A STUDY OF THE VOLATILE PROFILES OF SEVERAL CHEDDAR-TYPE ENZYME MODIFIED CHEESES

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DEDICATION

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ABSTRACT

Enzyme modified cheese (EMC) can add a very desirable cheese component to natural cheeses and products made from them. EMCs may be used to increase product uniformity, functionality, and the nutritional content of a food product. EMCs have been used for decades in food products such as snack foods and frozen meals, yet little research has compared the volatile profile across EMCs of a singular cheese type.

The aromatic profile of nine Cheddar EMCs was extracted using solvent-assisted flavor evaporation (SAFE) and was evaluated using a trained panel of seven sniffers, gas chromatography-olfactometry (GC-O), and gas chromatography-mass spectrometry. In this study, seventy-four unique odor-contributing chemicals were identified among all samples and given intensity ratings. The total number of volatiles per EMC ranged from 22 to 48. Of these, twelve chemicals provided an olfactory stimulus in only one EMC and only two – butyric acid and δ-octalactone – were perceived in all nine EMCs. Free fatty acids (FFA) were the most prevalent (area %) chemical group in all samples except one in which acetoin was most abundant. Six of the nine EMCs contained FFAs in a quantity of over 97% of the total odor-contributing volatiles. Most non-acid odorants were ethyl esters, δ-lactones, and 2-ketones, however, despite their low concentration, panelists labeled either γ-decalactone or γ-dodecalactone as one of the most intense non-acid odorants in eight of nine EMCs.

Through the understanding of the variations in the aromatic profiles of nine different Cheddar-type EMCs, product formulation can be improved increase to meet a customer's or a consumer's needs faster and more thoroughly.

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DCM	dichloromethane
DEE	diethyl ether
EMC	enzyme modified cheese
FFA	free fatty acids
FID	flame ionization detector
GC-MS	gas chromatograph-mass spectrometry
GC-O	gas chromatograph-olfactometry
ISTD	internal standard
LOL	Land O'Lakes, INC.
SAFE	solvent-assisted flavor extraction

1. CHAPTER I: LITERATURE REVIEW

1.1 INTRODUCTION

Enzyme-modified cheeses (EMCs) are cheeses which have undergone controlled, accelerated ripening periods through use of various enzymes and processing techniques (Salum et al. 2019). This accelerated ripening can provide a cheaper cheese ingredient with a more potent flavor and less fat. EMC's are made from a natural cheese of the same name and can have a similar or slightly altered texture to the unmodified cheese (Moskowitz and Noelck 1987). The final form of an EMC can be as either a paste or powder, and its application can be expanded for countless product-types. EMCs are GRAS-approved and have no limitations on proximate composition (Moskowitz and Noelck 1987; Freund 1993a). In general, EMC pastes contain 40-60% moisture while powders contain approximately 5% (Moskowitz and Noelck 1987).

1.2 ENZYME-MODIFIED CHEESE

1.2.1 Benefits of EMCs

Cheese has longtime been a desired ingredient and flavor in food products. However, variations in cheesemaking influence the performance and application of the final product. For many producers, cheese functionality must be consistent for texture, application, melt and spread (Guinee and Kilcawley 2017). EMCs offer intense flavor and make performance and application goals easier to meet. Product consistency also provides better control during product development and use (Christau 1993). EMCs are up to 30 times more potent than natural cheeses which allows for more flavor with less product (Moskowitz and Noelck 1987). By rapidly enhancing the natural flavor-producing pathways, aroma intensity is reached economically and can potentially be manipulated easily to fit the consumer preferences in a consistent and uniform manner (Kilcawley et

al. 1998; Guinee and Kilcawley 2017). Production costs decrease through enzyme-modification up to 40-80% compared to traditional ripening processes (Christau 1993). Although EMCs require additional processing steps, production occurs rapidly—eliminating the need for extended storage—and at a higher yield. Because of its increased yield and high potency, EMCs are used in much lower quantities in application than typical mature cheeses. The stability of EMCs are higher than that of traditional cheeses, possibly extending the expiration of products in which they are used (Christau 1993). This price reduction is also due in part to the use of cheaper ingredients such as stabilizers, vegetable oil or other milk ingredients, for their ability to add bulk which increases product yield. (Guinee and Kilcawley 2017). The main reason for consistency differences is because of the effect of seasonality on milk composition, although slight temperature or time changes in the ripening process can have a dramatic effect on the final flavor profile (Missel 1996).

EMCs are generally used at levels of 0.1-2% of a final product formulation but can be used up to 5%, depending on the intensity of the EMC and its application (Moskowitz and Noelck 1987). Aside from reduced cost, as previously discussed, reducing the amount of cheese used decreases the fat, cholesterol and calorie content of the product which can be beneficial in some cases for labeling, nutritional claims or functionality (Freund 1993b; Missel 1996). In the case of consumer opinion, reduced fat content can be desirable; many consumers view fat, especially saturated fat which comprises about 60-70% of the total fat in cheese, as a negative attribute in a food product (MacGibbon and Taylor 2006; Shingfield et al. 2008). Saturated fat has a negative association with cardiovascular disease and obesity; however, only C12:0, C14:0, and C16:0 seem to have negative physiologic effects. In fact, many of the fatty acids in milk, including short-chain, conjugated linoleic acid and vaccenic acid have been shown to boost health in numerous

animal studies (Parodi 2006; Shingfield et al. 2008). Nonetheless, consumer opinions drive sales. EMCs are a cost-efficient solution to help produce flavorful low-fat and no-fat cheese-flavored products (Freund 1993b)

1.2.2 Production of EMCs

The details of EMC production vary widely between manufacturers and cheese types, each requiring a different combination of techniques and ingredients to produce unique flavors. Additionally, most EMC production parameters are proprietary company knowledge and thus unpublished. Nevertheless, general EMC production is similar despite differences by facility. Every decision or change regarding processing parameters including time, temperature, pH, and ingredient additions, among others will affect the final flavor. Therefore, to produce a consistent product, it is essential for a producer to continuously monitor processing parameters and to keep a rigid schedule (Kilcawley et al. 1998).

A curd or shredded immature cheese of the same cheese type as the EMC is generally used as the starting material. A more mature, ripened cheese can be used; however, this increases the cost. Immature cheeses are cheaper than mature cheese of the same type, but use depends on the producer's preference (Kilcawley et al. 1998). The consistency and quality of the initial cheese substrate is of great importance to provide the same base materials in the same amounts and to give the most authentic flavor (West 1996).

Additional ingredients are added to the base cheese mix to improve product functionality, flavor, or microbial safety. Fat or protein may be added as substrate to promote increased flavor production or for texture or composition (Guinee and Kilcawley 2017). Common fat and protein sources include butterfat or cream. Flavor enhancers, including yeast extract, monosodium glutamate and diacetyl are commonly added to increase flavor strength.

Depending on the ingredient used and its amount, labeling requirements may deter use for certain customers (Anon 1996; West 1996; Kilcawley et al. 1998). EMCs intended to be used as a paste may need protection against undesirable microbial growth such as yeasts or coliforms. Nitrates, sorbic acid, nisin and potassium sorbate are often added for this purpose (Dulley 1976; Mann 1981). Prevention of phase separation is another concern for many producers, so emulsifiers and phase stabilizers may be added. Ingredients used to prevent phase separation include phosphate salts, mono- and diglycerides, citric acid, xanthan gum and antioxidants (Kilara 1985). Emulsifiers prevent phase separation by solubilizing protein, thereby increasing its ability to emulsify fat (Kilcawley et al. 1998). Emulsification also increases lipolysis by increasing the surface area of fat droplets (Kilcawley and Beresford 2002; Guinee and Kilcawley 2017). The most effective emulsifiers, therefore, are emulsifying salts which bind Ca^{2+} weakly and, if desired, provide an optimal pH to increase enzyme efficiency (Shrimp 1985; Guinee and Kilcawley 2017). Whatever the purpose, all ingredients are added with water to make a slurry for further processing.

Pasteurization of the cheese substrate inactivates any remaining microorganisms or enzymes present from previous processing or the environment. This reduces flavor inconsistencies from variations in starting composition of biologically active materials in the slurry. In addition, it becomes essential that all equipment used from this point on must be sterilized for the same reason (Kilcawley et al. 1998; Guinee and Kilcawley 2017). Pasteurization parameters vary according to the manufacturer but can range from 10 minutes at 72°C to 30 minutes at 82°C, before cooling (Kilcawley et al. 1998; Tamime 2010). Homogenization of this pasteurized substrate may be desirable to reduce phase separation and to ensure homogeneity for an even enzyme reaction. Homogenization also

aids in increasing surface area of fat droplets to increase lipolysis. (Guinee and Kilcawley 2017)

At this point, enzymes and starter cultures, if needed, are added to the pasteurized cheese matrix. Typical enzyme classes used include proteinase, peptidase, lipase and carboxylase, although any class can be used, depending on the desired outcome. The use of lipase and proteinase is essential, however, to obtain the characteristic cheesy aroma regardless of cheese type (Tamime 2010). Starter cultures can provide acidity for enzyme efficiency or flavor development and can participate in secondary reactions, increasing flavor production. The incubation temperature again depends on the producer's preference and the desired flavor profile of the final product. A high temperature can increase the reaction speed but a temperature that is too high will kill starter cultures or inactivate enzymes prematurely. In general, the cheese slurry is incubated at temperatures between 40-55°C for a few days. The use of more heat-stable enzymes and cultures may allow for shorter incubation times (as short as 8 hours) at 80°C (Tamime 2010). As with any parameter in the manufacture of EMC, consistency in manufacture is critical for a consistent and quality product, including the timing of ingredient, enzyme, or culture additions (Kilcawley et al. 1998). During incubation, hydrolysis of protein, fat and, in some cases, carbohydrate proceeds at a rapid rate. To maintain homogeneity, continuous agitation is accomplished with slow-moving scrapers (Kilcawley et al. 1998).

Once the desired flavor profile is reached, the mixture must again undergo heat treatment, to inactivate the microorganisms and enzymes within the product to prevent further compositional changes. This step is essential to maintain product stability and quality and to increase shelf life. Processing parameters will again differ between manufacturers, but the time and temperature combination serve the same purpose: to kill any remaining cultures and to inactivate all enzymes (Kilcawley et al. 1998; Tamime 2010; Guinee and

Kilcawley 2017). It is also important to not destroy the developed flavor through overcooking. A producer can monitor residual enzyme activity through amylase or proteinase activity (West 1996; Kilcawley et al. 1998). Residual enzymes or cultures will affect both the final EMC product composition and the product in which the EMC has been used. In either case, uncontrolled product changes are undesirable.

This heat-treated EMC paste can be homogenized again or can be directly packaged after cooling (Guinee and Kilcawley 2017). EMCs are packaged as a paste or powder, with the choice depending on its application or customer preference (Tamime 2010). Powdered EMCs have a longer shelf life than pastes and the powdered form can increase the ease of application (e.g. on chips, in dry blends). Spray-drying is the most common method used to make EMC powder from EMC paste. This requires additional ingredients as carriers, e.g. whey and maltodextrin might be added to facilitate the spray drying of the EMC paste. The addition of any ingredient dilutes the flavor intensity of the EMC powder, but the final product would still be stronger than a natural cheese powder of the same type (Kilcawley et al. 1998; Guinee and Kilcawley 2004). The final infeed mixture for the spray drier will vary between producers and cheese type, but a solids content of at least 35% is considered desirable. The preferred particle size of an EMC powder is 100-150 μm which increases its dispersibility, increasing ease of use (Kilcawley et al. 1998).

1.2.4 EMC Applications

EMCs are the main alternative to natural cheeses in food products, especially where a cheesy flavor predominates (Missel 1996). EMCs are especially useful in low-fat and no-fat cheese products. The technology used to make EMCs can be applied to any cheese, but the most common types include Cheddar, Swiss, Blue, Mozzarella, Provolone, Feta and Parmesan (Moskowitz and Noelck 1987). As previously mentioned, EMCs are generally added to foods at 0.1-2% of the total formulation by weight but can be added up

to 5% (Moskowitz and Noelck 1987). Application in food products is generally as a powder, but pastes are used as well. EMC powders offer increased shelf life and a strong flavor in products such as instant mashed potatoes or macaroni compared to paste forms. Powders are also easily applied to crackers and chips. EMC pastes are more often used as ingredients in processed cheese or cheese sauces. In many cases, EMCs can offer better functionality and product stability than natural cheese during freeze-thaw cycles due to their inability to coagulate, such as in the case of frozen cheese sauces (Missel 1996). Other products which commonly use EMCs include soups, salad dressings, cheese analogs and bakery products, among many more (Freund 1993a; Buhler 1996; Missel 1996; Guinee and Kilcawley 2017).

1.3 AROMA DEVELOPMENT

1.3.1 The Olfactory System

Flavor perception consists of a combination of the experiences of taste, aroma, mouthfeel, appearance and chemesthesis of a food. The olfactory system, responsible for aroma, has a detection limit as low as 10^{-19} moles for certain volatiles and is often much more sensitive than our most sensitive analytical equipment (Reineccius 1994; Curioni and Bosset 2002). The concentration, character, and odor threshold of individual compounds present in a food determines whether the final aroma will be pleasant or undesired. While there are exceptions, often 75-95% of what is perceived as flavor originates in the olfactory system as aroma (Spence 2015). Therefore, an understanding of the volatile fraction of EMCs is of great importance for product consistency, improvements, and use. This study explores the volatile compounds of various Cheddar-type EMCs and thus flavor development during Cheddar cheese and Cheddar-type EMC production will be discussed.

1.3.2 Flavor Development in Enzyme-Modified Cheese

Like most cheeses, the majority of Cheddar flavor and its EMC derivative forms during the ripening process. Although an EMC can be produced without the use of its title cheese, full character aroma is unlikely to be reached without it (Kilcawley et al. 1998). Therefore, the entire processing line from the manufacture of the cheese substrate used for EMC production to the final application of the EMC itself must be considered when determining all methods of flavor development. Flavor production begins with the cow, is altered with each processing parameter, and is finished with cultures, starter or adjunct, and enzyme slurries. The enzymes consist primarily of lipase and protease, but carboxylase, esterase, phosphatase, and peptidase may also be added (Fox 1993, 2011; McSweeney 2004). The degradation of milk constituents leads to a limited number of compounds which contribute directly to cheese aroma including short chain fatty acids, δ-lactones and ethyl esters. The majority of cheese aroma comes from complex biological processes that occur synergistically between microbes and exogenous enzymes (Singh et al. 2003). Some of the changes include deamination, decarboxylation, esterification and β-oxidation (Fox 1993). These secondary reactions are largely what produce top notes and give the characteristic title flavor (Singh et al. 2003).

1.3.2.1 During Manufacture

Consistency during manufacture is extremely important to producing a consistent product. Any deviation in manufacture can have a drastic impact on the types and rates of reactions which will occur during ripening and lead to the final aroma (McSweeney 2004). These parameters include processing temperatures, water activity, pH, salt, time, and moisture content (Fox 1993; Singh et al. 2003). These parameters affect not only aroma but the overall flavor and texture of the cheese as well. For example, salt levels can affect culture activity, influencing acid production and pH. As a result, the quantity of

nonvolatile free fatty acid salts increases, directly reducing their contribution to aroma (Fox 1993; Singh et al. 2003).

Cheese aroma starts with the cow; it's influenced by her diet, breed, microflora, environment, and the time of year (Fernández-García et al. 2002; Muñoz et al. 2003; Andiç et al. 2015). The specific fatty acid profile and protein composition shapes the base milk flavor (Foda et al. 1974). Pasteurization can help to eliminate some inconsistencies by inactivating most of the indigenous microbes and their enzymes, but it can also slightly heat-denature whey protein, increasing the volume of whey proteins retained in the final cheese product. This whey may increase the accessibility of casein to proteinases, increasing hydrolysis and volatile precursors (Lau et al. 1991).

Moisture loss during Cheddaring or any other step of manufacture may occur (Walstra et al. 2006). For example, changes to the height or width of Cheddar blocks can increase or decrease moisture loss due to syneresis (Fox 1993). Changes in moisture can directly influence the rate of primary and secondary reactions, therefore altering the final aroma profile. In addition, the time of whey drainage during Cheddaring determines the mineral and salt content, the final pH, and the moisture to casein ratio (Lawrence et al. 1983). Finally, the time at which exogenous enzymes and cultures are added is crucial, as is the strain and source of those ingredients (di Palma et al. 1987; Seitz 2010). Sources can be animal, microbial or plant and each may have its own impurities such as sugars, salts, preservatives, and traces of undesirable enzymes (Fox 2011).

1.3.2.2 Proteolysis

Proteolysis, which occurs up to 4 times more in an EMC than in the named cheese, generates high levels of amino acids and peptides (Fox 2011). Some of these compounds can contribute directly to the overall flavor of Cheddar, but most are

nonvolatile and are important contributors to the characteristic savory taste of Cheddar. Raw milk contains the native protease, plasmin, which likely doesn't contribute much to the overall aroma after pasteurization (Visser 1993). It can have some impact, though, depending on the amount of time the milk is aged before pasteurization. Chymosin and plasmin are responsible for the hydrolysis of casein into large molecular weight peptides, which are further broken down into small peptides and amino acids by starter or adjunct bacteria enzymes (Fox et al. 1994, 1995; Singh et al. 2003). Endopeptidases are commonly used for their broad affinity for high molecular weight casein peptides, however any enzyme which cleaves peptides may be used (Fox 2011).

1.3.2.3 Lipolysis

Cheddar cheese has a high fat content, typically around 30%. This fat is critical to the aroma development as fat is both a source of and a carrier of flavor compounds (Renner 1993). Without fat, a full characteristic aroma will not be reached (Ohern and Tuckey 1969). Like any other food, the fat in Cheddar is subject to various degradation reactions including lipolysis by enzymatic hydrolysis and oxidation (Singh et al. 2003). Oxidation in cheese is limited and therefore isn't typically a concern for flavor development (Adda et al. 1982). Lipolysis, however, plays an essential role in cheese flavor, largely for its contribution of free fatty acids which contribute both directly and indirectly (secondary reactions) to the volatile fraction. Triglycerides compose 98% of the fat in cheese and are broken into free fatty acids, mono- and di-glycerides and glycerol (Singh et al. 2003). This hydrolysis typically occurs at the S_n1 and S_n3 positions, favoring short and medium chain fatty acids, leading to a much larger concentration of butyric acid, as compared to the other acids (Singh et al. 2003). Lipolysis can occur 1,000 times faster at the oil-water interface, so emulsification is often employed to increase this surface area during the manufacture of EMCs (Fox 2011).

