ABSTRACT

SMITH, SAMUEL Sensory Characteristics of Whey and Whey Permeate from Different Sources and the Use of Permeates as Salt Substitutes. (Under the direction of Dr. MaryAnne Drake).

Dried whey products are valuable food ingredients, but some whey and whey permeate sources are underutilized. The flavor and flavor stability of Cheddar and Mozzarella wheys have been thoroughly researched, however, little work has been done to compare these whey sources to acid and rennet wheys. Flavor characteristics, specifically salty taste, of Cheddar and de-lactosed Cheddar whey permeates have also been evaluated, though characteristics of permeates from alternative sources have not been researched. The overall goal of this thesis was to evaluate whey and whey permeates from different sources and to establish a baseline of information for the viability of processing underutilized wheys and whey permeates into value added ingredients.

The objective of the first study was to characterize and compare flavor and flavor stability among cheese, rennet and acid wheys. Full-fat, and fat-free Cheddar, rennet and acid casein, Cottage and Greek yogurt wheys were manufactured in triplicate. Wheys were processed using standard manufacturing procedures and evaluated for compositional, volatile and descriptive analysis immediately and after 24 and 48 hours storage at 4 C. Protein, solids and fat contents were not different between wheys with the exception of Greek yogurt which only contained trace amounts of true protein and therefore a lower overall solids content. Fresh liquid wheys contained sweet aromatic and cooked milky flavors. Sweet wheys (Cheddars and rennet casein) were characterized by diacetyl/buttery and sweet aromatic flavors and sweet tastes while acid wheys (Cottage,

acid casein and Greek yogurt) contained distinct sour aromatic flavor and sour taste. All wheys increased in cardboard flavor and decreased in sweet aromatic and buttery flavors over storage. Volatile compound composition was distinct among wheys. Cultured wheys were less stable over storage than non-cultured wheys as indicated by an increase in lipid oxidation compounds. Among the cultured wheys, whey made with thermophilic starter culture (Greek) was more stable than those made with mesophilic starter culture (Cheddars and Cottage) over storage. Fat-free Cheddar was also more stable than full-fat Cheddar. These results provide baseline information for the viability of processing underutilized wheys into value-added ingredients.

The objective of the second study was to evaluate the sensory and compositional characteristics of different milk and whey permeate streams and a low sodium application made from them. Milk, Cheddar, Mozzarella, and Cottage permeates were manufactured in triplicate and de-lactosed whey permeate was obtained in triplicate from a commercial supplier. Composition (fat, protein, solids and minerals), organic acid, and volatile compound analyses were conducted on the permeates. A trained sensory panel documented sensory attributes of the permeates and cream of broccoli soup made with or without the addition of permeates or salt. Consumer acceptance testing was also performed on each of the soups. Cottage permeate was higher in lactic acid content and therefore contained a higher salty taste than all other non-reduced lactose permeates. All permeates had distinct flavor profiles, however, all permeates contained salty taste. Sweet whey permeates were characterized by sweet aromatic and milky flavors and sweet tastes while Cottage permeate contained caramel and potato/brothy flavors and sour taste. The de-lactosed permeate contained a beefy/brothy flavor the highest level of cardboard

flavor which was attributed to a higher aldehyde content. All permeates contributed salty taste to the soups. The salt control soup was perceived as the saltiest and was liked the most while the Cottage permeate soup was the least liked of all soups. All other permeate soups scored at parity for overall liking ($p<0.05$). These results demonstrate the potential for the use of permeates and de-lactosed permeates from different sources as salt substitutes in product applications.

Sensory Characteristics of Whey and Whey Permeate from Different Sources and the Use of Permeates as Salt Substitutes

by
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DEDICATION

To my parents who instilled a love of learning in me and to my wife for her never-ending support.

BIOGRAPHY

Samuel Smith was born in Corpus Christi, TX. Shortly thereafter, he moved to Wilmington, NC, where he grew up as the youngest of 5 children. In the fall of 2006, Sam began his undergraduate studies at Brigham Young University, Idaho. After freshman year, he spent two years serving on a mission for the Church of Jesus Christ of Latter-day Saints in Switzerland, Germany and Austria. Upon completion of his mission, he continued his education at Brigham Young University, Provo where he began to study Food Science. After completion of his Bachelors degree in the spring of 2012, he married Jessica Clites, a dance education major, also at BYU-Provo. At this time, Sam started his Food Science career as a quality assurance technologist for Nestle Stouffers in Springville Utah. After one year in QA, he decided to return to school in order to get a Masters in Food Science from North Carolina State University. Sam began his Masters degree in the Fall of 2013 under the direction of Dr. MaryAnne Drake.

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I would first like to thank my parents who raised me to be the man that I am now. They instilled in me a genuine love for learning and the importance of a good education. My mother always took the chance to spend time with me in the kitchen which gave me a love for cooking. From my father, I gained a genuine appreciation of the sciences. I never would have found my love for food science without them. They have always shown me their never-ending love and support throughout my entire life.

Next I would like to thank my wonderful wife who has kept me going through this whole process. She is honestly my better half and I would be lost without her.

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I also have to thank the other members of the Drake lab. They are the only ones who really know what it's like and without them, this thesis would have been impossible.

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CHAPTER 1

Literature Review

Introduction

Cheese or yogurt are manufactured by the coagulation of milk, the liquid byproduct of this process is known as whey. Cheese manufacture typically yields 1 parts cheese to 9 parts whey, therefore, a large quantity of whey is produced worldwide. The constituents of whey typically include water (approximately 93%), lactose (4.5-5% w/v), soluble proteins (0.6-0.8% w/v), lipids (0.4-0.5% w/v), and ash (0.5% w/v) though this composition varies based on the source of the whey (Kosikowski and Mistry, 1997). The proteins found in whey include β-lactoglobulin, α-lactalbumin, bovine serum albumin, and immunoglobulins, whereas, cheese is composed mostly of the milk protein casein (Moor et al., 1993). Due to its composition, whey exhibits a very high biochemical oxygen demand which, if not treated properly and is disposed of directly into streams or lakes, poses a serious threat to local aquatic wildlife (Siso, 1996). This problem and the expense associated with disposing of fluid whey led to the development of many uses for whey, particularly in the food industry.

Whey can be broken down into two different main varieties; sweet and acid wheys, though distinct differences also exist between (sweet) cultured and rennet set wheys (Campbell et al., 2011b). Sweet wheys characteristically have a pH of around 6-7, and acid wheys characteristically have a pH of less than 5 (Siso, 1996). Common sources of sweet wheys are Cheddar, Gouda, Mozzarella, Swiss cheese and rennet casein. Alternately, sources of acid whey include Cottage cheese, Greek yogurt, and acid casein.

The flavor of fluid whey changes based on cheese production parameters including milk origin, heat treatment, bacterial starter cultures, coagulation method and pH (Gallardo-Escamilla et al., 2005; Liaw et al., 2011; Campbell et al., 2011a, 2011b). Pasteurization of the fluid whey, storage and fat removal also subsequently influence flavor (Liaw et al., 2010; Whitson et al., 2011). These impacts on fluid whey flavor directly influence the flavor of any subsequent dried whey ingredient (Evans et al., 2010; Whitson et al., 2011; Jervis et al., 2012; Campbell et al., 2011b; Jervis et al., 2015). As such, flavor variability and flavor stability of fluid whey are important to maximize flavor quality of dried whey ingredients.

Usage of Whey

Whey is an important product for many food applications. In the past, much of liquid whey was treated as a waste product and used as animal feed, sprayed onto fields as fertilizer or disposed of by discharging it into water systems or through the sewer system. Presently, liquid whey is concentrated and dried to create whey powder, or is further processed using filtration techniques into spray-dried, protein-rich products known as whey protein concentrates (WPC) which contain 30-80% of total solids (w/w) protein or whey protein isolate (WPI) which contains up to 95% of total solids (w/w) protein (Siso, 1996; Moor et al., 1993). These protein-rich products are used to add nutritional value to foods and also provide functionality to foods such as gelation, foam formation, emulsification and thermal stability (Foegeding et al., 2002; Moor et al., 1993). Dried whey and whey protein products are often used in infant formulas, health foods, frozen foods, drinks, supplements, and other medical and pharmaceutical purposes (Moor et al.,

1993; Smithers et al., 2008; Eugster et al., 2012; USDEC, 2015). Deproteinized whey (also called permeate) can be further processed to extract and concentrate lactose which is most often used in the pharmaceutical industry but can also be used in the food and candy industries as well. Other uses for whey include using it for fermentation processes to produce ethanol, lactic acid, biomass and other organic chemicals (Eugster et al., 2012).

Currently, research is underway to identify uses for acid wheys including acid casein whey, Greek yogurt whey, and cottage cheese whey. Most acid whey is currently underutilized because of its high acidity of the product which leads to a highly sour taste. McGugan et al. (1979) explained that there was immense environmental pressure for the use of acid whey, though because of the undesirable flavors and sour taste, it was not used in foods. Now over 30 years later, the same environmental pressures exist and the same problems still persist. Another problem with acid whey is the difficulty manufacturers have with spray drying. Because of the high lactic acid and the lower levels of the α-monohydrate form of lactose, which is less hydroscopic, acid whey tends to stick to the walls of spray dryers. This is problematic because of the shorter production runs caused by down time required by manufacturers to clean the walls of the spray dryers. Attempts to alter the pH or mineral content of acid whey before spray drying have succeeded in creating a less hydroscopic powder that was easier to spray dry; however, these attempts produced powders with less desirable flavors (chalky, astringent, and bitter). Increasing the temperature of the spray dryer, and employing multi-stage dryers

have also helped to improve drying performance of acid whey products, though this often costs extra money in new equipment and equipment modifications (Modler and Emmons, 1977; Nassauer et al., 1996, Kosikowski and Mistry, 1997).

The recent growth of the Greek yogurt market has increased the problems associated with acid whey disposal. Greek yogurt became popular in the US around 2005. In 2012, Greek yogurt sales constituted 25% of all yogurt sales in the USA. According to the US Census Bureau (2012), yogurt sales were at 5.2 billion USD by the end of May 2011 which was a 7% growth over yogurt sales in 2009. Chandan and Kilara (2012) stated that the growth of the yogurt market was stimulated by Greek yogurt sales alone. This growth is consistent with past growth from 1980 to 2009 when total yogurt production increased from 260 million kilograms to 2 billion kilograms. That is nearly an 8 fold increase over 30 years. The US Census Bureau (2012) showed that yogurt consumption increased 400% per capita in the past 30 years. This large increase in acid whey production due to the increased manufacture of Greek yogurt further necessitates the need to find an economic use of this underutilized whey.

Whey Manufacture

Although dried and fluid whey ingredients contribute many useful functional and nutritional benefits, in order for them to be accepted for use in food applications, the flavor profile of these ingredients must not negatively affect the products in which they are used. Fresh whey and dried whey ingredients are expected to have a bland, delicate flavor that does not carry over into the finished product applications (Campbell et al.,

2011b). This is however, not the case for the majority of whey products. The flavor of the fluid whey directly influences flavor of the final spray dried product. The flavor of fluid whey is largely determined by the cheese make procedure and actual composition of the whey and therefore it is important to understand how different varieties of wheys are made.

Sweet Wheys

Sweet wheys are typically produced using the addition of rennet to cultured or uncultured, pasteurized cow's milk. Rennet cleaves the phenylalanine-methionine bond at the 105-106 position in κ-casein. κ-casein is a negatively charged, hair-like structure which surrounds the outside of the casein micelle, not allowing them to interact. When the κ-casein is cleaved, casein micelles are then capable of aggregating and forming a gel (Kosikowski and Mistry, 1997). These wheys have a higher pH than wheys made from only the addition of starter culture. Cheddar and Mozzarella cheese wheys are examples of wheys produced in this manner with the addition of mesophilic and thermophilic culture (respectively) in order to produce the specific flavors, textures and characteristics specific to the cheeses that these wheys come from. If annatto coloring was added to the cheesemilk, the whey will also contain residual annatto. Rennet casein is a non-cultured, rennet-coagulated skim milk product that produces a sweet whey similar to Cheddar and Mozzarella wheys (without the addition of starter culture).

Acid Wheys

Acid wheys are produced by reducing the pH of milk to the isoelectric point which causes the casein to aggregate and form a gel. This can either be done through the direct

addition of acid or through fermentation with the addition of starter culture. Acid casein can be produced through either of these methods. The acid produced by the starter culture replaces the hydrochloric or sulfuric acid used in the manufacture of mineral acid casein, and the casein precipitates when the pH reaches 4.6. In both cases, an acidic whey is produced. Greek yogurt and Cottage cheese wheys are other examples of acid wheys. Greek yogurt is made through the same process as traditional yogurt but typically contains an additional step at the end to increase the protein content. 21 CFR 131.200 gives the standard of identity for yogurt but there is no standard of identity for Greek yogurt specifically. Greek yogurt is made by heat treating milk to very high temperatures before homogenizing, cooling and inoculating the milk with thermophilic starter culture. The purpose of the higher heat treatment is to denature whey proteins to enhance the yogurt gel formation. After inoculation, the milk is then incubated until the pH decreases to 4.5-4.6. After incubation Greek yogurt is strained in order to remove much of the whey and increase the solids content. Traditionally, this was done by hanging the yogurt in cloth bags in a cool place for 24 to 48 hours. Modern techniques instead use centrifugation or ultrafiltration to remove the whey (Chandan and Kilara, 2012). Additionally, Instead of straining, manufacturers may also add dried ingredients such as milk or whey protein concentrates and hydrocolloids in order to achieve the desired thickness and texture. These yogurts are typically referred to as Greek-style or fortified yogurts. Depending on how the Greek yogurt is made and strained, the flavor of the yogurt, and therefore the whey varies.

Cottage cheese is an acid-set, cultured cheese (mesophilic culture) that can be produced with or without the addition of a small quantity of rennet (Teubner and Mair-Waldburg, 1998). After a long incubation time, the curds are cut and the whey is drained once a pH of 4.65-4.75 is reached. The process also includes a high cook temperature (53-57 ^{o}C) which helps to firm the curd and separate the whey.

After wheys are collected they are fat separated and pasteurized. Fat separation must be employed in order to remove excess fat from the whey. If this is not done, the fat to protein ratio will be far too high in order to make a low-fat, high-protein product such as WPC. Also, much more substrate for lipid oxidation is present which would lead to increased levels of off flavors in the final product. Pasteurization is used to kill the starter bacteria that was added to the cheese milk during the cheese making process. This must be done or else fermentation will continue during storage or further processing of the whey. After these two steps, wheys are further processed and fractionated into individual components, typically a high-protein concentrate powder in the case of sweet wheys; however processes to extract lactose and minerals can also be applied.

WPC/WPI Manufacture

Whey is commonly consumed in the form of dried WPC (25-85% protein) and WPI (>90% protein). The processing steps used to produce these products incorporate additional problems to the whey industry. Dried whey proteins (up to 80-85% protein) are produced by membrane filtration (ultrafiltration, diafiltration). Whey protein isolate then

requires an additional membrane filtration step or another process called ion exchange to further reduce fat and minerals. Ion exchange extracts whey proteins from whey by passing the whey through a column over negatively charged beads or resin which bind to the positively charged whey proteins. After absorption, a high pH solution is used to desorb the proteins and collect them (Kinsella and Whitehead, 1990; Doultani et al., 2006). Ultrafiltration processes rely on the large size of the protein molecules to separate them from smaller molecules such as lactose and minerals in the whey as the whey is passed through a filtration membrane. The protein concentrate is located in the filtration retentate, while the majority of the lactose, minerals and other small molecules pass through the membrane small pores and reside in the filtration permeate. The permeate can then be further processed to remove much of the lactose through crystallization or nanofiltration. This process creates a product called delactosed permeate which increases the mineral content of the product significantly (Kinsella and Whitehead, 1990; Kosikowski and Mistry, 1997; Frankowski et al., 2014). After filtration, the protein concentrates and permeates are typically spray dried to form a powder. Sweet whey powder is simply whey that has had water removed. This product is produced by evaporation or by reverse osmosis followed by spray drying.

If annatto coloring was used during the cheese make procedure, then residual annatto coloring will also be present in the whey in the form of the water-soluble carotenoid norbixin. The norbixin present in the whey does not contribute any flavor to the whey, however, the norbixin colorant must be removed from the whey in order to produce a

desirable white WPC or WPI (Campbell et al., 2011a). This is most commonly accomplished through chemical bleaching. The two chemicals that are currently approved by the US Food and Drug Administration include hydrogen peroxide (HP) and benzoyl peroxide (BP) though the enzyme lactoperoxidase (LP) present in milk has also been used in conjunction with small amounts of HP and thiocyanate to effectively destroy much of the norbixin in whey. All of the different bleaching methods currently used contribute oxidative compounds to the whey which is the cause of the norbixin destruction, however, these oxidative compounds lead to lipid oxidation. This results in a large increase in off flavors in the final product (Croissant et al., 2009). The use of BP to bleach whey is in controversy in the US and in other areas around the world. For instance, whey bleached with BP may not be used in infant formula and is not allowed in many countries including China and Canada. This is due to the breakdown product, benzoic acid, being considered potentially harmful (Kang et al., 2010; Campbell et al., 2012; Smith et al., 2014). In other areas, such as the EU, whey from cheese that was colored with annatto cannot be used in infant formula at all, regardless of bleaching. These countries rely on whey produced from uncolored cheeses to produce infant formula (ADPI, 2015).

Another type of dried dairy protein is serum protein concentrate (SPC). Milk which has had the milk fat globules and casein micelles removed through a microfiltration process is called milk serum. Milk serum proteins consist of the soluble milk proteins, predominantly β-lactoglobulin and α-Lactalbumin. These proteins are whey

proteins that are directly removed from milk rather than being subjected to cheese manufacture . Once removed from milk, these proteins can be concentrated like fluid whey to produce SPC. SPC is similar in composition to WPC, however, SPC does not contain glycomacropeptide, which is cleaved from the casein micelle with the addition of rennet. It also does not contain any starter cultures or chemical additions used during the cheese make and therefore it tends to have a milder flavor and distinct functional properties (Evans et al., 2009; 2010).

