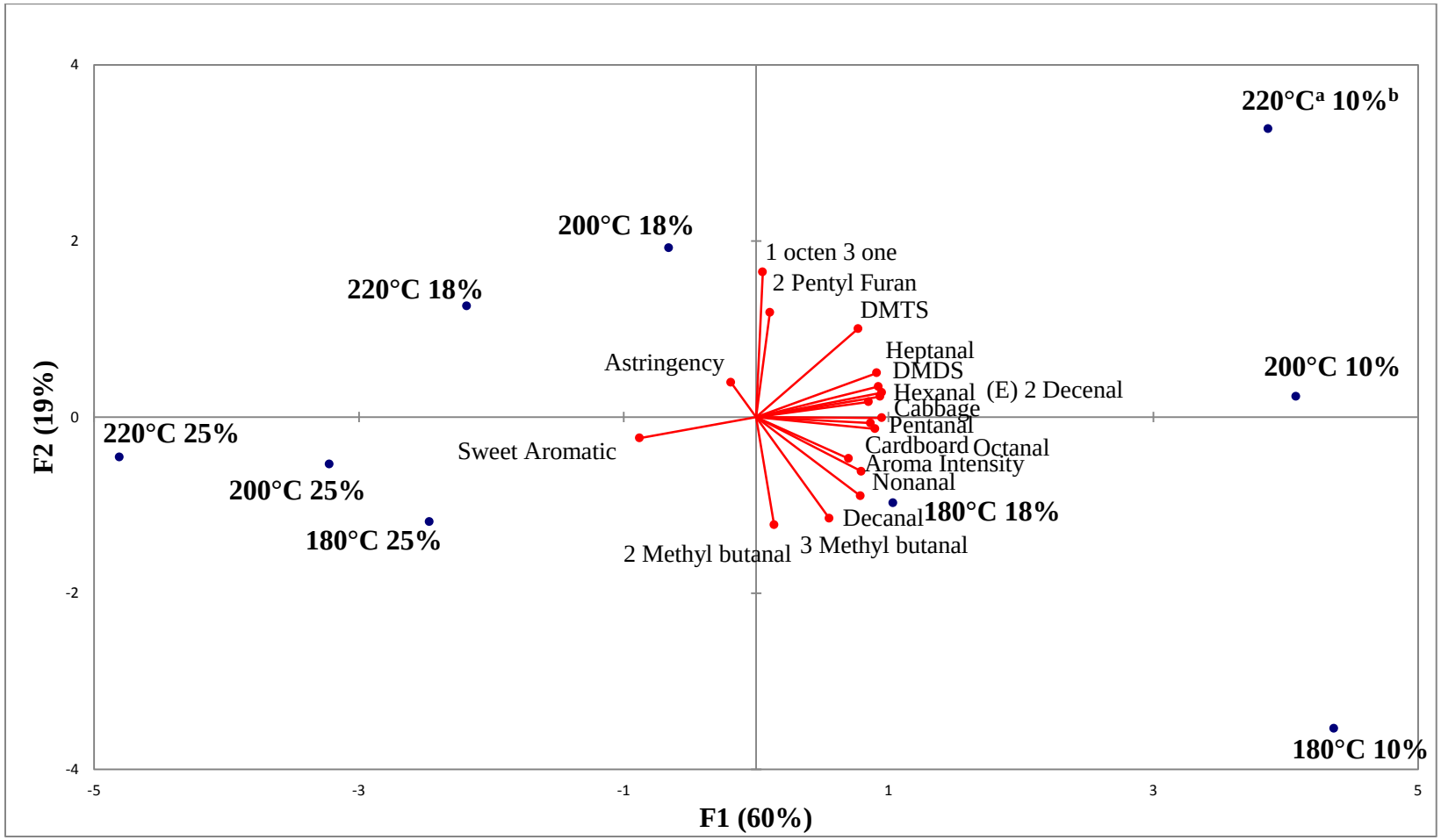

Figure 3.1 Principal component biplot of sensory and volatile compound analyses of rehydrated WPC80

[a]Denotes the inlet temperature during spray drying. [b]Denotes the percent feed solids concentration during spray drying.