Endogenous lipoprotein lipase can begin hydrolysis if the milkfat globule membranes are disrupted during processing. Milk also contains native esterases as do the cheese microflora which can act on damaged milkfat globule membranes (Deeth and FitzGerald 1983; Olivecrona et al. 1992; Singh et al. 2003). After pasteurization of the cheese slurry, additional cultures are added to kick-start lipolysis while increasing acidity, exogenous enzymes, and secondary cultures to accelerate lipolysis. Most lipases used in EMCs are of animal or bacterial origin. Microbial lipase tends to be cheaper and are vegetarian and kosher, but are not free of contaminants. However, their wide range of specificity to produce varying fatty acid profiles continues to make them a popular choice (Fox 2011).

1.3.2.4 Starter and Non-Starter Microbes

Microorganisms in cheese serve two purposes: to lower pH and to produce volatile and nonvolatile flavor chemicals typical of Cheddar cheese. They generally come from two sources: the primary, or starter lactic acid bacteria, and the secondary microflora. Starter cultures rapidly reduce pH so secondary microbes can begin volatile formation during the ripening process (Andiç et al. 2015). However, starter cultures can also participate in the ripening process. Many *Lactobacilli* contain various proteolytic enzymes which are largely responsible for the production of free amino acids and small peptides (O'keefe et al. 1978; Atlan et al. 1993). Some species, *lactobacilli*, *pediococci*, *lactococci*, and *micrococci*, are known to drastically improve Cheddar flavor and are thus commonly added as secondary microorganisms (McGregor and White 1988; Saker and White 1988). Each species or strain will produce a different mix of compounds due to their differences in rate of hydrolysis and affinity to flavor molecules. However, some researchers found that differences in the proteolytic activity of *Lactobacilli* had only a

small effect on flavor, possibly due to differences in acid production (di Palma et al. 1987; Seitz 2010).

Acidification occurs as lactic acid bacteria, primarily *Lactococcus lactis* spp *cremoris* and ssp *lactis*, converts lactose into lactic acid (Singh et al. 2003). The acid enables optimum enzymatic and adjunct culture activity to further hydrolyze peptides and to create volatile precursors and their subsequent products. Some manufacturers may add exogenous lactic acid and acetate to generate a specific aroma character or to rapidly reduce pH (Kilcawley et al. 2010).

1.3.3 *Aroma Formation During Accelerated Ripening*

Although specific aroma compounds and their concentrations will vary between Cheddar cheeses from different producers, they all share the same base components: fatty acids, esters, lactones, ketones, aldehydes, furans, alcohols and sulfur-containing compounds (Moskowitz and Noelck 1987; Maarse et al. 1989; Singh et al. 2003). The main pathways of creation for each compound class will be discussed.

1.3.3.1 Fatty Acids

Free fatty acids comprise the major proportion of the volatile fraction of Cheddar cheese and their distribution from milk triglycerides greatly influences the final flavor. Most short-chain free fatty acids (C:4 to C:10) are produced primarily as a result of lipolysis of triglycerides (Stark and Adda 1972). Lower molecular weight acids (C:2 to C:6) are also created through lactose and amino acid degradation and through the oxidation of ketones, esters, and aldehydes (Molimard and Spinnler 1996). These short-chain acids, including even-chain acids up to C:12, are generally the biggest contributors of the acids to cheese flavor because of their low-odor threshold. In general, their odors are described as sharp, goat, rancid and vinegar. Longer-chain fatty acids (those over C:12) play a small role in

the overall flavor, despite their relatively high quantity; high odor thresholds can make their contribution low (Curioni and Bosset 2002; Collins et al. 2003). The intensity of free fatty acids depends not only on their concentration but also on their distribution in the fat phase and on the concentration of salts. An increase in salt content or pH can decrease the volatility and odor intensity of free fatty acids through changing ionization (Adda et al. 1982; Brennand et al. 1989). Free fatty acids provide strong aromas typical to Cheddar cheese but they (C:4 to C:10) also serve as building blocks for numerous other important compounds, such as ketones, alcohols, lactones and esters (Curioni and Bosset 2002).

1.3.3.2 Amino Acids

Amino acids are nonvolatile but provide important precursor materials for volatile formation. Because of this, they are often studied as a key part of cheese flavor (Singh et al. 2003). Some researchers found that the rate of conversion of amino acids to volatile compounds was more important to the rate of flavor production than the increased amino acid content during proteolysis (Yvon et al. 1998). Secondary products of proteolysis include aldehydes, esters, carboxylic acids, sulfur-containing compounds, and acids and methanethiol (Hemme et al. 1981; McSweeney and Sousa 2000; Kranenburg et al. 2002). Phenylalanine, tyrosine, tryptophan, leucine, isoleucine, valine and methionine are believed to be the amino acids most responsible for these products (McSweeney and Sousa 2000).

1.3.3.3 Esters

Esters are produced from the reaction of an acid and an alcohol. In the case of cheese, ethanol, produced by starter lactic acid bacteria, reacts with the carboxylic acid of free fatty acids, creating ethyl esters (Arora et al. 1995; Urbach 1997). These typically have a sweet, fruity aroma with floral notes, giving cheese its fruity notes (Bills et al. 1965; Curioni

and Bosset 2002). However, an over-production of esters can create overly fruity cheese which is considered an off-flavor (Bills et al. 1965; Morgan 1970; Liu et al. 1998). This is believed to be caused by an excessive alcohol production of *Lactobacilli*. Ester formation is also affected by the salt concentration and water activity while minimally affected by pH or temperature (Liu et al. 2004).

1.3.3.4 Lactones

Lactones in cheese are cyclic esters produced by the intramolecular esterification of hydroxy acids and subsequent ring closure (lactonization) (Jolly and Kosikowski 1975; Molimard and Spinnler 1996). Their aroma contribution is small and is believed to only contribute background flavor. Odor descriptors characteristic of lactones include peach, apricot, coconut, and caramel (Jolly and Kosikowski 1975). Both δ-and Ɣ- lactones have been identified in Cheddar cheese but δ- lactones are more prevalent (Wong et al. 1973).

1.3.3.5 Ketones

Oxidative degradation of free fatty acids, mainly C:6 to C:12, leads to the formation of methyl ketones (Collins, Y et al. 2003). Ketone odor changes from fruity to floral with increasing molecular weight (Reineccius 2006). Contribution of methyl ketones to the overall Cheddar aroma is low, and likely just part of background or top-note flavors (Hawke 1966; Singh et al. 2003). Methyl ketones can also be reduced to secondary alcohols, thus reducing the aroma contribution of ketones (Hawke 1966).

1.3.3.6 Alcohols

Alcohols are formed from secondary and tertiary reactions of amino acids and fatty acids. (Jolly and Kosikowski 1975; Hemme et al. 1981; Molimard and Spinnler 1996; Andiç et al. 2015). Alcohols can also be formed through lactose fermentation by lactic acid bacteria. As a results of lactic acid bacteria fermentation and continued hydrolysis, ethanol is the

alcohol most abundantly formed during ripening (Arora et al. 1995). However, ethanol does not typically contribute directly to cheese flavor; instead, it reacts quickly with free fatty acids to create ethyl esters with free fatty acids, which contribute a great deal to the volatile profile.

Alcohols decreases in polarity and solubility with increasing chain length which can affect their expression in the final aroma; those with increased solubility may be lost with water during processing while those with an increased affinity for fat may be concentrated in the fat portion. Alcohols exhibit an array of odors. The unsaturated series of alcohols begin with harsh and very unpleasant odors. As they increase in size, green and earthy notes emerge (Reineccius 2006).

1.3.3.7 Aldehydes

Aldehydes are transamination products of amino acids and are considered transitory compounds, meaning that they can be reduced or oxidized to alcohols or acids. This can occur rapidly in the ripening process via enzymatic catalysis (Keeney and Day 1957; Moio et al. 1993; Andiç et al. 2015). β-oxidation of unsaturated fatty acids can also lead to aldehyde formation. The odors of straight-chain aldehydes is characterized by green, grassy and herbaceous aromas, becoming more floral with higher molecular weight, and their contribution is considered pleasant until their concentrations exceed certain thresholds (Collomb and Spahni 1996; Curioni and Bosset 2002; Reineccius 2006).

1.3.3.8 Sulfur-Containing Compounds

Sulfur-containing compounds have low-odor thresholds and typically exhibit strong garlic and ripe cheese odors. Their production is believed to be a result of methionine catabolism by either a lyase deamination and demethylation or by transamination from *lactococci* ssp aminotransferases (Alting et al. 1995; Gao and Steele 1998; Berger 1999).

1.3.4 Aroma Profile of Cheddar Cheese

Despite decades of analysis into Cheddar cheese aroma, a character impact compound has yet to be determined. This supports the idea that Cheddar flavor is a complex mix of volatile and nonvolatile compounds as opposed to the presence of a single characterizing compound. Odor analysis of Cheddar cheese, as in any fermented food product, becomes more difficult with the differences of flavor development during production; aroma compounds found in one Cheddar may be a great deal different from those in another. Additionally, the length of ageing can cause increases or decreases in different compounds, increasing or reducing their aroma contribution (Hannon et al. 2006). This makes it difficult to provide a definite list of volatile compounds and their concentrations that are found in Cheddar. Many of the studies reviewed reported different volatile compositions, but some compounds were often found in Cheddar cheeses (Table 1).

The most prevalent and abundant odorants in Cheddar cheeses are fatty acids. Saturated fatty acids from C:2 to C:18 have been identified in various concentrations, with even-chain acids being in the majority. Butyric acid is often the predominating acid in quantity with caproic acid following. Overall, though, the proportion of fatty acids, aside from butyric acid, would be similar to that of the milk used (Siek et al. 1969, 1971; Kilcawley et al. 2010).

Both Ɣ- and δ- lactones have been found in all Cheddars while δ- decalactone and δ-dodecalactone may be key odorants in certain types of Cheddar (Wong et al. 1973; Jolly and Kosikowski 1975). Researchers found various low molecular weight (C:12 and fewer) aldehydes and 2-ketones were found by many researchers, most notably nonanal and 2-butanone, and in addition, acetoin (Keen et al. 1974; Curioni and Bosset 2002). Of the ethyl esters, ethyl butyrate and ethyl caproate are considered the most important

contributors of the class, but esters up to ethyl laurate have been identified (Arora et al. 1995; Curioni and Bosset 2002).

Perhaps the most important set of compounds to Cheddar aroma are sulfur-containing compounds. Cheddar cheese flavor is dependent on the presence of sulfur compounds, but there has not been a single compound whose concentration varies with Cheddar flavor. It is hypothesized that the sulfur compounds observed may be breakdown products or precursors of other important volatile compounds. An additional theory suggests that the sulfur compounds and neighboring volatiles together produce the Cheddar flavor (Urbach 1993). Methanethiol is most commonly associated with a good Cheddar cheese flavor. Additional sulfur-containing compounds of note in Cheddar flavor include methional, hydrogen sulfide, dimethyl sulfide and dimethyl trisulfide (Lindsay and Rippe 1986; Urbach 1995; Milo and Reineccius 1997).

Table 1. Volatiles previously identified in Cheddar cheese by two or more authors

Acetic acid[1, 2, 3, 9, 11, 12, 16]	ϒ-decalactone[2, 11, 13]	Pentanal[4, 14]
Propanoic acid[1, 16]	δ- dodecalactone[2, 11, 13, 16]	Hexanal[3, 4, 11, 14, 16]
Butyric acid[1, 2, 3, 9, 10, 11, 12, 16]	Ethanol[4, 5, 9, 12, 13, 14]	Heptanal[4, 6, 11]
Valeric acid[1, 2, 16]	1-propanol[4, 15]	Acetaldehyde[3, 13]
Caproic acid[1, 9, 11, 12, 16]	1-pentanol[4, 6]	α-pinene[1, 12, 14]
Caprylic acid[2, 11, 12, 16]	2-propanol[4, 6]	Homofuraneol[3, 11]
Capric acid[1, 10, 11, 16]	Acetoin[4, 6, 7, 11, 12, 13]	Skatole[3, 11]
Lauric acid[1, 16]	Acetone[4, 5, 7, 12, 13, 15]	Methional[2, 3, 5, 11, 13, 16]
Ethyl acetate[1, 4, 6, 9, 11, 12, 13]	2-butanone[4, 5, 6, 7, 12]	Dimethyl sulfide[3, 4, 5, 11, 12, 13, 15, 16]
Ethyl butyrate [1, 2, 4, 6, 9, 11, 12, 13, 14, 15, 16]	2-pentanone[4, 5, 6, 7, 12, 13]	Dimethyl disulfide[13, 14, 15]
Ethyl caproate[1, 2, 4, 6, 16]	2-heptanone[7, 12, 13]	Dimethyl trisulfide[3, 4, 11, 13, 14, 16]
Ethyl caprylate[2, 12]	1-octen-3-one[1, 2, 3, 11, 16]	Dimethyl tetrasulfide[3]
δ- octalactone[13, 16]	2-nonanone[7, 12, 13]	Methanethiol[5, 12, 13]

δ- decalactone[2, 3, 11, 12, 13, 16]	Diacetyl[1, 3, 4, 5, 6, 7, 9, 11, 12, 13, 15]	Hydrogen sulfide[5, 8, 15]

[1](Christensen and Reineccius 1995); [2](Suriyaphan et al. 2001); [3](Milo and Reineccius 1997); [4](Arora et al. 1995); [5](Manning and Robinson 1973); [6](Arora et al. 1995); [7](Walker and Harvey 1959); [8](Walker 1959); [9](Dacre 1955); [10](Urbach 1997); [11](Drake et al. 2010); [12](T. Elizabeth et al. 2018); [13](Perret 1978); [14](Wood 1989); [15](Lindsay and Rippe 1986); [16](Avsar et al. 2010)

1.4 SUMMARY

Cheddar cheese aroma is undoubtedly a complex mix of volatile compounds which is greatly influenced by its processing parameters. Any deviation in manufacturing conditions, including the length of time of each processing step, pH, salt, and moisture or the inputs such as enzymes, microbes, or other ingredients, can cause flavor defects and inconsistencies.

Cheddar cheese aroma can be created faster with the production of EMCs, which provides an economical option for cheese flavors. Its base flavor comes from the shreds or curds that are used in production which can be from a young or mature cheese. Any added enzymes or microbes use the protein, fat, carbohydrates, and volatile chemicals from the cheese slurry to produce an aromatic profile. Proteolysis and lipolysis are the major pathways for the flavor formation of Cheddar-type EMCs. These pathways produce hundreds of different volatile compounds which are important for Cheddar flavor, especially fatty acids, esters, lactones, ketones, aldehydes, furans, alcohols and sulfur-containing compounds.

2. CHAPTER II: OBJECTIVES, MATERIALS AND METHODS

2.1 OBJECTIVES

This study provides a complete aroma profile of seven commercial Cheddar-type EMCs and two Cheddar-type natural cheeses through semi-quantification and identification of volatile aroma compounds using Gas Chromatography-olfactory-flame ionization

detection and gas-chromatography-mass spectrometry analysis. Nine powdered cheese systems composed of various amounts of EMC samples were similarly analyzed for its overall aroma profile.

2.2 MATERIALS

2.2.1 Chemicals and Gases used in Analysis

Chromatography-grade dichloromethane (DCM) and reagent-grade sodium hydroxide were purchased from Fischer Scientific (Geel, Belgium) and hexyl ether (ISTD 1) was purchased from Tokyo Chemical Industry Co., LTD (Tokyo, Japan). Undecane (ISTD 2) and the anhydrous magnesium sulfate used for sample drying were sourced from Sigma-Aldrich (St. Louis, MO, USA). The liquid nitrogen and all gases used (nitrogen, hydrogen [ultra-high purity], and compressed air) were purchased from Matheson (Basking Ridge, NJ, USA).

2.2.2 Chemicals used in the Preparation of Olfactory References

The following chemicals were used for preparation of olfactory references: heptanal (Abcam, Cambridge, UK), decanal, nonanal, octanal (Acros Organics, Geel, Belgium), 2-heptanone, 2-nonanone, 2-octanone (Alfa Aesar, Haverhill, MA, USA), 2-decanone, 2-undecanone (Beantown Chemical, San Diego, CA, USA), 2-pentadecanone, 2-tridecanone (Frontier Scientific, Logan, UT, USA), acetoin, acetol and ethyl octadecenoate (Tokyo Chemical Industry Co., LTD, Tokyo, Japan). All other chemicals used for odor preparation were purchased from Sigma Aldrich (St. Louis, MO).

2.3 METHODS

2.3.1 Sample Preparation

This method was modified from the work of *Milo and Reineccius* (1997). Three hundred g of frozen EMC was ground using a KitchenAid shredder attachment. The metal collection bowl and grinding tools were chilled ten minutes before use to prevent sticking of cheese shreds.

Three hundred mL of DCM was added to two 1 L Erlenmeyer flasks separately with a large stir bar (1 x 7 cm). Twenty-six µl of a 10,000 ppm internal standard stock solution, hexyl ether in DCM, was added to each flask and the mixture was stirred.

2.3.2 Volatile Extraction

Cheese shreds (150g per flask) were slowly added to each of the solvent-containing flasks and then were stirred for one hr. After filtering (Whatman grade 1, 240 mm), the volatile compounds were re-extracted using the same technique with 200 ml DCM per Erlenmeyer. The extracts were combined and concentrated to 300 ml through fractional distillation with a Vigreux column (40 cm x 2 cm) at a maximum temperature of 40°C. The non-volatiles were collected using a solvent-assisted flavor evaporation (SAFE) system as described by Engel et. al. (ECHA 2021). The water bath was set to 60°C and vacuum of 10^{-5} Torr was maintained for the duration of the extraction. The sample was added dropwise at a rate no faster than 100 ml per hour and a liquid nitrogen bath was used to chill the collection flask.

2.3.3 Volatile Fractionation

The EMC extract was brought to room temperature and divided into two fractions (1:5). A second internal standard, undecane, was added to each fraction (14 µl of a 1600 ppm

stock solution in DCM). The smaller solvent fraction was dried with anhydrous magnesium sulfate for 10 min and concentrated under nitrogen to a final volume of 0.2 mL using a Kuderna-Danish vial (sample FA). The larger fraction was extracted in a separatory funnel with 2 N NaOH (3 x 32 ml) to remove free fatty acids. The sample was again dried with anhydrous $MgSO_4$ and concentrated under the same conditions to a final volume of 0.2 mL (sample NFA). Gas Chromatography-Olfactory (GC-O) and Gas Chromatography-Mass Spectrometry (GC-MS) were used to analyze the extracts.

2.3.4 Sensory Panelists for GCO – Selection, Training and Odor Identification

2.3.4.1 Panelist Selection

Twelve panelists were used for odor identification on the GC-O work. Recruited panelists were members of the University of Minnesota (UMN) Sensory Center's email list of the Sensory Center and/or students, faculty and staff in the UMN Department of Food Science and Nutrition. Panelists were informed of the study opportunity via email and/or flier posted in the UMN Food Science and Nutrition building on the UMN St. Paul campus. Screening parameters included being of age 18-65 and being absent of any olfactory disorder.