Whey Composition

The composition of whey is determined by several factors though the largest factor is cheese type. The composition of Cheddar, Cottage, strained yogurt and rennet casein wheys is given in Table 1.1.

The low protein content of yogurt whey is due to the high heat treatment applied to milk for yogurt manufacture. Chandan and Kilara (2012) stated that the high heat treatment denatures the whey proteins, causing them to unfold and assist with yogurt gelation. As such, whey proteins are retained with the yogurt mass. The mineral content of acid wheys such as Cottage, lactic and mineral acid casein is much higher. Calcium is at levels 2-3 times higher in acid whey than sweet whey (0.4-0.6 vs 1.2-1.6 g/L in sweet whey vs acid whey respectively) (Panesar et al., 2007). Large differences in calcium between wheys are due to the method of setting the curd. In sweet wheys, rennet is used which causes calcium caseinates to form, thus binding the calcium. In the acid precipitated wheys,

ionized (soluble) calcium is formed which favors the whey rather than binding to the curd (Wong et al., 1978; Hill et al., 1985).

Whey permeate varies in composition based on several processing parameters and cheese whey variety; however, the typical dry weight composition of whey permeate powder consists of 3-5% moisture, 65-85% lactose, 8-20% ash, 3-8% protein and less than 1.5% fat. The composition of delactosed whey permeate also varies largely based on permeate source and method and degree of lactose removal, though the process of lactose removal concentrates the other components of the permeate (USDEC, 2015).

Flavor of Fresh Liquid Wheys

Many studies have been performed on the sensory properties of Cheddar cheese whey. Flavors documented in fresh sweet wheys such as Cheddar and rennet casein include cooked milk, buttery, milky, caramelized milk, cardboard, oaty, milk fat, musty/soil-like, metallic, sweet aromatic, sour aromatic and sweet taste. (Carunchia Whetstine et al., 2003; Gallardo Escamilla et al., 2005; Liaw et al., 2010; 2011; Campbell et al., 2011b). However, flavor variation was reported between different types of wheys and between different manufacturers (Carunchia Whetstine et al., 2003; Gallardo Escamilla et al., 2005).

Figure 1.1 shows the flavor of fluid wheys from different varieties of cheese. Thirty sensory odor and flavor characteristics were documented in the eleven types of fluid whey. The flavor profile of rennet casein whey was comparable with other sweet wheys,

though differences existed. Karagül-Yüceer et al. (2003b) explained that some of the most characteristic flavors and aromas present in rennet casein were ‘cooked milky’, ‘sweet aromatic’, ‘animal/wet dog’, ‘potato/brothy’, and ‘cardboard’. Many of these flavors and others, specifically overall aroma intensity, sweet aromatic, buttery, and sour aromatic were lower in rennet casein compared to cultured sweet wheys (Campbell et al., 2011b). Acid wheys were characterized by stale, dirty, chemical, pungent, rancid, acid, astringent, chalky, metallic and chemical flavors and salty and bitter tastes (Gallardo-Escamilla et al., 2005). Little research has been performed on the sensory characteristics of Cottage cheese whey, though due to its composition and low pH it is compared with other acid whey products such as yogurt whey and acid casein whey in regards to flavor characteristics (Gallardo-Escamilla et al. 2005). Branger et al. (1999) characterized Cottage cheese whey as ‘salty’, ‘buttery’, and ‘cheesy’ though further research must be done to identify other sensory characteristics and the volatile composition of Cottage cheese whey.

Volatile composition of fresh liquid whey

The flavors in liquid whey are caused by volatile compounds that are present. Karagül-Yüceer et al. (2003a) documented the flavor of liquid Cheddar cheese whey using gas chromatography olfactometry and sensory evaluation. They documented that volatiles dimethyl triulfide, 2,3-butanedione, 2-butanol, hexanal, 2-acetyl-1-pyrroline, and methional played an important role in the flavor of liquid whey. They also reported that aldehydes hexanal, 1-octen-3-one, (Z)-and (E)-2-nonenal, (E,Z)-2,6-nonadienal, (E,E)-2,4-nonadienal, and (E,E)-2,4-decadienal may contribute to cardboard or metallic flavors.

Other volatiles in liquid wheys included ethyl acetate which is characteristic of kimchi and wine, and 2-heptanone, and 2-nonanone which are characteristic of fermented milk products. Musty and earthy flavors were documented and attributed to pyrazines such as 2-methoxy-3-isopropylpyrazine and 2-isobutyl-3-methoxypyrazine which exhibit oiled potato, and geranium earthy flavors. Other volatiles identified in Cheddar whey were 2-methyl butanal and 3-methyl butanal which have been identified to contribute malty and nutty flavors (Liaw et al., 2010). The major flavor volatiles reported in rennet casein whey were propionic acid, dimethyl sulfide, diacetyl, 2-butanedione, acetaldehyde, and 1,2 propanediene (Gallardo-Escamilla et al., 2005). Though Mozzarella, Cheddar and rennet casein wheys all contain many of the same volatile compounds, differences exist between them. Liaw et al. (2011) and Campbell et al. (2011b) reported that fresh Mozzarella and Cheddar liquid wheys had very different sensory profiles and observed higher overall aldehyde levels in Cheddar whey than Mozzarella whey. Gallardo-Escamilla et al. (2005) also documented the differences between these wheys. They documented higher levels of acetaldehyde and heptanal in Mozzarella wheys than Cheddar wheys though storage of wheys was not taken into account which would affect these results. Rennet casein was also distinct in volatile profile. Campbell et al. (2011b) documented that wheys produced without culture did not contain diacetyl (butter flavor) and were much lower in lipid oxidation products than either Cheddar or Mozzarella wheys.

Little work has been performed on the volatile compositions of various acid wheys. However, some of the characteristic volatile flavor compounds documented in acid casein whey included propan-1-ol, acetaldehyde, 2-butanone, propionic acid, heptanone and 2-furfural (Gallardo-Escamilla et al., 2005). Much of the flavor in Greek yogurt carries over into yogurt whey. Two main volatile compounds present in Greek yogurt are acetaldehyde (characteristic of green apple and plain yogurt flavors) and diacetyl (Gallardo-Escamilla et al., 2005; Hruskar et al., 1995). The formation of acetaldehyde is due to threonine metabolism in Lactobacillus bulgaricus (which is required to be present in yogurt by the FDA) via the enzyme threonine aldolase which converts threonine to glycine and acetaldehyde (Hruskar et al., 1995). Diacetyl is produced during citrate metabolism of many lactic acid bacteria (Hugenholtz, 1993). Diacetyl is in much lower concentrations in Greek yogurt than acetaldehyde. Though these two volatiles are the largest characterizing flavors in yogurt, methional, ethanol, acetoin, and butanon-2 also may contribute flavor to the yogurt mass and therefore the whey.

Off flavors that occur in whey are largely due to lipid oxidation compounds formed from fat present in milk and whey. Lipid oxidation is a complex process that is broken down into 3 distinct phases: initiation, propagation, and termination. It is an irreversible process that breaks down fats into smaller compounds such as aldehydes and ketones which impart flavors in foods. Major contributors to lipid oxidation include UV light, transition metal ions, and enzymes (Coupland and McClements, 1996). Temperature and fatty acid composition also contributed to the rate of lipid oxidation (Frankel, 1983).

Lactoperoxidase is a heat stable enzyme found intrinsically in bovine milk that can contribute to the breakdown of lipids, causing lipid oxidation as well (Campbell et al., 2012). Liaw et al. (2011) and Campbell et al. (2011b) documented differences in lipid oxidation initially and with storage between Cheddar and Mozzarella wheys based on total aldehyde concentrations in the wheys. They also documented that fat removal in Mozzarella and Cheddar wheys slowed down lipid oxidation.

Storage of liquid whey

It was originally believed that processing into dried ingredients was the cause of off flavor formation in whey; however Tomaino et al. (2004) documented that lipid oxidation initiated during the production of liquid Cheddar whey. They also reported that starter culture addition was directly related to the oxidative stability of whey (Tomaino et al., 2004). Further research confirmed that storage of liquid Cheddar and Mozzarella wheys increased cardboard flavor directly sourced to increased volatile lipid oxidation compounds (Tomaino et al., 2004; Whitson et al., 2010; Campbell et al., 2011b; Liaw et al., 2010; 2011). Campbell et al. (2011b) also documented that these changes in lipid oxidation compounds increased with cultured wheys compared to rennet set wheys and that differences documented among cultured wheys were attributed to differences with starter cultures. Storage of fluid wheys led to higher levels of cardboard flavor in wheys made from mesophilic Cheddar starter cultures compared to wheys made from thermophillic Mozzarella starter cultures. Over storage, cardboard flavor increased in liquid Cheddar whey as buttery and cooked milky flavors decreased (Campbell et al., 2011b; Liaw et al., 2011). Increased levels of volatile lipid oxidation products were

consistent with sensory data in these studies. Though aldehydes typically increased during the storage of liquid whey products due to lipid oxidation, Campbell and others (2011b) and Liaw and others (2010) reported that some aldehydes such as 2,6 nonadienal, decanal, heptanal and octanal decreased over 6 d storage. This observation was attributed to breakdown of these aldehydes into other compounds, binding with proteins, reacting with sulfur compounds, or reacting with amines to produce Schiff bases. Whitson et al. (2011) evaluated the effects of storing liquid retentate held for 48 hours before spray drying. They documented that longer liquid retentate hold times caused higher intensities of cardboard, serumy, fatty, and aroma intensities in the finished dried WPC80 and WPI compared to WPC80 and WPI made from retentate that was spray dried within 12h of manufacture. This study also confirmed that flavors in fluid wheys carryover into flavor of finished spray dried product.

Serum protein concentrate flavor

SPC is characterized by many of the same flavors as WPC and other dried dairy ingredients. Evans et al. (2009; 2010) compared spray dried SPC with spray dried WPC34 and WPC80. They documented that the SPC contained sweet aromatic, cereal, and cooked milky flavors and sweet taste, much like fresh WPC. Due to the lack of starter culture, fermentation flavors and degradation products such as diacetyl, methional (potato brothy), acetic acid (vinegar) and butanoic acid (cheesy) were absent or in lower quantities in SPC than WPC. Cardboard off flavors were also present in WPC but not SPC, signifying higher lipid oxidation rates in WPC. This was confirmed by the volatile compound composition of these two products. WPC was higher in lipid oxidation

aldehydes, hexanal, heptanal, pentanal, octanal and nonanal as identified using solid phase headspace microextraction (SPME). Peach flavored protein beverages made with SPC80 contained cereal, free-fatty acid, and soapy flavors and bitter tastes, while the same beverages made with WPC contained all of these flavors with the exception of free-fatty acid but also contained cardboard flavor. Consumer testing on protein beverages made with WPC and SPC showed that consumers preferred the SPC beverages for their aroma, appearance and mouthfeel, however, consumers preferred the WPC for overall liking and flavor.

Dried whey ingredient flavor and volatile composition

Dried whey ingredients such as WPC80 and WPI are expected to have a bland, delicate flavor much like those previously mentioned to describe fresh whey; however, this is not the case for many concentrated whey ingredients. The flavors identified in WPC and WPI included flavors both associated with and not commonly associated with dairy. The dairy flavors consisted of sweet aromatic, cooked/milky, and buttery while the non-dairy flavors consisted of cardboard, animal/wet dog, cucumber, cereal, burnt, metallic, brothy, vitamin, and soapy. Differences were documented between WPC80 made from different processing facilities, indicating that processing parameters affected the flavor of the products. Though WPC80 and WPI were similar in flavor characteristics, some differences were also documented between WPI and WPC80 powders. WPI contained animal/wet dog, soapy, and cucumber flavors and bitter taste that were not found in WPC80 (Drake et al., 2003; Carunchia-Whetstine et al., 2005). Studies have documented that many lipid oxidation and sulfur degradation products such as aldehydes and ketones

have been linked to off flavors in dried whey and milk powders. (Carunchia Whetstine et al., 2003, 2005; Drake et al., 2003; Liaw et al., 2010 Campbell et al., 2011a, 2011b**).** Whitson et al. (2010) documented the volatile chemical components of cardboard off flavor IN whey proteins and attributed it to a combination of lipid oxidation compounds, pentanal, heptanal, nonanal, 1-octen-3-one, in the presence of the sulfur degradation product, dimethyl trisulfide (DMTS). DMTS was also responsible for cabbage flavors in WPI (Wright et al., 2006). Potato/brothy flavors have been reported in fluid and dried whey ingredients and are attributed to methional (Karagul-Yuceer et al., 2003a; Mahajan et al., 2004; Carunchia Whetstine et al., 2005). Beefy/brothy flavor has been documented in Cheddar cheese and other fermented dairy products such as yogurt and sour cream and has been attributed to 2-methyl-3-furanthiol (Cadwallader et al., 2006; Shepard et al., 2013; Desai et al., 2013). The animal/wet dog flavor present in dried dairy ingredients has been linked to a combination of volatile compounds including hexanoic acid, indole, guiacol, and p-cresol (Drake et al., 2003; Karagul-Yuceer et al., 2003b).

Off flavors in dried whey ingredients tend to carry over into final product applications such as acidified beverages, however differences in beverage processing steps alter the flavor of the finished product (White et al., 2013). This study documented differences in acidified, heat treated, and acidified and heat treated WPI on the flavor of protein beverages. They documented that acidification of non-instantized WPI produced higher levels of potato/brothy flavors and sour taste and that heating increased cooked/sulfur and animal/tortilla flavors. Acidification and heating together caused beverages to increase in

cardboard, potato/brothy, soapy and malty flavors and sour taste. Evans et al. (2010) evaluated the flavor of peach beverages made with the addition of WPC80. They documented similar flavors such as cardboard, cereal, and soapy. Oltman et al. (2015) tested consumer perception of apple flavored protein beverages made with WPI and also documented sulfur/brothy, cardboard, and soapy flavors from WPI that carried over into the final beverage.

Whey Permeate Flavor and Volatile Composition

Whey permeate has many of the same flavors and volatiles present in liquid and spray dried whey protein products. Frankowski et al. (2014) documented the flavor and volatile composition of commercial whey and delactosed whey permeates. Permeates were characterized by sweet aromatic flavors and sweet and salty tastes. The salty tastes identified in permeates were higher than that of fluid whey or milk. They documented that liquid permeates had higher intensities of cooked milky and buttery flavors and sweet taste whereas spray dried powders had higher intensities of graham cracker and cardboard flavors. Delactosed whey permeates were much saltier and sourer than regular permeate products, and also contained distinct savory aromatics such as potato, beefy and vitamin flavors. The volatile composition of permeates were similar to, but in lower concentrations than that of fluid and spray dried whey. The major volatile components of permeates included aldehydes, sulfur containing compounds and diacetyl. As expected, delactosed whey permeates were higher in these compounds than regular whey permeates due to the concentration of these compounds during removal of lactose, and the longer processing and storage time required to remove lactose.

Effects of Spray Drying on Flavor and Volatile Composition

Spray drying of whey also has been attributed to flavor differences in WPC and WPI. Whitson et al. (2011) documented that dried whey concentrates and isolates were more intense in flavor than liquid whey protein retentate. The study suggested that the difference in flavor, specifically an increase in cardboard flavor, was due to spray drying. Other studies have also documented that Maillard browning reactions occur during spray drying due to the high heat levels that the whey is exposed to, which leads to flavor development (Ferretti and Flanagan, 1971;1972; Labuza and Saltmarch,1981; O'Brien and Morrissey, 1989). Whitson et al. (2011) explained that the drying process and storage of the dried whey can also add more lipid oxidation and sulfur degradation products such as dimethyl disulfide and dimethyl trisulfide. Park et al. (2014a) examined different spray drying parameters and how they affected flavor of subsequent dried dairy ingredients. Higher temperatures during spray drying (220 ^{o}C as opposed to 180 ^{o}C or 200 ^{o}C) reduced lipid oxidation volatile compounds (pentanal, hexanal, heptanal, nonanal, and (Z)4-heptenal) in WPC80. They also documented that higher feeds solids concentration resulted in a larger average particle size which decreased lipid oxidation compounds in spray dried WPC80 as well. Park et al. (2014b) documented that WPC acidified to pH 3.5 exhibited lower levels of lipid oxidation compounds and therefore lower levels of cardboard flavor. They attributed the lower levels of lipid oxidation compounds to two possible causes. First, β-LG, which is the main protein responsible for binding flavors in whey protein, exists as a monomer at low pH rather than a dimer at neutral pH. Hydrophobic regions in the interior of the protein have more solvent accessibility in the

monomeric conformation and are more readily available to bind to flavor compounds, most of which are also hydrophobic. While bound to the β-LG, the volatile compounds are less capable of being released, thus causing an overall lowering of cardboard flavor in the WPC. The second proposed mechanism for decreased cardboard flavor in WPC made from whey that was acidified was based on the higher amount of protonation at low pH of the volatile compounds which are not already bound to proteins. This causes these compounds to be less soluble in water and therefore more volatile, thus lowering the final concentration of these compounds in the finished spray-dried powder. Evans et al. (2009) evaluated differences in freeze dried and spray dried WPC. They documented that cooked flavor was lower in spray dried powders, while sweet taste was higher in freeze dried powders. They also reported that cardboard flavor was present in both varieties. Because the flavors of the two different drying methods were so similar, freeze drying is an acceptable alternative to spray drying, though spray drying is often a much less costly process and is therefore typically preferred.

