Factors Affecting Flavor Quality of Bovine Milk and Dairy Products

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Dedication

This thesis is dedicated to all those in search of the Truth.

"The aim of natural science is not simply to accept the statements of others, but rather to investigate the causes that are at work in nature." - St. Albert the Great

Abstract

Milk is an important food staple consumed around the world. Fluid milk is consumed as a standalone beverage, used in meal preparation, serves as the fundamental ingredient for fermented foods (yogurt, kefir, cheese, etc.), and is used for processed dairy products such as butter, ice cream, coffee creamer, etc. Indeed, it is this very versatility that necessitates that milk have the highest possible flavor quality to maintain and expand market share of dairy products, as well as provide nutritive benefits and better hedonics for consumers.

In this work, two individual studies were undertaken to better understand drivers of dairy quality as a result of volatile and nonvolatile sensory stimuli respectively, and both were directly related to addressing current needs in the dairy industry. The first study focused on the elucidation of potential causes of a documented off-flavor defect, spontaneous oxidized flavor (SOF) in milk. The second study aimed to provide an improved understanding of the contribution of nonvolatile small-molecular weight compounds on textural attributes as related to creaminess perception in dairy products.

For the first study, the principal objective was to identify the causative off-flavor compounds in SOF milk. Comparative aroma analysis between a reported SOF milk and clean milk sample without noted off-flavors (control) were investigated using various volatile analytical techniques, including solvent extraction, solvent assisted flavor evaporation (S.A.F.E.), and gas chromatography-olfactometry/mass spectrometry (GC-O/MS), utilizing the OSME method for aroma discrimination. Key differences in the aroma profiles of the off-flavored milk were found in several very strongly rated “green,

musty" terpenoid compounds that were completely absent from the control. These compounds were positively identified as endo-borneol, 2-methylisoborneol, and α-terpineol. After quantification studies, the impact of these compounds was validated using aroma recombination analyses. A degree of difference sensory test incorporated a control clean milk (same as reference), a clean milk spiked with day 0 levels of the terpenoid off-flavor compounds (endo-borneol 0.95 µg/L; 2-methylisoborneol 0.0037 µg/L; α-terpineol 0.84 µg/L), and a clean milk spiked with day 14 levels of the same compounds (endo-borneol 0.20 µg/L; 2-methylisoborneol 0.017 µg/L; α-terpineol 0.62 µg/L). The control milk was rated 0.75, the day 0 milk rated 1.67, and the day 14 milk rated 2.0 (α=0.05, critical difference value=1.0897), showing that the addition of the terpenoid "musty" compounds resulted in a significantly different flavor profile when compared to a reference clean milk. Panelists described similar "unclean" perceptions as were found in the preliminary tasting of the original milk samples. As a result, it was confirmed that the identified terpenoid compounds were responsible for flavor defects observed in the initial off-flavor milk. While the milk screened for this study was identified as 'SOF' in nature, more research would be needed to verify that this was indicative of SOF or simply another off-flavor issue. Regardless, these results illustrated a benefit to the dairy industry to conduct more directed off-flavor analyses and determine the root causes of product off-flavors on a per sample basis.

The aim of the second study was to provide greater understanding of molecules that contribute to texture attributes associated with creaminess perception in dairy products. Heavy whipping cream was selected from several dairy product extracts as a

characteristically ‘creamy’ product. Optimized food grade solvent extraction, HPLC multidimensional fractionation, and descriptive analysis using a five-term sensory lexicon and references were employed to identify fractions of interest with the strongest textural attributes. UPLC/MS/MS analyses and NMR afforded the positive identification of five compounds associated with textural attributes, falling into three categories: vitamin complex (orotic acid, pantothenic acid) hippuric acid (hippuric acid, 2-methylhippuric acid), and sulfate compounds (p-cresol sulfate).

Quantitative measurements of the individual compounds were undertaken to assess their presence across a variety of nonfat, reduced fat, and full fat dairy products, and concentrations obtained from skim milk and whole milk were used for further recombination sensory experiments. A control skim milk was compared with a skim milk spiked with the five texture-active compounds at levels found in whole milk (orotic acid 120 mg/kg; pantothenic acid 1.3 mg/kg; hippuric acid 1.6 mg/kg; 2-methylhippuric acid 300 mg/kg; p-cresol sulfate 9.5 mg/kg). The 2-AFC sensory evaluation tests indicated the spiked skim milk had a significantly “creamier, fuller bodied flavor” when compared with the control skim milk ($p=1/2$, $n=20$, $\alpha=0.01$). This verified the sensory relevance of the identified compounds, indicating the contribution of these compounds to creaminess perception. None of these five compounds have been previously reported in literature to exhibit textural effects, nor effects on creaminess quality in dairy.

In summary, elucidation of the objectionable flavors present in a purported SOF whole milk sample indicated the cause of off-flavor was from a microbial source or a flavor ‘taint’ not caused by spontaneous oxidation. This indicated the dairy industry

would benefit from a more bottom-up approach in identifying responsible objectionable flavor compounds to make informed decisions about minimizing off-flavors in fluid milk. The second study provided novel understanding of compounds that contribute to textural attributes and creaminess perception. Identification of these compounds provides an improved basis for product developers to positively influence creamy texture and target creamy quality enhancement in dairy products. In acknowledging the role that texture compounds play alongside the larger picture of creaminess obtained through previous research, developers may create high-quality nonfat, reduced fat, and full fat dairy products for a variety of markets, consumers, and purposes.

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Chapter 1

Introduction

Milk History

The species of animals commonly acknowledged as the ancestor to the modern cow was first domesticated around 8000 BCE in several different locations around the world, including Europe, India, and the Middle East. Ancient Indo-Europeans were already herding around 3000 BCE, when they migrated and filled areas of Europe and Asia with their agricultural traditions and culture; this is reflected in many creation myths and cultural stories that incorporate dairy, herding, and agricultural practices across these different parts of the world. Cows and milk symbolized nurturing, bounty, and survival in these cultures.[1, 2] Roaming and herding eventually transitioned to the practice of stabling cows in Europe, and greater confinement of the animals led to an overall loss of muscle mass and horn density. While still used for meat, this meant that cows could be selectively reared for milk quality and yield, leading to staple breeds for milk production such as Jerseys, Guernseys, and Holsteins.[1]

With the rise of the Industrial Revolution in the mid-eighteenth century, newly expanded methods of transportation and large scale processing brought change to the dairy industry. New technologies like steam power led to improvements in farming equipment, allowing cows to be more relegated to milk production, rather than hauling and laborious farm work. Machines were also developed for better milk processing including churning, milking, and separation of cream. Efficient transportation from rural

areas to urban centers meant fresher milk and dairy products could be enjoyed in much more distant markets, as well as with higher quality.[1]

In the mid-1800s, Louis Pasteur helped champion the *pasteurization* process to effectively kill pathogenic bacteria, as well as provide a greater foundation for understanding the standardized addition of cultures for dairy fermentation. By 1886, another agricultural scientist, Franz Ritter von Soxhlet, developed instrumentation capable of efficiently completing the pasteurization process.[3] These and other advances meant that safer and higher quality milk could reach and be enjoyed by a larger consumer base. Soon, milk moved beyond its predominant association as food for infants and children to recognition as a good source of nutrition for adults.[2] This fueled greater interest in nutritive studies and processing methods for other dairy products. Yogurt, for example, was sold in pharmacies as a medicine at the beginning of the 1900's. It was eventually commercialized by Isaac Carasso, who pioneered producing yogurts with jams for different flavors. Together with his son Daniel, the two founded Danone (Dannon) in France after fleeing their native Spain for France during World War II. Yogurt laboratories and factories opened in France as early as 1932, with the United States following in 1941.[4] Other processed dairy products, like heavy whipping cream, were much more laborious to make prior to the 1900's. With the invention of the French centrifugal separation method of isolating the cream layer from milk, it became much less time intensive and more profitable to produce than previously used methods. Eventually, changes in technology and automation led to the creation of aerosolized whipped cream in canisters, something that would have been impossible just a few decades earlier.[1]

These advances in dairy chemistry, processing, and nutritive understanding have continued to inform current dairying standards, best practices, and innovation in the industry today.

Milk composition and nutrients

In the United States, the standard of identity of milk is determined by the Code of Federal Regulations, CFR Sec. 131.110: "Milk is the lacteal secretion, practically free from colostrum, obtained by the complete milking of one or more healthy cows. Milk that is in final package form for beverage use shall have been pasteurized or ultrapasteurized, and shall contain not less than 8.25 percent milk solids not fat and not less than 3.25 percent milkfat."[5] Milk in its natural form evolved over time as a method of providing nutrient-rich, quality sustenance to newborn and developing mammals. Milk is an excellent source of a variety of both macro and micronutrients important for general human nutrition and health. It is important to remember that animal diet also plays an important factor in the nutritive quality of milk. Animals are fed nutrient dense diets to encourage rapid growth, including standardized levels of silage or grass that contain various levels of proteins, carbohydrates, lipids, vitamins, and minerals. As such, the levels of nutrients intrinsic to the animal's diet may be influenced according to environmental conditions, and these play a role in what will be passed on to the milk of the lactating animal. These can also vary between species and even breeds (see Table 1 below).

Table 1, The Composition of Various Milks[1]

The Compositions of Various Milks

The figures in the following table are the percent of the milk's weight accounted for by its major components.

Milk	Fat	Protein	Lactose	Minerals	Water
Human	4.0	1.1	6.8	0.2	88
Cow	3.7	3.4	4.8	0.7	87
Holstein/Friesian	3.6	3.4	4.9	0.7	87
Brown Swiss	4.0	3.6	4.7	0.7	87
Jersey	5.2	3.9	4.9	0.7	85
Zebu	4.7	3.3	4.9	0.7	86
Buffalo	6.9	3.8	5.1	0.8	83
Yak	6.5	5.8	4.6	0.8	82
Goat	4.0	3.4	4.5	0.8	88
Sheep	7.5	6.0	4.8	1.0	80
Camel	2.9	3.9	5.4	0.8	87
Reindeer	17	11	2.8	1.5	68
Horse	1.2	2.0	6.3	0.3	90
Fin whale	42	12	1.3	1.4	43

The primary proteins in milk consist of approximately 80% caseins and 20% whey proteins. Caseins exist in four principal forms: α_{s1}, α_{s2}, β, and κ caseins. The majority of milk caseins naturally exist in micellular form. Many proposed conformations related to casein micelle structures exist, but the most commonly accepted variations in recent years include that of a larger micelle composed of smaller sub-micellular structures. Generally, casein micelles are thought to exist with the more hydrophobic α_{s1}, α_{s2}, and β caseins in the central core of the sub-micelles. The more hydrophilic κ-caseins extend out preferentially on external portions of the micelle, where they interact with each other as well as the bulk aqueous phase of the fluid milk (see Figure 1).[6]

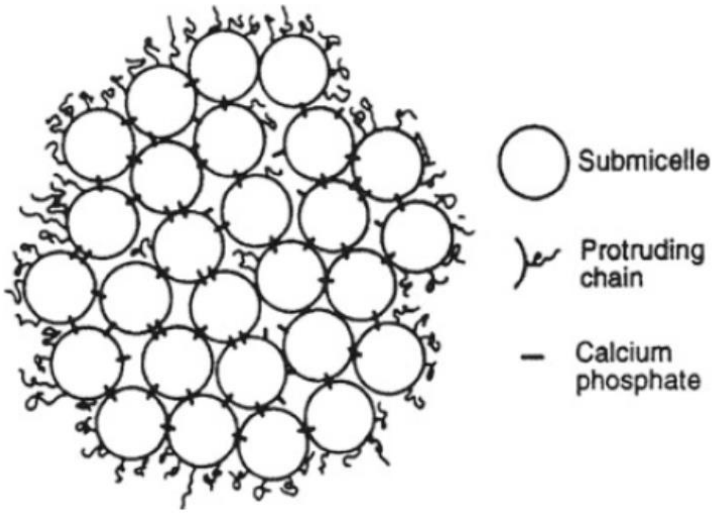

Figure 1, Example of casein micelle substructure[7]

Whey proteins, in contrast, can be defined as those proteins that remain in the bulk milk even after isoelectric precipitation of caseins at pH 4.6. Whey proteins consist of soluble lactalbumin and insoluble lactoglobulin fractions. Two more minor proteinaceous species inherent to milk include proteose peptones (non heat-denatureable proteins) and non-protein nitrogen. These typically have very low commercial value; nevertheless, they play roles in basic molecular interactions within milk. Proteose peptones, for example, have been widely asserted as one of the staple features responsible for frothing properties in skim milk.[8]

The lipid portion of milk primarily consists of triacylglycerols in the form of lipid globules dispersed in an aqueous bulk phase. Milk also contains di- and monoacyl glycerides, free fatty acids, phospholipids, and cholesterols. The key fatty acids present in milk are palmitic acid (16:0) at 43%, followed by myristic (14:0, 13%), stearic (18:0,

11%), and oleic acids (18:1, 11%).[9] Microbes in the digestive system of ruminants are responsible for converting existing unsaturated fatty acids from grasses to more saturated fats in milk, thereby acting as a source of either mono- or diacylglycerols. They also may form much smaller (and occasionally volatile) short chain fatty acids, which may impact flavor quality.

The predominant sugar in milk is lactose, a reducing sugar and disaccharide comprised of glucose and galactose. In bovine milk, lactose varies in concentration between 0 and 10%. Other sugars also exist in milk, albeit in smaller quantitative levels. These include glucose, N-acetylglucosamine, and various oligosaccharides, which can occur in either linear or branched configurations.[10]

Milk generally contains a wide range of micro nutrients, including both vitamins and minerals. Milk contains a good balance of many vitamins, including fat soluble A, D, E, and K, and B vitamins.[11, 12] Common minerals inherent to milk include major salts like calcium, phosphorus, magnesium, iron, zinc, copper, manganese, as well as minor trace elements such as selenium, iodine, molybdenum, cobalt, fluoride, boron, chromium, arsenic, nickel, silicon, vanadium, etc. (Figure 2).[13] Some minerals like calcium and phosphorus are more intrinsic for micellular interaction and stability in the protein fraction of milk. Milk is often fortified with extra vitamin D. Of the major micronutrients, milk is lacking primarily in vitamin C and iron.[1]

Cationic	Concentration		Anionic	Concentration	
	mg l^{-1}	mmol kg^{-1}		mg l^{-1}	mmol kg^{-1}
Calcium	1040–1280	26–32	Carbonate (including CO_2)	~200	~2
Magnesium	100–150	4–6	Chloride	780–1200	22–34
Potassium	1210–1680	31–43	Citrate	1320–2080	7–11
Sodium	350–600	17–28	Total phosphorus (PO_4) (all forms)	930–1000	30–32
			Inorganic phosphorus (as PO_4)	1800–2180	19–23
			Sulphate	~100	~1

Figure 2, Relative mineral concentrations in milk[14]

Consumption and current trends

Over the past 40 years in the United States, the dairy industry has moved from steady, slow growth to a more industrialized, market-driven, and fluctuating market presence. After World War II, dairy production was predominantly located in the Northeast, Great Lakes, and Midwestern regions of the United States. Overall the dairy industry was fairly stable and experienced slow expansion, however new production areas emerged over the next 20 years in regions on the west coast, such as California and the northern Pacific coast. Perhaps the most significant shift came in the form of widespread industrialization as large-scale milking operations developed. Even small milk producers bought into updated technologies such as milking machines and automated feeding systems. New practices were introduced, like the shift from pasture to pre-bought or grown silage feeds and indoor milking parlors. In addition, changes in milking expectations per animal changed. From 1950 to 2000, the number of milk cows in the United States decreased approximately 58%, despite doubling of milk production

between 1950 to 1975 and increasing another 75% by the year 2000, as seen in Figure 3 below.[15]

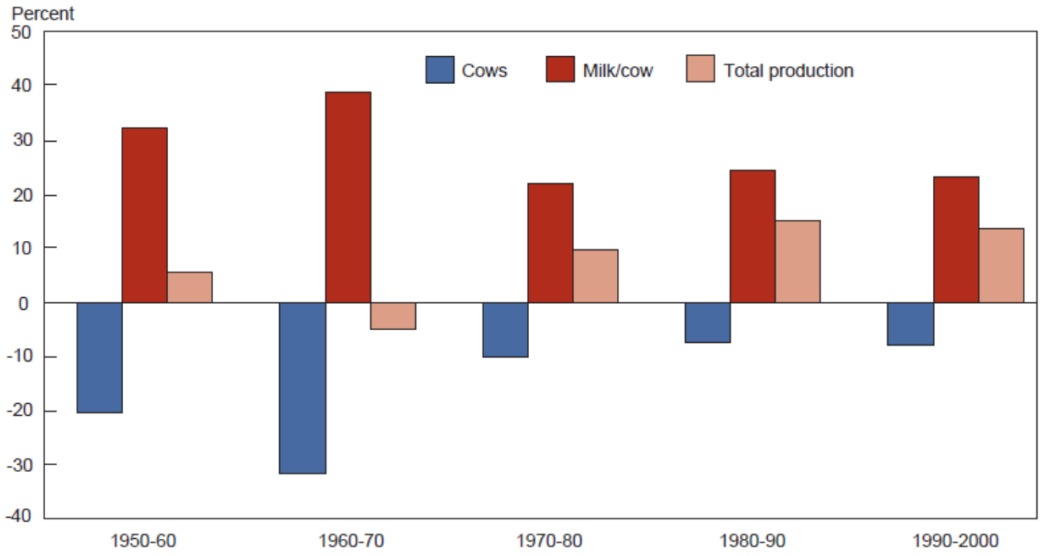

Figure 3, USDA Regional share of U.S. milk production between 1975 and 2000[15]

Despite the higher production, over the past several decades there has been a lower demand for fluid milk among the U.S. population, a trend which has been ongoing since at least 1940. Since 1970, average adult consumption of milk has decreased from an average of 0.96 cups to 0.61 cups daily. In contrast, low fat alternatives like 1%, and 2% milk purchases now account for 70% of dairy purchases in the market.[16]

Consumers regularly point to four key areas that primarily influence their food purchasing decisions: sensory enjoyment, health and wellness, convenience, and safety.[17]

Contemporary successes and increasing market share for dairy products often find more direct relatability to consumer needs in value-added dairy products like yogurt. From 2012 and onward, the yogurt market in particular has expanded rapidly. This is in no small part due to the rise of Greek yogurt, accounting for one-third of the market. Dairy products incorporating both pre- and probiotics for digestive wellness and other benefits remain desirable to consumers. Recently, added protein has become of even greater interest to the dairy and food industries. Consumers are more health-conscious than ever, and they frequently cite nutrition as a key determinant in choosing dairy products for breakfast, snacks, and other meals.[18] However, they are also unwilling to compromise on flavor quality, and this creates challenges for the food industry in creating products that contain both added ingredients and equal, or better, flavor profiles for consumers. To this end, researches have extensively investigated the volatile profiles of a variety of dairy products, as well as those with altered fat levels, processing parameters, and other ingredients like starch and protein. Many come with their own unique benefits and challenges.

General Milk Volatile Flavor Profiles

Studies enumerating the specific flavor volatiles present within dairy products have been researched for decades. The flavor profile of milk, as a main ingredient, acts as the foundation for volatile profiles of many other dairy and ready-to-eat products. Thus, any issues related to flavor quality or off-flavor formation and contamination can have further ramifications on flavor profiles in subsequent food products. The compounds that

contribute to milk flavor frequently have very low odor thresholds and result from naturally occurring fats, as well as various other flavors resulting from the animal's metabolism, feed sources, etc.[19, 20] In general, the flavor profile of milk consists of low concentrations of many different classes of volatiles, including 2-alkanones (e.g., 2-pentanone, 2-heptanone, 2-nonanone, etc.); alcohols (1-pentanol, 1-octen-3-ol, dodecanol, etc.); aldehydes (pentanal, hexanal, nonanal, etc.); esters (ethyl butanoate, ethyl octanoate); nitrogenous compounds (indole, skatole); lactones (δ-decalactone, γ-dodecalactone); and others.[21] Fresh milk has been found to contain such key volatile compounds as ethyl hexanoate (fruity, "sweet" notes), dimethyl sulfone (sulfur, burned, roasted), nonanal (green, oxidized lipid), ethyl butyrate (vegetable, green, fruit), 1-octen-3-ol (mushroom, earth), and indole (green, jasmine).[20] This wide array of compounds often results from lipid oxidation of certain fatty acids characteristic to ruminant animals, including oleic acid, palmitoleic acid, linoleic acid, palmitic acid, myristic acid, stearic acid, and smaller saturated fatty acids.

The pasteurization process also influences the flavor profile of milk. Varying levels of time and temperature treatment result in the formation of different volatiles and even color changes. Heating of milk results in a host of different volatile rearrangements and formation, including creation of compounds like methyl ketones, 3-keto esters, lactones, and hydroxyl esters.[22] After decades of research, some flavors have actually become recognizable as a result of proper pasteurization treatment of milk. High temperature, short time (HTST) pasteurization parameters (161°F for 15-20 seconds) result in formation of 1-octen-3-one (mushroom, earthy), dimethyl sulfide (sulfur, milk),

hexanoic acid (rancid, floral), acetic acid (pungent, vinegar), 1-nonen-3-one (mushroom, earthy), dimethyl trisulfide (sulfury, fecal), 2,3-butanedione (buttery, vanilla, cream), 2-methyl thiophene (plastic), and 3-methyl-2-butenal (green, metallic).[20] The more extreme parameters used for UHT milk processing (275-300°F for 4-15 seconds) typically result in milk with much more prominent 'cooked' flavors and darker colors. Researchers have pointed out that these cooked notes are largely due to Maillard reaction products, such as 2-acetyl-1-pyrroline and furaneol, as well as sulfur-containing compounds like methional and 2-acetyl-2-thiazoline.[23]

Lipid Volatiles and Oxidation

Lipids are incredibly influential on flavor profiles of foods, and dairy products are no exception. Fat derived volatiles in dairy typically produce flavors described as "sweet," "buttery," "creamy," and "rich" that derive from fatty acids, lactones, carbonyls, and fatty acid esters. Lipids may also interact with proteins, carbohydrates, and other ingredients in food matrices to produce a wide array of complex and extensive flavor profiles. Lipids can occasionally suppress off-flavors by acting as a nonpolar solvents that inhibit subsequent availability for perception.[24] In this same manner, lipids may also serve to prolong volatile release over time, particularly as the food product increases in temperature upon gustation, thereby allowing retronasal aroma perception.

Lipid oxidation in dairy can produce more pronounced flavor complexity, as well as aroma defects. Some compounds like 1-octen-3-one, hexanal, and Z-4-heptenal apparently contribute to a full, rounded dairy flavor when present at lower concentrations.

Higher concentrations of these compounds, however, can result in negative attributes such as aged, metallic, green, and cardboard-like notes.[25] Higher temperatures may also lead to an exacerbation of lipid oxidation off-flavor development, specifically during storage, although improper processing conditions can also lead to off-flavor formation. In combination with lipolysis, a natural occurrence in milk, oxidation may lead to higher concentrations of free fatty acids, particularly if combined with abusive processing or storage conditions. Smaller free fatty acids are often volatile and may contribute to the overall aroma profile and potential increase of rancidity in milk.[9]

Photooxidation can also occur in dairy products as a result of improper storage or exposure to light, e.g. during transportation, displays at the point of purchase, consumer handling, etc. In particular, methionine may be degraded to other odor-impact compounds such as 3-ethylthiopropanal. In combination with naturally present (and increased) levels of sulfurous compounds such as methanethiol, milk flavor could already be significantly affected at the time of purchase and consumption by the customer.[25]

Another off-flavor commonly recognized in industry is known as “hardened flavor.” This defect can occur in a variety of foods, but it is especially notable in foods like dairy with delicate flavor profiles and hydrogenated fats. Specific flavor compounds responsible have varied among several conflicting studies, encompassing a variety of unsaturated lipid oxidation compounds, alcohols, and lactones.[26] It is generally believed that hardened flavor consists of compounds that result from oxidation of linoleic and oleic acids, such as E-2-nonenal, 10-octadecenoic acid, and 11-octadecenoic acid.[27] In

other food matrices, studies have pointed to unsaturated lipid volatiles such as 6-trans-nonenal.[28]

Enzymatically derived off-flavors may also form by naturally occurring lipoxygenase in dairy products as well. While the pasteurization process largely negates this off-flavor route through enzyme denaturation, its presence may indicate an error in proper processing protocols. Lipoxygenase may also affect raw milk prior to pasteurization, leading to off-flavor compounds that could lead to subsequent off-flavor pathways within the milk. Examples of enzymatic off-flavors include dimethylsulfide, 2- and 3-methylbutanal, methylpropanal, (E,Z)-2,6-nonadienol, ethyl esters, and higher levels of various short chain fatty acids from naturally occurring dairy fats.[25, 29]

Lastly, milk flavor may also be affected by numerous other factors common to the food industry, including improper handling conditions, improper processing, microorganism derived off-flavors, inappropriate packaging, etc.[30] These routes and others may affect milk and related dairy products anywhere along the production and supply chains. It is important to incorporate quality standard checkpoints to mitigate these occasions as much as possible.

Processed Dairy Products

A whole host of processed dairy products can result from whole fat milk, including milks with different fat concentrations, fermented dairy products like yogurts, heavy whipping cream, and other products like cheese, ice cream, frozen yogurt, kefir, and countless others. Very generally, whole fat milk is used as the starting product, after

which it may be adjusted for needed specifications, thermally processed for safety or sterility as needed, and then further treated based on the desired final product. This can include inoculation with microorganisms, addition of milk solids, pasteurized cream, or flavors for such foods as cheeses, yogurts, ice creams, butters, flavored milks, etc. While there are many and varied dairy products available in the contemporary marketplace, it is worth presenting aspects of flavor and processing conditions for several staple dairy products that were included in the creaminess study, which will be discussed later. These products include milks, yogurts, and heavy whipping cream.

Milk

The flavor quality of whole milk typically follows the general volatile profile discussed above, and it contains approximately 3.3% milkfat. Processing conditions include preheating for greater efficiency when subjected to cream separation processes (e.g. centrifugal separation) to rapidly and effectively isolate and remove the bulk fat from the milk. Using this bulk fat, producers can then adjust the lipid levels for milk, whipping cream, yogurt, etc. to their respective specified final product requirements, such as readdition of 1.5-2% fat for low fat milks. Next, the desired pasteurization and homogenization conditions are employed, followed by addition of vitamins, minerals, or milk solids lost, further processing, aseptic filling, or use in other processing streams for creation of other dairy products.[31]

Yogurt

General yogurt processing incorporates lactic acid bacterial fermentation of milk, most commonly with the strains *Streptococcus thermophilus* or *Lactobacillus bulgaricus*.