2.3.4.2 Odor Selection and Training

Fifty odors were selected for odor training (Table 2) based on preliminary GC-O analytical analyses of four EMC samples. The fifty compounds selected were chosen for their prevalence in the four EMCs used in preliminary testing, as well as in studies of other Cheddar cheeses, and based on their availability as pure reference compounds. Odorants were organized by their retention times, as determined under the GC-O method used, and then grouped into five sets of ten odors; the ten training odors with the lowest retention times comprised the first set of ten odors, the ten odors with the next lowest retention times comprised the second set of ten odors, and so forth. Each odorant was prepared for

sensory analysis by adding two or three drops of each chemical to a cotton ball that was in a 30 ml (25mm x 95mm) glass vial, which was then tightly sealed with a black plastic lid. The vials were labeled with a randomly assigned three-digit code; the three-digit codes differed across odors and training sessions.

Table 2**.** Chemicals used for odor training for GC-O followed by retention time (min) in parenthesis, which are separated into five sets of ten.

Odor Set	Odorants
A	2-hexanone (2.843), Et[1] butyrate (3.426), hexanal (4.049), 2-heptanone (4.136), Et valerate (5.063), heptanal (6.122), Et caproate (7.482), 2-octanone (9.064), octanal (9.013), acetoin (9.146)
B	Acetol (9.698), Et heptanoate (10.561), nonanal (12.458), 2-nonanone (12.488), Et caprylate (14.046), acetic acid (14.696), 2-decanone (16.140), decanal (16.119), Et nonanoate (17.469), propanoic acid (17.554)
C	2-undecanone (19.280), butyric acid (19.864), Et caprate (20.313), 2-dodecanone (21.936), valeric acid (22.739), Et undecanoate (22.786), 2-tridecanone (24.337), δ- hexalactone (24.344), Et laurate (25.028), caproic acid (25.091)
D	δ- heptalactone (25.963), heptanoic acid (27.253), δ- octalactone (27.916), 2-pentadecanone (28.551), Et myristate (29.060), caprylic acid (29.284), δ-nonalactone (30.040), ϒ-decalactone (31.032), nonanoic acid (31.198), δ-decalactone (32.050),
E	Et palmitate (32.653), capric acid (33.021), δ- undecalactone (34.002), undecanoic acid (34.764), δ- dodecalactone (35.858), Et octadecenoate (36.012), lauric acid (36.433), tridecanoic acid (38.040), myristic acid (39.588), palmitic acid (42.541)

[1]ethyl

Training instructions were given through the SIMS software program (Sensory Computer Systems, Berkley Heights, NJ). Panelists were first instructed to select a designated vial by number, smell the odor and select the correct name of the odor from a list of potential compound names; the list of potential names included the actual name of the chemical, as well as the names of some of the other chemicals included in odor training. Upon selection, panelists were immediately notified of the correct label and instructed to repeat the name aloud while re-smelling the odor. The next odor was then presented following

the sample procedure. Odors of one odor set were presented in random odor without repeat until all odors were presented in the trial. For an odor set, this labelling procedure was repeated 15 times during the first training session in which the odor set was presented and 3 times in subsequent training sessions with the odor set. In sessions with multiple odor sets, panelists completed all labeling trials for an odor set prior to beginning labelling trials for another odor set.

Training took place across 23 days with panelists completing one training session per day. Throughout training, the number of odor sets presented in a session and the number of potential labels presented with each odor progressively increased. Table 3 outlines the design of the odor training program, including the individual odor sets presented, the number of labelling trials completed for each odor set, the order in which odor sets were presented, and the number of labels presented with each odor stimulus in a session. A brief summary of the training program design is given below.

In training sessions 1 through 13, panelists selected the correct name for each odor in a given odor set among a list of 5 potential labels; the potential labels provided varied among odors and included the odor ‘s name as well as the names of 4 other odors in the odor set. In training sessions 14 through 18, each odor was an odor set was presented with 10 potential labels which represented the correct name for the odor as well as the names of the nine other odors in the odor set. In sessions 19 through 23, each odor is presented with 15 potential labels which represented the correct name for the odor, as well as the names of the seven training odors which eluted immediately before and after the odor.

In session 1, panelists completed 15 labelling trials for one odor set. In sessions 2 through 5, panelists completed 3 labeling trials for one odor set prior to completing 15 labelling trials for a second odor set. Panelists completed 15 labeling trials for odor set A, B, C, D, and E in session 1, 2, 3, 4, and 5, respectively. Following session 5, only three trials were

completed for each odor set presented in a session. In session 6, sessions 7 through 11, sessions 12 through 16, and sessions 17 through 23, three trials were completed for two, three, four, and five odor sets, respectively.

Table 3. Design of the odorant training program.

Session	Odor Set[1]					Number of labels[2]	Odor set presentation order within a session[3]
	A	B	C	D	E		
1	15*					5	A
2	3	15				5	A, B
3		3	15			5	B, C
4			3	15		5	C, D
5				3	15	5	D, E
6	3				3	5	E, A
7	3	3	3			5	A, B, C
8		3	3	3		5	B, C, D
9			3	3	3	5	C, D, E
10	3			3	3	5	D, E, A
11	3	3			3	5	E, A, B
12	3	3	3	3		5	A, B, C, D
13		3	3	3	3	5	B, C, D, E
14	3		3	3	3	10	C, D, E, A
15	3	3		3	3	10	D, E, A, B
16	3	3	3		3	10	E, A, B, C
17	3	3	3	3	3	10	C, D, E, A, B
18	3	3	3	3	3	10	D, E, A, B, C
19	3	3	3	3	3	15	E, B, C, A, D
20	3	3	3	3	3	15	A, C, E, D, B
21	3	3	3	3	3	15	B, D, A, E, C
22	3	3	3	3	3	15	D, E, B, C, A
23	3	3	3	3	3	15	C, A, D, B, E

[1]the ten odorants in each odor set are listed in Table 2

[2]number of potential labels which panelists selected from when labelling each odor stimulus during the labelling trials for a session.

[3]the order in which subjects completed the labelling trials for each odor set in a session. Presentation order should be read from top to bottom.

*Number of labelling trials completed for the odor set in the specified session.

Table courtesy of Sara Kleba

After odor training was complete, panelists participated in an introductory GC-O run in which the operating principle of the GC and olfactory port were explained. Each panelist

then experienced the sniffing procedure with a solution of isoamyl acetate, eugenol, citral, cinnamaldehyde and allyl isothiocyanate; each odor was chosen based on its likely absence from the EMC samples. A brief introduction to the GC-O was given to panelists before their training with the sniffing port (Appendix 1).

2.3.4.3 Odor Identification

"FA" and "NFA" extracts of each EMC were evaluated by six panelists; panelists were selected from the pool of panelists that had completed odor training. Each panelist assessing a sample sat through the entirety of one GC-O run, which was one hour. A total of six runs were completed for each sample type per EMC with each panelist completing one run. Panelists that completed odor identification varied among samples and were chosen based on their availability. Sample "FA" was smelled first and sample "NFA" was smelled at a subsequent session. Exit and entry into the sniffing room was prohibited to everyone except to the GC-O operator and the panelist and all were instructed to refrain from eating or drinking (besides water) for one hr before testing, as well as from wearing perfume, lotion or other scented products. Following the SIMS software on a tablet, panelists were prompted upon olfactory stimulus to select the chemical identified and its intensity. If unable to identify the odor by name, he or she was instructed to select "other" for its identification and to verbally describe the odor to be captured via audio recording. Labels of all 50 chemicals trained were provided in order based on retention time. The data from the questionnaire was compiled and matched to chromatographic peaks using the mass spectrum chromatogram and the Kovats indexes (aka Linear Retention Indices) and odor descriptors given in literature. Compounds in which two-thirds of (or four out of six) panelists responded to an olfactory stimulus were noted; compounds in which fewer than two-thirds of panelists could detect were discarded and not included in the total aroma profile for that sample.

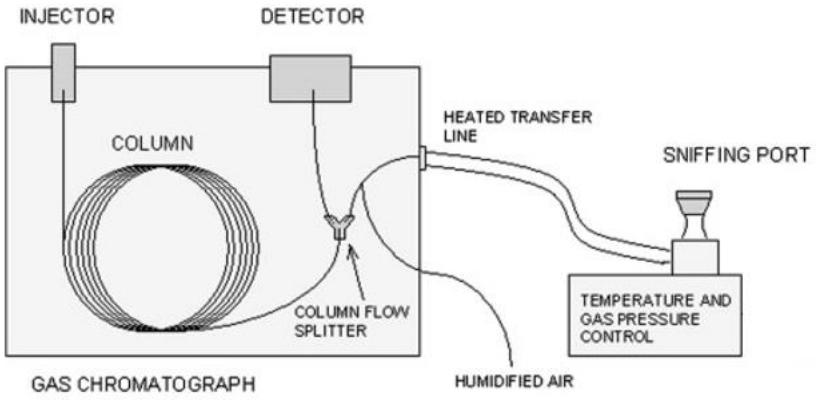

Figure 1. Internal structure of the GC equipped with an olfactory port. (Plutowska and Wardencki 2008)

2.3.5 Gas Chromatography-Olfactory

A Hewlett-Packard Model 5890 Series II GC with a flame ionization detector (FID) was used to separate and semi-quantify the volatile compounds in each EMC sample. The column was a J&W Scientific DB-Wax column (30 m x 0.25 mm x 0.25 µm film thickness). The temperature of the injection port and of the FID detector was set at 250 °C. The oven was set to an initial temperature of 40 °C and was raised at a rate of 3 °C per min until it reached 85 °C and then at a rate of 5 °C until a final temperature of 220 °C at which it was held constant for 18 min. An inlet split ratio of 20:1 with a split flow of 28.2 ml per min was set using hydrogen as the carrier gas. Additional run parameters included a column head pressure of 8.74 psi, linear velocity of 38 cm per sec and a flow of 1.41 ml per min with constant flow. Before each run, the column was cleaned for 20 minutes at 200 °C and an instrument blank was completed before the first sample of the day. A 1 m sniffing port was attached to the column with an internal diameter of 0.18 mm and an external diameter of 0.34 mm and the flow was diverted for sniffing in a ratio of 1:1 (0.7 ml/min). Humidified air was simultaneously run through the sniffing port to provide comfort for the sniffer at a rate of 40 ml/min.

2.3.6 Gas Chromatography-Mass Spectrometry

An Agilent Technologies Model 6890N Network GC System with an Agilent Technologies Model 5973 quadrupole Mass Selective Detector (GC-MS) was used to identify volatile compounds in each cheese sample. A J&W Scientific DB-Wax column (30 m x 0.25 mm x 0.25 µm film thickness) was used and set to an initial temperature of 40 °C then raised by 3 °C per minute until 85 °C and then 5 °C until constant at 220 °C for 18 min. The injection port was held at 250 °C and a split flow of 47.5 ml per minute and a split ratio of 20:1 was used. The MS gas flows were set as being operated under vacuum with constant flow and a column head pressure of 7.9 psi, a linear velocity of 83 cm per second and a flow of 2.4 ml per minute. The carrier gas was hydrogen. The column was cleaned for 20 minutes at 200 °C before each run. Additionally, an instrument tune and blank were completed before the first sample of the day.

3. CHAPTER III: METHOD DEVELOPMENT

3.1 SAMPLE PREPARATION OF EMC PASTES

3.1.1 Solvent

The initial extractions were done with diethyl ether (DEE). DEE has a slight polarity (vs a hydrocarbon e.g. pentane) and a low boiling point which improves extraction efficiency and volatile retention during concentration, respectively. In the first attempt, EMC shreds were placed in an Erlenmeyer flask and the solvent, DEE, was added. The EMC shreds quickly clumped and stuck to the bottom of the flask, which prevented stirring and lead to reduced surface area and in turn poor extraction. In the next attempt, EMC was gradually added to stirring diethyl ether which increased stir time but did not prevent the EMC from sticking to the bottom and clumping together in one mass, although a period without clumping occurred. In a final attempt to use diethyl ether as the solvent, the grinding tools

were chilled for ten minutes before grinding to delay softening of the EMC. This too delayed clumping for thirty minutes after addition of EMC, however, this is too short of an extraction time, according to the referenced method.

To reduce clumping/matting, DCM was substituted for DEE as a solvent. DCM has a higher density which was thought to support the shredded EMC thereby not allowing it to settle to the bottom and matt. Similar to DEE, it also has high volatility and some polarity compared to hydrocarbon solvents. Two separate extractions were completed with EMC and DCM. In both experiments, the grinding tools were first chilled, and EMC was gradually added to the stirring solvent. In one experiment, anhydrous magnesium sulfate was mixed with EMC shreds prior to extraction to prevent clumping. The EMC in both extractions behaved similarly, and no major difference was observed between the two experiments. The solvent change increased extraction time and delayed the onset of clumping. In addition, when clumping did occur, EMC formed in curds about one centimeter in diameter. During re-extraction, larger curds, about one and a half centimeters in diameter, formed. With both solvents, the EMC clumped, decreasing surface area and extraction efficiency. The most efficient extraction technique was determined to be by gradually adding shredded EMC into the DCM.

3.1.2 Removal of Fatty acids

The fatty acids were removed following the method outlined in Milo and Reineccius (1997), however, the necessity of this removal was not known. The free fatty acids provide much of the overall aroma profile, but there was concern that the presence of high levels of free fatty acids could hide the presence of other compounds. Removal of the fatty acids has its downsides, though. Smaller and more polar volatiles are more likely to be co-extracted during FFA removal, distorting the observed quantity. It is possible, however, that the extent of this loss would be relatively standardized across samples, but this is unknown

and unlikely due to the varying concentrations of numerous volatiles. Ultimately, the Project Leads requested that the full aroma profile of each EMC be tested, requiring each cheese to be sniffed twice (sample FA and sample NFA) and the fatty acids to be removed. Removal of the free fatty acids allows the less abundant volatiles, or those with a higher odor threshold, to be identified.

The even-chain fatty acid profile of each EMC was provided by LOL. Fatty acids dominated the chromatograms generated by GC-O and GC-MS which often prevented the detection/measurement of various less abundant aroma compounds, both by the FID and by the olfaction. Following the method described in Milo and Reineccius (1997), EMC D, the EMC with the highest free fatty acid content, was washed three times with a 0.5 M solution of sodium bicarbonate (3 x 50 ml). This did not effectively remove all FFA. The even-chain fatty acid composition of each EMC was provided by the supplier with qualitative data on each acid in parts per million (ppm) (Appendix 2). Upon inquiry, odd-chain fatty acids were not quantified during this external analysis simply because the analysts did not choose to quantify them. It is likely that this decision was based on the low levels of odd-chain fatty acids in milk from which cheese is made (Givens and Shingfield 2006). With this assumption, the analysts may have assumed that their flavor contribution might be insignificant. The total fatty acid content and the corresponding amount of 2 N NaOH needed to remove the fatty acids were calculated with the external data from the supplier that outlined the quantity of each even-chain FFA (ppm). From this data it is possible to calculate the volume of 2 N NaOH that is needed to remove these FFAs. The ratio of even- to odd-chain FFAs varies per sample but the quantity of even-chain FFAs is higher than that of odd-chain FFAs in every EMC sample (Appendix 2). To correct for the unknown excess of FFAs, 2 N NaOH was used in excess to ensure the adequate removal of FFAs. The excess volume of 2 N NaOH was calculated based on the

EMC sample with the highest quantity of even-chain FFAs (EMC D) for complete FFA removal.

To better understand the process of extracting free fatty acids from the solvent extract of each EMC, the effect of the use of a 2 N NaOH solution during fatty acid removal was evaluated in EMC D, the EMC with the highest fatty acid content. (Appendix 2). The amount of 2 N NaOH required to neutralize 150 g of EMC D was calculated to be 93.7 ml. Maintaining the ratio of EMC to solvent, 225 g EMC D was combined with 450 ml DCM and ISTD (51 µl 5-methyl-3-heptanone, - 12,500 ppm), following the method steps outlined in section 2.3.

The DCM extract was separated into three equal portions and each was treated in one of the following ways before concentration to 0.5 mL: a. no treatment, b. water wash (3 x 50 ml water), and c. treatment with 2 N NaOH (3 x 31.5 ml). Treatment with water removed acetic acid entirely and up to half of the quantity of butyric acid. Shorter chain fatty acids are more polar than longer chain fatty acids (ECHA 2021). This increased polarity allows for solubility in water. The partition coefficients of each are 0.17 and 0.79, respectively (ECHA 2021). Removal of all fatty acids was achieved after three washes of 31.5 ml 2 N NaOH, as calculated by the ppm values in Appendix 2, including 0.8 ml of excess base. This excess was included for ease of measurement of three equal volumes 31.5 ml instead of three volumes of 31.2 ml.

As expected, acetic acid was fully removed with the water wash due to its high solubility in water. Butyric acid was also reduced (47%) in the water wash. 96 ml of 2 N NaOH removed all free fatty acids present. This experiment contained the internal standard, 5-methyl-3-heptanone (Log P: 2.40) (GmbH 2016). A proper internal standard is one which is not present in the sample and one that follows similar chemical reactions and processes as the non-acid volatiles. It was found that the quantity of this compound was decreasing

with fatty acid removal and the associated extraction. It is believed that this reduction is due to the aldol reaction which is the reaction of a ketone with a base such as sodium hydroxide (Carey and Giuliano 2017). This resulted in the reduction of the quantity of the internal standard. Thus, a different internal standard had to be chosen. Ethers are reasonably stable compounds, so they were considered as an internal standard. Hexyl ether (Log P: 4.98) was found to be separated from other EMC volatiles and thus was chosen as ISTD 1 (Ribo 1988). A stock solution of 10,000 ppm was prepared and 100 µl was added in the first step of the sample extraction of EMC I. This concentration was too high and was lowered (26 µl of the 10,000 ppm solution).

A second internal standard, undecane (ISTD 2; Log P: 6.42), was added to the SAFE distillate fractions (samples FA and NFA) in equal amounts (Ribo 1988). Its purpose was to compare the two samples of each EMC (samples FA and NFA) and ultimately to compare EMCs to each other after the removal of FFAs. The intent was to standardize compound quantities for semi-quantification of all volatiles in each EMC sample. A stock solution of 1,600 ppm undecane in DCM was prepared and 100 µl was added after SAFE extraction to each fraction of the sample extraction of EMC I. This concentration was again too high and so further extractions were completed until the appropriate amount was found (14 µl of the 1600 ppm solution per fraction).