Effects of Different Bleaching Methods on Flavor and Volatile Composition

Other processing parameters such as bleaching methods have also been shown to contribute off flavors to WPC. Because HP and BP are non-specific oxidizing agents, as they break down the annatto to remove the color, they also cause lipid oxidation products. Several studies have shown that bleaching causes higher levels of off flavors and higher levels of volatile lipid oxidation compounds (Croissant et al. 2009; Kang et al., 2010; Listiyani et al. 2011; 2012; Campbell et al., 2012; Jervis et al., 2012; Smith et al. 2014). Differences in efficacy, volatile compound composition and flavor exist between

different bleaching methods. HP bleached whey was higher in lipid oxidation compounds hexanal, heptanal, octanal, nonanal, decanal, 1-octen-3-one and dimethyl disulfide and cardboard and fatty off flavors than BP bleached whey (Croissant et al., 2009; Jervis et al., 2012). Jervis et al. (2012) and Listiyani et al. (2011) documented that BP bleached wheys were whiter than wheys bleached with HP, though other studies have shown that other parameters can be used to maximize bleaching with different bleaching agents (Li et al., 2012; Listiyani et al., 2012). Campbell et al. (2012) documented that whey bleached with LP and the addition of 20 mg of HP/kg at 35 and 50°C resulted in 99% norbixin destruction which was much higher than whey bleached with 250 mg of HP/kg (32 and 47% at 35 and 50°C respectively), signifying that native LP with the addition of small amounts of HP may be a viable alternative to BP or HP bleached whey. This study documented that WPC80 bleached with HP and LP both were much higher in cardboard intensity than a control WPC80 and that LP bleached WPC contained a noticeable cabbage off flavor while the HP bleached WPC contained a distinct fatty flavor. No work has been performed at this time on the effects of bleaching on different whey varieties.

Conclusion

In conclusion, there are two main varieties of whey; sweet and acid. Between these two varieties, many differences exist. Sweet wheys such as Cheddar and rennet casein whey tend to exhibit mild, ‘sweet’, ‘milky’ flavors but may also contribute off flavors such as ‘cardboard’ and ‘wet dog’ to the food system it is added to. Acid wheys, such as Cottage cheese, Greek yogurt, and acid casein wheys, are even more difficult to add to food systems because of their low pH and because of off flavors such as ‘stale’, ‘rancid’, and

‘sour aromatic’. Due to the abundance of whey in dairy production facilities, and especially the growth in some fields such as Greek yogurt, it is necessary to determine new and improved methods for utilizing whey into more novel food systems. This can be achieved if the undesirable flavors are reduced or removed through optimization of processing parameters such as bleaching and spray drying. Further research must be done on all of these whey varieties in order to better understand their chemical make-up and the degradation products that cause off flavors to occur. The objectives of this thesis were to document compositional and flavor differences in various whey and permeate products using different manufacturing procedures in order to provide a baseline for investigating their potential use in product applications.

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TABLES

Table 1.1 Composition of Cheddar, Cottage, Yogurt and Rennet Casein Wheys
([1]Kosikowski and Mistry, 1997; [2]Smith et al., 2014 [3]Eugster et al., 2012)

Component	Whey Type			
	Fluid Cheddar[1]	**Fluid Cottage[1]**	**Strained Yogurt[2]**	**Rennet Casein[3]**
Solids % w/v	6.35	6.5	5.5	6.5-6.8
pH	5.7-6.5	4.0-4.6	4.5	6.5
Fat %	0.5	0.04	0.03	0.2-0.5
Protein %	0.8	0.75	0.11	0.4-0.5
Carbohydrate %	4.85	4.9	3.9	5.0-5.2
Ash %	0.5	0.8	0.64	0.4-0.5

FIGURES

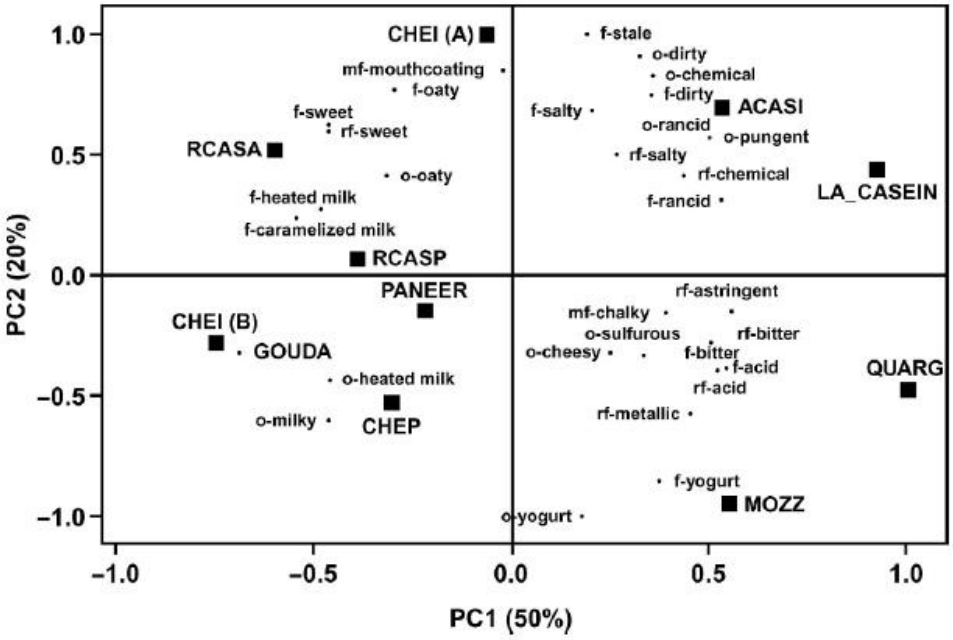

Figure 1. Results of principal component analysis of sensory attributes of wheys showing the first 2 principal components (PC1 and PC2). o- = odor, f- = flavor, mf- = mouthfeel, rf- = residual flavor (after swallowing); yogurt = natural yogurt; heated milk = heated or boiled milk. Whey samples: ACASI = acid casein; CHEI(A) = Cheddar, company A; CHEI(B) = Cheddar, company B; CHEP = Cheddar, pilot plant; GOUDA = Gouda cheese; LA_CASEIN = lactic acid casein; MOZZ = Mozzarella cheese; PANEER = Paneer cheese; QUARG = Quarg cheese; RCASA = rennet casein, company A; RCASP = rennet casein, pilot plant.

Figure 1.1 PCA diagram of flavors in 11 wheys (*Gallardo Escamilla et al., 2005)*

CHAPTER 2

Short Communication: The Flavor and Flavor Stability of Cheese, Rennet, and Acid Wheys

Short Communication: Flavor and flavor stability of cheese, rennet and acid wheys

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ABSTRACT

Dried whey ingredients are valuable food ingredients, but potential whey sources are underutilized. Previous work has established flavor and flavor stability differences in Cheddar and Mozzarella wheys, but little work has compared these whey sources to acid or rennet wheys. The objective of this study was to characterize and compare flavor and flavor stability among cheese, rennet and acid wheys. Full-fat and fat-free Cheddar, rennet and acid casein, Cottage cheese, and Greek yogurt fluid wheys were manufactured in triplicate. Wheys were fat separated and pasteurized followed by compositional analyses and storage at 4 C for 48 h. Volatile compound analysis and descriptive sensory analysis were evaluated on all liquid wheys initially and after 24 and 48 h. Greek yogurt whey contained almost no true protein nitrogen (0.02% w/v) while other wheys contained 0.58% ± 0.4% w/v true protein nitrogen. Solids and fat content were not different between wheys with the exception of Greek yogurt whey which was also lower in solids content than the other wheys (5.6% vs 6.5% w/v, respectively). Fresh wheys displayed sweet aromatic and cooked milk flavors. Cheddar wheys were distinguished by diacetyl/buttery flavors, acid wheys (acid casein, Cottage cheese, and Greek yogurt) by sour aromatic flavor. Acid casein whey had a distinct soapy flavor and acid and Greek yogurt wheys had distinct potato flavor. Both cultured acid wheys contained acetaldehyde flavor. Cardboard flavor increased and sweet aromatic and buttery flavors decreased with storage in all wheys. Volatile compound profiles were also distinct among wheys and changed with storage, consistent with sensory results. Lipid oxidation aldehydes increased in all wheys with storage time. Fat free Cheddar was more stable than full fat

Cheddar over 48 h storage. Uncultured rennet casein whey was the most stable whey as exhibited by the lowest increase in lipid oxidation products over time. These results provide baseline information for the viability of processing underutilized wheys into value-added ingredients.

Introduction

When cheese or yogurt is produced by coagulating the casein from milk, the liquid byproduct is known as whey. Whey and whey protein powders are important ingredients but may contribute undesirable flavors to finished products (Wright et al., 2009; Evans et al., 2010; Oltman et al., 2015). The flavor of fluid whey changes based on cheese production parameters including milk origin, heat treatment, bacterial starter cultures, coagulation method and pH (Gallardo-Escamilla et al., 2005; Liaw et al., 2011; Campbell et al. 2011a; 2011b). Pasteurization of fluid whey, storage and fat removal also subsequently influence flavor (Liaw et al., 2010; Whitson et al., 2011). These impacts on fluid whey flavor directly influence the flavor of any subsequent dried whey ingredient (Jervis et al., 2012; Campbell et al., 2011b; Jervis et al., 2015). As such, flavor variability and flavor stability of fluid whey are important to maximize flavor quality of dried whey ingredients.

Whey is divided into two main categories, acid whey and sweet whey, though distinct differences also exist between cultured and rennet set wheys (Campbell et al., 2011b). Fresh sweet whey flavor has been characterized by the sensory terms sweet aromatic, cooked milky, and diacetyl/buttery (Gallardo-Escamilla et al., 2005; Liaw et al., 2010; 2011). Few studies have addressed sensory properties of acid whey. Gallardo Escamilla et al. (2005) reported rancid and stale flavors in acid casein whey but the impact of storage time was not addressed so it is unclear if other confounding factors influenced the sensory properties reported. Storage of Cheddar and Mozzarella wheys increased cardboard flavor directly sourced to increased volatile lipid oxidation

compounds (Whitson et al., 2010; Campbell et al., 2011b; Liaw et al., 2011). Campbell et al. (2011b) demonstrated that these changes in lipid oxidation compounds were increased with cultured wheys compared to rennet set wheys and that differences also documented among cultured wheys were attributed to differences with starter cultures.

Little work has addressed underutilized acid wheys such as Cottage cheese and Greek yogurt wheys. A direct comparison of sweet and acid, and cultured and non cultured liquid wheys over storage time has yet to be studied and would facilitate additional applications for those products. The objective of this study was to evaluate the flavor and flavor stability of 6 different liquid wheys over 48 h storage. The whey varieties evaluated were white Cheddar, fat-free white Cheddar, rennet casein (sweet wheys), acid casein, Greek yogurt and Cottage cheese wheys (acid wheys). This study served as a baseline understanding of the sensory and stability characteristics of these 6 wheys and may also determine potential uses for Greek yogurt whey.

Methods and Materials

Each replicate of the experiment was conducted over 2 days. On day 1, 3 of the wheys were manufactured and on day 2, the other 3 wheys were manufactured. 1 lot of milk was used for each replicate of all wheys. The order of which wheys were made on which days was randomized across all 3 replicates. Approximately 75 L of milk was used for each replicate of each whey. All wheys were manufactured in triplicate. Wheys were manufactured using standard procedures (Varnam and Sutherland, 1994; McAuliffe et al., 1999; Campbell et al., 2011b; Maragkoudakis et al., 2006). Raw bovine milk was

obtained from the North Carolina State Univ. Dairy Research and Education Unit (Raleigh, NC). Wheys were manufactured from skim milk with the exception of Cheddar which was made from whole milk. Skim milk was used since it is the milk source commonly used for these whey sources. Whey from regular (whole milk) uncolored Cheddar cheese milk has been previously characterized and served as a control (Liaw et al., 2010; 2011; Campbell et al., 2011b). Milk for wheys except Greek yogurt was HTST pasteurized at 72 C for 16 s using a plate heat exchanger (model T4 RGS-16/2, SPX Flow Technology, Greensboro, N.C., U.S.A.). For Greek yogurt whey, raw skim milk was heated to 95 C for 5 min in a water jacketed pasteurization vat and then immediately cooled to 37 C using the same vat (Model MPD1050, Micro Process Design, D&F Equipment Co, McLeansville, N.C., U.S.A.). All wheys were subjected to fat separation and pasteurization at 63 C for 30 min followed by cooling to 10 C using a glycol bath. Cooled, pasteurized wheys were dispensed into autoclaved amber glass containers (VWR International P.A., U.S.A) and stored at typical refrigeration temperatures (4 C). Wheys were sampled for immediate analysis and then following 24 and 48 h in order to determine the stability of each whey.

Cheddar and fat-free Cheddar were produced in the same manner: milk was heated to 31 C and was inoculated with mesophilic starter culture (Choozit^{TM} MA 11, Danisco, New Century, N.J., U.S.A.) at a rate of 50 DCU / 454 kg milk. Calcium chloride was then added at 0.39 mL / kg of milk (50% w/v, Dairy Connection Inc., Madison, Wis., U.S.A.). The milk was ripened for 60 min under constant agitation. Milk was then coagulated for 30 min with double strength recombinant rennet (Dairy Connection)

which was added at a rate of 0.09 mL / kg of milk. The coagulum was cut into approximately 2.5 cm cubes and allowed to rest for 5 min, followed by gradual heating to 39 C over the course of 30 min with gentle agitation. Cheddar and fat-free Cheddar wheys were then drained, fat separated, pasteurized and held at 4 C.

For Cottage cheese whey manufacture, pasteurized skim milk was heated to 37 C and inoculated with mesophilic starter culture (F-DVS Fresco 1000-21, Chr. Hansen, Milwaukee, WI, U.S.A.) at a rate of 5 g / 25 L milk. After 30 min, double strength recombinant rennet (Dairy Connection) was added at a rate of 0.2 mL / 100 L of milk. The milk was then incubated for approximately 4-5 h, until a pH of 4.6 was reached. Afterwards, the curd was cut into 2.5 cm cubes and allowed to rest for 10-15 min. The curd was then heated gradually to 55 C over 90 min, drained through a sieve, separated, and pasteurized.

For Greek yogurt whey, raw skim bovine milk was heated to 95 C for 5 min in a vat pasteurizer (Model MPD1050, Micro Process Design, D&F Equipment Co, McLeansville, N.C., U.S.A.) and then cooled to 40 C and inoculated with thermophilic starter cultures (DVS YF-L702 Chr. Hansen) at a rate of 1 g / 5 L milk. The milk was then incubated at 40 C for 4 h until the pH reached 4.6. The coagulum and whey were then transferred to a plastic strainer lined with two layers of fine cheese cloth and the whey was allowed to drain into a collection container for 16 h at 4 C followed by separation and pasteurization (Maragkoudakis et al., 2006).

Both acid and rennet casein were produced without the addition of starter culture. Acid casein whey was manufactured by addition of dilute (1 N) hydrochloric acid (Sigma

Aldrich, St. Louis, Mo., U.S.A.) at a rate of approximately 3.75 L / 75 L skim milk to bring the pH to 4.6. The milk was then heated to 50 C. The whey was drained from the curd, fat separated and pasteurized. Rennet casein whey was manufactured by heating skim milk to 29 C and adding double strength recombinant rennet (Dairy Connection Inc.) at a rate of 0.09 mL / kg of milk. The milk was then allowed to rest for 1 h before the curd was cut and the whey was drained, separated and pasteurized (Varnam and Sutherland, 1994).

Proximate analyses were conducted in triplicate. Total nitrogen (TN) and non-protein nitrogen (NPN) were analyzed by Kjeldahl (AOAC 2000; method numbers 991.20;33.2.11 and 991.21;33.2.12). True protein nitrogen (TPN) for each whey was determined by subtracting NPN from TN. TPN values were obtained by multiplying nitrogen values by a conversion factor of N x 6.38 (Barbano and Lynch, 1992). Fat content was determined using Mojonnier fat extraction (AOAC 2007; method number 989.05), and total solids were determined using air oven drying (AOAC 2007; method number 990.20). The pH was measured on all wheys using a pH meter at all time points (model VWR Symphony SB70P, Radnor, P.A. U.S.A.) and titratable acidity was determined at all time points using AOAC 1996; method number 947.05-1947. Sodium, potassium, calcium, and magnesium were determined by inductively coupled plasma spectrophotometry (ICP) by the North Carolina State Univ. Analytical Services Laboratory (Raleigh, N.C.). Lactose was measured using a method modified from Upreti et al. (2006). 100 uL of each sample was extracted in 900 uL 0.013 N H_2SO_4, vortexed and centrifuged at 8000 x G for 5 min. The top layer was removed and filtered using

0.45-um nylon syringe filters (VWR International, West Chester, PA). 20 uL of each sample was injected on the HPLC (Waters 1525 Binary Pump, Waters, Milford, MA) using an autosampler (Waters 2707 Autosampler) onto the column (0.8 mL / min, 300 – x 7.8 ion exclusion, Bio-Rad Labs, Richmond, CA, 55 C). Each replicate of each whey was injected in duplicate. The temperature of the injector was 4 C and a refractive index detector (Waters 2414 Refractive Index Detectore, 30 C) was use. A standard curve was created for lactose. Standards were obtained from Sigma Aldrich (Sigma Aldrich, St. Louis, MO).

Descriptive analysis (DA) was performed on wheys at each time point. A trained panel (n = 8, 6 females, 2 males, ages 22 - 47 y) with > 100 hours of experience using an established sensory lexicon for fluid whey and whey ingredients and the Spectrum™ method evaluated the wheys (Meilgaard et al., 2007; Liaw et al., 2010, 2011). Wheys were dispensed into lidded soufflé cups and evaluated at 15 C. Each panelist evaluated each whey in triplicate. Compusense Five version 5.2 (Compusense, Guelph, Canada) was used for data collection.

At each timepoint, selected volatile compounds were extracted from wheys using headspace solid phase microextraction (SPME) and gas chromatography mass spectrometry (GC-MS). The methods were modified from Liaw and others (2010) and Campbell and others (2011b). All injections were made on an Agilent 7820A with 5975 inert MSD with a ZB-5ms (5% diphenyl / 95% dimethylsiloxane) (30 m X 0.25 mm ID X 0.25 μm) column (Phenomenex, Torrance, Calif., U.S.A.). Samples were injected using a CTC Analytics CombiPal Autosampler (CTC Analytics, Alexandria, Va., U.S.A.).

