ABSTRACT

RIZZO, PETER VINCENT. Sensory and Instrumental Analysis of Flavors That Drive Liking in Dairy Products. (Under the direction of Dr. MaryAnne Drake).

Flavor is often cited as the most important attribute of food products to consumers. The flavor of food products can be studied by many methods including volatile flavor analysis, trained panel tasting, and consumer evaluation. These methods work together to provide food manufactures with important perspectives when developing new food products. Volatile flavor analysis, in conjunction with trained panel tasting, provides insight into important chemical flavor markers in foods, as well as contributes to the understanding of sensory differences between food products. In the first study, the flavor chemistry of Cheddar cheeses smoked with hickory, cherry, or apple woods was investigated. The objective of this work was to characterize the flavor differences among Cheddar cheeses smoked with these woods, and to identify aroma-active compounds that contribute to differences in sensory perception among cheeses. The flavor chemistry of the wood smokes was first examined using solid-phase microextraction (SPME) gas chromatography-olfactometry (GCO) and gas chromatography-mass spectrometry (GCMS). Compounds belonging to categories including phenolics, carbonyls, furans, and others were identified. Subsequently, commercial Cheddar cheeses smoked with hickory, cherry, or apple woods were obtained in duplicate lots, along with unsmoked control cheeses (n=14). These cheeses were evaluated by a trained sensory panel followed by volatile compound analysis.

Hickory smoked cheeses were characterized by high intensities of smoke aroma, overall smoke intensity, and meaty/smoky flavor. Cherry wood smoked cheeses contained fruity flavor, which did not appear in other cheeses. Similarly, the panel identified waxy/green flavor exclusively in apple wood smoked cheeses. Phenol, guaiacol, 4-methylguaiacol, and syringol were identified as the most important compounds contributing to characteristically “smoky”

flavors. Benzyl alcohol was identified as a major contributor to fruity flavor in cherry wood smoked cheeses. Finally, 2-methyl-2-butenal and 2-ethylfuran contributed to waxy/green flavor in apple wood smoked cheeses. The results from this study identified flavors and aroma-active compounds associated with Cheddar cheeses smoked with different wood sources and can aid the dairy industry in manufacturing smoked Cheddar cheeses with a desirable flavor profile.

In the second study, consumer perceptions and preferences for lactose-free dairy milk (LFM) were characterized with the objective of identifying consumer desires for LFM that lead to a successful product. Three 1.5 hour focus groups (n=25), an online survey (n=331), trained panel descriptive analysis of commercial LFM, and a consumer acceptance test of commercial LFM (n=160) were conducted with LFM consumers to achieve this objective. From focus groups, the most desirable attribute of LFM was a low price. Furthermore, the online survey identified that the ideal LFM was ultrapasteurized in a half-gallon cardboard package and had the same sweetness and texture as traditional milk. Cluster analysis of consumer acceptance results revealed three consumer segments with distinct preferences for LFM. High sweet taste was a driver of liking for the overall population and high viscosity was a driver of dislike. The results of this study provide actionable insights for new LFM product development which is important to the dairy industry for producing desirable products in this growing market segment.

Sensory and Instrumental Analysis of Flavors That Drive Liking in Dairy Products

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A thesis submitted to Graduate Faculty of
North Carolina State University
in partial fulfillment of the
requirements for the degree of
Master of Science

Food Science

Raleigh, North Carolina
2020

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BIOGRAPHY

Peter Vincent Rizzo was born on November 27th, 1995 in Raleigh, North Carolina to Thomas and Nina Rizzo. Peter grew up in Apex, North Carolina, and attended high school in Cary, North Carolina, at Panther Creek High School. Following high school, he attended college at North Carolina State University and obtained a B.S. in Food Science in 2018. For three years during his undergraduate career, Peter worked as an undergraduate lab assistant and research assistant for Dr. MaryAnne Drake in the sensory and flavor lab at NC State. He also completed two summer industry internships. The first was with Edlong Dairy Technologies (Chicago, IL) in 2016 where he developed their sensory flavor lexicon and learned about the basics of flavor formulation. The second was with PepsiCo (Valhalla, NY) in 2018 where he was a member of the Mountain Dew product development team and worked on a new Mountain Dew beverage. Following his undergraduate degree, Peter immediately pursued an M.S. degree in Food Science at North Carolina State University under the supervision of Dr. MaryAnne Drake. After graduation, Peter plans to move to Atlanta, GA, to work for Coca-Cola on the Analytical Services team, focusing on flavor analysis. Away from school, Peter enjoys cooking, movies, ping pong, and basketball.

AKNOWLEDGEMENTS

I have so many people in my life to thank for their continued support as I pursued my master's degree. To my parents, thank you for teaching me the importance of perseverance and hard work. Mom, thank you for taking a job as a contract taster in our lab five years ago. Back then, I never realized what a blessing it would be to see you twice a week and feel your constant support. Dad, thank you for talking me through my chemistry and research topics throughout my undergraduate and graduate careers. More importantly, thank you for showing me what it truly means to be a successful man in all aspects of life. To my older brother, Victor, thank you for giving me a place to live for my last year of school, but also for your friendship and guidance throughout all of my six years at NC State. I am so glad I got to spend my last year in North Carolina with you. I would also like to thank my closest friends, Charles and Faith Scott, for their unending support and love over the years. To their beautiful daughter and my goddaughter, Ivy Scott, thank you for bringing me joy every time I saw you.

I want to thank my committee members, Dr. Drake, Dr. Hanson, and Dr. Stevenson for their guidance throughout my degree. Dr. Drake, working for you for the past five years has influenced my life in a tremendous way. I have learned so much from you, and I can't thank you enough for pushing me to go above and beyond what was required. It is from your mentorship that I am the professional I am today. Finally, I would like to thank the members of the entire Drake lab, both past and present over the last five years. Whether I helped you with your research as an undergraduate lab assistant, or you helped me with mine when I became a graduate student, I truly appreciate everything I have learned from each of you.

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INTRODUCTION

Smoked cheese is a specialty category of cheese that is currently growing in the United States. Although smoked cheese is gaining popularity today, smoked foods have been a part of the human diet for thousands of years due to the preservative properties of smoke and the characteristic flavor it imparts onto food products. It is not clear exactly when smoked cheese was first created, but it is manufactured using two main methods today. Cheese can be naturally cold smoked by smoldering wood or other plant material, or liquid smoke flavorings can be applied to the cheese or cheese milk to impart the smoky flavor. As smoked cheese grows in popularity, it will become important to the dairy industry to fully understand the flavor associated with smoked cheese from a chemical perspective. In order to understand the flavor chemistry of smoked cheese, one must investigate the factors that can affect the flavor chemistry of cheese itself, as well as those that can affect the flavor of wood smoke that is eventually imparted onto the cheese. This literature review will explain the history and process of manufacturing smoked Cheddar cheese. Additionally, the factors that can affect the flavor chemistry of both Cheddar cheese and wood smoke will be reviewed, as well as various methodologies that are employed when studying the flavor of smoked Cheddar cheese.

SMOKED FOODS – HISTORY AND USES

The discovery of fire is considered one of the most important and influential discoveries in the history of mankind. Shortly after fire was used by humans to cook food, the smoke created from the fire was also put to use (Rozum, 2009). It is believed that food, likely meat products, was hung over fires in ancient times to protect them from being eaten by other roaming animals. This is how the beneficial effects of smoking food would have first been discovered (Ledesma et al., 2017). Smoking foods was originally used as a method of food preservation. Wood smoke is

rich in organic acids and phenolics. These compounds, as they adsorb onto the surface of food, act as antimicrobials and antifungals to extend the shelf life of the food (Cadwallader, 2007). While smoking foods was a very useful technique for food preservation, it also greatly enhanced the sensory quality of the food product, which is its major function in modern times considering many other food preservation methods are well understood and available. Smoking food obviously changes the flavor of the final food product, but can also have a significant impact on the color and texture of the product (Ledesma et al., 2017). It is of no surprise that cultures all over the world employ this technique to enhance foods. Some examples include hibachi in Japan, kebobs in the Middle East, and barbeque in the United States (Rozum, 2009).

Smoke and Food Preservation

The preservative effect of smoke was likely the major reason ancient cultures employed the technique, and one of the reasons it is still used today (Ledesma et al., 2017). One of the first ways that smoking foods contributes to preservation is the drying effect that takes place during the smoking process. Drying food lowers its water activity, creating a hurdle for bacterial growth or inhibiting it all together (Ledesma et al., 2017). Drying mostly takes place in smoked meats and seafood. In addition to drying, smoke is comprised of several different organic compounds that contribute substantially to its antimicrobial capability. Smoke has been shown to suppress the growth of bacteria such as *Listeria monocytogenes*, *Salmonella spp*., *Escherichia coli*, and *Staphylococcus spp*., all of which are known food pathogens (Lingbeck et al., 2014). The phenolic fraction of wood smoke is believed to contribute the most to food preservation (Rozum and Maurer, 1997). Phenolic compounds disturb the cell membranes of bacterial cells, creating holes and causing the intracellular fluids to leak, destroying the cell (Rozum and Maurer, 1997). Carbonyls are another major component of wood smoke and have been shown to possess an

antimicrobial effect. Carbonyls are able to penetrate bacterial cell walls and inactivate essential enzymes in the cell cytoplasm and membrane (Painter, 1998). Carbonyls condense with amino groups in important polypeptides within enzymes, which inhibit them from bonding with their substrate. They can also condense on the substrate itself, again hindering bonding with an enzyme. Carbonyls also inhibit growth outside of the cell by interfering with nutrient uptake (Painter, 1998). The mechanism entails the inactivation of extracellular enzymes excreted by bacteria, through similar means of inactivating enzymes within the cell. It is also possible that wood smoke constituents act as antioxidants to prevent against certain chemical spoilage in addition to microbial spoilage. This process includes reducing rancidity in the fat of meat and seafood (Lingbeck et al., 2014). With these various antimicrobial and anti-spoilage activities, it is no surprise that smoking food was a very important preservative technique especially before refrigeration was so easily accessible.

Sensory Impact of Smoking Food

Anyone who has tasted or seen a smoked food product next to an unsmoked version of the same product understands the overall sensory impact of smoking foods. The most obvious sensory characteristic of food that is affected by smoke is flavor. The flavor of smoke is very complex and will be overviewed in detail later in this review; however, smoke is comprised of a complex mixture of flavor-active organic compounds that are generated by the pyrolysis of wood polymers (Maga, 1987). There are several factors that can affect the sensory perception of wood smoke including the type of wood used, smoldering temperature, air flow during pyrolysis, and wood moisture content. Some of the most important classes of flavor compounds found in wood smoke are phenolics and carbonyls (Rozum, 2009). When food is exposed to smoke, these flavor compounds adsorb onto the surface of the food and even penetrate inside the food product. These

flavor compounds can contribute a wide array of flavors onto a final food product. Jaffe et al. (2017) evaluated many smoked food products in the effort to generate a smoked foods lexicon that could potentially be applied to describe the smoky flavor of any smoked food product. The food evaluated by the trained panel included sauces, meats, fish, cheeses, vegetables, and smoke flavorings themselves. The sensory descriptors that were generated by the trained panel included: overall smoky, ashy, woody, musty/dusty, musty/earthy, burnt, acrid, pungent, petroleum-like, creosote/tar, cedar, and metallic (Jaffe et al., 2017). Clearly through these descriptors, one can see the range of flavors that smoke can impart onto food. The final flavor of the smoked food product is heavily affected by the time and temperature conditions during the smoking process, as well as the smoking technique used (Ledesma et al., 2017). A study was conducted to determine the effect that smoldering temperature of the wood chips had on the overall sensory perception of smoked sausages (Pöhlmann et al., 2013). It was determined that smoldering below 500ºC was unacceptable, producing a smoke flavor that was too intense in the final sausage. Smoldering temperatures above 750ºC were also unacceptable but for the opposite reason, producing a smoke flavor that was insufficient in intensity. These results suggest that ideal smoked sausages are produced from wood that is smoldered between 500ºC and 750ºC. There was another study conducted on smoked Cheddar cheese, and the effects of smoking the cheese at different points during its ripening period (Rehman et al., 2003). All smoked Cheddar cheeses exhibited a “smoky” flavor, but those that were smoked later in their ripening periods (after 6 and 9 months of ripening) had a distinct “skunky” flavor.

While the effect of smoking on the flavor of food is very obvious, smoking drastically affects the color of food products as well. Many studies have instrumentally investigated the effect of wood smoke on the color of foods. In general, smoking a food product tends to decrease

the L* value of the color reading (Ledesma et al., 2017). L* is the "lightness" value. A decrease in the L* value indicates that the food has become darker as a result of smoking. This would be logical to anyone who has seen a smoked food product next to an unsmoked variation of the same product, as the smoked food tends to appear darker and browner. Rhia and Wendorff (1993) conducted a study evaluating the color of smoked Cheddar and Swiss cheeses by instrumental analysis, descriptive sensory analysis, and consumer acceptance. The lightness to darkness readings of the instrumental analysis and the descriptive sensory analysis were very highly correlated. Based on the amount of time the cheeses were exposed to the smoke, the color of the cheeses varied significantly, ranging from a light golden brown to a deep mahogany or walnut brown. It was concluded that consumers liked being able to distinguish a smoked cheese from an unsmoked cheese based on its outward appearance, but they preferred the light golden brown cheeses over the much darker brown cheeses (Riha and Wendorff, 1993). Smoke also heavily affects the appearance of meat. Like smoked cheese, smoking meat tends to lower the L* value of the meat and makes the appearance darker (Ledesma et al., 2017). However, smoke also has a curing effect when it is applied to meat and has been shown to give meat a more pink hue (Poligné et al., 2002). Meat that has been exposed to nitrites will develop a pink color that is indicative of a cured meat. Smoking meat has a similar effect, which was demonstrated in a study on smoked pork. After being exposed to smoke for 9 hours, the a* values of the pork increased. The a* value in a color reading indicates "redness." Therefore as the a* value increases, the redness of the meat increases (Poligné et al., 2002).

Finally, when smoke is applied to food products, it can also affect texture. As stated previously, the smoking process often dries the food product, especially meat and fish. This water loss has a significant impact on the final product, usually making it tougher in certain

respects (Ledesma et al., 2017). However, the specific smoking process can affect texture in different ways. While hot smoking generally dries the product and creates a tougher texture, cold smoking does not dry the food, and thus creates a softer texture due to other textural effects like denaturation of tissues. It is possible cold smoking has a softening effect on cheese as well. In a study investigating the texture of smoked and unsmoked cheeses, with the exception of one outlier, all smoked cheeses evaluated by a trained panel and an instrumental texture analyzer were found to be "softer" and "creamier" than many other unsmoked cheeses (Adhikari et al., 2003).

Types of Smoked Foods

Smoked foods have been a part of the human diet for thousands of years. The list of different smoked food products is quite extensive (Ledesma et al., 2017). Meats are some of the most popular smoked food products. Countries all over the world smoke meat, most of which can be categorized into sausages and hams. Smoked meats are very common in American and European cultures, such as bacon, country ham, and prosciutto. However, the list of smoked meat products found in the review by Ledesma et al. (2017) displays smoked meats from countries all over the world including Bulgaria, Brazil, Estonia, Bosnia, and many more. Considering the popularity of smoked meat products, it is no surprise that there have been many studies centered around various aspects of smoked meats. In 1998, a study was conducted on the texture properties of smoked poultry sausage. The authors found that the amount of water and wheat protein added to the sausage heavily affected texture attributes such as hardness, springiness, and cohesiveness of mass (Li et al., 1998). Another study investigated the smoking treatment of dry-cured Iberian salchichon sausages on consumer acceptance. They found that as smoking time, and thus smoke flavor intensity, increased, the liking scores of younger consumers

tended to increase, while the liking scores of older consumers tended to decrease (Carrapiso et al., 2015).

Like meat, smoked fish and seafood products are also very popular throughout the world and have many variations from hundreds of cultures. Much of the smoked fish in the world is produced and consumed by African and Asian countries. Eighty percent of the fish processed in Africa is smoked or heat preserved (Ledesma et al., 2017). Smoked fish is traditionally produced by direct smoking techniques, as well as application of liquid smokes (Rozum, 2009). Salmon and herring are the two most commonly smoked fish. Smoked salmon is very common in the United States and Europe. A study from 2004 investigated the sensory, chemical, physical, and microbiological properties of smoked salmon available in the European market, which were sourced from several countries including Belgium, Denmark, France, Germany, Italy, and the United Kingdom (Cardinal et al., 2004). The study concluded that smoked salmon in the European market could be classified into eleven different groups based on their sensory properties. The most distinguishing factors of the smoked salmon samples were color, smoke flavor intensity, characteristic of smoke flavor, amine flavor, and saltiness (Cardinal et al., 2004). Another study characterized the flavor chemistry of fresh salmon versus smoked salmon. Forty-nine aroma-active volatile compounds were detected in fresh salmon, while 74 were identified in smoked salmon (Varlet et al., 2006). Heptanal and (E,Z)-2,6-nonadienal were the most important compounds identified in fresh salmon, and were responsible for its typical fishy odor. Phenolic compounds like cresol and guaiacol, as well as furan derivatives and carbonyls, were some of the most important compounds found in smoked salmon and contributed to its characteristic flavor (Varlet et al., 2006). A study was conducted on smoked herring, which investigated the effects of different smoking methods (temperature variations of traditional smoking and voltage variations

of electrostatic smoking) on the sensory perception of the fish. Additionally, the authors tried to generate linear models to illustrate the relationship between sensory perception and the concentrations of 10 phenolic compounds. The study determined that the smoked herring treatments could be clearly distinguished by a trained panel based on the smoking method, but the linear relationships between sensory perception and the 10 phenolic compounds were inconclusive (Cardinal et al., 2006).

One of the lesser known categories of smoked foods is smoked beverages. The most popular and well-known smoked beverages are whisky, tea, and beer, whisky being the most popular of the three (Ledesma et al., 2017). Unlike traditionally smoked foods, smoking beverages is exclusively for flavor enhancement purposes, not preservation. Smoked whisky usually excludes the "e" at the end of "whiskey" because it is most commonly Scotch whisky that is smoked or "peated" as it is often referred to (Bringhurst and Brosnan, 2014). The burning of peat produces dried malted barley to be used as a substrate in the fermentation process, but also produces many flavor characteristics. Most notably, it produces Scotch whisky's characteristic phenolic, smoky, and rubbery notes. Other than water, tea is the most widely consumed beverage in the world (Ledesma et al., 2017). One of the most commonly smoked teas is yerba mate tea, which is indigenous to South America. When yerba mate tea is manufactured, the leaves are slowly dried by directly applying wood smoke. Other than to help dry out the tea leaves, this process provides the final product with a slightly smoky flavor that is rather unique to this particular type of tea (Heck and De Mejia, 2007).

Spices and flavorings are also typically smoked food products throughout the world. These spices are most commonly smoked salt, smoked garlic, chipotle (smoked jalapeño), and smoked paprika. Smoked paprika is the most popular smoked spice and is found often in

American and European cuisines (Ledesma et al., 2017). Paprika is a spice made from grinding the dried red pepper fruits of a plant originating in America. These fruits are dried by applying natural smoke for a period of several days (Gallardo-Guerrero et al., 2010). It is during this time that many flavor compounds are transferred from the smoke to the dried fruits that will eventually become the paprika spice (Mateo et al., 1997). While paprika is often used as a flavoring agent in many food products, it is also used as a coloring agent due to its attractive and vibrant red color (Ledesma et al., 2017). A study was conducted in 2010 investigating the effects of the time and temperature during the smoking process on the carotenoid content of paprika, the components responsible for paprika color. They determined that higher exposures of smoke and higher smoking temperatures decreased the amount of carotenoids in paprika, while a milder time and temperature combination left most of the carotenoids intact (Gallardo-Guerrero et al., 2010). Another study analyzed the volatile flavor compounds found in smoked Spanish paprika. Some of the most abundant compounds were acetic acid, 3-methylbutanal, ethyl acetate, and dimethoxyphenol, most of which were believed to have originated from the smoke. These compounds, along with other phenols, methyl-branched aldehydes, acids, and carbonyls contributed most to the overall flavor of the smoked paprika (Mateo et al., 1997).

Smoked cheese is the final product category of popular smoked foods and will also be the focus of the remainder of this review. Like many of the other food products mentioned in this list, smoking cheese likely began as a preservation method before refrigeration was so easily accessible (Ledesma et al., 2017). However, today, cheese is mostly smoked to create a unique and artisan flavor (Rehman et al., 2003). There have been several studies that characterized the effects of the smoking process on cheese. Some of these studies have already been mentioned. For instance, the investigation of Adhikari et al. (2003) on the textural properties of smoked

cheeses, and the study by Rehman et al. (2003) that examined the effects of smoke application on the ripening process of Cheddar cheese. However, there have also been several more studies conducted. A study conducted in 2011 investigated changes that occurred throughout processing on the volatile, sensory, and microbial aspects of Oscypek cheese, a cheese made from sheep's milk traditionally manufactured in Poland (Majcher et al., 2011). Fifty-four volatile compounds were identified in the cheese, which could be separated into nine different categories. It was determined that most of the free fatty acids, esters, ketones, alcohols, aldehydes, and sulfur compounds originated from biochemical reactions within the cheese, while most of the furans and phenols were introduced by the smoking process. Also, it was the smoking process that was the main influence on the overall flavor of the cheese (Majcher et al., 2011). Another study investigated the aroma-active volatile compounds present in smoked goat cheese but took it one step further, analyzing the exterior portions as well as the interior portions of the cheese. They found that the exterior of the cheese had much higher concentrations of smoke-derived flavor compounds than the interior of the cheese, and only compounds that were in relatively high concentrations on the exterior could also be found in the interior. The classes of flavor compounds that were determined to be the most important in the overall cheese flavor were acids and phenolics (Guillén et al., 2004).

SMOKED CHEDDAR CHEESE PROCESSING

Smoked Cheddar cheese is traditionally produced by two different methods: direct application of natural wood smoke through a cold smoking process, or by the addition of liquid smoke flavorings to the cheese milk before manufacturing (Mcilveen and Vallely, 1996). For the cold smoking process, typically, an entire finished block of cheese is smoked, which produces a flavor gradient with high intensities of smoke flavor on the outside of the cheese and lower

intensities on the inside (Guillén et al., 2004; Sikorski and Sinkiewicz, 2014). The lack of a smoke flavor gradient is the advantage of liquid smoke in smoked Cheddar cheese production. Since the liquid smoke is added to the cheese milk, a consistent smoke flavor throughout the cheese block is typically seen (Mcilveen and Vallely, 1996). Recently, a new published method of cheese smoking has been documented. Here, the fresh Cheddar cheese curds are smoked in a vacuum chamber before being formed into a finished block of cheese. This produces a consistent smoke flavor intensity throughout the cheese, with the benefits of the natural cold smoke process (McLeod, 2017).

Natural Cold Wood Smoking

Although there are warm and hot smoke methods, cold smoking is used for smoked cheese production to protect against melting during processing. Cold smoking is usually conducted between 15ºC and 25ºC and typically does not exceed 30ºC (Rozum, 2009). The same browning reactions occur in cold smoking as hot smoking, but take more time to develop (Rozum, 2009). Most industrial cold smoking uses smoke generators, which burn sawdust or wood chips on a heated plate or grate at a controlled temperature. The air that is needed for combustion and smoldering is added from below the heating instrument (Sikorski and KoKołakowski, 2010). The volume of smoke produced, its temperature, and humidity, can be controlled electronically in modern smoke generators, usually by sending the smoke through a water spray. The smoke is also filtered to remove toxins and tars. The burning wood and the cheese that the smoke is being applied to are usually kept in two separate chambers with piping to direct the smoke from one chamber to the other. The flow of smoke from one chamber to the other is forced mechanically at a controlled rate. Separating the smoke generator from the cheese allows for better control over environmental conditions such as temperature, humidity, and air

circulation (Mcilveen and Vallely, 1996). Each of these factors can affect the final product. All cheeses must be arranged in the smoking chamber to receive uniform smoke exposure, temperature, and air circulation (Anonymous, 2001). Since temperature is usually kept low and constant during smoked cheese production, humidity and air circulation are generally more of a concern. Consistent air circulation throughout the chamber creates a consistent amount of smoke exposure for all of the cheese within the chamber, leading to a uniform smoke flavor intensity. If the chamber is very humid, water can condense on the cheese and allow water soluble smoke aroma-compounds to adhere to the cheese (Mcilveen and Vallely, 1996). Twenty times more phenolic compounds tend to absorb onto wet surfaces than dry surfaces (Sikorski and KoKołakowski, 2010). This can drastically affect the final sensory perception of the smoked cheese, especially considering the importance of phenolic compounds to smoke flavor (Maga, 1992). Along with humidity, smoke flavor compounds also absorb onto the surfaces of the cheese based on the electrostatic nature of the compound and the protein matrix of the cheese (Sikorski and KoKołakowski, 2010). As smoke flavor compounds stick to the surface of the cheese, they slowly begin to diffuse inside the cheese block based on the concentration gradient that is created on the outside of the cheese versus the inside. The rate at which the compounds diffuse inward is highly temperature dependent. The electrostatic makeup of the cheese protein structure, as well as the chemistry of the compound itself, also heavily affect the diffusion rate (Sikorski and KoKołakowski, 2010).

Liquid Smoke Application

Liquid smoke is defined has a colloidal suspension of aqueous condensate that is produced from smoldering wood chips or sawdust (Cadwallader, 2007). It is generated from producing wood smoke in a very similar method as described above. The sawdust or wood chips

are smoldered under controlled conditions to produce gaseous smoke, which is quickly chilled in a condenser to produce liquified smoke. The liquid smoke then moves through refining vats and filters to remove impurities like tars and carcinogens. Finally, many liquid smokes are aged to mellow their pungent initial flavor (Lingbeck et al., 2014). Due to the filtration and aging steps, liquid smoke is believed to be healthier than traditional smoke (Ledesma et al., 2017). This is due to the removal of polycyclic aromatic hydrocarbons, which are carcinogens, during liquid smoke filtration. During traditional wood smoking, these carcinogens are applied to the final food product since there is nothing to filter out these harmful compounds. The European Union has a safety limit of 5ppb of the most common polycyclic aromatic hydrocarbons in a traditionally smoked food product. For a product treated with liquid smoke, the limit is just 0.03ppb (Rozum, 2009). Liquid smoke is not only used to make smoked cheese, it is also used in a wide array of food products including meats, fish, sauces, seasonings, baked beans, and many more (Cadwallader, 2007). Liquid smoke has many advantages over traditional smoking, most notably consistent smoke flavor and application on the product. A consistent application on the food product is one of the major reasons that liquid smoke is used in so many different food products. These different application methods include direct mixing, atomization, drenching/showering, internal addition, or topical application/dipping (Rozum, 2009). During smoked Cheddar cheese production, the two methods most commonly used are direct mixing and dipping (Mcilveen and Vallely, 1996). In some cases, liquid smoke is added directly to the cheese milk prior to production. This eliminates the flavor gradient that is typically found in traditionally smoked cheeses, creating a consistent smoke flavor throughout the entire finished cheese block. However, cheese blocks can also be dipped in liquid smoke after production as another method of manufacturing smoked Cheddar cheese. This method mimics traditional

smoking by creating a flavor gradient with a high smoke flavor intensity on the outside of the cheese and a milder flavor on the inside. The reason a smoked cheese manufacturer would use the dipping method as opposed to the traditional smoking method is because the liquid smoking method is more environmentally friendly, is much cleaner, and produces a healthier product for the consumer (Cadwallader, 2007).