The fatty acids were removed following the method outlined in Milo and Reineccius (1997), however, the necessity of this removal is not known. The free fatty acids provide much of the overall aroma profile, but there is concern that the presence of high levels of free fatty acids could hide the presence of other compounds. Removal of the fatty acids has its downsides, though. Smaller and more polar volatiles are more likely to enter the aqueous phase during fatty acid removal, distorting the observed quantity. However, the extent of this loss should be relatively standardized across samples because the same volume of

base is used. Ultimately, the Project Leads requested that the full aroma profile of each EMC be tested, requiring each cheese to be sniffed as is and again without the fatty acids (sample FA and sample NFA). Removal of the free fatty acids allows the less abundant volatiles, or those with a higher odor threshold, to be identified.

3.1.3 Final aroma isolate concentration

Due to the uniqueness of the EMC profiles and intensities, it was necessary to determine a final concentrate volume that allowed for adequate sniffing concentrations and identification via MS. The volumes under consideration were 0.2 and 0.1 ml. 0.2 ml was a more desirable volume for syringe and injection handling to allow a buffer for sample evaporation, but it was uncertain whether the larger volume would be too dilute for the less potent cheeses. Therefore, EMC H, one of the weakest samples based on data obtained by the sensory team, was sniffed at both volumes and sniffing data were compared. Two panelists sniffed EMC H at the sniffing port of the GC-O at a concentration of 0.2 ml and 0.1 ml. Odor descriptors and retention times were matched to peak identity based on GC-O and GC-MS data. Two odors detected in the less concentrated sample were not found in the more concentrated sample. The identity of the missing odors is not of importance; instead, the quantity of odorants perceived indicates the acceptability of the final concentrated volume. Similarly, three compounds found in the more concentrated sample were not identified by MS in the less concentrated sample. Although the more concentrated sample contained three compounds that were not identified in the less concentrated sample, the more concentrated sample might allow for increased identification. However, because the less concentrated sample also contained two compounds that were not smelled in the more concentrated sample, it is not reasonable to use this justification. Because of this, it was concluded that there was no compelling

benefit to concentrating the samples to the smaller volume. Therefore, 0.2 ml was the preferred final volume due to a slightly lower risk of sample loss resulting from evaporation.

In this phase of development and for this sniffing experiment, 150 g of EMC was used for each sample (sample FA and sample NFA). The final method splits a total of 300 g of EMC into a 1:5 ratio (total profile: FFA removed) after SAFE extraction. This change increased the volume of volatiles in the sample NFA which was the sample more likely to have hidden odors. In fact, this increase of cheese per final concentration would increase the odor profile experienced in the sample NFA which allows for increased odor perception. The total analyte concentration in sample FA was decreased, reducing the olfactory perception of volatiles with high sensory thresholds. However, this is of little concern in sample FA due to the abundance of FFA so it was not seen as necessary to repeat this experiment.

3.2 SAMPLE PREPARATION OF EMC FORTIFIED POWDERS

The objective of this thesis is to evaluate and identify the aromatic profile of 9 EMC pastes. However, the larger objective of this project is to understand the application and contribution of the nine EMC pastes in application i.e., powder and process cheese. Method development for the extraction and analysis of EMC fortified cheese powder was completed during experimentation of the EMC pastes and will be discussed, however, volatile analysis of the powder was not completed at the time of this writing so the results will not be discussed.

3.2.1 Method development

Spray-dried powders are encapsulated products; fat and flavor components are enclosed by a hard, dehydrated shell made of cheese solids. It was hypothesized that the quantity of extracted fat directly relates to the recovery of volatiles due to their similar extraction

efficiencies as lipid. Thus, the yield of fat recovered in experimentation was used as a guide for flavor extraction. Although there was concern that the encapsulated fat would not be extracted from the powder without prior hydration, the method previously used for the EMC pastes was used as a starting point upon which to compare recovery of fat from the powdered cheese samples. (Note: for the sake of time, the volatiles in many of the following experiments were extracted only once; two extractions are certain to yield a higher fat recovery.) The cheese powders tested in this phase were not the cheese powders to be used in analysis but were cheese powders of similar composition and aromatic intensity.

In this method, cheese powder in two similar experiments was added to DCM at 40% solids (% w/v); this solution was stirred vigorously for 1 and 24 hr, (Appendix 3.1) filtered, concentrated, and the fat was measured gravimetrically. These experiments gave a 31 and 36% yield, respectively. A yield this low is troublesome for we cannot be certain that the volatiles encapsulated in the particle core have been extracted. To improve extraction efficiency, ultrasonic assisted extraction (UAE) (Appendix 3.2) was explored to increase solvent penetration into the encapsulated core; ultrasonic waves create small depressions on the core's surface, increasing the surface area available for solvent entry. This experiment was run on a 40% solids slurry in DCM. Before UAE, the mixture stirred for 15 min to begin volatile extraction. In two separate experiments, UAE was run for 20 min and for 2 sets of 20 min before continuing extraction with a stir bar for 1 hr. In the sample run for 2 x 20 min, the sample flask was swirled between runs. With the available instruments, UAE could not be completed in a closed system, therefore some volatiles may have been lost during the 20 min extractions. Both methods recovered a 20% yield. It would be desirable to extend the UAE time but there was not a reasonable solution to retain lost volatiles.

Water increases the hydration of encapsulated powders which in turn opens the encapsulated structure, releasing lipids and volatiles. It was hypothesized that the use of water in conjunction with DCM could allow for a greater fat yield. Water (Appendix 3.3) was added to EMC cheese powder until a smooth paste was reached and DCM was added to the paste. The cheese paste was too gummy to incorporate DCM for extraction. In an identical preparation, the cheese paste was then frozen with the intent of grinding the solid cheese mixture into smaller particles, much like the EMC method. After freezing overnight, the frozen paste was too soft to grind (Appendix 3.3). This method was again completed but a 1:1 ratio of water to DCM was used to make the paste. An emulsion was immediately formed and did not break overnight (Appendix 3.4). All three methods were discarded.

The methods developed were subsequently designed to either break or to prevent an emulsion from initially forming. Two mixtures (Appendix 3.5) of 20% cheese powder solids in water were prepared in two Erlenmeyer flasks. Using 1 N HCl, the pH of the solutions was brought to 5.2 and 4.6, the isoelectric point of whey and casein, respectively. (Casein is insoluble at pH 4.6 when warm; heat can promote flavor profile changes so the experiment was run at ambient temperature (Post et al. 2012). DCM was added in a 1:1 ratio to water and stirred for 1 hr. The viscosity of both samples increased over time, presumably due to pH-induced protein coagulation and a gel, was formed. Both samples were filtered overnight; the gelled sample at pH 5.2 did not separate and the organic phase was not recovered. In a similar experiment, (Appendix 3.6) samples were brought to pH 4.6 and were centrifuged at 2000 x g for 4 hrs which resulted in the separation of water with no solvent recovery.

To further impede or destabilize gel or emulsion formation, sodium chloride (Appendix 3.7) (1 M) was added to 5% cheese solids in water and then the pH was brought to 4.6. An emulsion was formed and attempts to break the emulsion included centrifugation, physical

disruption with a glass stir rod, the addition of solvent and extended filtration time with no success in achieving phase separation. The addition of salt increased the density of the aqueous phase closer to that of DCM thus promoting instead of destabilizing the emulsion.

The addition of both acid and salt pose concerns for the recovery of a representative flavor profile. In other words, both salt and acid can affect the net charge of certain volatiles. This in turn can affect their solubility in either the aqueous or organic phase which could result in a change in the analyzed flavor profile. The final approach (Appendix 3.8) utilized to combat these challenges while preventing emulsion or gel formation used a rotating plate. DCM was carefully added via pipette in equal volume to four aqueous solutions of 5, 10, 20 and 30% solids. The rotational speed of the plate was optimized for maximum interfacial contact without emulsification. After 20 hr, the solvent phase was removed via pipette and the powder was re-extracted for 15 hr with an equal amount of DCM. Despite the recovery of the solvent phase from all four samples, no fat was extracted.

Liquid-liquid continuous (Appendix 3.9) extraction with DEE was next evaluated for its efficiency in fat extraction of cheese powder in a 5 % cheese solids solution of water. An emulsion was formed over the 2 hr extraction window and the emulsion began to clog the bubbler and enter the collection flask. The emulsified portion in the collection flask could not be separated into phases. Regardless of emulsion formation, the collected ether was colorless, and it was assumed fat was not recovered in an adequate yield; in this experiment a colored (yellow) solvent phase is indicative of the level of fat extracted.

The final experiment in (Appendix 3.10) the method development of the powder samples combined techniques from previous experiments. Two solutions of 23% solids in 1 M NaCl were prepared. The pH of one sample was brought to 4.2 with 1 M HCl and the pH of the remaining sample stayed at its native value of 5.36. DEE was added to both samples in a 1:2 ratio with 1 M NaCl. After 1 hr stir time, the samples were centrifuged and the fat was

weighed. More fat (46% yield) was recovered from the sample with no pH adjustment. Because this method had the highest fat recovery, it was assumed that this method also yielded the most volatiles. The scale-up of this experiment was unsuccessful, however, as the solvent phase could not be recovered.

Effort returned to the original method used for EMC samples. Solutions of 9, 17 and 25% solids were prepared and stirred for 2 separate extractions with DCM; fat yields of 75, 73 and 70% were recovered. A second trial of this method was completed before its acceptance as the final method (section 3.2.2) for the volatile extraction of the cheese powders.

After volatile extraction of the powder, the method followed the steps outlined above for the volatile extraction of EMC paste. Two modifications to this method were made: the amount of each internal standard and of 2 N NaOH (section 3.2.2).

3.2.2 Method for volatile extraction from cheese powder

3.2.2.1 Sample Preparation

This method was modified from the work of *Milo and Reineccius* (1997) and again from the method used in volatile extraction of EMC pastes as outlined earlier.

Three hundred and twenty-five mL of methylene chloride was added to two 1 L Erlenmeyer flasks separately with a large stir bar (1 x 7 cm). Sixty µl of a 10,000 ppm internal standard stock solution, hexyl ether in methylene chloride, was added to each flask and the mixture was stirred.

3.2.2.2 Volatile Extraction

Three hundred g of frozen cheese powder (2 x 150g) was slowly added to each of the solvent-containing flasks and then were stirred for three hr. After filtering, the volatile

compounds were re-extracted using the same technique with 225 ml methylene chloride per Erlenmeyer. The extracts were combined and concentrated to 300 ml through fractional distillation with a Vigreux column (40 cm x 2 cm) at a maximum temperature of 40°C. The non-volatiles were removed from the solvent extract using the solvent assisted flavor evaporation system (SAFE) as described by Engel et al. The water bath was set to 60°C and vacuum of 10^{-5} Torr was maintained for the duration of the extraction. The sample was added dropwise at a rate no faster than 100 ml per hour and liquid nitrogen was used to chill the collection flask.

3.2.2.3 *Volatile Fractionation*

The SAFE distillate was brought to room temperature and divided into two fractions (1:5). A second internal standard, undecane, was added to each fraction (50 µl of a 1600 ppm stock solution in methylene chloride). The smaller fraction was dried with anhydrous magnesium sulfate for 10 min and concentrated under nitrogen to a final volume of 0.2 mL using a Kuderna-Danish vial (sample FA). The larger fraction was extracted in a separatory funnel with 2 N NaOH (3 x 10 ml) to remove free fatty acids. The sample was again dried and concentrated under the same conditions to a final volume of 0.2 mL (sample NFA). Gas Chromatography-Olfactory (GC-O) and Gas Chromatography-Mass Spectrometry (GC-MS) were used to analyze the extracts.

3.2.3 Analytical Methods

Gas chromatography-olfactory (GC-O) and gas chromatography-mass spectrometry (GC-MS) were used as the main analytical tools. Time was spent optimizing the operating parameters of both instruments to limit the differences in retention time. It is desirable to have the same compound in elution times between two instruments to associate orders with identities. Unfortunately, it was not possible to set the operating parameters up for

the two different instruments in a manner that gave the same elution times; the GC-MS operates under vacuum while the GC-O does not. Differences in retention were kept within a minute as can be seen below in Table 4.

Table 4. The differences (min) of the retention of a series of alkanes on a gas chromatograph-olfactometer (GC-O) and a gas chromatograph-mass spectrometer (GC-MS)

Alkane	GC-O[1] (min)	GC-MS[2] (min)	Difference[3] (min)
decane	2.888	<2	<0.888
undecane	4.286	3.48	0.806
dodecane	6.517	5.776	0.741
tridecane	9.503	8.888	0.615
tetradecane	12.905	12.453	0.452
pentadecane	16.371	16.107	0.264
hexadecane	19.356	19.286	0.07
heptadecane	21.81	21.842	-0.032
octadecane	24.079	24.257	-0.178
nonadecane	26.129	26.416	-0.287
eicosane	28.098	28.519	-0.421
heneicosane	29.905	30.408	-0.503
docosane	31.651	32.243	-0.592
tricosane	33.333	34.012	-0.679
tetracosane	34.93	35.68	-0.75
pentacosane	36.489	37.318	-0.829
hexacosane	37.993	38.895	-0.902
heptacosane	39.429	40.392	-0.963
octacosane	40.843	41.87	-1.027

[1]the retention time (min) of various alkanes on the GC-O using the run parameters outlined in section 2.3.5
[2]the retention time (min) of various alkanes on the GC-O using the run parameters outlined in section 2.3.6
[3]the difference (min) of retention time between the GC-O and GC-MS; a negative value indicates that the alkane eluted first from the GC-O, a positive value the GC-MS

3.2.4 Sample Blank

A sample blank was run to identify contaminants from the laboratory environment and from the analytical equipment. DCM, ISTD 1 and ISTD 2 (the same volumes used in sample extraction) were run through the entire extraction method (aside from cheese grinding due to solvent incompatibility with the equipment material). Like sample extraction, the sample blank was split in a 1:5 ratio (FA Blank: NFA Blank). Before injection

into the GC-O and GC-MS, a blank run with no injection was completed on both instruments. Column contamination was observed in the GC-O as two peaks which eluted at times 1.547 and 54.267 min. These contained no odor and were therefore not a concern for this analysis. The only contamination observed on the GC-MS was a column contamination of butylated hydroxytoluene (BHT) at 26.584 min.

Sample FA Blank contained 5 chromatographic peaks, none of which contained an odor. Of these, 3 eluted before the solvent peak (2.196) at 1.901, 1.954, and 2.044 min. The additional peaks (3.892 and 5.872 min) are important to note as they elute around volatile compounds of interest. Sample NFA Blank also contained 5 chromatographic peaks. Both samples contained the same impurities aside from one peak: the peak at 3.892 min was not found in sample NFA blank. A peak at min 36.510 was instead present. Similarily, none of these peaks exhibited any aromatic presence and were therefore not of concern. It is important to note any odor contribution by contamination so as not to mistakenly believe that it is part of the flavor profile of a sample. Additional peaks in both samples included the two internal standards, undecane and hexyl ether, which eluted at roughly times 4 and 11 minutes.

4. CHAPTER VI: RESULTS AND DISCUSSION

4.1 PRECISION AND LIMITATIONS

4.1.1 Precision

A coefficient of variation (CV) of <10% on replicates (analysis of the same sample) were considered good precision. CV was calculated for each volatile in a sample using equation 1 where σ is the standard deviation and μ is the mean.

Equation 1. The coefficient of variation

$$CV = 100\ \% * \frac{\sigma}{\mu}$$

The peak area of the FFAs from the same sample extract varied considerably between chromatograms; the CV of FFAs in two-thirds of EMC samples were above 23%, suggesting very poor experimental duplication. However, the CV of most of the non-acid volatiles trended less than 10% which indicated that the FID and the injection onto the column of the GC-O delivered reproducible results. There were exceptions, i.e. ten compounds had a CV greater than 10% in three or more EMC samples. These include benzene ethanol, 2-tridecanone, ethyl laurate, indole, isophorone, γ-decalactone, δ-nonalactone, δ-undecalactone, δ-dodecalactone, and δ-undecalactone (Appendix 4).

Samples FA (one part) and NFA (five parts) are derived from the same EMC extraction which would be expected to result in a uniformly proportionate concentration of volatile compounds between the two fractions. The difference between the two fractions is that the FFA were removed from the NFA extract by washing the solvent extract with w N NaOH – this would remove the FFAs from the sample NFA. One would expect that additional manipulation of a sample would increase volatile loss, especially of the shorter chain, more water-soluble compounds when a solution of 2 N NaOH is used. However, the analytical results of the non-acid volatiles were acceptable, thus eliminating the extraction method itself as a cause of poor reproducibility of the peak areas of the FFAs. It is then necessary to evaluate other sources of poor precision.

Manual injection of each sample into the GC-O would lead to peak area discrepancies as the injection volume would vary slightly thereby altering the quantity but not the proportion of each volatile. However, the overall acceptable precision of the non-acid volatiles supports the rejection of this hypothesis; if true, poor precision would have been seen

more evenly across all volatiles. It is unlikely that samples FA and NFA would be injected onto the column with such inconsistency when all injections were completed by the same experienced operator.

Lastly, each series of GC-O injections of a single EMC sample took place over the course of two weeks in accordance with panelist availability. The volatile loss, if any, of short chain FFAs would occur at a higher rate than that of long chain FFAs again distorting the recorded peak area.

4.1.2 Data Normalization

After SAFE distillation, each EMC extract was split into a 1:5 ratio at which point an equal concentration of ISTD 2 was added to each fraction. This internal standard enabled the two extracts to be compared quantitatively without overshadowing of the FFAs to the panelist or to the GC. The average ISTD 2 by sample type was also used as the baseline upon which all chromatographic areas were normalized to limit human variability in the sample injection on the column. Once all peak areas were normalized within a dataset, peak areas of sample FA were multiplied by 5 to mirror their concentration in scale with sample NFA.

Although two internal standards were added during extraction, only one was used during data analysis. The first, ISTD 1, was added during the initial extraction phase with the intent to evaluate extraction efficiency and to compensate for odorant loss throughout experimentation. It was later decided that ISTD 2 by itself was sufficient to provide results of semi-quantification because it was added in equal parts to both sample FA and sample NFA. Normalization increased precision by compensating for the inaccuracies of manual injections (Table 5).

Table 5. The standard deviation and coefficient of variation (%) of three compounds within a single EMC sample type pre- and post-normalization, as described in section 4.1.

Compound	Pre-normalization		Post-normalization	
	Standard Deviation	Coefficient of Variation (%)	Standard Deviation	Coefficient of Variation (%)
2-heptanone	2095	33	72	1
ethyl butyrate	1421	33	78	2
δ-octalactone	423	36	65	6

4.1.3 Outliers

For any suspected outliers, a Q test (equation 2) was run with a confidence interval of 95%. In the case of three or more identified outliers on any given chromatogram, the data from that chromatogram was omitted.