Volatile analysis samples were prepared in triplicate by adding 5 mL of liquid whey to 20 mL amber SPME vials (MicroLiter Analytical Supplies Inc., Suwanee, Ga., U.S.A.) followed by addition of 0.5 g NaCl and internal standard (10 µL of 81 ppm 2-methyl-3-heptanone in methanol; Sigma Aldrich, St. Louis, Mo., U.S.A.) to each vial. Vials were frozen at -80 C and added to the autosampler 3 at a time, 60 - 120 min prior to injection in order to reduce off flavor formation due to storage time but providing ample time for the samples to thaw. Each vial was equilibrated for 25 min at 40 C with 4 s pulsed 250 rpm agitation. All analyses for each replicate were performed using the same DVB / Carboxen / PDMS 1 cm SPME fiber (Supelco, Bellefonte, Pa., U.S.A.). The fiber was exposed in the sample vials at a depth of 3.1 cm for 40 min before it was retracted and injected at 5 cm for 5 min into the GC inlet. Single ion monitoring (SIM) was used to search for selected volatile flavor compounds based on previous studies (Karagül-Yüceer et al., 2003b; Gallardo-Escamilla et al., 2005; Campbell et al., 2011b). Scanning from 35 to 350 m/z to evaluate a mass collection of all volatiles was also simultaneously performed.

The GC method used an initial oven temperature of 40 C and after 3 min ramped to 250 C at a rate of 10 C / min and held for 5 min. SPME fibers were introduced into the split / splitless injector at 250 C and a constant flow rate of 1 mL / min was used. The purge time was set at 1 min. The MS transfer line was maintained at 250 C with the quad at 150 C and source at 250 C. Identification of compounds was performed using the NIST 2005 library of spectra and comparison of spectra of authentic standards injected under identical conditions. Relative abundance for each compound was determined using the

calculated recovery of the internal standard concentration to determine relative concentrations of each compound. An alkane series (Sigma Aldrich, Milwaukee, Wis., U.S.A.) was used to calculate retention indices (Van den dool and Kratz, 1963).

Selected volatile fatty acids were also analyzed using SPME and GC-MS for each time point using a modified method from Tomaino et al. (2004). All injections were made on an Agilent 6890N with 5973 inert MSD with a ZB-FFAP ms (30 m X .25 mm ID X .25 μm) column (Phenomenex, Torrance, Calif., U.S.A.). Samples were injected using a CTC Analytics CombiPal Autosampler (CTC Analytics, Alexandria, Va., U.S.A.). Volatile analysis samples were prepared in triplicate by adding 5 mL of liquid whey to 20 mL amber SPME vials (MicroLiter Analytical Supplies Inc., Suwanee, Ga., U.S.A.) followed by addition of internal standard (40 μL of 317 ppm heptadecanoic acid in ether; Sigma Aldrich, St. Louis, Mo., U.S.A.) to each vial. Vials were frozen at -80 C and added to the auto sampler 3 at a time, 60 - 120 min prior to injection in order to reduce off flavor formation due to storage time but providing ample time for the samples to thaw. Each vial was equilibrated for 10 min at 100 C with 4 s pulsed 250 rpm agitation before 40 min fiber exposure (22 mm vial penetration) of a 30 μm polydimethylsiloxane fiber (PDMS) (Supelco, Bellefonte, Pa., U.S.A). The fiber was then retracted and injected at 5 cm for 10 min into the GC inlet. The GC method used an internal temperature of 100 C for 2 minutes with a ramp rate of 10 C / min up to 245 C and is held for 13.5 min. Helium flow rate was 1 mL / min and the fiber was injected into the split/splitless injector at 250 C. Scanning from 35 to 350 m/z to evaluate a mass collection of all volatiles was performed. Identification of compounds was performed using the NIST 2005 library of

spectra and comparison of spectra of authentic standards injected under identical conditions. Relative abundance for each compound was determined using the calculated recovery of the internal standard concentration to determine relative concentrations of each compound.

One way analysis of variance with means separation was conducted on proximate analysis results, and two way analysis of variance (whey type by time) with means separation was conducted for sensory and volatile compounds. Principal component analysis (PCA) was also conducted on sensory and volatile data to visualize differences. Statistical analyses were conducted with XLSTAT (Version 2013.5.03 Addinsoft, New York, NY).

Results and Discussion

Proximate analysis results were consistent with previous studies (Kosikowski and Mistry, 1997; Gallardo-Escamilla et al., 2005; Campbell et al., 2011b) with the addition of Cottage cheese whey and Greek yogurt whey. All wheys contained 6.5% ± 0.4% solids with the exception of Greek yogurt whey which contained 5.6% solids ($p < 0.05$). The lower solids content in the Greek yogurt whey was attributed to its low true protein content (0.02%). The low true protein content of Greek whey was likely due to the high temperatures applied to the milk for the production of the Greek yogurt. The high heat treatment denatures the whey proteins, causing them to unfold and contribute to gel structure. As such, whey proteins are retained with the yogurt mass. This finding suggests that Greek yogurt whey is likely not a viable source of protein for concentrated whey protein applications. Sweet wheys did not differ in true protein content, averaging 0.58%

± 0.4%. Acid casein and Cottage cheese wheys were not different from each other, averaging 0.48% ± 0.4% ($p > 0.05$), though acid casein whey was lower ($p < 0.05$) in true protein compared to sweet wheys (0.45 vs 0.58% respectively). The lower true protein content of these acid wheys was attributed to the pH of these wheys being closer to the isoelectric points of the whey proteins α-lactalbumen and β-lactoglobulin (4.2 and 5.1, respectively) which would cause them to be more likely to associate with the casein micelles and be retained with the curd (Vasbinder and de Kruif, 2003). NPN values were also calculated for all wheys. Cottage cheese whey contained 0.053% NPN. Cheddar, Greek yogurt, rennet casein and fat-free Cheddar whey were not different ($p > 0.05$) in NPN (0.038 ± 0.002%), and acid casein contained 0.026% NPN. Milk typically contains approximately 0.025 to 0.028% NPN. Wheys that are produced from cheeses using rennet (Cottage, Cheddars, and Rennet casein) retain glycomacropeptide in the NPN fraction, thus increasing the NPN value of these wheys (Turhan and Etzel, 2004). Hill et al. (1985) also documented higher NPN values in cultured, low pH wheys than high pH wheys and attributed the effects to proteolysis caused by the lactic culture during the longer fermentation time. This would explain why Greek yogurt whey was higher in NPN than acid whey though neither contained rennet, and why Cottage was higher than Cheddar and rennet casein given that all three of these wheys used rennet.

Fat content of all wheys were not different, averaging 0.012% ± 0.008% ($p > 0.05$). The pH (6.5 ± 0.05 and 4.58 ± 0.12 for sweet and acid wheys, respectively) and titratable acidity (0.15 ± 0.03% for sweet wheys, 1.0% for Cottage and Greek yogurt, and 0.72% for acid casein wheys) did not increase over storage ($p > 0.05$), indicating that

there was no microbial growth over storage. Lactose concentration was not different between sweet wheys and acid casein whey, averaging 5.55% ± 0.15% ($p > 0.05$). Greek yogurt whey and Cottage whey contained lower levels of lactose (3.79 and 3.25% respectively; $p < 0.05$). This is due to the long fermentation times of these two wheys which give the lactic acid bacteria more time to convert lactose to lactic acid (Kandler, 1983). Minor differences between wheys for sodium, potassium and magnesium were observed (Table 2.1); however, large differences in calcium were documented between sweet wheys and acid wheys (0.35% ± 0.04 and 1.18% ± 0.04% respectively; $p < 0.05$). The large difference in calcium was due to the method of setting the curd. In sweet wheys, rennet is used which causes calcium caseinates to form, thus binding the calcium. In the acid precipitated wheys, ionized (soluble) calcium is formed which favors the whey rather than binding to the curd (Wong et al., 1978; Hill et al., 1985).

Consistent with previous studies, sensory differences were documented between sweet and acid wheys, and between cultured and non-cultured wheys (Gallardo-Escamilla et al., 2005; Campbell et al., 2011a; 2011b). The main effects on whey flavor were whey type and storage time (Table 2.2). Sweet wheys were characterized by sweet aromatic and cooked milky flavors and sweet tastes. Greek yogurt, Cottage cheese and acid casein wheys were characterized by sour aromatic flavor and sour and umami tastes. They also had low intensities of cooked milky flavor and sweet taste. Greek yogurt and acid wheys contained a distinct potato/brothy flavor. Greek yogurt and Cottage cheese wheys also had distinct acetaldehyde flavor and higher aroma intensities compared to other wheys. Acid casein was the only whey to display a soapy flavor initially. Little work has

documented flavors of Greek yogurt, acid casein and Cottage wheys, though many of the predominant flavors in these types of wheys (potato, sour aromatic, acetaldehyde, free fatty acid, and soapy) are flavors documented in Cottage cheese and Greek yogurt (Drake et al., 2009; Desai et al., 2013).

Wheys were expected to increase in off flavors due to lipid oxidation occurring during storage. Several studies have documented these changes in Cheddar, Mozzarella and rennet-set wheys (Campbell et al., 2011b; Liaw et al., 2011) though no work has been done on flavor of acid whey over storage. Full-fat Cheddar whey contained the highest intensities of cardboard flavor among sweet wheys following 48 h ($p < 0.05$; Table 2.2, Figure 2.1). Acid casein contained the highest intensities of cardboard flavor initially and after 48 h ($p < 0.05$). Fat-free Cheddar whey had the same amount of cardboard flavor as full-fat Cheddar whey initially, but had a lower increase in cardboard flavor than full-fat Cheddar whey. This is likely due to less free fat in the skim milk capable of oxidizing during the cheese making process. Lipid oxidation initiates during the cheesemake procedure (Campbell et al., 2011b). Efficient fat removal from whey lessens the amount of fat available to oxidize, therefore reducing the overall content of lipid oxidation products, but will not eliminate it (Liaw et al., 2011). Cheddar whey from whole milk likely had more initiation of lipid oxidation due to higher amounts of fat present in the cheese milk. All wheys increased in cardboard flavors and all sweet wheys decreased in cooked milky and sweet aromatic flavors over storage time ($p < 0.05$). Due to the lack of starter culture, rennet casein whey was the most stable with the smallest increase in cardboard flavors after 48 h and the lowest initial levels of lipid oxidation

compounds. This is consistent with previous work performed by Campbell et al. (2011b) who demonstrated that lipid oxidation was accelerated with the addition of starter culture compared to whey without starter culture.

Volatile compound analysis results were consistent with sensory results (Table 2.3, Figure 2.2). Aldehydes including hexanal, heptanal, octanal, and nonanal have been attributed to cardboard flavor in liquid whey (Karagül-Yüceer et al., 2003a; 2003b; Gallardo-Escamilla et al., 2005; Liaw et al., 2010; Whitson et al., 2010; Campbell et al., 2011b). Rennet casein whey had the lowest quantity of volatile aldehydes at all time points, consistent with sensory profiles ($p < 0.05$). Full-fat Cheddar whey contained the highest levels of volatile aldehydes of the sweet wheys after 48 h. Fat-free Cheddar contained lower levels of lipid oxidation compounds hexanal and heptanal after 48 h than full-fat Cheddar whey. All wheys had lipid oxidation compounds at time 0 though uncultured sweet and acid wheys had lower initial levels of lipid oxidation than cultured sweet and acid wheys respectively, consistent with results reported by Campbell et al. (2011b) that lipid oxidation was accelerated by the addition of starter culture. Sweet wheys and uncultured acid whey had low initial levels of volatile aldehydes, and increased levels of these compounds after 48 h (Table 2.3, Figure. 2.2). Cottage cheese and Greek yogurt wheys had the highest levels of aldehydes initially and Cottage cheese whey displayed the highest increase of volatile aldehydes between time 0 and 48 h ($p < 0.05$). Acid casein whey also increased in volatile aldehydes over 48 h ($p < 0.05$) with the largest increases in both acid casein and Cottage cheese wheys occurring between 24 and 48 h. Greek yogurt whey was fairly stable and did not increase in volatile aldehydes

as much as other acid wheys over storage ($p > 0.05$), possibly due to the 16 h required for manufacture by straining. This whey was already "older" or "stored" upon collection. Since most commercial Greek whey is manufactured by centrifugation, these storage stability results may not be indicative of current commercial Greek whey. The distinct potato / brothy flavor detected in Greek yogurt was likely due to elevated levels of methional which is a Strecker degradation product formed by the interaction of the amino acid methionine with heat initiated Maillard reaction intermediates. Methional has been identified and documented in fluid Cheddar whey and whey protein concentrates/isolates using gas chromatography-olfactometry and solvent assisted flavor evaporation but was not positively identified in this study by headspace (SPME) analysis (Carunchia Whetstine et al., 2005; Liaw et al., 2011). Cottage cheese and Greek yogurt wheys were the only wheys containing acetaldehyde, also consistent with sensory results.

Volatile free fatty acid results were also consistent with sensory results (Table 2.4). The acid wheys contained higher levels of all volatile free fatty acids than sweet wheys, consistent with higher levels of sour aromatic flavors and sour taste. Volatile free fatty acids in acid wheys did not increase over time ($p < 0.05$). Cottage whey contained the highest levels of decanoic and dodecanoic acid, which have been documented to contribute to soapy flavor (Mahajan et al., 2004).

Collectively, these results are consistent with previous studies but also contribute new findings. Gallardo-Escamilla and others (2005) noted that different volatile compound compositions determined most of the distinct sensory attributes of Cheddar, acid and rennet casein wheys. These authors did not evaluate or account for effects of

storage on flavor nor did they evaluate lipid oxidation volatiles which are primarily responsible for off flavors in whey and dried whey ingredients. Campbell et al. (2011b) and Liaw et al. (2011) reported increases in aldehydes during 4 C storage concurrent with increased off flavors in liquid whey, though only Cheddar and Mozzarella wheys were evaluated. Campbell et al. (2011b) later reported that wheys made from mesophilic Cheddar starter cultures contained higher levels of volatile aldehydes, and more off flavors than wheys made with thermophilic Mozzarella starter cultures. Greek yogurt whey was the only whey in the current study made with thermophilic starter cultures and was more stable over 48 h than Cottage cheese whey, made with mesophilic starter cultures. Gallardo-Escamilla et al. (2005) documented rancid and cheesy flavors in acid casein wheys, concurrent with results found in this study. Liquid storage time increased lipid oxidation compounds and off flavors in all wheys confirming that storage time in liquid form should be reduced in all (pasteurized) wheys prior to utilization.

This study also demonstrated that Greek yogurt whey, due to its low protein content, is not a viable source of protein for concentration. Possible uses for Greek whey could be further fractionation into lactose and minerals. Frankowski et al. (2014) demonstrated that potassium and organic acids enhanced salty taste of sweet whey permeates. Greek yogurt whey has a composition similar to permeate but with higher acidity and may be a viable salt substitute. Cottage cheese and acid casein wheys also contained distinct potato/brothy and soapy flavors initially and after storage and therefore evaluation of protein concentrate powders from these sources should be addressed with these flavors in mind. One possible emerging use of acid whey permeate following

protein separation may also be as a salt substitute. Whey permeate is currently a waste product and is often used as animal feed or fertilizer. These applications would provide a beneficial use for acid whey as a value added ingredient in product applications.

Conclusions

This study served as a baseline comparison of the sensory, volatile, and compositional characteristics of fluid sweet and acid wheys. Previous studies have demonstrated that culture addition, cheese type, processing steps and storage time all impact the flavor of liquid whey and subsequent whey powder (Gallardo-Escamilla et al., 2005; Liaw et al., 2010; Whitson et al., 2010; Campbell et al., 2011b; Whitson et al., 2011). The current study confirmed these previous differences in flavor and flavor stability of Cheddar and Mozzarella wheys and compared them to Greek yogurt, Cottage cheese, acid and rennet wheys. Cottage cheese and acid casein wheys behaved similarly to Cheddar with increases in volatile aldehyde composition and concurrent increases in off flavor intensity with storage. Greek yogurt was the most stable of the acid wheys while rennet casein was the most stable of the sweet wheys. Rennet casein produced a mild flavored whey that showed little change in flavor over storage and rennet casein whey would have good potential for use as a source of whey protein ingredients. This study provides a platform for increased evaluation of applications for non-traditional wheys and acid wheys.

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TABLES

Table 2.1. Mineral analysis results of wheys

Sample	Ca (g/L)	K (g/L)	Mg (g/L)	Na (g/L)
Acid	1.16 [a]	1.40 [a]	0.11 [a]	0.41 [bc]
Cottage	1.15 [a]	1.34 [b]	0.10 [a]	0.49 [a]
Greek	1.23 [a]	1.47 [a]	0.11 [a]	0.44 [ab]
FF Cheddar	0.31 [b]	1.18 [b]	0.06 [c]	0.34 [c]
Cheddar	0.40 [b]	1.35 [ab]	0.08 [b]	0.42 [ab]
Rennet	0.35 [b]	1.44 [a]	0.08 [b]	0.42 [ab]

means in the same column not sharing a common superscript are different ($p<0.05$).

Table 2.2. Means separations for sensory attributes with significant F-statistics and interactions from descriptive sensory analysis of liquid wheys (Whey type*Time).