FACTORS CONTRIBUTING TO THE AROMA-ACTIVE COMPOUND PROFILE OF CHEDDAR CHEESE

Cheddar cheese flavor is very complex and has been widely studied in the past. There are many factors that influence the aroma-active compound profile of Cheddar cheese, and thus the overall sensory perception. Cheese flavor can usually be traced back to the degradation of milk macronutrients like lactose, milk fat, and casein proteins (Singh et al., 2003). The biochemical actions of glycolysis, lipolysis, and proteolysis are responsible for basic textural and flavor components of Cheddar cheese. While the starter bacteria contribute significantly to these processes in Cheddar cheese production, most of the flavor development in Cheddar cheese occurs during ripening and can be attributed to the biochemical activity of the secondary bacteria and their enzymes. Secondary, or non-starter bacteria, are cultures added to the cheese (or present in the cheese milk) for reasons other than acidification such as flavor and texture development (Singh et al., 2003). All different cheese varieties have a different makeup of casein proteins (peptides and amino acids), milk fats (fatty acids, triglycerides), and carbohydrates (lactose). The final flavor of the cheese depends heavily on the macronutrient content of the cheese during ripening.

Contribution of Glycolysis and Carbohydrate Metabolism to Cheddar Cheese Flavor

Initially, lactose is metabolized by the starter bacterial culture to produce lactic acid, which lowers the pH of the milk to aid in protein coagulation and the formation of cheese curds. The metabolism of lactose through glycolysis eventually leads to many important flavor compounds in Cheddar cheese, namely diacetyl and acetic acid (Murtaza et al., 2014). In Cheddar cheese, the majority of the lactic acid formation occurs in the vat, prior to salting and molding of the curd (Singh et al., 2003). The pH of the curd continues to reduce in the vat until it reaches about 5.0 for Cheddar type cheeses. After the curd has reached the desired pH, it usually contains 0.8%-1.5% lactose before molding (Huffman and Kristoffersen, 1984). The curds are then salted to help inhibit additional pH depression caused by continued fermentation of lactose by the starter culture. The non-starter bacteria (usually pediococci and mesophilic lactobacilli) are more salt tolerant than the starter bacteria, and will therefore take over the fermentation of the remaining lactose (Fox et al., 1990; Singh et al., 2003). Here, more lactic acid and lactate are produced by the non-starter bacteria. Lactate can be further metabolized into carbon dioxide and acetate. Acetate, especially when in its conjugate acid form (acetic acid), plays a very important role in cheese flavor by providing a sour aromatic flavor (Murtaza et al., 2014).

Citrate is another carbohydrate derived molecule that is present in low levels in Cheddar cheese (0.2%-0.5% w/w), but can be metabolized by non-starter bacteria to produce two very important flavor components: diacetyl and acetate (Singh et al., 2003). Diacetyl gives Cheddar cheese a characteristic buttery flavor, and as described above, acetate provides a sour aromatic flavor. Citrate in Cheddar cheese usually reduces to zero after about 6 months of ripening, at which time it has been fully metabolized (Fox et al., 1990). It is for this reason that buttery flavor from diacetyl is usually associated with young Cheddar cheeses. Other flavor compounds created

as byproducts of citrate metabolism are acetoin and 2,3-butandiol (Cogan, 1995). These compounds are very similar in structure to diacetyl (2,3-butanedione). Therefore, it is no surprise that they also provide a similar flavor. Acetoin is buttery, but also provides a slight sour aromatic flavor. 2,3-butandiol also creates a buttery flavor but is also slightly fruity and alcohol-like.

Contribution of Lipolysis and Fatty Acid Metabolism to Cheddar Cheese Flavor

Cheddar cheese is a high-fat food, containing 30.5% fat or greater (Singh et al., 2003). While of course the fat in Cheddar cheese plays a big role in the overall texture of the cheese, it also is very important to the flavor. Cheddar cheese that is made with fat free milk does not develop a full aroma, even after 12 months of ripening, indicating its importance to cheese flavor (Carunchia Whetstine et al., 2006). This is likely because flavor components are dissolved and held inside the lipid matrix of the cheese (Singh et al., 2003). Triglycerides make up 98% of the fat in cheese. The breakdown (lipolysis) of triglycerides leads to free fatty acids, which are very important to the aroma of Cheddar cheese. Shorter chain fatty acids, between four and six carbons in length, have very sharp and rancid aromas. Medium chain fatty acids, between seven and nine carbons, have a sharp and goaty flavor. Finally, longer chain fatty acids, between ten and twelve carbons and beyond, have soapy and coconut-like aromas (Murtaza et al., 2014). Only moderate amounts of lipolysis are desirable in Cheddar type cheeses. Fatty acids in high concentrations would be considered rancid in Cheddar cheese, which is a flavor defect, but is desirable to other cheeses like Feta (Singh et al., 2003). Lipases, or enzymes that break down lipids, are produced by both starter and non-starter bacterial cultures in Cheddar cheese production. However, milk also has an indigenous lipase called lipoprotein lipase, which is believed to contribute to lipolysis during cheese production even more than lipases produced by starter bacteria (Reiter and Sharpe, 1971). Therefore, while the bacterial culture certainly

contributes to free fatty acid flavor production in Cheddar cheese, native milk lipases do as well (Olson, 1990).

Fatty acids generated from lipolytic enzymes can be further metabolized by non-starter bacteria to produce other important flavor compounds in Cheddar cheese. One class of compounds that results from further fermentation of free fatty acids is esters. Esters can result directly from lipolysis but are also developed by secondary chemical reactions (Harper et al., 1980). Esters are usually associated with fruity flavors, responsible for aromas from banana to pineapple. These compounds play a background role in the flavor of Cheddar cheese, but are very important to the overall flavor of other cheeses like Parmesan, which has a distinct pineapple aroma (Singh et al., 2003). Similar to esters are lactones, however, lactones are not generated from metabolism of free fatty acids. Lactones are cyclical esters, formed through a non-enzymatic reaction involving the intramolecular esterification of a hydroxy fatty acid within a triglyceride molecule. A study was conducted on lactone formation in Gouda cheese but considering the similar manufacturing process of Gouda and Cheddar cheeses, the mechanism of formation is likely the same in Cheddar cheese (Alewijn et al., 2007). After adding acid and base to the cheese fat and seeing no change in the concentration of lactones, it was determined that the hydrolysis of triglycerides into fatty acids is not involved in lactone formation. Instead, the mechanism proposed involved the trans-esterification of a hydroxy fatty acid within a triglyceride molecule. When the molecule is in the correct confirmation, the reaction begins with a nucleophilic attack of the hydroxy group of the fatty acid on the carbonyl carbon, forming a free lactone and a diglyceride (Alewijn et al., 2007). γ- and δ-lactones are aroma-active and are believed to play an important role in the overall aroma impact of Cheddar cheese (Wong et al.,

1975). Lactones display a wide range of flavors but are usually associated with coconut and milky aromas.

Contribution of Proteolysis and Amino Acid Metabolism to Cheddar Cheese Flavor

The major proteins present in Cheddar cheese are caseins and the proteolysis of caseins takes place heavily throughout Cheddar cheese production from curd formation to ripening (Singh et al., 2003). Non-starter bacteria play a large role in the proteolysis of caseins during cheese maturation, but before Cheddar cheese is matured, there is another major proteolysis step at the beginning of manufacturing, cheese renneting (Fox, 1989). Rennet is a proteolytic enzyme that specifically cleaves the phenylalanine (105) – methionine (106) bond in the casein micelle, cleaving κ-19casein from the casein micelle. K-casein is largely hydrophilic and therefore keeps the casein micelle in the milk suspension. After the rennet cleaves κ-casein, the micelles fall out of suspension and coagulate to form curds (Fox, 1989). The cleaved κ-casein that did not get washed out after whey draining remains in the curds and, along with the casein micelles, can be further hydrolyzed by enzymatic reactions during ripening. The initial products of casein proteolysis are peptides and free amino acids, which are believed to contribute slightly to Cheddar cheese taste but are not aroma-active. In fact, high concentrations of amino acids can lead to bitterness, which is a flavor defect in Cheddar cheese (Singh et al., 2003). However, compounds that are produced from further amino acid metabolism are aroma-active and considered to be important to Cheddar cheese flavor. It is the amino acid composition of the cheese that leads to the development of certain flavor compounds. Therefore, the proteolytic enzymes present and their specificity in the peptide bonds they hydrolyze are of extreme importance to the final flavor of the cheese (Fox, 1989).

Amino acids act as precursors to a wide range of flavor compounds found in Cheddar cheese including amines, aldehydes, alcohols, and more (Law, 2001). Considering the wide range of flavors metabolized amino acids can contribute to Cheddar cheese, there have been studies to determine if amino acid fortified cheese will develop more complex flavor. However, this was not the case, indicating that the rate-limiting factor of amino acid derived flavor development is the biochemical reactions that convert amino acids to flavor compounds, and not the availability of the amino acids themselves (Yvon et al., 1998). The amino acids Valine, Leucine, Isoleucine, Phenylalanine, Tyrosine, Tryptophan, and Methionine seem to be the most important precursor amino acids to flavor compounds (Murtaza et al., 2014). 2-methylpropanal is a product of Valine degradation and is responsible for malty and nutty flavor. 2-methyl-1-propanol is another Valine degradation product and produces an alcohol and wine-like flavor. Similarly, degradation of Leucine forms 3-methylbutanal which has a nutty and malty aroma. 2-methylbutanal however, is formed from Isoleucine degradation, but has a similar toasted nutty flavor. These Strecker aldehydes contribute to the nutty flavor that is sometimes present in Cheddar cheese. Avsar et al. (2004) investigated the flavor chemistry of Cheddar cheeses containing nutty flavor and others without nutty flavor to determine the volatile compounds responsible. After conducting a series of analyses including high vacuum distillation of direct solvent extracts of Cheddar cheese, gas chromatography-olfactometry, and gas chromatography-mass spectrometry, higher concentrations of 2/3-methylbutanal and 2-methylpropanal were found in Cheddar cheeses containing nutty flavor than Cheddar cheeses without nutty flavor (Avsar et al., 2004). Model system analysis with mild Cheddar cheeses confirmed that these compounds caused nutty flavor. Methionine is a sulfur containing amino acid, and thus produces many sulfur containing flavor compounds after being metabolized by non-starter bacteria. These

compounds include dimethyl sulfide, dimethyl disulfide, and dimethyl trisulfide, all of which produce a sulfurous, cabbage, and onion-like aroma. Methanethiol has a similar sulfurous cabbage aroma. Methional is also produced from Methionine degradation and it has a distinct potato brothy aroma that can be detected in Cheddar cheese (Singh et al., 2003; Murtaza et al., 2014). Phenylalanine contains a benzene ring in its structure, and therefore produces many flavor compounds containing the same aromatic ring. Some notable compounds created from Phenylalanine metabolism are phenyl acetaldehyde, phenylethyl alcohol, and phenyl acetic acid, all of which provide a rosy and floral aroma to Cheddar cheese. A study conducted in 2005 specifically investigated these flavor components in Cheddar cheese and based on their concentrations within Cheddar cheese, determined that phenyl acetaldehyde and phenyl acetic acid were responsible for the perception of rosy and floral flavors in Cheddar cheese (Carunchia Whetstine et al., 2005). Tyrosine also contains an aromatic ring but after being metabolized, produces less pleasant compounds such as p-cresol, which smells like animal and medicinal. However, flavor compounds derived from Tryptophan are likely the most unpleasant of all. These compounds include indole and skatole, which are attributed to aromas such as fecal and mothball (Singh et al., 2003; Murtaza et al., 2014). Clearly the metabolism of free amino acids can produce a wide array of flavors that are very important to the overall sensory perception of Cheddar cheese.

FACTORS CONTRIBUTING TO THE AROMA-ACTIVE COMPOUND PROFILE OF WOOD SMOKE

Smoke created from the pyrolysis of wood is rich with a complex profile of hundreds of flavor compounds (Maga, 1987). There are many factors that determine the exact make-up of the aroma-active compound profile of wood smoke. For instance, the type of wood burned plays a

large role. Softwood vs. hardwood will produce different flavor compounds. Even different varieties of wood within hardwood and softwood can have a significant effect (Maga, 1992). Another factor is the smoldering temperature of the wood. There are three major structural constituents of wood: hemicellulose, cellulose, and lignin. Each of these polymers thermally degrades in a different temperature range (Rozum, 2009). The availability of molecular oxygen and airflow is another factor that can affect the final flavor chemistry of wood smoke. If oxygen is available or unavailable to participate is various reactions during pyrolysis, various flavor compounds can be formed (Wasserman and Fiddler, 1969). Finally, the moisture content of the wood is another significant condition that affects the aroma-active compound profile of the smoke produced. Water allows certain reactions to begin, while also preventing others if it remains present for too long (Maga, 1987). Each of these factors are extremely important and must be considered when investigating the flavor chemistry of wood smoke.

Wood Source

The fact that the wood source used for smoke generation has an effect on the overall sensory perception of the smoke is logical and is one of the major factors that influence product development of smoked foods. As stated before, there are two major classes of wood: hardwood and softwood. Although softwoods (Pine and Cedar) are used to smoke several food products, hardwoods (Hickory, Cherry, Apple, Maple, etc.) are much more commonly used for smoke generation in the food industry (Maga, 1987). This has to do with the fact that in general, hardwoods produce more consumer-accepted aromas than do softwoods, however this is not the case in all situations.

The different combination of aromatics produced by the type of wood come from the specific polymer make up of each wood source: cellulose, hemicellulose, and lignin (Maga,

1992). Cellulose is ubiquitous throughout the plant kingdom and is a very important structural wood polymer as it is usually responsible for most of the mass of wood. Cellulose is a long chain of about 9,000-15,000 glucose molecules with each glucose linked by β-(1,4) acetal bond (Rozum, 2009). Cellulose degradation begins with depolymerization by the cleavage of the glycosidic linkages between glucose molecules in the chain. These free sugars and oligosaccharides then participate in Maillard reactions, producing associated flavor compounds. The pyrolysis of cellulose produces many volatile compounds, but most importantly produces aliphatic acids and aldehydes. Acids, such as acetic, propionic, and butyric acids, provide smoke with a sour aromatic flavor and aid in the antimicrobial properties of smoke (Rozum, 2009). Aldehydes contribute heavily to the flavor of wood smoke as well. Many experts believe carbonyl compounds are some of the most important aroma-active compounds that contribute to wood smoke flavor and even consider them to be just as important as phenolics (Maga, 1987). Hemicellulose is more complex than cellulose and is made up of a series of five and six carbon sugars such as arabinose and xylose, and glucose, mannose, and galactose, respectively. These sugars are also bound by β-(1,4) acetal bonds as well, and therefore break down similarly to cellulose to participate in Maillard reactions (Rozum, 2009). The thermal degradation of hemicellulose is mainly responsible for the furan derivatives, carboxylic acids, and some alcohols present in wood smoke. Lignin is the last major wood polymer and is a phenolic-based chain. Thermal degradation of lignin results mainly in the phenolic fraction of wood smoke aroma, including phenols, guaiacols, and syringols (Rozum, 2009). Based on their characteristic smoky aromas, relative abundance in wood smoke, and low odor thresholds, these compounds are believed to be some of the most important aroma compounds in wood smoke (Maga, 1987).

Some of the most important compounds generated from lignin pyrolysis are guaiacol, 4-methyl guaiacol (or creosol), and 2,6-dimethoxyphenol (or syringol).

An entire study was conducted on classifying the aroma contributions of just these three compounds (guaiacol, 4-methylguaiacol, and syringol) to wood smoke flavor in terms of their concentrations in wood smoke, their odor thresholds, and their sensory properties and acceptance (Wasserman, 1966). Lignin also degrades to form ferulic acid, which can undergo further degradation to form compounds like vanillin (Maga, 1992). These structural polymers are found in different ratios depending on the wood source. For instance, hardwoods typically have a higher ratio of five carbon sugars to six carbon sugars in their hemicellulose fractions compared to softwoods. However, softwoods usually have more lignin than hardwoods do (Maga, 1992). Baltes et al. (1981) conducted a study to compare the overall make-up of the phenolic fraction of hardwood versus softwood smoke. Based on percent abundance, they found that hardwoods tended to have lower concentrations of guaiacol and guaiacol derivatives (4-methylguaiacol in particular) than softwoods, while softwoods had lower concentrations of syringol and syringol derivatives than hardwoods (Baltes et al., 1981). This study concluded that the type of wood used for smoking a food product (hardwood or softwood) could be determined by analyzing the guaiacol to syringol ratio within the smoked product.

The specific type of hardwood or softwood also plays a big role in the overall flavor chemistry, and thus the sensory profile, of smoke. Most, if not all, aroma-active compound profiles of various wood sources are made up of the same constituents: phenolics, carbonyls, acids, alcohols, etc., and variation in abundance and concentrations of these constituents plays a large role in distinguishing the flavor of one type of wood from another (Fujimaki et al., 1974). Fujimaki et al. (1974) compared the volatile compound profiles of smoke from Cherry, Pine,

Bamboo, Cedar, and two types of Oak wood and concluded that the major differences in smoke aroma appeared to come from differing ratios of flavor constituents, rather than the presence or absence of a specific aroma compound. In a study by Maga (1992), the aroma intensity of various aroma compound fractions (phenolic, basic, carbonyl, and neutral) from smoke of different wood sources (Apple, Chestnut, Cherry, Red Oak, and White Oak) were compared to one another. Cherry wood smoke had the highest aroma intensity in the phenolic fraction, while Red Oak smoke had the highest carbonyl aroma intensity. The differing aroma intensities of each smoke fraction could be traced back to the structural polymer make up of each individual wood (Maga, 1992). There have been other studies that have demonstrated the effects of differing wood sources on volatile compound composition of wood smoke. Maga and Chen (1985) identified different concentrations of eight pyrazines from 17 wood sources. Hickory wood had the highest amount of total pyrazines generated and redwood had the lowest. Due to their low odor thresholds and relative concentrations, these pyrazines were believed to contribute to the overall sensory perception of smoke (Maga and Chen, 1985). Another study from Guillén et al. (2000) demonstrated the differing concentrations of polycyclic aromatic hydrocarbons resulting from liquid smoke flavorings of various wood sources. In this study, smoke from poplar wood was found to have the highest concentrations of polycyclic aromatic hydrocarbons, while cherry smoke had the lowest (Guillen et al., 2000).

Smoldering Temperature

The temperature of the burning wood can have a significant effect on the resulting smoke aroma, and thus its volatile compound profile. While different types of wood all contain different amounts of hemicellulose, cellulose, and lignin, these three wood constituents also thermally degrade at different temperatures (Maga, 1992). Hemicellulose degrades first at temperatures

ranging from 200-260ºC. Once the temperature surpasses 260ºC until 310ºC, cellulose degrades. Finally, lignin is the last wood polymer to degrade between 310ºC and 500ºC. Especially considering the flavor compounds that are generated by the thermal degradation of each of these polymers, such as phenolics from lignin degradation, the final temperature of the burning wood has a significant role in the flavor chemistry of the smoke generated. For instance, if the wood is burned at a low temperature, it is possible that hemicellulose will degrade fully, but not cellulose or lignin, resulting in a very different overall sensory profile than if higher temperatures were achieved (Maga, 1992). Temperature and type of wood are both important considerations when producing smoked food products. As stated before, softwood tends to have higher amounts of lignin than hardwoods; however, softwood also tends to burn faster and at much lower temperatures than hardwood. Therefore, although softwood contains more lignin, the temperature at which it burns has a significant impact on the overall flavor chemistry of the smoke (Rozum, 2009). Maga (1992) also demonstrated the impact of smoldering temperature on wood smoke flavor by measuring the concentration of phenolic compounds in the wood smoke depending on the smoldering temperature of the wood pellets. The concentration of phenolics increased with temperature, which makes sense considering that lignin degrades at the highest temperature and is responsible for most of the phenols in wood smoke flavor (Maga, 1992). This same trend was seen in another study where the total concentration of pyrazines in Hickory wood smoke increased as the temperature of the wood was increased from 290ºC to 450ºC (Maga and Chen, 1985).

Oxygen Availability

The amount of oxygen present during pyrolysis can have an impact on the resulting wood smoke flavor chemistry. First, oxygen is required for combustion reactions to take place, and

thus for wood to burn and produce smoke (Rozum, 2009). Additionally, molecular oxygen has also been shown to participate in Maillard reactions, which produce many important flavor compounds in wood smoke after the degradation of cellulose and hemicellulose (Dyer et al., 1991; Rozum, 2009). In the study referenced previously by Maga and Chen (1985), the impact of the presence of air on pyrazine concentration was examined. While the concentration of each individual pyrazine was affected in different ways based on the presence of air, overall, significantly more pyrazines were generated in the absence of air. Another study examined the abundances of other important wood flavor constituents: furfuryl alcohol, cyclotene, and guaiacol. The abundance of each of these compounds was heavily affected by the abundance of oxygen present. The abundance of furfuryl alcohol decreased as oxygen increased. However, cyclotene and guaiacol were at relatively low concentrations when oxygen was absent, as well as when it was very abundant (50% of the air in the system). It was at 10% oxygen at which both cyclotene and guaiacol were at their highest concentrations in the wood smoke (Wasserman and Fiddler, 1969).

Moisture Content of the Wood

Moisture content is another aspect of wood that can affect the final aroma-active compound profile of resulting wood smoke after pyrolysis. Like oxygen, water is important in the Maillard reaction. Here, water acts as a solvent to allow the other reactants (amino acids and sugars) to come together and start the reaction (Rozum, 2009). However, as soon as water has brought the reactants together, the reaction must take place in the absence of water. This might suggest that there is an ideal amount of wood moisture content that produces the best volatile compound profile. While this has not been explicitly studied, information published by Gorbatov et al. (1971), with some further analysis by Maga (1987), demonstrates the concept. Gorbatov et

al. (1971) reported that the lowest moisture content woods produced smoke with the highest concentrations of phenols, acids, and carbonyls, while the medium moisture woods produced the most acceptable sensory properties. However, Maga (1987) provided further analysis on this study due to the fact that differing yields of wood smoke condensates were obtained from the woods with varying moisture contents. By normalizing the amount of smoke condensate across all wood moisture contents, the medium moisture woods had the highest proportions of volatiles in their wood smoke condensates (Maga, 1987). This extra calculation by Maga demonstrates the importance of wood moisture content in the formation of flavor compounds in wood smoke. It additionally lends credence to the idea that there is an ideal concentration of moisture in the wood that will produce the most desirable flavor compound profile in wood smoke. Finally, wood moisture content was another variable measured in the pyrazine study by Maga and Chen (1985). At 4% moisture the total abundance of pyrazines in Hickory wood smoke were about twice that of wood with a moisture content of 20% or 30% (no other woods with moisture content between 4% and 20% were tested). There were no significant differences in the pyrazine content of the smoke generated from the 20% and 30% moisture woods. Since wood moisture content has an impact on the final flavor chemistry of the wood smoke, woods used for smoking are usually dried to different moisture contents after harvest depending on the desired use and flavor profile. Usually, wood used to generate smoke in the food industry does not exceed 35% moisture (Maga and Chen, 1985; Guillén and Ibargoitia, 1999).

APPROACH TO SENSORY AND VOLATILE FLAVOR ANALYSIS OF CHEESE

For many years, studies have been conducted to understand the sensory and flavor chemistry of foods. These analyses can be incredibly complex, but they all combine aspects of objective sensory analysis with instrumental techniques. All good chemical flavor analyses either

begin with trained descriptive sensory analysis, or at least involve it somewhere along the way. Descriptive analysis can be one of the most sophisticated and useful tools of a sensory scientist (Lawless and Heymann, 2010). This technique provides the sensory scientist with a complete understanding of the sensory descriptions associated with food products and can determine which sensory attributes are the most important when characterizing the flavor of foods. Gas chromatography-olfactometry (GCO) takes sensory evaluation of foods even further. This is the next step in truly understanding the flavor of a food product. It is the bridge between descriptive sensory analysis and chemical flavor analysis of foods (Delahunty et al., 2006). Gas Chromatography-Mass Spectrometry (GCMS) is the final tool used to characterize the flavor and flavor chemistry of food products. Instead of using the human nose to detect the compounds coming off the GC, as is the case with GCO, GCMS uses a mass spectrometer as a chemical detector. A mass spectrometer is able to analyze and detect compounds with such accuracy that the investigator, with the aid of outside libraries or authentic chemical standards, can determine the exact chemical compounds that are present in the food (Gross, 2017). All of these tools used in conjunction are very powerful and aid scientists in fully characterizing the flavor of food products.

Descriptive Analysis

Descriptive analysis is a very powerful tool when characterizing the flavor profile of foods. Descriptive analysis is an objective or analytical sensory evaluation, where the data is intended to be interpreted as if it came from an analytical instrument. There should be no subjective viewpoints or measures of "liking" coming from a descriptive sensory analysis panel (Drake, 2007). Descriptive analysis is conducted by a panel of trained tasters, usually 6-12, depending on the method used. These tasters are trained to detect flavor attributes in foods and

scale the intensity of these flavors using a quantitative scale. The data generated is very powerful because it can be statistically analyzed and combined with many other analyses including consumer acceptance as well as instrumental flavor analysis (Murray et al., 2001; Lawless and Heymann, 2010). One of the most important aspects of a descriptive analysis panel is that they are trained to identify and scale flavors. This training can be brief, lasting only a few hours, if the attributes are few or if the panelists already have many hours of experience participating in descriptive analysis, or it can last several weeks if the food and flavors of interest are numerous and/or particularly complex (Drake, 2007). One key component of training panelists is the development or use of an already established flavor lexicon. A flavor lexicon is a widely used tool that displays the sensory language that will be used by the panel during evaluation of the product, a description of the attribute's meaning, and usually a food or chemical reference to help the panel identify and/or scale the correct sensory attribute (Drake and Civille, 2003). Depending on the product of interest, these lexicons can be simple, or quite complex. For example, a flavor lexicon for Cheddar cheese was developed in 2001, which had 27 different sensory attributes only to describe the flavor of Cheddar cheese without even taking into account texture attributes (Drake et al., 2001).

There are several different types of descriptive analysis methods. Some of the most well-known methods include the flavor profile method, the texture profile method, quantitative descriptive analysis (QDA), and the Spectrum™ method (Lawless and Heymann, 2010). The flavor profile method was the first documented descriptive analysis technique used by sensory scientists. It is a consensus technique where rating sessions are conducted through group discussions. Using the flavor profile method, the panel considered the overall flavor of the product, as well as certain detectable flavor components (Murray et al., 2001). The flavor profile

method is not typically used in descriptive analysis today. Similarly, the texture profile method was based off of the flavor profile method and was developed about twenty years later. As the name suggests, this method is used to document the texture of food products from the first bite throughout mastication. QDA is a much more popular descriptive analysis method, which was developed in the 1970s (Murray et al., 2001). One of the major differences between QDA and previous methods is that there was a much bigger emphasis on panel member selection for QDA than other methods. QDA uses a product specific scale, so therefore members of the panel were usually selected based on their familiarity with the product. The thought process was that those who are much more familiar with the product or are regular consumers of the product will have an easier time discriminating the more intricate aspects of the product (Sawyer et al., 1962). Although the panel is more selective, many hours of training are still required for a QDA panel to be successful. A QDA panel uses an unstructured line scale to scale the intensity of flavors or textures to avoid biases (Lawless and Heymann, 2010). Another distinguishing aspect of a QDA panel is that the panel leader is not an active participant in the panel evaluation. The biggest limitation of descriptive analysis using the QDA method is that it is very difficult to compare the results of one panel working on a certain product to another panel working on another product, or even the same type of product. This is due to the fact that QDA scales are very product specific and agreed upon by that specific panel of tasters (Murray et al., 2001).