Equation 2. Q-test

$$Q = \left| \frac{(x_q - x_c)}{(x_h - x_l)} \right|$$

Q = experimental Q-value; x_q = suspect value; x_c = value closest to x_q; x_h = highest value; x_l = lowest value

Outliers were identified in the sample FA fraction of EMCs B, E, and H and in the sample NFA fractions of EMC B and D. Upon removal of the outliers, CV improved, as was expected. To further improve CV across samples, it is possible that a reduction of the confidence interval may identify additional outliers. The limit of the confidence interval is at the discretion of one who wishes to analyze this data, particularly the project sponsor.

4.1.4 Comparison of SAFE Data to That Provided by LOL for FFA

The SAFE method was designed for the recovery of a broad range of aromatic compounds yielding an extract suitable for GC-O and panelist sniffing – not necessarily for exact quantification. In fact, this methodology is likely biased toward shorter-chain FFA and poorly extracting long-chain FFAs. During the SAFE extraction (high-vacuum distillation),

compounds with high volatility will be recovered more efficiently from the solvent extract than those with low volatility. This is in part due to chain length but also to the matrix which they are leaving; the sample feeding the SAFE apparatus is a mixture of DCM, non-acid volatiles, FFAs, and triglycerides, among other non-volatiles. Low volatility coupled with the hydrophobicity of longer-chain FFAs would have discouraged the separation of long-chain FFAs from the lipid material, thus preventing their extraction and analysis.

Using a method adapted from the work of *Bateman and Jenkins (1997)*, our sponsors provided the total quantity of FFAs for each EMC sample (ppm, Appendix 2). Our sponsors did not measure all odd chain FFA because of their low quantities. However, we identified several odd chain FFA that are significant odor contributors, particularly propionic, valeric, nonanoic, and undecanoic acids. Additionally, only specific even chain FFAs were routinely analyzed which ignored unique acids such as 9-decenoic acid. It is a very good method for measuring the major FFAs in EMCs. Extracting FFA from EMCs using hexane and isopropanol (as our sponsors did) supports the extraction of both polar and nonpolar FFAs due to their difference in polarity (Table 6). This solvent blend may also enhance the separation of triglycerides from fatty acids, especially of long-chain fatty acids, compared to an extraction with DCM alone.

The use of different analytical methods produced drastically different results in the quantification of FFAs. Using DCM alone, butyric acid and palmitic acid were quantified in a 64:0.07 ratio but the sponsor's method yielded a 17:25 ratio. Both methods provide essential information yet fail to provide all the desired data. However, the two methods when used in tandem may provide the most accurate quantification of odor contributing FFAs. For the purpose of this report, the chromatographic data gathered during the volatile extraction of EMC pastes will be used to provide the volatile profiles of each sample. Distillation with the SAFE apparatus is largely accepted as a sensitive mode of flavor

extraction (Engel et al. 1999) and so it was believed that this data set better follows the objectives of this study due to its sensory contributions while providing an acceptable volatile profile.

Table 6. Relative polarity of solvents used in the quantification of FFAs of EMC samples and in the volatile extraction of EMC samples

Chemical	Relative Polarity[a]
hexane	0.009
DCM	0.309
isopropanol	0.546
acetic acid	0.648
formic acid	0.728
water	1

[a](Reichardt and Welton 2011)

4.1.5 Challenges in Compound Identification

4.1.5.1 Instrumental Challenges

Two instruments (GC-O and GC-MS) and sensory descriptors were utilized in the identification of GC peaks. Preliminary identification was completed through the analysis of ion fragmentation on the GC-MS. Additionally, all samples NFA were further concentrated under nitrogen to 50 µl and injected into a GC-MS previously used (Agilent) and into a GC-TOF-MS (LECO). Both GC-MS instruments were operated under the same run parameters. This additional MS step aided in the identification of two unidentified compounds. Ion fragments, when combined with panelist descriptors and retention indexes helped to narrow compound possibilities. These potential compounds were obtained to provide odor descriptors and to match peak elution.

Despite the effort to identify all odor-contributing compounds, seven unknown compounds remain. For simplicity, unknown compounds were labeled with their approximate elution

time. For example, an unknown compound with a retention time of 11 min was labeled “unknown 11”.

Although the GC-O and the GC-MS run parameters remained unchanged throughout experimentation, the elution order and timing of volatile elution was slightly different between the two instruments; slight variations in retention time were observed in the GC-O depending on the quantity and specific volatiles present whereas retention time remained fairly unchanged in the GC-MS. Variations in retention can create uncertainty in peak selection. One expects minor discrepancies in elution time since one GC system is operated at a positive pressure and the other under a partial vacuum. An attempt was made to run both instruments under conditions that resulted in exact agreement in retention time to facilitate relating sniffing data (times) to MS identification. This was not possible.

Peak separation also varied occasionally between the two instruments. For instance, two peaks with clear separation on one instrument may coelute or partially coelute on the other. When coelution occurred on the GC-MS, it was sometimes possible to identity the two compounds through ion fragmentation analysis. However, if the reverse were true, an accurate peak measurement could not be recorded as there would be no indication of separation.

In some instances multiple panelists identified a particular compound through odor recognition and odor descriptors yet no peak was observed on the GC-O or GC-MS. Often, these compounds could be detected through ion fragmentation on the GC-MS however, this typically lead to difficulty in peak identification. Compounds with low odor thresholds such as those just described can be potent in extremely low quantities. For example, δ-nonalactone can be detected at a level of 0.065 ppm (Stahl 1987) and was often described by panelists but was rarely easy to identify via MS; extensive ion searching led to its

verification in these EMCs. In fact, in one sample, all six panelists selected δ-nonalactone in place of caprylic acid (odor threshold: 3 ppm (Stahl 1987)), the chemical which eluted immediately before and in much higher quantity. The odor of δ-nonalactone overshadowed and masked any obvious contribution of caprylic acid; this instance will be discussed in detail in the analysis of EMC E.

4.1.5.2 Challenges in the use of Human Subjects

Inconsistencies in sensory panelist data collection lead to challenges in identification. When gathering time-dependent data from human subjects, it is important to remember the consequence of reaction time, especially when coupled with any decision-making process. For example, a panelist must first smell an odor, recognize that an odor was smelled, analyze, and determine odor descriptors, identify the compound from the list given in the questionnaire and then select the odor by name. The time at which a panelist selects an odor is recorded and is used in the same manner as the retention time of a chemical from the GC-O. Any delay in odor identification especially when a panelist is unsure of his or her answer can affect the reported time of selection. Occasionally, odor selection was delayed over 30 s which can drastically affect the researcher's ability to match an odor to a chromatographic peak. On occasion, panelists delayed odor selection without providing any verbal indication of the odor.

Coelution of two odorants can also impact odor perception. When this happens, the resulting odor is classified either as homogeneous or heterogeneous. A mixture is considered homogenous when the combination of two volatiles yield a new odor called a blending mixture (Thomas-Danguin et al. 2007). Analytical data of the unidentified compound, unknown 30, suggests that this type of homogenous odor was created in EMC I (section 4.2.1.2.5). A chemical mixture is also considered homogeneous when the intensity of one odorant masks or completely overshadows other odorants (Kay et al.

2005). It is possible that in the example described above, δ-nonalactone and caprylic acid were present in this type of homogenous mixture. In the case where portions of the odor quality of the less intense odorant is perceived, a mixture is considered heterogeneous with partial overshadowing (Berglund and Olsson 1993). In a heterogeneous mixture, partial odor lending can also occur, resulting in a mixture of odor qualities which represent the individual odorants and a blended aroma (Kay et al. 2005).

Humans perceive aroma quality differently and can vary greatly in their sensitivity to certain odorants (Amoore 1967). For this reason, any compound detected by at least two panelists was considered an odor-contributing compound in this study. It was then hypothesized that the number of panelists who smelled a particular odorant was not the most important factor to consider when analyzing the flavor profile of a food; instead, the combined evaluation of both panelist recognition and his or her intensity rating of a particular odorant may be more telling of the average perceived aroma. Olfactory perception is influenced by age, race, genetics, experience, health, culture and environment, among others, which can make it difficult to predict the perception of an odorant mixture in foods (Wysocki and Beauchamp 1984). Perception is also influenced by the time of day, past associations, mood or mood disorders; one study found that depressed patients perceived odorants significantly less pleasant (67%) than their non-depressed peers (Atanasova 2012).

4.2.1 Discussion of EMC Flavor Profiles

4.2.1.1 Overview

The flavor profiles of 9 Cheddar-type EMCs were created with the aid of GC-O, GC-MS, panelist odor recognition and Kovats indices. The number of total volatiles per EMC ranged from 22 to 48 chemicals but seventy-four unique volatile compounds were

identified as odor-contributors to the studied EMCs. Of these, 12 compounds were found uniquely in only one EMC and two were odor contributors in all 9 EMCs: butyric acid and δ-octalactone. Butyric acid was the most abundant volatile in 7 EMCs; in the remaining samples, acetoin and benzoic acid were most abundant. FFAs were the most prevalent chemical class of volatiles across all samples except the sample in which acetoin was most abundant. In fact, FFAs comprised over 97% of the total quantity of contributing volatile compounds in 6 EMCS.

It is difficult to compare the odor intensities of isolate fractions, i.e. Sample FA and Sample NFA since the final sample volume of these samples were in a 1:5 ratio; compound quantity and intensity do not linearly correlate and so a prediction of intensity across isolate fractions cannot be accurately made. The comparison of EMCs by intensity must therefore be limited to between similar fraction types. Of the FFAs in Sample FA for all EMCs, butyric acid was most often rated as the most intense acid, followed by caproic acid. Odor intensity of the compounds in Sample NFA varied more widely across samples.

4.2.1.2 Unidentified Compounds

4.2.1.2.1 Unknown 11

Panelists detected unknown 11 (~11.6 min) in both EMC A and H. Unknown 11 was perceived in EMC A by 4 panelists with an average intensity of 5.2 and in EMC H by 2 panelists with an average intensity of 4.4. Odor descriptors included barn, malt, copper, metal, and stale French fries but no clear chromatographic peak was found. Unknown 11 is likely a chemical with a very low odor threshold – one which cannot be detected at its concentration in the EMCs.

4.3.1.2.2 Unknown 14

The odor qualities reported by panelists for compounds eluting between minutes 13 and 15 were difficult to decipher. Four compounds, 1,3-di-tert-butylbenzene (13.292 min), benzyl ethyl ether (~13.9 min), ethyl caprylate (14.046 min) and 1,2,3,4-tetra-methylbenzene (15.081 min) were found to elute in this retention window and are believed to be responsible for odor descriptors in all EMCs (Table 7). The quantities of 1,3-di-tert-butylbenzene, benzyl ethyl ether, and 1,2,3,4-tetra-methylbenzene were often just above the detection limit of the GC-O and GC-MS, yet their unique odor qualities could be perceived. The retention time of 1,2,3,4-tetra-methylbenzene was often later than the response time of panelists. The time recorded by the GC-O is the time at which the peak area is at the apex of the peak and does not necessarily account for peak width. It is possible that olfactory detection began in the initial stages of chemical elution. Additionally, the described quality of unknown 14 occasionally matches that of 1,2,3,4-tetra-methylbenzene and its presence has been confirmed through mass spectrometry. Panelists occasionally perceived these compounds instead of ethyl caprylate which in general was one of the non-acid volatiles in higher concentration. Of course, the odor perception of these compounds varied by panelist and by sample. Effort was made to classify and match olfactory response with chromatographic peak. However, in the case where peak assignment could not be confidently completed, the group of odorants were classified as “Unknown 14”. The specifics of each case will be discussed in the evaluation of each EMC as necessary.

Table 7. Elution time (min) from the GC-O and odor descriptors of the four odorants potentially responsible for the identify of unknown 14

Odorant	Elution time (min)	Odor descriptors
1,3-di-tert-butylbenzene	13.292	grease
Benzyl ethyl ether	13.311	fruity, pineapple, tropical
ethyl caprylate	14.046	fruity, winey, sweet, apricot, banana, brandy, pear
1,2,3,4-tetra-methyl benzene	15.081	camphor, sweet, plastic, gasoline

4.2.1.2.3 Unknown 17

The odorant referred to as "unknown 17" was perceived by two or more panelists in sample NFA of EMCs G, H, and I. A full description of panelist responses can be found in Table 8 but the most common descriptors include earthy and carrot juice. The chromatographic peak assigned to unknown 17 can be found in the chromatograms of all three samples at 17.07 min. The only compound detected by the GC-MS at this time was tetradecamethyl-cycloheptasiloxane. No odor descriptors or threshold values were found in literature for this compound, however, due its high molecular weight (519 g/mol) it is unlikely to have a significant odor.

Table 8. Odorant intensity, area and descriptors of unknown 17 in enzyme modified cheese (EMC) G, H, and I.

EMC	Intensity	Quantity (%)	Comments
G	2.5	0.036	floral, earthy, carrot juice
H	5.6	0.232	cardboard, not sweet, not fruity, grain, earthy, like ethyl nonanoate
I	6.3	0.188	carrot juice with a hint of cinnamon or tomato juice, earthy, organic, waxy, crayon

4.2.1.2.4 Unknown 23

Three panelists recorded an olfactory stimulus in sample NFA of EMC I at 22.950 min with an intensity of 4.0. Unknown 23 is most likely a non-acid volatile, however, its exact identity is not known. All three panelists provided unique yet similar odor descriptors: salty, corn flour, masa, fried tortilla chip; corn, grain; wheat, toast, rancid. This odorant is

probably the GC-MS peak at 22.729 min as a potential match for unknown 23. The only chromatographic peak detected by the GC-O and GC-MS was naphthalene however, the odor descriptors given by panelists do not directly support this conclusion. As the identity of unknown 23 is not known, it is reasonable to assume that this compound could be one with a low odor threshold which is present at a concentration below the sensitivity of the FID.

4.2.1.2.5 Unknown 30

Three panelists recorded two separate odor stimuli 20-30 s apart between 29.883 and 30.150 min during their session smelling sample NFA of EMC I. All three panelists recorded both odorants and no other panelist recorded stimuli to either aroma and in each case, panelists selected “unknown” when prompted to identify the odorant by name. Even though panelist responses (Table 9) suggest that unique volatiles are responsible for each stimulus, a 20-30 s response gap, coupled with delayed response time, is not wide enough to assume that the odor quality represents that of one compound which evolved in characteristic through its elution. This theory had been expressed by panelists in more than one instance i.e. descriptors of caprylic acid in EMC C and caproic acid in EMC G and H.

Table 9. Panelist responses including time of response, intensity and comments for the unidentified compound, unknown 30, as observed during GC-O runs of EMC I

Panelist	Stimulus 1			Stimulus 2		
	Time (min)	Intensity	Comments	Time (min)	Intensity	Comments
1	29.9	2	cat urine	30.2	3.1	wet dog
3	29.5	1.9	n/a	30.0	3.2	earthy, organic, dirt, seaweed, swamp
7	29.8	8.9	melted butter	30.1	4.9	fishy

Panelist 1 also selected δ-nonalactone by name in a delayed response time at 30.817 min; δ-nonalactone eluted at time 30.314 min during this run. Odor descriptors (fruity, sweet) given by panelist 1 are consistent with those of δ-nonalactone as found in literature (coconut, sweet, creamy, coumarin) (Appendix 6) but it is important to note that the descriptors are not an exact match. The quantity of δ-nonalactone present in EMC I is comparable to that detected by panelists in other EMCs so it is reasonable to assume that more than one panelist would have detected it.

The odor descriptors provided by other panelists do not coincide with those of δ-nonalactone. It is hypothesized that a homogenous, blended odor quality was perceived around 30 min and it is anticipated that δ-nonalactone is involved. Other contributing compounds to the odor quality around 30 min remain undetermined.

4.2.1.2.6 Unknown 38

During sniffing runs of sample NFA of EMC B, three panelists responded to the odor stimulus of a chemical at 38 min. Panelist intensity ratings averaged 6.4 and sensory responses were matched to the chromatographic peak at 37.881 min. Due to the lack of odor quality descriptors and the inconclusive mass spectrum of this peak, the identity of unknown 38 could not be determined. The mass-to-charge ratios of note include (mass-to-charge ratio: relative abundance) 42:60, 43:100, 55:60, 56:85, 69:35, 84:50, 99:60, 113:30, 128:10, 145:10, 159:10, and 193:5.

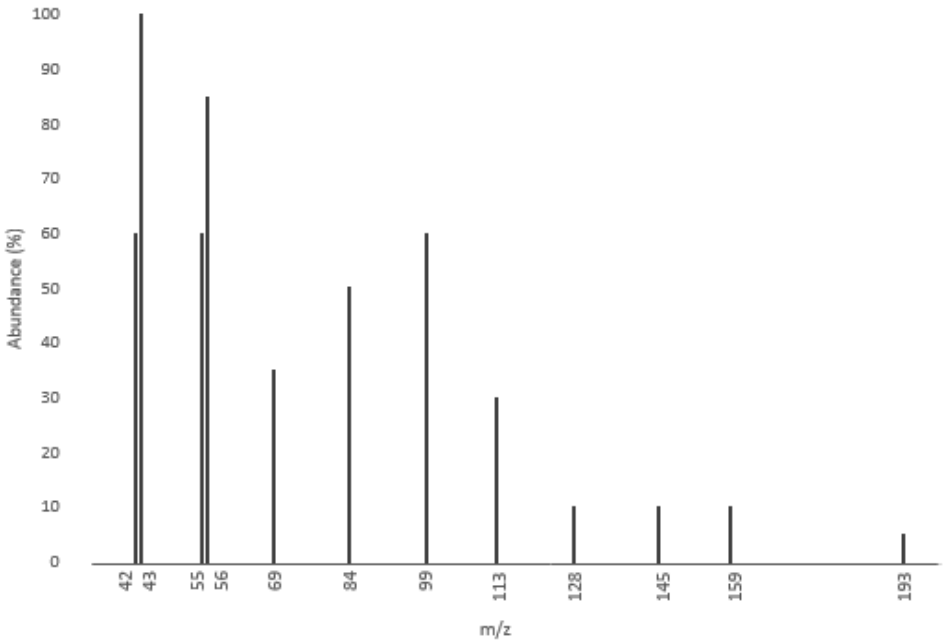

Figure 2. Mass spectrum showing the major ions for unknown as determined from the GC-MS

4.2.1.2.7 Unknown 41

Two panelists recorded an odor during the GC-O run of sample NFA EMC A at 41.5 min (intensity 1.4); both incorrectly labeled the odorant by selecting either lauric acid (36.433 min) or palmitic acid (42.541 min). Neither panelist provided odor descriptors but selected chemical names. It was, therefore, assumed that the odor quality of unknown 41 was similar to both lauric acid (mild, fatty, coconut, bay oil) and palmitic acid (waxy, creamy, candle), as found in literature (Appendix 5). A corresponding chromatographic peak was not found which suggests that the odorant has an odor threshold below the detection limit of the FID. However, it is important to note this sniffing session was the second session for all panelists. It is possible that panelists responded to an external odor, as panelists were still becoming familiar with the ambient odors.