		Aroma intensity	Cardboard	Potato brothy	Cooked milky	Acetaldehyde	Sour aromatic	Sweet aromatic	FFA/ Soapy	Sweet	Umami	Sour
Whey	**Acid**	2.0bc	2.2a	1.1a	1.0c	ND	0.5b	ND	1.1a	1.4b	1.8a	1.5b
	Greek	2.1ab	1.2c	1.1a	1.5b	1.2a	1.7a	ND	ND	1.2b	2.2a	2.1a
	Cottage	2.3a	1.2c	ND	1.6b	1.3a	1.6a	ND	ND	1.3b	1.9a	1.8ab
	FF Cheddar	1.9c	1.5b	ND	2.4a	ND	ND	1.0b	ND	2.0a	0.9bc	ND
	Cheddar	2.1bc	1.4b	ND	2.5a	ND	ND	1.2a	ND	1.8a	1.3b	0.5c
	Rennet	2.0bc	1.2c	ND	2.7a	ND	ND	0.8b	ND	2.0a	0.7c	ND
Time	**0**	2.1	1.1c	ND	2.2a	0.5	0.7	0.7	ND	1.7	1.5	1.0
	24	2.1	1.5b	0.5	1.8b	0.5	0.9	ND	ND	1.6	1.6	1.1
	48	2.0	1.8a	0.5	1.7b	ND	0.7	ND	ND	1.6	1.3	0.9
Interaction - Whey * Time	**Acid*Time-0**	1.9cd	2.0bc	1.2a	1.0gh	ND	ND	ND	0.8b	1.3defgh	1.7bcd	1.5bc
	Acid*Time-24	1.9cd	2.0bc	0.8a	1.2fgh	ND	0.7cde	ND	1.2a	1.5def	1.8bcd	1.5cd
	Acid*Time-48	2.2abc	2.7a	1.3a	0.8h	ND	0.6cde	ND	1.3a	1.3efgh	1.8bcd	1.6abc
	Greek*Time-0	2.1bcd	0.7j	0.8a	1.7d	1.2ab	1.8ab	ND	ND	1.2fgh	2.4abc	2.2abc
	Greek*Time-24	2.3ab	1.3efg	1.4a	1.3efg	1.5a	2.0ab	ND	ND	1.1gh	2.5ab	2.3a
	Greek*Time-48	2.0bcd	1.6def	1.2a	1.5def	0.9b	1.4abc	ND	ND	1.4defgh	1.7bcd	1.7abc
	Cottage*Time-0	2.5a	0.6j	ND	1.7d	1.6a	2.1a	ND	ND	1.1h	2.7a	2.3ab
	Cottage*Time-24	2.4ab	1.2ghi	ND	1.6de	1.0b	1.3bcd	ND	ND	1.5defg	1.7cd	1.6abc
	Cottage*Time-48	2.1bc	1.7cd	ND	1.4defg	1.2ab	1.4abc	ND	0.5b	1.4defgh	1.4de	1.5cd
	FF Cheddar*Time-0	2.1bcd	1.2fghi	ND	2.9ab	ND	ND	1.3b	ND	2.2a	0.7e	ND
	FF Cheddar*Time-24	2.0cd	1.6de	ND	2.3c	ND	0.6de	0.6d	ND	1.7bcd	1.4de	0.6e
	FF Cheddar*Time-48	1.7d	1.3efg	ND	2.6bc	ND	ND	1.0bc	ND	2.0ab	0.7e	ND
	Cheddar*Time-0	2.1bcd	0.9hij	ND	3.1a	ND	ND	2.0a	ND	2.2a	0.7e	ND
	Cheddar*Time-24	2.1bc	1.4efg	ND	2.3c	ND	0.7cde	1.0bc	ND	1.6cde	1.6d	0.7de
	Cheddar*Time-48	2.1bc	2.1b	ND	2.2cd	ND	0.5e	0.7cd	ND	1.7bcd	1.6d	0.7e
	Rennet*Time-0	2.2abc	0.9ij	ND	3.1a	ND	ND	0.8cd	ND	2.0abc	0.7e	ND
	Rennet*Time-24	1.9cd	1.2fgh	ND	2.7bc	ND	ND	0.9cd	ND	2.0ab	0.7e	ND
	Rennet*Time-48	1.8d	1.5defg	ND	2.3c	ND	ND	0.7cd	ND	2.1a	0.7e	ND

a-j means in the same column not sharing a common superscript are different ($p<0.05$).
ND = Not detected.
Means shaded in gray are not different ($p>0.05$)
Attributes were scored using a 0-15 pt universal spectrum scale (Meilgaard et al., 2007). Fluid whey attributes fall between 0-3 on this scale (Liaw et al., 2010, 2011; Campbell et al., 2011b)

Table 2.3 Means separations for selected volatile compounds identified on a ZB-5 column with significant F-statistics and interactions from volatile analysis of wheys in ppb.

Sample	hexanal	acetaldehyde	heptanal	DMS	diacetyl	2-heptenal	DMDS	2-3pentanedione	2-4dimethylfuran
Acid	6.75 c	ND	0.85 b	0.33	0.17 b	0.01	3.70	0.03 c	0.44
Cheddar	5.66 c	ND	0.99 b	0.40	3.87 b	0.01	4.53	0.04 c	0.35
FF Cheddar	1.32 c	ND	0.25 c	0.68	4.55 b	0.01	3.85	0.03 c	0.99
Cottage	37.1 b	5.73 b	10.0 a	0.54	16.8 ab	0.15	4.07	5.68 b	0.84
Greek	79.3 a	9.14 a	5.04 b	0.51	32.9 a	0.03	7.05	19.2 a	0.55
Rennet	0.94 c	ND	0.15 b	0.97	0.31 b	0.01	4.33	0.03 c	0.15
0	14.3 c	9.16	2.39	0.33	11.4	0.02	4.33	5.44 a	0.24
24	27.3 b	7.15	3.25	0.68	10.1	0.03	4.60	4.98 b	0.65
48	36.2 a	6.00	4.59	0.65	10.8	0.09	5.28	4.55 c	0.51
Whey-Acid*Time-0	3.14 cd	ND	0.51 d	0.17	0.06 b	0.01	4.76	0.05 f	0.14 b
Whey-Acid*Time-24	5.00 c	ND	0.91 cd	0.12	0.08 b	0.01	2.67	0.02 f	0.10 b
Whey-Acid*Time-48	12.1 c	ND	1.12 cd	0.71	0.37 b	0.01	3.66	0.02 f	1.09 ab
Whey-Cheddar*Time-0	3.78 cd	ND	0.15 d	0.10	1.25 ab	0.01	3.30	0.05 f	0.03 b
Whey-Cheddar*Time-24	5.77 c	ND	1.19 bc	0.75	4.71 ab	0.01	3.57	0.03 f	0.16 b
Whey-Cheddar*Time-48	7.42 c	ND	1.64 bc	0.34	5.64 ab	0.02	6.71	0.03 f	0.87 ab
Whey-FF Cheddar*Time-0	0.97 d	ND	0.02 d	0.89	5.90 ab	0.01	3.98	0.04 f	0.04 b
Whey-FF Cheddar*Time-24	1.30 d	ND	0.28 d	0.42	4.03 ab	0.01	5.50	0.04 f	2.53 a
Whey-FF Cheddar*Time-48	1.68 d	ND	0.47 d	0.74	3.73 ab	0.02	2.07	0.02 f	0.39 ab
Whey-Cottage*Time-0	12.8 bc	8.44 ab	8.04 abc	0.60	19.6 ab	0.03	4.94	6.77 d	0.45 ab
Whey-Cottage*Time-24	33.9 bc	5.48 ab	9.47 ab	0.32	14.4 ab	0.06	5.05	5.15 e	1.91 a
Whey-Cottage*Time-48	64.7 ab	3.28 b	12.5 a	0.69	16.3 ab	0.36	2.22	5.11 e	0.17 b
Whey-Greek*Time-0	51.2 abc	9.87 a	3.13 bc	0.57	35.9 a	0.02	5.31	20.3 a	0.56 ab
Whey-Greek*Time-24	90.9 a	8.82 ab	4.55 abc	0.58	31.2 ab	0.04	7.23	19.7 b	0.90 ab
Whey-Greek*Time-48	95.7 a	8.73 ab	7.44 abc	0.40	31.5 ab	0.03	8.62	17.6 c	0.18 b
Whey-Rennet*Time-0	0.87 d	ND	0.09 d	0.19	0.35 b	0.01	3.33	0.05 f	0.03 b
Whey-Rennet*Time-24	0.72 d	ND	0.13 d	1.60	0.23 b	0.01	4.49	0.03 f	0.17 b
Whey-Rennet*Time-48	1.22 d	ND	0.22 d	1.13	0.36 b	0.02	5.17	0.01 f	0.23 ab

means in the same column not sharing common lettering are different ($p<0.05$)
means shaded in gray are not different ($p>0.05$)
[1]dimethyl sulfide
[2]dimethyl disulfide

Table 2.3 (Continued)

Sample	nonanal	octanal	2-butanone	2-heptanone	2- methyl butanal	Z-4-heptenal
Acid	0.018 b	0.021 a	8.48	0.06 c	0.21 b	2.30 b
Cheddar	0.004 b	0.008 a	6.89	0.04 c	0.21 b	0.82 bc
FF Cheddar	0.005 b	0.006 b	18.62	0.025 c	0.21 b	0.62 bc
Cottage	0.125 a	0.006 b	7.64	0.80 a	0.47 ab	2.03 bc
Greek	0.036 ab	0.004 b	9.75	0.43 b	0.61 a	27.1 a
Rennet	0.003 b	0.004 b	7.69	0.02 c	0.22 b	0.55 c
0	0.015	0.009	9.81	0.27	0.20 b	1.77 b
24	0.017	0.01	7.05	0.28	0.33 ab	7.69 a
48	0.08	0.012	7.42	0.26	0.42 a	7.23 a
Whey-Acid*Time-0	0.007 b	0.005 b	8.31	0.05 b	ND	1.68 c
Whey-Acid*Time-24	0.010 b	0.005 b	8.65	0.07 b	0.32 bc	2.84 c
Whey-Acid*Time-48	0.038 b	0.008 ab	8.47	0.07 b	0.32 bc	2.39 c
Whey-Cheddar*Time-0	0.001 b	0.002 b	14.0	0.03 b	0.10 bc	0.26 c
Whey-Cheddar*Time-24	0.005 b	0.005 b	4.57	0.04 b	0.29 bc	1.76 c
Whey-Cheddar*Time-48	0.007 b	0.004 b	2.06	0.04 b	0.23 bc	0.44 c
Whey-FF Cheddar*Time-0	0.002 b	0.004 b	20.6	0.01 b	0.25 bc	0.45 c
Whey-FF Cheddar*Time-24	0.003 b	0.006 b	18.8	0.03 b	0.18 bc	0.93 c
Whey-FF Cheddar*Time-48	0.010 b	0.007 ab	16.5	0.03 b	0.19 bc	0.49 c
Whey-Cottage*Time-0	0.044 b	0.014 ab	5.50	0.79 a	0.38 bc	1.23 c
Whey-Cottage*Time-24	0.041 b	0.017 ab	6.91	0.81 a	0.51 b	1.96 c
Whey-Cottage*Time-48	0.290 a	0.023 a	10.5	0.80 a	0.52 b	2.90 c
Whey-Greek*Time-0	0.019 b	0.020 ab	10.7	0.45 ab	0.29 bc	6.83 b
Whey-Greek*Time-24	0.028 b	0.022 a	9.45	0.44 ab	0.51 b	37.4 a
Whey-Greek*Time-48	0.060 b	0.021 ab	9.13	0.40 ab	1.02 a	37.0 a
Whey-Rennet*Time-0	0.003 b	0.003 b	10.5	0.02 b	0.20 bc	0.19 c
Whey-Rennet*Time-24	0.002 b	0.004 b	5.66	0.02 b	0.20 bc	1.28 c
Whey-Rennet*Time-48	0.003 b	0.005 b	6.91	0.01 b	0.25 bc	0.18 c

Table 2.4. Means separations for selected volatile fatty acids identified on a ZB-FFAP column with significant F-statistics from volatile analysis of wheys in ppb.

Sample	Acetic	Butyric	Hexanoic	Octanoic	Decanoic	9 decenoic	Dodecanoic	Oleic
Acid	411 b	165 bc	202 bc	240 b	816 a	1999 b	2302 a	742 b
Cottage	1221 a	199 b	254 b	324 a	942 a	27285 a	2530 a	727 b
Greek	976 a	331 a	396 a	363 a	510 b	2307 b	1104 b	2043 a
Cheddar	522 b	141 bc	124 cd	80 c	223 c	319 b	716 b	ND
FF Cheddar	456 b	101 bc	91 d	67 c	270 c	379 b	1076 b	457 c
Rennet	420 b	84 c	64 d	48 c	193 c	287 b	878 b	530 c

means in the same column not sharing common lettering are different ($p<0.05$)
means shaded in gray are not different ($p>0.05$)

FIGURES

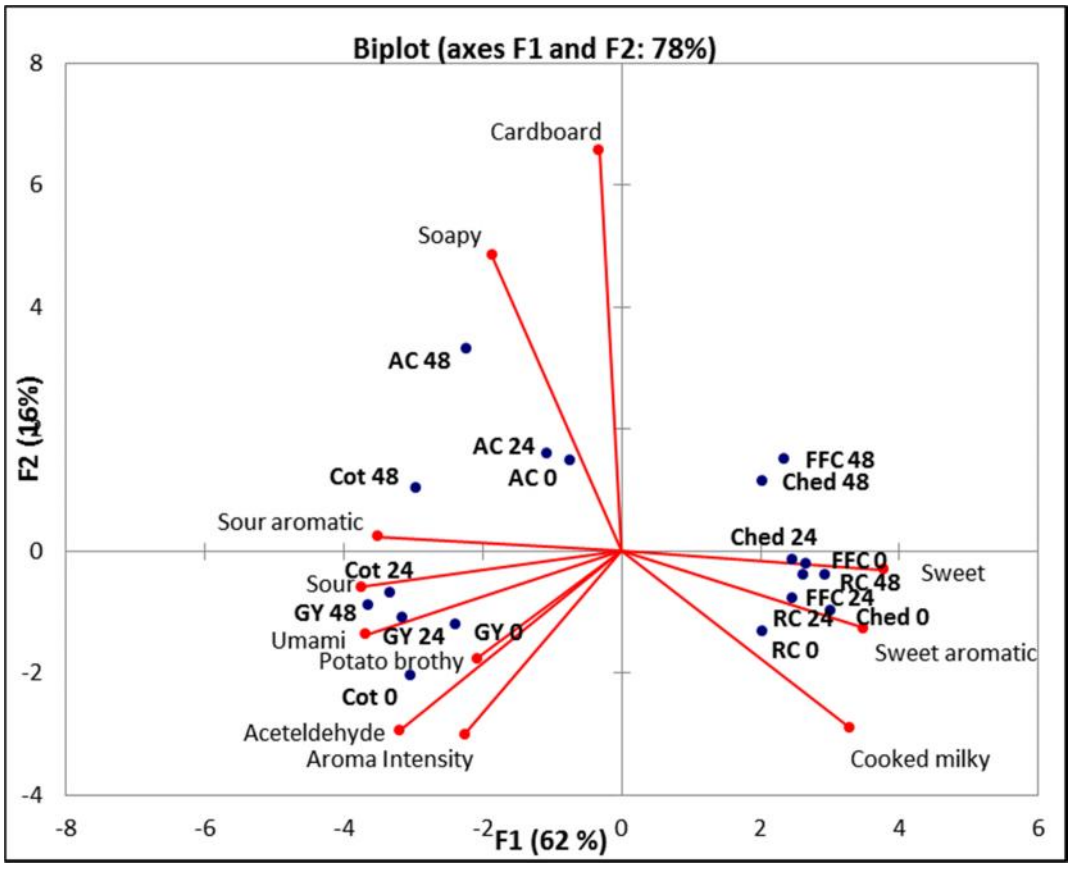

Figure 2.1. Principal component analysis (PCA) biplot of descriptive analysis of liquid wheys. *Cottage cheese (Cot), Greek yogurt (GY), Acid casein (AC), Cheddar cheese (Ched), fat free Cheddar cheese (FFC), rennet casein (RC). Numbers (0, 24, 48) following sample codes denote hours of storage.*

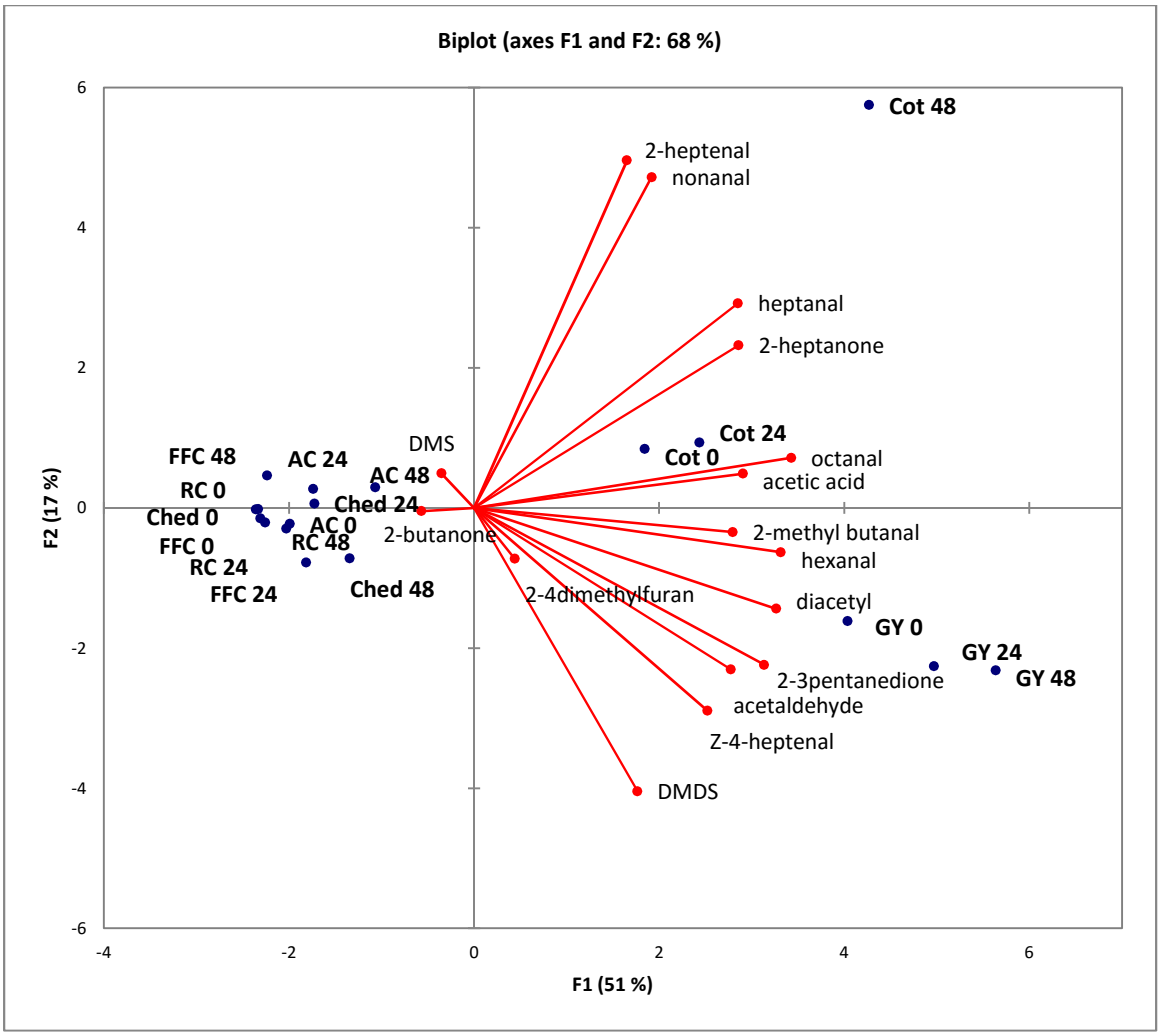

Figure 2.2. Principal component analysis (PCA) biplot of volatile compound analysis results of liquid wheys. *Cottage cheese (Cot), Greek yogurt (GY), Acid casein (AC), Cheddar cheese (Ched), rennet casein (RC). Numbers (0, 24, 48) following sample codes denote hours of storage.*

CHAPTER 3

Evaluation of Whey, Milk, and Delactosed Permeates as Salt Substitutes.