The Sensory Spectrum™ Method was developed by Gail Civille and is the most widely used descriptive analysis method today (Lawless and Heymann, 2010). The major limitation of the QDA method, is the main benefit of the Spectrum™ method. The Spectrum™ method uses a universal scale to record the intensities of flavors and textures in food products. The increments of the scale are standardized across all attributes. For this reason, the Spectrum™ method

employs an extensive use of reference points for both identifying and scaling flavor components in food. Therefore, the data recorded by one laboratory can be compared by another on the other side of the world (Lawless and Heymann, 2010). Additionally, data can even be compared between different food products. For instance, sensory data scaled using the Spectrum™ method of analysis can compare the sweetness of ice cream to the sweetness of barbeque sauce. This can be an extremely important tool when generating and analyzing descriptive sensory data (Murray et al., 2001). Although the Spectrum™ method is very powerful and versatile, it requires an extensive amount of training. To start a new descriptive sensory panel with the Spectrum™ method, one will likely need to spend 15-20 hours discussing terminology, 10-20 hours learning the universal scale, 15-40 hours of practicing profiling foods depending on their complexity, and then several more hours actually profiling the food to generate reproducible data (Murray et al., 2001). Regardless of the enormous time requirement to use the Spectrum™ method properly, studies have been published using the this method to profile cheese (Avsar et al., 2004; Carunchia Whetstine et al., 2005; Jo et al., 2018).

Gas Chromatography – Olfactometry

Descriptive sensory analysis documents the sensory attributes present in the food and their intensities. These results illustrate how the food is perceived by human senses. The next stage in a complete chemical flavor analysis is to understand why the food tastes the way it does, or in other words, what compounds cause certain flavors to occur in food. GCO analysis is the next step in understanding the flavor of food products. GCO analysis is the act of sniffing the chemical effluent of a gas chromatograph, thereby using the human nose as the detector rather than a typical chemical detector (Van Ruth, 2001). The human nose is much more sensitive to aroma-active compounds than chemical detectors and GCO also allows the investigator to

understand the aroma description and perceived intensity of each flavor compound. The relevance of a flavor compound to the overall sensory perception of the food is mainly due to its odor sensory threshold, and not necessarily its concentration. Therefore, the intensity of the aroma detected by the GCO operator is much more telling than the relative abundance obtained from a chemical detector (Dekker, 1997).

There are several different GCO methods that are typically used. The detection frequency method assesses the frequency an aroma is detected by a number of sniffers. This method suggests that the number, or frequency, of sniffers that are able to detect a certain aroma in the GC effluent are directly related to the importance of the compound to flavor (Van Ruth, 2001). However, one of the major issues with this method is the amount of time it takes to conduct. A full GCO analysis can last from 15 to 60 min with time in between each run for sample preparation and GC equilibration. With a minimum of four or more sniffers, evaluating just one sample can be rather time intensive (d'Acampora Zellner et al., 2008). Another method of GCO analysis is a time intensity method called Osme. When using this method, each sniffer records the amount of time he/she detects a certain aroma. It is believed that the amount of time that the aroma is detected is correlated with its importance in the overall flavor of food (Van Ruth, 2001). However, this approach is not regularly used in GCO analyses today. The charm analysis is a GCO technique that is similar to Osme but uses a computer interface (Acree et al., 1984). The underlying assumption that the length of each aroma event correlates with each compound's importance to flavor still holds true, but additional measurements are taken by the computer. The computer records the retention time of the aroma event, an aroma description based on a key stroke of the operator using a predetermined code, and the amount of time it took for the operator to select an aroma description (Acree et al., 1984). Other GCO methods are dilution analyses, the

most common of which is aroma extract dilution analysis (AEDA) (Delahunty et al., 2006). To use this method, the investigator must have performed a solvent flavor extraction of the food product. This flavor extract is then sniffed on the GCO with the compound aromas recorded as they elute from the GC, just as in any other GCO analysis. However, once the flavor extract has been sniffed once, the extract is then serially diluted with solvent usually at a 1:2 or 1:3 ratio. Once the extract has been diluted, it is sniffed again on the GC. This process is repeated until the flavor extract has been diluted to the point where aromas are no longer detected during the GCO analysis. The theory behind this method is that the longer a certain aroma persists through multiple dilutions, the more important it is to the overall sensory perception of the food product (Van Ruth, 2001). AEDA is very commonly used in chemical flavor analysis, but its major setback is the time required to conduct this analysis. Finally, there are direct intensity GCO methods, the most common of which is post peak intensity. For this method, the sniffers are trained to use an intensity scale, similarly to how panelists are trained to use an intensity scale when using the Spectrum™ method of descriptive analysis. After detecting an aroma when sniffing the effluent of the GC, the assessor records a description of the aroma along with the highest intensity that he/she smelled the aroma. There are usually multiple sniffers, most commonly two, used for this method and an average of the intensities recorded are used when reporting the final aroma intensity of flavor compounds detected (Delahunty et al., 2006). This method is commonly used in studies investigating the sensory perception and flavor chemistry of food products because the data that is generated can be correlated with descriptive sensory data that uses intensity scaling (Van Ruth and O'Connor, 2001). Like descriptive analysis, direct intensity GCO methods require a considerable amount of training. Nevertheless, direct intensity GCO methods have been used extensively in studies characterizing the flavor and flavor

chemistry of cheese. This technique was used to aid in the identification of rosy/floral flavor in Cheddar cheese (Carunchia Whetstine et al., 2005). Similarly in 2004, this method was used to understand nutty flavor in Cheddar cheese (Avsar et al., 2004). Both of these studies also employed the use of AEDA. By combining post peak intensity and AEDA, the investigators were able to identify rosy/floral and nutty aroma compounds respectively, while also determining which contributed most to flavor. GCO direct intensity methods were also used in combination with sensory and other instrumental analyses to characterize the flavor and flavor chemistry of Gouda cheese (Jo et al., 2018).

Gas Chromatography – Mass Spectrometry

GCMS is the last important piece of equipment that is necessary for understanding the flavor chemistry of foods. After understanding sensory perception by conducting descriptive analysis and identifying aroma active volatiles by sniffing them from GC effluent during GCO, the retention times of aroma events can be recorded during GCO and matched to corresponding retention times from a GCMS (Cadwallader and Singh, 2009). When studying flavor chemistry, GCO and GCMS are almost always used in conjunction (Avsar et al., 2004; Carunchia Whetstine et al., 2005; Jo et al., 2018). A mass spectrometer is a chemical detector that is able to determine the identity of chemical compounds with the aid of chemical libraries and authentic chemical standards (Gross, 2017). After volatile compounds are separated by gas chromatography, they pass from the column to the mass spectrometer where they are ionized and fragmented after being bombarded with an electron beam. These ions then pass into the mass analyzer, which measures the mass to charge ratio (m/z) of each ion. The mass analyzer can be set to accept or reject certain ions of interest from moving to the detector; however, during general flavor analysis, the mass analyzer is often set to allow a very wide range of ions through to the detector

(Gross, 2017). Quadrupole mass analyzers are regularly used for flavor chemistry analyses (Arora et al., 1995; Guillén et al., 2004; Jo et al., 2018). The reason for this is because quadrupole mass analyzers are versatile in that they can analyze, as well as transmit, large amounts of ions all at the same time (Gross, 2017). After passing through the mass analyzer, the ions are detected by the chemical detector. Electron multiplier detectors are usually paired with quadrupole mass analyzers, because like quadrupoles, they are robust to detecting many ions at once (Gross, 2017). The detector sends a signal to the computer to determine the size of the compound's resulting peak and imbeds its corresponding m/z, which was measured by the mass analyzer, to aid in identification of the compound. The data gathered from a GCMS is very powerful in the identification of chemical compounds because compounds tend to fragment and ionize in the mass spectrometer consistently and predictably. Therefore, the fragmentation and resulting ion m/z's create a "fingerprint" for each compound (Gross, 2017). Using this "fingerprint" and the time at which the compound was detected relative to an alkane series, allows the investigator to determine the identity of the compound. When paired with GCO, GCMS allows the investigator to match the chemical identity of a compound to an aroma event, giving insight into the flavor contributions of the volatile compound.

While the mass spectrometer is extremely important in identifying the compounds that elute off the GC column, there is another important step that must occur before compounds reach the mass spectrometer or are sniffed by GCO. This is the chemical extraction of flavor compounds from food. Flavor compounds can be extracted in a number of ways including direct solvent extraction. Here, a solvent is applied directly to the food product. Typical solvents used for direct solvent extraction are ethyl ether, dichloromethane, and sometimes alcohol (Cadwallader and Singh, 2009). The selection of solvent is based on the solubility of the target

flavor compounds. Most flavor compounds are non-polar, making ethyl ether an effective solvent for extraction. In addition, ethyl ether easily evaporates, allowing for efficient concentration of the flavor extract. Dichloromethane is more polar than ethyl ether, making it a suitable solvent when targeting more polar compounds like acids (Cadwallader and Singh, 2009). Flavor extracts can be directly injected into the GC (depending on the product), or the extract can be further refined prior to injection (Cadwallader and Singh, 2009). However, a relatively new method, and one that has gained a considerable amount of popularity in recent years, is headspace solid phase microextraction (SPME). SPME uses a fused silica fiber that is coated with a stationary phase that will allow volatile compounds in the headspace above a food product to adsorb onto the fiber when exposed to it (Kataoka et al., 2000). Once the SPME fiber has been exposed to volatile compounds for a sufficient amount of time (usually 30 minutes), it is ready for injection onto the GC where the compounds are separated by the column and analyzed by the mass spectrometer. SPME has become very popular because it reduces sample preparation time, eliminates the need to purchase solvents and conduct chemical extractions, and has been shown to improve to detection limits especially with low molecular weight compounds (Xu et al., 2016). However, with high molecular weight compounds, SPME is sometimes not the best method of extraction because it relies heavily on a compound's ability to volatilize in the headspace of the food product. High molecular weight compounds are less likely to do so (Xu et al., 2016).

SPME, in conjunction with GCMS, has been used in countless flavor chemistry analyses of food products. SPME-GCMS was used to extract and identify the most important flavor components in smoked goat cheese. The main flavor compounds identified were acids and phenolics (Guillén et al., 2004). Considering how different the chemistry is of acids and

phenolics, the versatility of SPME as an extraction method is clear. Similarly, SPME was also used to extract flavor compounds from Oscypek cheese, which is a type of sheep's cheese. The compounds identified were various types of free fatty acids, esters, ketones, alcohols, aldehydes, furans, phenols, sulfur containing compounds, and terpenes (Majcher et al., 2011). These results demonstrate even further how robust SPME is as an extraction method. Considering how quickly SPME can extract these compounds (usually between 20-30 minutes) and the fact that the technique can be easily integrated with an autosampler, it is clear how important SPME is to the chemical flavor analysis of dairy products and food (Souza-Silva et al., 2015).

Finally, GCMS is an important tool in flavor chemistry because it allows for accurate and precise quantification of flavor compounds in foods. The use of GCMS to quantify flavor compounds has been demonstrated in numerous studies. There are several methods used to quantify flavor compounds with GCMS, but the two most common are relative abundance to an internal standard and by generating external standard curves (Cadwallader and Singh, 2009). In a study on smoked goat cheese, Guillén et al. (2004) quantified compounds of interest by relative abundance to an internal standard. In this method, a known amount of an authentic chemical standard is added to each sample before analysis. The compound that is used must not be naturally present in the sample to ensure its peak response from the GCMS is entirely due to the known amount of the compound that was added (Gross, 2017). The peak response from the internal standard is then compared as a ratio to that of the compound of interest. Multiplying this ratio with the concentration of the internal standard in the sample yields the concentration of the target compound (Guillén et al., 2004). External standard curves are also commonly used in flavor analyses on cheese (Carunchia Whetstine et al., 2005; Jo et al., 2018). Carunchia Whetstine et al. (2005) and Jo et al. (2018) used five-point external standard curves to quantify

flavor compounds of interest with GCMS. These curves are created by analyzing five different known concentrations of the target compound with GCMS and using the peak response of each to create a line equation. Once this equation has been established, the peak response of the compounds of interest within the sample can be substituted into the line equation to yield the true concentration of the compound in the sample (Carunchia Whetstine et al., 2005; Jo et al., 2018). Stable isotopes of flavor compounds are also commonly used with relative abundance and external standard curves for volatile compound quantification. An isotope is a compound containing one or more atoms that have a different number of neutrons than is most commonly present in nature (Gross, 2017). For example, some of the most common isotopes of flavor compounds contain a deuterium in place of a hydrogen (deuterium contains one neutron and hydrogen contains zero) or a carbon-13 instead of the typical carbon-12 (carbon-13 contains 7 neutrons and carbon-12 contains 6). These isotopes are used to very accurately quantify compounds that are native to the food matrix. Rychlik and Bosset (2001) used stable isotopes to quantify 2/3-methylbutanal, methional, dimethyltrisulfide and several other compounds in Gruyere cheese. Since each of these compounds is native to Gruyere cheese, the authors obtained stable isotopes of each target compound. The stable isotopes provided an internal standard that was non-native to the cheese matrix and chemically behaved exactly as the compound of interest did within the matrix (Rychlik and Bosset, 2001). Stable isotopes can be used as an internal standard for relative abundance, or to generate external standard curves (Gross, 2017).

OBJECTIVES

The objectives of this thesis are to investigate the flavor chemistry of wood smoke alone, as well as Cheddar cheeses that have been smoked using various wood sources (Hickory, Cherry, or Apple) to gain an understanding of the chemical components that lead to differences in

sensory perception. Smoked foods are an important staple in the human diet and have been for thousands of years. As the popularity of smoked Cheddar cheese increases, it is important to understand its flavor chemistry. This work will provide smoked Cheddar cheese manufacturers and the flavor industry with important information regarding the chemical makeup of these products and specific compounds that significantly impact sensory perception, aiding the product development of similar cheeses. In addition to the flavor chemistry of smoked Cheddar cheese, consumer desires and perceptions of lactose-free milk will be examined. This work will also support product development in the dairy industry.

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CHAPTER 2:
IDENTIFICATION OF AROMA-ACTIVE COMPOUNDS IN CHEDDAR CHEESE IMPARTED BY WOOD SMOKE

Identification of Aroma-active Compounds in Cheddar Cheese Imparted by Wood Smoke

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INTERPRETIVE SUMMARY

Cheddar cheese is the most popular cheese in the United States and demand for smoked cheese is rising. This study investigated the flavor chemistry of smoked Cheddar cheese. Distinct flavors and aroma-active compounds were identified for Cheddar cheeses smoked with hickory, cherry, or apple woods. Guaiacol, 4-methylguaiacol, and syringol were important to all cheeses and contributed to high smoke flavor intensities. Benzyl alcohol contributed to fruity flavor in cherry smoked cheeses, and 2-methyl-2-butenal and 2-ethylfuran contributed to waxy/green flavor in apple smoked cheeses.

ABSTRACT

Cheddar cheese is the most popular cheese in the United States, and the demand for specialty categories of cheese, like smoked cheese, are rising. A sensory and chemical flavor analysis of Cheddar cheeses smoked with different wood sources can aid the dairy industry to manufacture desirable smoked Cheddar cheese products. The objective of this study was to characterize the flavor differences among Cheddar cheeses smoked with hickory, cherry, or apple woods, and to identify important aroma-active compounds contributing to these differences. First, the aroma-active compound profiles of hickory, cherry, and apple wood smokes were analyzed by solid-phase microextraction (SPME) gas chromatography-olfactometry (GCO) and gas chromatography-mass spectrometry (GCMS). Seventy-eight aroma-active compounds were identified in wood smokes. Compounds belonged to important categories which included phenolics, carbonyls, and furans. After wood smoke aroma-active compounds were determined, commercial Cheddar cheeses smoked with hickory, cherry, or apple woods, as well as an unsmoked control, were obtained in duplicate lots. Cheeses were evaluated by trained panel descriptive sensory analysis and by SPME GCO and GCMS to identify aroma-active compounds. The trained panel distinguished hickory smoked cheeses by high intensities of flavors associated with characteristic "smokiness" like smoke aroma, overall smoke intensity, and meaty/smoky flavor. Cherry wood smoked cheeses were distinguished by the presence of a fruity flavor. Apple wood smoked cheeses were characterized by the presence of a waxy/green flavor. Ninety-nine aroma-active compounds were identified in cheeses. Phenol, guaiacol, 4-methylguaiacol, and syringol were identified as the most important compounds contributing to characteristic "smokiness." Benzyl alcohol contributed to the fruity flavor in cherry wood smoked cheeses, and 2-methyl-2-butenal and 2-ethylfuran were responsible for the waxy/green

flavor identified in apple wood smoked cheeses. These smoke flavor compounds, in addition to diacetly and acetoin, were deemed important to the flavor of cheeses in this study and were quantified using 5 pt standard curves. Results from this study identified key compounds contributing to differences in sensory perception among Cheddar cheeses smoked with different wood sources.

Key words: smoked Cheddar cheese, descriptive analysis, gas chromatography, aroma-active compound

INTRODUCTION

Cheese consumption in the United States has steadily increased. Between 1975 and 2008, total cheese consumption in the United States increased from less than 20 pounds per capita to about 35 pounds per capita (Davis et al., 2010). Today, Cheddar cheese is the most popular cheese in the United States. In 2017, Cheddar cheese made up 22.0% of the volume of cheese sold in the United States (Dairy Management Inc, 2017). As cheese consumption has grown in general, smoked cheese demand has also risen (Market Research Future, 2019). A recent study surveyed smoked food consumers to determine the types of smoked foods they purchased regularly (Del Toro-Gipson et al., 2020a). Smoked cheese was selected by 66.9% of consumers and smoked Cheddar was one of the most popular cheese types selected, second only to smoked Gouda.

Smoked cheese is generally produced using one of two methods: (1) cold smoking cheese blocks by smoldering wood, or (2) applying liquid smoke or liquid smoke flavorings to cheese milk (Mcilveen and Vallely, 1996). There are advantages and disadvantages to each method. Cold smoking exposes cheese to natural wood smoke and is more conceptually appealing to consumers (Del Toro-Gipson et al., 2020a), but produces a smoke flavor gradient since smoke flavor compounds cannot penetrate throughout the interior of the cheese block (Guillén et al., 2004; Sikorski and Sinkiewicz, 2014). Conversely, the advantage of adding liquid smoke to the cheese milk is that no flavor gradient is produced (Mcilveen and Vallely, 1996). Liquid smoke is also filtered to remove potentially harmful compounds from the smoke (Ledesma et al., 2017). Recently, a new method of smoked cheese manufacture has emerged that combines the advantages of cold smoking and liquid smoke. This process involves cold smoking fresh cheese

curds in a chamber before the cheese block in formed (McLeod, 2017). By using this method, the final cheese block has a consistent smoke flavor throughout, without the need for liquid smoke.

Smoked foods have been a part of the human diet for thousands of years (Ledesma et al., 2017). Humans first smoked foods to take advantage of preservative effects of smoke, but as food preservation techniques like refrigeration have become widely available, today, foods are typically smoked to create a unique and characteristic flavor. Many studies have investigated the flavor chemistry of wood smoke (Fiddler et al., 1970; Maga, 1987; Maga, 1992; Cadwallader, 2007). One of the most distinguishing factors that affects the flavor chemistry of wood smoke is the wood source used (i.e. hickory, mesquite, cherry, apple, etc.). Flavor compounds found in wood smoke are the products of the pyrolysis of wood polymers such as cellulose, hemicellulose, and lignin. Different types of woods naturally have different compositions of wood polymers, leading to differences in the flavor chemistry of their respective smokes (Rozum, 2009). While the flavor chemistry of wood smoke has been widely studied, there have been very few studies that have investigated the impact of wood smoke on the flavor chemistry of cheese. A study examined the aroma-active compounds in Palmero cheeses, a goat cheese smoked with pine needles as the source, by gas chromatography-mass spectrometry (**GCMS**) with headspace solid-phase microextraction (**SPME**) (Guillén et al., 2004). Volatiles originating from both the cheese and smoke were identified, indicating that volatile compounds from the smoke were transferred to the cheese during the smoking process. Smoke-derived compounds that were identified included alcohols, hydrocarbons, carbonyls, furans, phenolics, and several others. The most important classifications of compounds that contributed to the flavor of Palmero cheeses were acids originating from the cheese and phenolic compounds originating from the smoke (Guillén et al., 2004). Important phenolics that were imparted from the smoke were guaiacol derivatives

(methoxyphenols) and syringol derivatives (dimethoxyphenols) (Guillén et al., 2004). These compounds are widely believed to be some of most important aroma-active compounds in wood smoke (Wasserman, 1966; Maga, 1987; Cadwallader, 2007).

The aroma-active compound profile of cross sections of the Palmero cheeses were also investigated (Guillén et al., 2004). As expected, the exterior of the cheese had a greater abundance of volatile compounds than the interior, and compounds were more concentrated on the exterior than the interior. The only compounds that were identified in the interior of the cheese were those that were highly concentrated on the exterior (Guillén et al., 2004). Another study explored the flavor chemistry of Oscypek cheese, which is a Polish smoked cheese made from ewe (sheep) milk, using GCMS with headspace SPME (Majcher et al., 2011). The authors determined that furans and phenolic compounds were the most influential contributors from the smoke to the overall flavor of the cheese (Majcher et al., 2011). These studies examined the volatile compounds present in Palmero and Oscypek cheeses using GCMS, but the role of different wood smokes was not evaluated nor were aroma-active compounds identified.

Although Cheddar cheese is the most popular cheese in the United States (Dairy Management Inc, 2017), and smoked Cheddar is very popular among smoked cheese consumers (Del Toro-Gipson et al., 2020a), to our knowledge there have not been any studies investigating the impact of different wood sources on the flavor chemistry of Cheddar cheese. Rehman et al. (2003) studied the flavor effects of smoking Cheddar cheeses at different points in the ripening process. Three-kilogram blocks of Cheddar cheese were smoked at 3, 6, and 9 mo into the ripening process followed by sensory analysis. The 3 mo smoked Cheddar cheese had the best smoke flavor and the authors concluded that it was best to smoke Cheddar cheese early in the ripening process (Rehman et al., 2003). Del Toro-Gipson et al. (2020b) evaluated the sensory

aroma properties of different wood smokes. Trained panelists distinguished nine wood smokes based on their aromas and characterized the smokes using a projective mapping exercise (Del Toro-Gipson et al., 2020b). Cheddar cheese was subsequently exposed to four of the most distinct smokes. Trained panelists characterized the flavor of Cheddar cheeses smoked with different wood sources (Del Toro-Gipson et al., 2020b). Consumer liking of Cheddar cheeses smoked with different wood sources was investigated (Del Toro-Gipson et al., 2020a). Consumers distinguished differences among the Cheddar cheeses smoked with different wood sources and had a preference for cherry wood smoked Cheddar cheese (Del Toro-Gipson et al., 2020a). The objective of our study was to identify flavor differences among Cheddar cheeses smoked with hickory, cherry, or apple woods and to determine the flavor compounds responsible for these differences. This objective was accomplished by volatile compound analysis of hickory, cherry, and apple wood smokes using both gas chromatography-olfactometry (**GCO**) and GCMS with headspace SPME to gain an understanding of the aroma-active compounds present in each of these wood smokes. Subsequently, Cheddar cheeses smoked with these wood sources were evaluated by a trained panel and the flavor chemistry of the smoked Cheddar cheeses was analyzed by SPME GCO and GCMS to identify the specific aroma-active compounds responsible for flavor differences.

MATERIALS AND METHODS

Aroma-active Compound Analysis of Wood Smoke

Volatile Flavor Extraction. To fully characterize the sources of flavor in smoked Cheddar cheese, the flavor components of wood smoke itself (without being applied to cheese) were evaluated first. This step assisted with determining whether flavor compounds identified in the smoked Cheddar cheeses originated from the wood smoke or the Cheddar cheese. Volatile

compound extraction of wood smoke was accomplished by suspending divinylbenzene/carboxen/polydimethylsiloxane (**DVB/CAR/PDMS**) SPME fibers (Supelco, Bellefonte, PA) from the inside ceiling of a cocktail smoking box (Fortessa Tableware Solutions, LLC, VA). Wood smoke was applied to the sealed cocktail smoking box using a handheld food smoker (Polyscience Smoking Gun Handheld Food Smoker, Polyscience Culinary of Breville USA Inc., CA) through 0.63cm natural latex tubing (Abbot Rubber Company Inc., IL). Hickory, cherry, and apple wood chips (Camerons Superfine Wood Chips, Camerons, Colorado Springs, CO) were used. Each wood source had its own latex tubing to avoid cross contamination of aromatics during the smoking process. Additionally, the cocktail smoking box and the handheld food smoker were cleaned thoroughly with deodorized soap after each use. 0.5g of wood chips were burned to generate smoke for each extraction. SPME fibers were exposed to 23°C smoke for 3 min. After exposure to the wood smoke, SPME fibers were immediately injected onto the GC for analysis.

GCO. Aroma-active volatile compounds in wood smokes were identified by GCO. The SPME fibers used were 2.0cm in length with DVB/CAR/PDMS coating (Supelco). All injections were made on an Agilent 6850 GC with a flame ionization detector (**FID**) and olfactometer attached (Agilent Technologies Inc., Santa Clara, CA). Fibers were injected at a depth of 30mm into the GC inlet at 250°C on a ZB-5ms column (30m length x 0.25mm internal diameter x 0.25µm film thickness; Phenomenex, Torrance, CA) for 5 min, with a constant flow rate of 2mL/min. The GC oven was held at 40°C for 3min and ramped at a rate of 10°C/min to 150°C. The rate then increased to 30°C/min to 200°C, which was held for 10min. The GC effluent was split 1:1 between the FID and the olfactometer port using deactivated fused silica capillaries (1m length x 0.25mm internal diameter; Phenomenex). The FID was held at 300°C and the GC

effluent was mixed with humidified air in the olfactometer port. Each wood smoke was evaluated in duplicate by two sniffers, each with over 75 h of experience GC sniffing. For each aroma event, sniffers recorded the retention time, aroma description, and perceived intensity using a 5 pt intensity scale. This process was replicated twice for each wood smoke for GCO and for GCMS.

GCMS. Aroma-active volatile compounds in wood smokes were identified by GCMS. The SPME fibers used were 1.0cm in length with DVB/CAR/PDMS coating (Supelco). All injections were made on an Agilent 7890B GC with a 5977B MSD attached (Agilent Technologies Inc.). Samples were manually injected at a depth of 5.0cm into a splitless GC inlet at 250°C onto a ZB-5ms column (30m length x 0.25mm internal diameter x 0.25µm film thickness; Phenomenex) with a constant flow rate of 1mL/min of Helium. SPME fibers were injected for 5min in the GC inlet. The GC oven was initially set to 40°C, which was held for 3min. The temperature ramp began at a rate of 10°C/min to 90°C, then slowed to 5°C/min to 200°C, which was held for 10min. The temperature ramp then began again at a rate of 20°C/min to 250°C, which was held for 5min. The purge time was set for 1min. The MS transfer line was held at 250°C, with the quadrupole and source held constantly at 150°C and 230°C, respectively. Wood smokes were analyzed using a method scanning from 30-400 m/z in tandem with selective ion monitoring mode. Volatile aroma-active compounds from wood smokes were identified using their aroma properties, the National Institute of Standards and Technology mass spectral database (NIST, 2017), authentic chemical standards, and retention indices, calculated relative to an alkane series (Van den Dool and Kratz, 1963). Each wood smoke was analyzed in triplicate with two experimental replications.

Smoked Cheddar Cheese

Smoked Cheddar cheeses were manufactured by the Hilmar Cheese Company (Hilmar, CA, USA) using a patented process in which fresh cheese curds were exposed to cold wood smoke in a chamber (McLeod, 2017). There were six smoked Cheddar cheeses manufactured from duplicate Cheddar cheese lots for this study (6 smoked cheeses + unsmoked control per lot, 2 lots, 14 cheeses total). Three different wood sources were used for smoking (hickory, cherry, and apple) and each Cheddar cheese was smoked using only one wood source. For each wood source, a Cheddar cheese with a high intensity of smoke flavor and a low intensity of smoke flavor was manufactured. The high intensity smoked cheeses were exposed to smoke for a longer period of time than the low intensity smoked cheeses (McLeod, 2017). Cheeses were 2 mo old upon arrival at North Carolina State University and were stored at 4°C. Each cheese was also subsampled for instrumental analyses and stored at -80°C. For the remainder of this manuscript, cheeses will be referred to using the following format: "Wood Source-Smoke Intensity". For example, the hickory wood smoked cheese with high intensity smoke flavor will be referred to as "Hickory-High." The unsmoked Cheddar cheese will be referred to as "Control."