While the fermentation pathway depends on the strain of microorganism selected for the product and desired characteristics, generally the process follows the glycolysis pathway for formation of lactic acid. Fermentation may be conducted in-container (set yogurts) or in vats and mixed prior to filling (stirred, "Swiss style" yogurts). Greek yogurts, in contrast, might have higher volumes of water or whey protein removed from the yogurt prior to the fermentation process, making them less watery and thicker. Yogurt inoculants are commonly incubated at 1.5-3.0% of the yogurt and cultured at approximately 45°C for several hours. Yogurt is a low pH product, and acidity levels are monitored until they reach a pH of approximately 4.5. Given certain product lines and specifications, added fruits or flavors may also be incorporated. Generally, yogurt has been found to include important volatiles such as 1-octen-3-one, 1-nonen-3-one, and lipid oxidation products such as E-2-nonenal, linoleic acid, and others.[32]

Heavy whipping cream

Whipping cream is created from defatted milk, after the isolation processes mentioned above. Various cream-based products may then be created after standardization of the cream, pasteurization, etc. Heavy whipping cream usually contains 30-36% milkfat, and the dairy industry typically verifies the product quality in measuring proper whipping characteristics and emulsion stability post-whipping.[33] Some volatiles previously reported to be important in whipped cream include 2,3-butanedione, 3-hydroxy-2-butanone, dimethyl trisulfide, 2-nonanone, butanoic acid, acetic acid, dimethyl sulfide, and 2-butanone.[34]

Overall, flavor quality plays a central role in consumer acceptance and ultimately choice in the contemporary competitive marketplace. Understanding the flavors and off-flavors, volatiles and nonvolatiles present in milk as the initial source of flavor quality is key to all subsequent aspects of flavor in the final product, whether fluid milk, yogurt, whipping cream, cheese, etc. As a result, this will determine creaminess perception in dairy foods, which combines all these dimensions for what consumers identify as an indicator of high quality in dairy products. These are the aspects that can be manipulated and employed by the food industry to create a variety of dairy products that, nevertheless, retain the characteristics and qualities that consumers desire and expect.

Considerations and Research Objectives

The number of dairy products available within the contemporary marketplace—kind of product, low and full-fat options, flavor profiles, and nutritive qualities—reflects the complexity that dairy research brings to the industrial and academic spaces. In understanding modern dairy flavor challenges, there is a wide range of foundational research from which one may draw important knowledge and resources. While this research can illuminate areas for further exploration, it can also occasionally provide conflicting views on causes and effects of flavor development and impact in dairy products. This is particularly the case when it comes to two key areas of dairy flavor research: off-flavor analysis and inhibition, and understanding dairy flavor quality.

Spontaneous Oxidized Flavor in milk is one such example of a contemporary off-flavor challenge for the industry. The off-flavor defect, commonly asserted to be the

result of oxidative flavor development, has been acknowledged in dairy products for several decades. As the ‘spontaneous’ label suggests, however, there is still exceedingly little consensus in literature as to the causes. There is even less known about the volatile flavor compounds directly responsible for the off-flavor. Most have attributed SOF development from various concentrations or identities of certain fatty acid precursors, trace metals, or pro-/antioxidant levels. In discovering the identities of impactful off-flavor compounds, the conflicting assertions of previous studies may be understood in a wider, more applied context. Understanding off-flavor mechanisms and methods of inhibition will offer the dairy industry a solution for an increasingly acknowledged off-flavor issue, protecting clean milk from contamination and increasing the flavor quality of dairy products as a whole.

Just as necessary is the greater understanding and improvement of basic dairy flavor quality. One high-value area of dairy quality consumers routinely identify is creaminess. While ‘creaminess’ as a descriptor can be elusive, there has nevertheless been significant research in understanding creaminess as it relates to certain areas, particularly lipid concentration, bulk viscosity/rheology, and volatile research. However, there has been exceedingly little work conducted in the area of small molecular weight compound contributions to either taste or texture sensations. In providing solutions for the creation of higher quality zero, low-, and full fat creamy products, it is necessary to investigate alternatives that may not necessarily follow the typical routes of investigation. This could lead to obtaining a more comprehensive view of dairy systems and ingredients and provide further insight on the molecular basis of creaminess perception. This will

afford tools with which the dairy industry may provide a variety of high quality, nutritive creamy dairy products for consumers.

Study 1 Objectives:

1. Characterize key off-flavor compounds in select SOF identified milk samples
2. Investigate precursors of off-flavor compounds, and if applicable propose methods for monitoring or inhibiting SOF development

Study 2 Objectives:

1. Characterize creamy flavor compounds by sensory guided fractionation techniques in products with milk fat (i.e. whole milk)
    a. Determine the key nonvolatile (texture, taste) creamy-contributing compounds
2. Conduct recombination studies to validate the impact of the identified compounds on the perception of creaminess

Chapter 2

Literature Review

Spontaneous Oxidized Flavor

Over the past several decades, there has been increasing acknowledgment in the literature of a particular off-flavor phenomenon characteristic to whole fat bovine milk, commonly referred to as "spontaneous oxidized flavor," or SOF. First attributed to areas of northern Europe and Scandinavia, the characteristics of this off-flavor have frequently been described as reminiscent of oxidized dairy flavor, with cardboard, spoiled, and fish-like flavor defects. However, milk identified as containing SOF typically has no apparent single instigating factor that produces the off-flavor. In recent years, there has been an increased association of SOF type flavor defects within milk supplies in various parts of the world, including New Zealand, Canada, and the Midwestern United States. Typically, patterns of SOF at particular farms and locations indicate that it is seasonal in nature and yields an overall drop in milk flavor quality, occurring between late autumn and mid-spring in the Midwestern United States. It is commonly accepted that SOF increasingly worsens over time; this can be noticeable at the farm, processing, and supermarket levels, or even after it has reached consumers. It is capable of contaminating otherwise clean milk, hence the common allusion to oxidative mechanisms in literature. General points of overall off-flavor contamination in the dairy industry include transportation from the farm in tanker trucks or at the point of bulk tank mixing prior to pasteurization, which in

the case of SOF is believed to contaminate otherwise acceptable milk.[35] Therefore, at the time that the milk is tasted by the consumer, it is already too late to evaluate, mitigate, or counteract the off-flavor. After decades of research, more work is needed to understand this off-flavor issue and the potential causes to provide viable solutions for the dairy industry.

SOF research began in earnest in the mid-twentieth century. It has largely focused on testing various hypothesized instigators of the off-flavor, rather than identifying the impactful volatile compounds responsible. In large part, this pattern has carried over to contemporary mitigation efforts. Due to the lipid oxidative descriptors commonly associated with SOF, it has been predominantly accepted in literature that SOF likely follows a lipid oxidative mechanism, such as that of the radical mechanism.[9] Enzymatic relevance for SOF has been largely disproven, as lipoxygenase is negated due to deactivation during pasteurization. Previous research can be categorized into three general areas of investigation and effects on off-flavor and lipid oxidation: trace metal concentration; the balance of polyunsaturated fatty acids (PUFA) in milk, resulting principally from bovine diet; and the presence and effects of pro- or antioxidant levels in milk. Some studies investigate only one of these categories, while others have investigated all to various degrees. It is important to note that in the body of research presented here, "SOF" is the term used for the specific flavor defect in question, however because of the lack of positive identification of specific off-flavor compounds, "oxidized flavor" may also be included occasionally. In this way, it is possible to draw parallels with the oxidative mechanisms to which much more general milk literature refers. The

specific literature available for the SOF phenomenon is limited; therein lies the difficulty in truly assessing and identifying "spontaneous oxidized flavor," and thus the necessity of this study.

Trace metals and SOF instigation

Trace metal catalysis is known to affect both lipid concentration, oxidation rates, and subsequent lipid oxidation products in milk, particularly in regard to volatile flavors.[9] To our knowledge, the first study to investigate trace metal catalysis and effects specifically on SOF appeared in 1959. The researchers [36] studied whether copper naturally present in pasture or silage bovine feeds could result in SOF, as compared to feeding regimens supplemented with copper sulfate. Milk from control feeds did not contain significantly different levels of copper. Switching diets from silage to pasture grazing over a period of five days reduced copper concentration in the cows, but had no appreciable effect on SOF flavor. However, cows treated with copper sulfate supplements in their diets did produce significantly higher levels of SOF flavor. In this early research, it was also hypothesized that catalytic activity leading to SOF might form a dynamic interaction with trace metals in the body. Another notable paper [37] studied the relationship between xanthine oxidase, a naturally occurring oxidative enzyme, and copper in milk. However, SOF was found to develop independently of enzyme levels. Others [38] have claimed that trace metals can vary during particular stages of lactation. When coupled with the presence of copper supplemented through food and drinking water, it has been asserted that significant correlation effects exist with the tendency of

milk to produce SOF earlier in lactation. Curiously, the same group claimed that of all the dairy products their group tested, nonfat milk contained the highest overall incidence of oxidized flavor. On its surface, this would appear to confound oxidative mechanism hypotheses, since the full-fat milk counterparts produced less overall SOF defects than the nonfat milks. Later, Juhlin et al. [35] found similar results in SOF variability according to lactation cycle. In their study, copper concentration had a notable impact on SOF formation in milk, thought to occur because of free radicals and subsequent oxidative instability. In their sample pool, this occurred in the first 1-3 weeks of the lactation cycle. After that time, the copper concentration remained relatively low in comparison to the prior three weeks, however higher SOF levels indicated other factors could have been at work. In particular, the group hypothesized that genetic factors in certain cows could play a role in SOF development. In contrast,[39] another study found that intravenous injections of cows with copper glycinate increased measured copper levels in both blood and milk, and SOF developed in milk within the first 24 hours of supplementation. However, there was no evidence that SOF was present or increased after that initial 24 hour period.

Polyunsaturated fatty acid (PUFA) presence and SOF effects

PUFA concentration has also been linked to increased off-flavor presence in SOF flagged milk samples. It has been previously reported [40] that short chain fatty acid synthesis as well as lipid hydrogenation occurs in the rumen of cows. Protective methods have been developed to delay or drastically reduce the hydrogenation of unsaturated fatty acids present in bovine feed, i.e. encapsulation. The typical goal is to limit the levels of

available PUFA, so that they may pass unaffected through the digestive tract and directly to the milk. However, this may have further ramifications as unsaturated flavor compounds are then available in the milk that would not otherwise have been naturally present, potentially introducing other flavor issues.

Others have investigated whether certain feeds can either instigate or inhibit particular fatty acid levels, as well as resultant off-flavors in milk. One group [41] found that higher concentrations of PUFA as a result of roasted soybean feed, when given in the presence of copper, led to evident oxidative flavor defects. Milk fatty acid profiles did not change over time, but oxidative organoleptic properties were perceived in samples that had higher levels of linoleic and linolenic acids in milk. The group also produced data suggesting that higher levels of antioxidants showed greater resistance to overall SOF presence. In 2000, Morales et al. [42] further found that oxidative flavor development could be affected directly, based on manipulating the dietary conditions of cows. In contrast to the previous research mentioned above, they found that fatty acids released from soybeans were actually somewhat protected from digestive hydrogenation and oxidation in the milk. However, they reiterated the viewpoint that higher copper presence in the milk mitigated this benefit, which likely led to greater risk of SOF formation. Other studies [35, 43] have also tangentially noted that PUFA levels could contribute to the SOF problem as well. In any case, most related research has reached consensus that SOF cannot be explained solely through particular kinds or levels of PUFA in milk, nor from particular feeding regimens. PUFA in SOF research have almost universally been observed in combination with other factors at work in the milk, given the oxidative

implications for SOF formation and PUFA as the obvious source of reactants for oxidative off-flavors.

Antioxidants

Antioxidant research in relation to SOF has been decidedly conflicted as well. Antioxidants are widely known to inhibit lipid oxidative flavor formation.[23] Historically, SOF researchers have focused on vitamins naturally present in milk, primarily α-tocopherol (vitamin E). Clausen et al. [44] found that oxidative stress in milk may proceed rapidly as a result of high initial lipid peroxide concentrations, as well as depletion of small molecular weight antioxidant compounds during oxidation of milk over time. In spiking higher levels of these low molecular weight compounds into SOF sensitive milk, they found increased resistance to SOF. While the group cited previous copper-related research when referring to causes of SOF formation, they found no data to suggest that it played a role in the SOF formation in their particular milk samples. Another group [38] that had incorporated antioxidant screening protocols found that SOF sensory defects were inversely related to antioxidant levels: feeds that contained higher levels of natural antioxidants were capable of inhibiting SOF formation. In 2007, Panda et al. [45] observed that supplementation of antioxidants like α-tocopherol in buffalo milk showed correlated quantitative increases of the antioxidant at the milk level, as well as subsequent reduction in copper levels and what they believed to be SOF defects. St-Laurent et al. [46] found that herds fed a silage diet, when changed to pasture grazing and supplemented with α-tocopherol for four weeks, generally contained less SOF than prior to changing diets. The

group suggested that those cows that did not have decreased SOF may have been genetically predisposed to be susceptible. Finally, Franke et al. [47] increased concentrations of another antioxidant, selenium, two- to threefold higher than occurred naturally in cows. However, they did not find that SOF susceptibility was reduced. In contrast, higher levels led to greater SOF occurrence, corresponding to potential prooxidant effects when antioxidant levels were abused.

One notable paper by Juhlin et al. [48] conducted a comprehensive study of all three aspects of typical SOF investigation: copper concentration, fatty acid composition, and antioxidant effects. They found that higher levels of unsaturated fatty acids in cows' diets led to correlated increases of PUFA in milk. There seemed to be a significant correlation between the resultant PUFA content and copper levels in forming SOF. In contrast to previous research, they were unable to find any inhibitory effects between α-tocopherol and SOF occurrence. Given some observed trends that were not fully explained by their results, the group also theorized that SOF could be a genetic issue among cows, particularly as it could relate to variable bioavailable copper levels from animal to animal.

Despite several research groups proposing genetic variability and breeding strategies to produce less SOF-susceptible and higher quality milk, very little work has been completed related to studying bovine genomes specifically in relation to SOF. Given a general scale of animal heritability between 0.0 (no genetic effects) and 1.0 (completely resultant from genetics), several studies have found relationships of higher incidence of oxidized flavor and genetic factors. In 1967, Kratzer et al. [49] reported a

heritability for general oxidized flavor defects of 0.26±0.17, and Neimann-Soerensen et al. [50] reported the heritability TBA-value (oxidation of milk) in their genetic study as between 0.17±0.04 to 0.47±0.06. Juhlin et al. [51] reported a value of 0.15 for SOF defects. While these values may indicate genetic variability and oxidative flavor presence, this still does not fully provide a complete picture. The 1967 study [49] in particular mentioned how breeding strategies to eliminate flavor defects would likely reverse the significant progress that had been made up until that point to produce higher milk yield.

As is evident by the body of literature, there are many who have attempted to explain the SOF phenomenon. Often, these studies have built on past work and contemporary knowledge of feeding regimen, milk conditions, and the environment. Many researchers have also employed relative increases or decreases in general organoleptic properties to assess SOF presence or absence within their milk samples, with little sensory data to substantiate the claims. However, to our knowledge no one has studied SOF in relation to the particular causative flavor compounds involved. As one study [43] aptly noted: "The explanation of the increase in SOF is obscure, probably not being due to one single parameter but rather to a number of contributing factors." There is assuredly potential for a greater fundamental understanding of specific SOF volatile flavor compounds. Positive confirmation of off-flavor compounds would afford much needed information for the dairy industry. More powerful analytical techniques and knowledge of flavor generation mechanisms that have been developed over the past six decades can provide insight of the causative factors of SOF and potential mitigation strategies for better bulk milk quality.

Creaminess in Dairy Products

Consumers often cite creaminess as a key factor in purchasing decisions as well as increased hedonic scores when consuming dairy products.[52] Creaminess encompasses two primary dimensions: volatile (ortho- and retronasal perceptions) and nonvolatile stimuli (gustatory mouthfeel/texture and taste perceptions).

Prior work has predominantly focused on the areas of volatile flavors and texture in relation to bulk rheology. Volatile profiles of dairy products have been extensively investigated at least as far back as the mid-twentieth century. Over time, researchers have provided the food and flavor industries ubiquitous volatile profiles and a variety of tools to utilize when attempting to define what constitutes high-quality creamy flavor. Historically, texture studies in relation to creaminess typically focus on the types and concentrations of dairy lipids or added starches present, as well as their resultant effects on overall product viscosity, rheology, and perceived texture.

Role of Volatiles

Volatiles in dairy products are primarily influenced by the flavor profile of the milk used, and thus hinge on the quality of the flavor profile received from the ruminant animal. Milk flavor quality, discussed above (see **General Milk Volatile Flavor Profiles**), is of the utmost importance as the starting point for quality creaminess. Flavor defects can occur based on cow nutrition (e.g. onion or garlic off-notes; antioxidant levels and resulting instigation of/resistance to catalyzed, oxidized flavors), processing contaminations (e.g. sanitation in processing plants), or metabolism, all of which can

affect and influence the final volatile flavor profile of the milk. In addition, this can have further ramifications if that milk is then used as an ingredient or processed into subsequent dairy products.

While "creaminess" may certainly be used to describe food products that are non-dairy in nature, there are nevertheless several volatiles that have been recognized as having notable influence on creamy flavor perceptions within dairy. Two key classes include lipid oxidation products (saturated and unsaturated fatty acids, aldehydes, ketones) and lactones. A study from 2001 [53] identified the principal volatile compounds of full-fat cream as γ-decalactone, δ-decalactone, δ-dodecalactone, (Z)-4-heptenal, and (E,E)-2,4-nonadienal. Likewise, another group [54] identified 2,3-butanedione, 3-hydroxy-2-butanone, dimethyl trisulfide, 2-nonanone, butanoic acid, acetic acid, dimethyl sulfide, and 2-butanone as key odorants for high-quality creamy dairy flavor. Schlutt et al. [34] reported that a few of the above mentioned volatile compounds have been found to induce creaminess in low-fat dairy foods specifically, including (Z)-4-heptenal, tetradecanone, and various free fatty acids, aldehydes, and lactones. The group also identified a series of volatile and semivolatile lactones present in cream that appeared to induce creamy flavor retronasally when added above threshold levels. However, it was evident from their work that no single aroma compound was solely responsible for creamy perception. The compounds that were most effective in inducing creamy flavor were derived from long-chained fatty acid triglycerides containing at least one short-chained fatty acid. In corresponding sensory studies, cream spiked with semi-volatile lactones was found to be significantly creamier without nose clips, while no significant

creaminess was perceived with nose clips. It was noted that bitterness and mouth drying increased in the presence of long-chain fatty acids.

While it has been accepted that the presence of certain staple lipid oxidation and lactone volatiles in dairy products are uniquely responsible for fatty flavors and overall creaminess, other works in the past several years have found less-studied, novel compounds that also impart creamy sensations. Sarrazin et al. [55] cited the role of some typical lactones and their sensory importance for full-fat cream, however they also noted the discovery of several new lactones as potential creamy enhancers, the most relevant of which was 4-methyldecan-5-olide. Lawless and Clark [56] found that adding general vanilla flavor to dairy samples also increased overall creaminess perception. Recently, another group suggested that volatiles with caramel-related notes from the Maillard browning reaction may actually play a role in certain creamy flavor profiles. They found 2,5-dimethyl-4-hydroxy-3-(2H)-furanone, and to a lesser extent dihydromaltol, maltol, and 5-ethyl-4-hydroxy-2-methyl-3(2H)-furanone respectively as the most intense novel contributors to quality creamy flavor in Ryazhenka kefir products.[57]

Despite near-universal acknowledgement of volatile flavors as inherently necessary for creaminess, research incorporating the more complex interplay between volatile and non-volatile contributions to creaminess perception has been much more limited. Tepper et al. [58] conducted experiments with a milk model system with various levels of oil (0%, 5%, 10% w/v) and natural cream flavor (0%, 0.5%, or 1% w/v). Panelists judged samples based on three attributes: fat content, mouth coating, and thickness. The samples with 5% fat content and the highest concentrations of flavor (1%)

were found to be similar in overall perception to that of 10% fat content and 1% flavor. Therefore, the higher flavor concentrations appeared to simulate a somewhat higher fat content in their samples. The group also proposed a psychophysical model of fat perception in dairy products that included viscosity, fat particle size and distribution effects, and volatile flavor concentration. Later, another study conducted by Visschers et al. [59] observed the effects that either ortho- or retronasal volatile stimulation could have on cross-modal sensory perception within a variety of food textures. The group developed a method whereby panelists would taste water, custard, or protein gels of various textures without added flavor. Then, the group would add strawberry volatiles ortho- or retronasally between 0.5 and 6.5 seconds after swallowing. The 6.5 second time delay appeared to intensify the volatile perception of the strawberry flavor. As firmness of the food sample increased, volatile intensity was diminished, but there did not appear to be a significant difference between ortho- or retronasal aroma delivery on this interaction. As a result, the researchers concluded that differences in texture could have an effect on volatile intensity and perception due to cross-modal interactions.

Recognizing the importance of nonvolatile stimuli on flavor profiles is a relatively young aspect of flavor research when compared to volatile work, only having been researched over the past fifteen years or so. As a result, there is a distinct lack of research conducted on volatile and nonvolatile profiles' mutual occurrence and cross-modality in foods. As advances are made in analytical, technological, and statistical processes, investigating the impact of volatile and nonvolatile stimuli and their interactions on flavor

perception will likely become more routine. This will allow understanding food flavors more holistically within a given food product, in situ.

Role of Nonvolatiles: Texture, Lipids, and Taste

Biological perception of texture. Texture (sometimes referred to as mouthfeel in some literature) has been studied in many foods, particularly within the last decade or so as the food industry has increasingly recognized the importance of texture to the overall food experience. In acknowledging texture, research has often focused on sensations such as audio/visual cues, bulk rheology, and viscosity during and after consumption (Figure 4).[60]

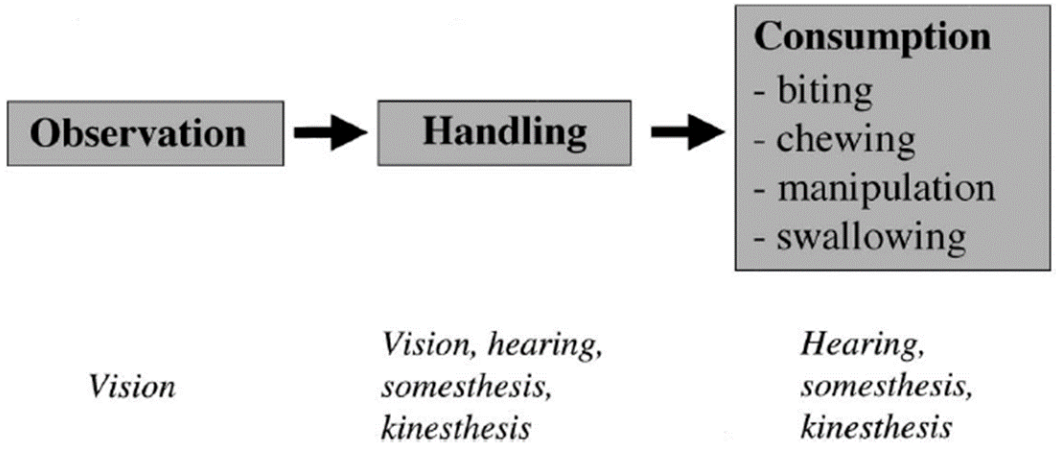

Figure 4, Involvement of the senses in texture perception during the process of food consumption (adapted from [60])

Upon entry into the mouth and subsequent mastication, food is broken down into smaller pieces through physical handling; shear and tensile forces; wetted; lubricated; further

broken down through enzymatic processes such as salivary α-amylase; and finally swallowed.[61] The mouth contains somatosensory tissues that vary widely in distribution and location among the different surfaces of the mouth. The roof of the mouth, the soft pallet, the tongue, the gums, etc. all have different nerve receptors, concentrations of these receptors, and various recognition thresholds based on location, receptor type, and tissues in the mouth.[62] In general, different textural receptors may be either large or small, as well as slow or rapidly adaptive. A rapidly adaptive receptor would be more important upon initial discovery and perception of a food when placed in the mouth; in contrast, a slower adaptive receptor would be important in recognizing a prolonged sense of textural change in the mouth.[60, 63, 64] Indeed, the tongue contains all these types of receptors, which comes perhaps much more heavily into play during food consumption as a result of the tongue's core role for mobility and food manipulation in the mouth. Rapidly adaptive receptors are important for external physical perception in the mouth, including for "self-touch" as the tongue moves across the body's own tissues in the oral cavity. In contrast, the slower and more deeply buried adaptive receptors are more important for proprioception, the body's self-recognition of its own internal movements.[62, 65, 66] Other receptors that also play a role in general oral sensory recognition include thermal and pain receptors, and while these assuredly influence perception of many foods, this review is primarily concerned with the mechanoreceptors as key contributors to texture in relation to creaminess.

Certain textural sensations have been more regularly studied over the past several decades, such as astringency. Several mechanisms for astringency and puckering

sensations in the mouth have been elucidated, most notable of which suggest a decreased lubrication in the mouth from precipitated salivary proteins as a results of the presence of astringent compounds.[67, 68, 69, 70] Others have suggested alternative mechanisms including increased friction in the mouth, or cross-linking of proteins with the oral epithelium during consumption.[71] Initial preferential interaction of astringent compounds with salivary proteins was also suggested to result in the formation of more extensive networks in the mouth. This would further create astringent perceptions as a result of interactions with other compounds in salivary solution. [72]

Classifications of texture. Initial classifications of texture of food began in earnest in 1963, when Szczesniak [73] grouped textural characteristics into three main categories: mechanical (encompassing hardness, cohesiveness, viscosity, elasticity, adhesiveness), geometric (food size and shape, food shape and orientation), and "other" characteristics (primarily moisture and fat content) (see Table 2 below). In conducting analytical tests, the group found that two descriptors in a product were not necessarily mutually exclusive; foods encompass a wide range of descriptors and product-specific sensations. In 1966, Guinard and Mazzucchelli [64] presented a good summary of sensory perception of texture and 'mouthfeel' with a slightly more thorough perspective, including "creaminess." Their review, while examining "creaminess," "astringency," "smoothness," and "viscosity," also included other descriptors like "juiciness" and "carbonation." Indeed, texture has perhaps been more commonly recognized and investigated in relation to creaminess perception. For the purposes of understanding

creaminess, it is worth noting that research often classifies descriptors like “smoothness” and “viscosity” both before and after consumption as sensory sub-attributes of creaminess, rather than distinct, separate categories.