The following sections discuss each EMC and display the odor-contributing volatiles in each by order of intensity, starting with the most. The volatiles are split into two sections: acids and non-acids. It is not possible to compare the intensity of the acids and non-acids

as sample FA and NFA are not proportionate to the total aromatic profile. One cannot predict the intensity of an odorant at different concentrations because the quantity and intensity of a volatile do not share a linear relationship. Further, human perception of odorants and their intensities varies by individual, as does one's odor threshold. However, one can evaluate the abundance of odorants as a percentage of the total number of odor-contributing compounds in each EMC (quantity %). An odor-contributing compound was classified as any compound which was detected by two or more panelists. Odor-contributing volatiles are sorted in order of abundance in Appendix 4. Appendix 4 also shows odorants sorted by chemical group for each EMC.

4.3 AROMATIC PROFILES OF EACH EMC

Panelists were trained to identify various odor stimuli by chemical name to reduce discrepancies between odor descriptors. However, odorants were mislabeled during GC-O sniffing over 50% of the time. Published odor descriptors of the incorrectly selected odorant often aided the identification of the correct compound through the comparison of odorants. It was also common for a panelist to select a similar sounding odorant (i.e. heptanal for 2-heptanone) or an odorant of the same chemical group (i.e. lauric acid for undecanoic acid). This too helped to narrow the list of the possible odorants responsible for the stimulus. After the first 20 GC-O runs, panelists were instructed to describe the stimulus of every odor that was perceived instead of only those which he or she could not name. These descriptors are shown under "comments" in various tables throughout this discussion and are compared to odor descriptors for each compound as found in published sources in Appendix 6. Odor descriptors from different panelists are separated with a semicolon. Panelists were unable to describe the stimulus of all odorants and so the number of panelists who reported a stimulus may not match the number of comments per odorant.

4.3.1 EMC A

Fifty-one odorants were detected by panelists in EMC A, the most volatiles for any EMC. This sample was the only one in which panelists responded to the olfactory stimulus of unknown 41 and 2-hexanone – this latter compound was present in other samples but at a concentration below its odor threshold. Five odorants in this EMC present in only one other EMC: δ-hexalactone (EMC I), ethyl palmitate (EMC B) palmitic acid (EMC E), 2-pentadecanone (EMC *C),* and unknown 11 (EMC H). All other odorants were detected in three or more EMCs.

This EMC is one of three in which butyric acid was not identified as the most intense acid even though it accounted for 64% of the total odor-contributing chemicals by quantity. 9-decenoic acid was rated as the most intense odorant in EMC A with a rating of 9.1 and was followed by butyric acid (8.9), caproic acid (8.2), benzoic acid (7.5), and lauric acid (7.2). As mentioned, butyric acid was the most abundant and was followed by caproic acid (25%), caprylic acid (5%), capric acid (3%), and lauric acid (0.8%). Despite its high odor intensity, 9-decenoic acid was only present in a quantity of 0.3%.

Non-acid odorants of importance include ethyl palmitate, which was the most intense non-acid with a rating of 8, followed by isophorone (7.5), 1,2,3,4-tetra-methylbenzene (6.3), ethyl laurate (5.9) and ethyl caprylate (5.8). Four of the top five non-acid volatiles in highest abundance were the ethyl esters of four of the most abundance fatty acids: ethyl caprate (0.08%), ethyl caprylate (0.07%), ethyl caproate (0.03%) and ethyl laurate (0.2%). Of the five most abundant non-acids, acetoin (0.03%) was the only volatile which was not derived from the major fatty acids.

The sum of the intensity ratings for the acids and non-acids of EMC A was the highest of any other EMC. This suggests that the odorants present in EMC A has the most-intense odor of the EMCs. The comparison of odorant intensity as a sum can be seen in Table 10.

Table 10. The sensory intensity and analytical quantity (%) of all odor-contributing volatiles in enzyme modified cheese A including the number of panelists who responded to its olfactory stimulus and their comments, sorted by the most intense odorant

Compound	Panelists	Intensity	Quantity (%)	Comments
Acids				
9-decenoic acid	3	9.1	0.316	potpourri; cucumber
butyric acid	6	8.9	63.953	
caproic acid	6	8.2	24.626	mix between chocolate and tridecanoic acid
benzoic acid	2	7.5	0.188	
lauric acid	5	7.2	0.806	floral
capric acid	6	6.7	3.124	watermelon but more tart; jolly rancher watermelon
valeric acid	5	6.5	0.687	
caprylic acid	6	6.0	5.109	caramel; burnt sugar
acetic acid	6	5.8	0.101	
nonanoic acid	3	5.6	0.077	
tridecanoic acid	3	5.4	0.007	
heptanoic acid	3	5.1	0.186	
myristic acid	4	5.1	0.255	spicy
undecanoic acid	3	4.7	0.021	pepper, acidic, old, stale
propanoic acid	5	3.1	0.082	
palmitic acid	2	2.8	0.072	
Non-Acids				
Et[1] palmitate	2	8.0	0.002	
isophorone	2	7.5	0.001	
1,2,3,4-tetra-methyl benzene	6	6.3	n/a	earthy; plastic, pet store, bird food pellets; strong malt
Et laurate	5	5.9	0.023	wheat but sweet, clean, and fruity; oatmeal with a little cinnamon
Et caprylate	4	5.8	0.067	barn; cheesy, Cheddar, wheat; sweet like potato agar or garlic potato
benzene ethanol	4	5.4	0.001	floral
2-pentadecanone	3	5.4	0.001	French fries, salty, oily
γ-dodecalactone	4	5.3	0.003	
indole	4	5.2	0.001	cow manure
unknown 11	4	5.2	x	barn, malt
acetol	4	5.1	0.007	

δ-decalactone	6	5.1	0.020	
δ-dodecalactone	5	5.0	0.010	Like undecanoic acid but milkier
Et 9-decenoate	3	4.9	0.009	grain; milk licorice flavor
Et nonanoate	4	4.9	0.002	
Et undecanoate	3	4.7	0.001	licorice; fennel
hexanal	2	4.7	0.001	
δ-hexalactone	3	4.7	0.001	grain; milk licorice flavor
Et caproate	6	4.6	0.031	
2-tridecanone	4	4.6	0.006	
Et caprate	4	4.6	0.076	flowers
Et butyrate	6	4.5	0.013	aromatic; bubblegum
δ-octalactone	4	4.5	0.003	coconut
Et myristate	3	4.3	0.007	
Et valerate	4	4.2	0.001	
2-octanone	3	4.1	0.000	musty, mushroom
δ-nonalactone	4	3.9	0.003	
acetoin	2	3.5	0.031	crayon, oxidized
γ-decalactone	4	3.5	0.001	
2-undecanone	6	3.5	0.012	
2-nonanone	3	3.3	0.013	rice
2-heptanone	2	2.8	0.019	
δ-tetradecalactone	2	2.5	0.016	
2-hexanone	4	1.8	0.008	
unknown 41	2	1.4	n/a	

[1]ethyl

4.3.2 EMC B

EMC B is the only sample in which panelists found unknown 38 and was one of two samples in which 1,2,3,4-tetra-methyl benzene (EMC F) and decanal (EMC G) were perceived. In total, 44 odorants were noted by panelists. Butyric acid (9.2), caproic acid (7.7), capric acid (7.1), nonanoic acid (6.6) and caprylic acid (6.5) were the most intense acids and ethyl heptanoate (8.0), γ-decalactone (7.2), acetoin (6.7), δ-undecalactone (6.5) and unknown 38 (6.4) were the most intense non-acid odorants in EMC B.

In order of abundance, butyric acid (56%), caproic acid (26%), caprylic acid (8%), capric acid (5%), and lauric acid (1%) were the five most abundant acid odorants in EMC B; this pattern of decreased abundance by even-chain length did not continue after lauric acid. The peak area of the acid odorants was over 99.7% of the total quantity of odor-

contributing volatiles. The five most abundant non-acid odorants in EMC B were acetoin (0.06%), ethyl caprate (0.05%), ethyl caprylate (0.04%), ethyl caproate (0.02%), and δ-decalactone (0.02%).

The odorants listed in Table 12 represent those which could be matched to a chromatographic peak. Compound identification of unknown 14 could not be completed with panelists' odor descriptors or odor selection; either too little information was provided by the panelist or that which was given could not be matched to a chemical. All four panelists selected "unknown" when prompted to identify the odorant by name, therefore, it is important to consider for the possibility of a delayed response time. Ethyl caprylate, benzyl ethyl ether and 1,2,3,4-tetra-methylbenzene were all found in EMC B based on GC-MS data. The odor description (Table 11) of panelist 1 matches with that of 1,2,3,4-tetra-methylbenzene (Table 7), however, the response time, assuming no delay in panelist selection, is approximately 44 s before its elution which makes 1,2,3,4-tetra-methylbenzene unlikely to be responsible for this stimulus. The descriptors given by panelist 3 suggest the presence of 1,3-di-tert-butylbenzene. A delayed response could be the reason for the time difference between its known elution time and panelist response time but 1,3-di-tert-butylbenzene was not found by GC-MS analysis. It is possible that 1,2-di-tert-butyl-benzene is present at a concentration below the sensitivity of the MS. The final descriptor (barn, malt) which was provided by panelist 2 does not match the published odor descriptors of any of the four compounds.

Table 11. Panelist intensity ratings, response time and comments for unknown 14 as recorded during the GC-O run of EMC B sample NFA

Panelist	Intensity	Response time (min)	Comments
1	2	14.27	new, cheap plastic, plastic toy
2	11.6	14.40	barn, malt
3	6.7	14.70	car grease, machinery grease
7	6	14.70	N/A

Table 12. The sensory intensity and analytical quantity (%) of all odor-contributing volatiles in enzyme modified cheese B including the number of panelists who responded to its olfactory stimulus and their comments, sorted by the most intense odorant

Compound	Panelists	Intensity	Quantity (%)	Comments
Acids				
butyric acid	6	9.2	55.918	
caproic acid	6	7.7	26.178	sour then chocolate; grains, haystack
capric acid	6	7.1	5.090	vomit, acidic
nonanoic acid	3	6.6	0.154	butter popcorn flavor; intense butter
caprylic acid	5	6.5	7.706	
heptanoic acid	3	6.3	0.299	
tridecanoic acid	3	5.7	0.037	grain; chocolate
valeric acid	6	5.3	0.658	mild licorice
propionic acid	4	5.3	0.085	
9-decenoic acid	4	5.3	0.544	floral, plants, field; strong floral
lauric acid	4	5.3	1.432	
heptanoic acid	3	5.1	0.186	
myristic acid	4	5.1	0.255	spicy
Non-Acids				
1,3-di-tert-butylbenzene	unknown	unknown	0.000	
1,2,3,4-tetra-methylbenzene	unknown	unknown	0.000	
Et[1] heptanoate	2	8.0	0.001	corn flakes
γ-decalactone	5	7.2	0.000	buttered popcorn flavor
acetoin	2	6.7	0.062	
δ-undecalactone	2	6.5	0.000	
unknown 38	3	6.4	0.001	no description
indole	4	6.3	0.001	cow manure with chlorine and butter; unpleasant
unknown 14	4	5.9	x	new cheap plastic, plastic toy; barn, malt; car grease, machinery grease
δ-decalactone	2	5.7	0.018	
δ-dodecalactone	2	5.6	0.010	
γ-dodecalactone	4	5.5	0.004	like undecanoic acid but more fruity than soapy
Et palmitate	2	5.5	0.001	
δ-nonalactone	4	5.3	0.000	
Et myristate	2	5.3	0.004	
δ-octalactone	5	5.2	0.003	warm snickerdoodle
acetol	2	5.2	0.002	
Et caprate	4	5.1	0.048	mushy, moldy, grain; chocolate, floral

Et butyrate	5	5.0	0.013	vanilla
Et laurate	2	4.9	0.015	
Et nonanoate	2	4.5	0.001	carrot juice
2-decanone	3	4.4	0.001	rotten fruity but not so fermented
Et caproate	6	4.0	0.022	lights, sweet; mild licorice; barley
Et caprylate	2	3.7	0.035	pet store, pet food, hamster cage
Et valerate	4	3.7	0.000	sweet smell with light body
2-nonanone	5	3.4	0.012	formaldehyde and peaches; dairy
Et undecanoate	2	2.4	0.001	black licorice or anise; malt
2-undecanone	3	2.4	0.012	grassy, slight watermelon; mild but fresh
Et 9-decenoate	3	2.2	0.006	minty, not peppermint; grocery store bakery buttercream frosting on cookies

[1]ethyl

4.3.3 EMC C

All 39 odor-contributing volatiles in EMC C were identified. EMC C did not contain any unique compounds but three of its odorants were identified in only one other EMC: heptanal (EMC G), decanal (EMC B), and 2-pentadecanone (EMC A). Butyric acid was both the most abundant (52%) and the most intense compound (9.1). Following in abundance were caproic acid (30%), caprylic acid (8%), capric acid (5%), benzoic acid (1.3%), lauric acid (1.1%), valeric acid (0.7%) and myristic acid (0.6%). The quantity of even-chain FFA decreased in order of chain length from C:4-C:14 (Table 13); the only FFA to disrupt this pattern was valeric acid. Despite being the acid in the lowest concentration, undecanoic acid was the third most intense acid. In order from most to least, butyric acid (9.1), benzoic acid (6.7), undecanoic acid (6.6), capric acid (6.5) and caproic acid (6.5) were the five most intense acids, according to panelists.

Ethyl 9-decenoate (6.9), γ-decalactone (6.6), γ-dodecalactone (6.4), ethyl laurate (6.1), and δ-decalactone (6) are the five most intense non-acid volatiles detected by panelists. Similarly, ethyl caprylate (0.2%), ethyl caprate (0.2%), ethyl caproate (0.1%), 2-hetanone (0.05%), and ethyl laurate (0.04%) are the five most abundant non-acid odor-contributing compounds.

The mass spectrum of heptanal was found "hidden" within the front portion of the peak of 2-heptanone (6.199 min). According to the ion fragmentation, 2-heptanone was present in higher quantity than heptanal, although the proportion could not be determined on either the GC-O or the GC-MS. Four panelists responded to 2-heptanone two of which also smelled and identified heptanal by name. For this reason, the presence of heptanal – although not clearly visible – was confirmed however it was not given an area value. It can therefore be assumed that the reported area of 2-heptanone includes that of heptanal, and more of 2-heptanone is more abundant.

Table 13. The sensory intensity and analytical quantity (%) of all odor-contributing volatiles in enzyme modified cheese C including the number of panelists who responded to its olfactory stimulus and their comments, sorted by the most intense odorant

Compound	Panelists	Intensity	Quantity (%)	Comments
Acids				
butyric acid	6	9.1	51.999	rancid dairy
benzoic acid	3	6.7	1.335	sharp, astringent; craft store, potpourri, grandma's house; potpourri, floral, artificial
undecanoic acid	3	6.6	0.055	milky; butter popcorn flavor
capric acid	6	6.5	4.508	acidic, vomit
caproic acid	6	6.5	30.099	sour chocolate, cadmium chocolate; rancid, cardboard, old barn; acidic but coconut
nonanoic acid	2	6.4	0.150	buttery, sweet, brown sugar; butter and brown sugar or just butter and sugar
propanoic acid	2	6.1	0.089	
acetic acid	6	6.0	0.077	
caprylic acid	5	5.4	7.965	super sweet followed by spit up
lauric acid	5	5.4	1.115	coconut; melted butter and a little grass

valeric acid	5	4.9	0.712	
9-decenoic acid	3	3.5	0.483	watermelon
myristic acid	2	3.5	0.571	
Non-Acids				
Et[1] 9-decenoate	2	6.9	0.022	mint
γ-decalactone	3	6.6	0.001	doctor's office, fresh, medical, minty – Extra brand gum in a pool but not chlorine smell, just amount of water
γ-dodecalactone	4	6.4	0.002	super sweet; milky, fragrance of hot or steamed milk with sweet flavor of lactose
Et laurate	3	6.1	0.042	
δ-decalactone	4	6.0	0.015	coconut, pineapple, creamy, pina colada; bad popcorn, fake butter flavor
1,3-di-tert-butylbenzene	6	5.9	0.000	barn, malt; salty, potato chip; strong cracker, cheese, wheat; car grease; fermentation
Et caproate	6	5.7	0.146	pineapple, fresh, fruity, tangy; similar to 2-octanone but more of a sweet flavor; slight licorice
δ-undecalactone	4	5.6	0.000	
indole	3	5.5	0.003	moth balls, doesn't smell good; old lady
Et valerate	4	5.5	0.001	
2-decanone	3	5.4	0.001	fresh, Febreze
Et caprate	5	5.3	0.187	floral then rancid, honey; organic, not in a good way
Et nonanoate	6	5.2	0.005	carrot, carrot juice; crayon, waxy; earthy
acetoin	4	4.7	0.026	
Et butyrate	6	4.6	0.039	pineapple, light, airy, fresh; sweet
heptanal	2	4.6	0.000	
2-tridecanone	2	4.5	0.016	
2-undecanone	4	4.4	0.027	green, sour; coconut
δ-nonalactone	3	4.3	0.008	coconut, tropical
2-heptanone	4	4.3	0.046	
δ-octalactone	4	3.9	0.000	coconut, tropical; coconut
2-pentadecanone	3	3.4	0.006	
δ-dodecalactone	2	3.2	0.008	
Et undecanoate	3	3.0	0.003	licorice-like
2-nonanone	3	2.7	0.030	
Et caprylate	2	2.5	0.207	dairy

[1]ethyl

4.3.4 EMC D

EMC D was the EMC with the most unique odorants which were not found in any other EMC i.e. 3-octanone, γ-hexalactone, octadiene-2-one, and propyl caprylate. 39 odor-contributing chemicals were detected – assuming only one odorant was responsible for the quality of unknown 14. We can offer no opinion as to the identity of unknown 14; neither 1,3-di-tert-butylbenzene nor 1,2,3,4-tetra-methylbenzene could be confirmed as present and panelist descriptions do not match the fruity or sweet notes of benzyl ethyl ether or ethyl octanoate (Table 7). Additionally, the odor qualities provided by panelists (Table 14) do not match those of any of the four odorants (Table 7) suspected to contribute to unknown 14, based on MS results.

The five most intense acids in EMC D were butyric acid (9.30, caproic acid (8.2), tridecanoic acid (7.0), undecanoic acid (7.0), and caprylic acid (6.6). Combined, butyric acid (48%) and caproic acid (36%) made up 83% of the total quantity of odor-contributing volatiles. Following these in decreasing abundance were caprylic acid (7%), capric acid (4%), and valeric acid (0.8%). Despite their high intensity, tridecanoic acid and undecanoic acid were present only at a concentration of 0.008% and 0.35%.