Composition Analysis of Smoked Cheddar Cheese

Fat, moisture, and pH analyses were conducted on cheeses. Since all cheeses within a single lot were manufactured from the same lot of milk and the smoking process does not affect the fat content of foods (Ledesma et al., 2017), fat analysis was only conducted on the control cheese. However, both moisture and pH can be affected by smoking (Ledesma et al., 2017), so all cheeses were evaluated for these properties. All composition measurements were based on modified methods from Hooi et al. (2004). Fat content was measured by a modified Mojonnier extraction method using 1.0g of finely grated cheese. Fat extracts were dried in a vacuum oven

set to 90°C and -100kPa for 10min. Moisture content was measured by drying 2-3g of finely grated cheese in a vacuum oven set to 100°C and -100kPa for 5h. To measure pH, cheeses were finely grated to create a uniform sample and were packed tightly around the pH electrode to ensure proper contact and an accurate reading (Hooi et al., 2004). An InLab Solids glass electrode (Mettler-Toledo GmbH, Schwerzenbach, Switzerland) was used for all pH measurements at an ambient temperature of 21.5°C. Prior to use, the probe was calibrated using pH buffers at pH 4, 7, and 10 (Thermo Scientific, Chelmsford, MA). All composition analyses were conducted in duplicate with two experimental replications.

Descriptive Analysis of Smoked Cheddar Cheese

All cheeses were evaluated by trained panel descriptive sensory analysis. Each panelist had over 150 h of tasting experience evaluating the flavor of dairy products including Cheddar cheese (n=6; 3 females, 3 males; 23-54y). Panelists were provided deionized water and unsalted crackers to cleans their palates between sample evaluations. Cheeses were evaluated using an established Cheddar cheese flavor lexicon (Drake et al., 2001), with smoke terms added (Del Toro-Gipson et al., 2020b), using a 0-15pt universal intensity scale consistent with the Spectrum Method™ (Meilgaard et al., 2007). Cheeses were cut into $2cm^3$ cubes in a dark room to prevent light-induced oxidation of lipids. Cheeses were served in 60 mL lidded plastic soufflé cups (PFS Sales Co., Raleigh, North Carolina) labeled with random 3-digit codes. Panelists evaluated cheeses in duplicate and paper ballots were used for data collection.

Aroma-active Compound Analysis of Smoked Cheddar Cheese

GCO. Aroma-active compounds in smoked and unsmoked Cheddar cheeses were analyzed by GCO. Six grams of finely grated cheese were added to 40mL screw top amber vials. Cheeses were equilibrated to 40°C for 30min using a Reacti Therm TS-18821 heating instrument

(Thermo Scientific, Waltham, MA). SPME fibers were exposed to the cheese headspace at a depth of 20mm for 30min at 40°C. The SPME fibers, GC instrument, and GC oven program settings were the same as they were for the wood smoke analysis. The same group of sniffers evaluated the smoked Cheddar cheese that evaluated the wood smokes, and aroma events were recorded in the same manner.

GCMS. The volatile aroma-active compounds in smoked and unsmoked Cheddar cheeses were analyzed by GCMS. Three grams of finely grated Cheddar cheeses were added to 20mL amber glass vials with Teflon silicon septa lids (Supelco). An internal standard of 20µL of 2-methyl-3-heptanone (81mg/kg in methanol) was added to each vial prior to SPME fiber exposure. The same type of SPME fibers used for wood smoke flavor analysis were also used for smoked Cheddar cheese analysis. Fiber exposure and injection were accomplished using a CTC Analytics Combi PAL autosampler (Leap Technologies, Carrboro, NC). Vials were equilibrated to 40°C for 25min under 4 second pulsed 250rpm agitation. The SPME fiber was then exposed to the cheese headspace at a depth of 3.1cm for 30min. The fiber was retracted from the vial and injected into the GC inlet at a depth of 5.0cm for 5min. The GC instrument, GC oven program, and MSD settings were the same as they were for wood smoke analysis. Flavor-active compounds were identified in the same manner as they were for wood smoke analysis. All smoked Cheddar cheeses were analyzed in triplicate from two experimental replications.

Chemicals and Quantification of Aroma-active Compounds in Smoked Cheddar Cheese

Aroma-active volatile compounds identified in smoked Cheddar cheeses were selected for quantification based on assessment of current literature (Wasserman, 1966; Maga, 1987; Singh et al., 2003) and their aroma properties in relation to the characteristic flavor profiles identified by sensory analysis of each cheese. Selected compounds for quantification included:

diacetyl (buttery, cheese and smoke), acetoin (buttery/sour, cheese), 2-methyl-2-butenal (green/waxy/leafy, smoke), 2-ethylfuran (waxy/green, smoke), phenol (rubber/phenolic, smoke), benzyl alcohol (fruity/cherry, smoke), guaiacol (smoky/meaty/sweet, smoke), 4-methylguaiacol (marshmallow/campfire, smoke), and syringol (sweet/woody/smoky, smoke). These compounds were quantified using 5pt external standard curves with internal standard calibration (minimum $R^2 \geq 0.92$). The standard curves were built using the unsmoked control Cheddar cheese as the matrix. Since all cheeses (smoked and unsmoked) in each lot were manufactured from the same lot of milk, the unsmoked control cheese provided an ideal matrix to create the standard curves of smoke-derived aroma-active compounds.

To create the standard curves, 3g of unsmoked control Cheddar cheese was added to 20mL amber glass vials with Teflon silicon septa lids (Supleco). A series of these vials were dosed with known and increasing concentrations of each selected compound and a known amount of internal standard (2-methyl-3-heptanone at 81mg/kg in methanol). The vials were analyzed in triplicate using the same type of SPME fiber, GC instrument, GC oven program, and MSD settings that were used for the smoked Cheddar cheese GCMS analysis. The area under the peak generated from the compound of interest was compared to that of the internal standard as a ratio. This ratio was plotted to create a standard curve for each compound individually. Selected compounds were then quantified in the Cheddar cheeses by determining the area under the peak of the target compound relative to the area under the peak of the internal standard and inserting this ratio into the line equation generated from the standard curve of the same compound. In addition to quantifying the concentrations of selected compounds among the cheeses, this method was also used to quantify and compare selected compounds between the exterior and interior of individual smoked cheeses. According to the patent by which the cheeses were

manufactured (McLeod, 2017), the smoke process generated a consistent smoke flavor throughout the cheese block (±10% smoke flavor intensity). Upon receipt of the smoked cheeses, this item was confirmed via sensory evaluation and by quantification of key aroma-active smoke-derived compounds (phenol, guaiacol, 4-methylguaiacol, and syringol) from the exterior and interior of each smoked cheese block.

With the exception of diacetyl and acetoin, authentic standards of selected compounds and the internal standard were purchased from Sigma-Aldrich (St. Louis, MO). Since the Control Cheddar cheese matrix naturally contained diacetyl and acetoin, stable isotopes of each were sourced and used to create standard curves. Stable isotopes were used because they achieve peak separation from the corresponding native compound in Cheddar cheese, allowing them to be accurately quantified (Gross, 2017). A deuterium labeled diacetyl standard was purchased from CDN Isotopes (Pointe-Claire, Quebec, Canada) and a carbon-13 labeled acetoin standard was purchased from Toronto Research Chemicals (North York, Ontario, Canada).

Statistical Analysis

All statistical analyses were conducted using XLSTAT software (version 2019; Addisoft, New York, NY). Proximate analysis results, descriptive analysis results, and concentrations of selected aroma-active compounds were analyzed using analysis of variance (**ANOVA**) at 95% confidence and Fisher's least significant difference as a post hoc test. Descriptive analysis and the GCO aroma intensities of selected compounds in smoked Cheddar cheeses were further analyzed by principal component analysis (**PCA**) to determine chemical drivers of flavors in smoked Cheddar cheeses.

RESULTS AND DISCUSSION

Aroma-active Compound Analysis of Wood Smokes

There were 112 aroma events recorded during wood smoke GCO analysis including 63 in hickory wood smoke, 55 in cherry, and 64 in apple, with some overlap between each smoke. Aroma-active compounds corresponding to 78 of the aroma events were identified by GCMS, and 34 were unknown (**Table 1**). Fifty of the aroma events were identified in multiple woods, meaning 72 aroma events were identified only in a single smoke. The compounds identified belonged to several different compound classifications including phenolics, benzene derivatives, carbonyls (aldehydes and ketones), furans, alcohols, acids, esters, and lactones. These compounds have been identified in many types of wood smoke including hickory, cherry, apple, oak, maple, mesquite, and many more (Fiddler et al., 1966; Wasserman, 1966; Fujimaki et al., 1974; Baltes et al., 1981; Maga, 1987; Maga, 1992). Compounds according to these classifications are found in all types of wood smoke, but usually at different concentrations among smoke types.

Flavor compounds found in wood smoke are the products of the pyrolysis of wood polymers (cellulose, hemicellulose, and lignin). All wood smoke flavor compounds can be traced back to the thermal degradation of one of these polymers (Rozum, 2009). The polymer chain of cellulose is made up of glucose monomers which participate in Maillard reactions as cellulose depolymerizes during pyrolysis. Maillard reactions from cellulose monomers produce aliphatic acids and aldehydes (Rozum, 2009). Hemicellulose is more complex than cellulose. Hemicellulose is a polymer made up of several five-carbon and six-carbon sugar monomers including glucose, mannose, arabinose, galactose, and xylose. Similarly to cellulose, these sugars depolymerize from the long chain during pyrolysis and participate in Maillard reactions to

produce flavor compounds. These reactions also produce acids and aldehydes as they do during cellulose pyrolysis, but furan derivatives are also formed (Rozum, 2009). Finally, the thermal degradation of lignin, a complex phenolic-based wood polymer, is responsible for creating phenolic compounds in wood smoke. The phenolic fraction of compounds is comprised of benzene derivatives, phenol derivatives, guaiacols, and syringols (Maga, 1992; Cadwallader, 2007; Rozum, 2009). Although all of the compounds that were identified in hickory, cherry, and apple wood smokes potentially impact flavor, phenolics are considered the most important contributors to smoky flavor due to their characteristic smoke-like aromas, usually low aroma thresholds, and high concentrations in wood smoke (Wasserman, 1966; Maga and Fapojuwo, 1986; Maga, 1992).

Phenolic compounds were identified in all wood smokes analyzed (**Table 1**). Some of the most commonly identified phenolic compounds were guaiacol derivatives (2-methoxyphenols), and syringol derivatives (2,6-dimethoxyphenols). These compounds have a characteristic smoky aroma and are likely responsible for some of the most distinguishing flavors of wood smoke in general. While most aroma-active compounds were associated with smoke-related aromas like smoky, ashy, and charcoal, they also possessed a wide range of other aromas such as woody, spicy, vanilla, nutty, marshmallow, and many others. Notable phenolic compounds that were identified in all wood smokes were phenol, guaiacol, 4-methylguaiacol, and syringol. The aroma of phenol was described as phenolic. Guaiacol and 4-methylguaiacol were described with smoke-related aromas including smoky/meaty/phenolic and marshmallow/campfire, respectively. Syringol was described as smoky/rubber/woody. The detection and identification of these compounds in wood smoke was expected because they have been identified in many wood smokes including hickory, cherry, and apple (Fiddler, 1966; Maga, 1987; Maga, 1992).

Wasserman (1966) determined that due to their low odor thresholds and high concentrations in wood smoke, guaiacol, 4-methylguaiacol, and syringol were some of the most important flavor-active compounds in wood smoke, with 4-methylguaiacol possibly being the single most important compound contributing to the flavor of wood smoke. However, the specific type of wood smoke used in the study by Wasserman (1966) was not stated.

Various carbonyl compounds including aldehydes and ketones were detected by GCO and identified by GCMS for hickory, cherry, and apple wood smokes. Like phenolics, these compounds are also believed to be important to wood smoke flavor (Fiddler et al., 1970; Fujimaki et al., 1974; Kostyra and Baryłko-Pikielna, 2006). In studies investigating the effects of the carbonyl fraction of wood smokes (oak, cherry, pine, cedar, and bamboo) and wood smoke flavorings, carbonyl compounds had a modifying effect on the phenolic compounds and contributed to the complexity of smoke aroma (Fiddler et al., 1970; Kostyra and Baryłko-Pikielna, 2006). The carbonyl compounds identified in this study had a wide range of associated aromas. For example, the aroma of 2-methyl-2-butenal was described as floral and green, while the aroma of various cyclopentenone derivatives were generally described as caramel and nutty. Benzaldehyde derivatives were associated with nutty and fruity flavors, with varying ratios of nutty to fruity flavor intensity depending on the specific compound. Acetophenone was also identified in hickory, cherry, and apple wood smokes and was described as floral and honey. As outlined by the studies previously mentioned, it is clear that carbonyl compounds do not necessarily have a distinct “smoky” aroma but contribute a wide range of flavors to wood smoke. Although their aroma descriptions are not usually associated with wood smoke, the frequency by which carbonyls were detected in all wood smokes indicates their importance to wood smoke flavor.

Several furans were identified in wood smokes. Similar to carbonyls, furans are believed to have a modifying effect on the phenolic fraction of the wood smoke, which has been described as "softening" the phenolic pungency (Kim et al., 1974). These compounds also provided a wide range of flavors to the wood smoke. Furfural was identified in all smokes with an aroma of caramel/grain. Similarly, 5-methylfurfural was detected in all smokes and was described as smoky/chemical/sweet. There were several other furans identified that were not present in all three smokes but contributed a similar flavor profile including furfuryl alcohol (hickory and cherry), 2(5H)-furanone (hickory and cherry), and 2-methylbenzofuran (hickory), which were described as sweet/milky/nutty, woody/burnt, and sweet/vanilla, respectively. Furans were also responsible for several other flavor profiles. 2-furylethylketone was described as fruity/cinnamon. Other furans, 2,3-dihydrobenzofuran and 5-hydroxymethylfurfural, contributed a green/waxy and cleaner/leather aroma to wood smokes, respectively.

Carboxylic acids, esters, and alcohols were also identified when analyzing the flavor chemistry of hickory, cherry, and apple wood smokes. The most notable acid detected in all three wood sources was acetic acid. Acetic acid has a pungent/sour/vinegar-like aroma and was detected at relatively high intensities by GCO. Acids, including acetic acid, are known to provide wood smoke with a sour aromatic flavor, as well as contribute to its antimicrobial properties (Cadwallader, 2007; Rozum, 2009; Ledesma et al., 2017). Few esters and alcohols were identified during wood smoke analysis in the current study. These results were expected because in most types of wood smoke, esters and alcohols are not as numerous as phenolics, carbonyls, and furans (Maga, 1992). Aromas associated with esters and alcohols in the current study ranged from metallic/sweaty to fruity/cherry.

As stated previously, all wood smoke aroma-active compounds originate from the pyrolysis of wood polymers cellulose, hemicellulose, and lignin. Since different types of wood have different relative amounts of these wood polymers, the aroma-active compound profiles of their corresponding smokes are comprised of different concentrations of flavor compounds, which lead to differences in overall sensory perception (Maga, 1992). There were several aroma events recorded in a single smoke including 23 aroma events exclusively identified in hickory wood smoke, 19 in cherry, and 20 in apple. While major contributors to smoke flavor like guaiacol, 4-methylguaiacol, and syringol were identified in all smokes, these compounds contributed to the flavor modification of each individual smoke.

When examining the compounds detected exclusively in hickory wood smoke, most of their associated aromas are reminiscent of smoke and/or Maillard reaction products. For example, 5-methylguaiacol and 4-methylsyringol were described as smoky/ashy and smoky/woody, respectively. There were also several compounds described as nutty, vanilla, and/or caramel including 2-cyclopenten-1-one, benzaldehyde, 2-methylbenzofuran, and vanillin. These results reflect the findings of Del Toro-Gipson et al. (2020b) where trained panelists distinguished hickory wood smoke by a high intensity of smoky, ashy, and woody/bark aromas. It is possible that many of the nutty aroma compounds identified in the current study contributed to the woody flavor of hickory smoke in addition to compounds like syringol and 4-methylsyringol that are associated with woody flavor.

Cherry smoke had several aroma events described as fruity/cherry that were not detected in other smokes. The most notable aroma-active compound described as fruity/cherry was benzyl alcohol with a distinct cherry-like aroma. Trained panelists similarly distinguished cherry wood smoke by a fruity/cherry aroma (Del Toro-Gipson et al., 2020b).

Apple wood smoke also contained aroma events described as fruity; however, most of these were associated with green fruit-like. For example, the aromas of 2-nonen-1-ol and decanal were described as apple. Similarly, there were compounds that provided a waxy/green aroma to apple wood smoke including 2,3-dihydrobenzofuran, 3,4-dimethoxytoluene, and 2-methyl-2-butenal. Although 2-methyl-2-butenal was also detected in hickory wood smoke, the aroma event was recorded at a higher intensity in apple wood smoke. These compounds likely contributed to the plastic and resinous/terpene flavors detected by trained sensory panelists in apple wood smoke (Del Toro-Gipson et al., 2020b).

Physical and Sensory Analysis of Smoked Cheddar Cheeses

Composition Analyses. The unsmoked control cheese had a pH of 5.53±0.00477, a moisture content of 38.9%±0.271, and a fat content of 28.5%±1.06 **(Table 2**). The pH of smoked Cheddar cheeses ranged from 5.27±0.0102 to 5.34±0.0225 and moisture content ranged from 37.0%±0.359 to 38.1%±0.618. The smoking process seemed to have an effect on the pH and moisture of cheeses because both measurements were lower in smoked cheeses than the Control cheese.

Descriptive Analysis. Sensory differences among Cheddar cheeses, smoked and unsmoked, were recorded by a trained panel (**Table 3**), published previously by Del Toro-Gipson et al. (2020a). Cheeses differed in all attributes except for sour and salty tastes. In general, low-intensity smoked cheeses had lower intensities of smoke-derived flavors and higher intensities of cheese-derived flavors than the high-intensity smoked cheeses. These results indicated that in general, low-intensity smoked cheeses more closely resembled the flavor of unsmoked cheese compared to high-intensity smoked cheeses, which was expected. Considering the high-intensity smoked cheeses take on more intense smoke-derived flavors, it is most

effective to examine these cheeses when comparing the flavor impact of the different wood sources on Cheddar cheeses.

Hickory-High had significantly higher intensities of smoke aroma, overall smoke intensity, and meaty/smoky flavor than Cherry-High and Apple-High ($p<0.05$). In addition to having relatively high intensities of campfire/marshmallow and ashy flavor, Hickory-High was mainly characterized by a high intensity of flavors associated with characteristic "smokiness." Cherry-High was characterized by the highest intensity of campfire/marshmallow flavor ($p<0.05$) and the presence of fruity flavor, which was not detected in any other smoked Cheddar cheese. Phenolic flavor was also detected in Cherry-High, which was not detected in Hickory-High or Apple-High. Apple-High was characterized by the highest intensity of ashy flavor ($p<0.05$) and the presence of waxy/green flavor, which did not appear outside of apple wood smoked cheeses. Outside of hickory wood smoked cheeses, meaty/smoky flavor was only detected in Apple-High. Considering the fruity flavor in Cherry-High and the waxy/green flavor in Apple-High did not appear in the unsmoked Control or other smoked cheeses, these flavors are likely smoked-derived and are exclusive to their corresponding smoke. These results support those of the GCO analyses of cherry and apple wood smokes. Several aroma events from cherry wood smoke analysis were described as fruity, such as benzyl alcohol. Similarly, several aroma events from apple wood smoke were described as green and/or waxy, such as 2-methyl-2-butenal.

Flavor Characterization of Smoked Cheddar Cheeses

There were 145 aroma events recorded during GCO analysis of Cheddar cheeses, with compounds originating from both the cheese and wood smoke (**Table 4**). The aroma-active compounds corresponding to 99 of these aroma events were identified by GCMS and 46 were

unknown. Out of the total 145 aroma events recorded for all cheeses, 39 were documented just in the unsmoked control Cheddar cheese. The flavor chemistry of Cheddar cheese has been widely investigated and is well understood. The majority of complex flavors in Cheddar cheese are developed during ripening (Singh et al., 2003). Since the Cheddar cheese used in this study was young (2 mo), its flavor was relatively simplistic by sensory and volatile compound profile compared to an aged Cheddar cheese. Young Cheddar cheese is desirable when manufacturing smoked Cheddar cheese because young cheese leads to a more desirable smoke flavor (Rehman et al., 2003). Aroma-active compounds found in Cheddar cheese largely originate from the biochemical reactions of the bacterial culture during initial fermentation and ripening. As microbes metabolize milk carbohydrates, lipids, and proteins, various flavor compounds are created as byproducts of the fermentation pathways. Similar to how each aroma-active compound in wood smoke can be traced back to the pyrolysis of a single wood polymer (cellulose, hemicellulose, and lignin), aroma-active compounds in Cheddar cheese can be traced back to the microbial metabolism of milk carbohydrates, lipids, or proteins (Singh et al., 2003).

Analysis of cheese-derived flavor compounds. Diacetyl, acetoin, and 2,3-butanediol were detected in Cheddar cheeses. While diacetyl was also detected in wood smoke, it is considered to be important to young Cheddar cheese flavor, along with acetoin and 2,3-butanediol (Murtaza et al., 2014). These compounds have similar buttery/dairy aromas (**Table 4**). These three compounds were produced as byproducts of glycolysis (fermentation of lactose) by the starter or non-starter bacterial culture (Singh et al., 2003). More specifically, they were produced by the metabolism of citrate (Cogan, 1995). Citrate is in relatively low concentrations in Cheddar cheese (0.2%-0.5% w/w) and is usually fully metabolized after 6 mo of ripening. For

this reason, diacetyl, acetoin, and 2,3-butanediol are considered important to the flavor of young Cheddar cheeses (Fox et al., 1990).

Other cheese-derived compounds detected included organic acids, esters, and lactones, which were created by the metabolism of lipids during ripening. Organic acids that were detected included acetic acid, butyric acid, hexanoic acid, nonanoic acid, and decanoic acid. Like diacetyl, acetic acid was detected in both wood smoke and Cheddar cheese. Free fatty acids in Cheddar cheese are generated by the enzymatic breakdown of triglycerides, the main component of fat in cheese (Singh et al., 2003). Organic acids play an important background role in the flavor of Cheddar cheese. Short chain fatty acids like acetic acid and butyric acid usually provide pungent and sharp aromas and were described as vinegar and free-fatty-acid/butyric, respectively, in the current study. Medium chain fatty acids, hexanoic acid and nonanoic acid, were described as free fatty acid/acidic and cheese/free-fatty-acid/floral, respectively in the current study. Finally, longer chain free fatty acids like decanoic acid usually have waxy/soapy aromas (Murtaza et al., 2014) and was described as green/waxy in this study (**Table 4**). Two esters were identified, methyl hexanoate and ethyl decanoate, and were described as floral/oil and waxy/sweet, respectively. Esters are usually associated with fruity/floral/sweet aromas and are the result of further metabolism of fatty acids by non-starter bacteria (Harper et al., 1980). Esters usually play a background role in the flavor of Cheddar cheese. Similar to esters are lactones. Lactones are formed through the intramolecular esterification of a hydroxy fatty acid within a triglyceride (Alewijn et al., 2007). Lactones usually have coconut/milky/peach aromas. Only one lactone was detected in Control, δ-octalactone, which was described as sweet/cooked. Like esters, lactones play a background role in young Cheddar cheese flavor (Singh et al., 2003).

There were also several compounds identified that originated from the metabolism of cheese amino acids, some of the most notable being dimethyl sulfide, methional, and acetophenone. The sulfur-containing amino acids, dimethyl sulfide and methional, are known to be in Cheddar cheese and originate from the metabolism of Methionine, a sulfur-containing amino acid (Singh et al., 2003; Murtaza et al., 2014). Dimethyl sulfide was described as sulfur, and methional was described as potato/brothy. These compounds play a role in the milky and brothy flavors associated with Cheddar cheese. Similar to several other compounds mentioned, acetophenone was identified in wood smoke and Cheddar cheese. In Cheddar cheese, it is generated by the metabolism of Phenylalanine, which is likely where it obtains its aromatic ring (Singh et al., 2003).

Analysis of smoke-derived flavor compounds. Although cheese-derived flavor compounds are important to the flavor of smoked Cheddar cheese, only 39 of the 145 aroma events were present in the unsmoked control cheese, including some overlap with smoked cheeses. The remaining aroma events were recorded solely in smoked Cheddar cheeses. Considering the amount of smoke-derived aroma events and that the trained panel mainly distinguished the cheeses by smoke-derived flavors, it is clear that aroma-active compounds originating from smoke are the most important to consider when characterizing the flavor of Cheddar cheeses smoked using different wood sources. Many of the same compounds that were detected during wood smoke analysis were also detected during smoked Cheddar cheese analysis, indicating that many flavor compounds from the smoke are imparted onto the cheese during the smoking process without causing secondary reactions on the cheese surface. Many of the notable phenolic compounds identified in wood smoke were detected in smoked Cheddar cheese including phenol, guaiacol, 4-methylguaiacol, and syringol (**Table 4**). These compounds

are important to the overall flavor of smoked Cheddar cheese. Other compounds identified in smoked Cheddar cheeses that are believed to be important to flavor were 2-methyl-2-butenal and 2-ethylfuran for apple wood smoked cheese, and benzyl alcohol for cherry wood smoked cheese (**Table 4**).

The phenolic compounds, phenol, guaiacol, 4-methylguaiacol, and syringol, provided the smoked Cheddar cheeses with its characteristic smoky flavor. This is logical considering the importance of these compounds to the flavor of wood smoke as determined by previous literature (Wasserman, 1966; Fiddler et al., 1970; Fujimaki et al., 1974; Maga, 1992). 2-methyl-2-butenal and 2-ethylfuran were determined to be important to apple wood smoked Cheddar cheese based on their associated aromas in relation to the green/waxy flavor identified by the trained panel in Apple-Low and Apple-High. 2-methyl-2-butenal was described as green/waxy/leafy and was only detected in Apple-Low and Apple-High. Similarly, 2-ethylfuran was described as waxy/green and was detected at the highest GCO intensities in Apple-Low and Apple-High. Benzyl alcohol played an important role in the flavor of cherry wood smoked Cheddar cheese. Benzyl alcohol likely contributed to the fruity flavor detected by the trained panel in Cherry-High. Benzyl alcohol had a distinct fruity/cherry aroma and was aroma-active only in cherry wood smoked Cheddar cheeses. Unlike apple and cherry wood smoked cheeses, there were no additional compounds identified in hickory wood smoked cheeses that significantly contributed to flavor, outside the phenolic compounds listed previously. This is in line with the findings of the trained panel profile of hickory smoked cheeses, which did not identify specific flavors unique to hickory wood smoked cheeses.