Table 2, Classification of textural characteristics [64, 73]

Table 1. Classification of textural characteristics[a]

Mechanical characteristics: Primary	**Secondary**	**Examples**
Hardness		Soft, firm, hard
Cohesiveness	Brittleness	Crumbly, crunchy, brittle
	Chewiness	Tender, chewy, tough
	Gumminess	Short, mealy, pasty, gummy
Viscosity		Thin, viscous
Elasticity		Plastic, elastic
Adhesiveness		Sticky, tacky, gooey
Geometric characteristics: Class		**Examples**
Particle size and shape		Gritty, grainy, coarse
Particle shape and orientation		Fibrous, cellular, crystalline
Other characteristics: Primary	**Secondary**	**Examples**
Moisture content		Dry, moist, wet, watery
Fat content	Oiliness	Oily
	Greasiness	Greasy

Texture and creaminess perception. Some researchers have attempted to determine the roles that particular lipids and other ingredients have on creamy sensations

and overall product acceptability. In 1988, Mela [74] conducted a study that compared whole milk, half-and-half, heavy cream, and a mixture of half-and-half and heavy cream in relation to perceived fat content, creaminess, and pleasantness by a sensory panel under a variety of conditions (lack of visual stimuli, noseclips to eliminate olfaction, etc.). The group added various starches to samples, mimicking the mouthfeel and viscosity of full-fat dairy products. The increased viscosities seemed to enhance the "fat content" rating of the lower fat samples. It did not, however, drastically change the overall perceived creaminess or pleasantness between samples. Their results illustrated that "fat content" could be somewhat more textural in nature, as well as being capable of recognition via oral stimulation.

Daget et al. [75] found that in prepared model dessert creams with various fat and thickener concentrations, creaminess was found to depend on both thickener and fat content, but more so on thickener concentration. The group developed a quadratic formula for product rheology and perceived creaminess and hedonic scores. However, their formula suggested that optimum liking did not match maximum creaminess; rather, it depended on thickener and fat content, which they developed based on response surfaces for optimization of hedonic scores for the desserts (Table 3).

Table 3, Optimum viscosity, shear thinning, and sensory scores for creams at different fat levels[75]

OPTIMUM VISCOSITY AND SHEAR THINNING FOR EACH FAT LEVEL

% fat content	Optimum Liking Consistency			Highest Creaminess		
	Viscosity (mPas)	Flow Beh. Index n	Sensory Score	Viscosity (mPas)	Flow Beh. Index n.	Sensory Score
3.5	812	0.16	18.5	880	0.15	17.5
10	812	0.15	18.7	1064	0.14	20.5
20	916	0.20	17.9	1808	0.11	22.6
30	880	0.26	15.6	7480	0.04	26.9

Another study aimed to observe the physical and physiological effects of structural breakdown on creaminess perception in semisolid foods. Weenen [76] found that in the presence of an α-amylase inhibitor, a 0% fat dessert was described as increasing in creaminess by approximately 59%, whereas a 3% fat dessert was not affected. The group attributed this result to the possibility that starch breakdown and disruption of the continuous phase of the product affected perceived roughness and fat surfacing in the mouth. The group also attempted to define the "roughness" described in their analyses, whether by friction, perceived particles by tactile movement, vibrations in the mouth, or a combination of these factors. Their tests found a correlation of "roughness" descriptors with particles approximately 70 to 80 μm in diameter, about the size of a starch granule. They further attributed "thickness" and "melting" sensations to the breakdown of the starch network upon exposure to amylase in saliva during gustation, although if true, this

would predominantly affect products where a significant starch presence contributed to the texture of the overall product.

Other studies have incorporated sensory panels and experimental designs attempting to reproduce consumers' experiences of creaminess. One such study [77] evaluated creaminess in dairy products in terms of fattiness, texture, flavor, and pleasantness. Twelve different dairy products were sampled, including soft white cheeses, cream desserts, set yogurts, stirred yogurts, *mousse de lait*, and *crème de yaourt*, both common French desserts. Three main categories were outlined: texture, fattiness/natural flavor, and sweetness. Based on panelist responses, creaminess was reported to be a combination of smoothness, homogeneity during gustation, and softness, and the panel mainly associated creaminess with texture and overall pleasantness. In another review,[78] it was found that three individual sensory attributes—thickness, smoothness, and slipperiness—were responsible for approximately 74% of the variance in creaminess among 16 products rated for their rheological properties. However, the group noted that in dairy products specifically, these descriptors could be refined solely to thickness and smoothness. Creaminess was reportedly enhanced when the bulk food product was characterized to have a minimal physical breakdown after mechanical stress. The same group also studied fat content in relation to perceived creamy or fatty mouthfeel, finding that foods with fat contents under 15% resulted in both creaminess and fattiness perceptions, although creaminess was identified at higher levels of fattiness. At fat contents above 15%, fattiness replaced creaminess as the more dominant perception, and both leveled off at higher concentrations (see Figure 5 below, adapted

from Wijk et al.[79]). In that study, greater creaminess perception was related to a higher initial stiffness of the products, followed by low shear stress after the product began to flow after breakdown.

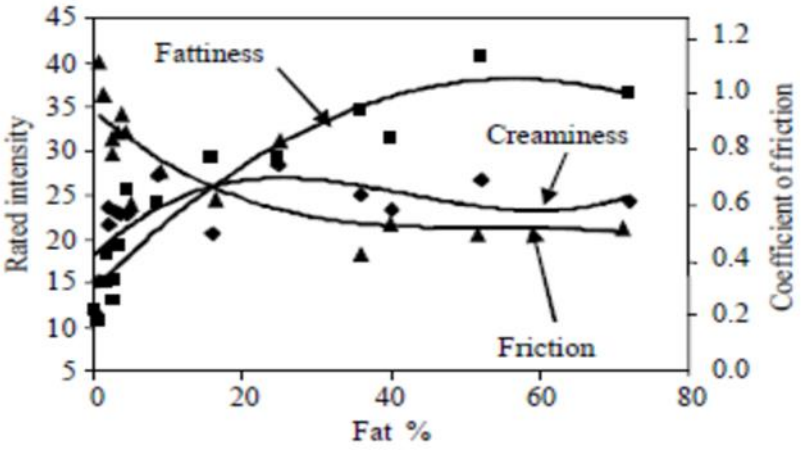

Figure 5, Fat content vs. sensory intensity of creaminess (diamonds), fattiness (squares) and instrumental friction (triangles)[79]

In 1999, Elmore et al. [80] studied eight puddings with various degrees of thickness, mouthcoating, rate of melt, and smoothness using defined kinds and levels of starches, milk fat, and salts. Using descriptive analysis techniques for appearance, texture, and flavor of the products, they found that descriptors like “airy, thickness (visual and oral), denseness and mouth coating, dairy flavor and aftertaste” were all positively associated with creaminess; negative descriptors included “surface shine, rate of melt, sweet flavor and sweet aftertaste.” Overall, they found ‘thickness’ to be an important descriptor for

consumer acceptance of creamy texture. Creaminess was found to be higher for products that were "smoother" and seemed to contain more "dairy flavor." Hedonic scores correlated with thicker, higher fat puddings than thinner, low fat samples. Later, Weenen et al. [81] employed quantitative descriptive analysis and principal component analysis to elucidate what they asserted were six different textural dimensions in commercial semisolid foods. The foods observed included mayonnaise, dressings, custard desserts, and warm sauces, and panelists developed six textural dimensions including viscosity, surface feel, bulk homogeneity, adhesion/cohesion, wetness-dryness, and fat. Two other nontextural attributes deemed important were perceived temperature of the products and oral irritation. In contrast with previous studies,[82] they found that descriptors "heavy, light, gummy, and slippery" were not important in the products they studied. Later, the same research group [83] reported that thick, airy, smooth, and fatty sensations were positively associated with creaminess in mayonnaise, custard desserts, and sauces, while rough, heterogeneous, melting, and grainy sensations were more negatively associated. The group found that orthonasal aroma and trigeminal sensations did not contribute to creaminess, although various kinds and concentrations of retronasal flavor could positively or negatively contribute to the mouthfeel of creaminess as well.

In 2003, de Wijk et al. [84] aimed to describe the texture of semi-solid vanilla custard dairy desserts and resulting sensory effects. A descriptive analysis panel identified two key textural scales: one ranging from *melting* to *thick*, and the other from *rough* to *creamy-soft*. After presenting several types of custard, thickener, and fat content, the researchers found that the melting/thick dimension developed by the panel related

predominantly to thickener type, and the rough/creamy-soft dimension was related to fat content. It was suggested that higher fat products were correlated with higher sensory scores due to increased lubrication in the mouth and release of volatiles. Later, the same group [85] hypothesized that the dominance of the *creamy-soft* dimension could be attributed to friction in the mouth, particularly since the samples that were rated highest in their previous study were correlated with products containing higher levels of fat. They found that starch-based custards with lower fat levels had higher roughness ratings, lower ratings of creaminess, and higher measurements of oral friction. The opposite held true when the custards contained higher fat levels. They also found that physical and interactive effects of fat droplets could affect friction. Smaller fat droplets allowed for more enhanced surface area interaction of the lipids with the rest of the mouth, and less overall friction. In general, they asserted that fat was successful in reducing friction at levels between 0% and 5% in their food products, although in some cases as high as 10%. These appeared to decrease in effectiveness at levels up to 20%, however. Later, they attempted [86] to integrate a quantitative prediction model for creamy mouthfeel in custard desserts and validate the protocol in commercially available yogurts. They had previously acknowledged [81] texture of semi-solid foods as incorporating six categories, including attributes related to viscosity, surface feel, bulk homo-/heterogeneity, ad-/cohesion, wetness/dryness, and fatty sensations. In the end, they obtained descriptors like “thick,” “airy,” and “fatty” mouthfeel which were positively correlated with creaminess, while “rough” and “dry” mouthfeel were negatively correlated.

In 2007, Frøst et al. [87] presented a review of four of their previous studies incorporating descriptive sensory and rheological analyses of different dairy products: acidified milks, vanilla yogurts, plain stirred yogurts, and cream cheese. Their compiled results showed that creaminess sensory properties were partially described by rheology, but there were other missing factors as well. Visual cues were important in their samples (glossy, grainy, etc.). Overall, 'smoothness' was intrinsically important for creaminess, as it was important in all four products. Viscosity and fatty aftertastes also seemed important. In contrast, graininess, chalky mouth feeling, stickiness and drying were negatively correlated to creaminess ratings. They found that astringency was an important factor for creaminess in their acidified milk samples, yet negatively with all the other dairy samples. This could have been explained by other properties present in the milks, including high levels of aroma, viscosity, and fattiness. Frøst's previous work [88] in acidified milks showed that smoothness and creaminess were somewhat related. Creaminess was directly measurable with descriptors regarding appearance, aroma, taste, flavor and texture, while "smoothness" was only moderately correlated to creaminess. In their vanilla yogurt study,[89] the group experimented with variable sample texture with microparticulated whey proteins, taste with various levels of sugar, and aroma with vanilla flavors. Vanilla yogurts were most positively related to sweetness, volatiles (coconut, cream, caramel, vanilla descriptors), viscosity, and smoothness. This was similar even in plain stirred yogurts.[90] Yogurts were generally considered more creamy when viscosity was high. Finally, cream cheese was positively correlated with

smoothness and high meltdown rate upon gustation. Additionally, other descriptors like cream and butter aromas were also related.[91]

Each of these approaches—lipid and ingredient effects on texture, physical breakdown of foods during gustation, and sensory experimentation to determine creaminess characteristics—all have their place in bringing much-needed context to the definition and physical parameters encompassed by creaminess. What is evident, however, is that they all vary according to what creaminess aspects are most important in the specific foods studied, or according to certain evaluation panels. They recognize phenomena correlated with creaminess, but frequently approach it from the same general sources (e.g. lipids create a defined threshold of creaminess), and may include particular descriptors that others do not. Whether this is due to fundamental product differences, processing conditions, or ingredients, can often be inferred. In any case, these may have more relevance in helping evaluate what descriptors are associated with creaminess, or even what creaminess is conceptually, rather than providing specific solutions for a range of dairy products.

Analytical and mathematical optimization of texture. A comprehensive review of much of the food textural analysis research of the 20th century was published in 1987. In it, Kokini [92] presented various mathematical and descriptive approaches his group had developed over several years for understanding texture and viscosity in foods in relation to creaminess. The three key terms the group proposed included ‘thick,’ ‘soft,’ and

'slippery.' In applying these terms to a three dimensional textural space, they developed the following regression equation:

$$\log creamy = thick^{0.539} + smooth^{0.728} + slippery^{0.220} \ (^{82})$$

The group [93] attempted to define thickness in particular as the shear stress on the tongue, the "motion of the tongue whereby the liquid is squeezed between the tongue and the roof of the mouth…" In dairy products specifically, they further refined their equation to:

$$\log creamy = thick^{0.540} + smooth^{0.840} \ (^{92})$$

Using their equation, creaminess scores were able to be plotted in dairy and other products using a regression line presented below in Figure 6:

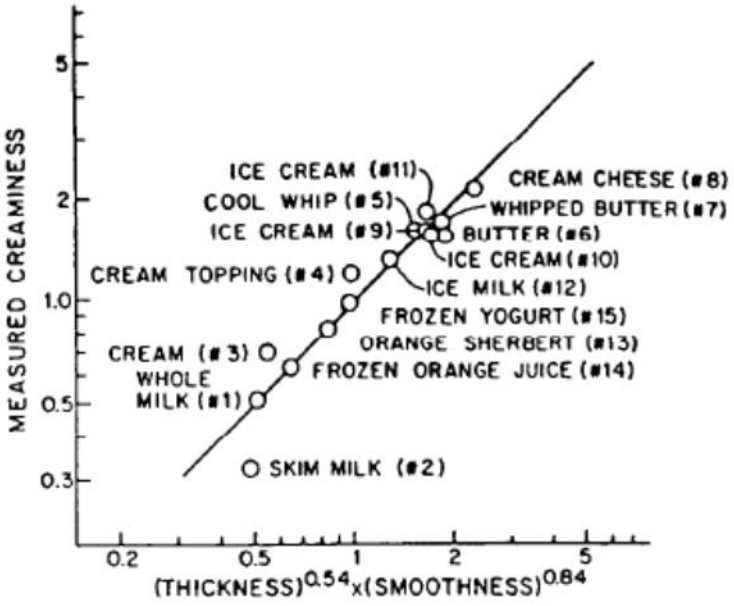

Figure 6, Magnitude estimate of sensory creaminess scores vs. log(creamy)[92]

However, later research suggested that this equation appeared to be unique to certain kinds of dairy products, as other products had significantly lower R^2 values. This suggested that 'creaminess' encompasses more than just thickness and smoothness during gustation. Kokini [94] also presented several other generalized mathematical models for understanding thickness in foods. This included equations for Newtonian foods, non-Newtonian foods, non-Newtonian foods with time-dependent factors, and foods that melt in the mouth. Various "universal curves" were developed to illustrate a variety of food products in relation to shear stress and normalized spreadability.

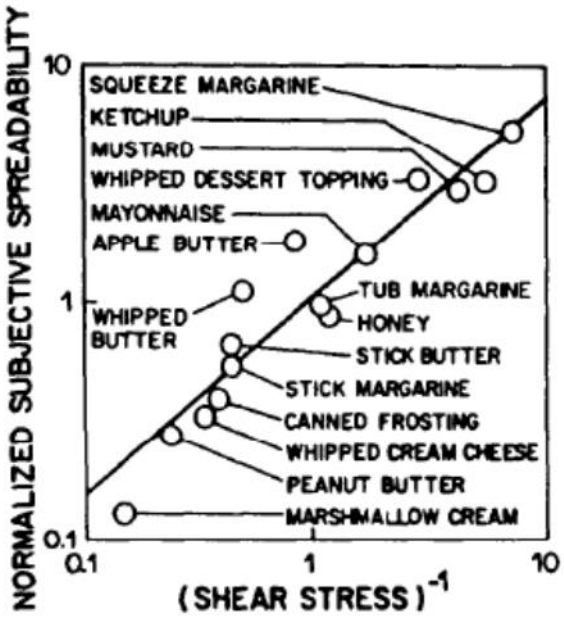

Figure 7, Subjective spreadability vs. transient shear stress on knife[94]

Mathematical models for creaminess may be useful to a point, but because of inherent differences in products due to lipids, added ingredients, variations in flavor profile (volatile and nonvolatile), etc. it may be difficult to correlate equations with tangible sensory responses. Some of these studies do not mention follow-up sensory evaluation to ascertain relevance in actual food products. In general, mathematical models may be useful as a starting point for product development, or even in initially screening or rating samples for creaminess. However, using these studies as an encompassing solution for creaminess quality may prove troublesome once applied to certain products.

Particle size. Particle size has also been investigated in relation to smoothness and texture. In 2002, Kilcast and Clegg [95] investigated whether solid (calcium carbonate or alumina), liquid (oil or fat droplets), or gaseous particles (air bubbles) could impart creaminess characteristics to model systems of chocolate mousses and artificial creams. They found that creaminess increased upon addition of small solid particles, while larger particles lent themselves to greater grittiness perception by their panelists. However, they were not able to draw solid conclusions from the small particle addition due to an increase in sample viscosity as well. Their suggested particle size cutoff for creaminess impact was between 4 and 7 μm. In their liquid cream systems, results suggested a greater creaminess perception imparted from texture, but not much in terms of creamy flavors. Their artificial creams increased creaminess perception with greater fat content, but also contradicted some previous literature [85] in that they also found enhanced creaminess with a greater fat droplet size. However, they attributed these results to a greater concentration of solid fat in their system, and not exclusively to the oil-in-water liquid emulsions incorporated. Finally, their aerated mousse models reinforced the concept that air bubble size was a key driver of creaminess perception. Additionally, they found total air content as a potential secondary factor of creaminess, but this may have been due to viscosity changes in the products as a result of more or less air present in the samples.

In general, particle size studies have found features of value in understanding creaminess, especially in offering ranges of particles with mouthfeel activity. It is important to remember that, based on the target formulation, levels of fat, etc., some of

these methods may be more helpful or relevant than others. Even so, there is also some contradiction between particle size hypotheses on mouthfeel in the oral cavity, as well as complexities added through ingredient addition (i.e. air for whipped or aerated dairy products) that may not allow these phenomena to perform universally in every food.

Investigations of product-specific creaminess. Less commonly, researchers have approached understanding texture within the context of specific products to inform theories about creaminess perception. For example, Muir et al. [96] studied commercially available strawberry flavored fermented milks to ascertain the predominant sensory perceptions present and classify them according to product composition. They found that panelists associated fat content with creaminess (defined in their study as aroma, flavor, and mouthfeel). However, they also associated creaminess with the presence of protein, including flavor, mouthfeel, and mouthcoating. They noted that samples with higher protein were categorized with descriptors such as acid flavor, chalky mouthfeel, and aftertaste. Interestingly, they also found that samples containing gelatin apparently had higher overall creaminess ratings, the effects of which were dampened somewhat when combined with starches in the formulations. Unfortunately, they did not distinguish what particular factors were responsible for these textural and creaminess descriptors, only drawing parallels to bulk ingredients present in the commercially available products.

Others have focused on the mouthfeeling present in milk itself. Dunkley [97] found that full fat whole milk was a richer source of desirable mouthfeel attributes than skim milk. Obviously, given similar processing conditions, the immediate differences between

these food products are the presence of milkfat, differing by approximately 2.7-3.0%. However, even small differences in fat concentration and emulsion properties, as noted above, can affect the mouthfeel and overall texture of dairy products. Furthermore, variables such as cow breed, diet, season and other environmental conditions have also been shown to affect mouthfeel and texture properties of milk. Pastured cows have previously been found to have a less "harsh" milk than those that were given dry silage diets.[22] There could be other textural properties at work that distinguish the dairy products other than the bulk fat. Bulk rheological properties can seemingly account for only part of the textural properties present in dairy.

Lipids

Lipids typically influence the sensory characteristics of a food system in a variety of ways. Volatile effects from lipids have been discussed earlier, however fats also play a major role in nonvolatile perceptions in food, such as mouthfeel and flavor profile richness. In terms of both volatility and nonvolatility, lipids can mask off-flavors, and they also allow for diffusion of a variety of flavor components between the aqueous and lipid phases in a food, producing a balanced flavor profile.

For the past several decades and particularly arising in the 1990s, there has been an increasing association of dairy product texture with dairy product quality. With the rise of low- and zero-fat dairy products in the marketplace, fat replacers were incorporated to attempt to provide higher quality dairy products while meeting consumer demand for "healthier" dairy product selections. Various methods for replacing fats

included the addition of other functional ingredients called fat mimetics. Some of these replacers included short chained fatty acids, sucrose polyesters and polymers, and micro-particulate proteins.[98] Carbohydrates and polymers can be used to absorb water and provide added moisture and texture to mimic sensory losses from fat removal. Micro-particulate proteins can absorb moisture, as well as provide a host of hydrophilic and hydrophobic sites for emulsification properties and other ingredient interactions.[24]

Fat extenders and fat substitutes were also developed. Extenders typically include emulsifiers that incorporate some of the beneficial aspects of lipids while having a lower overall caloric value and still offering a similar functionality as the full fat. Fat substitutes, in contrast, serve to replace the normal functions of fat in food while also offering decreased or zero caloric value. Modified lipids can become less absorbed by the body, also providing lower overall caloric values.[24]

Over the years, several methods have been proposed for qualitative and quantitative evaluation of lipid contributions and effects on texture in food. In 2006, Prinz et al. [99] generated two quantification methods for determining degree of lubrication (fat content) and viscosity (starch content) in semi-solid vanilla custards. One method employed measuring the turbidity of two post-consumption rinses with water to evaluate residual oral coating after swallowing the custards. The group found this method was sufficient for representing the viscosity, fat content, thickness, creaminess, and fattiness of the respective samples. In general, the first rinse was more highly correlated with aspects of both lubrication and viscosity: terms were used such as “thickness, melting, creaminess, and fattiness.” This was likely due to the removal of the outermost layer of

lipid and starch coating on the tongue, directly correlated with the fat and starch content of the custards. The second rinse was correlated predominantly with fat content alone, and the group hypothesized that the lack of exposure to the atmosphere of the lipids immediately adjacent to the tongue's surface may indicate a greater importance of this fraction for taste and trigeminal sensations, rather than for volatiles. Overall, the differing ingredient concentrations of these rinses pointed to the potential dynamics of non-homogeneity in relation to food matrices, ingredients, and resultant effects on oral coating. The second method involved quantification with opto-electronic reflection sensors of the tongue surface; however, the method did not adequately correspond with sensory results. Later, Pivk et al. [100] sought to examine the role that mouthcoating had on overall fatty mouthfeel. The group presented three distinct methods to quantify lipid retention in the mouth, including the determination of lipid mass after oral rinsing, the evaluation of the thickness of mouthcoating by measuring fluorescent dye spiked into the lipids, and the measurement of localized lipid thickness on the tongue with filter paper. Other methods included analyzing the turbidity of expectorated samples, semi-quantitation of lipids using mouth swabs, and models illustrating that pure oils could become emulsified in saliva during oral processing. The group found that lipid coating on the surface of the mouth affected mouthfeel and oral perception, which their data showed to be an important quality in reduced fat foods. The group also found [101] that samples containing medium length triglycerides and resultant coating in the oral cavity increased logarithmically with higher lipid concentrations. After a certain point, however, mouthfeel was not found to increase. Lipid perception primarily accumulated on the

tongue, not on the other surfaces of the mouth. Neither did the lipid deposition retain for long, having mostly disappeared after two minutes of gustation.

Others have approached lipid perception in relation to bulk rheology and product-specific physical characteristics. In 2006, de Wijk et al. [78] suggested that in various starch-based custard desserts, lipid contributions in food matrices could be attributed to two factors: bulk rheological properties of the food and the surface properties of the bolus. It was hypothesized that during gustation, fat globules could be released and enhance both lubrication in the mouth and the release of lipid-soluble flavors, thereby enhancing creaminess. The group found that select sensory dimensions identified by their panelists—creaminess/fattiness, stimulus viscosity, surface airiness, and bulk viscosity heterogeneity—showed both clear differences and some commonalities among custards. Due to the non-fat rheological basis of the custards they used, these results were important in understanding bulk viscosity and alternatives for low-fat food products, as well as how even minimal amounts of fat could play a role in overall texture perception. Drewnowski [102] observed the sweetness, creaminess, and fat content ratings of liquid (skim milk, whole milk, half and half, heavy cream) and solid dairy products (cream cheese, cottage cheese) with varying amounts of fat. His results showed that sweetness and fat content were easily discernible for liquid milks and heavy cream but were more difficult to differentiate when rated in the solid foods. Interestingly, despite inconsistent sensory responses, panelists consistently rated stronger hedonic scores for the samples with higher sugar and fat even in the solid foods, despite not being able to rate them accurately.

Still others have investigated the effect that lipids, in the form of emulsions, may have on taste, thickness, and creaminess descriptors. Mela et al. [103] found that panelists' perceptions of emulsions created with higher pressures and decreased fat globule sizes created greater perceptions of fat content and smoothness. A higher degree of fatty acid saturation was shown to produce higher perceived fat contents as well. The group suggested that fat concentration appeared to have a sizable contribution to perceived fat, which was independent of rheological effects. Richardson and Booth [104] studied the effects that individual panelists' performances and abilities had on discriminating emulsion viscosity, lipid globule size, and between-globule distances. They found that some individuals were able to distinguish differences in fat-globules based on distribution, size, and distance down to 0.5-3.0 μm. They also found that some individuals were able to sense variations in product viscosity as small as 1.0 mPa. Overall, panelists varied in their ability to discern creaminess based on these factors, as well as whether a high-fat milk or cream was best as a standard of comparison. The researchers noted that globule size and distribution were not sufficient to completely describe the physical parameters of creaminess perception in their products. In 2005, another group [105] studied how emulsions with varying oil droplet size, concentration, and added thickeners would affect overall creaminess. Sensory results showed that taste, thickness, and creaminess were all significantly increased with either higher viscosity or fat content. Creaminess perception in particular was increased more through a higher net viscosity than by a higher oil volume in the emulsions. While the researchers noted that studies had found various effects of lipid droplet size on textural effects and fat

perception, they found that an increased oil droplet size from 0.5 μm to 2.0 μm had no effect on the different creaminess descriptors.

There is evidence that optimized emulsions may also be able to contribute to fatty oral perception and thus greater creaminess in dairy products. Richardson and Booth [106] found this to be the case. In their 1993 study, higher concentrations of fat globules contributed to creaminess. Smaller and more uniform fat globules produced greater levels of smoothness, especially in combination with greater viscosity values in solution, likely as a result of the increased surface area for the emulsions. Others have also pointed to this phenomenon as well.[85] In 2006, Akhtar et al. [107] found that in butter oil-in-water emulsions with sodium caseinate as an emulsifier, 'thickness' and 'creaminess' were influenced primarily by emulsion viscosity. Fat content also contributed to these sensations as well. Two different thickeners, maltodextrin and xanthan, were used for constant defined solution viscosities with shear rates of 50 s^{-1}, and both resulted in markedly different levels of creaminess ratings. However, they found that added flavor increased hedonic score but did not contribute to thickness or creaminess. Despite this, in their models viscosity was not in itself sufficient to fully describe the thickness or creaminess ratings either.

Lipids assuredly offer a major component of quality creaminess in dairy products, and they are frequently one of the most commonly identified aspects that consumers perceive when evaluating creaminess. Nevertheless, despite many studies regarding lipid concentration, mouthcoating, bulk rheology, type of product, and emulsion effects on lipid perception, no single parameter is capable of generating the complete picture of

creaminess. On their own, lipids may impart certain aspects of creaminess (i.e. slickness, slipperiness, bulk rheology in the mouth), but without the added context of volatiles, these descriptors could easily become associated with negative descriptors like greasiness or oiliness. Furthermore, the lack of fat in zero and low-fat dairy products is a major hurdle that is difficult to overcome, even with the industry solutions that have been used over the past decade. Therefore, it is worth investigating alternate sources of nonvolatile contributions or textural perceptions to potentially work in tandem with, or even aid in replacing, fats in dairy products.