Evaluation of whey, milk, and delactosed permeates as salt substitutes.

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ABSTRACT

Whey and milk permeates are byproducts of high protein dairy powder manufacture. Previous work has shown that these permeates contribute to salty taste without contributing significantly to sodium content. The objective of this study was to explore the sensory characteristics and compositional analysis of permeates from different milk and whey streams and a low sodium product application made from them. Skim milk, Cheddar, Cottage, and Mozzarella cheese whey permeates were manufactured in triplicate, and de-lactosed whey permeate was obtained in triplicate. Composition (protein, fat, solids, minerals) was conducted on permeates. Organic acid composition was determined using high-performance liquid chromatography (HPLC). Volatile compounds were extracted from permeates by solid phase microextraction (SPME) with gas chromatography mass spectrometry (GCMS). A trained sensory panel documented sensory attributes of permeates and cream of broccoli soups with and without salt or permeates followed by consumer acceptance testing (n=105) on the soups. Cottage permeate contained a higher lactic acid content than other permeates which contributed to a higher salty taste. Cottage cheese whey permeate also contained potato/brothy and caramel flavors and sour and salty tastes while de-lactosed whey permeate had high levels of cardboard and beefy/brothy flavors and salty taste. Milk, Cheddar, and Mozzarella cheese whey permeates were characterized by sweet taste and cooked milky flavor. Permeates with higher cardboard flavor had higher levels of aldehydes. All permeates contributed to salty taste and to salty taste perception in soups; although the control soup with added salt was perceived as saltier and was preferred by consumers over permeate soups ($p<0.05$). Soup with permeate from Cottage cheese was the least

liked of all soups due to its sour taste. All other permeate soups scored at parity for liking ($p>0.05$). These results demonstrate the potential for milk, whey, and delactosed permeates from different whey streams to be used as salt substitutes in product applications.

Introduction

It is well known that the current American diet contains too much salt. The recommended daily allowance for sodium is 1500-2400 mg/day while Americans typically consume an average of 3000 mg/day, most of which comes from packaged, processed food items (USDA, 2010; Kim et al., 2012). High sodium intake levels have been linked to hypertension in adults. Hypertension, or high blood pressure, affects almost 50 million people in the US. Hypertension is a state where the blood pressure in the adult human body reaches and maintains a blood pressure level above 140/90 mmHg. Hypertension places people at higher risk for cardiovascular disease. A reduced sodium diet can lower the blood pressure of adults (Sacks et al., 2001, FDA 2010); however, studies have shown that many people don't understand all of the health risks associated with a high salt diet, and that many are resistant to change their sodium intake if it negatively impacts food flavor (Kim et al., 2012).

The food industry has developed many approaches to reduce sodium intake, though this has proven challenging because the primary driving forces behind the purchase intent and liking of foods are flavor and texture; both of which heavily correspond to salt content (Yates and Drake, 2007; Childs and Drake, 2009; Kim et al., 2012). The most common approach is by partial replacement of sodium chloride with

potassium chloride or another halide salt; however, these salts are often perceived as bitter and metallic (Murphy et al., 1981; Sinipoli and Lawless, 2012). Because of its bitter taste, KCl is often blended with NaCl which can reduce the bitter taste of the KCl (Breslin and Beauchamp, 1995; Monteiro et al., 2014). Another approach is through the use of flavor enhancers which increase the salty taste perception of product applications. Studies have demonstrated that flavor enhancers which increase salty taste perception include nucleotides such as monosodium and monocalcium glutamates, non-protein nitrogen compounds, and lactic, orotic, and citric acids (Yamaguchi and Takahashi, 1981; Roininen et al., 1996; Ball et al., 2002; Salles et al., 2002; Kang et al., 2007; Frankowski et al., 2014).

Due to the increased use of whey proteins in the food industry, whey permeate, also known as modified whey, deproteinized whey, or dairy product solids, is in abundance. Permeate, the byproduct of whey protein manufacture, contains approximately 65-85% lactose, 8-20% ash or minerals, 3-8% protein (mostly non-protein nitrogen), and no more than 1.5% fat (USDEC, 2011). The production of permeate increased by 17% per year between 2005 and 2009 (Gerdes, 2011). Currently, whey permeate produced today is mostly used in fermentation membrane bioreactors to produce lactic acid or ethanol (Mehaia and Cheryan, 1986; Gabardo et al., 2014), sprayed on fields as fertilizer, or is fed to animals (Scott and Krishnapillai, 2015; Woyengo et al., 2015). However, permeate can also be used in many food applications. Permeate works well in baked goods due to its high lactose content which is used in the Maillard reaction to produce a brown color and caramelized flavor. Permeate is also used in confectionary

icings and coatings, and dry mixes for dips and sauces as a bulking agent or milk solids replacer. Research has also demonstrated the use of Cheddar whey permeate as a salt substitute because of its high mineral and lactic acid content (Frankowski et al., 2014; USDEC, 2015).

Frankowski et al. (2014) analyzed the individual components of Cheddar whey permeate and de-lactosed permeates (DLC) from different suppliers to determine components of whey permeates that contributed to salty taste. Permeate and DLC compositions differed due to variations in processing parameters and storage. The main contributors to salty taste in permeates and DLC included NaCl, KCl, lactic acid and orotic acid, though the components that contributed the most salty taste, other than sodium, were lactic acid and KCl. Salty taste in DLC was higher than standard permeate due to minerals and organic acids concentrated during the lactose removal process. Frankowski et al. (2014) also reported flavors in permeates consistent with other whey and milk products such as cooked/milky, beefy/brothy, diacetyl, cardboard, and graham cracker. These flavors have been sourced to volatile compound components in the whey or milk (Carunchia Whetstine et al., 2003, 2005; Drake et al., 2003; Gallardo-Escamilla et al., 2005; Campbell et al., 2011b). Frankowski et al. (2014) hypothesized that permeates made from acid whey would be saltier than those made from sweet whey due to the higher organic acid composition in acid whey. The objective of this study was to compare composition and sensory differences of permeates from four different sources; milk, Cheddar, Mozzarella and Cottage cheese wheys, as well as de-lactosed permeate

(DLC). This study will help to determine appropriate ingredient applications for permeates from different whey sources.

Methods

Experimental Design

Permeates were manufactured across 2 d. On day 1, two of the permeates were manufactured, and on day 2, the other two permeates were manufactured. One lot of milk was used for each replicate of all permeates. The order of which permeates were made on which day was randomized across all three replicates. Approximately 190 L of milk was used for each replicate of each permeate. Cheddar, Mozzarella, and Cottage wheys were made using standard manufacturing procedures (Campbell et al., 2011b, McAuliffe et al., 1999). Wheys and skim milk were pasteurized, fat separated, ultrafiltered (UF), diafiltered, and then concentrated to 10-12% solids (wt/wt) using reverse osmosis (RO) and spray dried. Liquid Delac (37% solids) from Cheddar whey used in this study was obtained from a commercial supplier from three different production days. Following analytical tests, Cheddar, Mozzarella, Cottage, milk and delac permeates were evaluated with a salt and a no salt control in a cream of broccoli soup application.

Permeate production

Mozzarella and Cheddar wheys were produced in a similar manner: Raw whole bovine milk was obtained from the North Carolina State Univ. Dairy Research and Education Unit. Milk was HTST pasteurized at 72 C for 16 sec using a plate heat exchanger (model T4 RGS-16/2, SPX Flow Technology, Greensboro, N.C., U.S.A.). Milk was then heated to 31 C (Cheddar) or 35 C (Mozzarella) and inoculated with

mesophilic starter culture (ChoozitTM MA 11, Danisco, New Century, N.J., U.S.A.) at a rate of 50 DCU/454 kg milk (Cheddar) or thermophilic starter cultures (ChoozitTM LH100 and TA 61, Danisco) at rates of 20 DCU/454 kg milk for LH100 and 50 DCU/454 kg milk for TA61 (Mozzarella). Calcium chloride was then added at 0.39 mL/kg of milk (50% w/v, Dairy Connection Inc., Madison, Wis., U.S.A.). The milk was ripened for 60 min under constant agitation. Milk was then coagulated for 30 min with double strength recombinant rennet (Dairy Connection) which was added at a rate of 0.09 mL/kg of milk. The coagulum was cut into approximately 2.5 cm cubes and allowed to rest for 5 min, followed by gradual heating to 39 C (Cheddar) or 42 C (Mozzarella) over the course of 30 min with gentle agitation. Whey was then drained through a sieve and fat separated using a hot bowl centrifugal separator (Model SI600E, Agri-Lac, Miami, Fla., U.S.A). For Cottage cheese whey manufacture, pasteurized skim milk was heated to 37 C and inoculated with mesophilic starter culture (F-DVS Fresco 1000-21, Chr Hansen, Milwaukee, WI, U.S.A.) at a rate of 5g/25L milk. After 30 min, double strength recombinant rennet (Dairy Connection) was added at a rate of 0.2 mL/100L of milk. The milk was then incubated for approximately 4-5 h, until a pH of 4.6 was reached. Afterwards, the curd was cut into 2.5 cm cubes and allowed to rest for 10-15 min. The curd was then heated gradually to 55 C over 90 min, drained through a sieve, and fat separated.

Raw bovine skim milk was also obtained from the North Carolina State Univ. Dairy Research and Education Unit (Raleigh, NC). After pasteurization, milk was heated to 50 C using a water bath. Wheys and skim milk were then ultrafiltered using a pilot

scale ultrafiltration system (Model Lab 46, Filtration Engineering, Champlin, Minn., U.S.A.) using four spiral wound UF membranes (Snyder Filtration, Vacaville, Calif., U.S.A.; nominal cutoff. 10000 Da, Surface area 5.0 m^2). Deionized water was added for diafiltration at 40% of the original weight of the milk or whey. Before processing, the UF system was cleared of the soak solution (0.54% vol/vol, Hydrisoak, Hydrite Chemical, Brookfield, Wis., U.S.A.) using water until neutral pH was attained. Water was then drained from the system in preparation for the addition of whey or milk. Approximately 170 kg whey/milk was added to the system for processing. The operating inlet pressure was 210 kPa and the outlet retentate pressure was 140 kPa. Whey/milk was ultrafiltered and the permeate was collected in a holding tank. After ultrafiltration, permeates were pumped using a centrifugal pump to the RO system. The RO system used a high-pressure pump (model A96084762P10324US193, Grundfos Pumps Corporation, Olathe, KS) and two 10 cm outer diameter RO thin-film composite filter column membranes (model RO-SYN2-4040, Snyder Filtration, Vacaville, CA). Permeates were concentrated to 10-12% solids (wt/wt). Total percent solids was analyzed using the Smart System 5 moisture/solids analyzer (CEM). Concentrated permeates were spray dried (model Lab 1, Anhydro Inc., Soeberg, Denmark). Inlet temperature was 200 C and outlet temperature was 95 C. Spray dried samples were collected in mylar bags (TF-4000, Impak Corp., Central City, S.Dak., U.S.A.) and stored at -80 C until analysis.

Proximate Analysis

Proximate analyses were conducted in triplicate. Total nitrogen (TN) and non-protein nitrogen (NPN) were analyzed by Kjeldahl (AOAC 2000; method numbers

991.20,33.2.11 and 991.21;33.2.12). True protein nitrogen (TPN) for each whey was determined by subtracting NPN from TN. True protein values were obtained by multiplying nitrogen values by a conversion factor of N x 6.38 (Barbano and Lynch, 1992). Fat content was determined using Mojonnier fat extraction (AOAC 2007; method number 989.05), and total solids was determined using air oven drying (AOAC 2007; method number 990.20). The pH was measured on all permeates at 10% solids (w/v) using a pH meter (model VWR Symphony SB70P, Radnor, P.A. U.S.A.). Sodium, potassium, calcium and magnesium were determined by inductively coupled plasma spectrophotometry (ICP) by the North Carolina State Univ. Analytical Services Laboratory (Raleigh, N.C.).

Organic Acids and Lactose

Organic acids and lactose were extracted using a method modified from Upreti et al. (2006). Permeates were analyzed at 10% solids. 100 uL of each sample was extracted in 900 uL 0.013N H_2SO_4 (Sigma Aldrich, St. Louis, MO), vortexed and centrifuged at 8000xg for 5 min. The top layer was removed and filtered using 0.45-um nylon syringe filters (VWR International, West Chester, PA). Twenty uL of each sample was injected on the HPLC (Waters 1525 Binary Pump, Waters, Milford, MA) using an autosampler (Waters 2707 Autosampler) onto the column (.8mL/min, 300-x7.8 ion exclusion, Bio-Rad Labs, Richmond, CA, 55 C). Each replicate of each permeate was injected in duplicate. The temperature of the injector was 4 C and a photodiode array detector (Waters 2998) was used for organic acids and a refractive index detector (Waters 2414 Refractive Index Detectore, 30 C) was used for lactose. The maxima used for organic

acid calculation was 254 nm and 285 nm due to citric and orotic acids coeluting at 254 nm. Citric acid was not detected at 285 nm. A standard curve was created for each organic acid and lactose. Organic acid standards were obtained from Thermo Scientific Inc (Fisher Scientific Inc, Pittsburg, PA) and lactose standard was obtained from Sigma Aldrich (Sigma Aldrich, St. Louis, MO).

Descriptive Sensory Analysis of Permeates

Descriptive analysis (DA) was performed on all permeates (10% solids, w/v). Powders (manufactured permeates) were rehydrated to 10% (w/v) solids with deionized water. Liquid delac (37% w/v solids,) was diluted to 10% (w/v) solids with deionized water. A trained panel (n = 8, 6 females, 2 males, ages 22 - 49 y) each with > 100 hours of experience using an established dairy sensory lexicon and the Spectrum^{TM} method (Meilgaard et al., 2007; Frankowski et al., 2014) evaluated the permeates. Permeates were dispensed into lidded soufflé cups and evaluated at 15 C. Each panelist evaluated each sample replicate in duplicate. Unsalted crackers and room temperature deionized water were used for palate cleansing. Compusense Five version 5.6 (Compusense, Guelph, Canada) or paper ballots were used for data collection.

Volatile Compound Analysis

For each permeate, headspace solid phase microextraction (SPME) with gas chromatography mass spectrometry (GC-MS) was used to extract and separate selected volatile compounds. Methods were taken and modified from Liaw and others (2010) and Campbell and others (2011b). Injections were made on an Agilent 7820A with 5975 inert MSD with a ZB-5 ms column (30 m X .25 mm ID X .25 μm) (Phenomenex, Torrance,

Calif., U.S.A.) using a CTC Analytics CombiPal Autosampler (CTC Analytics, Alexandria, Va., U.S.A.). Three replicates of volatile analysis samples were prepared by adding 5 mL of liquid permeate (10% solids) to 20 mL amber SPME vials (MicroLiter Analytical Supplies Inc., Suwanee, Ga., U.S.A.) along with the addition of internal standard (10 µL of 81 ppm 2-methyl-3-heptanone in methanol; Sigma Aldrich, St. Louis, Mo., U.S.A.) and 0.5 g NaCl. Samples were equilibrated for 25 min at 40 C with 4 s pulsed 250 rpm agitation. Analyses were all done using the same DVB/Carboxen/PDMS 1 cm SPME fiber (Supelco, Bellefonte, Pa., U.S.A.) for each replicate of the study. The fiber was exposed at a depth of 3.1 cm for 40 min in the sample vials before being retracted and then injected at 5 cm for 5 min into the GC inlet. Based on previous studies, selected volatile compounds were searched for using single ion monitoring (SIM) (Karagül-Yüceer et al., 2003; Gallardo-Escamilla et al., 2005; Campbell et al., 2011b, Frankowski et al., 2014). Simultaneously, a mass collection of all volatiles was also evaluated by scanning from 35 to 350 m/z.

The GC method ramped the oven temperature from an initial 40 C to 250 C after 3 min at a rate of 10 C/min and held the temperature for 5 min. SPME fibers were introduced into the split/splitless injector at 250 C. A constant flow rate of 1 mL/min was used and a 1 min purge time was set. The MS transfer line was maintained at a temperature of 250 C with the quad at 150 C and source at 250 C. The NIST 2005 library of spectra and comparison of spectra of authentic standards injected under identical conditions were used for compound identification. Relative concentrations of each compound were calculated using the calculated recovery of the internal standard

concentration. Retention indices were calculated using an alkane series (Sigma Aldrich, Milwaukee, Wis., U.S.A.) (Van den dool and Kratz, 1963).