The importance of these compounds to the flavor of smoked Cheddar cheese is illustrated in the PCA biplot of trained panel DA means and GCO intensities of aroma-active compounds

(68.25% explained variability) (**Figure 1**). All high-intensity smoked cheeses loaded positively on principal component 1 (41.38% explained variability) and were mostly associated with smoke-related flavors. Additionally, sensory attributes such as cooked, whey, and milkfat/lactone, which were cheese-derived flavors, loaded heavily on principal component 1 in the negative direction. This demonstrates that as the intensity of smoke attributes and aroma-active compounds increase, the more smoked cheeses are associated with smoke-derived flavors instead of cheese-derived flavors. Thus, the high-intensity smoked cheeses can be used to distinguish the effects of the smoke wood source on the cheese. The correlations between the sensory attribute and aroma-active compound vectors give insight into the contributions of each compound on the sensory perception of the smoked cheeses. Hickory-High had the highest intensities of the sensory attributes smoke aroma, overall smoke intensity, and meaty/smoky. It also had the highest GCO intensities of guaiacol and syringol. Hickory-High, along with these sensory attribute and aroma-active compound vectors, were heavily loaded on principal component 1 in the positive direction. Considering the position of these sensory attributes, aroma-active compounds, and Hickory-High on the PCA biplot, it is clear that guaiacol and syringol played a large role in the development of these flavors in smoked Cheddar cheese. This is logical considering guaiacol and syringol were described as smoky/meaty/sweet and sweet/woody/smoky, respectively, during GCO analysis of smoked Cheddar cheeses. This effect can also be seen by examining the positions of the campfire/marshmallow and 4-methylguaiacol vectors. Cherry-High and Hickory-High had the most intense campfire/marshmallow flavor of all cheeses, which affected its position on the biplot. Cherry-High loaded positively on principal component 2 (26.88% explained variability), while Hickory-High loaded positively on principal component 1. Campfire/marshmallow and 4-methylguaiacol loaded positively on both principal

components 1 and 2. When also considering that 4-methylguaiacol was described as marshmallow/campfire during GCO analysis, it clearly contributes significantly to the campfire/marshmallow sensory attribute identified by the trained panel. Using this same technique, a similar conclusion can be drawn from the positions of Apple-High, waxy/green, 2-methyl-2-butenal, and 2-ethylfuran vectors, all of which loaded heavily on principal component 2 in the negative direction. These compounds are both associated with green and waxy aromas and likely contribute to the manifestation of this flavor in apple wood smoked cheeses. Finally, benzyl alcohol, fruity, and Cherry-High vectors all loaded heavily on principal component 2 in the positive direction. The fruity/cherry aroma associated with benzyl alcohol is likely contributing to the perception of fruity flavor in Cherry-High.

Quantification of Aroma-active Compounds

Important smoke-derived compounds (phenol, guaiacol, 4-methylguaiacol, and syringol) were quantified in the exterior and interior of each cheese block to ensure the smoke was evenly distributed throughout the cheese block, in line with the patent by which they were manufactured (McLeod, 2017). According to the patent, the smoke flavor intensity of the exterior and interior of the cheese block should be within 10%. Upon analysis, no significant differences in concentration were found for any of the compounds between the exterior and interior of the cheese block ($p>0.05$). These results confirm that the patented method by McLeod (2017) created a smoked cheese block with a consistent smoke flavor throughout.

Phenol, guaiacol, 4-methylguaiacol, syringol, 2-methyl-2-butenal, 2-ethylfuran, benzyl alcohol, diacetyl, and acetoin, were quantified in Cheddar cheeses because they were important to the flavor of the cheeses in this study (**Table 5**). As expected, and in line with GCO and sensory results, guaiacol and syringol were most concentrated in Hickory-High ($p<0.05$). Also as

expected, 2-methyl-2-butenal and 2-ethylfuran were most concentrated in Apple-High ($p<0.05$). Benzyl alcohol was in the highest concentration in Cherry-High but was at similar concentrations in Hickory-High ($p>0.05$). This result was unexpected because benzyl alcohol was not detected during GCO analysis of Hickory-High, nor were fruity flavors documented in hickory smoke or hickory smoked cheeses (Del Toro-Gipson et al., 2020b). It is possible that benzyl alcohol was not aroma-active in Hickory-High due to suppression by co-eluting compounds. In Table 4, 3-methyl-1,2-cyclopentanedione and 2,3-dimethyl-2-cyclopenten-1-one elute on either side and very closely to benzyl alcohol. Additionally, both of these compounds were detected in Hickory-High, but not in Cherry-High. Statistically significant differences were found between the concentration of 4-methylguaiacol among smoked cheeses, however its concentration did not differ as drastically as expected among smoked cheeses considering the differences in GCO intensity recorded among smoked cheeses.

One of the most unexpected results of quantification was that diacetyl and acetoin were at higher concentrations in Control than they were in any of the smoked cheeses ($p<0.05$). Considering diacetyl and acetoin are largely cheese-derived, the concentrations of these compounds were expected to be relatively similar among all cheeses, with diacetyl having the potential to be slightly more concentrated in smoked cheeses because diacetyl was also detected in wood smoke. The current results can potentially be explained by two different hypotheses. First, it is possible that the concentrations of diacetyl and acetoin are lower in smoked cheeses than Control because of SPME fiber adsorption competition. There were significantly more aroma-active compounds identified in the smoked cheeses than Control. Due to their relative concentration in the matrix, it is possible that some of the smoke-derived compounds out-competed diacetyl and acetoin when adsorbing to the SPME fiber, leading them to appear lower

in concentration in smoked cheeses. This scenario has been investigated, and it was determined that smaller and/or more volatile compounds were displaced more than large compounds when adsorbing to a SPME fiber (Trujillo-Rodríguez et al., 2017). Considering diacetyl and acetoin are smaller than most smoke-derived compounds like phenolics, this is a likely justification for these results. Another explanation could be that compounds from the wood smoke reacted with diacetyl and acetoin during the smoking process, which led to reduced concentrations of native cheese compounds and the production of potentially new aroma-active compounds. This is a possibility because there were several smoke-derived compounds identified in smoked Cheddar cheeses that were not identified in wood smoke. 2-ethylfuran is a good example. However, it is important to consider that these compounds could have been in the wood smoke but were not detected due to coelution off the GC column or that compounds were transferred to the cheese at different concentrations than they appear in the smoke due to interactions on the cheese curd surfaces during smoking. This needs to be considered because 2-ethylfuran has been detected in wood smoke previously (Maga, 1987). More research is needed to test these hypotheses definitively.

CONCLUSIONS

The flavor of hickory, cherry, and apple wood smokes were analyzed alone, as well as after they were applied to young Cheddar cheeses. Several compounds were identified in all smokes, while others were exclusive to a single smoke. Differences in the volatile compound profiles of each wood smoke are responsible for differences in overall sensory perception. The application of smoke significantly impacted the overall flavor of the cheese. Cheeses smoked with different wood sources could be distinguished from one another by trained panel descriptive analysis and volatile compound analysis. Hickory wood smoked Cheddar cheese was

distinguished by high intensities of flavors related to smoke in general including smoke aroma, overall smoke intensity, and meaty/smoky. The trained panel identified a fruity flavor that was only present in cherry wood smoked Cheddar cheese, making fruity flavor a distinguishing characteristic of this cheese. Similarly, a waxy/green flavor was detected exclusively in apple wood smoked cheeses, a distinct attribute of these smoked cheeses. Through volatile compound analysis, phenolic aroma-active compounds such as phenol, guaiacol, 4-methylguaiacol, and syringol, were determined to be largely responsible for the characteristic "smoky" flavor of smoked Cheddar cheeses. Benzyl alcohol likely contributed to the fruity flavor identified in cherry wood smoked cheese. Similarly, 2-methyl-2-butenal and 2-ethylfuran were determined to contribute to the waxy/green flavor in apple wood smoked cheeses. The results from this study can help smoked cheese manufacturers understand the flavors present in Cheddar cheeses smoked using different wood sources. Additionally, smoked cheese manufacturers will be able to understand which aroma-active volatile compounds contribute most to the overall sensory perception of Cheddar cheese smoked using a particular wood source.

ACKNOWLEDGEMENTS

Funding for this research was provided in part by Hilmar Cheese (Hilmar, CA), Dairy West (Meridian, ID), and the National Dairy Council (Rosemont, IL).

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Tables and Figures

Table 1: Aroma-active compounds identified in wood smokes by headspace solid phase microextraction gas chromatography olfactometry.

Retention Index ZB-5	Aroma-active Compound	Aroma Description	Method of ID	Hickory Smoke	Cherry Smoke	Apple Smoke
<600	unknown	stinky, sulfur	O			+
<600	unknown	ashy	O		+	
<600	sec-butylamine	pungent, acrid	O, RI, MS	+		
<600	methylglyoxal	vanilla, protein powder	O, MS	+	+	
<600	diacetyl	buttery	O, RI, MS	+	+	+
643	acetic acid	vinegar, sour	O, RI, MS	+	+	+
654	2,3-pentanedione	buttery, caramel	O, RI, MS	+		+
658	1-hydroxy-2-propanone	buttery, caramel	O, RI, MS	+		
724	3-penten-2-one	smoky	O, RI, MS		+	
761	toluene	smoky	O, RI, MS	+	+	+
771	unknown	smoky, meaty, rubber	O	+		
771	2-methyl-2-butenal	floral, green	O, RI, MS	+		+
783	unknown	phenolic, rubber, metallic	O		+	+
784	2,3-dimethylpentanal	rubber, glue-like	O,MS,RI	+		
795	unknown	phenolic	O		+	
810	unknown	cleaner, chemical	O	+		
819	2-methyl-2-pentenal	fruity, grassy	O, RI, MS	+	+	
827	furfural	caramel, grain	O, RI, MS	+	+	+
853	furfuryl alcohol	sweet, milky, nutty	O, RI, MS	+	+	
854	unknown	phenolic, butyric	O			+
856	ethylbenzene	metallic, phenolic	O, RI, MS		+	+
867	1-(acetyloxy)-2-propanone	grain, free fatty acid	O, RI, MS	+	+	+
875	unknown	free fatty acid	O			+

Table 1 (continued):

881	4-cyclopentene-1,3-dione	smoky, ashy	O, RI, MS		+	+
889	styrene	phenolic, metallic	O, RI, MS	+		+
902	2-methyl-2-cyclopenten-1-one	woody	O, RI, MS			+
903	2-cyclohexen-1-one	cherry, smoke, woody	O, RI, MS	+	+	
903	unknown	fruity, rosy	O		+	
907	furfuryl formate	metallic, phenolic	O, RI, MS		+	
912	unknown	rubbery, phenolic	O			+
915	2-acetylfuran	roasted, meaty	O, RI, MS	+		+
919	2-hydroxy-2-cyclopenten-1-one	smoky, buttery	O, RI, MS		+	+
919	2(5H)-furanone	woody, burnt	O, RI, MS	+	+	
925	unknown	butryic	O		+	+
930	2-cyclopenten-1-one	almond, roasted	O, RI, MS	+		
931	unknown	potato brothy	O	+		+
950	unknown	cleaner, floral, acidic	O	+	+	+
955	benzaldehyde	sweet, nutty	O, RI, MS	+		
959	5-methylfurfural	smoky, chemical, sweet	O, RI, MS	+	+	+
981	phenol	phenolic	O, RI, MS	+	+	+
988	methyl levulinate	sweet, smoky	O, RI, MS	+		
993	unknown	plastic, earthy	O		+	+
1008	unknown	fruity, sweet	O		+	
1008	unknown	metallic, phenolic	O	+		
1010	2-furylethylketone	fruity, cinnamon	O, RI, MS			+
1023	2-hydroxy-3-methyl-2-cyclopenten-1-one	sweet, woody, caramel	O, RI, MS	+		+
1026	3-methyl-1,2-cyclopentanedione	smoky, rubbery	O, RI, MS		+	+
1035	benzyl alcohol	fruity, cherry	O, RI, MS		+	
1054	2-hydroxy-3,4-dimethyl-2-cyclopenten-1-one	mollasses	O, RI, MS	+		
1055	2-methylphenol	smoky, earthy, woody	O, RI, MS	+		+
1064	acetophenone	floral, honey	O, RI, MS	+	+	+

Table 1 (continued):

1066	4-methylbenzaldehyde	floral, rosy	O, RI, MS	+		
1071	3-methylphenol	earthy, brown	O, RI, MS	+		+
1073	2-methylbenzaldehyde	floral, sweet, chemical	O, RI, MS	+	+	
1075	p-cresol	ashy, smoky	O, RI, MS			+
1092	guaiacol	smoky, meaty, phenolic	O, RI, MS	+	+	+
1098	2-nonen-1-ol	apple	O, RI, MS			+
1106	2-methylbenzofuran	sweet, vanilla	O, RI, MS	+		
1113	maltol	vanilla, smoky	O, RI, MS		+	+
1120	3-ethyl-2-hydroxy-2-cyclopenten-1-one	smoky, campfire, tobacco	O, RI, MS	+		+
1123	2,6-dimethylphenol	menthol, smoky	O, RI, MS	+		+
1124	unknown	smoky, ashy	O		+	
1141	3-ethylphenol	smoky, ashy, apple	O, RI, MS			+
1143	2,4-dimethylphenol	smoky, ashy, leather	O, RI, MS		+	
1143	2,5-dimethylphenol	roasted, tobacco, apple	O, RI, MS			+
1157	3-methylindene	bandaid, smoky	O, RI, MS	+		+
1165	4-ethylphenol	smoky, meaty, plastic	O, RI, MS		+	
1177	5-methylguaiacol	smoky, ashy	O, RI, MS	+		
1183	4-methylguaiacol	marshmallow, campfire	O, RI, MS	+	+	+
1184	naphthalene	smoky, ashy	O, RI, MS			+
1190	unknown	cucumber, coconut	O	+	+	+
1200	decanal	sweet, apple, cinnamon	O, RI, MS			+
1210	unknown	musty, smoky	O		+	
1212	2,3-dihydrobenzofuran	sweaty, green, waxy	O, RI, MS			+
1226	unknown	leather, smoky	O	+		
1227	5-hydroxymethylfurfural	cleaner, leather	O, RI, MS		+	
1233	3,4-dimethoxytoluene	waxy, soapy	O, RI, MS			+
1242	unknown	smoky, leather, phenolic	O		+	+
1255	2-methoxybenzenethiol	sweet, menthol	O, RI, MS	+		

Table 1 (continued):

1264	3,5-dimethoxytoluene	menthol, tobacco	O, RI, MS	+		+
1277	4-ethylguaiacol	smoky, spicy, vanilla	O, RI, MS	+	+	+
1284	unknown	cooked, spicy	O		+	
1293	2-methylnaphthalene	waxy, chemical, woody	O, RI, MS	+	+	+
1310	unknown	smoky, phenolic	O, RI, MS		+	
1313	4-vinylguaiacol	woody, spicy, phenolic	O, RI, MS	+	+	+
1331	unknown	medicinal	O			+
1352	syringol	smoky, rubbery, woody	O, RI, MS	+	+	+
1370	4-propylguaiacol	charcoal, smoky, spicy	O, RI, MS	+	+	+
1386	biphenyl	cleaner, soapy, menthol	O, RI, MS		+	+
1391	unknown	smoky, meaty	O		+	
1394	1-ethylnaphthalene	pine resin, floral	O, RI, MS	+		
1395	vanillin	vanilla, smoky	O, RI, MS	+		
1409	4-propenylguaiacol	woody, brown, ashy	O, RI, MS	+	+	+
1429	unknown	cleaner, apple	O			+
1443	trans-isoeugenol	smoky	O		+	
1443	3,5-dimethoxy-4-hydroxytoluene	smoky, woody	O, RI, MS	+		
1472	2,6-di-tert-butylbenzoquinone	smoky, roasted, tobacco	O, RI, MS			+
1473	unknown	smoky, lactone	O		+	
1497	unknown	tobacco, smoky, savory	O			+
1498	apocynin	vanilla	O, RI, MS	+		
1520	unknown	apple, smoky	O			+
1546	unknown	lactone, floral, slight smoky	O		+	
1548	unknown	woody	O	+		
1554	unknown	smoky, nutty	O	+		
1568	4-vinylsyringol	smoky, nutty	O, RI, MS	+		+
1569	acetoveratrone	grain-like	O, RI, MS	+		
1602	4-allylsyringol	smoky, spicy, savory	O, RI, MS	+	+	+

Table 1 (continued):

1624	unknown	floral, rosy, sweet	O		+	
1636	unknown	soil, earthy	O, RI, MS			+
1661	syringaldehyde	sweet, nutty, spicy	O, RI, MS	+	+	+
1701	trans-4-propenylsyringol	spicy, floral	O, RI, MS	+		+
1893	γ-tetradecalactone	resinous	O, RI, MS	+		

Plus sign (+) indicates the presence of the compound in the wood smoke; blank indicates that the compound is not present in the wood smoke

Retention indices are calculated as the mean of the retention indices from GCO and GCMS on a ZB-5ms column

Method of identification key: O – identified by olfactometer port; RI – identified by comparing the calculated retention index with previous literature; MS – identified by mass spectra obtained from GCMS.

Table 2: Proximate analysis of control and smoked Cheddar cheeses: pH, moisture content, and fat content

Sample	pH	Moisture Content (%)	Fat Content (%)
Control	5.53 ± 0.00477	38.9 ± 0.271	28.5 ± 1.06
Apple-High	5.27 ± 0.0125	37.2 ± 0.476	-
Apple-Low	5.30 ± 0.0113	37.6 ± 0.518	-
Cherry-High	5.27 ± 0.0102	37.0 ± 0.359	-
Cherry-Low	5.27 ± 0.00817	37.6 ± 0.0946	-
Hickory-High	5.31 ± 0.0160	38.1 ± 0.265	-
Hickory-Low	5.34 ± 0.0225	38.1 ± 0.618	-

pH was conducted at 21.5ºC

Fat content is assumed to be the same for all cheeses within a lot since each lot (6 smoked cheeses, 1 control) were manufactured from the same lot of milk

Table 3: Descriptive analysis trained panel means of control and smoked Cheddar cheeses

	Control	Apple-Low	Apple-High	Cherry-Low	Cherry-High	Hickory-Low	Hickory-High
Overall Aroma	2.6d	2.8cd	3.4b	2.3d	3.5b	3.1bc	4.4a
Smoke Aroma	ND	2.3c	3.1b	1.8d	3.4b	2.6c	4.0a
Overall Smoke Intensity	ND	2.3d	3.3b	1.7e	3.2b	2.8c	4.1a
Cooked	4.3a	3.4c	3.3c	3.7b	3.3c	3.7b	3.3c
Whey	3.3a	2.8b	2.5b	2.7b	2.5b	2.7b	2.4b
Milkfat/Lactone	3.6a	3.2b	3.2b	3.2b	3.1b	3.2b	3.1b
Campfire/Marshmallow	ND	1.6d	2.0bc	1.8c	2.5a	2.0c	2.2b
Meaty/Smoky	ND	ND	1.0b	ND	ND	1.1b	3.6a
Fruity	ND	ND	ND	ND	1.6	ND	ND
Waxy/Green	ND	1.0b	1.6a	ND	ND	ND	ND
Phenolic	ND	ND	ND	0.6a	0.6a	ND	ND
Ashy	ND	2.3c	3.2a	1.3d	2.3c	2.8b	2.9b
Sour	2.9a	3.0a	2.9a	2.9a	3.0a	2.9a	2.9a
Salty	3.1a	3.0a	2.9a	2.9a	3.0a	3.1a	3.1a
Sweet	2.0b	2.1ab	2.3a	2.2a	2.1ab	2.0b	2.0b
Umami	2.1b	2.7a	2.6a	2.7a	2.7a	2.7a	2.7a

Values published previously by Del Toro-Gipson et al. (2020a)
Attributes were scored on a 0 to 15pt scale where 0=imperceptible and 15=extremely intense
Different letters in rows indicate significant differences ($p<0.05$)
ND = not detected

Table 4: Aroma-active compounds identified in control and smoked Cheddar cheeses by headspace solid phase microextraction gas chromatography olfactometry.

Retention Index ZB-5	Aroma-active Compound	Aroma Description	Method of ID	Hickory-High	Hickory-Low	Cherry-High	Cherry-Low	Apple-High	Apple-Low	Control
<600	acetaldehyde	cleaner, solvent	O, RI, MS		+		+	+	+	
<600	dimethyl sulfide	sulfur	O, RI	+	+	+	+	+	+	+
<600	carbon disulfide	sulfur	O, RI, MS	+		+	+			
<600	diacetyl	buttery	O, RI, MS	+	+	+	+	+	+	+
606	unknown	buttery	O							+
621	2-methylfuran	nutty, milky, green	O, RI, MS	+		+	+		+	
623	acetic acid	vinegar	O, RI, MS	+	+	+	+	+	+	+
656	unknown	sweet, milky	O		+	+	+		+	
670	3-pentanone	lemon cleaner	O, RI, MS					+		
680	2-pentanone	green, fruity, sweet	O, RI, MS			+	+	+	+	
681	2-ethylfuran	waxy, green	O, RI, MS	+				+	+	
698	acetoin	buttery, sour	O, RI, MS	+	+	+				+
705	unknown	cooked, milky	O		+				+	
708	2-vinylfuran	waxy, smoky, phenolic	O, RI, MS	+			+	+	+	
719	3-methyl-3-buten-1-ol	fruity, candy, sweet	O, RI, MS		+					
740	unknown	green, grassy	O							+
746	2-methyl-2-butenal	green, waxy, leafy	O, RI, MS					+	+	
747	4-methyl-2-pentanol	meaty, savory, cooked	O, RI, MS	+		+	+	+		
769	prenol	plastic	O, RI, MS							+
771	toluene	phenolic, rubbery	O, RI, MS	+	+	+		+	+	
773	2-methyl-2-buten-1-ol	soil, earthy, leafy, green	O, RI, MS						+	
779	2,3-butanediol	milky, cooked, solvent	O, RI, MS	+		+	+	+		+
792	2,3-dimethylcyclopentanone	smoky, spicy, phenolic	O, RI, MS			+	+			
801	2-ethyl-5-methylfuran	phenolic, bandaid, metallic	O, RI, MS	+	+	+	+	+	+	
817	2,3,5-trimethylfuran	brothy, meaty, savory	O, RI, MS			+	+			
818	unknown	flowery, green, grassy	O							+
826	unknown	green	O						+	

Table 4 (continued):

833	2-cyclopenten-1-one	caramelized, grain, nutty	O, RI, MS	+	+	+	+				
837	unknown	green, waxy	O					+	+	+	
841	butanoic acid	FFA, butyric	O, RI, MS		+					+	
845	isopropyl butyrate	smoky, green	O, RI, MS			+		+			
856	furfuryl alcohol	sweet, smoky, vanilla	O, RI, MS	+	+	+	+				
862	ethylbenzene	cleaner, sweaty	O, RI, MS	+		+	+		+	+	
870	1,3-dimethylbenzene	rubber, metallic, FFA	O, RI, MS			+		+	+		
876	p-xylene	nutty, butyric	O, RI, MS		+		+			+	
876	pentanoic acid	butryic	O, RI, MS			+			+		
879	1-(acetyloxy)-2-propanone	sweet, buttery, nutty	O, RI, MS	+	+	+		+			
886	3-heptanone	FFA, cheesy	O, RI, MS	+							
886	o-xylene	green, floral, rosemary	O, RI, MS		+			+	+		
891	styrene	plastic, cleaner	O, RI, MS	+		+	+	+	+		
896	2-heptanone	milky, powdered cheese	O, RI, MS							+	
903	2-methyl-2-cyclopenten-1-one	brothy, milky, bread	O, RI, MS			+	+		+		
905	unknown	milky, waxy	O					+		+	
913	2-acetylfuran	phenolic, plastic	O, RI, MS	+	+	+	+	+			
919	dimethylsulfone	phenolic, brothy	O, RI, MS	+					+	+	
921	methyl hexanoate	floral, oil	O, RI, MS		+					+	
930	methional	potato, brothy	O, RI, MS	+		+	+	+	+	+	
933	2-cyclohexen-1-one	vegetable	O, RI, MS	+							
936	unknown	caramelized, cooked	O						+	+	
948	2-methylcyclohexanone	green, aldehydic, fresh car	O, RI, MS					+			
958	benzaldehyde	nutty (linger 20s)	O, RI, MS		+						
963	5-methylfurfural	brothy, caramelized	O, RI, MS	+		+	+				
966	3-methyl-2-cyclopenten-1-one	waxy, cleaner	O, RI, MS	+					+		
976	hexanoic acid	FFA, acidic	O, RI, MS				+			+	
983	phenol	rubber, phenolic	O, RI, MS	+	+	+	+	+	+		
986	unknown	nutty, green, fruity	O		+	+	+				
987	6-methyl-5-hepten-2-one	woody, green, waxy	O, RI, MS					+	+	+	

Table 4 (continued):

995	benzofuran	smoky, earthy	O, RI, MS	+						
1006	allyl valerate	green, leafty, rubber	O, RI, MS	+				+		
1013	o-cymene	sweet, nutty	O, RI, MS		+					
1017	4-methoxytoluene	fresh, cleaner, phenolic	O, RI, MS			+				
1018	p-cymene	metallic, cleaner	O, RI, MS				+		+	
1022	limonene	chemical, citrus, green	O, RI, MS		+					+
1025	3-methyl-1,2-cyclopentanedione	ashy	O, RI, MS	+						
1026	2-ethyl-1-hexanol	green, waxy, rubber	O, RI, MS					+		+
1032	benzyl alcohol	fruity, cherry	O, RI, MS			+	+			
1038	2,3-dimethyl-2-cyclopenten-1-one	savory, smoky, green	O, RI, MS	+	+			+	+	
1042	indene	smoky, phenolic	O, RI, MS		+					
1044	2-methylphenol	smoky, meaty, rubber	O, RI, MS	+				+		
1064	acetophenone	nutty, fruity, floral	O, RI, MS	+				+		+
1079	α,α-dimethylbenzyl alcohol	musty, waxy	O, RI, MS	+				+		
1088	guaiacol	smoky, meaty, sweet	O, RI, MS	+	+	+	+	+	+	
1095	δ-hexalactone	vanilla, sweet	O, RI, MS		+			+	+	
1101	nonanal	green, fatty, cheesy	O, RI, MS							+
1108	maltol	vanilla, sweet	O, RI, MS		+			+		
1109	2-methylbenzofuran	smoky, sweet, woody	O, RI, MS	+		+			+	
1110	4-ethylanisole	woody, sweet	O, RI, MS			+				
1110	3-ethyl-2-hydroxy-2-cyclopenten-1-one	smoky, caramel, woody	O, RI, MS				+	+		
1111	p,α-dimethylbenzyl alcohol	smoky, ashy, vanilla, sweet	O, RI, MS			+				
1118	unknown	smoked meat	O		+					
1121	methyl octanoate	sweet, green	O, RI, MS				+	+		
1122	unknown	vanilla, smoky	O					+	+	
1125	unknown	ashy, meaty, smoky	O			+	+	+		
1127	unknown	cooked, brown, maillard, cheesy	O							+
1132	unknown	vanilla, caramel, smoky	O					+	+	
1135	unknown	cheesy, brown	O							+
1138	2,6-dimethylphenol	smoky, meaty, ashy	O, RI, MS			+	+			

Table 4 (continued):

1141	2,4-dimethylphenol	smoky, meaty, grain	O, RI, MS	+					+		
1142	2,5-dimethylphenol	phenolic, coffee, fermented	O, RI, MS	+				+			
1145	2,3-dimethylphenol	campfire	O, RI, MS					+			
1154	2-ethylphenol	smoky, ashy, brown	O, RI, MS	+	+						
1168	unknown	smoky, meaty, caramelized	O				+	+			+
1169	5-methylguaiacol	smoke, meaty	O, RI, MS					+			
1173	cis-3-hexen-1-yl butyrate	cotton candy	O, RI, MS				+	+			
1182	naphthalene	ashy, rubber	O, RI, MS	+	+	+		+			
1201	decanal	green, floral, citrus, aldehydic	O, RI, MS								+
1202	4-methylguaiacol	marshmallow, campfire	O, RI, MS	+	+	+	+	+	+		
1220	unknown	smoky, bready, sweet	O			+			+		
1231	unknown	smoky, ashy, marshmallow	O	+	+			+			
1249	unknown	phenolic, smoky, ashy	O	+	+		+	+	+		
1250	caprolactam	vanilla, marshmallow, campfire	O, RI, MS			+	+				
1252	1-(4-methyl-2-thienyl)ethanone	smoky, apple?	O, RI, MS					+			
1259	nonanoic acid	cheesy, FFA, floral	O, RI, MS			+	+				+
1274	2,5-dihydroxyacetophenone	smoky, spicy	O, RI, MS	+							
1279	4-ethylguaiacol	waxy, phenolic, smoky	O, RI, MS			+		+	+		
1285	pentyl hexanoate	smoky, dried leaves, ashy	O, RI, MS		+						
1289	δ-octalactone	sweet, cooked	O, RI, MS			+		+	+		+
1302	unknown	smoky, metallic	O	+	+				+		
1316	4-vinylguaiacol	woody, soapy	O, RI, MS	+		+	+				
1348	syringol	sweet, wood, smoky	O, RI, MS	+	+	+	+	+			
1361	decanoic acid	green, waxy	O, RI, MS					+	+		+
1364	unknown	sweet, smoky	O		+	+	+				
1379	unknown	smoky, nutty, caramelized	O	+		+	+	+	+		
1392	ethyl decanoate	waxy, sweet	O, RI, MS		+						+
1400	unknown	ashy, smoky	O			+					
1415	caryophyllene	musty, phenolic, smoky	O, RI, MS					+			
1447	4-methylsyringol	meaty, smoky, ashy	O, RI, MS	+		+	+	+			

Table 4 (continued):

1451	α-methyl-p-isopropylhydrocinnamic aldehyde	meaty	O, RI, MS				+				
1468	unknown	caramelized, nutty, phenolic	O		+		+				+
1472	unknown	cooked, pasta	O								+
1476	unknown	sweet, brown, smoky	O	+		+				+	
1495	cis-3-methyl-γ-nonalactone	caramelized, cooked milk	O, RI, MS			+					
1498	δ-decalactone	sweet, charred	O, RI, MS	+			+	+			
1513	unknown	sweet, caramel, nutty	O	+	+						
1522	unknown	menthol, smoky	O			+		+	+		
1530	ethyl 4-ethoxybenzoate	ashy, minty	O, RI, MS			+	+				
1536	unknown	sweet, smoky	O	+	+			+			
1552	unknown	honey, sweet	O				+	+			
1553	dodecanoic acid	pine needles	O, RI, MS			+	+				
1573	unknown	brown, smoky	O	+	+	+		+	+		
1575	unknown	cooked, savory, caramelized	O								+
1580	ethyl dodecanoate	sweet, floral, toasted	O, RI, MS		+		+				
1592	unknown	plastic, cleaner	O	+		+					
1607	unknown	lactone, milky	O			+					+
1627	unknown	smoky, rubber, metallic	O	+				+	+		
1651	unknown	waxy, sweaty, metallic	O			+	+		+		
1661	unknown	nutty, sweet, ashy	O	+	+	+		+			
1696	unknown	waxy, phenolic, green	O	+		+		+	+		
1751	unknown	smoky, almond	O		+			+			
1773	unknown	caramelized, smoky	O			+		+			
1837	unknown	smoke, meat, roasted	O	+		+					
1862	unknown	brothy, brown	O	+	+	+	+		+	+	
1884	unknown	phenolic, metallic	O			+	+	+			
1897	unknown	smoky	O			+		+			
1975	unknown	cheesy, milky	O			+					+

Plus sign (+) indicates the presence of the compound in the wood smoke; blank indicates that the compound is not present in the wood smoke

Retention indices are calculated as the mean of the retention indices from GCO and GCMS on a ZB-5ms column
Method of identification key: O – identified by olfactometer port; RI – identified by comparing the calculated retention index with previous literature; MS – identified by mass spectra obtained from GCMS.