Taste and physiochemical sensations of creaminess

As mentioned above, dairy products encompass various concentrations and kinds of tastants depending on variables such as fermentation, processing, and the target flavor profile. Each of the five basic tastes can be included in dairy products, including sourness (lactic, acetic acids), bitterness (casein hydrolysate),[108] saltiness (sodium), sweetness (lactose), and umami (glutamate).[109]

Taste perceptions have been studied primarily in relation to general flavor profiles of dairy products, but very little has been investigated in relation to creaminess. Some research has shown that tastants and texture may actually affect overall perception of volatiles in dairy products. Lethuaut et al. [110] found that higher concentrations of sugar or starch in six different kinds of dairy products were generally found to be related to higher perceptions of aroma compounds, despite no significant changes in overall higher quantitative release of the compounds from the food. They found that samples

incorporating λ-carrageenan showed slightly higher aroma perception versus those products made with ι-carrageenan or κ-carrageenan, however they did not appear to affect quantitative aroma release. It is possible that cumulative interactions for thickeners or other molecular interactions such as these could very well affect typical 'creamy' volatiles, which would have an effect on the food system as a whole during consumption.

One notable paper [34] described certain compounds that imparted taste specifically in relation to creaminess perception, in combination with volatiles and semi-volatiles. Compounds that were isolated from fat isolates of full-fat cream included lactones with longer aliphatic fatty acids in their structures. These compounds were bitter, but as the carbon length of the chains increased, the group found that an oily mouthfeel was also imparted to the dairy products. Some samples exhibited an increased melting behavior upon whipping as well. Sweetness has also been associated with general creaminess quality in dairy as well.[75, 80] This is particularly relevant in dairy products, which typically have subtle sweetness presence due to the lactose naturally present in milk. In dairy processing, added sugars typically found in formulations for rheological, gustatory, or processing effects (e.g. microbial fermented dairy products) also lend sweetness to the overall flavor profile. Sweetness has indeed been found to be an important indicator of hedonic liking among creamy products, as well as sourness depending upon the product.[52]

Still others[111111] have studied how the viscosity of food products may also affect taste perception. Based on the viscosity and resultant diffusivity of tastants in a food system, tastants may be perceived slower in more viscous foods. A model proposed by

Kokini et al. [111] assumed that taste intensity in the mouth is virtually immediate upon interaction of the tastant with the taste bud. Therefore, the rate of diffusion of the tastant would be the limiting factor for taste within the food.

Over the past several years, others have attempted to link tastant perception with somatosensory and oral physicochemical perception of sensations such as creaminess. In creaminess research, this has predominantly been through understanding potential correlation of genetic predisposition and taste perception. In 2003, Kirkmeyer and Tepper [112] conducted a study that attempted to understand creaminess perception of dairy through free-choice profiling and panelist response to 6-*n*-propylthiouracil. They used ten confirmed nontasters and ten supertasters in combination with nine different dairy products to understand panelist variation in flavor or texture cues for creaminess perception. The descriptive scales incorporated in the study formed around a sweet/sour axis and a dairy flavor/texture axis. The group found that the basic techniques the two groups of tasters used in identifying creaminess were basically the same, although they differed predominantly in the number and quality of descriptors they used to portray the sensations they experienced. Nontasters placed equal emphasis on taste and flavor/texture sensations, while supertasters placed slightly more emphasis on the flavor/texture dimension. A few years later, Lim et al. [113] conducted further research attempting to observe PROP effects on taste and tactile sensations in the mouth, as well as creaminess perception of three different dairy products. They found that perceived intensities of PROP did not accurately predict perception of creaminess, nor did it describe ability to determine textures by oral tactile sensations. The group suggested that other biological

factors like tongue fungiform density may be more useful in assessing tactile oral abilities. This has been supported in some cases. For example, panelists in a separate study [114] were able to distinguish between fat content among different salad dressings, and data suggested that this could have been due to tongue papillae densities, which differed among nontasters, medium tasters, and supertasters.

In 2011 Kutter et al. [115] used a localized anesthesia to nullify the mechanoreceptors in the mouth of subjects, after which sensory panelists were asked to describe the sensations they perceived in custard desserts. Their study found that proprioception had no effect on the perception of thickness. Rather, the mechanosensors were apparently the dominant method that panelists used to distinguish viscosity differences between samples, particularly resulting from the bulk rheology of the food.

Lastly, it is worth mentioning that in 2015, a paper was published [116] asserting that a sixth taste may exist, which the group termed "oleogustus." The group used two different sensory sorting tasks to determine whether humans could experience taste sensations elicited from short, medium, and long chain fatty acid samples in comparison to other commonly used taste standards (sweet, sour, bitter, salty, and umami). They claimed that their data strongly suggested that non-esterified fatty acids have a gustatory taste sensation independent of the other five tastes. However, their data did overlap somewhat with both sour and umami tastes. While there was some indication of other novel sensations at work, it remains to be seen if this is indeed a new taste. While the group did verify the viscosity and particle size distributions were not significantly different between the samples, there could be other factors at play, such as trigeminal

sensations or mechanoreception that were not measured in the study. The group noted some hurdles, including how some panelists did not seem able to distinguish some of the test samples compared to the controls, the concentrations of fatty acids in their samples may have been higher than would be found in most naturally existing food products, and there may not have been adequate rinsing in some cases, leading to sample carryover. At a minimum, further work is needed before positively identifying their findings with a true "sixth taste."

Food is consumed in summation of its parts, nonvolatile and volatile, and greater understanding of both areas will provide a better basis for moving beyond model systems in the laboratory to application in actual food products. Creaminess in particular can be difficult to adequately define, since it encompasses both volatile nonvolatile profiles and sensations. The contributions of volatiles have been decidedly more well-studied in a variety of dairy products over the past sixty years. In comparison, the nonvolatile body of research, while extensive in certain areas like lipid perception threshold and product viscosity, is lacking in areas that might offer different, novel approaches to influencing texture in a way that is non-lipid based. This may have greater ramifications for zero and low-fat creamy dairy products in particular. In creating new knowledge related to the nonvolatile profile of dairy products, further strategies may be developed for understanding the creamy profile of dairy in its entirety. Understanding and introducing viable solutions for influencing texture and creaminess may put the wider picture of creaminess perception into greater context. This can only help in providing a variety of options for consumers and strategies for product developers.

Chapter 3

Off-flavor Analysis and Inhibition Strategies for Midwestern Bovine Milk

Abstract

Spontaneous oxidized flavor (SOF) has been cited in literature as a sporadic off-flavor problem in bovine milk from the Baltic region of northern Europe; however, recently there have been increasing occurrences around the world and particularly the Midwestern region of the United States. Over the past five decades, researchers have investigated factors that influence the SOF development in milk but few have been successful, limiting the ability of the dairy industry to monitor, control, and develop mitigation strategies for this milk off-flavor problem. In order to gain better insight on SOF development in milk, the causative off-flavor compounds in tainted milk samples obtained from a farm in the Midwestern United States were identified and pathways of off-flavor generation were proposed. Solvent extraction, GC/MS coupled with olfactometry, and OSME intensity values were employed. Key volatile compounds including endo-borneol, 2-methylisoborneol, and α-terpineol were identified in off-flavored milk samples stored for 14 days after HTST processing. Sensory recombination experiments were performed and degree of difference experiments indicated that milk samples spiked with the identified compounds were significantly different from control milks. The panelists described the milks as “green,” “musty” and “unclean,” which correlated with perceptions noted in the original off-flavored milk samples.

In past studies, these compounds have been previously documented as important off-flavor taints from various microbial sources. Identifying these particular off-flavor compounds in samples initially targeted for the SOF phenomenon indicates the need for better off-flavor screening and understanding in the milk industry. Labeling off-flavors as 'spontaneously oxidized' complicates understanding whether SOF is truly a specific oxidative off-flavor phenomenon, or rather has been broadly categorized as such despite potential for more individual, localized off-flavor issues. Knowledge of off-flavor pathways and precursors will ultimately provide an improved basis to mitigate milk flavor quality problems.

Introduction

Spontaneous oxidized flavor (SOF) in bovine milk has been identified in recent decades as a sporadic off-flavor problem affecting milk both before and after pasteurization. It is primarily characterized by "cardboard," "fishy," or "oxidized" flavors, and it is suggested to be capable of contaminating the flavor of otherwise acceptable milk during bulk tank mixing prior to pasteurization. The off-flavor was first identified in northern Europe, and during the last decade it has been recognized as a growing problem in the Midwestern United States. It has been frequently described as a seasonal off-flavor taint, appearing in the late autumn months and disappearing in early spring.[35] Since its characteristic off-flavors are reminiscent of lipid oxidation and affect the flavor of clean milk, a common assumption is that off-flavor development follows from the oxidative radical mechanism. Indeed, much of the previous work focusing on

SOF off-flavor has attempted to identify its origin and methods of inhibition through a reductionist approach. Researchers have frequently attempted to correlate sensory defects with changes in potential causative variables associated with oxidative reaction pathways. The most common examples include changes in bovine diet, the presence of specific polyunsaturated fatty acids (PUFA), and instigation or inhibition of SOF through various oxidizers, antioxidants, catalysts, etc. [35-39, 41-51]

Several studies have suggested that the presence of particular micronutrients such as copper may catalyze more extensive formation of lipid oxidation products in SOF milk. Juhlin et al. [35] claimed that the problem might be partially genetic, especially in the variability of copper present during the lactation of individual cows. In a later study,[48] the group also asserted that there was significant correlation between polyunsaturated fatty acids, copper levels, and SOF formation, although they noted that precisely defining levels of substrates, pro-, and antioxidants directly responsible was exceedingly complex and required further study. Timmons et al. [41] further described a link between increased SOF with higher levels of PUFA combined with a higher presence of copper within animals. Bruhn et al. [38] determined that there was a more complex dynamic between copper and alpha-tocopherol concentrations in cows which could lead to increased milk susceptibility to SOF formation; however, of the samples they studied, nonfat milk showed the greatest incidence of SOF, contradicting the typical lipid oxidation model.

Others have attributed SOF formation exclusively to lipid-antioxidant levels and interactions. Clausen et al. [44] found that higher concentration of initial lipid peroxidation products, in combination with a reduction of lower molecular mass antioxidants in the

initiation stages of lipid oxidation, could accelerate oxidized flavor defects. However, they only observed lipid oxidation products and did not relate their findings to sensory effects in the milks. The group also found that copper was not in itself directly related to the extent of milk oxidation, even with intravenous levels as high as 1.0 mmol L^{-1} in milk. St-Laurent et al. [46] additionally showed that high copper levels during early stages of lactation did not result in SOF type flavor formation; since the group found that supplemented vitamin E could reduce organoleptic oxidative perceptions, they hypothesized that the degradation of the antioxidant vitamin E in the bovine diet could be responsible for SOF susceptibility. The group asserted that intravenous injection of copper in cows led to a slight increase in SOF levels 24 hours after injection. It did not drastically increase SOF over a four week period despite higher copper levels in both blood and milk samples. However, their sensory assessments in determining SOF and drawing these conclusions were made simply with three trained panelists' ratings of the milks using a scale ranging from zero ("good" flavor) to three ("strongly oxidized" flavor). No specific off-flavor compounds of interest were identified. Others have suggested specific solutions such as vitamin E supplementation in cows, which could increase the concentration of antioxidants in cows' blood, resulting in reduced SOF and copper flavor in milk.[45] Granelli et al [43] investigated levels of PUFA, tocopherol, and β-carotene levels, concluding that the control herds showed lower antioxidant:PUFA ratios than experimental herds, yet did not develop any characteristic SOF flavor. They claimed that "the explanation of the increase in SOF is obscure, probably not due to one single parameter but rather to a number of contributing factors."

It is important to note, however, that none of these studies identified flavor compounds responsible for the observed SOF defects. To our knowledge, identification of specific flavor compounds and overall profiles have never before been incorporated in studies related to SOF analysis, instigation, or inhibition. Clearly, there is a range of proposed causative factors of SOF, as well as several suggested mitigation strategies. Due to the seasonal nature of the problem, it seems possible that a change in cows' homeostasis, possibly due to alterations in diet, living conditions, etc. could be affecting their milk production and negatively impacting flavor quality. Knowledge of the objectionable flavors responsible for flavor defects in the milk, particularly whether oxidative in nature, could lend further credibility to previous findings in SOF studies, or provide insight regarding another potential off-flavor source. The overall goal of this study was to identify volatile compounds responsible for the flavor defects in SOF screened milks. This approach will aid in the development of possible mitigation strategies and screening methods, which can be incorporated by milk producers prior to combining and mixing milk from different sources and thus avoiding off-flavor contamination.

Materials and Methods

Milk Samples.

Non-flavor defective (control) and off-flavored milk samples were obtained from a farm in rural Nebraska by the Midwest Dairy Foods Research Center. The farm had been flagged for several consecutive years as having SOF-type tainted milk during late

autumn and early spring. Both the control and off-flavored raw whole fat milk samples were shipped in chilled containers to the University of Minnesota Flavor Research and Education Center. Upon arrival, samples were evaluated (and expectorated) by experienced dairy judges at the University of Minnesota (Department of Food Science & Nutrition) to evaluate overall flavor quality. The control milk was noted as having typical flavors associated with high quality whole fat bovine milk, and the off-flavored milk was noted as having "unclean" flavor quality and aftertaste, as well as a faint metallic sensation. Milk samples were HTST pasteurized at the University of Minnesota Joseph Warthesen Food Processing Center (Saint Paul, MN) using a HTST/UHT Direct Steam Injection & Indirect Tubular Processing System (MicroThermics, Raleigh, North Carolina). Processing conditions included sanitation using a chlorine solution (100 ppm) followed by a clean water wash at 248°F (120°C) for one hour. Control and off-flavored milk samples were individually homogenized and processed using a final temperature of 165°F (74°C) for 20 seconds to ensure proper pasteurization. Previously sterilized glass bottles were filled with respective samples and cooled immediately to 60°F (16°C). Next, samples were tasted by three experienced dairy judges to evaluate flavor quality of the control and off-flavored samples post-pasteurization. The judges identified the control milk as "clean," or containing no objectionable volatile notes; the off-flavor sample, however, was identified as retaining the "unclean" flavor profile, as well as a metallic and objectionable aftertaste. The pasteurized samples were either immediately prepared for analysis (day 0 samples) or refrigerated storage (4.5°C) for 14 days before subsequent extraction and analysis (day 14 samples). A Standard Plate Count (SPC) methodology

was conducted during the two-week period using a Plate Count Agar (PCA, Neogen Corporation) to observe overall CFU/mL and ensure adequate pasteurization of samples. For both control and off-flavored milks, plate counts were initially calculated as too few to count (TFTC) and 2,400 CFU/mL, respectively; both fell within the < 20,000 CFU/mL limits for Grade A pasteurized milk as dictated by the United States Pasteurized Milk Ordinance. After 14 days of storage, plate counts were calculated as 1,800 CFU/mL and 49,000 CFU/mL for control and off-flavored milks, respectively.

Chemicals.

Authentic flavor standards used for quantification in this study included: E-2-decenal, 2-heptanone, nonanal, 2-nonanone, and 2-methylisoborneol (Aldrich, Milwaukee, WI); 2-methyl-3-heptanone and α-terpineol (Aldrich, St. Louis, MO); E,E-2,4-decadienal and 2-methylvaleric acid (SAFC, St. Louis, MO); and endo-borneol (Sigma, St. Louis, MO). Diethyl ether was obtained from Fisher-Scientific (Somerville, NJ).

Extraction Procedure.

A modified version of the methodologies reported by Karagul-Yuceer et al.[117] and Colahan-Sederstrom and Peterson[23] was incorporated for the extraction of control and off-flavored milk volatiles. Samples were extracted in duplicate after pasteurization, both fresh (day 0) and after two weeks of aging (day 14) to observe storage effects on any off-flavors present, particularly since SOF has been reported to exacerbate over time. One liter of redistilled diethyl ether was spiked with internal standard solutions: 3 µL of a 2-methyl-3-heptanone internal standard solution (50 µL/5mL diethyl ether) was added for

the neutral/basic volatile extract, while 3 µL of a 2-methylvaleric acid internal standard solution (33 µL/5mL diethyl ether) was added for the acidic volatile extract. One liter volumes of each respective milk sample were measured and divided into five individual Teflon-lined centrifuge bottles with Tefzel closures (Nalgene, Rochester, NY). Twenty grams of sodium chloride was added in each bottle, followed by addition of 40 mL of the redistilled diethyl ether spiked with the internal standards (Fisher Scientific, Fair Lawn, NJ). The milk in each bottle was individually extracted five times (40 mL/bottle, 5 x 200 mL extractions). After solvent addition, each bottle was gently overturned several times, followed by gentle stirring on an orbital shaking table at 30% maximum speed for twenty minutes, and gently overturned several times again to avoid emulsion formation (model 3540, Labline Instruments, Inc., Melrose Park, IL). The samples were centrifuged for 20 minutes at 4°C at 4000 rpm (Allegra X-22R Centrifuge, Beckman Coulter). After centrifugation, the solvent was carefully removed with Pasteur pipets and pooled, dried over anhydrous sodium sulfate, filtered, and stored overnight in a -20°C freezer. Samples were concentrated to approximately 100 mL using a fractional distillation column. The volatile fraction was then isolated using a solvent-assisted flavor evaporation (S.A.F.E.) system as described by Engel et al.[118] The ether distillates were further concentrated to approximately 20 mL, after which they were fractionated into neutral/basic and acidic fractions for analysis. To this end, the ether samples were washed with 1 M sodium bicarbonate (3 x 50 mL) and a saturated solution of sodium chloride in water (2 x 55 mL) in separatory funnels. The organic phase containing the neutral/basic volatiles was isolated and dried over anhydrous sodium sulfate and distilled to a volume of

approximately 1 mL. The aqueous fractions were combined and reacidified to a pH of 1.5 with 18% v/v of 12 M hydrochloric acid and extracted with repurified diethyl ether (3 x 50 mL) to isolate the acidic volatile fraction. The fraction was dried over anhydrous sodium sulfate, and distilled to approximately 1 mL. Extracts were stored at -80˚C prior to analysis.

Gas Chromatography-Mass Spectrometry (GC-MS)

Flavor extracts were analyzed with an Agilent 7890A GC equipped with a Leco Pegasus® 4D GCXGC 4D TOF-MS system, as well as a Hewlett Packard 6890 GC equipped with a Hewlett Packard 5973 MSD. Two μL of each sample was injected under splitless mode; inlet temperature was set at 250 °C, helium was used as a carrier gas with total flow rate of 1.0 mL/min. Compounds were analyzed on two different GC capillary columns with different chemistries: a DB-WAX (Agilent, 60 m x 0.25 mm x 0.25 μm) and HP5 (Agilent, 60 m x 0.25 mm x 0.25 μm). The temperature profile used for the DB-WAX column was 40°C for two minutes, then ramped to 220°C at 3°C/min and held for 3 minutes, whereas for the DB-5 the temperature profile was 40°C for 2 minutes, then ramped to 250°C at 3°C/min and held for 3 minutes. Mass spectral data was obtained in scan mode with a range of 30-350 amu. Confirmation of compounds was made using authentic standards coupled with a Shimadzu GCxGC system (GC-2010 Plus, GC-2010 Plus, GCMS-QP2010 Ultra).

Gas Chromatography-Mass Spectrometry-Olfactometry (GC-MS-O).

Flavor extracts were analyzed using gas chromatograph-mass spectrometry (Agilent Technologies, model 6890A and 5973) with an olfactometry port (Gerstel,

Baltimore, MD) equipped with either a DB-WAX or an HP5 column (60 m x 0.25 mm x 0.25 μm). Two μL of each sample was injected under splitless mode with the inlet temperature set at 250°C. The temperature profile used for the DB-WAX column was 40°C for two minutes, then ramped to 220°C at 7°C/min and held for 3 minutes, whereas for the DB-5 the temperature profile was 40°C for 2 minutes, then ramped to 250°C at 7°C/min and held for 3 minutes. The effluent of each column was split 1:1 after separation between an MS and olfactory sniffing port. Purified air was bubbled through distilled water and purged at the end of the sniffing port at a rate of 10 mL/min. Three panelists experienced in GC-MS-O analysis protocols identified aromas as they eluted from the heated olfactory sniffing arm (250°C). During olfactory runs, panelists recorded the elution time of the compound, the perceived odor descriptor, and an approximate intensity based on the OSME method for gas chromatography olfactometry. Intensity ratings were assigned numerical values as follows: 1, “very weak;” 2, “weak;” 3, “moderate;” 4, “strong;” and 5, “very strong.”

Data from each panelist was compiled after discussion and consensus, and values were averaged among all three individuals. Compounds were positively identified using mass spectral data, odor descriptors, and Kovats retention indices in comparison, with authentic standards. Compounds were labeled as “tentatively identified” if mass spectral matches were not able to be collected.

Quantitation of Selected Off-flavor Compounds.

The selected off-flavor compounds endo-borneol, 2-methylisoborneol, α-terpineol, 1-nonanal, 2-heptanone, 2-nonanone, E-2-decenal, and E,E-2,4-decadienal

were quantified in the off-favor and control milk samples. Analyses was performed on a GC/MS system (Agilent Technologies, model 6890A and 5973), using either scan mode (30-350 amu) or selective ion monitoring (SIM) mode for maximum sensitivity. Two μL of each sample was injected under splitless mode with the inlet temperature set at 250°C. The temperature profile used for the DB-WAX column was 40°C for two minutes, then ramped to 220°C at 7°C/min and held for 4 minutes. Helium was used as a carrier gas and set at constant flow at 1.0 mL/min. A combination of internal and external standard protocols was used. Quantitative analysis was completed using comparison of peak areas and the calculation of retention factors using the internal standards mentioned in "Extraction procedure" described above. Percent recovery studies were performed in triplicate and calculated for each of the individual compounds of interest, and the final calculated odorant concentrations were comparable to the concentrations obtained from independent five-point calibration curves for each compound ($r^2 > 0.97$ for all compounds). For SIM analyses, ions characteristic to the odorants of interest were selected and are reported in Table 1. Statistical significance was verified using the student's t-test. All analyses were performed with Statistix 10 (Analytical Software, Tallahassee, FL). Significance was established at $p < 0.05$ for all compounds.

Sensory recombination study

Approval for the sensory evaluation protocol was granted by the Ethics Committee, University of Minnesota (IRB # 1510E79045). An eleven-member sensory panel was convened, consisting of individuals familiar with sensory evaluation protocols for various food products. Panelists participated in a degree of difference sensory test.

They were provided with 30 mL of commercially available whole fat milk as a reference. Panelists were served sample milks randomly assigned with three-digit codes. For each test, panelists were presented with 3 different milk samples: one clean whole milk sample (same as the reference milk), one clean milk spiked with 'musty' compounds identified as important off-flavors after GC-MS-O analysis and added at levels quantified in the day 0 off-flavored milk sample; and one clean milk spiked with 'musty' compounds added at levels quantified in the day 14 off-flavored milk sample. In this study, "clean" milk was defined as a high quality, commercially-available whole bovine milk. Panelists were asked to taste the reference milk, followed by each of the 3 randomly coded samples from left to right and evaluate them for degree of overall flavor difference from the reference (not appearance or texture). Panelists were instructed to rate the milks on a 5-point scale, ranging from "0"- no difference, "1"- very slight difference, "2"- slight difference, "3"- moderate difference, "4"- large difference, or "5"- extremely large difference. Panelists were also asked to describe the perceived flavor differences noticed in the samples. Data was analyzed using a two-sided Dunnett's Multiple Comparisons test, using the statistical software package Statistix 10 (Analytical Software, Tallahassee, FL). Statistical significance was calculated using a significance value of $\alpha = 0.05$.

Results and Discussion

The volatile profiles of bovine milk and characteristic oxidation products have been well-established within the last several decades. Generally, whole fat bovine milk contains typical flavors like saturated and unsaturated aldehydes and ketones as a result

of lipid oxidation, lactones as a result of rearrangement during pasteurization, and pyrazines from more extensive thermal processing conditions. SOF research has repeatedly pointed to the presence of more pronounced 'oxidized' flavors as the source of off-flavor in flagged milk samples. As a result, methods of SOF identification and inhibition have focused on preventing the initiation and propagation of the typical lipid oxidation mechanism. Based on previously published research related to oxidative off-flavors, it was hypothesized that the off-flavored milks flagged as SOF in this study would contain greater lipid oxidation species than the control milks.

The off-flavored milk samples used in the present study were obtained from a farm in the Midwestern United States that had received seasonal complaints over two consecutive years that were associated with flavor profiles commonly associated with SOF. GC-MS-O analysis conducted for both control and off-flavor samples revealed a range of compounds commonly found in milk including fatty acids, aldehydes, ketones, and lactones. Many of these compounds are inherent to fresh, high-quality milk. For example, nonanal in whole fat bovine milk has been identified [21] as increasing in concentration as a result of adequate thermal treatment, as have select 2-alkanones. In the current study, the presence of common lipid oxidation products in milk were reported to have a similar presence between the control and off-flavored milk samples, and did not corroborate the hypothesis that flavor differences between SOF and control milk samples were primarily due to the presence of oxidation products.

The lists of volatile flavor compounds identified in control and off-flavored milk acidic fractions after 0 and 14 days of storage are shown in Tables 5 and 6 below. Despite

minor differences (isobutyric acid, pentanoic acid, and dodecanoic acid present in the control milk; nonanoic acid present in the off-flavor milk), no main differences in the profiles were observed between the peak areas and the odor intensities of the compounds. The acids identified in the acidic fractions are typical to milk and milk products, and they are associated with "fatty" or "cheese-like" aroma descriptors.

The volatile compounds identified in the neutral/basic fraction of the off-flavored milk samples also had many similarities with the control milk (Tables 7 and 8), however, several key differences in the GCO profile were identified. A distinct series of terpenoid compounds was identified in the neutral/basic fraction of the off-flavored milk, all with characteristic "green" and "musty" descriptors, including endo-borneol, 2-methylisoborneol, and α-terpineol (Table 8). These compounds were completely absent in the control milk samples, both on day 0 and after 14 days of storage, suggesting possible involvement in the off-flavor present in the tainted samples. Because of the long-standing association of off-flavors as a result of lipid oxidation in previous research, the chromatograms and GCO profiles for both control and off-flavor milk samples were also screened for differences in lipid oxidation products. Several volatiles known to result from lipid oxidation were further examined in off-flavored samples stored for 0 and 14 days. Quantification of lipid oxidation products followed when compounds fulfilled the following criteria: 1) absence in the control milks or 2) increase in peak area over the 14-day period of storage in the off-flavored samples. Most notably, the off-flavored milk samples stored for 14 days contained nonanal, 2-heptanone, 2-nonanone, E-2-decenal, and E,E-2,4-decadienal, while the day 0 off-flavor milk sample only contained nonanal

and 2-nonanone. GCO panelists described these lipid-derived compounds using similar terminology ("green," "fatty," etc.) as well as with similar OSME intensities for both the day 0 and day 14 samples. After quantitation, their contribution to oxidative off-flavor development in the milk samples was examined.