Cream of Broccoli Soup Preparation

Cream of broccoli soups used for consumer acceptance testing were prepared using a formula from the Dairy Research Institute website on permeate usage (DRI, 2015). The formula consisted of 50.2% low sodium chicken broth, 16.9% whole milk, 8.3% heavy cream, 8.2% broccoli, 4.1% unsalted butter, 4.1% diced onion, 3.0% all-purpose flour and 5.24% permeate. All percentages calculated on a w/w basis. All ingredients except permeates were purchased at a local grocery store (Raleigh, NC). For the salt control, the formula was adjusted to include 0.55% salt addition. For the no salt control, the formula omitted the addition of both salt and permeate. Soups were prepared in one large batch (approximately 42 L) on a stove top and subdivided into smaller batches before the milk, cream and permeate or salt were added to each batch.

Descriptive Sensory Analysis of Soup

Descriptive sensory analysis was performed on cream of broccoli soups made with the addition of Cheddar, Mozzarella, Cottage, milk or de-lactosed whey permeates, and salt and no salt controls. A trained panel (n = 8, 6 females, 2 males, ages 22 - 49 y) each with > 100 hours of experience using an established sensory lexicon and the Spectrum™ method (Meilgaard et al., 2007; Frankowski et al., 2014) evaluated the soups. Soups were dispensed into lidded 120 ml styrofoam cups labeled with 3 digit codes and evaluated at 60 C. Each panelist evaluated each sample replicate in duplicate.

Compusense Five version 5.6 (Compusense, Guelph, Canada) or paper ballots were used for data collection.

Consumer Acceptance Testing of Soup

Consumer acceptance testing was also performed on cream of broccoli soups made with the addition of Cheddar, Mozzarella, Cottage, milk and de-lactosed whey permeates, and salt and no salt controls. Consumer acceptance testing was approved (exempt) by the University Institutional Review Board for Human Subjects. Participants (n = 105) were self-proclaimed soup consumers who were gathered using fliers and university email listservs. Consumers were served approximately 75 mL of each soup at 60 C in 125 ml styrofoam cups labeled with three-digit blinding codes (PFS Sales Co., Raleigh, NC). The order of presentation for each of the samples was randomized and balanced among all 7 soups. Consumers were provided with unsalted crackers and ambient temperature de-ionized water as palate cleansers between samples. Consumers first gave informed consent to participate in the panel. Consumers then scored all products for appearance, overall liking, overall flavor, thickness and salty taste using a 9-pt hedonic liking scale where 9=like extremely and 1=dislike extremely. Consumers then answered a series of 5-pt just about right (JAR) questions regarding the salty taste, thickness, broccoli flavor and milky/creamy flavor where 1&2=not salty enough, too thin, too weak, and too weak respectively, 3=just about right, and 4&5=too salty, too thick, too strong and too strong respectively. A 3 min rest was enforced between each soup. After all samples were tasted, consumers were asked a series of demographic questions about themselves including gender, age, income and level of education.

Consumers received a food treat for participation. Compusense Five version 5.6 (Compusense, Guelph, Canada) was used for data collection.

Statistical Analysis

One way analysis of variance (ANOVA) with means separation was conducted on proximate analysis, sensory and volatile compounds. Principal component analysis (PCA) was also conducted on sensory and volatile data to visualize differences. Consumer panel JAR scores were evaluated using Chi-Square. Statistical analyses were conducted with XLSTAT (Version 2013.5.03 Addinsoft, New York, NY).

Results and Discussion

Proximate analysis results were consistent with previous studies and reported values (Frankowski et al., 2014; USDEC, 2011). Moisture concentration was not different between manufactured permeate samples (1.3 ± 0.54%, w/w) ($p>0.05$). True protein content was also not different between manufactured permeate samples (0.003 ± 0.003%, w/w) ($p>0.05$) though NPN values were higher in Cottage permeate than other manufactured permeates (3.6% vs. 2.5% ± 0.04%, w/w) ($p<0.05$). Delac permeate contained 37.3% ± .14% (w/w) solids, 0.01% ± .001% w/w true protein and 2.5% ± .2% w/w NPN. Higher NPN values have been reported in low pH wheys such as Cottage cheese whey due to proteolysis caused by lactic culture during the longer fermentation times of these wheys which led to higher soluble nitrogen values found in the whey (Hill et al., 1985). Potassium, magnesium and sodium concentrations were consistent between manufactured permeates, but calcium was higher in Cottage whey permeate than the other permeates ($p<0.05$) (Table 3.1). This difference was due to the method of setting of

the curd for the Cottage whey. In sweet wheys, rennet is used which causes calcium caseinates to form and bind the calcium. In the acid-precipitated Cottage whey, ionized calcium is formed which favors the whey rather than binding to the curd (Wong et al., 1978; Hill et al., 1985). Delac permeate was higher in NPN, true protein, and all minerals except calcium by 2 to 3 times ($p<0.05$). This is because the proportion of mineral solids to the rest of the solids in DLC permeates is increased as lactose is removed and is consistent with previous results (Frankowski et al., 2014). The pH was not different between Cheddar and Mozzarella permeates (6.2 ± 0.02) ($p>0.05$), pH of the milk, Cottage and delac permeates were 6.4 ± 0.02, 4.8 ± 0.03, and 5.4 ± 0.05 respectively.

The lactose concentration of DLC was the lowest of all permeates due to the mechanical removal of lactose from this permeate (Figure 3.1). The Cheddar, Mozzarella and milk permeates were higher in lactose than Cottage permeate ($p<0.05$) (Figure 3.1). The long fermentation time required to produce the Cottage whey allows for the lactic acid bacteria to convert much of the lactose to lactic acid, thus lowering the lactose content. This result also explains the higher lactic acid concentration in the Cottage permeate compared to the other permeates ($p<0.05$). Delac permeate also undergoes several processing steps to remove lactose, which allows sufficient time for the bacteria to convert lactose. Few differences were observed among citric and orotic acid results among all permeates with the largest difference observed in the DLC permeate ($p>0.05$) (Figure 3.2). Differences documented were likely a result of different starter cultures used or different rates of starter culture metabolism due to the cheese make procedures which

would produce organic acids at different rates (Kandler, 1983). The low citric acid levels in delac permeate were likely caused by the extra time required to remove the lactose in this permeate, which provided time for bacteria to utilize the citric acid for metabolism (Llano and Cuesta, 2008).

Trained panel profiling on permeates demonstrated that each permeate had distinct flavors, characteristic of the whey sources that they were made from or from milk in the case of milk permeate (Figure 3.3, Table 3.2). Delac permeate was the saltiest of the permeates followed by Cottage ($p<0.05$) and both permeates also had sour and umami tastes. Though all permeates were salty, the higher salty taste in the DLC and Cottage permeates was attributed to their mineral and acid compositions. The sodium, potassium and calcium concentrations and high levels of lactic acid all contributed to salty taste in the permeates (Frankowski et al., 2014; Lawless et al., 2003). Studies have also shown that the sweet taste of lactose suppresses salty taste, therefore the salty taste of the Cottage and delac permeates were expected to be higher than the Cheddar, Mozzarella and milk permeates due to their lower lactose concentrations (Frankowski et al., 2014; Pangborn 1959). The sour taste of the Cottage and delac permeates was due to the much lower pH of the Cottage permeate and the high levels of lactic acid present in these two permeates. Cheddar, milk and Mozzarella permeates were characterized by sweet aromatic flavors and sweet tastes, with low levels of cardboard flavor in the Cheddar and Mozzarella permeates. Consistent with previous studies on fluid whey, Mozzarella, Cottage and milk permeates had no or lower cardboard flavor than Cheddar with the Cottage and Milk permeates exhibiting no cardboard flavor at all. This result has been

attributed to the starter culture used in the cheese manufacture procedure (Campbell et al., 2011a; 2011b). Milk permeate did not contain any added starter culture and Mozzarella and Cottage used thermophilic starter cultures. Wheys produced without starter culture have been shown to exhibit lower cardboard flavors than wheys made with starter culture, and wheys made from thermophilic starter cultures have been documented as having lower levels of lipid oxidation compounds than those from mesophilic starter culture (Campbell et al., 2011a; 2011b). In addition to salty, sour and umami tastes, Cottage permeate was also characterized by potato brothy and caramel flavors, possibly due to the higher heat profile during cheese manufacture. Vitamin/brothy flavor was documented in the DLC permeates. Frankowski et al. (2104) previously evaluated de-lactosed permeates and reported similar results.

Volatile composition of permeates was consistent with sensory results (Table 3.3, Figure 3.4) and characteristic of those found in fluid whey and whey products (Carunchia Whetstine et al., 2003; 2005; Frankowski et al., 2014; Gallardo-escamilla et al., 2005; Karagul-Yuceer et al., 2003). All permeates contained lipid oxidation compounds which contribute to off flavors, delac permeate contained the highest levels of aldehydes, hexanal, octanal and decanal, which have been correlated with cardboard flavor ($p<0.05$) (Karagül-Yüceer et al., 2003; Gallardo-Escamilla et al., 2005; Liaw et al., 2010; Whitson et al., 2010; Campbell et al., 2011b). Aldehydes increase in liquid whey products with storage time and high heat processing steps (Vazquez-Landaverde et al., 2005; Liaw et al., 2011; Whitson et al., 2011). Delac permeate undergoes high heat treatment and longer processing time in order to remove the lactose, which likely caused the increased

aldehyde load. Cheddar, Milk, Mozzarella and Cottage permeates had lower levels of aldehydes, consistent with lower levels of cardboard off flavors. Cottage permeate contained higher levels of methional than other permeates ($p<0.05$), consistent with the low but distinct potato flavor of this permeate (Carunchia Whetstine et al., 2005; Liaw et al., 2011).

Trained panel profiling was performed to characterize the flavor and basic taste profiles of the cream of broccoli soups made with the addition of each of the permeate varieties and a salt control and a no-salt control (Table 3.4). Soups made with permeate had an approximately 60% decrease in sodium content. This varied slightly between samples based on sodium content of the permeates. Sensory results showed that each soup had a distinct flavor profile determined by the permeate added. All soups had similar aroma intensities and equal levels of vegetable/brothy and milky/creamy flavors ($p>0.05$), therefore the differences noted in the soups were attributed to differences in their basic taste profiles. The sweet taste of the Cheddar, Mozzarella and milk permeate soups was increased due to the addition of these permeates which contain high levels of lactose. The sour taste was much higher in the Cottage permeate soup than the other permeate soups ($p<0.05$), which was also expected because of the sour taste of this permeate which carried over into the soup. The sour taste of the Cheddar, Mozzarella, milk and delac permeate soups was lower than the no-salt control ($p<0.05$). This was due to the sweet taste of these permeates, suppressing the natural, slightly sour taste of the soup (Frankowski et al., 2014; Pangborn 1959). The umami taste was higher in the Cheddar, Mozzarella, Cottage and milk permeate soups than the two control and DLC

soups ($p<0.05$). The salt control was the saltiest of all of the soups, followed by the Cottage and DLC permeates, though all of the permeate soups were saltier than the no-salt control ($p<0.05$). A low but distinct metallic/bitter taste in the Cottage and DLC soups was also noted which carried over from the permeates.

Consumer acceptance testing was conducted with 105 consumers on the cream of broccoli soups. A salt control and a no-salt control were also evaluated alongside the permeate soups to serve as baselines for comparison. Not surprisingly, consumers scored the salt control the highest for overall liking, flavor liking, thickness liking and salty taste liking ($p<0.05$) (Table 3.5). This soup was the most similar to what the consumers expected the soup to taste like, and therefore, they liked it the most. The Cheddar, Mozzarella, milk and DLC permeate soups scored higher than the no salt control, which was liked more than the Cottage permeate soup. Consumers also scored the Cottage permeate soup lowest in salty taste liking. The low scores given to the Cottage permeate soup were likely due to the overall negative perception that consumers had for this soup rather than an actual lack of salty taste. Trained panel results documented that this soup was saltier than the other permeate soups which also scored higher than the Cottage permeate soup in salty taste liking. Studies have shown that disconfirmed consumer expectations for products negatively impact consumer perceptions of that product (Cardello at al., 1985; 1992). Because the Cottage permeate soup was sour, and consumers did not expect that a creamy soup would be sour, they did not like the soup. Because the Cottage whey permeate was saltier (by trained panel) than other sweet whey and milk permeates, another more congruent soup flavor with the accompaning sour taste

(e.g. cheese or broccoli cheese) may be more acceptable. The sweet taste of the Cheddar, Mozzarella and milk permeate soups identified by trained panelists also likely had an effect on the overall liking of these soups. Panelists likely did not expect that level of sweetness in these soups and this may have decreased the overall liking of these soups.

Consumers also evaluated the soups for salty taste, broccoli flavor, thickness, and milky/creamy flavor on a 5 pt. JAR scale, which indicated whether these attributes were too high, JAR, or too low for each soup (Table 3.6). The JAR scores indicated that a large proportion of consumers considered the salt control and Cottage permeate soups to be 'too salty' (33.3% and 25.7% respectively), whereas an even larger portion of consumers considered the Cottage permeate to be 'not salty enough' (41.0%) ($p<0.05$). This indicates that consumers may have been confused about the salty taste of the Cottage permeate soup with the other flavors/tastes (eg sour taste) of the soup and were unsure of how to score it. A higher number of panelists scored the no-salt control soup as being 'not salty enough' than the Cheddar, Mozzarella, milk, and Cottage permeate soups (57.1% vs. 41.0%, 45.7%, 42.9% and 41.0% respectively), further confirming that consumers perceived saltiness in these permeate soups was higher than in the no-salt control ($p<0.05$). An equal number of panelists scored the Cheddar, Mozzarella, and milk permeate soups as JAR in salty taste as the salt control ($p>0.05$), further evidence that the addition of the permeate increased the acceptability of the salty taste of this product application. Consumers also evaluated the broccoli flavor of the soups using the same scale. The results showed that fewer panelists considered the broccoli flavor to be 'too weak' for the Cheddar, Mozzarella and milk permeate soups than the no-salt control.

Because salty taste enhances the flavor of foods, it was expected that the salty taste would increase consumer perception of other flavors in the soup in addition to salty taste. All soups were at parity with one another for the thickness JAR question, indicating that all samples were consistent in perceived thickness ($p>0.05$). A higher number of consumers scored the Cottage, DLC and to a lesser extent no-salt control samples as being 'too weak' for milky/creamy flavor compared to the Cheddar, milk, Mozzarella and salt control samples ($p<0.05$). This result is likely due to the lack of sweet taste in these soups, especially in the Cottage and DLC varieties which panelists would perceive as milky/creamy flavor.

Conclusions

Permeates from different sources were viable options for use as salt substitutes. All permeates were salty but the Cottage permeate was the saltiest of the non-reduced lactose permeates due to its higher lactic acid and lower lactose concentrations than the other permeates. The Cottage permeate was also sour and contained a potato/brothy flavor. Milk, Mozzarella and Cheddar permeates all contained sweet taste and cooked milky flavor. DLC permeate contained the highest levels of salty taste due to the high proportions of minerals and organic acids caused by the removal of lactose. DLC permeate also contained the highest cardboard flavor of the permeates, attributed to the high aldehyde content in this permeate. As expected, permeates contributed specific flavors and tastes into the soups depending on the characteristics of the permeates and therefore special consideration must be taken when choosing the permeate source for a particular application. The milk, Mozzarella, and Cheddar permeates contributed a milky

flavor and sweet and salty tastes to the soup. The Cottage permeate contributed the highest level of salty taste of the non-reduced lactose permeates, however, this permeate also contributed a sour taste which consumers disliked with this flavor soup. This permeate would work well in a product application where sour taste is also desired or expected. Consumers preferred the salt control soup over the permeate soups; however, the addition of Cheddar, Mozzarella, Milk and DLC permeates increased the overall liking and salty taste scores over the no-salt control and provided a significant decrease in the sodium content of the soups. Because of the high salty taste profiles of the Cottage and DLCrmeates, they are best suited for use as salt substitutes in product applications. Future work on these permeates should focus on optimizing processing parameters to reduce/minimize cardboard flavor and perhaps reduce sour taste of Cottage permeate or to identify ideal applications for flavoring systems to enhance liking of reduced sodium permeate applications.