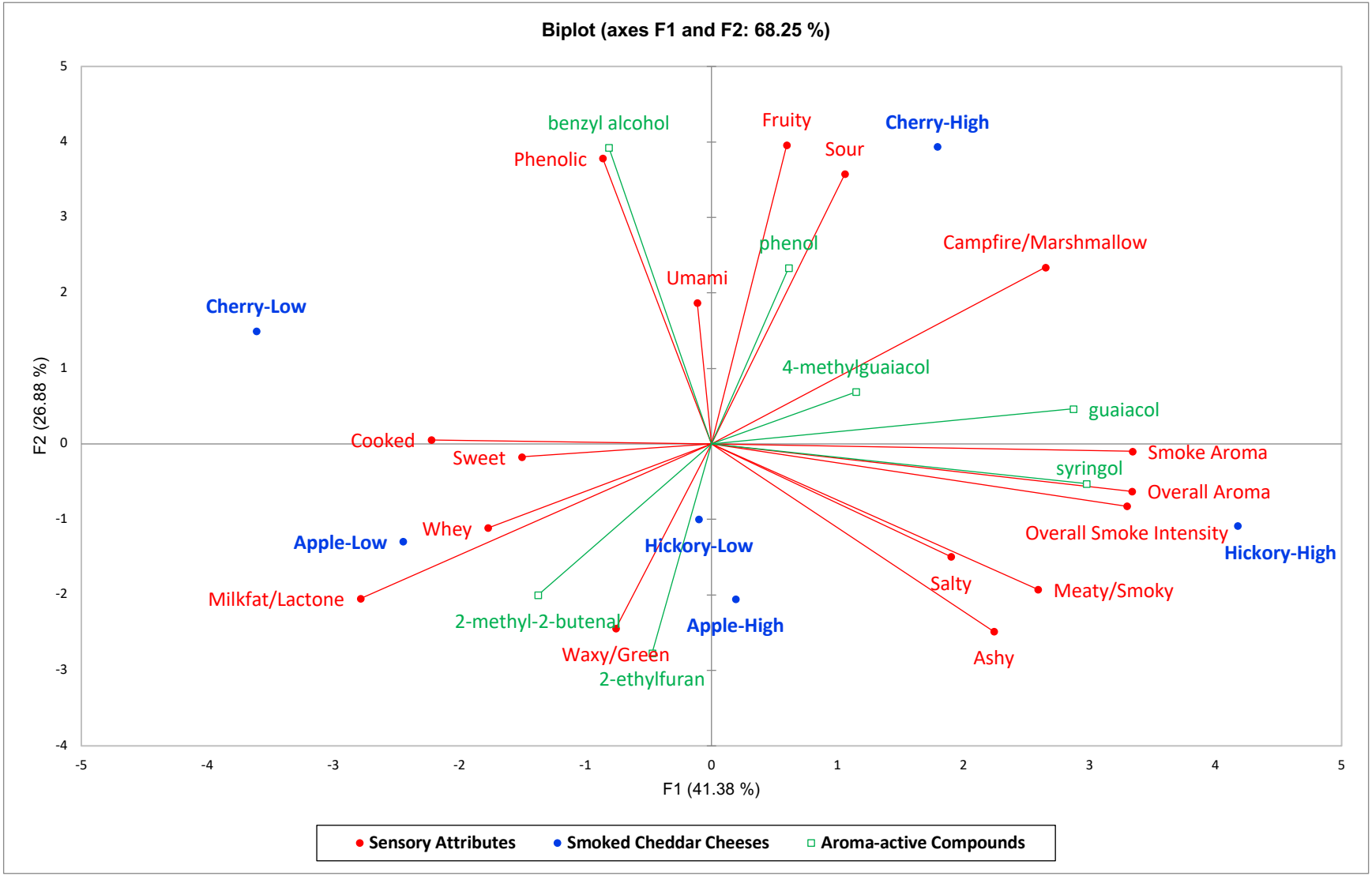

Figure 1: Principal component analysis biplot of descriptive analysis means for smoked Cheddar cheeses overlaid with GCO aroma intensities of selected smoke-derived aroma-active compounds

Table 5: Concentrations of selected aroma-active compounds in control and smoked Cheddar cheeses (mg/kg)

Aroma-active Compound	**Control**	**Apple-High**	**Apple-Low**	**Cherry-High**	**Cherry-Low**	**Hickory-High**	**Hickory-Low**
Diacetyl[1]	21.5a	6.58c	9.04b	3.40d	2.09d	5.90c	2.81d
Acetoin[2]	95.6a	13.8bcd	18.5bc	7.59cd	5.89d	23.6b	11.5cd
*2-methyl-2-butenal[3]	ND	34.8a	18.5bc	10.4cd	2.91de	20.7b	6.10de
2-ethylfuran[4]	ND	0.353a	0.298ab	0.190bc	ND	0.115c	ND
Phenol[5]	ND	0.492c	0.466d	0.522b	0.414e	0.562a	0.433e
Benzyl alcohol[6]	ND	0.426b	0.426b	0.456a	0.427b	0.451a	0.413c
Guaiacol[7]	ND	0.149b	0.089c	0.138b	0.057d	0.269a	0.076cd
4-methylguaiacol[8]	ND	0.454b	0.447c	0.454b	0.441d	0.470a	0.444cd
Syringol[9]	ND	0.982b	0.595c	0.995b	0.366c	1.86a	0.501c

Different letters in rows indicate significant differences ($p<0.05$)
*Concentrations in µg/kg
ND = not detected
LOQ – limit of quantification – estimated by comparing the target response of noise in the control cheese (multiplied by 10) with the target response of the lowest concentration used in the standard curve for the corresponding compound. The resulting ratio was multiplied by the known lowest concentration of the corresponding compound.
[1]LOQ: 5.28 ug/kg
[2]LOQ: 251 ug/kg
[3]LOQ: 0.256 ug/kg
[4]LOQ: 1.82 ug/kg
[5]LOQ: 143 ug/kg
[6]LOQ: 6.87 ug/kg
[7]LOQ: 12.8 ug/kg
[8]LOQ: 4.41 ug/kg
[9]LOQ: 41.3 ug/kg

CHAPTER 3:
CONSUMER DESIRES AND PERCEPTIONS OF LACTOSE-FREE MILK

Consumer Desires and Perceptions of Lactose-free Milk

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INTERPRETIVE SUMMARY

Fluid milk consumption has declined in the United States, but lactose-free milk consumption has increased. Three consumer clusters were identified with variable specific desires for lactose free milk. The overall preferred lactose-free milk was packaged in a half-gallon cardboard carton, ultrapasteurized with a sweet taste.

ABSTRACT

Fluid milk consumption has declined in the United States, but lactose free dairy milk (LFM) sales have steadily increased. It is important to understand how consumers perceive LFM and what consumers value when purchasing LFM. This study characterized consumer perceptions and preferences for LFM. Three 1.5 hour focus groups (n=25), an online survey (n=331), trained panel descriptive analysis, and a consumer acceptance taste test (n=160) were conducted with LFM consumers. Focus groups were evaluated by frequency of responses. From the focus groups, price was a primary choice driver of LFM. Habit and flavor familiarity with cow's milk were a major driver of selection of LFM over plant-based alternatives for consumers. Higher sweetness and lower viscosity were the primary sensory differences between LFM and traditional milk and were viewed negatively in general. The online survey included Kano questions, Maximum Difference Scaling (MaxDiff), and an Adaptive Choice Based Conjoint (ACBC). The data gathered from these techniques provided insight into the perceptions and purchase habits of consumers. Kano data demonstrated consumer attitudes towards the presence or absence of product attributes. MaxDiff scaled the importance of product attributes to consumers. ACBC provided insight into consumer purchase habits by simulating a purchase decision through an online interface. Attributes evaluated included price, packaging material, package size, lactose removal method, shelf life, sweetness, texture, nutrition claims, and label claims. Survey responses were analyzed by univariate and multivariate statistics. Survey results quantitatively confirmed many focus group observations. Price, texture, sweetness, shelf life, and package size were the most important attributes to LFM consumers. A low price, ultrapasteurized LFM in a half-gallon cardboard package was the ideal LFM. Descriptive analysis of nine commercial LFM followed by consumer acceptance testing was conducted. External preference

mapping was conducted with trained panel and consumer acceptance results. Consumer acceptance testing of commercial LFM revealed three consumer clusters with distinct preferences for LFM flavor and texture. High sweet taste was a driver of like for the overall population, and eggy flavor and viscosity were drivers of dislike. Knowledge of consumer preferences for LFM will provide actionable insights for new product development within the dairy industry for this market segment.

Key words: lactose-free milk, focus group, conjoint analysis, descriptive analysis, consumer acceptance

INTRODUCTION

Fluid milk sales have decreased in the United States by 3.7% from 2017 to 2018 (Dairy Management Inc., 2018). According to the United States Department of Agriculture, the total production weight of all fluid milk beverages decreased by nearly 10% from 1987 to 2018 (USDA, 2019). There are many issues that have led to this decline, such as higher frequency of animal rights activism and vegan diets, but one of the largest reasons consumers avoid dairy is due to a rise in the "diagnosis or perception of lactose intolerance" in developed nations (Zingone et al., 2017). It is estimated that more than 70% of the world's population has some degree of lactose intolerance (Messia et al., 2007). At over 146 million gallons, lactose-free dairy milk (**LFM**) currently accounts for 4.0% of the total volume of fluid dairy milk sold in the United States per year, and sales are increasing. LFM sales grew by 12% in 2017, with an additional 9% increase in 2018 (Dairy Mangaement Inc., 2018). Although LFM is rising in popularity among consumers, there is very little published research on LFM in general, and even less concerning consumer preferences and purchase habits of LFM. In light of its swift rise in popularity, it is important to understand consumer desires and perceptions surrounding LFM to support the dairy industry in providing LFM products that meet the needs of consumers.

Adhikari et al. (2010) conducted a consumer study on LFM to determine consumer acceptability of LFM compared to traditional milk. This study compared the sensory characteristics of LFM at different fat contents to the corresponding traditional milks using a trained descriptive analysis panel and consumer liking. LFM had a more intense sweet taste than traditional milk. In both traditional milk and LFM, consumer liking and fat content of the milk were directly related, meaning as fat content of the milk increased, consumer liking scores also increased (Adhikari et al., 2010). However, a full profile of consumer expectations and

perception of LFM was not conducted. Most notably, consumers were not screened to be actual LFM consumers, only milk consumers. LFM consumer purchase habits and preferences such as packaging, pricing, and labeling, were not investigated. These product attributes heavily affect product success in the marketplace (Harwood and Drake, 2018; McLean et al., 2017). Additionally, the objective of the study was to compare the sensory properties of LFM and traditional milk, not to investigate the specific sensory properties of LFM that lead to consumer acceptance.

While Adhikari et al. (2010) is the only LFM consumer study to our knowledge, there have been several studies involving LFM that have focused on the activity of β-galactosidase in milk, as well as its chemical effects on the quality of the milk. Nielsen et al. (2018) measured the proteolytic side-activity of β-galactosidase in ultra-high temperature (**UHT**) pasteurized LFM compared to unhydrolyzed UHT milk and found higher levels of free amino acids in the lactose-hydrolyzed sample, which indicated that β-galactosidase exhibited proteolytic activity in milk and can lead to a decreased product shelf life (Nielsen et al., 2018). Jansson et al. (2014) conducted a similar study with UHT lactose-hydrolyzed and unhydrolyzed milk, but the study was conducted over a nine-month storage time. They also found β-galactosidase to exhibit signs of proteolysis. Additionally, they observed higher concentrations of furosine and 2-methylbutanal in the lactose-hydrolyzed samples, indicating they were more prone to Maillard and other reactions involving sugars (Jansson et al., 2014). Messia et al. (2007) conducted a study focused on measuring Maillard products such as furosine and lactulose in lactose-hydrolyzed milk and unhydrolyzed milk. Over time, the concentration of furosine was significantly higher in lactose-hydrolyzed milk compared to traditional milk, with the opposite effect for lactulose. A higher concentration of furosine, which is a heat load indicator, suggests

that lactose-hydrolyzed milk is more prone to Maillard reactions than traditional milk. Therefore, lactose-hydrolyzed milk may contain Maillard reaction flavors (such as higher cooked or caramelized flavors) not present in traditional milk with the same heat load. Messia et al. (2007) concluded that lactose-hydrolzyed milk was less chemically stable than traditional milk, given the greater reactivity of glucose and galactose compared to lactose.

Focus groups are a popular qualitative data collection method which allow researchers to quickly learn consumer attitudes. Focus groups provide the opportunity to ask open-ended questions to gain useful insights into consumer perceptions. The process encourages the participants to determine the importance of product attributes using their own words and thoughts, which can enhance an understanding of consumer perspectives (Kitzinger, 1995). Focus groups also allow participants to interact with each other in open discussions that lead to information that a researcher might have never considered.

In addition to understanding consumer perception of LFM through focus groups, it is also important to identify consumer desires that influence purchase decisions. Conjoint analysis is an analytical technique that simulates this scenario (Orme, 2010). In contrast to the qualitative nature of focus groups, conjoint analysis is a quantitative technique and is often applied to data gathered from an online survey exercise. During a conjoint survey exercise, consumers are presented with hypothetical product concepts which are randomly generated by the software. Survey participants are requested to choose which hypothetical product concept is most appealing. By having consumers evaluate various product attributes within product concepts, the importance of each product attribute can be assessed. Although there are many different types of conjoint analyses, Adaptive Choice Based Conjoint (**ACBC**), which is a variant of traditional Choice Based Conjoint (**CBC**), has gained popularity and been used in studies both inside and

outside the food industry (Al-Omari et al., 2017; Harwood and Drake, 2018; Oltman et al., 2014). ACBC surveys adapt to the specific responses of each individual, creating a personalized survey for each participant (Jervis et al., 2012; Orme, 2010). For this reason, ACBC surveys can achieve consistent data with fewer overall participants than CBC. Furthermore, ACBC surveys are more reliable when price is included as an attribute (Chapman et al., 2009; Jervis et al., 2012; Harwood and Drake, 2018). ACBC techniques have been applied to many food products including bread, bacon, and fluid milk (Jervis et al., 2014; McLean et al., 2017; Harwood and Drake, 2018).

Conjoint analysis survey designs are often paired with other survey methods including Maximum Difference (**MaxDiff**) scaling and Kano questions. MaxDiff is similar to Conjoint analysis in that it requires participants to make trade-offs, but the trade-offs are based on single attributes or statements rather than entire product builds. MaxDiff exercises do not include product concepts with multiple attributes. Consumers are presented with a list of product attributes and are asked to choose the "best" and "worst" options in the list, rather than an ideal or acceptable multi-attribute concept, as is the case with ACBC. Kano questions are also now fielded via an online survey interface and differ from MaxDiff and ACBC by integrating both qualitative and quantitative data. Kano questions aim to identify consumer attitudes towards certain product attributes based on whether the attribute is present or absent from a product offering (Kano, 1984; Zacarias, 2015). Both Kano questions and MaxDiff have been used in a variety of food studies including chocolate milk, tomatoes, hot beverages, bacon, and fluid milk (Kim et al., 2013; Li et al., 2014; Oltman et al., 2014; McLean et al., 2017; Harwood and Drake, 2018).

While focus groups and survey methods are conceptual exercises, consumer acceptance taste tests require consumers to directly interact with the product. Blinded consumer acceptance tests have been shown to accurately assess consumer liking of foods. In conjunction with trained descriptive analysis data, consumer acceptance data can be used for preference mapping, which provides an understanding of objective sensory attributes that drive liking or disliking (Clark, 1998). To our knowledge, a collective examination of the LFM consumer has never been attempted prior to this study. Since LFM popularity is growing quickly in the United States, identifying drivers of like and purchase habits will be extremely valuable for new product development of lactose-free beverages in the dairy industry. The goal of this study was to apply focus groups, survey methods, and consumer acceptance tests to investigate consumer desires and perceptions of LFM.

MATERIALS AND METHODS

Experimental Overview

Three focus groups were conducted to identify consumer perceptions of LFM. The focus groups were the first analysis conducted to take advantage of unexpected information that is often gathered through open discussions with consumers. This qualitative exercise was necessary for a deeper understanding of LFM consumer perceptions to assist with development of the subsequent survey and consumer liking test ballot. The online survey was the next analysis conducted following the focus groups. The online survey consisted of an ACBC exercise, a MaxDiff exercise, and Kano questions. The objective of the online survey was to expand on the information obtained from the focus groups using a quantitative method. It also provided a means to reach many more consumers than would have been possible with focus groups. Additionally, it allowed for a quantitative analysis of the purchase habits and preferences of

LFM consumers. Subsequently, nine commercial 2% LFM products were evaluated by a trained descriptive analysis (**DA**) panel. Finally, LFM consumers evaluated the same nine LFM products across a two-day period. Trained panel DA and consumer acceptance testing of LFM were separate analyses from the focus groups and online survey and allowed for an understanding of LFM consumer preferences regarding actual sensory perception of commercially available LFM. External preference mapping was then applied with trained panel DA and consumer acceptance data to illustrate the sensory properties in LFM that drive liking and disliking among consumers. All sensory and survey testing procedures were conducted in compliance with the North Carolina State University (**NCSU**) Institutional Review Board regulations.

Focus Groups

Three 90-minute focus groups were conducted on the NCSU campus. Each focus group was comprised of seven to ten adult LFM consumers (n=25 total). All focus group participants were between the ages of 18-64y and were a total of 9 males and 16 females. Participants were recruited using a database of over 10,000 consumers maintained by the Sensory Service Center at NCSU. In order to qualify for the focus groups, consumers must have been between the ages of 18-64y, had an annual salary greater than $20k, and purchased and consumed LFM regularly (at least once per month). The focus groups were led by an experienced moderator, while a scribe observed remotely through video and audio online streaming. All focus group sessions were also video recorded for repeat-viewing purposes. During each focus group, the participants were asked a series of questions regarding the following focus areas: (1) *Purchase Habits*, (2) *Packaging and Labeling*, (3) *Milk Processing*, (4) *Sensory Characteristics*, (5) *Milk Applications* (**Figure 1**). All questions and responses were verbal in an open discussion format. For the *Milk Processing* focus area, questions were asked regarding participant knowledge of this subject.

Then, participants were given an information sheet outlining pasteurization and ultrapasteurization processes, and then asked if their perception changed after being educated on the subject. The data gathered from the focus groups was used to generate the online survey. Participants were compensated with a $35 gift card to a local store following participation in one of the focus groups.

Online Survey

An online survey was created based on the focus group results using Lighthouse Studio (Sawtooth Software version 9.5.3, Orem, UT). The survey was fielded using the same database used for focus group participant recruitment. Consumers over the age of 18y and had purchased and consumed LFM within the last six months (n=331) were able to participate in the survey. After answering preliminary screen questions, participants (55 males and 276 females) were directed to MaxDiff scaling, Kano questions, and an ACBC survey specifically focusing on LFM. Upon full completion of the survey, participants were entered into a drawing for gift cards to a local store that was comprised of one $100 gift card, two $40 gift cards, and eleven $20 gift cards.

The ACBC survey consisted of nine attributes with two to five levels per attribute (**Table 1**). Prices were based on LFM prices in the Raleigh/Durham, NC area. The survey contained one build-your-own (**BYO**) task and eight screening tasks with four product concepts per task. For each screening task, consumers were asked to choose which of the products presented were "a possibility" or "won't work for me." To ensure the exercise adapted to each survey participant, five unacceptable and four must-have questions were integrated into the screening portion, in compliance with the software recommendations (Orme, 2009). Subsequently, a 10-question choice task tournament section was conducted. Each choice task presented three randomly

generated LFM product concepts based on the levels and attributes specified in **Table 1**. The levels within each attribute of the product concepts differed from the participant's BYO product by two to four attributes. consumers were asked to choose which of the three product concepts was the "best option." Twenty product concepts was the maximum number that could be entered into the tournament. The ACBC exercise concluded after 10 choice tasks.

Preceding the ACBC exercise, consumers participated in MaxDiff scaling and Kano questions. The MaxDiff exercise consisted of 15 LFM attributes and was comprised of 11 questions with five attributes listed per question. Consumers were asked to choose the single "most important" and single "least important" attribute in each set. Kano questions were asked immediately after the MaxDiff scaling exercise. They were comprised of many of the same features as the ACBC survey, such as questions concerning packaging, flavor, texture, and claims. Consumers were asked all Kano questions in a functional manner (for example, "LFM that has the same sweetness as regular milk") and in a dysfunctional manner (for example, "LFM that does not have the same sweetness as regular milk"). The response options for each LFM attribute were: "I like it," "I expect it," "I don't care/neutral," "I can live with it," and "I dislike it."

Descriptive Analysis

Nine representative commercial 2% LFM products were evaluated by a trained sensory panel. The LFM were purchased on two different occasions, three weeks apart. All LFM were ultrapasteurized (**UP**). The trained panel consisted of five females and two males (ages 24-54 y), each with over 100h of experience with sensory evaluation of dairy products. Panelists documented sensory attributes of LFM using a 0 to 15pt universal scale consistent with the Spectrum^{TM} method (Meilgaard et al., 2007) and an established lexicon for milk (Lee et al.,

2017; McCarthy et al., 2017a). Milks were evaluated at 20ºC in coded 60mL soufflé cups (PFS Sales Co., Raleigh, North Carolina). The samples were prepared with the overhead lights off to avoid light oxidation. For each of the two sessions, panelists were calibrated with a commercial 2% UP milk and a commercial 2% UP LFM. Panelists expectorated samples after each evaluation and rinsed with deionized water. Each milk was evaluated by each panelist in duplicate. Paper ballots were used for data collection.

Consumer Acceptance Taste Test

Subsequently, LFM consumers (n=160) were recruited to evaluate the nine 2% LFM evaluated by the trained panel. Consumers were 18-64y, primary shoppers, purchased and consumed LFM at least once per month, and consisted of 46 males and 114 females. Each consumer evaluated all nine LFM samples over a two-day period. Five LFM were evaluated on the first day, and four LFM were evaluated on the second day. The order of presentation for all LFM was balanced and randomized over the two-day period, meaning each consumer could receive any five of the nine LFM on day one, and the remaining four on day two, in a randomized balanced order. Consumers were served 60 mL of each sample in 155mL clear plastic tumbler (PFS Sales Co., Raleigh, North Carolina) at 4ºC. Samples were coded with three-digit blinding codes.

Before tasting, participants were first asked to look at the sample and answer questions concerning: appearance liking, color liking, and aroma liking. Just About Right (**JAR**) questions for color and aroma were asked alongside their corresponding liking questions. Next, consumers were instructed to taste the LFM and were asked questions concerning overall liking, flavor liking, sweetness liking, thickness/mouthfeel/viscosity liking, and aftertaste liking. The aftertaste liking question was only asked of those who indicated that they perceived an aftertaste. JAR

questions for flavor, sweet taste, cooked flavor, thickness/mouthfeel/viscosity, and creaminess were asked alongside their corresponding liking questions. Liking questions were scored on a 9-point hedonic scale where 1 = dislike extremely, and 9 = like extremely. JAR questions were asked on a 5-point scale where 1 = much too little, 2 = too little, 3 = just about right, 4 = too much, and 5 = much too much. For each sample, quality and purchase intent questions were asked on a 5-point intensity scale. For the quality question, 1 = extremely low quality, 2 = low quality, 3 = neither high nor low quality, 4 = high quality, and 5 = extremely high quality. For the purchase intent question, 1 = definitely would not buy, 2 = probably would not buy, 3 = might or might not buy, 4 = probably would buy and 5 = definitely would buy. Consumers were instructed to cleanse their palates with deionized water and a bite of unsalted cracker between samples, and a 2 min rest was enforced between samples. Compusense Cloud (Guelph, Canada) was used to collect data. All consumers were compensated with a $30 gift card to a local store upon full completion of the consumer acceptance test.

Statistical Analysis

Online Survey. In the online survey, individual utility scores from the ACBC survey and the MaxDiff exercise were determined using Hierarchical Bayesian estimation. Cluster analysis of individual utility scores from the ACBC survey was conducted with XLSTAT (version 19.5.2018, Addisoft, Paris, France) using k-means clustering. Mean values of utility scores, importance scores, and MaxDiff scores were analyzed for significant differences by a one-way analysis of variance (**ANOVA**) with Fisher's least significant difference post hoc test at 95% confidence. Kano questions were analyzed according to the model proposed by Kano et al. (1984). LFM attributes were assigned to Kano classifications, which include: attractive (usually unexpected by the consumer and result in an increase in satisfaction when present); must-have

(expected by the consumer and result in dissatisfaction if not present); one-dimensional (there is a linear relationship between attribute presence and consumer satisfaction); rejecter (consumers are indifferent if the feature is absent and dissatisfied if it is present); and indifferent (consumer satisfaction is not affected whether the feature is present or absent). The MaxDiff and Kano data were analyzed using the same clusters determined by the ACBC utility score cluster analysis to further define each consumer group.