Terpenoid compounds such as those found in the off-flavored samples have been previously reported to occur from various bacterial and fungal sources. Specifically, 2-methylisoborneol has been documented to occur as a result of cyanobacteria (blue-green algae) in aqueous environments and myxobacteria from soil and water-based systems, as well as Actinomycetes and various other fungi. Both 2-methylisoborneol and geosmin have been previously reported [119] to bioaccumulate in catfish, resulting in sensory defects upon consumption as reported by sensory panelists. Another study [120] identified a wide range of "musty" compounds present in apple juice both before and after pasteurization including endo-borneol, 2-methylisoborneol, and α-terpineol. In that study, populations of various thermoacidophilic bacteria (*Alicylobacillus acidoterrestris*, *Actinomycetes* and *Streptomyces ssp.*) were found to quantitatively increase over time on the surfaces of apples, which corresponded with increasing sensory defects in apple juice made from the contaminated fruit. In general, terpenoid compounds have been noted in literature as having exceedingly low odor thresholds (endo-borneol at 140 μgL^{-1} in water, 2-methylisoborneol at 0.002-0.1 μgL^{-1}in water, and α-terpineol at 4.6 μgL^{-1} in water), as well as notable "green" and "musty" odors. The authors also found that the odor thresholds of the identified terpenoid compounds were often perceived at even lower

concentrations when observed within a food matrix than previously published odor thresholds in water.

Quantitative studies were subsequently conducted to evaluate the odor activity of the individual terpenoid compounds, and key differences were noted for the flavor compounds present in the off-flavor milk samples. The concentrations of the terpenoid compounds in off-flavored milk samples after day 0 and day 14 are shown in Figure 9 below. Over the 14-day storage period, endo-borneol and α-terpineol decreased significantly ($p < 0.05$), while 2-methylisoborneol increased significantly ($p < 0.05$) to 0.017 μg/L, approximately a 4-fold increase from its day 0 concentration. The concentration of 2-methylisoborneol was well above its published odor threshold of 0.002 μg/L in water.[120] The decrease in concentration of endo-borneol and α-terpineol indicates that these products may not have been stable over time in the milk samples. The survival of microbial strains in pasteurized milk (as indicated previously) may have resulted in the noted increase of 2-methylisoborneol during storage. It is uncertain whether the loss of endo-borneol and α-terpineol in the milk samples are directly related to the increase of 2-methylisoborneol over time, either through microbial metabolism or other conversion pathways. Further study would be necessary to elucidate these mechanisms. For the lipid derived compounds (Figure 10), nonanal and E,E-2,4-decadienal had a decreasing trend over the 14-day period, with nonanal showing no statistical significance. However, 2-heptanone, 2-nonanone, and E-2-decenal were not found in the day 0 samples but were present after 14 days. The concentrations of both 2-heptanone and 2-nonanone were below the lowest published literature odor threshold values in water (140 μg/L and 5

μg/L, respectively).[121] However, E-2-decenal was above the published aqueous odor threshold (0.3 μg/L).[122]

A preliminary sensory recombination study was conducted using commercially available flavor standards of the identified lipid oxidation and terpenoid compounds. Three judges experienced in dairy flavor evaluation tasted milk samples spiked with the same compound concentrations as the day 0 and day 14 off-flavored samples, containing either solely lipid oxidation products [2-heptanone, 2-nonanone, E-2-decenal, E,E-2,4-decadienal, nonanal], terpenoid compounds [endo-borneol, 2-methylisoborneol, α-terpineol], or a combination of all selected volatile compounds. The milk samples spiked with lipid oxidation products at quantified levels were not found to be objectionable by any of the judges, and thus the compounds were excluded from further sensory evaluation. Milks spiked with terpenoid compounds were perceived as tainted; sensory descriptors, as described by panelists, matched the initial sensory evaluation of the purported SOF milk samples received from the dairy farm. The clean milk with added terpenoids were regarded as "unclean" with distinctly unpleasant aftertastes. The samples containing all selected volatile compounds were primarily described in a similar manner as the samples only spiked with terpenoid compounds, with "musty" and "unclean" descriptors. Therefore, the terpenoid compounds were selected for further sensory evaluation.

A degree of difference (DOD) test was conducted to evaluate the magnitude of sensory difference between a milk sample spiked with the terpenoid compounds and a clean whole fat milk, allowing evaluation of the identified compounds' sensory

relevance. The sensory results are shown in Table 9. In comparison to the reference milk, the blind control milk was rated with a mean score of 0.75, with no sensory difference. The milks spiked with day 0 levels of the musty off-flavor compounds were found to have a mean rating of 1.67 when compared to the reference, and the milks spiked with day 14 levels received a mean score of 2.0, which was significantly different from the reference (α=0.05, critical difference value=1.0897). Panelists described the spiked milk samples using descriptors such as "stale," "pine," "dirty," "cardboard," "old," "not clean," "musty," "lacks freshness," "earthy," "grassy," and "metallic." These results indicated with statistical significance that an off-flavor sensory response was created from the addition of the musty off-flavored compounds. It is notable that a higher off-flavor perception than the control milk was created at the day 0 levels, followed by a higher difference rating and statistical significance at the day 14 levels; this occurred despite the overall lower concentration of both endo-borneol and α-terpineol observed after 14 days of storage, suggesting the key off-flavor role of 2-methylisoborneol. This was not unexpected, due to the much lower odor threshold of this terpenoid compound and the observed 4-fold increase after 14 days of storage. The descriptors used by the panelists, including terminology such as "stale," "cardboard," and "grassy," were descriptors that are often used when describing oxidation and SOF in food products.

This is the first study to suggest that SOF may be linked to a microbial flavor taint. As alluded to in prior research,[119, 120] it is possible that these musty off-flavor compounds could occur from similar microorganisms from aqueous or terrestrial sources within the milk supply chain, for example through animal feed or water sources, silage,

the milking process, comingled milk at the processing site, etc. SOF has long been associated with particular seasons of the year, which points to a potential variability in animal conditions or processing that could give rise to flavor profile variability. A seasonal alteration from one of these sources could result in microbial contamination, growth, and subsequent off-flavor contamination of milk. Additionally, if thermoacidophilic bacteria like these survive pasteurization, their propagation could cause further off-flavor development in milk. This would account for the increasing off-flavor development in the milk over time, as well as its ability to affect otherwise "clean" milk during bulk storage, particularly since these odorants can be perceived at extremely low concentrations. Unfortunately, the scope of this study could not identify if particular strains were responsible for off-flavor generation, but further study in the area could prove important in understanding causative strains and possible routes of contamination and mitigation.

In summary, off-flavor milks from a Midwestern farm flagged for SOF was found to contain the odor impact compounds endo-borneol, 2-methylisoborneol, and α-terpineol. These compounds were found to illicit a similar off-flavor sensory response when added in clean milk samples, including the generation of descriptors that matched previously published terms for SOF-type off-flavors. These terpenoid compounds have been previously identified as resulting from particular algal or microbial species, many of which have been found to be thermoacidophilic and have origins from water, soil, or silage. Lipid oxidation compounds, while identified in both the control and off-flavor milk samples in this study, did not sufficiently reproduce the off-flavor perceptions

identified in the original samples when spiked into control milks. More work is needed to understand whether the terpenoid compounds identified are unique to these samples, result from certain microbial strains, or are universally related to the off-flavor termed as SOF. This will also result in defining critical control points in dairy production to screen for sources of SOF contamination and the ability to propose methods of inhibition. Direct flavor analyses of off-flavor milk and dairy products in the dairy industry will lead to a better understanding of the specific volatiles and routes of formation that could be at work in this off-flavor development. This in turn will address whether SOF is related to a separate mechanism of off-flavor generation or could be the result of microbial contamination and subsequent off-flavor development.

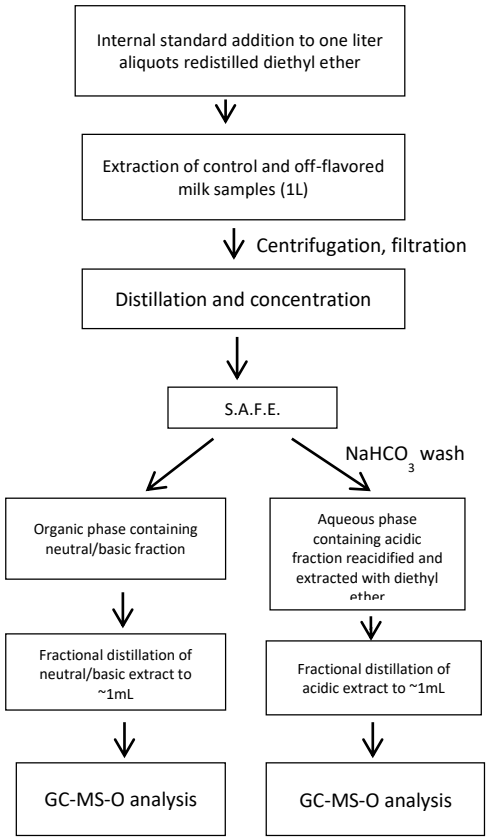

Figure 8, Schematic for the extraction protocol used for control and off-flavored milk samples prior to GC-MS and GC-MS-O analysis

Table 4, GC-MS SIM-mode Quantified Ions for Select Aroma Compounds

Compound name	Molecular weight	Monitored ions (SIM)
2-methyl-3-heptanone	128	128
nonanal	142	98, 142
endo-borneol	154	95, 154
2-methylisoborneol	168	95, 107, 168
α-terpineol	154	93, 154
E-2-decenal	154	97, 154
E,E-2,4-decadienal	152	81, 152

Table 5, Day 0 and Day 14 Acidic Fraction of Control Milk

Identified compound	DB-WAX	DB-5	Odor Descriptor	Average OSME Intensity (Day 0)	Average OSME Intensity (Day 14)
acetic acid	1490	594	fat, milk, cheese	2.33	2.17
isobutyric acid	1556	1195	fat, cheese, oil	2.83	2.50
butyric acid	1597	846	rancid oil	3.17	2.17
pentanoic acid	1722	976	cheese	2.33	2.17
hexanoic acid	1803	1022	soap, sweat	2.33	2.67
heptanoic acid	1979	1103	sweat, fat	2.67	2.83
octanoic acid	2061	-	fatty	2.25	2.50
decanoic acid	2296	1401	rancid, fat	2.50	2.00
benzoic acid	2434	-	fat, rancid	1.75	2.00
dodecanoic acid	2483	-	fat	1.75	2.50

Table 6, Day 0 and Day 14 Acidic Fraction of Off-flavored Milk

Identified compound	DB-WAX	DB-5	Odor Descriptor	Average OSME Intensity (Day 0)	Average OSME Intensity (Day14)
acetic acid	1436	689	sour	2.33	3.17
butyric acid	1623	847	fat, rancid, sweat	3.00	3.25
hexanoic acid	1835	1046	cheese, fat, grease	2.50	2.50
heptanoic acid	1941	1107	humid, sweaty, metal	2.83	2.25
octanoic acid	2060	-	fat	2.50	1.50
nonanoic acid	2185	-	cheese, fat, grease	1.50	1.50
decanoic acid	2270	1400	fat	1.83	2.00
benzoic acid	2413	-	fat, creamy	2.25	1.50

Table 7, Day 0 and Day 14 Neutral/Basic Fraction of Control Milk

Identified compound	DB-WAX	B-5	Odor Descriptor	Average OSME Intensity (Day 0)	Average OSME Intensity (Day 14)
acetic acid	1450	607	fat, cheese, pungent	-	2.83
pentanal	902	717	pungent, fat	-	1.67
isobutyl acetate	-	775	wheat, almond	2.00	1.67
hexanal	1084	804	wheat, dough, grain	-	2.17
1-nonen-3-one	1239	836	fat, milkfat	2.33	3.00
hexanol	-	879	cabbage, onion	1.67	-
mercaptomethylbutanol	-	974	brothy, bouillon	-	3.00
1-octen-3-one	1318	984	mushroom, brothy, roasted	2.33	2.83
pentylfuran	1245	999	earthy, green, rubbery	2.33	2.50
benzyl alcohol	-	1034	roasted, meaty	2.33	1.33
1-octanol	1547	1061	sulfury, pungent, barn	2.17	1.50
2-nonanone	1390	1091	milk, fat	2.17	3.00
nonanal	1400	1106	milk, fat, dairy	2.17	3.33
menthol	1646	1180	green	-	2.33
decanal	1450	1188	green, paper, rancid	2.50	2.50
Z-4-decenal	1522	1195	rancid oil	2.00	2.33
carveol	-	1220	musty, rancid, papery	3.00	2.50
γ-octalactone	1869	1260	oily, lactone	-	2.67
δ-octalactone	1921	1280	peach, dairy	2.50	2.67
thymol	2242	1293	herbal	2.33	2.83
geranyl acetone	-	1444	green, musty	2.50	2.83
δ-decalactone	2235	1453	sweet, vanilla	3.17	2.67
γ-decalatone	2135	1483	lactone, peach	2.83	2.67
methyl laurate	1786	1511	fatty	3.67	2.83

Table 8, Day 0 and Day 14 Neutral/Basic Fraction of Off-flavored Milk

Identified compound	DB-WAX	DB-5	Odor Descriptor	Average OSME Intensity (Day 0)	Average OSME Intensity (Day 14)
propanoic acid	1523	687	pungent, rancid	-	1.83
pentanal	-	732	pungent	2.17	2.00
hexanal	1107	777	oxidized, fat	1.67	1.00
butyric acid	-	816	cabbage, onion	-	1.67
hexanol	1347	844	hay, grass	1.67	1.83
2-heptanone	1195	882	fatty	1.67	2.33
3-octanol	1347	981	mushroom, earthy	2	2.00
benzyl alcohol	1872	1040	roasted, meaty	2.17	1.83
1-octanol	1550	1076	chemical, metal	2.33	1.67
2-nonanone	1435	1090	green	2.33	2.00
nonanal	1347	1112	fat, cream	2.00	2.33
endo-borneol	1657	1169	paper, musty	2.17	2.67
2-methylisoborneol	1573	1175	musty	2.33	3.67
octanoic acid	-	1179	sweat, cheese	2.33	2.50
naphthalene	1696	1189	musty	-	2.50
α-terpineol	1678	1192	musty	2.33	2.33
decanal	1550	1197	green, fat	2.33	2.17
E-2-decenal	1573	1240	cheese, fat	1.83	2.00
E,E-2,4-decadienal	1642	1252	paper, cardboard, bean	2.00	2.00
δ-octalactone	-	1270	peach, coconut	2.17	2.33
decanol	1696	1272	fat	2.00	2.67
δ-nonalactone	2163	1281	coconut	2.33	2.67
p-vinyl guaiacol	2280	1344	malty, mushroom	2.17	2.83
γ-nonalactone	-	1366	coconut, peach	1.83	2.33
decanoic acid	-	1373	rancid, fat	2.50	2.17
δ-decalactone	-	1500	lactone, cream	2.50	2.67
dodecanoic acid	-	2169	oil, soap	-	1.83

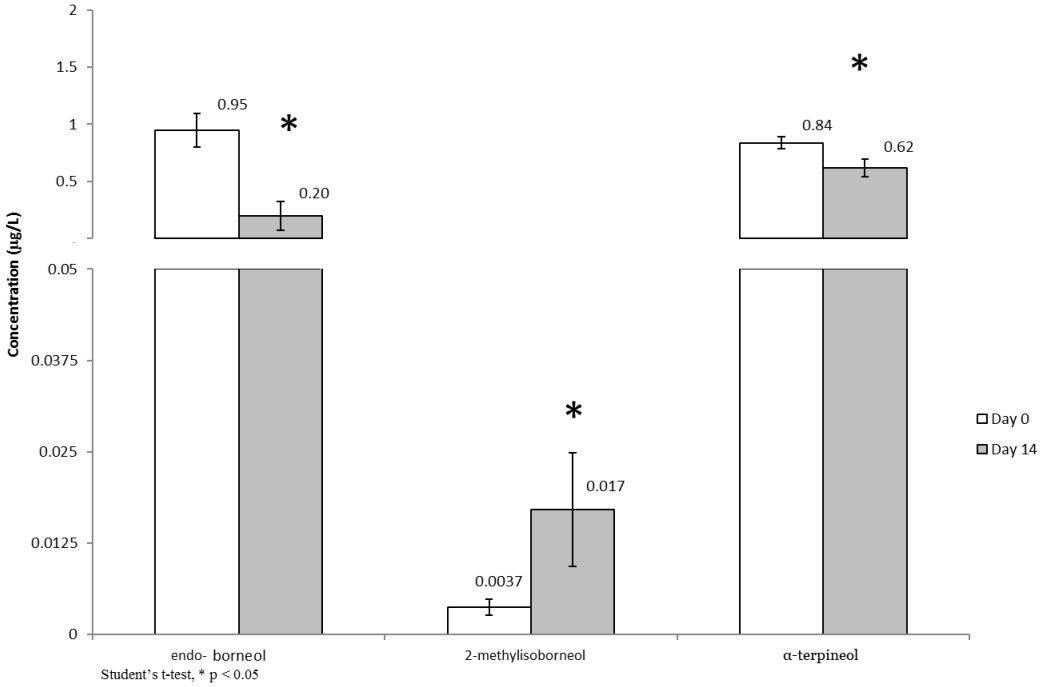

Figure 9, Concentrations of Microbial Compounds, SOF day 0 and Day 14

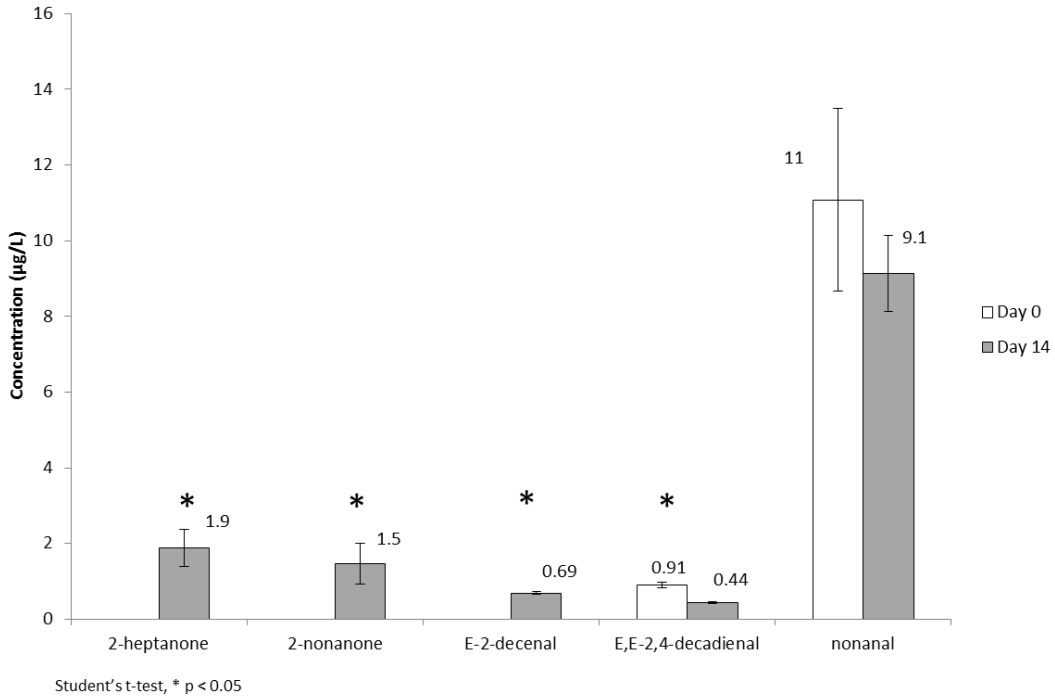

Figure 10, Concentrations of Lipid Oxidation Compounds, SOF day 0 and day 14

Table 9, Degree of difference test, $\alpha = 0.05$, Critical Value = 1.0897

Sample	**Mean Rating**
Control	0.7500
Musty Day 0	1.6667
Musty Day 14	2.0000*

Chapter 4

Characterization of Key Nonvolatile Mouthfeel and Taste Creamy-Contributing Compounds Responsible for Creaminess Perception in Dairy Products

Abstract

Creaminess is a distinct and desirable attribute of dairy products. In the current study, compounds that contribute to the overall mouthfeel of cream were characterized, and their contributions to creaminess perception were evaluated in fluid milk. An extraction protocol was developed and optimized to obtain nonvolatiles of interest from heavy whipping cream. Food grade extracts were profiled for mouthfeel active compounds utilizing multidimensional HPLC sensory guided fractionation techniques, and descriptive analysis protocols were developed using a 5-attribute lexicon (mouthfeel/thickness, astringency/drying, fatty texture, dairy mouthfeel, tingling/irritation), with appropriate references. Five mouthfeel compounds were selected and identified by UPLC/MS/MS-ToF and NMR as orotic acid, pantothenic acid, hippuric acid, 2-methylhippuric acid, and p-cresol sulfate. The concentrations of these five compounds were measured in eight different commercially available dairy products to evaluate their presence and potential formation trends across a variety of products. In particular, skim and whole milk quantitative data were used to develop sample recombinants for sensory evaluation. Using a 2-AFC sensory test, twenty panelists

reported that a skim milk spiked with levels of all five compounds as quantified in whole milk (recombinant) had a significantly "creamier, fuller body" when compared with skim milk itself (α=0 .01). As a result, it was shown that small-molecule, nonvolatile compounds can contribute to the creamy texture and body of dairy products. This lends insight to the overall context of texture in dairy, providing novel information regarding aspects of dairy matrices that have been decidedly less studied in relation to creaminess. Furthermore, this suggests that there exist routes through which the dairy industry could incorporate aspects of creaminess enhancement and targeted flavor improvement, offering consumers a variety of products while retaining a high level of quality creaminess.

Introduction

Dairy products are staple foods in many countries around the world. While sales of fluid milk in the United States have remained relatively static over the past several decades, international markets like China and India are increasingly looking to major producers like the U.S. and the European Union to meet the production demands that their dairy industries cannot currently fufill.[123] In a growing global market, health-conscious consumers are demanding dairy products with high nutritional quality (e.g. high-protein snacks and satiety-inducing foods) as well as sensory satisfaction. This trend is evident across the dairy product platform including condiments (whipped cream, sour cream, coffee creamers) and ready-to-eat foods (Greek yogurt, ice cream, puddings, etc.). Though consumers demand low fat and low sugar dairy based alternatives, flavor quality

and specifically creaminess still remain primary drivers of food choice and associations of overall product value.

Prior research focused on creaminess has routinely focused either solely on the volatile flavors or on the contribution of fats to creamy tastes and textures. Results have frequently been contradictory, particularly in how different components affect overall liking, mouthfeel, and texture in the final product.[34, 58, 74, 77] It is known that several key volatiles play an important role in creaminess perception; some of the main aroma compounds responsible for creaminess include γ-decalactone, δ-decalactone, δ-dodecalactone, (Z)-4-heptenal, and (E,E)-2,4-nonadienal.[53] In 2007, Schlutt et al. [34] also identified a series of lactones present in cream that seemed to induce retronasal cream flavor when added above their threshold levels. In general, the volatile flavor profiles of dairy products have been extensively studied and compounds responsible for creaminess in a range of dairy products have been well documented. However, volatile compounds are likely not the sole contributors of creaminess in dairy foods.

Aroma and volatiles have been studied more widely than other flavor modalities, such as taste or texture, with the latter two attributes becoming more recognized over the past decade as having a much greater effect than once thought on food flavor profiles. By far the most broadly studied nonvolatile contributors to creaminess in dairy have been lipids, particularly in relation to texture perception and the bulk viscosity behavior of a given product. In 1988, Mela [74] attempted to define the role that fat content had on sensory assessment of creaminess in several different dairy samples. Fluid dairy products with differing fat levels were evaluated, as well as those with added starches to simulate

thickness. Samples were rated for “fat content,” “creaminess,” and “pleasantness.” The samples that were rated more strongly for “fat content” and “creaminess” were found to coincide primarily with texture and viscosity effects. Other studies [79] have attempted to rate creaminess based solely on fat content or physical parameters such as fat globule size, with mixed results. In 2005, Weenen et al. [83] evaluated descriptors of creaminess in mayonnaise, custard desserts, and starch-based warm sauces. In their study, they found that rough, heterogenous, and grainy melting descriptors were negatively associated with consumers’ expectations of creaminess, while thick, airy, smooth, and fatty perceptions were more positively associated. There is conflicting rationale in these studies and others,[86] particularly on whether textures affect creaminess because of the presence and perception of certain fats and how these fats interact with volatile (aroma) perception. In particular, more in-depth work is needed to investigate nonvolatile factors contributing to creaminess, including whether these are intrinsic to milk fat or other components in milk to better understand the mechanisms of creaminess perception.

The main goal of this study was to further elucidate the role of dairy nonvolatiles on creaminess perception. Identification of creaminess inducing nonvolatile compounds naturally present in dairy would provide further information into origins and routes of creaminess perception. In addition, these insights will facilitate advancement of creaminess enhancement strategies in full, reduced, and low-fat dairy products, providing a more direct approach for creating high flavor quality and healthy alternatives for consumers.

Materials and Methods

Preliminary sensory evaluation.

Preliminary experiments were conducted to evaluate various dairy products to select a product that provided a nonvolatile isolate with the highest perceived creamy tastes and textures. Whole milk, half-and-half, and heavy whipping cream (Essential Everyday®, Cub Foods, Roseville, MN) were individually extracted with 50% ethanol/water, after which solvent was removed by rotary evaporation, diluted in nanopure water, and freeze dried twice to remove residual impurities. Precipitates were redissolved in 25 mL of nanopure water, and three panelists experienced in dairy product evaluation determined that heavy whipping cream was highest in characteristic "creamy" nonvolatile perceptions. An optimized extraction protocol of heavy whipping cream was conducted using aliquots of whipping cream with solvent ratios ranging from 50%, 75%, 87.5%, and 90% v/v in either ethanol or methanol and water, respectively. Extracts underwent rotary evaporation to remove residual solvent, after which precipitates were diluted in nanopure water and freeze dried twice to remove residual solvents. Final precipitates were diluted in 5 mL w/v aqueous aliquots for tasting by three individuals experienced with dairy sensory analysis. They rated samples according to perceived creaminess, both volatile and nonvolatile (with and without noseclips). Accordingly, heavy whipping cream extracted with 87.5% v/v methanol/water was selected as representative of a typical dairy product with a high-quality "creamy" flavor profile.

Nonvolatile extraction.

A generic brand of whipping cream was purchased locally (Essential Everyday®, Cub Foods, Roseville, MN) and 300 mL was freeze-dried for 96 hours, after which the solids were ground and extracted twice with an optimized ratio of methanol/water (175:25 v/v). The extract was filtered, and the solvent was removed by rotary evaporation. The precipitate was diluted in nanopure water and freeze-dried for 96 hours.