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TABLES

Table 3.1. Mineral composition of permeates (% w/w)

Permeate	%Ca	%K	%Mg	%Na
Cottage	2.09 a	2.51 b	0.19 ab	0.66 b
Cheddar	0.50 c	2.43 bc	0.13 c	0.65 bc
Milk	0.40 d	2.35 c	0.12 d	0.62 c
Mozzarella	0.48 c	2.51 b	0.13 c	0.66 b
Delac	0.84 b	7.54 a	0.27 a	1.85 a

a-d means in the same column not sharing a common superscript are different ($p<0.05$)

Table 3.2. Descriptive analysis results of permeates (10% solids)

Sample	Aroma Intensity	Sweet Aromatic	Cardboard	Potato Brothy	Beefy Brothy	Caramel	Sweet	Sour	Salty	Umami
Cottage	2.2a	1.0c	ND	0.5b	ND	1.7	ND	3.5a	3.3b	3.2a
Cheddar	1.8b	1.7ab	1.0b	ND	ND	ND	3.1ab	ND	1.8cd	1.1bc
Milk	1.0d	1.9a	ND	ND	ND	ND	3.2a	ND	1.3d	0.6c
Mozzarella	2.0ab	1.5abc	0.6c	ND	ND	ND	2.7b	ND	1.9c	1.1bc
Delac	2.0ab	1.8a	2.4a	ND	2.8	ND	2.0c	2.4b	7.4a	3.0a

ND = Not detected
a-d means in the same column not sharing a common superscript are different ($p<0.05$)
attributes were scored using a 0-15 pt. universal scale (Meilgaard et al., 2007)

Table 3.3. Relative abundance for selected volatile compounds in permeates (μg/kg)

Sample	Pentanal	Hexanal	Heptanal	Octanal	Nonanal	Decanal	3-methyl butanal	DMS	DMDS	Methional
Cheddar	0.107ab	0.131a	0.105	0.048b	0.094	0.180b	3.579a	0.244a	0.038bc	0.004bc
Cottage	0.745a	0.060ab	0.086	0.038b	0.357	0.148b	0.261b	0.071bc	0.255a	0.015a
Delac	0.080b	0.065ab	0.035	0.291a	0.298	0.561a	0.027b	0.014c	0.015c	0.008b
Milk	0.085b	0.011b	0.049	0.060b	0.118	0.064b	0.528b	0.242a	0.034bc	0.001c
Mozzarella	0.104b	0.098ab	0.051	0.046b	0.061	0.259b	0.047b	0.173ab	0.081b	0.002c

Sample	Diacetyl	Acetaldehyde	Acetic Acid	1-octen-3-one	2-methyl-3-furanthiol	4-octanone	benzeneacetaldehyde	2-pentanone	E,Z-2,6-nonadienal	benzaldehyde
Cheddar	1.692ab	2.160bc	0.202b	0.011bc	0.002a	0.054	0.046b	0.704ab	0.029a	0.097b
Cottage	2.172a	1.285c	0.555b	0.007c	0.002ab	0.060	1.290a	1.453a	0.011bc	1.067a
Delac	0.261b	4.164a	5.588a	0.058a	0.001abc	ND	0.180b	0.006b	0.003c	0.070b
Milk	0.259b	2.449bc	0.004b	0.029abc	0.001c	0.056	0.020b	0.590ab	0.042a	0.009b
Mozzarella	0.072b	2.642b	0.639b	0.050ab	0.001bc	0.079	0.081b	0.773ab	0.015b	0.129b

means in the same column not sharing common lettering are different ($p<0.05$).
means shaded in gray are not different ($p>0.05$)
[1]dimethyl sulfide
[2]dimethyl disulfide

Table 3.4. Descriptive analysis results of cream of broccoli soups made with and without permeate addition.

Sample	Aromatic intensity	Vegetable complex	Milky/Creamy	Sweet	Sour	Salty	Umami
Salt Control	2.1	2.0	3.0	2.0b	1.2bc	6.1a	2.3b
No Salt Control	2.2	2.0	3.2	2.0b	1.3b	3.0d	1.5c
Cheddar	2.0	2.2	3.1	3.5a	0.8c	4.0c	3.3a
Mozzarella	2.1	2.3	3.3	3.0ab	1.0bc	4.0c	3.5a
Milk	2.0	2.0	3.0	3.6a	0.8c	3.7c	3.2ab
Cottage	2.2	2.2	2.7	2.0b	3.0a	4.6b	3.5a
Delac	2.0	2.2	3.0	2.0b	0.5c	4.3bc	1.5c

means shaded in gray are not different ($p>0.05$)
a-d means in the same column not sharing a common superscript are different ($p<0.05$)
attributes were scored using a 0-15 pt universal spectrum scale (Meilgaard et al., 2007)

Table 3.5. Consumer liking scores for cream of broccoli soups with and without added salt or permeate

Sample Name	Salt	No Salt	Cheddar	Mozzarella	Milk	Cottage	Delac
Appearance	5.7 ab	5.7 ab	5.5 ab	5.8 ab	5.4 b	6.0 a	5.8 a
Overall Liking	6.1 a	5.3 c	5.6 b	5.6 b	5.5 b	4.2 d	5.4 b
Flavor	6.4 a	5.3 c	5.6 b	5.8 b	5.6 b	4.1 d	5.5 bc
Thickness	5.0 b	4.2 b	4.3 b	4.3 b	4.1 b	4.2 b	4.2 b
Salty Taste	5.8 a	5.0 bc	5.4 ab	5.1 b	5.3 b	4.6 c	5.1 b

Different lettering within each row indicates significant differences ($p < 0.05$)
Data represents 105 consumers
Liking attributes are scored on a 9-point hedonic scale where 1 = Dislike extremely and 9 = Like extremely

Table 3.6. Just about right scores for cream of broccoli soups with and without added salt or permeate

Attribute	Level	Salt	No Salt	Cheddar	Mozzarella	Milk	Cottage	Delac
Salty taste JAR	**Not salty enough**	7.6%c	57.1%a	41.0%b	45.7%b	42.9%b	41.0%b	55.8%a
	JAR	59.0%a	41.0%bc	50.5%ab	50.5%ab	48.6%ab	33.3%c	39.4%bc
	Too Salty	33.3%a	1.9%c	8.6%b	3.8%bc	8.6%b	25.7%a	4.8%bc
Broccoli Flavor JAR	**Too Weak**	27.6%c	51.4%a	30.5%bc	36.2%bc	31.4%bc	41.9%ab	38.5%abc
	JAR	69.5%a	45.7%cd	59.0%abc	52.4%bc	60.0%ab	33.3%d	54.8%bc
	Too Strong	2.9%c	2.9%c	10.5%b	11.4%b	8.6%bc	24.8%a	6.7%bc
Thickness JAR	**Too Thin**	68.6%a	73.3%a	71.4%a	74.3%a	77.1%a	75.2%a	71.2%a
	JAR	30.5%a	26.7%a	28.6%a	25.7%a	22.9%a	23.8%a	26.0%a
	Too Thick	1.0%a	0.00%	0.00%	0.00%	0.00%	1.0%a	2.9%a
Milky/Creamy Flavor JAR	**Too Weak**	23.8%c	38.1%ab	32.4%bc	34.3%bc	30.4%bc	51.4%a	50%a
	JAR	67.6%a	51.4%b	54.3%b	53.3%b	54.3%b	20.5%c	43.3%bc
	Too Strong	8.6%b	10.5%ab	13.3%ab	12.4%ab	15.2%ab	18.1%a	6.7%b

Data represents 105 consumers
Different lettering within each row indicates significant differences ($p < 0.05$)
JAR scales are scored on a 5-point scale where too little = 1 or 2, just about right =3 and too much = 4 or 5
Percentage of consumers that selected these options is presented

FIGURES

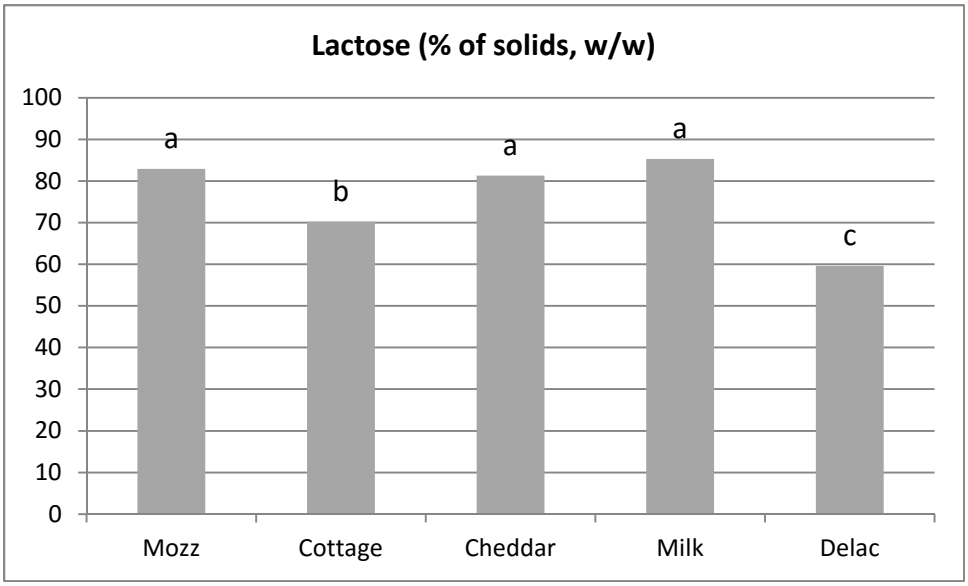

Figure 3.1. Lactose concentration of whey permeates - *columns not sharing a common superscript are different ($p<0.05$).*

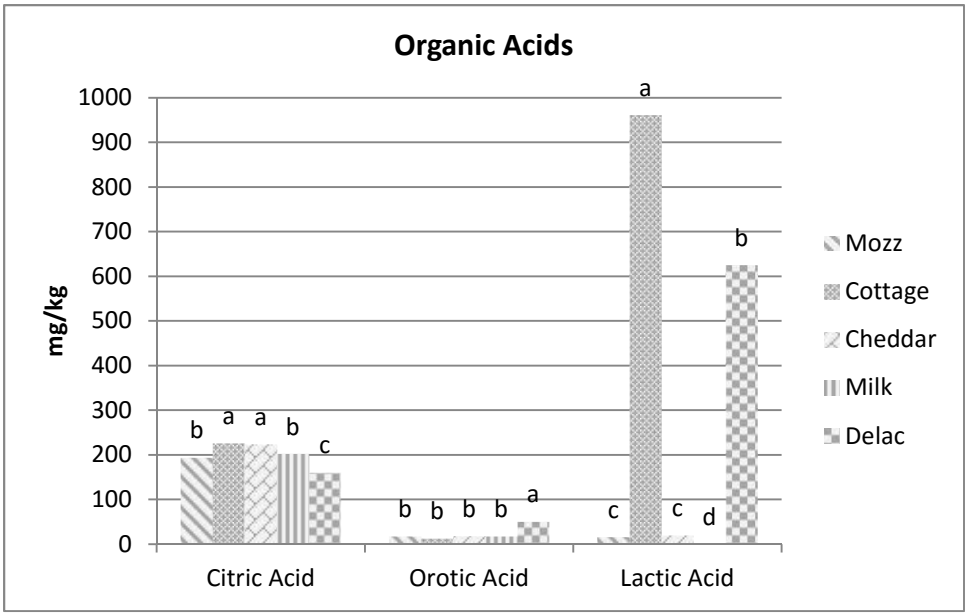

Figure 3.2. Organic acid concentration of whey permeates - *columns not sharing a common superscript within each group are different ($p<0.05$).*

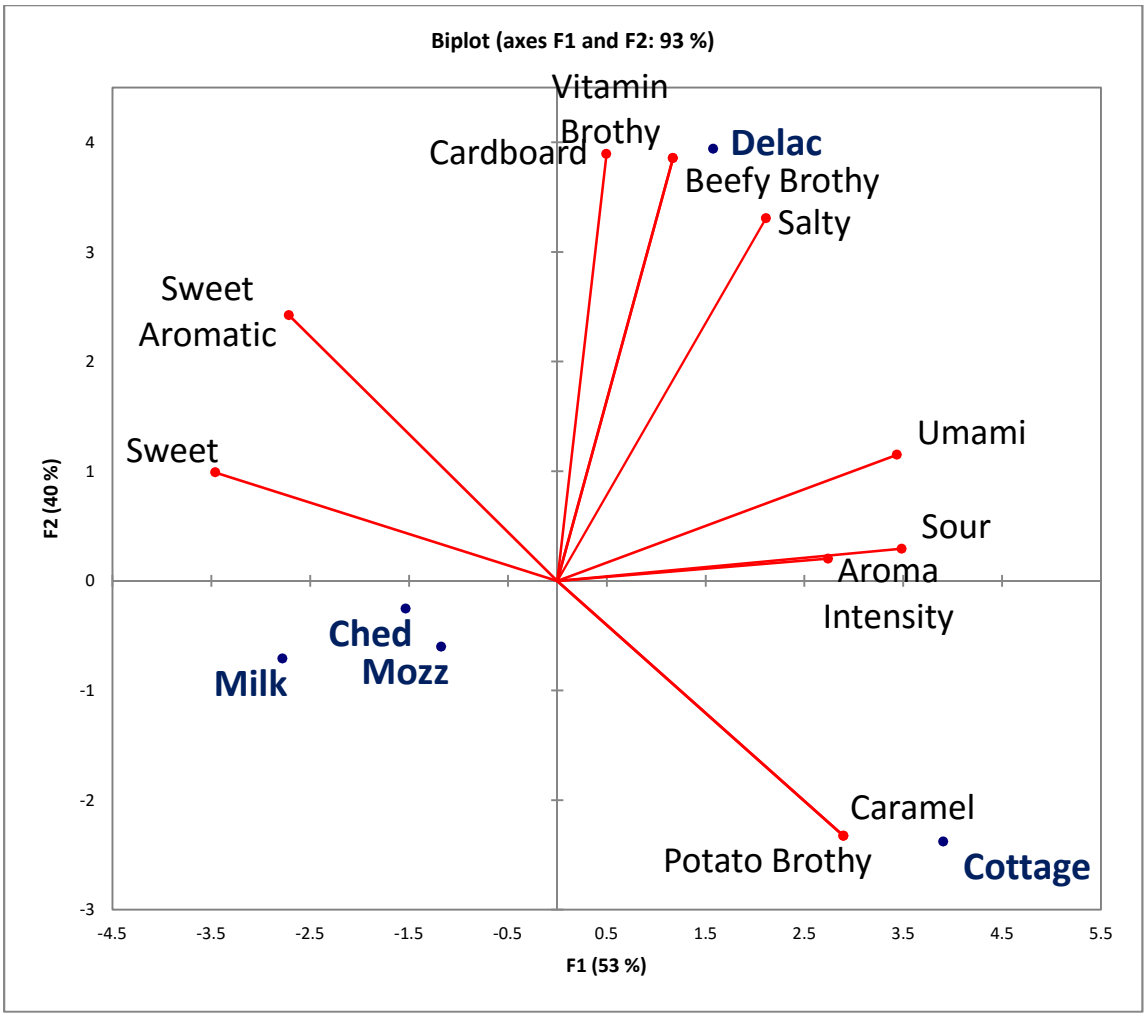

Figure 3.3. PCA biplot of descriptive analysis of whey and milk permeates. *Milk (milk permeate), Mozz (Mozzarella permeate), Cheddar (Cheddar permeate), Cottage (Cottage permeate) and Delac (de-lactosed whey permeate).*

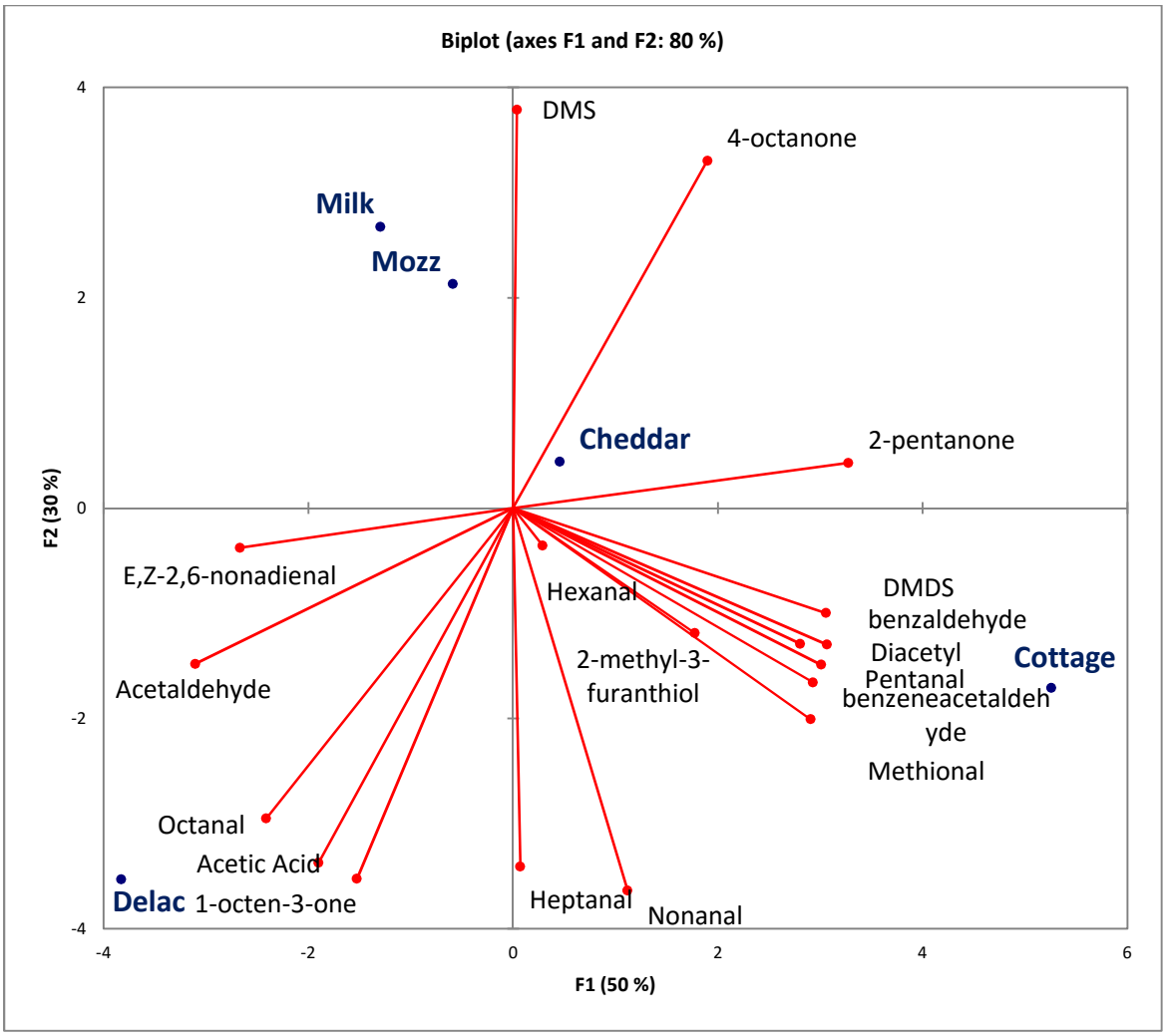

Figure 3.4. PCA biplot of volatile analysis of whey and milk permeates. *Milk (milk permeate), Mozz (Mozzarella permeate), Cheddar (Cheddar permeate), Cottage (Cottage permeate) and Delac (de-lactosed whey permeate).*