Descriptive Analysis. Descriptive analysis data were evaluated by one-way ANOVA with Fisher's LSD means separation. DA data were also analyzed using principal component analysis (PCA) with XLSTAT (version 19.5.2018, Addisoft, Paris, France).

Consumer Acceptance Test. In the consumer acceptance test, all 9-point liking questions were analyzed using a one-way ANOVA with Fisher's least significant difference post hoc test and means separation at 95% confidence. JAR questions were subjected to penalty analysis at a 20% threshold, and differences in selection proportion were determined via Chi-square test ($\alpha = 0.05$). Quality and purchase intent questions were analyzed using Kruskal-Wallis with Dunn's post hoc test ($\alpha = 0.05$). Partial Least Squares (**PLS**) regression was used for external preference mapping and was conducted with trained panel mean scores and consumer acceptance overall liking scores for the overall population and each consumer cluster. All statistical analyses were conducted using XLSTAT (version 19.5.2018, Addisoft, Paris, France).

RESULTS AND DISCUSSION

Focus Groups

Each focus group began by asking participants about their purchase habits of LFM. For the majority of participants, they first began purchasing LFM after they, or a member of their family, were diagnosed with lactose intolerance. For about half of the participants, once one

member of the family required LFM, the entire household consumed LFM exclusively. The remaining participants continued to purchase traditional milk in addition to LFM. Another major driver of purchase was the familiarity of dairy milk. Growing up drinking dairy milk and knowing its nutritional benefits led participants to purchase LFM instead of lactose-free plant-based alternatives. This is consistent with the results of a previous study by McCarthy et al. (2017b). Price was extremely important to participants. All participants were aware that LFM costs more than traditional milk, and this was viewed negatively. While participants were aware of several LFM brands, they most often chose to purchase store brands instead of name/national brands, citing price as the main driver of choice. Although the price of LFM was important to participants, all stated that dairy milk that does not cause stomach discomfort was worth paying a higher price.

Participants preferred half-gallon sized cardboard cartons. There was limited interest in package sizes that were bigger or smaller than half-gallon, even if the entire household drank LFM. Fat content, protein content, and the expiration date were cited as the most important information that could be found on the package, as well as "Lactose-free" prominently displayed on the label. Participants preferred packages where this information was easy to find. The color of the package was also important to participants. A colorful package, or one that used color to distinguish between fat content was more important to participants than being able to see the milk inside the package. Label claims on packages such as "high protein," "organic," and "high calcium" were appealing to participants, but generally only affected purchases if two LFMs were the same price.

Most participants were unfamiliar with LFM processing. After being presented with information regarding lactose removal methods, lactase enzyme and ultrafiltration, participants

did not have a preference, but stated their desire for whichever process resulted in the lowest final price, regardless of socio-economic status. Most participants were aware that pasteurization was a heat treatment applied for safety and shelf life but did not understand the differences between pasteurization and ultrapasteurization, or that ultrapasteurization resulted in a longer product shelf life. They also did not like the word "ultrapasteurization," stating that it sounded expensive and "scientific." After reading an information handout provided on pasteurization vs. ultrapasteurization, their opinions changed. The longer shelf life resulting from ultrapasteurization was seen as a good trade-off for the higher price of LFM; however, they still did not like the word "ultrapasteurization."

Next, participants were asked about the sensory characteristics of LFM compared to traditional milk, as well as some other perceptions of the product. All consumers thought that the biggest difference between LFM and traditional milk was the increased sweetness of LFM. Others also thought LFM had a lower viscosity than traditional milk. Sweetness was cited as one of the most distinguishing characteristics between different brands of LFM, along with flavor in general. When asked how participants would want LFM to taste, almost all responded that they would want it to taste exactly the same as traditional milk; however, when asked how the sensory properties of LFM could be improved, only a small minority indicated that the sweetness of LFM should be reduced to better resemble traditional milk. Most participants did not view LFM as a "natural" product, however having access to dairy milk they could consume without discomfort was more important. Finally, participants were asked about applications for LFM. All used LFM as a beverage, and about half also used it for cooking. Those who did not use LFM for cooking were skeptical about using it in recipes, indicating that they were not sure how the increased

sweetness of LFM would affect the dish. However, those who used LFM for cooking found it to be interchangeable with traditional milk in all recipes.

Adaptive Choice Based Conjoint Survey

Utility scores from hierarchical Bayesian analysis are reported as zero-centered values. More positive scores indicate more appealing levels within each attribute (Orme, 2010). The utility scores from the overall population (n=331) indicated that the ideal LFM product was low in price ($2.00-$4.00 per half gallon), packaged in a half-gallon sized cardboard carton, ultrapasteurized, and had the same sweetness and texture as traditional milk **(Figure 2),** consistent with focus group results. For the overall population, price was the most important attribute by a significant margin ($p<0.05$) (**Figure 3)**. Most of the results obtained from the ACBC survey reflected the findings of the focus groups, price being the most notable example. In a focus group setting with LFM consumers, many conversations led to the topic of price and its importance to participants. Survey results from LFM consumers indicated the same conclusion: price was extremely important to LFM consumers and heavily influenced purchase decisions.

As seen in **Figure 2**, cardboard half-gallon sized packages were the most preferred for LFM consumers, which was also supported by focus group discussions. Additionally, sweetness and texture that resembled traditional milk were most appealing ($p<0.05$). In focus groups, LFM consumers liked longer shelf life. UP was defined in the ACBC via shelf life. In contrast to traditional milk consumers, LFM consumers preferred ultrapasteurization over conventional pasteurization ($p<0.05$) (Harwood and Drake, 2018). These data suggest that the longer shelf life of LFM has value and is appealing to these consumers. Based on the importance scores of each attribute, shelf life was one of the most important LFM attributes after price. This, along with the

fact that ultrapasteurization was the most appealing pasteurization method, suggests that LFM consumers have come to expect LFM to be ultrapasteurized, given that all commercial LFM products are currently ultrapasteurized to our knowledge. However, it is clear that LFM consumers, like traditional milk consumers, do not prefer shelf stable milk. Although they view the extended shelf life of ultrapasteurization as a major benefit, LFM consumers expect milk to require refrigeration. Nutrition claims and label claims did not appear to be of importance to the overall population of LFM consumers when making purchase decisions compared to many of the other attributes ($p<0.05$).

Raw utility scores from the ACBC survey were segmented into three distinct consumer clusters based on participant responses and were named according to their results: the Bargain cluster (n=152), Performance cluster (n=103), and Pragmatic cluster (n=76). These clusters were mostly distinguished by their price importance scores. The Bargain cluster was defined by its high price importance score, the highest of all consumer clusters ($p<0.05$). By putting a vast quantity of importance on price, this consumer segment was not greatly affected by other LFM attributes when making purchase decisions. While the Bargain cluster did not constitute a majority of the survey participants, it was the largest consumer cluster, indicating a significant portion of LFM consumers were extremely price sensitive. The Performance cluster had the lowest importance score for the price attribute compared to the other two clusters and had the highest importance scores for all other attributes with package size as the only exception ($p<0.05$). These results indicate that consumers in this segment were not as price sensitive as other consumer clusters and tended to put more weight on product performance when making purchase decisions.

The Pragmatic cluster exhibited aspects of both the Bargain and Performance clusters, with a price importance score in between the other clusters ($p<0.05$), in addition to many other attributes. However, the Pragmatic cluster had the highest importance score for package size compared to the other clusters ($p<0.05$), and it was the second most important attribute of this consumer segment, following price. The Pragmatic cluster also had the highest importance score for package material, along with the Performance cluster, which scored at parity ($p>0.05$). These results indicate that a significant portion of the population heavily considers packaging attributes when making purchase decisions.

Maximum Difference Scaling

The results of the MaxDiff exercise closely resembled results from the ACBC survey and focus group discussions (**Table 2**). The most important LFM component to the overall population and each individual consumer cluster was "the fact that the milk is lactose-free" ($p<0.05$). This is logical and supports the findings of the focus groups: that the majority of consumers begin purchasing LFM because they or someone in their household is lactose intolerant. The first deviation in the MaxDiff results from the ACBC results is that "flavor of the milk" was the second most important LFM component, even above price ($p<0.05$), whereas price was the most important attribute found in the ACBC survey and sweetness was the third most important. One possible explanation for this difference is that in the ACBC survey, this attribute was presented as "sweetness" and in the MaxDiff exercise was presented as "flavor of the milk." This might indicate that LFM consumers interpret "flavor" differently than "sweetness" and see the flavor of LFM as more than just its sweet taste. The next most important LFM components for the overall population were "price" and "fat content," which scaled at parity ($p>0.05$). These results were also expected. Price is almost always one of the most important attributes for food

products. Fat content was not included in the ACBC survey but was also expected to be very important to LFM consumers. Most milk consumers are very strict about the milk fat content they purchase and are not usually willing to deviate from their selected fat percentage (McCarthy et al., 2017a). The importance of fat content was also noted by focus group discussions with LFM consumers in this study. Another difference between the MaxDiff results and the ACBC results is that "package size" was among the least important LFM components in the MaxDiff exercise, but one of the most important in the ACBC exercise. An explanation for this is that the MaxDiff exercise preceded the ACBC exercise in the survey, so the participants had not yet been exposed to all possible package sizes presented in the ACBC exercise (pint, quart, half-gallon, and gallon), and only drew from their prior purchase experiences with LFM when participating in the MaxDiff exercise. The vast majority of LFM is packaged in half-gallon sized packages, so it is possible LFM consumers had not considered other packaging options before participating in the ACBC survey.

Similar to the ACBC survey, the importance of price was a distinguishing factor among the three consumer clusters in the MaxDiff exercise with price being the most important to the Bargain cluster ($p<0.05$). Many of the other results from the MaxDiff cluster analysis were similar to the ACBC cluster analysis. In the MaxDiff, the Performance cluster had a higher score ($p<0.05$) for "organic" than the other two clusters, which mirrors the results of the ACBC and demonstrates the importance of other product attributes that consumers in the Performance cluster take into account when making purchase decisions. The Performance cluster had the highest score ($p<0.05$) for "brand," which was expected given the value-added mindset that this cluster demonstrated.

Kano Model

The results of the Kano Model indicate the overall population of LFM consumers were *indifferent* towards the majority of LFM attributes (**Table 3**). It is important to consider that the Kano Model classifies attributes into a rigid category and does not consider the total distribution of each classification's scores, meaning the *indifferent* classification had the highest score, but was not the only classification selected. Price was expected to be classified as *one-dimensional* considering consumer satisfaction tends to decrease linearly as price increases. Utility scores in the price attribute from the ACBC decreased as price increased (**Figure 2**). This trend was also observed when discussing price with LFM consumers during the focus groups. However, "milk that is lactose-free" was not expected to be classified as *one-dimensional*, but was expected to be classified as *must have*. Although the *one-dimensional* classification had the highest score for this attribute, it is important to note that less than 10% of the overall population separated it from being classified as *must have*. This stipulates that there is a significant portion of LFM consumers that consider lactose-hydrolysis as a must have for product satisfaction. One possible explanation for why the majority of survey participants classified this attribute as *one dimensional* is based on results from the focus groups. Only about half the LFM consumers that participated in the focus groups exclusively purchased LFM. The others indicated that they purchased traditional milk for other members of their family or for cooking applications. It is possible that in this context, milk does not have to be lactose-free in order for LFM consumers to derive satisfaction, leading to a *one-dimensional* classification for the attribute. We also learned from focus group discussions that there are varying degrees of lactose sensitivity among individuals, or at least the perception of variable sensitivity. With this in mind, it is possible that some less-sensitive LFM consumers do not require that all the lactose be removed, but will

derive more satisfaction as the lactose content decreases, leading to a *one-dimensional* classification.

Similar to the MaxDiff exercise results, the results from the Kano Model were segmented using the same consumer clusters derived from the ACBC survey. Each cluster mostly resembled the results of the overall population, with some notable differences. The only difference between the results of the Bargain cluster and that of the overall population is the attribute "milk that is lactose-free" was classified as a *must have.* This is an important distinction, given the Bargain cluster is the largest consumer cluster of the three, it demonstrates the importance of lactose-hydrolysis on consumer satisfaction of LFM. A distinction between the Performance cluster and the overall population was the attribute "milk that is low in price ($2.00-$4.00 per half-gallon)" was classified as *indifferent.* This was expected from the Performance cluster and reaffirms the results of the ACBC survey. Another difference between the Performance cluster and the overall population was that the attribute "milk that has the same viscosity/mouthfeel as regular milk" was classified as *one-dimensional* rather than *indifferent*. These results indicate that as the texture of LFM deviates from that of traditional milk, consumer satisfaction decreases. Since the Performance cluster was more concerned with sensory attributes of LFM than other clusters, this classification was anticipated and supports the results of the ACBC survey. This attribute was also classified as *one-dimensional* by the Pragmatic cluster, further explaining results from the ACBC survey that texture is an important attribute for LFM. The final distinction between the Pragmatic cluster and the overall population was the attribute "lactose-free milk that has the same sweetness as regular milk" was classified as *attractive* rather than *indifferent*. These results signify that consumers of the Pragmatic cluster do not expect LFM to have the same sweetness as traditional milk but will be pleased if it does. This falls in line with what is believed about the

Pragmatic cluster, that they will appreciate certain product attributes, but will not let these attributes heavily affect purchase decisions as much as price.

Descriptive Analysis

Sensory differences were documented among the nine commercial 2% LFMs evaluated (**Table 4**). The milks differed in all attributes except for milkfat flavor (**Figure 4**). Four of the milks had distinct paperboard flavor. It is important to note that all milks were packaged in a paperboard carton, except for LFM2. Only two milks, LFM2 and LFM4, exhibited eggy flavor. These results were somewhat unexpected considering that ultrapasteurized milk tends to have distinct intensities of eggy flavor due to the increased heat treatment. A recent study has also shown that the volatile sulfur compounds that create eggy flavor in milk result from Maillard reactions between reducing sugars (lactose) and cysteine and methionine amino acids (Jo et al., 2018). Considering lactose is hydrolyzed into glucose and galactose, which are both reducing sugars, an increase in eggy flavor might be expected in LFM compared to traditional milk. LFM6 was the only sample that exhibited grassy flavor, and this milk was an organic grass fed LFM, so grassy flavor was expected. The milks evaluated were different in viscosity ($p<0.05$). LFM2 and LFM6 were the most viscous milks, and LFM1 and LFM7 were the least viscous. The most distinguishing attribute between milks was sweet taste intensity, which was responsible for the most variation among milks. The median sweet taste intensity among LFMs evaluated was 3.2, but sweet taste intensities ranged from 1.5 (LFM2) to 4.2 (LFM9). This large variation in sweet taste intensity among the nine LFMs evaluated is likely due to the starting concentration of lactose in each milk. An important distinction for LFM2 is that it used a combination of lactase enzyme and ultrafiltration to remove lactose, whereas other milks solely used the lactase enzyme. Ultrafiltration would have greatly reduced the starting lactose concentration in this

sample, translating into a largely reduced sweet taste, and likely increased its viscosity, cooked flavor intensity, and eggy flavor intensity due to the increase in protein concentration.

Consumer Acceptance Taste Test

The same nine LFMs evaluated by the trained sensory panel were evaluated by n=160 LFM consumers (**Table 5**). Flavor and sweetness were the most important sensory attributes to LFM consumers, which can be concluded from considering that the milks that received the lowest overall liking scores (LFM2 and LFM8) also received the lowest scores for flavor liking and sweetness liking ($p<0.05$). Milks that received low overall liking scores also received the lowest quality and purchase intent scores.

Overall liking scores from the consumer acceptance test were used to segment consumers into distinct clusters based on which milks they most preferred, and each cluster was named accordingly: the Sweetness cluster (n=86), the Cooked Flavor cluster (n=42), and the Balanced cluster (n=32) (**Figure 5**). Similar to the ACBC survey, three distinct clusters were identified for the consumer acceptance test population, which is an interesting discovery especially when considering that the consumers that participated in the ACBC survey and the consumer acceptance test were not necessarily the same, but with the potential for some cross-over. These results might indicate that there are truly three distinct types of LFM consumers. Based on the consumer segmentation results, the overall liking score for LFM2 was the major factor for determining consumer segmentation.

A high overall liking score for LFM2 was the most distinguishing characteristic of the Cooked Flavor cluster. Although LFM2 received the lowest overall liking score for the overall population ($p<0.05$), the consumers from the Cooked Flavor cluster scored LFM2 a 7.1 in overall liking, their most preferred sample. Conversely, the Balanced cluster was mainly characterized

by an extremely low overall liking score for LFM2 (2.7). Finally, the Sweetness cluster had the highest overall liking score for all LFMs except for LFM2. These results possibly indicate that these consumers either were not able to distinguish samples from one another unless there was a large difference in sweetness (as was the case with LFM2) or they are much more easily pleased than consumers from other clusters. It is important to consider that the Sweetness cluster was the largest consumer cluster and constituted a majority of the consumers that participated in the consumer acceptance test. This might indicate that many more consumers are much more easily pleased by various LFM products, based purely on sensory perception, than would have been anticipated from the focus group and online survey results. For example, the MaxDiff exercise found flavor to be one of the most important LFM attributes that could affect purchase decisions, yet the majority of consumers (the Sweetness cluster) were generally pleased by all LFM evaluated. Even LFM2, which received the lowest overall liking score from the Sweetness cluster, was still "liked" with an overall liking score of 5.8.

The external preference map from PLS (**Figure 6**) helps to further understand distinctions between consumer clusters, based on overall liking scores. As seen in **Figure 5**, the Cooked Flavor cluster is largely associated with a high overall liking score for LFM2. **Figure 6** illustrates that through their relationship with LFM2, the Cooked Flavor cluster can also be characterized by preference for LFM that are high in viscosity, eggy flavor intensity, and cooked flavor intensity. One of the most important discoveries learned from **Figure 6** is the market potential for LFM products similar to LFM2 among consumers. LFM2 was an ultrafiltered, high protein, low sugar LFM. Although LFM2 received the lowest overall liking score for the overall population ($p<0.05$), it is clear that there is significant market potential for this product in the Cooked Flavor cluster consumers. Additionally, **Figure 6** demonstrates sweetness to be a driver

of dislike for the Cooked Flavor cluster, while it was a driver of like for the Sweetness and Balanced clusters. The Balanced cluster can also be characterized by its drivers of dislike: high viscosity, eggy flavor, and cooked flavor. This can be seen most clearly in **Figure 5**. The Balanced cluster scored LFM2 at 2.7 in overall liking, which was the only milk that possessed these drivers of dislike and lacked sweetness, a driver of like. Although, sweetness was a driver of like for the Sweetness cluster, this cluster did not appear to a have a specific driver of dislike, especially when considering its overall liking score of LFM2 (**Figure 5**). The Sweetness cluster scored LFM2 at 5.8, which was the lowest score given by the Sweetness cluster, but still indicates slight liking. The lack of sweetness is likely most responsible for the reduced overall liking score.

An important discovery learned from the consumers acceptance test is the importance of sweet taste to the overall population of LFM consumers as a driver of like. A good example of this can be seen when comparing LFM2 and LFM4. As seen in **Figure 4** and **Table 4**, LFM2 and LFM4 had a very similar overall sensory profile. The major difference between the two milks was their sweet taste intensity. When taking into account the consumers acceptance test results, LFM2 received the lowest overall liking score, while LFM4 received the highest score. Considering these milks were very similar in all other sensory attributes except for sweet taste intensity, they clearly demonstrate the importance of sweetness to the average LFM consumer. This is an important discovery especially when considering the findings of the focus groups and online survey, where consumers overwhelmingly stated that they would most prefer LFM that has the same sweetness as traditional milk.

Many of the results of this study reflect those of previous studies on fluid milk. Most notably, Harwood and Drake (2018) noted that price was the most important attribute of

traditional fluid milk to consumers making purchase decisions. This principle appears to be even more extreme for LFM consumers, likely due to the higher price point of LFM compared to traditional fluid milk. Fat content played a large role in purchase decisions for LFM, also confirmed in previous studies with milk (McCarthy et al., 2017b; Harwood and Drake, 2018). Harwood and Drake (2018) also found that flavor of the milk was very important to consumers, even more so than price. This result was also recorded in this study, but only in the MaxDiff exercise. After conducting a consumer acceptance test of UP LFM compared to UP traditional milks, Adhikari et al. (2010) found the increased sweetness of LFM compared to traditional milk to be a driver of dislike for consumers, the opposite finding of this study. Adhikari et al. (2010) did not specifically recruit LFM consumers to participate in their consumer acceptance test, as this study did. LFM is generally sweeter than traditional milk; therefore as this study proposes, LFM consumers have likely grown accustomed to the increased sweetness of LFM, and that it has become a driver of like specifically for LFM consumers. However, fluid milk consumers in general are not used to the sweetness of LFM, and would likely score sweeter LFM lower than traditional milk, as seen in the study by Adhikari et al. (2010).

Consumers have stated many reasons for avoiding dairy milk including animal rights and vegan diets, but concerns about lactose consumption is one of the largest reasons that cause consumers to avoid dairy milk (Zingone et al., 2017). LFM is a good alternative for consumers trying to avoid lactose, because it will also provide a rich source of nutrients that are not provided by plant-based dairy alternatives. On average, plant-based milks supply less than half of the protein found in dairy milk (Chalupa-Krebzdak et al., 2018). With the exception of soy milk, plant-based milks are not a source of complete proteins. Additionally, calcium found in dairy milk is much more easily absorbed by the body compared to calcium fortified plant-based

milk (Chalupa-Krebzdak et al., 2018). These are examples of the many reasons why plant-based milk alternatives are not recommended as a complete nutritional replacement to dairy milk. When considering LFM is nutritionally the best alternative to traditional milk for consumers trying to avoid lactose, this makes understanding consumer desires and perceptions of LFM extremely important.

CONCLUSIONS

LFM purchasers are largely comprised of lactose intolerant individuals or individuals who are related to someone lactose intolerant. Considering it is estimated that 70% of the world's population has some degree of lactose intolerance, the market potential for LFM is significant (Messia et al., 2007). Price is the most important attribute of LFM for purchase decisions. LFM consumers view the extended shelf life provided by ultrapasteurization as a benefit. LFM consumers also prefer half-gallon sized cardboard cartons, even over gallon sized plastic jugs. Although label and nutrition claims like "high protein," "high calcium," and "organic" were the most popular claims that could be found on a package, they were not very important when making the overall purchase decision for LFM. High sweet taste is a driver of liking for the majority of LFM consumers. This is important to consider during future product development and consumer testing for LFM consumers. Although they may think of traditional milk as being the "gold standard" target for LFM, reducing sweetness may lead to reduced consumer acceptance. The drivers of dislike for the overall population of LFM consumers are high viscosity and high eggy flavor intensity. Products with these attributes led to decreased acceptability for the majority of LFM consumers. However, it is important to consider that a segment of the population considers these attributes appealing, indicating a market potential for similar high protein, low carbohydrate milks. While this study presents many insights into LFM

consumer perceptions and desires, a limitation is that the focus groups and consumer acceptance test were populated entirely with consumers from the Raleigh/Durham area of North Carolina. It is possible that different regional preferences exist between LFM consumers in the United States. Understanding LFM consumer perceptions, drivers of like, and drivers of dislike, can help to guide product development in the dairy industry to better meet the needs of LFM consumers in today's market.

Acknowledgements

Funding was provided in part by the National Dairy Council (Rosemont, IL).

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Moderator Guide for Lactose-free Milk

Focus Area 1: Purchase Habits

- Why do you purchase LFM? For whom do you purchase it?
- Do you also purchase traditional milk?
- Why do you purchase dairy LFM instead of plant-based milk?
- Which brands of LFM do you purchase? Are there differences between brands?
- Which fat percentrage of LFM do you buy?
- How does the price of LFM compare to that of regular milk?

Focus Area 2: Packaging and Labeling

- What kind of LFM package do you most prefer (material, size)?
- Which LFM packaging elements are most important to you? Color? Picture? "Lactose-free" label?
- Are there any label claims that you would like to see on a package of LFM? (*probe for: organic , rBST-free, high protein, high calcium, etc.*)

Focus Area 3: Milk Processing

- How does the processing of LFM compare to that of traditional milk?
- Do you know how lactose is removed from LFM?
- What is pasteurization? What is ultrapasteurization?
- (*Pasteurization/Ultrapasteurization explanation handout is given to the participants*) Now that you know more about pasteurization and ultrapasteurization, has your perception of these processes changed? Positively or negatively?

Focus Area 4: Sensory Characteristics

- Are there differences between LFM and traditional milk?
- Do you want LFM to taste the same as traditional milk?
- How do different brands compare with one another?
- Is LFM natural? Is it more or less natural than traditional milk?

Focus Area 5: Milk Applications

- Do you purchase LFM for drinking only, or also for use in recipes?
- How versatile is LFM in cooking?
- Can it be used as a direct replacement for traditional milk in recipes?
- Do you think cooking with LFM affects any recipes you use it in?