Ultrafiltration and Solid Phase Extraction.

The solid precipitate was combined with an ethanol/water mixture (50:50 v/v) and underwent ultrafiltration with an Amicon 8200 ultrafiltration cell (Millipore, Bedford, MA, USA) using a 3 kDa cutoff membrane (Millipore) and a nitrogen pressure of 30 psi. After ultrafiltration, both the permeate and retentate were evaluated for creaminess perceptions. The selected permeate fraction of interest underwent further clean up and desalting using a solid-phase extraction method (Discovery® DSC-18 SPE, 2g). The SPE cartridge was pretreated with 12 mL of methanol and 12 mL of DI water. The permeate was dissolved in 30 mL of nanopure water and loaded onto the cartridge. The sample was washed with 30 mL of nanopure water. Final elution was performed with 36 mL of methanol, after which organic solvent was removed by rotary evaporation. The SPE aqueous eluent and resulting organic precipitate were individually taken up with 10 mL of DI water and freeze-dried. Both the aqueous and organic isolates were tasted, and the organic phase was found to contain creaminess perceptions of interest, so it was selected for further fractionation techniques. A second solid-phase extraction was used to concentrate the sample prior to injection onto the HPLC. Finally, the sample was syringe

filtered (Millex® 13 mm, 0.20 μm nylon membrane) before injection onto the HPLC system.

First dimensional semi-preparative fractionation sensory screening in water and dairy matrices.

Methods for LC fractionation were adopted from a similar protocol used previously by Kokkinidou and Peterson.[124] The sample was injected (500 μL) on a semi-preparative Shimadzu HPLC binary pumping system (Shimadzu, LC-10ADvp) equipped with a manual injector (SIL-10ADvp), variable-wavelength diode array detector (Shimadzu, SPD-10Avp), fraction collector (FRC-10A), and a semi-preparative reverse phase C18 column (250 mm x 10 mm, Pinnacle, Restek, Bellefonte, PA, USA). Absorption was monitored between 190-800 nm. Fraction collection occurred in tandem with UV monitoring. Chromatography was conducted with a binary solvent system of water with 0.1% formic acid (solvent A) and methanol (solvent B). A linear gradient was used starting with solvent B at a 10% concentration (5 min), increased to 100% (5-30 min), held at 100% B (30-40 min), and finally decreased back to 10% (40-43 min). The flow rate of the mobile phase was set at 3 mL/min, and the effluent was collected over 43 fractions (3 mL/fraction). Solvent was removed under vacuum, and fractions were freeze-dried twice. The resulting solids were subsequently evaluated for sensory perceptions in both aqueous and dairy matrices. Portions of the fractions were dissolved in DI water in 1:1 ratios (reconstituted volume to the initial volume of the whipping cream, 30 mL/fraction), the pH was adjusted to approximately 6.7 (milk pH) and evaluated for mouthfeel/texture and dairy/creaminess by sensory analysis. Three expert panelists wore

noseclips and placed 2 mL of each fraction on their tongues, rating each on a scale of "threshold," "weak," "moderate," and "strong" as compared to DI water. Another portion was dissolved in 1% milk to evaluate creaminess perceptions as a result of dairy matrix effects. Precipitates were diluted in 1% milk in 1:2 sample ratios (reconstituted volume to initial volume). Three expert panelists evaluated the fractions using a paired comparison test, without noseclips. Samples were labeled with randomized three-digit codes. Panelists were asked to compare the 1% milk samples spiked with the fractions with control 1% milk samples, and subsequently rated the milk sample they considered "creamier."

First dimensional preparative scale separation.

One liter of whipping cream was extracted using one liter of a methanol/water mix (875 mL and 125 mL, respectively) and prepared for fractionation using methods as described above. A preparative Shimadzu HPLC binary pumping system (Shimadzu, LC-8A) was used for fractionation and higher sample throughput. Approximately 0.5 g of the final whipping cream precipitate was dissolved in 5 mL of a 10% v/v methanol/water solution for injection. The system was equipped with a manual injector, UV-Vis detector (Shimadzu, SPD-10Ai), fraction collector (FRC-10A), an Agilent Pursuit 5 C18 column guard (30x21.2mm), and a reverse phase Agilent Pursuit 5 C18 column (150 mm x 21.2 mm). Chromatography was performed using a binary solvent system of deionized water spiked with a concentration of 0.1% formic acid (solvent A), and methanol (solvent B). A linear gradient was used starting with solvent B at 10% (5 min), increased to 100% B (5-30 min), held at 100% B (30-45 min), and decreased back to 10% B (45-50 min). The

flow rate of the mobile phase was 10 mL/minute, and the effluent was collected over 50 fractions (10 mL/fraction) (Figure 15). The absorbance of the effluent was monitored using two different wavelengths at 254 nm and 280 nm. Bulk solvent was removed under vacuum and samples were diluted with DI water and freeze-dried twice to remove any impurities. The resulting solids were finally reconstituted in DI water to volumes half that of their original concentrations (1:2 ratios, 56 mL), adjusted to milk pH (approximately 6.7) and evaluated by a descriptive analysis panel.

Descriptive sensory analysis.

A panel of 12 individuals experienced with descriptive sensory analysis was formed to identify the various mouthfeel and texture sensations present in the fractions resulting from the preparative separation of the whipping cream extract. The panelists were provided with isolated fractions assigned a randomized three-digit code. They were asked to rinse with deionized water prior to sampling, subsequently place 1 mL aliquots of sample on their tongue using food-grade pipettes, and wait for several seconds to ascertain all tastes or textures associated with the fraction. After discussion, panelists reached consensus regarding the descriptors encompassing the various sensory attributes present in the fractions, including mouthfeel/thickness, astringency/drying, fatty texture, dairy mouthfeel, and tingling/irritation, leading to the development of an appropriate lexicon. Five key references were used to compare textural sensations in the samples:

1. Mouthfeel/thickness: Dilutions of 0.5 g cornstarch suspended in 300 mL of deionized water

2. Astringency/drying: Dilutions of 3 Lipton® tea bags steeped for 5 minutes in heated 500 mL of deionized water and cooled[125]
3. Fatty texture: Dilutions of whole milk
4. Dairy mouthfeel: Dilutions of skim milk
5. Tingling/irritation: 200 mL of deionized water stirred with one copper penny over a 24-hour period[126]

Panelists rated the fractions using the developed lexicon ("mouthfeel/thickness," "astringency/drying," "fatty texture," "dairy mouthfeel," "tingling/irritation") as well as any new terms decided upon by group consensus.

Second dimension sensory guided fractionation.

Fractions 2, 4, and 9 as seen in Figure 16 from the first-dimension preparatory separation were selected for further purification based on ratings provided from a subset of the large panel, consisting of 6 members. A second-dimension separation with the same HPLC column with UV directed fractionation setup as described above was utilized; however, the aqueous mobile phase was spiked with a 10 mM ammonium acetate buffer (pH 7) to provide further separation based on potential ionizable differences between compounds within the fractions of interest. Time/UV directed fractionation was employed, and after cleanup steps and pH adjustment to approximately 6.7, the fractions underwent sensory evaluation. Those that were most texture active as perceived by a four-member sensory panel were selected for preliminary identification analyses, as seen in Figure 17. Injections for fraction 2 of the first dimension were collected over six fractions, of which fraction 3 was found to be the most sensory active.

Likewise, Fraction 4 was split into seven fractions with fraction 2 being the most sensory relevant. Finally, seven fractions were collected for fraction 9 and fraction 4 was isolated as containing the most sensory activity from its second dimension.

UPLC/MS/MS analyses of nonvolatile fractions.

Fractions of interest strongest in textural perceptions were redissolved in 1.5 mL of nanopure water and analyzed using a Waters Xevo™ G2 QTOF system for accurate mass spectroscopic structural interpretation. Prior to analyses, the instrument was calibrated using a reference sodium formate solution to verify instrumental mass accuracy and elemental composition estimates to sub 5 ppm resolution. Analytes were detected using positive and negative MS^e analyses using Leucine-Enkephalin (555.62268 g/mol) as a reference standard from a mass range of 50-1200 Da. A CORTECS™ UPLC® C18+ 1.6µm column (2.1x100mm, Waters) equipped with a CORTECS™ UPLC® C18+ 1.6µm column guard (Waters) was used for all experiments. All experiments were performed in triplicate. Mass spectrometric conditions were as follows: desolvation temperature, 500°C; source temperature, 120°C; capillary voltage, 2.7kV; desolvation gas, 700 L/hr; cone gas, 65 L/hr. UPLC runs were conducted using ultrasonicated nanopure water spiked with 1% formic acid as the aqueous phase and acetonitrile spiked with 1% formic acid for the organic phase. Chromatography was performed using a flow rate of 0.550 mL/min with the following linear gradient: solvent B at 2% (0-0.30 min), increased to 15% (0.30-0.50 min), increased to 35% (0.50-3.50 min), increased to 65% (3.50-4.00 min), increased to 99% (4.00-4.50 min), held at 99% (4.50-5.25 min), decreased to 2% (5.25-5.26 min), and held at 2% (5.26-6.50 min).

Compound isolation and purification.

Based on UPLC/MS/MS TOF analyses, several compounds of interest were identified within the fractions. Chromatographic and fractionation experiments were subsequently performed to further purify and obtain bulk concentrations of the compounds of interest from the second dimension extract using a Shimadzu HPLC binary pumping system. The system was equipped with an autoinjector (SIL-10ADvp) and binary pumps (LC-10ADvp). Two semiprep columns were used for initial isolation and subsequent purification of select compounds, including an Ultra AQ C18 5µm column (250x10mm, Restek) and a Luna 5µm Phenyl-Hexyl column (250x10mm, Phenomenex®), respectively. Downstream from the column was a split diverting 1:10 flow to a Waters Micromass Quattro *micro*™ API mass spectrometer, and a 9:10 flow to a Waters Fraction Collector III. Mass spectrometric parameters were as follows: desolvation temperature, 350°C; source temperature, 120°C; capillary voltage, 2.20 kV; desolvation gas, 450 L/hr; cone gas, 55 L/hr. Analyses were conducted in scan mode from 100-1200 Da. HPLC runs on the C18 column and the Phenyl-Hexyl column were conducted using ultrasonicated nanopure water spiked with 1% formic acid as the aqueous phase and methanol spiked with 1% formic acid for the organic phase. Chromatography was performed using a flow rate of 4.0 mL/min with the following linear gradient: solvent B at 10% (0-5.00 min), increased to 100% (5-20.00 min), held at 100% (20-27.00 min), 10% (27.10 min), and held at 10% (27.10-35.00 min). Isolated compounds obtained in the fractions were pooled, subjected to rotary evaporation, diluted in nanopure water and freeze dried twice.

Verification of purity and NMR analyses.

After isolation of select compounds of interest, relative purity of each compound (> 95%) was verified using peak areas obtained after performing analyses with the same ESI positive and negative UPLC/MS/MS methodologies mentioned above. Bulk solvent was removed under vacuum and samples were diluted with DI water and freeze-dried twice to remove impurities prior to tasting. Samples were diluted in water and tasted after pH adjustment to approximately 6.7. The same four-member panel tasted the fractions, and textural sensations were similar as those experienced previously. After further cleanup steps, the samples were prepared for subsequent NMR analyses.

Additionally, authentic flavor standards used for structural confirmation, quantification and sensory verification in this study included: orotic acid (Sigma, St. Louis, MO); D-pantothenic acid hemicalcium salt (SUPELCO, Bellefonte, PA); hippuric acid (Sigma, St. Louis, MO); 2-methylhippuric acid (Aldrich, St. Louis, MO); and p-cresyl sulfate (APExBIO, Houston, TX). Purity of sensory standards were either confirmed or further purified via HPLC fractionation and verified by electrospray ionization (ESI) positive and negative UPLC/MS analyses, as well as 1D-HNMR experiments prior to sensory analysis.

Various NMR experimentation for identity verification based on mass spectral assignments included 1-D NMR techniques, ^{1}H, ^{13}C and 2-D NMR experiments including Heteronuclear Single Quantum Coherence (HSQC), Heteronuclear Multiple Bond Coherence (HMBC), and COrrelation SpectroscopY (^{1}H-^{1}H COSY)]. These protocols were conducted on the taste actives using 0.2-10 mg of the purified analytes of

interest dissolved in 150 μL of D_2O and a Bruker 900 MHz instrument (results not shown).

Quantification of compounds of interest in eight dairy products.

Eight individual dairy products were purchased locally, extracted, and evaluated for the quantitative presence of the seven nonvolatile compounds of interest. The dairy products included: Essential Everyday® skim milk, 2% milk, whole milk, and heavy whipping cream (Cub Foods Roseville, MN), and Old Home® Fat Free Plain Yogurt, Plain Yogurt, Nonfat Greek Yogurt, and Greek Yogurt (Cub Foods, Roseville, MN). All yogurts had no added flavors or sweeteners. Approximately 50 g of each product was freeze dried to remove bulk water from the product. 1200 mL of a bulk solvent mix of 87/13 (v/v) methanol/water was spiked with 0.0233g of methyl-4-hydroxybenzoate as an internal standard. Each aliquot of freeze dried product was extracted with 35 mL of the bulk mix and shaken on an orbital shaking table at 120 rpm for 2 hours (MaxQ High Performance Orbital Shaker, ThermoScientific). After the extraction, each sample was centrifuged for 15 minutes at 4°C and 4000 rpm (Allegra X-22R Centrifuge, Beckman Coulter). After this, each sample underwent solid-phase extraction as a cleanup and concentration step (Discovery® DSC-18 SPE, 2g). Each SPE cartridge was pretreated with 10 mL of methanol and 10 mL of DI water. 10 mL of each sample was loaded onto the cartridge, and the final samples were eluted in 2 mL of methanol, after which each sample was syringe filtered (Millex® 13 mm, 0.20 μm nylon membrane) prior to injection via UPLC/MS. The UPLC/MS system used was a Waters triple-quadrupole mass spectrometer (Waters Quattro Premier XE) equipped with an ESI probe. The system

included an ACUITY UPLC sample organizer, binary solvent apparatus, and a CORTECS™ UPLC® C18+ 1.6µm column (2.1x100mm, Waters). Mass spectrometric conditions were: desolvation temperature, 270°C, source temperature, 120°C, capillary voltage 3.30V. MRM methods, ion transitions, and cone voltages were manually optimized for the internal standard and each compound of interest, listed in Table 10 below. Statistical significance was verified using analysis of variance (ANOVA) followed by Tukey's HSD all pairwise comparison. All analyses were performed with Statistix 10 (Analytical Software, Tallahassee, FL). Significance was established at $p<0.05$.

2-Alternative Forced Choice (AFC) Test

A twenty-member sensory evaluation panel was convened to ascertain potential sensory relevance of the five identified textural compounds. Samples included a control skim milk and a skim milk sample spiked with levels of all five compounds of interest found in whole milk. Samples were blinded with randomized three digit codes. Panelists wore noseclips and were presented with two sets of samples, after which they were asked to choose the "creamier, full-bodied sample." They were instructed to rinse well with water and to take a short break to recalibrate between sample sets.

Results and Discussion

Creaminess perception of dairy products is multimodal, variable, and dependent on the product at hand. An initial screening procedure was used to select a dairy product with known creaminess attributes. Three different dairy products—whole milk, half-and-half, and heavy whipping cream—were extracted to evaluate for creaminess sensations. Because previous research [34] had alluded to potential semivolatile effects on retronasal creamy perception, food grade extracts were evaluated for any creamy tastes/textures or aromas, with and without noseclips respectively. From these preliminary evaluations, heavy whipping cream was selected as the product highest in representative creamy nonvolatile sensations, after which an optimized extraction protocol was developed to obtain the highest possible textural perceptions prior to compound isolation, purification, and analytical protocols.

During the initial method development and throughout the entire nonvolatile extraction procedure, food-grade extracts were tasted after each analytical sample prep step to verify the presence of desired taste, texture, and overall creamy perceptions. The initial nonvolatile solid precipitate of the heavy whipping cream extract had a creamy, fatty, and dairy-type mouthfeel and texture after solvent removal and cleanup. After a first-dimension fractionation, the solvent free isolates were taken up in water and evaluated by panel for mouthfeel and textural sensations present while wearing noseclips. Mouthfeel and textural results obtained from 43 fractions are presented in Figure 12. Displayed ratings show consensus of three trained panelists experienced in dairy sensory evaluation, using a five-point categorical scale: no perception, “threshold,” “weak,”

"moderate," and "strong." No "moderate" or "strong" taste and texture attributes were reported; however, panelists identified 18 fractions with "threshold" and 15 regions with "weak" mouthfeel/texture. Panelists were also asked to describe "dairy/creamy" sensations that they tasted in the samples (Figure 13). Several fractions appeared to have recognizably creamy/dairy attributes when tasted while wearing nose clips; 12 fractions were rated as "threshold," 9 as "weak", and 4 were rated as having "moderate" creaminess. In general, the panelists observed an increasing trend for these perceptions upon removal of the nose clips, suggesting the increased impact of volatiles still present in the crude isolation on overall creaminess perception. Panelists also noted taste attributes associated with several fractions. Fractions 15, 17, 22, 26, 28, 30, 31, 34, 35, and 40 were found to be bitter and astringent, while fractions 32, 40, and 42 had sour tastes associated.

A replicated series of fractions was reconstituted in 1% fat milk to examine their sensory contributions to creaminess within a dairy matrix. A trained panel compared sets of samples that included a control 1% fat milk sample and fractions of interest reconstituted in 1% fat milk. A directional paired-comparison test was used to rank which sample was "creamier." Ranking was performed without noseclips to observe any potential semivolatile effects still present in the fractions, given that the aqueous fractions had occasionally yielded dairy-like texture and what seemed to be weak volatile perception upon removal of the nose-clips. Results of the sensory evaluation are shown in Figure 14, which revealed 12 fractions with increased creaminess when compared to the corresponding control 1% milk samples. Upon comparing the previous aqueous

fractions to those spiked in a dairy matrix, several fractions identified as having creaminess and textural attributes overlapped. Fractions 11, 13-14, 23, 32, 37-38, and 43 in Figure 14 all corresponded with "weak" mouthfeel/texture ratings from the aqueous sensory study mentioned above. Due to the 3 kDa cutoff membrane used in the extraction protocol, larger bulk lipids and proteins were eliminated from the whipping cream, and these were assumed not to be responsible for the textural differences observed between the fraction samples and the control samples. It was hypothesized that compounds of lower molecular mass present in the fractions contributed to texture, and thus increased creaminess when added to the dairy matrix (1% milk).

Combining the sensory data obtained from these various fractions in both aqueous and dairy matrices offered valuable insight into important sensory attributes for creaminess perception and particularly the relationship between creaminess and textural perceptions of nonvolatile compounds. However, before continuing with secondary separation, isolation, and structural elucidation of compounds with relevant sensory attributes, it was important to ensure that all panelists were in agreement regarding the textural attributes present within the samples. A descriptive analysis panel was undertaken to develop a lexicon of sensory descriptors and definitions of sensory qualities present within the samples. This would provide a source of common textural vocabulary and references for future fraction evaluation throughout the duration of the study.

Collected fractions were used for an initial screening panel consisting of 12 individuals experienced with sensory evaluation of food products. All panelists were able

to identify texture stimuli among several fractions and an aqueous control, as well as threshold differences or "no difference" for a blank sample; thus, all 12 individuals were selected for further descriptive lexicon development. Panelists were provided aqueous fractions from the whipping cream extract, and they were asked to describe any texture or taste that they perceived. The panelists' feedback for attribute term development resulted in five key categories with applicable references: "mouthcoating," the sensation associated with dilutions of nongelatinized cornstarch suspensions; "astringency/drying" associated with dilutions of black tea at room temperature; "fatty texture," as represented by consuming a whole fat milk, "dairy mouthfeel," as illustrated by the sensation of consuming skim milk; and "tingling/irritation," the sensation associated with nanopure water that had been stirred overnight with a copper penny.[126] Overall, the panelists agreed that the selected lexicon terms adequately described the sensory attributes present in the samples. They were able to begin assigning numerical scores using sensory scales developed with the references.

After the development of an appropriate lexicon, a smaller trained 6-member subset was selected to evaluate the first-dimension fractions for any texture sensations according to the established references; fractions of interest and their sensory ratings are shown in Figure 16 below. Fractions 2, 4, and 9 were found to be the most intense in total number of textural attributes perceived, in comparison to the rest of the fractions tasted. These three fractions then underwent a second-dimension separation for further purification and subsequent sensory evaluation. The corresponding chromatograms and the relevant descriptors are shown in Figure 17. The sensory descriptors matched

consistently with the references determined previously. The panel found that the fractions highest in intensity for cumulative texture activity included fraction 3 (from the first dimension fraction 2), fraction 2 (from the first dimension fraction 4), and fraction 4 (from the first dimension fraction 9).

For identification and structural elucidation of the compounds with sensory activity, these second dimension fractions highest in textural ratings were further examined via high resolution mass spectroscopy. Results revealed seven sensory active compounds of interest. Further separation and fractionation was employed in order to achieve bulk isolation and higher purity for confirmation of sensory relevance of individual compounds. Five key compounds were identified as the most texture active and were selected for further structural elucidation.

After bulk isolation of the compounds, ESI positive and negative experiments were conducted to verify relative purity by UPLC/MS/MS peak area calculations. Purity of approximately 95% or greater was achieved for all compounds, after which the same sensory panel evaluated each of the compounds spiked into aqueous aliquots and adjusted to the pH value of milk (6.7). The panel found textural and taste sensations that matched with the previous lexicon descriptions among these compounds. MS experiments were conducted to understand potential structural characteristics. These results, coupled with comparison to literature libraries and values of nonvolatile mass spectral data, allowed potential identities of the five unknown compounds to be assigned. After purchase of authentic standards and further mass spectral confirmation (high elemental composition matches < 5 ppm), all five were positively identified and found to belong to three general

classes of compounds, including vitamin complex compounds, hippuric acids, and sulfated analytes. 1D-HNMR and 2D NMR experiments were conducted on the isolated, purified compounds to verify their identities (all compounds matched the spectra reported in the literature). The five compounds of interest and their structures are shown in Figure 18 below, and the corresponding second dimension UV chromatograms and UPLC/MS chromatograms of interest are shown in Figure 19.

The first two fractions contained orotic acid (a pyrimidine carboxylic acid), and pantothenic acid (vitamin B5). The UPLC/MS results of orotic and pantothenic acid are shown in Figure 20 below, compared to their respective authentic standards. Both of these compounds are common to dairy products and occur naturally in milk. Additionally, the third and last fraction contained the aromatic, carboxylic acids hippuric acid and 2-methyl hippuric acid (Figure 21), and the sulfated analyte p-cresol sulfate (Figure 22). Retention times and mass spectral transitions matched between all five of these compounds and their respective analytical standards. ESI negative scans and MRM experimental transitions for the orotic, pantothenic, hippuric, and 2-methylhippuric acids showed transitions of 44 m/z between parents and sibling ions, characteristic of CO_2 losses from carboxylic acid groups. Pantothenic acid in ESI positive mode also yielded characteristic water losses from its structure, matching well with its corresponding standard.

The positively identified sulfate compound, p-cresol sulfate, also matched with its reference standard and exhibited the characteristic ion loss of 80 m/z for sulfate. As seen in Figure 19, two other unknowns were present in the chromatogram just upstream from

p-cresol sulfate. Preliminary sensory tests of these unknowns indicated these also exhibited some textural characteristics, albeit in weaker overall ratings than the other confirmed compounds. With molecular ions of 241 m/z and 243 m/z respectively, they also contained losses of 80 m/z and high elemental composition matches for sulfates in their structures. However, their elemental compositions also showed the presence of nitrogen, suggesting that they may have resulted from other available nitrogenous sources in dairy. The sulfate class of compounds may play a broader role in textural characteristics of dairy, but more work would be needed to confirm these identities, in addition to screening for other sulfated compounds with relevant textures. These unknowns notwithstanding, the five confirmed compounds have never before been attributed to either texture or creaminess perception in dairy.

After structural elucidation of compounds with textural activity, a larger quantitative study was undertaken to evaluate the presence of these five textural compounds across other zero-, low-, and full-fat dairy products. Doing so in a variety of dairy products and with different fat levels, processes, fermentation, etc. could provide insight for targeted industrial applications. Eight dairy products were selected for quantitative analysis including skim milk, 2% milk, whole milk, fat free plain yogurt, full fat plain yogurt, fat free Greek yogurt, full fat Greek yogurt, and whipping cream. The various concentrations of the compounds in the products can be seen in Figures 23-25 below.

Orotic acid is an intermediate in the mechanistic pathway of pyrimidine synthesis in animals,[127] and concentrations of orotic acid can vary in dairy products. Levels

typically found in dairy can range based on the particular breed of cow, thermal treatment, type of product, and reported analytical method. For example, one study [128] found levels at approximately 75 mg/L for whole milk, 79 mg/L for skim milk, and 66 mg/L in yogurt. Others [129] have found fairly high variability even within the same sample pool. Orotic acid is reasonably stable to more extreme thermal processing conditions such as UHT processing,[129] which could make it particularly relevant for optimization in dairy products subjected to more extreme thermal treatments. Previous work [130] reports orotic acid as a growth factor for lactobacilli strains in dairy products, and thus fermented products like yogurts generally have lower amounts of orotic acid present as a result of *Lactobaccillus spp.*[131, 132] It has been found to decrease in concentration over fermentation time and decreasing pH in cold stored yogurts, particularly as lactic acid increases in concentration.[133] In another such study,[129] the concentration of orotic acid decreased by as much as 45% during typical fermentation incubation times. The levels observed across the dairy products profiled in this study appeared to correspond with previously published values. A significantly lower level of orotic acid was observed in all of the yogurts, as opposed to the higher fat milks and whipping cream. Interestingly, the highest levels of orotic acid were observed in the higher fat milks (2% and whole) and the whipping cream, compared to the yogurts and skim milk. The presence of this compound in higher fat products was notable, as it not necessarily related to lipid presence or bioconversion in nature. This might indicate its importance as a key textural component of full-fat dairy, which is distinctly lacking in lower fat or fermented dairy products.

More work would be needed to understand this correlation, but orotic acid may be a viable contributor to texture if it could be incorporated in low-fat dairy products.