It is noteworthy that the quantity of FFAs in sample FA was so great that the final sample volume was 0.35 ml instead of 0.2 ml; almost all the solvent had been evaporated and only odorants remained. Sample FA of EMC D could not be concentrated further without risk of creating a disproportionate sample; it is more likely for small chain FFAs to evaporate at a faster rate than long chain FFAs such as myristic acid. For this reason, the intensity of the acids in EMC D cannot be compared to those in other EMCs directly but can be compared through trends. Sample NFA was not affected by this deviation and neither was the final calculations of abundance.

The four most abundant non-acid odorants were the ethyl esters of the four most a fatty acids abundant ethyl caproate (0.6%), ethyl butyrate (0.6%), ethyl caprylate (0.3%), and ethyl caprate (0.2%). The most intense non-acid odorants follow a similar pattern as the most abundant: three of the most intense non-acid odorants are the ethyl esters of three of the most abundant acids. In order of intensity, γ-dodecalactone (7.0), 2-nonanone (7.0), ethyl caproate (6.4), ethyl butyrate (6.2), and ethyl valerate (6.1) were found to be the most intense non-acid odor contributors of EMC D.

Table 14. Panelist intensity ratings, response time, and comments for unknown 14 as recorded during the GC-O run of EMC D sample NFA

Panelist	Intensity	Response time (min)	Comments
1	1	14.50	melted butter
2	8	14.53	barn, malt
3	5	14.43	iron, mineral
10	4	14.45	fermentation or potato agar

Table 15. The sensory intensity and analytical quantity (%) of all odor-contributing volatiles in enzyme modified cheese D including the number of panelists who responded to its olfactory stimulus and their comments, sorted by the most intense odorant

Compound	Panelists	Intensity	Quantity (%)	Comments
Acids				
butyric acid	6	9.3	47.811	acidic smell in dairy products
caproic acid	5	8.9	36.005	oxidized, rancid, sweaty armpits; hay, barn
tridecanoic acid	6	7	0.008	cat urine with cocoa; strong hay, straw
undecanoic acid	3	7	0.035	sweet
caprylic acid	6	6.6	7.245	urine, sweaty armpits; fermented
capric acid	6	6.4	3.809	light fermented
myristic acid	2	5.9	0.449	sweet but soapy
valeric acid	4	5.8	0.829	
9-decenoic acid	4	5.2	0.384	fruity, sour watermelon smell; strawberry, fresh
lauric acid	3	4.4	0.789	
acetic acid	6	4.3	0.039	barn, malt
propionic acid	3	4.2	0.132	sharp acidic

benzoic acid	2	4.1	0.187	potpourri; slight butter smell with potpourri, Michael's craft store
nonanoic acid	5	3.7	0.144	rancid; acidic; not sweet, industrial, metal; buttered popcorn and dust
Non-Acids				
γ-dodecalactone	3	7.0	0.003	clear milky with a little bit sweet
2-nonanone	2	7.0	0.088	fruity, grassy
ethyl caproate	3	6.4	0.612	heavy, sweet
ethyl butyrate	4	6.2	0.600	sweet mixed with fermented
ethyl valerate	4	6.1	0.011	very sweet; clear, sweet smell; fruity, ester
ethyl caprylate	2	6.0	0.347	not too sweet
indole	3	5.6	0.002	chlorine, butter, and cow manure; clear light smell
unknown 14	4	5.6		iron, mineral; fermentation or potato agar; melted butter; barn, malt
3-octanone	2	5.6	0.002	leaves, mint
δ-octalactone	4	5.4	0.003	
γ-decalactone	3	5.4	0.001	light fermented with sweet; bad buttered popcorn
propyl caprylate	2	5.3	0.001	earthy
δ-undecalactone	3	5.1	0.000	
δ-dodecalactone	3	5.1	0.008	bad buttered popcorn
Et[1] heptanoate	3	4.9	0.014	strong popcorn; starch or rice
Et laurate	2	4.9	0.029	fruity, coconut
δ-tetradecalactone	3	4.7	0.010	
2-heptanone	4	4.7	0.143	light aldehyde
octadien-2-one	3	4.0	0.001	green or watermelon; very sweet, similar to ethyl nonanoate
δ-nonalactone	3	3.6	0.000	
2-tridecanone	3	3.6	0.032	mild, sweet, fruity; oatmeal
2-octanone	2	3.5	0.003	mushroom; light
δ-decalactone	2	3.1	0.014	
γ-hexalactone	2	2.9	0.001	Mild oat then black licorice or anise; black licorice
ethyl caprate	2	2.7	0.209	

[1]ethyl

4.3.5 EMC E

Thirty-one odorants were identified as odor-contributing volatiles in EMC E (Table 16). Acids were the most abundant chemical group which comprised over 99% of the total

quantity of odor-contributing volatiles. Of these, five acids were in abundance over 1%: butyric acid (62%), caproic acid (24%), caprylic acid (6%), capric acid (5%), and lauric acid (1%). While palmitic acid was present at a very low level (0.1%), it was the fourth most intense acid with an intensity of 6.2. Intensities preceding palmitic acid include butyric acid (7.5), lauric acid (7.0), and caproic acid (6.6).

In order of abundance, 2-methyl-2-butanol (0.049%), δ-dodecalactone (0.037%), δ-decalactone (0.0%), ethyl butyrate (0.034%) and acetol (0.028%) were the five non-acid odor-contributing volatiles in highest quantity. Interestingly, only δ-dodecalactone was reported to be one of the top five most intense non-acid volatiles which in order of intensity includes indole (6.5), γ-decalactone (6.2), 1,3-di-tert-butylbenzene (5.5), δ-dodecalactone (5.3) and γ-dodecalactone (5). Of similar interest, three of the most intense compounds were among the six least abundant compounds: indole (0.008%), γ-decalactone (0.002%), and 1,3-di-tert-butylbenzene (0%), the last of which was present in a quantity below the sensitivity of the FID.

EMC E contained no unique odorants but did contain four volatiles which were perceived as odor-contributing by two or more panelists in only one other EMC: palmitic acid (EMC A), 2-methyl-2-butanol (EMC I), diacetyl (EMC G) and decanal (EMC G).

Table 16. The sensory intensity and analytical quantity (%) of all odor-contributing volatiles in enzyme modified cheese E including the number of panelists who responded to its olfactory stimulus and their comments, sorted by the most intense odorant

Compound	Panelists	Intensity	Quantity (%)	Comments
Acids				
butyric acid	6	7.5	61.447	acidic
lauric acid	4	7.0	1.084	milky with cow manure; bad buttered popcorn; musty
caproic acid	5	6.6	23.881	
palmitic acid	2	6.2	0.094	weak acidic; floral
benzoic acid	3	5.9	0.133	bad or fake buttered popcorn flavor
caprylic acid	5	5.8	6.061	caramel; sour lemon; nice roasted caramel apples

9-decenoic acid	3	5.2	0.451	watermelon jolly rancher
myristic acid	3	4.9	0.269	office supplies, paper, envelope adhesive
acetic acid	5	4.6	0.800	
undecanoic acid	3	4.4	0.024	slightly more floral than usual
capric acid	4	4.2	4.559	soapy
valeric acid	4	4.2	0.625	light fruit
nonanoic acid	2	3.5	0.294	woody but musty
Non-Acids				
indole	3	6.5	0.008	moth balls; grandma's cupboard, musty, mothball, gross; fruity, soapy, buttery
γ-decalactone	4	6.2	0.002	light fermented milk, sweet
1,3-di-tert-butylbenzene	5	5.5	0.000	car grease, grain; wheat, cheese; mashed potatoes but not with butter; salty like braised pork rice
Et[1] laurate	2	5.4	0.003	
δ-dodecalactone	2	5.3	0.037	lactone, dairy
γ-dodecalactone	5	5.3	0.013	fresh, floral, laundry softener; milky and fermented; dragon fruit vitamin water: fruity on top of soapy smell
δ-octalactone	4	5.1	0.010	almond
δ-decalactone	4	5.1	0.036	light acidic
Et undecanoate	4	4.9	0.001	tortilla chip, fried corn and lard; popped popcorn; light, sharp 2-dodecanone
2-methyl-2-butanol	3	4.0	0.049	
Et caprate	3	4.0	0.011	earthy, fruity, ester
2-undecanone	3	3.9	0.011	weak pear, fruity; green but not sour, light, and grassy
diacetyl	2	3.6	0.010	melted butter or margarine; buttery
decanal	3	3.3	0.002	fresh, Febreze; dew on grass, clover, not sharp
Et butyrate	3	3.2	0.034	light sweet and fermented smell and texture; pineapple, tropical
acetol	2	3.2	0.028	mildly acidic
2-tridecanone	3	2.6	0.009	light fermented; tortilla chip with brightness or fruitiness, maybe lime chips
Et caproate	5	2.5	0.015	sweet but stronger

[1]ethyl

4.3.6 EMC F

All 31 odor-contributing volatiles in EMC F were identified (Table 17). EMC F did not contain any odorants which were not detected in other samples although it did contain two which were found in only one other EMC: benzaldehyde (EMC I) and benzyl alcohol (EMC G). Butyric acid (68%), caproic acid (26%), capric acid (3%), lauric acid (0.8%), and valeric acid (0.7)% were the five most abundant odorants in EMC F; δ-tetradecalactone (0.02%), ethyl caprylate (0.02%), ethyl caproate 90.01%), δ-decalactone (0.01%) and ethyl butyrate (0.01%) were the five most abundant non-acids.

Again, butyric acid was ranked top in intensity (9.4) and was followed by benzoic acid (8.2), undecanoic acid (6.4), capric acid (6.1) and myristic acid (5.9). None of the most intense non-acid odorants were present in a concentration of more than 0.02%. These include δ-dodecalactone (8.4), δ-decalactone (7.4), γ-dodecalactone (6.8), indole (6.6) and 1,2,3,4-tetra-methylbenzene (6.5). EMC F had the lowest quantity of non-acids (0.095%) of all EMCs and thus the highest quantity of acids (99.9%).

Table 17. The sensory intensity and analytical quantity (%) of all odor-contributing volatiles in enzyme modified cheese F including the number of panelists who responded to its olfactory stimulus and their comments, sorted by the most intense odorant

Compound	Panelists	Intensity	Quantity (%)	Comments
Acids				
butyric acid	6	9.4	67.931	rancid cheese; strong acidic
benzoic acid	2	8.2	0.225	potpourri, eucalyptus
undecanoic acid	4	6.4	0.003	creamy, milky, slight soapy and fatty
capric acid	3	6.1	3.463	soapy; baby formula, pheromones
myristic acid	2	5.9	0.218	
acetic acid	6	5.8	0.335	acidic; vinegar
caproic acid	6	5.8	25.557	sweaty, rancid; sour, cheesy, Velveeta
9-decenoic acid	3	5.4	0.343	
tridecanoic acid	2	5.3	0.007	rancid glue
lauric acid	4	5.0	0.779	office, shredded paper, printer, a little like paste
propionic acid	2	4.0	0.119	rancid cheese; strong acidic

valeric acid	4	3.7	0.701	rancid cheese, sharp but not too strong
heptanoic acid	2	3.6	0.224	light and weak fermented milk odor
Non-Acids				
δ-dodecalactone	2	8.4	0.005	fresh, floral
δ-decalactone	4	7.4	0.011	creamy, sweet but coconut or pineapple; bad buttered popcorn, sweet; sweet, pear
γ-dodecalactone	4	6.8	0.003	milk and fermented lactose, sweet; soapy, fruity, fruity shower gel; acidic
indole	3	6.6	0.008	grandma's cupboard, moth balls, moldy; moth balls, very unpleasant; cow manure, grassy and buttery, unpleasant
1,2,3,4-tetra-methylbenzene	4	6.5	0.000	cracker, butter, wheat, cheese, a little metal; malt; French fry, potato; wheat, grain, smells like baking
Et[1] caprylate	4	5.8	0.015	sweet and smelly; started watery then went to metal, iron; dry erase marker
δ-octalactone	3	5.4	0.002	sunscreen with coconut oil, pina colada; sweet, almond; fruity, sweet
γ-decalactone	3	5.0	0.000	wet, ocean breeze with a little rancid
δ-nonalactone	2	4.8	0.000	Styrofoam but sweet, coconut, pineapple, a little smokey/grassy
Et caproate	5	4.5	0.014	anise, black licorice; pear or apple; pineapple, acidic; rubber then cracker
Et butyrate	4	4.5	0.009	sweet; sweet, blueberry
δ-tetradecalactone	3	4.3	0.017	sweet, paper, office supplies; acidic; rancid like caproic acid but also a combo of dog urine
benzene ethanol	4	4.3	0.000	hand lotion; fruity; cinnamon, sweet; floral
benzaldehyde	2	4.2	0.000	grassy; hot sauce, siracha
δ-undecalactone	2	4.0	0.001	fresh, sweet, floral, sugary; cream, sweet, vanilla
Et laurate	3	3.9	0.007	rancid, sweaty, cheese; marker, similar to octanal; soapy, oat

Et valerate	3	3.3	0.000	weak and light, sweet; sweet; fruity, ethyl
Et heptanoate	3	3.1	0.000	wheat thin, cardboard; sharp, similar to 2-dodecanone; similar to 2-nonanone

[1]ethyl

4.3.7 EMC G

Panelists responded to the stimulus of 30 odorants in EMC G of which four were only present in one other EMC: heptanal (EMC C), diacetyl (EMC E), naphthalene (EMC H), and decanal (EMC E). EMC G was the only sample in which the top 5 most intense and most concentrated share no similarities. The most important non-acid odorants which contribute to the aroma of EMC G are, by order of intensity, ethyl laurate (7.3), γ-dodecalactone (7.2), ethyl caprate (6.7), indole (6.1), and 1,3-di-tert-butylbenzene. At 25% abundance, acetoin was the most concentrated non-acid and the second most concentrated odorant overall in EMC G and was followed by δ-decalactone (1%), diacetyl (0.6%), δ-octalactone (0.5%), and hexanal (0.4%).

Butyric acid was both the most abundant (28%) and the most intense (5.8) acid in EMC G. Following in abundance was caproic acid (14%), capric acid (12%), caprylic acid (8%), and acetic acid (4%). After butyric acid, capric acid (5.4), nonanoic acid (5.2), caproic acid (4.9) and acetic acid (4.9) followed in intensity.

Descriptors of caprylic acid suggest the coelution with a fruity or sweet compound. The odor descriptions during the elution of caprylic acid were both pleasant and unpleasant: fruity, musty urine with a background of strawberry, burnt BBQ with acidity, and fruity and sweet. Initially δ-nonalactone was thought to contribute to this heterogeneous mixture however, neither panelist stimulus nor mass spectra could support this theory. Instead, it is possible that caprylic acid (29.284 min) and ethyl myristate (29.343 min; sweet, waxy, violet, orris) created a heterogeneous mixture with partial odor lending qualities.

Table 18. The sensory intensity and analytical quantity (%) of all odor-contributing volatiles in enzyme modified cheese G including the number of panelists who responded to its olfactory stimulus and their comments, sorted by the most intense odorant

Compound	Panelists	Intensity	Quantity (%)	Comments
Acids				
butyric acid	6	5.8	28.367	acidic, unpleasant
capric acid	2	5.4	11.595	acidic; musty, watery
nonanoic acid	2	5.2	0.532	acidic; stimulating and acidic; doctor's office, latex
caproic acid	4	4.9	14.044	stimulating acidic; soapy, glycerin followed by sweet oatmeal; waxy, rancid, goat
acetic acid	3	4.9	4.094	fermented, sweet; cracker, cheese
lauric acid	3	4.5	2.293	milky, creamy; acidic, pheromone; musty, moth ball
caprylic acid	4	3.9	7.999	fruity; musty urine, background of strawberry; burnt, BBQ, acidic; fruity and sweet
undecanoic acid	2	2.3	0.529	sweet; soapy, shower product, clean
Non-Acids				
Et[1] laurate	3	7.3	0.079	started bar soap then sweet oatmeal; matcha green tea
γ-dodecalactone	4	7.2	0.327	soapy, shower gel; Febreze, floral, lavender; acidic; sweet, lactone-y and fruity
Et caprate	2	6.7	0.229	lavender; hay, straw
indole	3	6.1	0.123	musty grandma, moldy; manure, butter, and chlorine pool all together; moth balls, bad artificial butter popcorn flavor
1,3-di-tert-butylbenzene	5	5.8	0.204	cracker, sweet; barn, malt; French fry, potato, oily, carbohydrate; earthy
hexanal	3	5.7	0.427	mixture of hot glue and alcohol; waxy, rancid, sweet
Et caproate	3	5.3	0.130	earthy; fishy, rancid
δ-octalactone	3	5.3	0.502	coconut, sunscreen; sweet, sunscreen, palm tree, artificial coconut
δ-decalactone	2	5.3	1.350	
acetoin	2	5.0	25.457	mild butter
γ-decalactone	3	4.8	0.077	slightly acidic, earthy, metal, sweet, syrup, fruit

naphthalene	3	4.6	0.172	light anise
benzene	3	4.3	0.076	floral; fruity, coconut; Greek yogurt, sour and dairy
ethanol				
δ-hexalactone	3	4.3	0.301	tortilla chips, salty, corn, sour chips like lime tortillas; organic
δ-tetradecalactone	2	4.3	0.068	
isophorone	3	3.8	0.049	cherry candy, sweet but a little sour; propionic acid, very brief, acidic but not too acidic; artificial watermelon, sour, slightly sweet
2-octanone	3	3.5	0.000	mushrooms, earthy; earthy, mushrooms; slightly fruity but musty overtone, wet-mushroom or fungi
Et caprylate	3	2.9	0.046	comment about RT and odor recognition times flipped
Et butyrate	2	2.9	0.054	fruity, ester, similar to γ-decalactone
Et myristate	2	2.9	0.033	
unknown 17	2	2.5	0.036	floral, earthy; carrot juice
dimethyl glutarate	3	2.2	0.226	buttercream frosting with a little black licorice
diacetyl	2	1.6	0.581	fruity and buttery; butter

[1]ethyl

4.3.8 EMC H

EMC H was the only sample in which no ethyl esters contributed to its aroma and was the only sample in which styrene was detected by panelists. Benzyl alcohol (EMC I), dimethyl glutarate (EMC I), limonene (EMC I), naphthalene (EMC G), and unknown 11 (EMC A) were the odorants that EMC H shared with only one other EMC. Only four acids were perceived by panelists and none of them were in a concentration of more than 10% or were given an intensity of more than 6. In order of intensity, the acids in EMC H were benzoic acid (6.0; 7%), caproic acid (4.9; 7%), butyric acid (4.3; 10%), and myristic acid (2.2; 3%). It is interesting that myristic acid was perceived by two or more panelists while other, more volatile acids were present but not smelled (acetic acid, nonanoic acid, capric acid, and lauric acid). Sensory intensity depends both upon the sensory threshold and

compound concentration. It is likely that these other acids were present at concentrations below their odor thresholds while myristic acid was not.