Figure 1: Focus group moderator guide

Table 1: Attributes and levels used in ACBC survey on lactose-free milk

Price	Package Material	Package Size	Lactose Removal Method	Shelf Life	Sweetness	Texture	Nutrition Claims	Label Claims
$2.00/0.5gal	Cardboard	Pint	Lactase Enzyme	Conventional Pasteurization-20 days under refrigeration	Less sweet than regular dairy milk	Thinner than regular dairy milk	High Protein	Organic
$3.00/0.5gal	Clear Plastic	Quart	Filtration/ Ultrafiltration	Ultrapasteurization-60 days under refrigeration	Same sweetness as regular dairy milk	Same as regular dairy milk	High Calcium	Low Carbon Footprint
$4.00/0.5gal	Opaque Plastic	Half-gallon		Shelf Stable-9 months without refrigeration	More sweet than regular dairy milk	Thicker than regular dairy milk	Reduced Sugar	Ethically Sourced
$5.00/0.5gal		Gallon					None	Grass Fed
$6.00/0.5gal								None

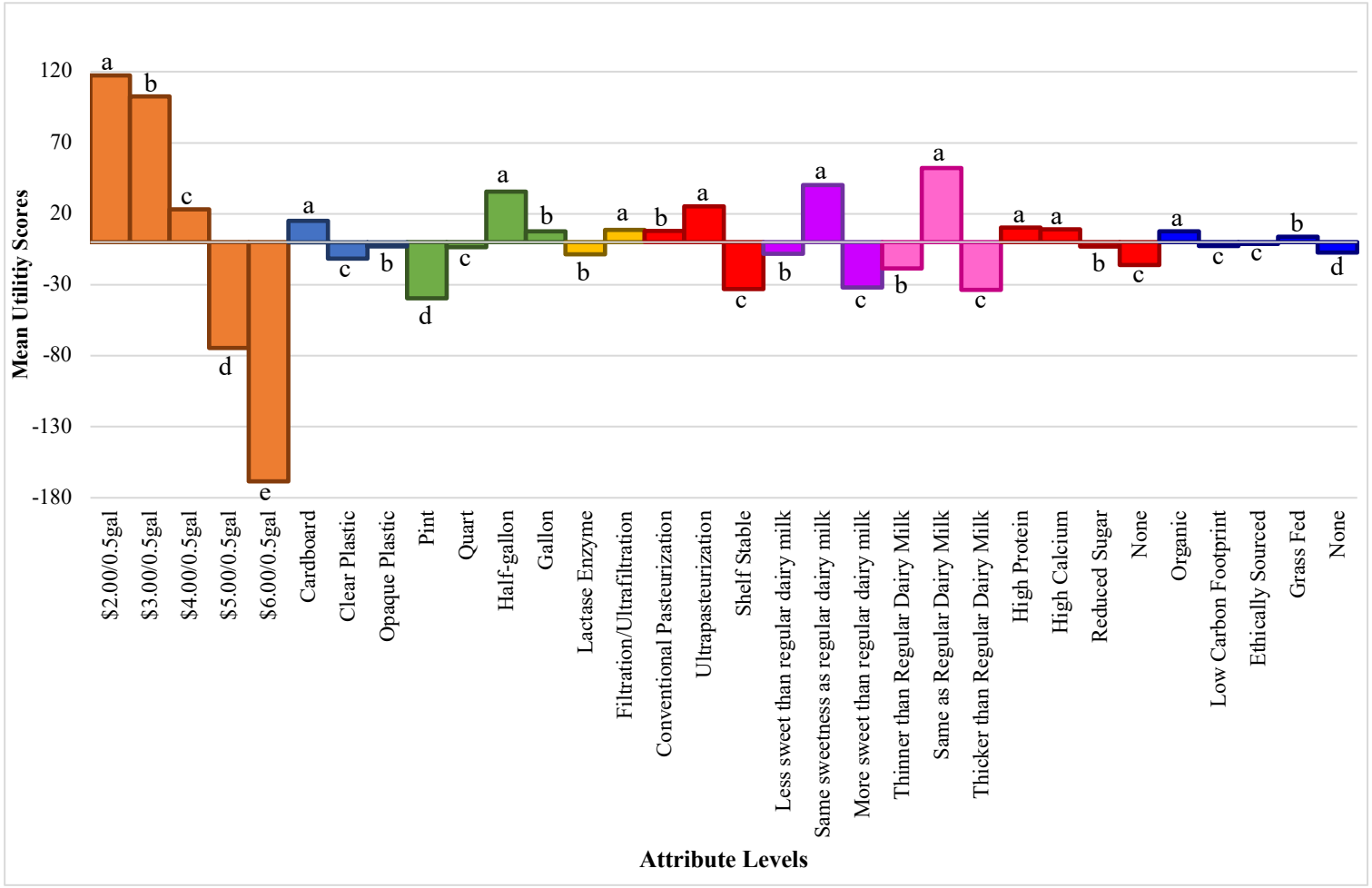

Figure 2: Mean ACBC utility scores for the overall population (n=331) for lactose-free milk attributes
Different letters over levels within the same attribute indicate significant difference ($p<0.05$)

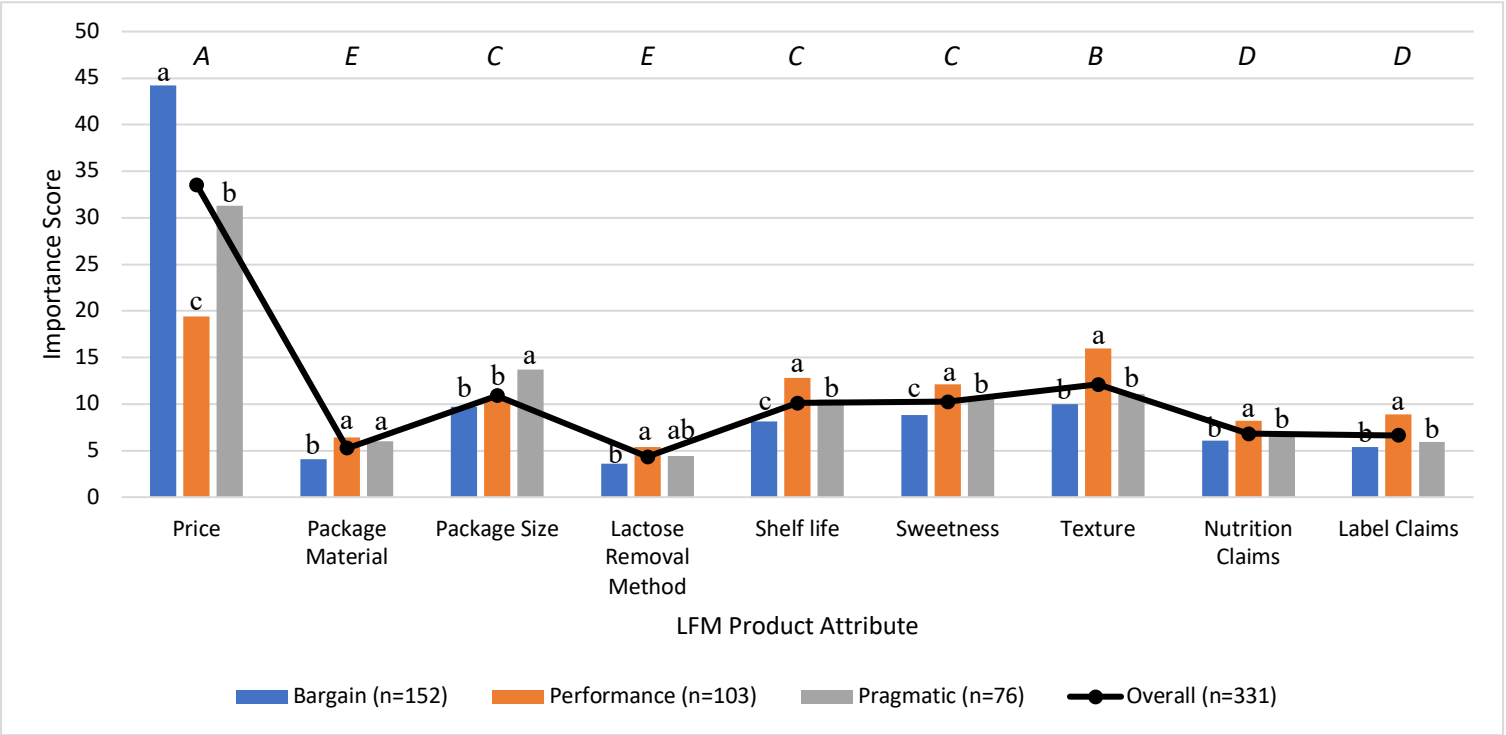

Figure 3: Importance Scores for lactose-free milk attributes from the ACBC survey for consumer clusters and the overall population. Different lower-case letters indicate significant differences between each consumer cluster within a single attribute ($p<0.05$). Different upper-case italicized letters over each attribute indicate significant differences between attribute importance scores for the overall population ($p<0.05$).

Table 2: MaxDiff scores for lactose-free milk for consumer clusters and overall population

LFM Attribute	Overall Population (n=331)	Performance Cluster (n=103)	Pragmatic Cluster (n=76)	Bargain Cluster (n=152)
Price	12.01*C*	9.91b	11.24b	13.81a
The fact that the milk is lactose free	15.85*A*	15.25a	16.77a	15.79a
Brand	1.91*H*	2.62a	1.30b	1.74ab
Flavor of the milk	14.39*B*	14.64a	14.14a	14.33a
Texture of the milk	9.39*D*	10.38a	8.78a	9.03a
Shelf life	8.68*D*	8.24a	8.42a	9.10a
Protein Content	4.37*F*	5.02a	4.16a	4.04a
Calcium Content	4.48*F*	4.67a	4.65a	4.26a
Package color/design	0.48*I*	0.52a	0.21a	0.58a
Lactose Free prominently displayed on the package	5.32*E*	5.69a	6.39a	4.52a
Package size	3.27*G*	2.54b	3.46ab	3.67a
Packaging material (cardboard, plastic, etc.)	1.06*I*	0.92a	1.27a	1.06a
Organic	3.11*G*	4.71a	2.61b	2.27b
Vitamin A and D fortified	4.30*F*	4.65a	4.19a	4.12a
Fat content (skim, 1%, 2%, whole)	11.40*C*	10.23b	12.41a	11.69ab

Different lower-case letters in rows indicate significant differences between scores from consumer clusters for a single LFM attribute ($p<0.05$)

Different upper-case italicized letters in the overall population column indicate significant differences between attributes for the overall population ($p<0.05$)

Sum of values in each column is 100 total points, results are interpreted as ratio-scaled values, and higher values indicate a more important score

Table 3: Kano classifications for consumer clusters and the overall population for lactose-free milk

	Bargain Cluster (n=152)	Performance Cluster (n=103)	Pragmatic Cluster (n=76)	Overall Population (n=331)
Milk that is low in price ($2.00-$4.00 per half-gallon)	D	I	D	D
Milk that is lactose-free	M	D	D	D
Milk that is a store brand	I	I	I	I
Milk that is a name/national brand	I	I	I	I
Milk that has a long expiration date (>50 days, unopened in refrigerator)	I	I	I	I
Milk that has a high protein content	I	I	I	I
Milk that has a high calcium content	I	I	I	I
Milk that has an attractive package	I	I	I	I
Milk that comes in a half-gallon sized package	I	I	I	I
Milk that comes in a gallon sized package	I	I	I	I
Milk that comes in a cardboard carton	I	I	I	I
Milk that comes in a clear plastic jug	I	I	I	I
Milk that comes in an opaque plastic jug	I	I	I	I
Milk that is organic	I	I	I	I
Milk that is vitamin fortified	I	I	I	I
Milk that is the fat content I usually buy	I	I	I	I
Lactose-free milk that uses filtration to remove lactose	I	I	I	I
Lactose-free milk that uses enzymes to remove lactose	I	I	I	I
Lactose-free milk that has the same sweetness as regular milk	I	I	A	I
Milk that has a reduced sugar content	I	I	I	I
Milk that has as low carbon footprint	I	I	I	I
Milk that is ethically sourced	I	I	I	I
Milk that comes from grass fed cows	I	I	I	I
Milk that has the same viscosity/mouthfeel as regular milk	I	D	D	I

Kano classifications were calculated according to the method of Kano et al. (1984)
D indicates “one-dimensional” Kano classification
I indicates “Indifferent” Kano classification
M indicates “must-have” Kano classification
A indicates “attractive” Kano classification

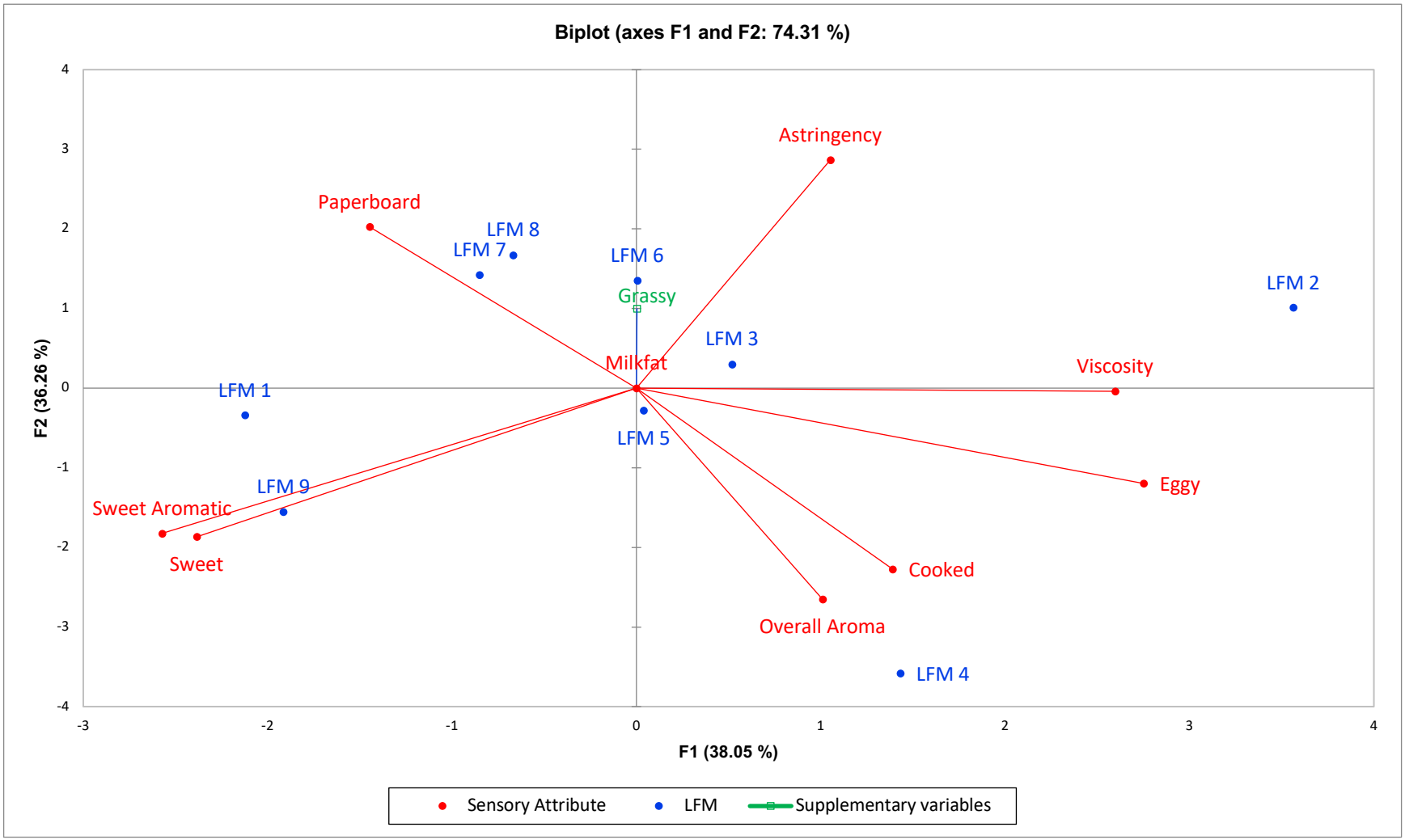

Figure 4: Principal component analysis biplot of descriptive analysis data for nine lactose-free milks (LFM)

Table 4: Descriptive analysis trained panel means of lactose-free milk

Sample	LFM 1	LFM 2	LFM 3	LFM 4	LFM 5	LFM 6	LFM 7	LFM 8	LFM 9
Overall Aroma	2.0a	2.0a	2.0a	2.1a	2.0a	2.0a	2.0a	2.0a	2.0a
Sweet Aromatic	1.4a	0.8b	1.0ab	1.2ab	1.0ab	1.0ab	1.0ab	1.0ab	1.4a
Cooked	4.0c	4.1bc	4.2b	4.5a	4.4a	4.0c	4.0c	3.8d	4.0c
Paperboard	1.0c	ND	ND	ND	ND	1.4a	1.3ab	1.2bc	ND
Eggy	ND	0.9a	ND	0.8a	ND	ND	ND	ND	ND
Milkfat	2.0a	2.0a	2.0a	2.0a	2.0a	2.0a	2.0a	2.0a	2.0a
Grassy	ND	ND	ND	ND	ND	1.0a	ND	ND	ND
Sweet	3.3bcd	1.5f	3.2cde	3.6b	3.4bc	3.2cde	2.9e	3.0de	4.2a
Astringency	1.9b	2.0a	2.0a	1.8b	2.0a	2.0a	2.0a	2.0a	1.8b
Viscosity	1.9d	2.20a	2.1b	2.1b	2.0cd	2.15ab	1.9d	2.0c	2.0c

Attributes were scored on a 0 to 15-point scale, with 0=not intense at all and 15=extremely intense
Different letters in rows indicate significant differences ($p<0.05$)
ND – not detected

Table 5: Consumer acceptance liking means for lactose-free milks (n=160).

Question		LFM 1	LFM 2	LFM 3	LFM 4	LFM 5	LFM 6	LFM 7	LFM 8	LFM 9
Appearance Liking		7.2a	7.2a	7.2a	7.2ab	7.1ab	6.9b	7.3a	7.1ab	7ab
Color Liking		7.1a	7.1a	7.0a	7.1a	7.0ab	6.7b	7.0a	7.0ab	6.9ab
Color JAR (%)	Too Light	1.3a	7.5a	2.5a	2.5a	1.9a	3.8a	1.9a	3.8a	0.6a
	JAR	85.6a	85.6a	83.1a	85.6a	86.3a	76.3a	90.6a	83.1a	83.1a
	Too Dark	13.1a	6.9a	14.4a	11.9a	11.9a	20.0a	7.5a	13.1a	16.3a
Aroma Liking		5.7abc	5.9ab	5.9abc	5.9ab	5.7bc	5.6bc	6.1a	5.5c	5.8abc
Aroma JAR (%)	Too Weak	20.6a	21.3a	15.6a	21.3a	21.3a	18.8a	29.4a	19.4a	13.8a
	JAR	59.4a	63.8a	66.3a	64.4a	60.0a	56.3a	61.3a	56.3a	62.5a
	Too Strong	20.0a	15.0a	18.1a	14.4a	18.8a	25.0a	9.4a	24.4a	23.8a
Overall Liking		6.6a	5.5c	6.7a	6.7a	6.6a	5.9bc	6.7a	5.4c	6.2b
Flavor Liking		6.6a	5.4de	6.5ab	6.6a	6.5ab	5.8cd	6.6a	5.3e	6.1bc
Flavor JAR (%)	Not Enough Flavor	6.9b	46.3a	11.3b	9.4b	11.3b	6.9b	10.0b	11.3b	8.8b
	JAR	69.4a	45.0b	67.5a	70.6a	70.0a	50.0ab	70.6a	45.6a	60.6ab
	Too Much Flavor	23.8abc	8.8c	21.3bc	20.0bc	18.8bc	43.1a	19.4ab	43.1a	30.6ab
Sweetness Liking		6.3ab	5.3d	6.3ab	6.4a	6.4a	5.8bc	6.4a	5.5cd	6.1ab
Sweetness JAR (%)	Not Sweet Enough	8.1b	55.0a	10.6b	12.5b	15.0b	11.3b	10.0b	20.0b	15.6b
	JAR	61.3a	44.4a	63.1a	64.4a	63.8a	52.5a	63.8a	45.6a	55.6a
	Too Sweet	30.6a	0.6b	26.3a	23.1a	21.3a	36.3a	26.3a	34.4a	28.8a
Cooked Milky Flavor JAR (%)	Not Enough Flavor	6.9a	19.4a	9.4a	6.3a	6.3a	6.9a	7.5a	8.1a	10.0a
	JAR	73.1ab	61.3abc	71.9ab	80.0a	75.0ab	59.4bc	76.9ab	50.6c	68.1abc
	Too Much Flavor	20.0bc	19.4bc	18.8bc	13.8c	18.8bc	33.8ab	15.6bc	41.3a	21.9abc
Thickness/Mouthfeel/Viscosity Liking		6.8ab	6.31cd	6.7ab	6.7abc	6.9a	6.6bcd	6.9ab	6.29d	6.6abcd
Thickness/Mouthfeel/ Viscosity JAR (%)	Not Thick Enough	12.5a	20.6a	13.8a	20.6a	11.9a	10.6a	10.0a	13.8a	9.4a
	JAR	80.0a	71.9a	79.4a	73.8a	80.6a	73.8a	82.5a	76.9a	81.3a
	Too Thick	7.5a	7.5a	6.9a	5.6a	7.5a	15.6a	7.5a	9.4a	9.4a
Creaminess JAR (%)	Not Creamy Enough	10.0b	32.5a	16.9ab	18.8ab	8.8b	13.8b	15.0ab	21.3ab	13.1b
	JAR	84.4a	60.0b	74.4ab	70.0ab	81.9a	74.4ab	76.3ab	69.4ab	75.6ab
	Too Creamy	5.6a	7.5a	8.8a	11.3a	9.4a	11.9a	8.8a	9.4a	11.3a
Aftertaste Yes (%)		43.1ab	38.1ab	35.6a	39.4ab	43.8ab	50.0ab	44.4ab	58.8b	49.4ab
Aftertaste Liking		5.5ab	5.0bc	6.0a	5.4ab	5.9a	5.0b	5.7a	4.4c	5.0b

Table 5 (continued):

Quality	3.7a	3.1b	3.6a	3.6a	3.7a	3.4ab	3.7a	3.1b	3.4ab
Purchase Intent	3.6a	2.9cd	3.6a	3.5ab	3.6a	3.1bcd	3.7a	2.7d	3.2abc

Different letters in rows following means indicate significant differences ($p<0.05$)
Liking attributes were scored on a 9-point hedonic scale where dislike extremely = 1 and like extremely = 9
Just about right (JAR) questions were scored on a 5-point scale where 1 or 2 = too little, 3 = just about right, and 4 or 5 = too much. Percentage of consumers that selected these options is presented.
Quality was scored on a 5-point scale where 1 or 2 = low quality, 3 = neither high nor low quality, and 4 or 5 = high quality
Purchase Intent was scored on a 5-point scale where 1 or 2 = would not buy, 3 = may or may not buy, and 4 or 5 = would buy

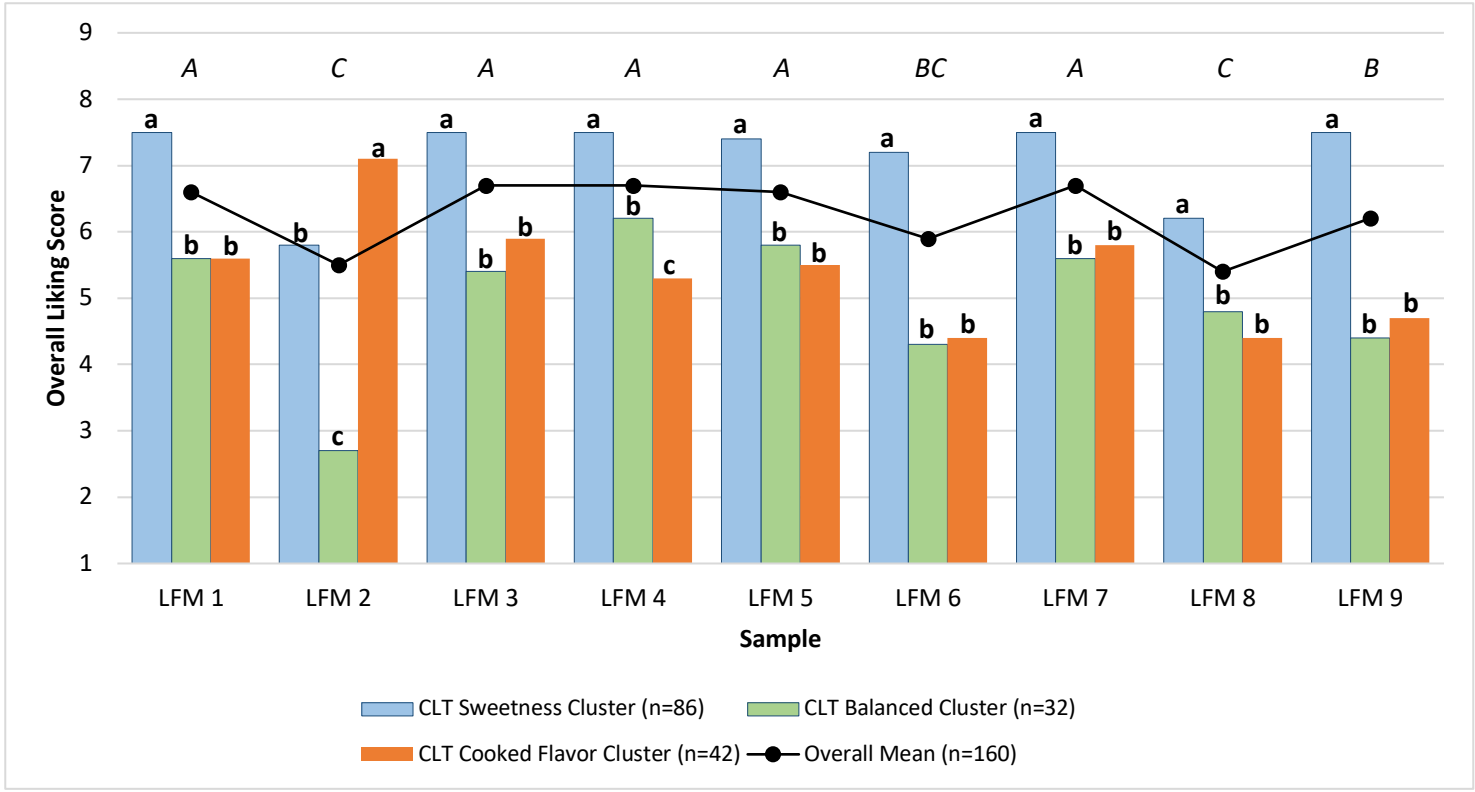

Figure 5: Mean overall liking scores for nine lactose-free milks (LFM) for the overall population (n=160) and by consumer cluster. Different lower-case letters within each LFM sample indicate significant differences between each consumer cluster ($p<0.05$) Different upper-case italicized letters over each LFM sample indicate significant differences between each attribute's importance score for the overall population ($p<0.05$)
Overall liking was scored on a 9-point hedonic scale where dislike extremely = 1 and like extremely = 9

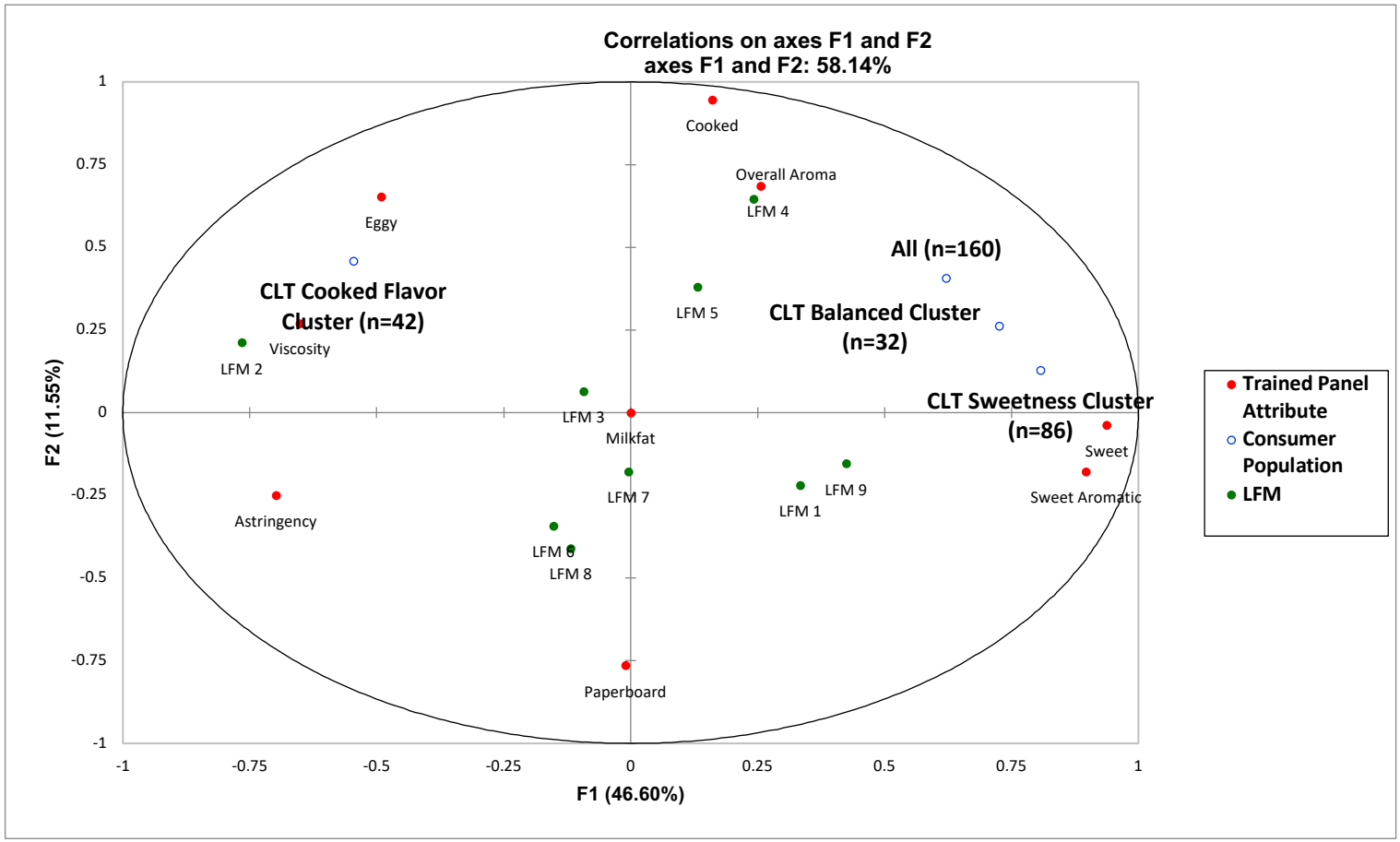

Figure 6: Partial least squares regression biplot of consumer clusters and descriptive analysis results for lactose-free milk.