Pantothenic acid, commonly referred to as vitamin B5, is a water-soluble vitamin found in a range of foods, including meats, whole grains, fruit, vegetables, and dairy. It is fundamental to coenzyme A and thus is intrinsic to bovine metabolism such as amino acid synthesis, as well as fatty acid oxidation. There are no current set standards in the United States for pantothenic acid supplementation in dairy cattle, since they have been found to metabolically synthesize sufficiently high quantities naturally.[134] The highest levels of pantothenic acid observed in our samples were present in 2% milk, full fat plain yogurt, and full fat Greek yogurt, followed by fat free plain yogurt. However, while some of these levels were significantly different between products, overall concentrations did not vary as widely as some of the other profiled compounds, only ranging between 0.46±0.014 mg/kg (whipping cream) and 2.6±0.78 mg/kg (plain Greek yogurt). This was seemingly comparable to concentrations found in previously published works, [135] where orotic acid was measured in milk products at approximately 3.5 mg/kg. Higher levels have been found in yogurt as well.[136] While this could indicate that the concentration of pantothenic acid and creaminess perception might not be as highly correlated, it is possible that certain kinds of products (plain Greek yogurt, plain yogurt, 2% milk, whole milk) may be more likely to contain slightly higher levels of pantothenic acid. More aqueous and therefore more relatively polar dairy products (2% and whole milk) appeared to have higher concentrations of pantothenic acid than compared to heavy whipping cream, a much more lipid-dense, nonpolar product. Bearing in mind the

hydrophilicity of pantothenic acid, it is possible that products that are more conducive to pantothenic acid dissolubility could have greater methods of retention and incorporation of the compound in final dairy products.

The formation pathway of hippuric acid in dairy is widely acknowledged in literature. Phenolic acids incorporated from feeds serve as precursors for benzoic acid upon microbial fermentation in the rumen.[137] Hippuric acid results from metabolism and interaction of aromatic compounds such as benzoic acid with the amino acid glycine in the liver of ruminant animals.[138] Hippuric acid has been previously recognized in milk studies going back to the early 1950's.[139] Ruminant animals have been found in several studies to contain significantly higher levels of hippuric acid if fed grass and organic diets, respectively, as compared with those fed silage or non-organic type diets.[140, 141, 142] Another case study [143] suggested that organic bovine milks contained hippuric acids at concentrations of approximately 30-40 mg/L, while conventional milks contained approximately 20-30 mg/L. Others [144] have measured hippuric acid levels as high as 50 mg/L. Some [145] have suggested that these phenomena are due primarily to the higher phenolic presence in grasses than silage. Likewise, aging of plants in feed sources and resultant degradation of phenols may directly lead to lower levels of hippuric acid in the final milk product.[146] Still others [147] have suggested that hippuric acid could be used as an indicator of a diet high in fruit and vegetables. Just as with orotic acid, hippuric acid has also been found to decrease in concentration over time in fermented products. This was previously reported to be the case,[144] in correlation with an increase in benzoic and lactic acids in fermented milk products as a result of conversion via lactic acid bacteria. In

yogurts, hippuric acid has been reported to decrease over fermentation time, along with increased formation of benzoic acid.[133] Similar results were observed in the profiled dairy products in this study. Hippuric acids were not found at appreciable levels in any of the yogurt samples, seemingly in line with previous research. There were significant differences among the other four products: skim milk, 2% milk, whole milk, and heavy whipping cream. The highest levels of hippuric acid were measured in the lower fat milks. While all milks came from the same manufacturer, whether these quantitative differences were a result of different lipid levels, or the result of differently sourced milks, would require further study.

Over the past several decades, 2-methylhippuric acid has been most commonly analyzed in urine studies as a marker indicative of human exposure to various organic solvents, particularly toluene. Key metabolites in these studies typically include isomers o-, m-, and p-cresol, as well as p-xylene and 2-, 3-, and 4-methylhippuric acid.[148] O-xylene in particular has been identified as forming minute amounts of 2-methylhippuric acid.[149] There is precedent for understanding its relation to 2-methylhippuric acid presence in food sources, however. Xylene may exist in minute amounts in foods, including meat, dairy, vegetables, and grains, offering a potential source of reactants for 2-methylhippuric acid formation.[150] It has been identified as a minor component of fatty acid metabolism, as well as from phenolic acid metabolism and fermentation pathways.[151] One group in particular [152] identified both hippuric and 2-methylhippuric acids in pigs as a result of metabolism when the animals were fed diets higher in plant phenolic acids. The group also suggested that these could be used as biomarkers of an

animal diet rich in polyphenols. Still others [151] found that supplementation of animal feeds with an enriched diet containing specifically arabinoxylans yielded higher levels of bioavailable hippuric acid and 2-methylhippuric acid, as well as pantothenic acid. Therefore, it seems viable that specific diets or feed supplementation may be capable of naturally elevating levels of these compounds in animals and passing them on to dairy products. In comparison to these previous studies, the quantitative analysis yielded significantly higher levels of 2-methyl hippuric acid in this study, most notably in the full fat plain and Greek yogurts (approximately 1700±310 and 1700±200 mg/kg, respectively). Both lower fat yogurts were comparable to the remaining four dairy products in 2-methylhippuric acid concentration. This may indicate not only the importance of initial bioavailability of the compounds from the animal, but also their potential synthesis or bioconversion upon further dairy product fermentation.

Compounds with sulfate groups in their structures have been previously identified in dairy research. One research group [153] identified free sulfate present at concentrations as high as 100 mg/L in whole fat cow's milk. However, they acknowledged that the acid digestion used in their study to mimic stomach conditions may have overestimated the levels of naturally occurring free sulfate in milk; in actuality, there were likely more complex structures present as well, such as amino sulfonates, nitric oxide-sulfonates, sulfuryl-sulfonates, oxy-sulfonates, etc. While it is conceivable that sulfate compounds could have textural sensory aspects when spiked into pure water, it is also possible that these same compounds could interact with the larger matrix of dairy products, such as in the presence of dairy peptides, lipids, and other textural components. This might account

for the kinds of interactions previously observed in this study, in which creaminess was enhanced in 1% milk in the presence of certain whipping cream fractions, possibly due to dairy matrix effects (Figure 14).

Phenols have been previously identified in fresh milk, existing particularly in conjugated forms such as phosphates and sulfates. Upon liberation by microbial degradation and bovine metabolism, phenols like p-cresol may undergo phosphorylation or sulfation in the liver or kidney prior to excretion. It is possible that p-cresol could become available for volatile perception once liberated, for example upon hydrolysis of conjugated groups during metabolism.[154] Indeed, p-cresol has been acknowledged in previous literature [155] as an important contributor to high-quality dairy volatile profiles, such as butter. At high enough levels, it has occasionally been found to be a source of off-flavor as well.[156] p-cresol has been previously reported [157, 158] to form in dairy products as a result of some specific bacterial strains, *Bacillus circulans* and *Lactobacillus*. It is understood to occur from bacterial biosynthesis and conversion of phenolic compounds and aromatic amino acids.[152] One study [151] identified p-cresol sulfate and other sulfate compounds as a result of feeding regimen in pigs. The group found that the levels of these compounds could increase upon supplementation with arabinoxylans. Furthermore, the group hypothesized that fermentation pathways could be responsible for conversion and subsequent bioavailability of the sulfated compounds, particularly as a result of phenolic acid metabolism and fermentation in the gut. In the current study, p-cresol sulfate was found to occur approximately four times higher in full fat plain yogurt than any of the extracted milks, full fat Greek yogurt, or heavy whipping

cream. The products lowest in p-cresol sulfate concentration were fat free Greek yogurt and heavy whipping cream. Similar to 2-methylhippuric acid, this may indicate potential for fermentative processes in formation of this texturally active compound. Even solely in the comparison of plain yogurt to Greek yogurt levels of p-cresol sulfate, opportunity may exist for further optimization according to specific dairy processing and fermentation parameters.

To evaluate the impact of these five compounds on 'creaminess' perception, a 2-Alternative Forced Choice (2-AFC) sensory evaluation test was conducted to determine if the identified compounds had a significant sensory effect when evaluated in a dairy matrix. The five compounds were spiked into a skim milk at concentrations determined in whole milk and compared to the original skim milk. Twenty panelists wore nose clips to eliminate volatile stimulation and were asked to identify the "creamier, fuller bodied sample" when compared with a control skim milk. They found that the spiked sample was significantly different compared with the control skim milk ($p=1/2$, $n=20$, $\alpha=0.01$), indicating that the five compounds were able to provide an enhanced body and creamier nonvolatile perceptions in a low fat milk product. It is important to note that there did seem to be some volatile contribution as a result of the p-cresol sulfate, likely as a result of free p-cresol present within the sample. Upon removal of the noseclips, panelists mentioned that they perceived retronasal perceptions reminiscent of the "stale, oxidized" notes believed to be associated with the volatile p-cresol. While this would have an impact on the overall sensory experience of the creamy dairy product, as we noted above, the concentration, bioavailability, and conjugation of naturally occurring p-cresol sulfate

in the dairy product itself would likely eliminate this somewhat. However, further study would be needed to elucidate the mechanics of either volatile liberation or retention as a result of natural occurrence in milk.

In summary, five novel textural compounds were identified from extracts of heavy whipping cream. These five compounds encompassed three individual structural classes, including vitamin-related conjugated aromatic compounds (orotic acid, pantothenic acid), hippuric acids (hippuric acid, 2-methylhippuric acid), and sulfates (p-cresol sulfate). Concentrations of all five compounds were measured across eight different dairy products, and the presence of each suggested that the quantitative significance of the compounds varied across parameters such as lipid concentration, processing parameters, and fermentation pathways. Findings in previous literature strongly suggest that there are routes available for optimization or incorporation of these textural compounds even in lower fat products. Some possibilities include inclusion of different feed sources for ruminants such as grasses for higher levels of hippuric acids, or supplements like arabinoxylans for increased presence of in vivo fermentation compounds like 2-methylhippuric acid or p-cresol sulfate. Our quantitative work suggests dairy fermentation may also contribute to these compounds' presence as well, i.e. in yogurts. Others possibilities could include ingredient addition. Fortification of vitamin complex compounds in animal feeds or dairy products, for example, would be relatively easy to incorporate as these compounds are natural and ubiquitous in the environment. Furthermore, consumers frequently view vitamin fortification positively as a method of ensuring quality nutrition in the food supply. More work would be needed to identify

what optimized methods would yield the highest analytical or sensory results in specific products or from different milk sources.

Given the textural perceptions described in this work, as well as the presence of these compounds across a variety of dairy products and fat levels, there are continued opportunities in flavor research for further creaminess understanding. Exploring texture and creaminess perceptions from these and previously studied creamy attributes like volatiles and bulk rheology could lead to higher quality creaminess and offer consumers a variety of healthy, flavorful products. The novel analytical and sensory techniques employed resulted in positively identified small molecules endogenous to dairy foods (natural products). In some cases, these compounds have been measured and studied for several decades, however this is the first time that they have been associated with textural queues and resultant effects on creaminess perception. There is already previous research suggesting the sources of these compounds' formation, as well as trends and mechanisms associated with their presence or disappearance over time. Thus, concrete opportunities exist to incorporate them as natural ingredients to maintain dairy nutritional quality, clean labels, and overall high quality creamy flavor profiles in dairy products.

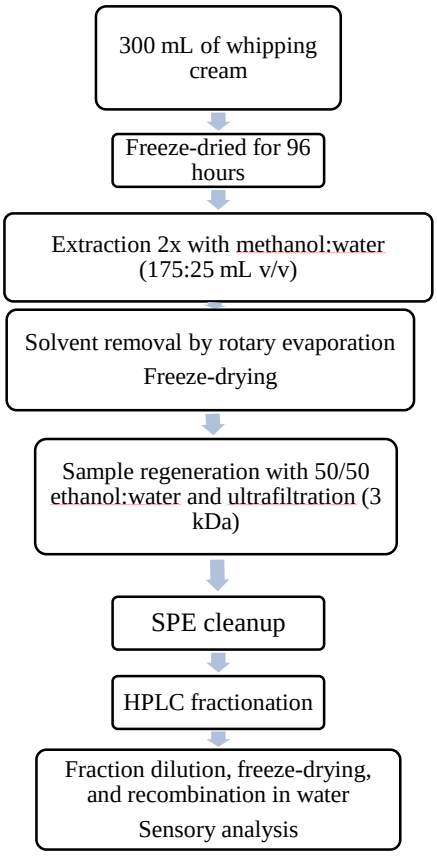

Figure 11, Procedure for Extraction, Cleanup, and Sensory Evaluation of Whipping Cream

Table 10, MRM transitions and capillary voltages for quantification of seven nonvolatile compounds. a = ESI negative, b = ESI positive, c = SIM (adapted from [159, 160])

Compound	Parent Ion	Daughter Ion	Cone voltage
Methyl-4-hydroxybenzoate[a] (ISTD)	151	136	40
Orotic acid[a]	155	111	30
Pantothenic acid[b]	220	202	35
Hippuric acid[a]	178	134	40
2-methylhippuric acid[ac]	192	148	25
240[a]	240	160	40
242[a]	242	162	40
p-cresol sulfate[a]	187	107	40

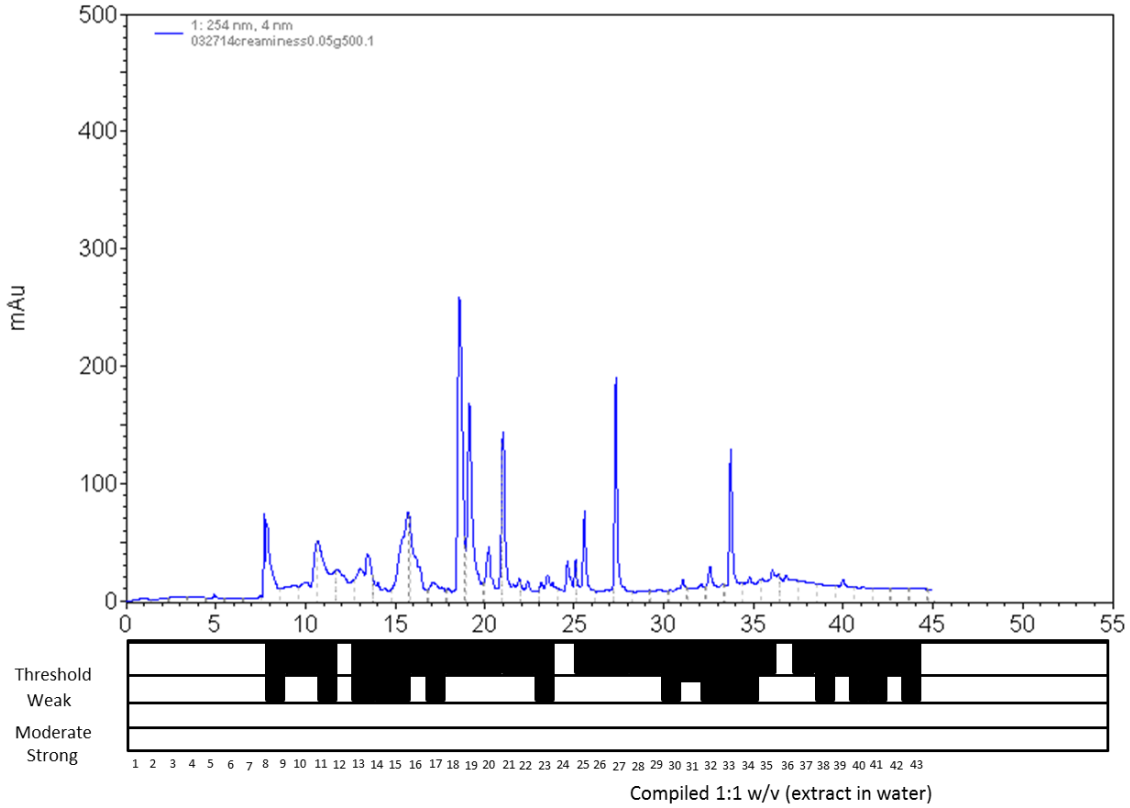

Figure 12, Mouthfeel/texture of fractions in water (compiled from three panelists)

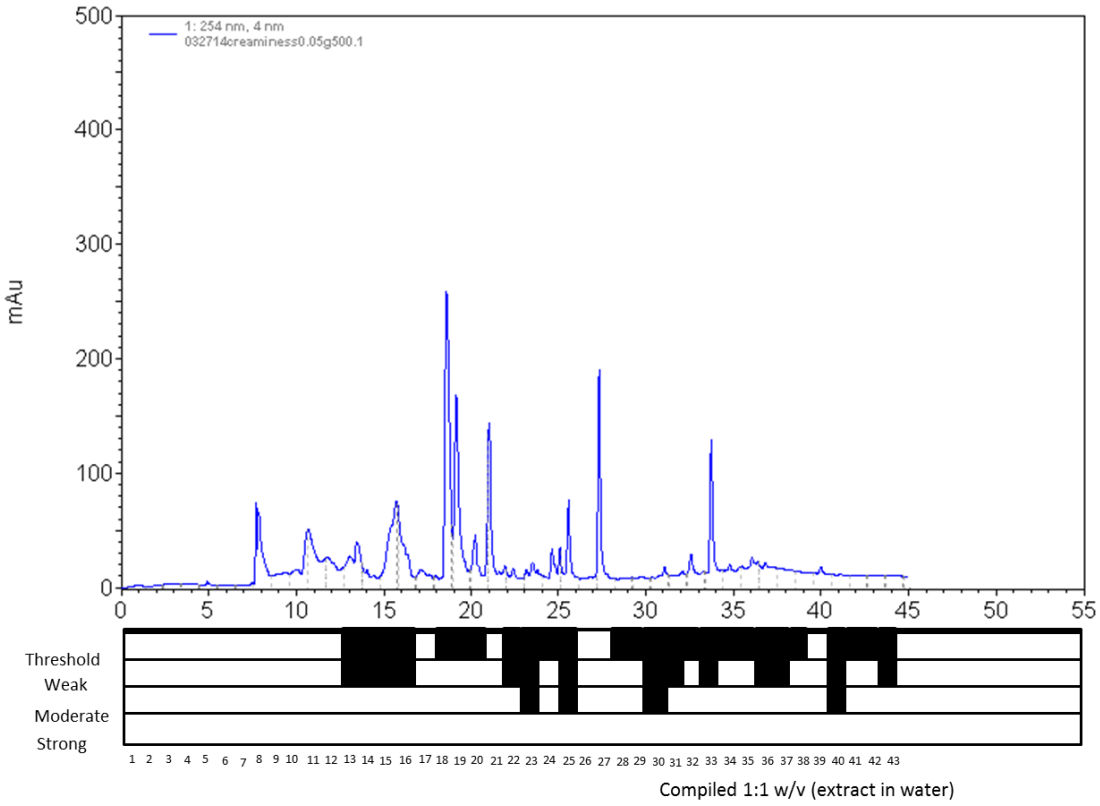

Figure 13, Dairy/creamy tastes from fractions in water (compiled from three panelists)

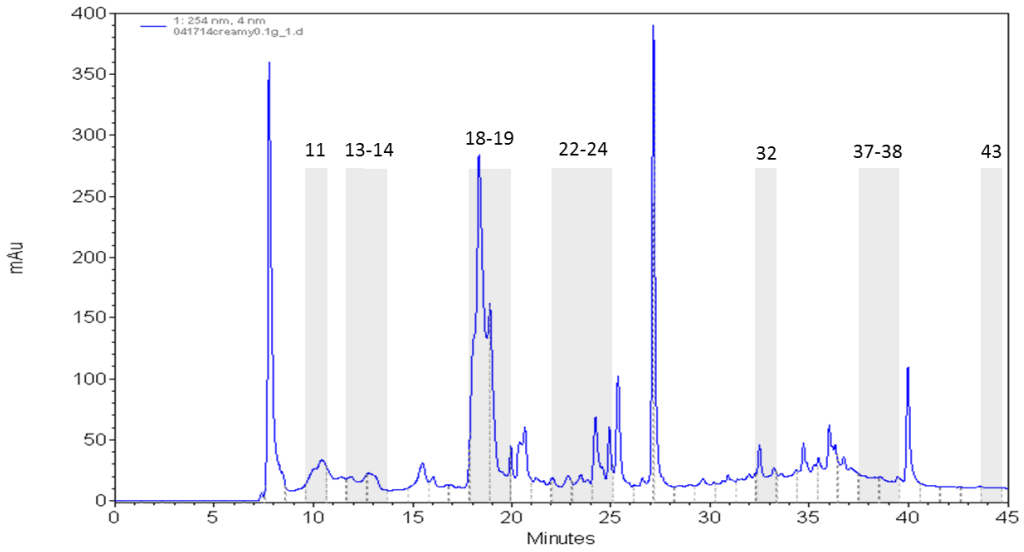

Figure 14, Directional paired comparison test of dairy/creaminess sensory ratings. Samples were spiked into 1% milk and compared with control milks for differences in overall creaminess perception

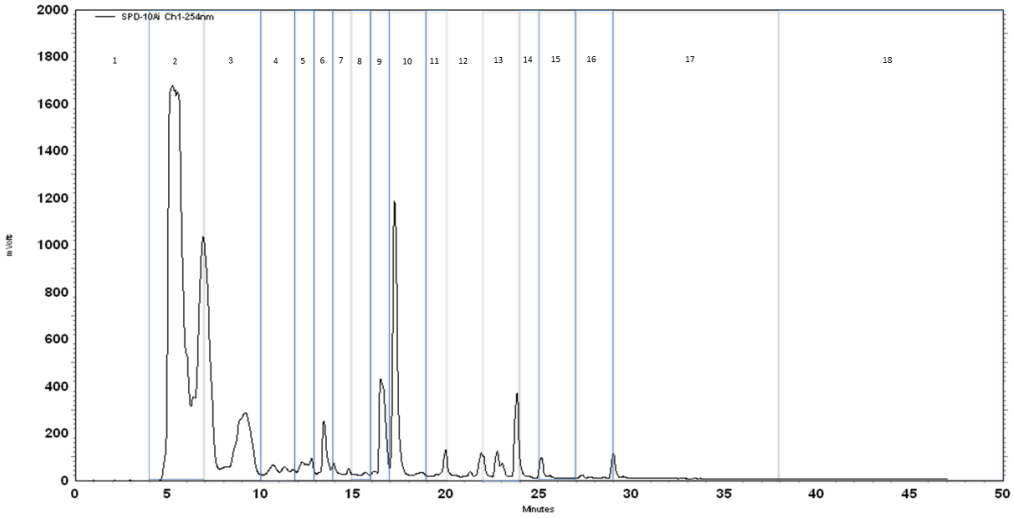

Figure 15, Preparatory-scale chromatogram and fractions of the whipping cream extract (monitored at 254 nm)

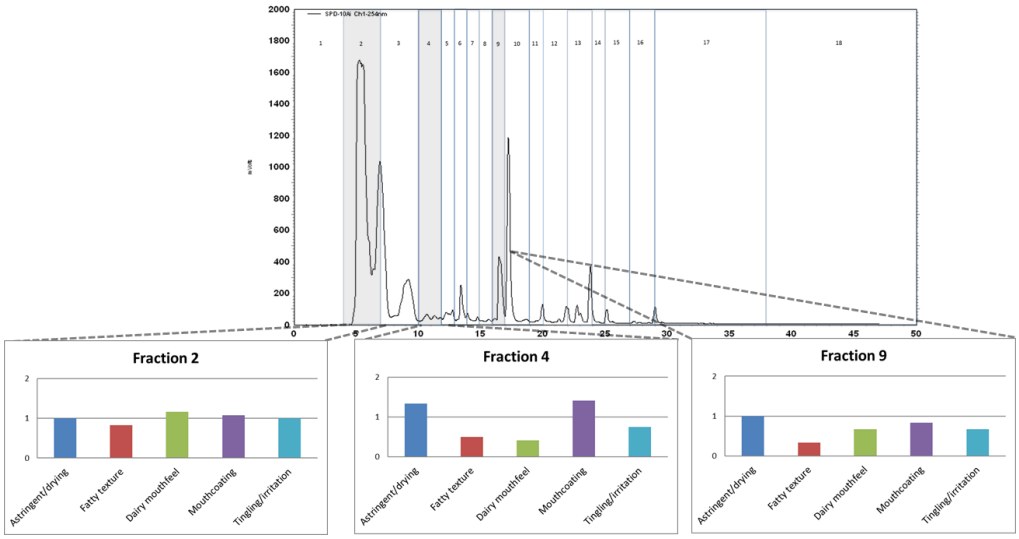

Figure 16, First dimension sensory descriptors and ratings for fractions highest in texture/mouthfeel activity. Perceptions were evaluated using a 7-point scale: 0- “No perception,” 1- “Very slight perception,” 2- “Slight/moderate perception,” 3- “Moderate perception,” 4- “Moderate/large perception,” 5- “Large perception,” and 6- “Very Large perception”

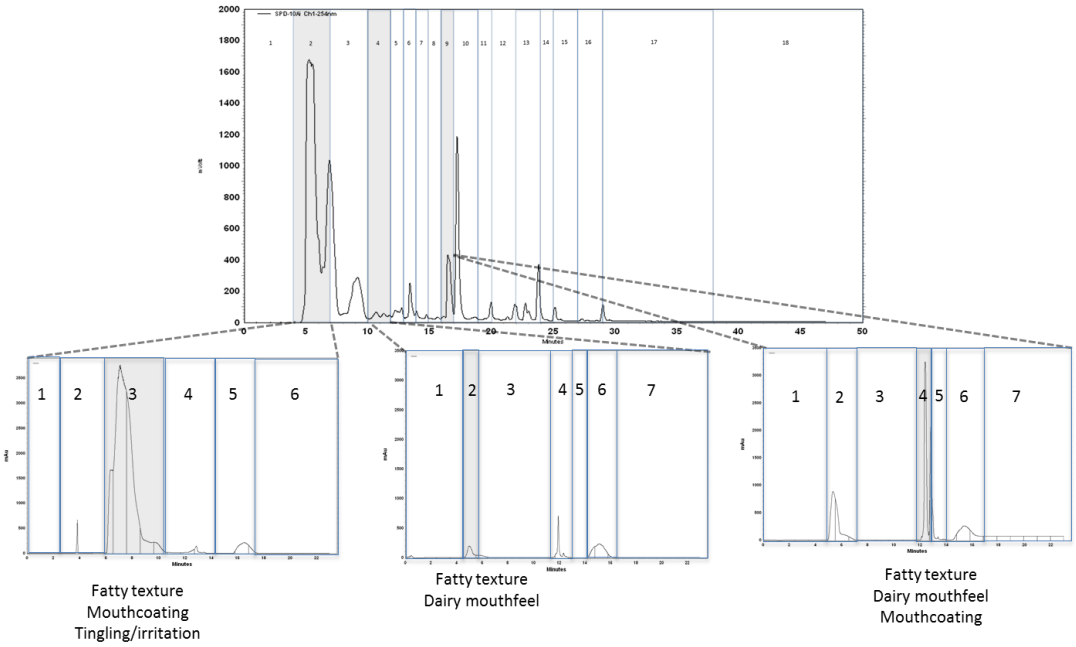

Figure 17, Key second dimension sensory descriptors for fractions highest in texture/mouthfeel activity