Table 3.2 Relative abundance (µg/kg) of selected volatile compound in rehydrated WPC80 spray dried at different solids concentrations and inlet temperatures

Compound	180°C 10%	180°C 18%	180°C 25%	200°C 10%	200°C 18%	200°C 25%	220°C 10%	220°C 18%	220°C 25%
Pentanal	4.48^{a}	4.08^{ab}	2.63^{cd}	4.90^{a}	3.30^{bc}	2.21^{de}	4.32^{a}	2.44^{cd}	1.50^{e}
Hexanal	77.8^{b}	75.5^{b}	54.8^{c}	92.6^{a}	71.4^{b}	52.7^{c}	86.2^{a}	59.3^{c}	39.8^{d}
Heptanal	3.70^{bc}	3.75^{bc}	2.57^{ef}	4.43^{ab}	3.43^{cd}	2.59^{ef}	4.62^{a}	2.79^{de}	1.97^{f}
(Z)4-Heptenal	0.982^{abc}	0.933^{bc}	1.11^{a}	0.852^{cd}	0.899^{bcd}	1.05^{ab}	0.849^{cd}	0.761^{d}	0.871^{cd}
Octanal	2.89^{a}	2.95^{a}	2.61^{ab}	2.95^{a}	2.75^{ab}	2.52^{ab}	2.85^{a}	2.44^{ab}	2.20^{b}
Nonanal	9.96^{a}	9.34^{ab}	7.99^{abc}	8.16^{abc}	7.72^{bc}	7.32^{bc}	8.82^{abc}	7.08^{c}	6.66^{c}
Decanal	0.230^{a}	0.151^{b}	0.107^{d}	0.149^{b}	0.114^{cd}	0.104^{d}	0.137^{bc}	0.097^{d}	0.091^{d}
(E)2-Decenal	0.044^{a}	0.038^{ab}	0.024^{cd}	0.043^{a}	0.036^{ab}	0.025^{cd}	0.044^{a}	0.032^{bc}	0.020^{d}
DMTS	0.283^{bc}	0.264^{bcd}	0.146^{cd}	0.523^{a}	0.382^{b}	0.085^{d}	0.735^{a}	0.215^{cd}	0.072^{d}
DMDS	6.03^{ab}	4.40^{bc}	2.34^{cd}	7.53^{a}	3.90^{bc}	1.32^{d}	7.16^{a}	2.53^{cd}	0.990^{d}
2,4-Nonadienal	0.041^{a}	0.037^{ab}	0.031^{bcd}	0.033^{bc}	0.030^{cd}	0.028^{cd}	0.029^{cd}	0.026^{d}	0.025^{d}
1-octen-3-one	0.260^{d}	0.455^{bc}	0.445^{bc}	0.516^{bc}	0.557^{b}	0.488^{bc}	0.699^{a}	0.537^{bc}	0.423^{c}
2-methyl-3-furanthiol	0.083^{a}	0.093^{a}	0.097^{a}	0.078^{a}	0.081^{a}	0.094^{a}	0.073^{a}	0.080^{a}	0.086^{a}
2,4 Decadienal	0.040^{a}	0.018^{b}	0.010^{bc}	0.014^{bc}	0.013^{bc}	0.009^{bc}	0.012^{bc}	0.009^{bc}	0.006^{c}
3-Methyl butanal	0.344^{a}	0.313^{ab}	0.304^{ab}	0.283^{ab}	0.258^{ab}	0.241^{ab}	0.243^{ab}	0.225^{ab}	0.216^{b}
2-Methyl butanal	0.785^{ab}	0.900^{a}	0.844^{ab}	0.738^{ab}	0.605^{ab}	0.756^{ab}	0.591^{ab}	0.569^{b}	0.629^{ab}
2-Pentyl Furan	6.80^{a}	8.63^{a}	7.87^{a}	7.56^{a}	8.59^{a}	7.59^{a}	8.63^{a}	8.00^{a}	7.02^{a}

Statistical analysis with means separations using Fisher's (LSD) with 95% confidence interval. Means in a row followed by a different letter are different ($p \leq 0.05$).

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