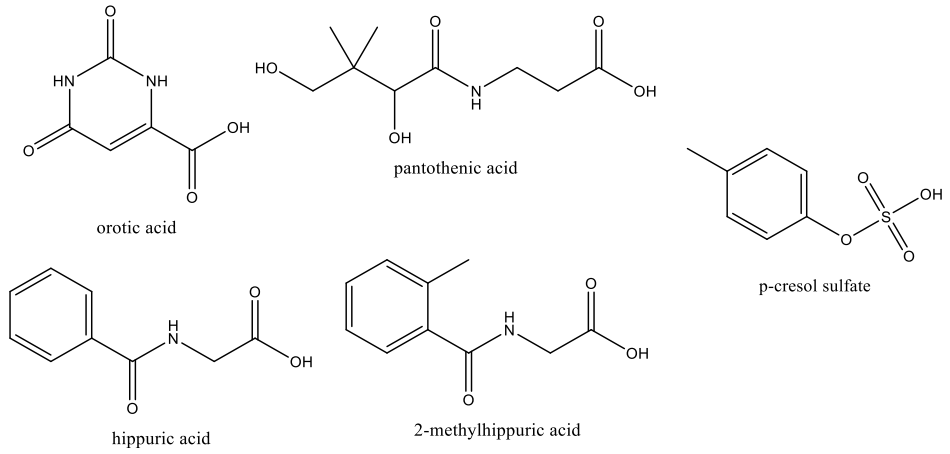

Figure 18, Select nonvolatile textural compounds identified in heavy whipping cream

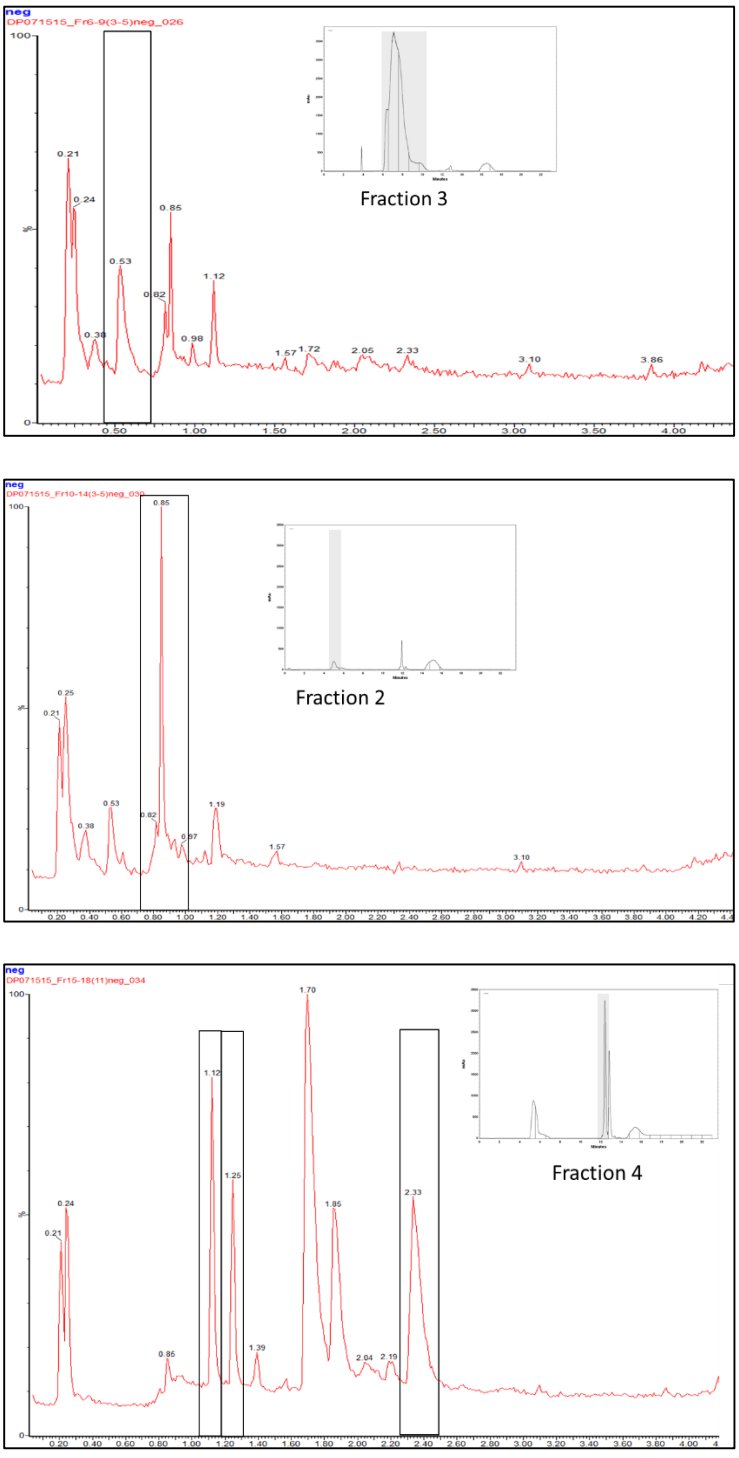

Figure 19, UPLC/MS ToF chromatograms and corresponding compounds from second dimension fractions. Top: Orotic acid. Center: Pantothenic acid. Bottom: Hippuric acid, 2-methylhippuric acid, p-cresol sulfate

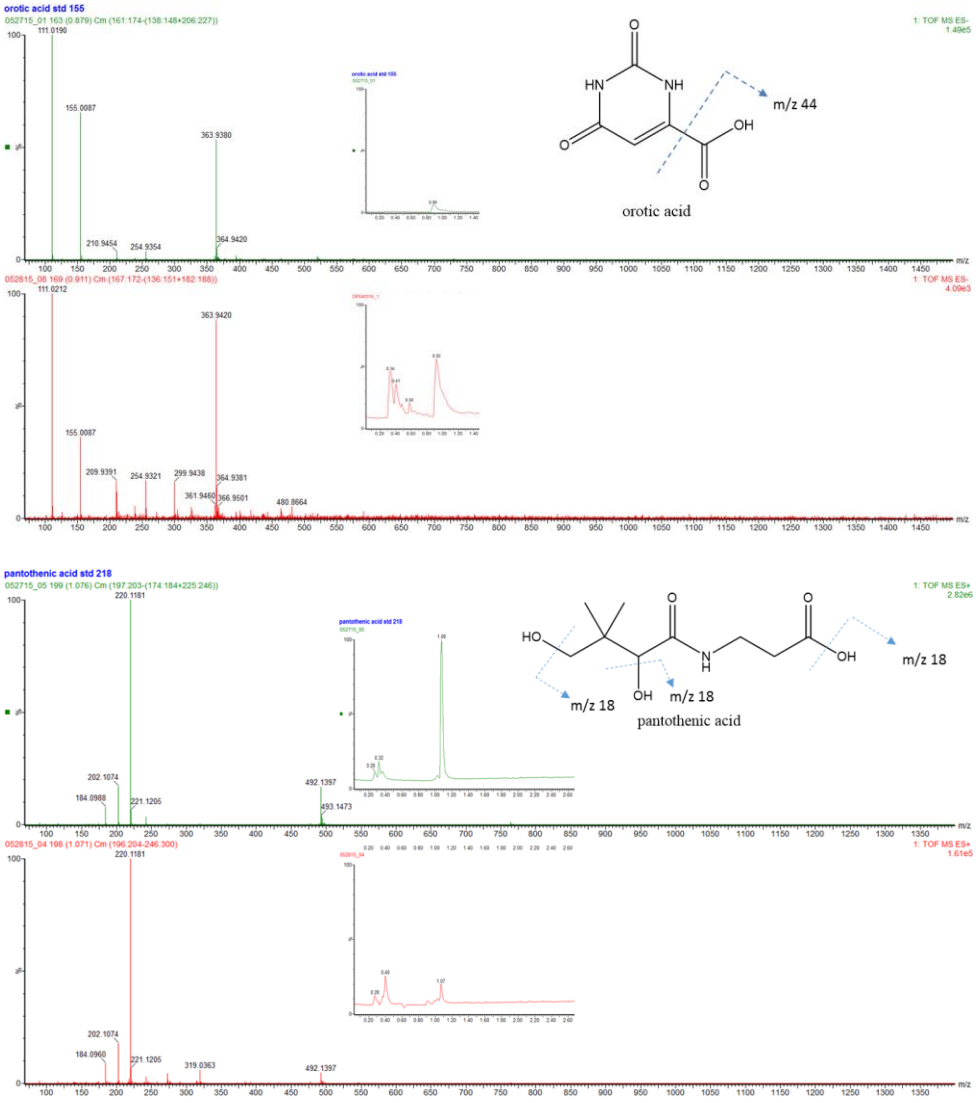

Figure 20, UPLC/MS ToF spectra of orotic acid and pantothenic acid. Top chromatograms and spectra (green) from pure standards; bottom chromatograms and spectra (red) from heavy whipping cream extract

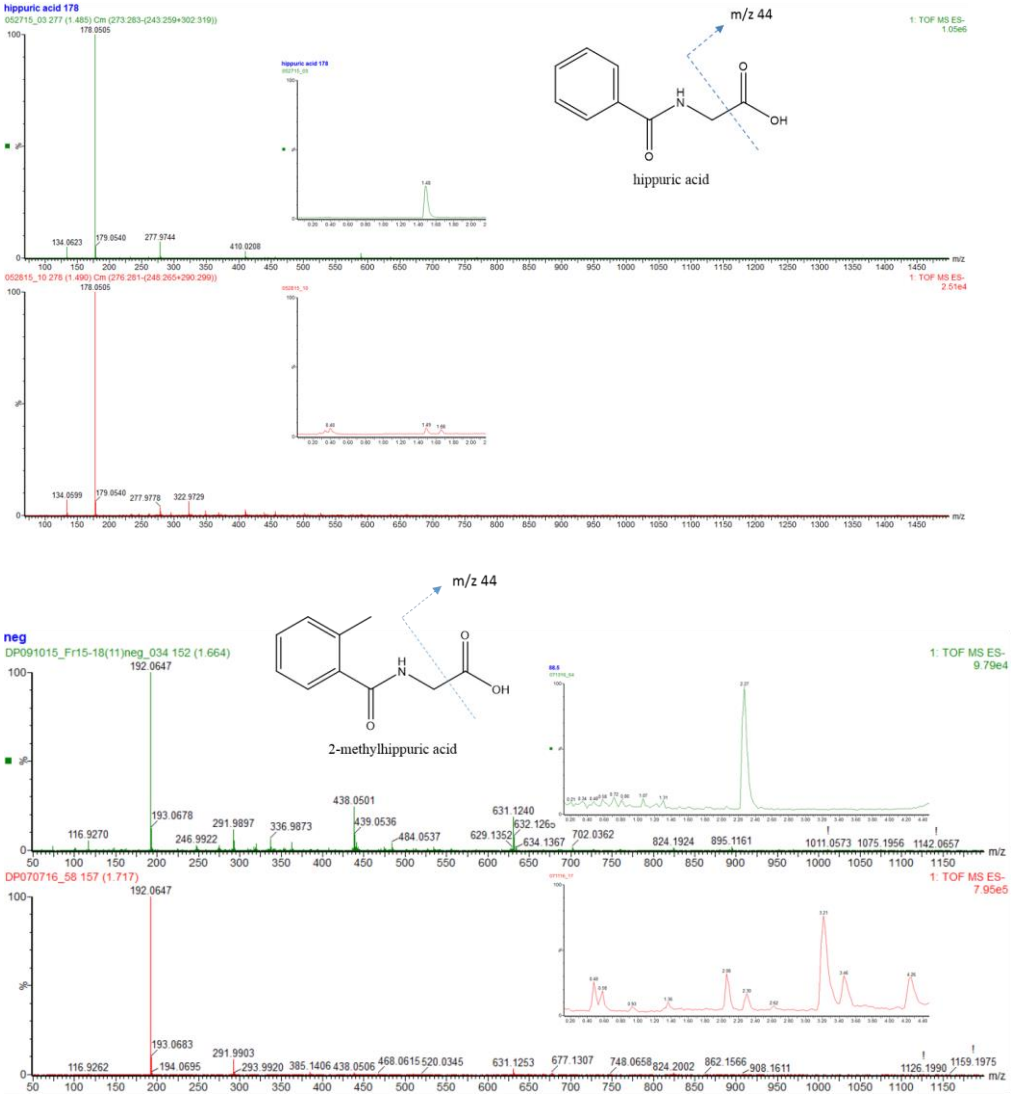

Figure 21, UPLC/MS ToF spectra of hippuric acid and 2-methylhippuric acid. Top chromatograms and spectra (green) from pure standards; bottom chromatograms and spectra (red) from heavy whipping cream extract

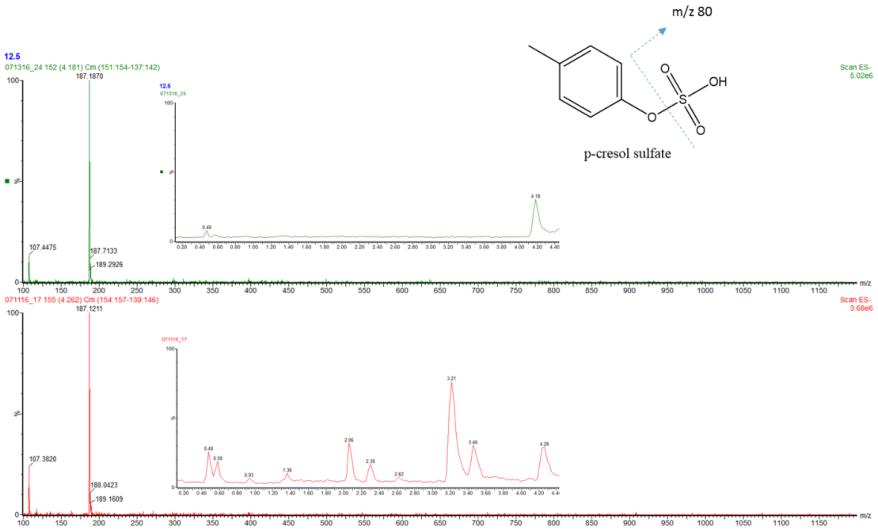

Figure 22, UPLC/MS ToF spectra of p-cresol sulfate. Top chromatogram and spectrum (green) from pure standards; bottom chromatogram and spectrum (red) from heavy whipping cream extract

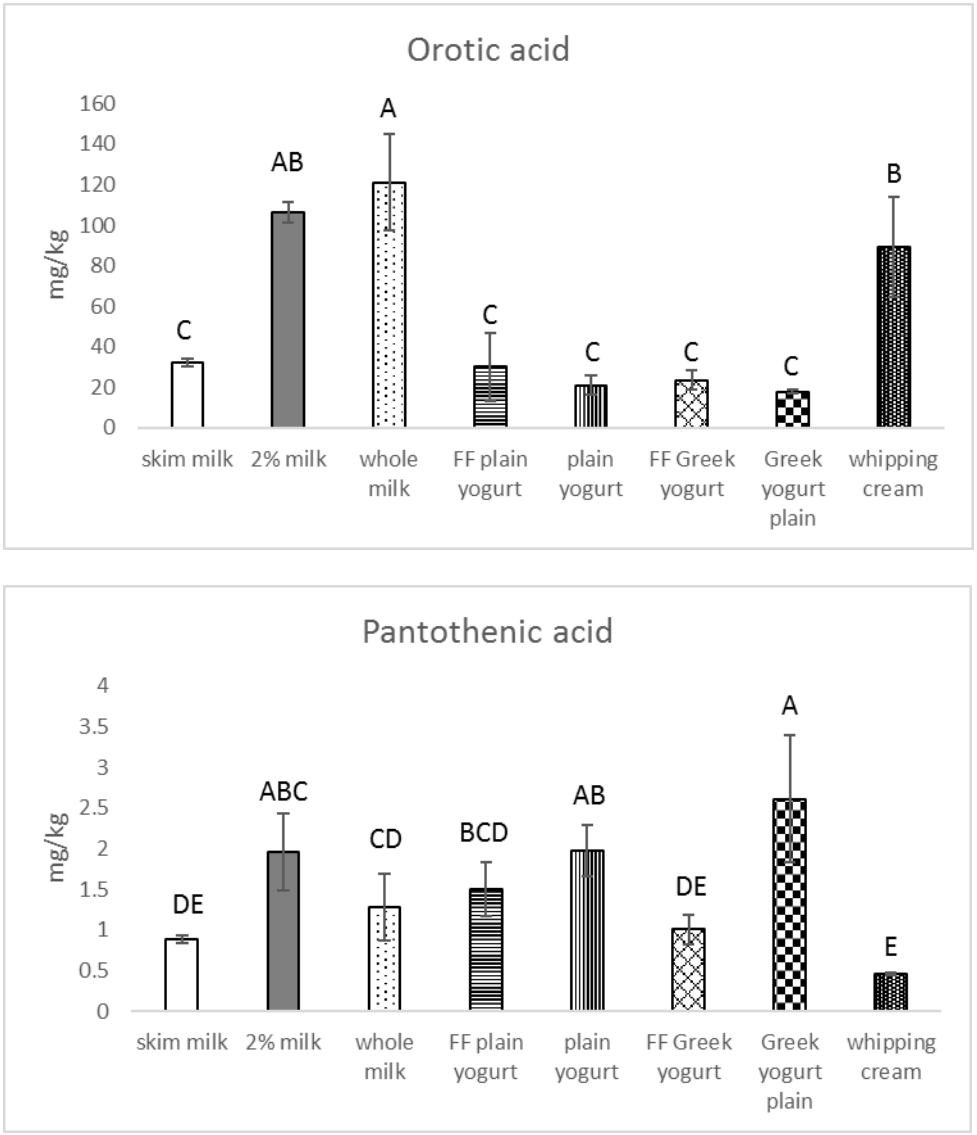

Figure 23, Quantitative results for vitamin-related structures in skim milk, 2% milk, whole milk, fat free plain yogurt, plain yogurt, fat free Greek yogurt, Greek yogurt, and heavy whipping cream. Top: Orotic acid, Bottom: Pantothenic acid

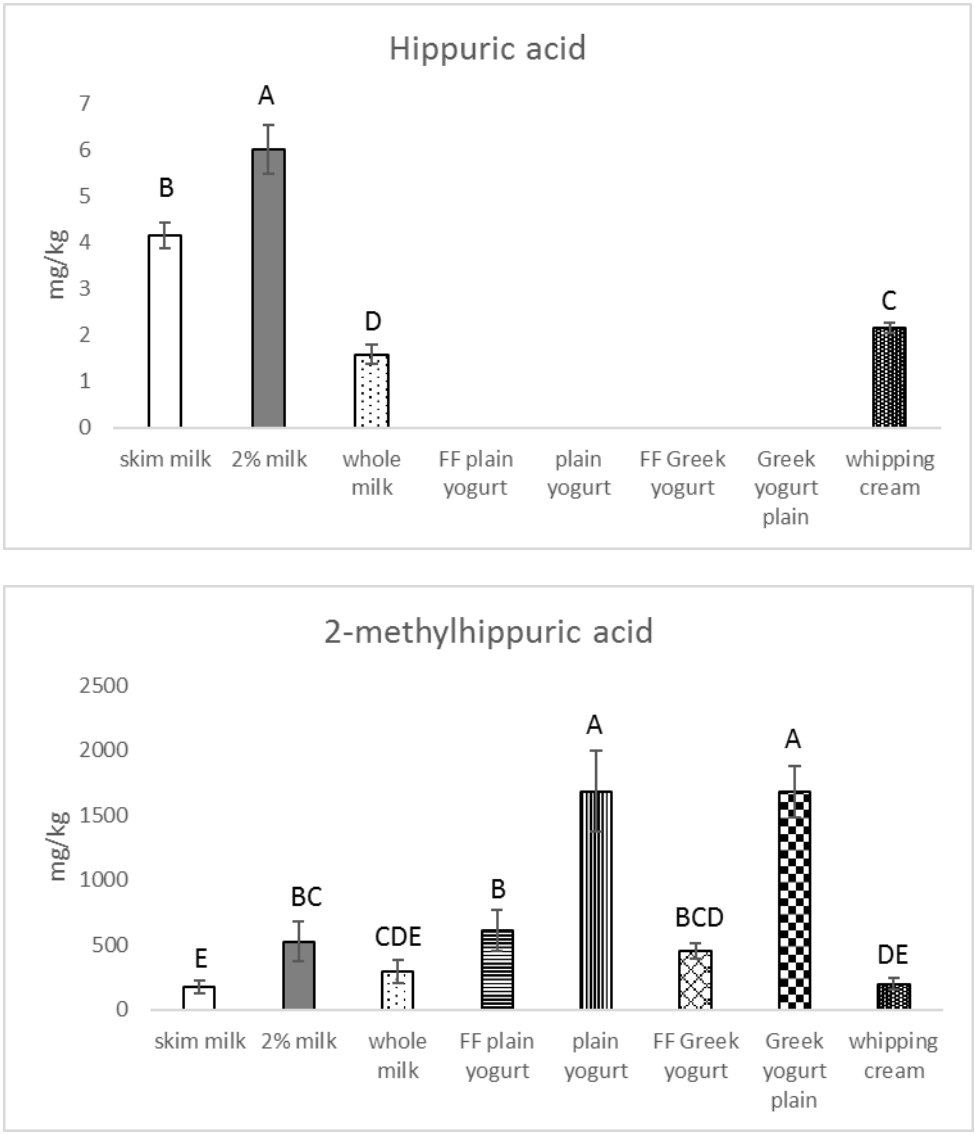

Figure 24, Quantitative results for hippuric acids in skim milk, 2% milk, whole milk, fat free plain yogurt, plain yogurt, fat free Greek yogurt, Greek yogurt, and heavy whipping cream. Top: Hippuric acid, Bottom: 2-methylhippuric acid

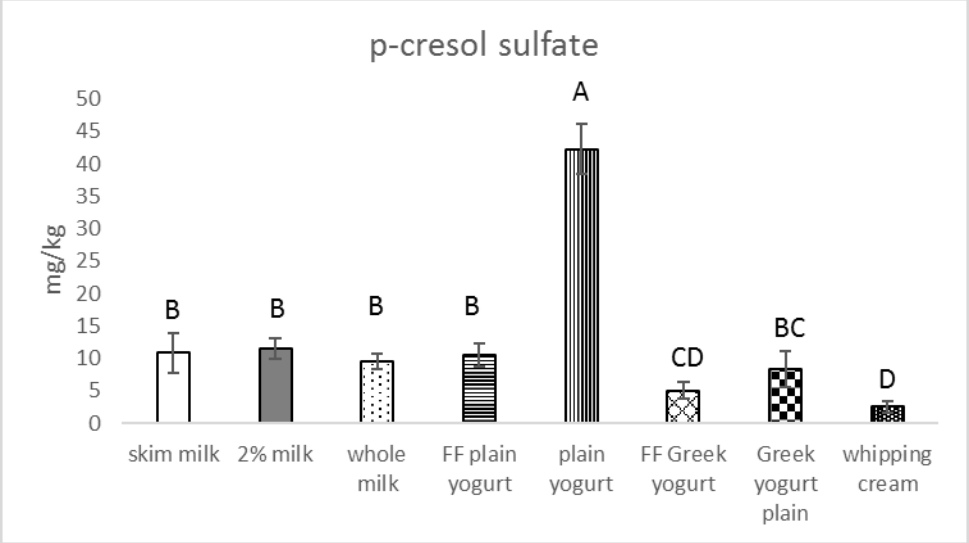

Figure 25, Quantitative results for p-cresol sulfate skim milk, 2% milk, whole milk, fat free plain yogurt, plain yogurt, fat free Greek yogurt, Greek yogurt, and heavy whipping cream

Chapter 5

Suggested Future Work

Much research has been conducted in understanding flavor quality in fluid milk, as well as an ubiquitous number of other dairy products with milk as a primary ingredient. Dairy research can include identification and inhibition of off-flavors, as well as the understanding and instigation of positive aspects of dairy flavor in enhancing product quality. The projects presented in this thesis incorporate these two different aspects, with the aim of providing greater insight for flavor improvement of dairy products and the development of potential industrial solutions. Several key areas are suggested for future work.

In the first study, off-flavor milk samples were screened for potentially containing the off-flavor contamination, "spontaneous oxidized flavor." Key volatiles in the off-flavored milk were identified and verified according to sensory protocols. However, these volatiles did not appear to be directly correlated with lipid oxidation products, in contrast to what much of prior literature has asserted are the causative factors of SOF. No research has been conducted in identifying specific volatile compounds, so more screening of 'SOF' flagged milks would be necessary to verify whether the terpenoid compounds in the study were truly representative of the SOF phenomenon. To this end, air, water, silage, or feed samples could be selected and analyzed for off-flavor formation when samples characteristic to SOF have been identified at the farm level. Additionally,

microbial assays could confirm whether certain microbial species are directly responsible for the terpenoid volatiles identified. Overall, these findings are also indicative of a greater need for product-specific volatile analyses to truly understand the causes of off-flavor in dairy. Attempts to understand SOF solely through off-flavor instigation have largely produced conflicting reports, and volatile analysis can provide needed context for responsible compounds and potential contaminations in dairy. This would allow the dairy industry to address specific concerns on a case-by-case basis, providing faster results and direct solutions to address milk quality.

On the other hand, in understanding dairy flavor quality, the creaminess study identified five small molecular weight contributors related to textural perception in dairy products. Optimization of these compounds based on animal supplementation, feeding regimens, product processing conditions, etc. could provide greater understanding in these specific compounds' formation pathways from various environmental and processing factors. Advances in analytical and statistical technology could allow greater profiling techniques to study both volatile and nonvolatile markers of interest across dairy products. Indeed, it would be valuable to put these particular compounds of interest into context with the overall picture of creaminess research that has come before: volatile aromas, bulk viscosities, lipid globule size and concentration, and textures. To that end, other optimization strategies could include techniques like response surface methodologies to evaluate volatiles, tastes, and textures at work in the products to produce the greatest possible sensory results for a variety of consumer products.

The same nonvolatile extraction protocols could be applied to other cream dairy products such as airy whipped creams, ice creams, etc. to assess these compounds' presence and contributions across more diverse dairy platforms, as well as whether other novel compounds may contribute as well. Other fractions found during fractionation experiments that contained confirmed, albeit weaker texture sensations as well. It is possible that the combination of other small compounds in solution could have different, potentially combinatory textures when studied in combination with the dairy matrix in its entirety.

More work could also be done observing effects in other non-dairy 'creamy' products. Similarities, differences, or parallels could be observed in combination to bring a greater understanding to other food systems in which texture plays an important role for sensory hedonics.

There are opportunities for more project-specific work as well. The sensory methodologies used in this study to describe texture incorporated descriptive analysis and incorporation of references to reflect panelists' experiences with fractions of interest. More work could be conducted in this area, particularly in refining textural nuances or building textural libraries to provide better methods for ensuring that panelists are properly trained in describing food texture. Additionally, further investigation is needed to observe what effects small molecular weight compounds may have when interacting with the tongue. This could provide context for how these compounds relate to previously conducted studies regarding astringency models, taste bud interactions, and physical sensations as they relate to creaminess and overall flavor quality.

These novel approaches will provide much-needed insight to how the fundamental behavior of small compounds in the oral cavity may contribute to texture and its context in overall creaminess perception. This opens up possibilities for greater understanding of natural ingredients at work in a food, as well as employing them in improved flavor profiles. This will provide solutions to the food industry while meeting consumers' demands for more nutritive, desirable attributes in the foods they enjoy.

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