Acetoin was both the most intense (6.5) and most abundant (42%) non-acid odorant in EMC H. Following in intensity were γ-dodecalactone (6.5), δ-decalactone (6.4), benzyl alcohol (5.7) and styrene (5.6). After acetoin, the non-acid volatiles in highest concentration were δ-decalactone (12%), δ-dodecalactone (6%), hexanal (3%), and δ-octalactone (3%). The quantity of non-acid odorants equates to 70% of the concentration of odorants, the most of any EMC.

Table 19. The sensory intensity and analytical quantity (%) of all odor-contributing volatiles in enzyme modified cheese H including the number of panelists who responded to its olfactory stimulus and their comments, sorted by the most intense odorant

Compound	Panelists	Intensity	Quantity (%)	Comments
Acids				
benzoic acid	2	6.0	6.961	strong bad butter popcorn
caproic acid	4	4.9	7.230	oats, grain, sweet; soapy followed by oatmeal
butyric acid	4	4.3	9.735	acidic; rancid, cheese, mold
myristic acid	2	2.2	2.581	slightly sweet but also acidic
Non-Acids				
acetoin	2	6.5	41.570	sweaty
γ-dodecalactone	2	6.5	0.415	soapy, milky
δ-decalactone	3	6.4	12.495	floral, coconut, creamy, fresh; δ-nonalactone, warm buttery, brown sugar, bad butter popcorn flavor;
benzyl alcohol	4	5.7	0.829	green tea, matcha; soapy, oatmeal; straw, hay, fodder; toasted grain
styrene	3	5.6	2.684	fishy, rancid; not musty but earthy and organic, not so mineral
unknown 17	3	5.6	0.232	cardboard, like ethyl nonanoate; not sweet,

				not fruity, is earthy, grainy
hexanal	4	5.4	3.454	hot glue or melted plastic; organic, earthy, mineral; waxy but sweet, rancid
isophorone	5	5.1	0.504	floral, fruity, sweet; artificial watermelon; cucumber; rancid
1,3-di-tert-butylbenzene	2	4.6	0.591	not sweet; barn, malt
unknown 11	2	4.4	0.000	copper, metal; stale French fries
dimethyl glutarate	4	3.8	0.440	sugary, frosting, grocery store buttercream frosting, slightly sweet with darker note; sweet onion, fruity or floral
δ-octalactone	2	3.6	2.776	coconut lotion, creamy, tropical, a little pineapple
δ-dodecalactone	3	3.2	5.755	light and weak milk texture; milky, bad butter popcorn, not sweet; smells like a lactone
2-tridecanone	2	2.5	0.746	tortilla chip, salty, fatty, corn chip' buttery, nutty
limonene	2	2.3	0.165	grass, sour, cut grass; similar to 2-heptanone
naphthalene	2	2.3	0.836	tea; vinegar but rancid
δ-nonalactone	4	0.0	0.000	Dairy, something related to lactones; wet dog; chlorinated water; creamy, fresh

4.3.9 EMC I

Panelists responded to the stimulus of 23 odorants, three of which could not be identified: unknown 17, unknown 23, and unknown 30 (Table 20). EMC I contained four unique odorants: unknown 23, unknown 30, delta hexalactone, and 1-decene. Additionally, four of its odorants were only detected in one other EMC: limonene (EMC H), 2-methyl-2-butanol (EMC E), benzyl alcohol (EMC H), and benzaldehyde (EMC F). The only acids that were perceived by panelists were butyric acid (37%) and caprylic

acid (12%) which had intensities of 6.1 and 4.6. Acetoin was the second most concentrated odorant in EMC I and was present in a quantity just under that of butyric acid (0.2 area units lower). In descending order, 1,3-di-tert-butylbenzene (4%), 2-methyl-2-butanol (3%), 1-decene (2%), and δ-undecalactone (6%) were the four next-most abundant non-acid odorants. The only similarity between the five most concentrated and the five most intense non-acids was acetoin which the second most-intense with a rating of 6.8. The most intense odorant was γ-decalactone (9.9) and the remaining non-acid odorants of note include δ-octalactone (6.4), benzyl alcohol (6.4), and unknown 17 (6.3). Non-acid odorants made up 50% by quantity of the total volatile profile, the second most of any EMC.

Table 20. The sensory intensity and analytical quantity (%) of all odor-contributing volatiles in enzyme modified cheese I, including the number of panelists who responded to its olfactory stimulus and their comments, sorted by the most intense odorant

Compound	Panelists	Intensity	Quantity (%)	Comments
Acids				
butyric acid	5	6.1	37.445	rancid, cheesy, sharp; sharp, disgusting, expired dairy products; stinky cheese
caprylic acid	2	4.6	12.075	acidic, coconut
Non-Acids				
γ-decalactone	2	9.9	0.172	fresh, Febreze, tropical, creamy
acetoin	2	6.8	37.244	dairy, lactones
δ-octalactone	3	6.4	0.906	anise, dry erase marker, sweet; coconut, fresh, tropical, creamy
benzyl alcohol	2	6.4	0.122	oat; green tea, matcha, watery
unknown 17	4	6.3	0.188	carrot juice with a little tomato or cinnamon; earthy, organic; waxy, crayon
isophorone	3	5.4	0.155	sweet, fruity; cucumber
δ-undecalactone	5	5.4	1.002	lactone, dairy; shower gel, fragrant soap; fruity, fresh

1,3-di-tert-butylbenzene	4	4.8	3.516	potato, French fry; barn, malt; earthy, metallic; cracker, wheat, baked goods
ethyl laurate	2	4.8	0.123	soapy, oatmeal at the end
1-decene	3	4.7	2.180	slightly fruity
limonene	2	4.6	0.136	cut grass, sour, green; grassy, fresh cut grass, lemon undertones
indole	3	4.4	0.166	manure; slight moth ball; moth balls, musty, grandma's cupboard; sanitary, doctor's office, fresh
dimethyl glutarate	3	4.1	0.175	salty, play-doh, moved to sweet, sugary, buttercream frosting; dairy, acidic sting in nose
hexanal	4	4.0	0.127	burning or melting plastic, hot glue; grassy, rancid, a little sweet, waxy, crayon; earthy, organic
unknown 30		4.0		cat pee; melted butter; wet dog; earthy, organic, dirt and seaweed or swamp scent; fishy
unknown 23	3	4.0	0.169	salty, corn flour, masa, fried tortilla chip; corn, grain; wheat, toast, rancid
2-methyl-2-butanol	2	3.6	2.811	sweet, fruity
benzaldehyde	3	3.2	0.192	carrot juice; a mildly sweet plant; fresh, Febreze, light
δ-hexalactone	3	3.1	0.572	acidic, lactic acid; fermentation
δ-nonalactone	1+	3.0	0.254	fruity, sweet, panelist associates with formaldehyde
2-tridecanone	2	3.0	0.177	earthy, organic, not too sweet; tortilla chip, salty

4.3.10 Summary of Results

Tables 21 and 22 each display a summary of the intensity and quantity of the odorants by acid and non-acid groups to provide a comparison between EMC samples. Table 22 also shows the abundance of all unidentified volatiles as a percentage of the total aromatic profile. Mass spectrometric data suggests that all the unidentified odorants are non-acid (section 4.2.1.2). Tables 23 and 24 compare the most important acid and non-acid odorants for all EMCs including their intensity ratings.

Additional summary Tables can be found in Appendices 5 and 7 which include information on the standard deviation, coefficient of variation and Kovats indices of each compound.

Table 21. The sum of the mean intensity ratings of acid and non-acid odorants, and the sum of the intensity of all odorants for any given enzyme modified cheese (EMC)

EMC	Acids	Non-Acids	Sum	Overall sensory intensity[1]
A	87	160	247	6.5
B	73	136	209	6.5
C	73	126	199	7.3
D	69	124	193	6.5
E	65	79	144	5.8
F	63	93	156	7.7
G	32	98	130	5.5
H	15	73	88	4.9
I	11	101	112	6.3

[1]As judged by a sensory panel, courtesy of Sara Kleba

Although the intensity of acid and non-acid odorants cannot be directly compared, one can subjectively evaluate the overall intensity of EMC samples. In Table 21, the sum of the GC-O intensity ratings in both the FA and NFA samples for each EMC are presented. (There are no data, or necessarily logic, to suggest that the arithmetic sum of the intensities of aroma compounds of a given GC-O run indicates/predicts the sensory intensity of a given composite, however, it was considered of interest to do so.) to better visualize any relationships, the data in Table 21 are plotted in Figure 3.

The total GC-O intensity of sample FA in all EMCs decreased with each EMC (alphabetically) which suggests a decrease in lipolysis with descending EMC (in Table 21). The remaining fraction of each EMC (sample NFA) follows a similar pattern of descending GC-O intensity through EMC E. The total GC-O intensity of both fractions were combined for a subjective comparison of overall intensity. The overall intensity of EMC A was 38 units above all other samples and was followed by order of most intense to least: EMC B, C, D, F, E, G, I, and H.

Descriptive sensory analysis of all nine EMCs was completed by Sara Kleba, whose work was unpublished at the time of this writing. Kleba was kind enough to share with us the results of her study in which 11 trained panelists determined that the average intensity rating of EMCs in order from most to least intense EMC was F (7.7), C (7.3), A (6.5), B (6.5), D (6.5), I (6.3), E (5.8), G (5.5) and H (4.9) (See Table 21). Kleba's study used each EMC paste "as-is" and did not use any modified pastes (i.e. no fatty acids were removed). In both methodologies, EMC H was the least intense EMC overall. Both methods can provide a different interpretation of the results.

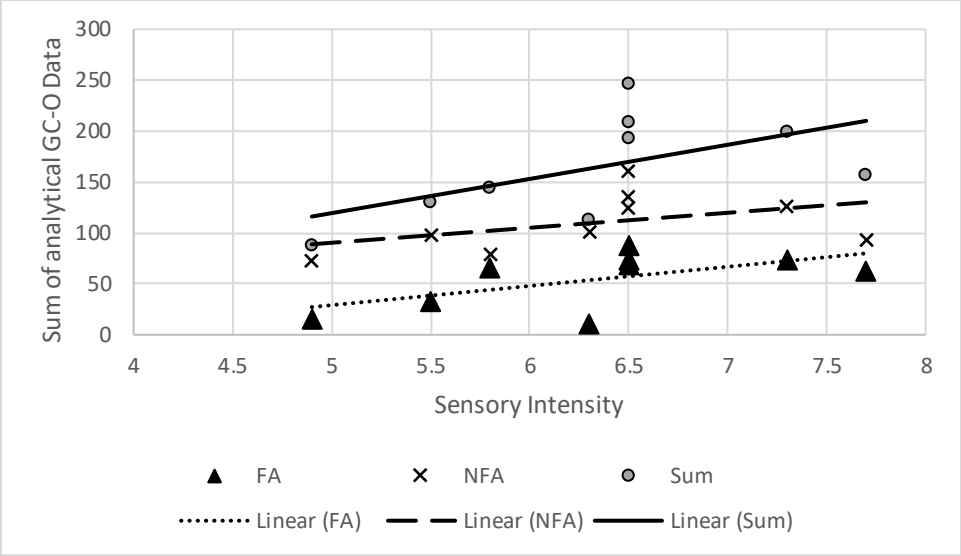

Figure 3. The relationships between the sums of GC-O data (FA, NFA and sum of both-Y axis) and overall sensory intensity from sensory profile analysis (X axis).

Table 22. The quantity of acids, non-acids and unknown compounds in each enzyme modified cheese (EMC) by area percent of total odor-contributing compounds.

EMC	Acids (%)	Non-Acids (%)	Unknown Volatiles (%)
A	99.6	0.39	0.00
B	99.6	0.27	0.09
C	99.2	0.84	0.00
D	97.9	2.13	0.00
E	99.7	0.28	0.00
F	99.9	0.10	0.00
G	67.3	32.54	0.12
H	26.5	70.04	3.45
I	49.5	50.26	0.22

Table 22 shows the sum of the concentrations of all acid and non-acid odorants for each EMC by percent of GC-O peak area. Acids are the greatest contributors to intensity for EMCs A-F while non-acid volatiles play a greater role in determining total aroma intensity of EMCs G-I. The remaining column (*Unknown Volatiles)* displays the percent quantity of all unidentified odorants by sample. Although their identity is not known, it is

unlikely that any of the unknown chemicals are acids (section 4.2.1.2) and they play a minor role in influencing odor character.

Table 23. The five most intense ACID odorants and their mean intensity ratings in order of most intense in each enzyme modified cheese (EMC)

EMC	Most Potent Non-Acid Volatiles				
	1	2	3	4	5
A	9-decenoic acid (9.1)	butyric acid (8.9)	caproic acid (8.2)	benzoic acid (7.5)	lauric acid (7.2)
B	butyric acid (9.2)	caproic acid (7.7)	capric acid (7.1)	nonanoic acid (6.6)	caprylic acid (6.5)
C	butyric acid (9.1)	benzoic acid (6.7)	undecanoic acid (6.6)	capric acid (6.5)	caproic acid (6.5)
D	butyric acid (9.3)	caproic acid (8.2)	tridecanoic acid (7.0)	undecanoic acid (7.0)	caprylic acid (6.6)
E	butyric acid (7.5)	lauric acid (7.0)	caproic acid (6.6)	palmitic acid 96.2)	benzoic acid (5.9)
F	butyric acid (9.4)	benzoic acid (8.2)	undecanoic acid (6.4)	capric acid (6.1)	myristic acid (5.9)
G	butyric acid (5.8)	capric acid (5.4)	nonanoic acid (5.2)	caproic acid (4.9)	acetic acid (4.9)
H	benzoic acid (6.0)	caproic acid (4.9)	butyric acid (4.3)	myristic acid (2.2)	
I	butyric acid (6.1)	caprylic acid (4.6)			

Table 24. The five most intense NON-ACID odorants and their mean intensity ratings in order of most intense in each enzyme modified cheese (EMC)

EMC	Most Potent Non-Acid Volatiles				
	1	2	3	4	5
A	(8.0) Et[1] palmitate	(7.5) isophorone	(6.3) 1,2,3,4-tetra-methyl benzene	(5.9) Et laurate	(5.8) Et caprylate
B	(8.0) Et heptanoate	(7.2) γ-decalactone	(6.7) acetoin	(6.5) δ-undecalactone	(6.4) unknown 38
C	(6.9) Et 9-decenoate	(6.6) γ-decalactone	(6.4) γ-dodecalactone	(6.1) Et laurate	(6.0) δ-decalactone
D	(7.0) γ-dodecalactone	(7.0) 2-nonanone	(6.4) Et caproate	(6.2) Et butyrate	(6.1) Et valerate
E	(6.5) indole	(6.2) γ-decalactone	(5.5) 1,3-di-tert-butylbenzene	(5.3) δ-dodecalactone	(5.3) γ-dodecalactone
F	(8.4) δ-dodecalactone	(7.4) δ-decalactone	(6.8) γ-dodecalactone	(6.6) indole	(6.5) 1,2,3,4-tetra-methyl benzene
G	(7.3) Et laurate	(7.2) γ-dodecalactone	(6.7) Et caprate	(6.1) indole	(5.8) 1,3-di-tert-butylbenzene
H	(6.5) acetoin	(6.5) γ-dodecalactone	(6.4) δ-decalactone	(5.7) benzyl alcohol	(5.6) styrene
I	(9.9) γ-decalactone	(6.8) acetoin	(6.4) δ-octalactone	(6.4) benzyl alcohol	(6.3) unknown 17

[1]ethyl

Table 1 displays odorants that have been found in Cheddar cheese by two or more independent researchers from a sample of 16 publications. 23 of the 52 chemicals listed in Table 1 were identified to be odor-contributors in at least one of the 9 EMC samples. These include: acetic, propionic, butyric, valeric, caproic, caprylic, capric, and lauric acid, ethyl butyrate, carproate and caprylate, δ octalactone, decalactone, and dodecalactone, γ decalactone, acetoin, 1-octen-3-one, 2-butanone, 2-pentanone, 2-heptanone, 2-nonanone, diacetyl, and hexanal.

4.4 FUTURE WORK

This project offers many avenues for additional interpretation, some of which support the work that has been previously discussed and others which may offer new insights.

4.4.1. Expand FFA Analysis

Perhaps the most direct continuation of this research is to combine the method used in this experimentation (section 2.3) with that used by our sponsors (section 4.1.4). The combination of the two methods would allow for the determination of all odor-contributing compounds in a sample, including odor intensity and descriptions, and would provide the quantification of all acid odorants.

4.4.2. Method Reproducibility

The reproducibility of the volatile profile of EMC sample extracts is unknown. Ideally, multiple extractions would have completed for each EMC to determine reproducibility of this method. However, the time-intensive extraction and panelist sniffing of nine EMC samples limit what can be studied. The re-extraction and instrumental analysis of even one EMC would help provide an estimate of the precision of this method.

4.4.3 Manufacturing Variability

The analysis of EMC extracts of different lots would provide information on the variability of manufacture. As previously stated, any deviation in ingredients (amounts or quality) or manufacturing practices – large or small – can potentially have a significant influence on the final flavor profile of an EMC. Through the volatile analysis of EMC samples from various lots, the reproducibility of its aromatic profile could be evaluated. In the case where two lots develop different profiles it may be necessary to study multiple samples to provide a more representative dataset.

4.4.5 Impact of EMCs on Final Products

EMCs are produced for use as ingredients and not to be consumed independently. A logical progression of this study is to evaluate the flavor contribution of a product with

added EMC based on product type or EMC concentration. Such products could include process or powdered cheese, cheese sauce, or cheese shreds. This type of study could help to guide the formulation of products which contain EMCs.

4.4.6. Determining How GC-O Data Predict Sensory Profiles of Cheese Products

Last, and perhaps most valuable, it would be worthwhile to invest another student in simply working with the GC-O data and the sensory panel data to understand how one predicts the other. One could consider: the sensory thresholds of each chemical found and amount present to potentially develop a better understanding of how chemical composition data can be used to predict sensory panel data, or one might combine data on similar chemical classes or similar sensory properties and determine if this allows better sensory predictions based on analytical data. There is a great deal of both analytical and sensory data that can be “mined” to provide learning. This type and quantity of data are seldom generated.

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6. APPENDICES

APPENDIX 1. Instructions given to panelists for GC-O sniffing prior to his or her training run

Rules: no phone, no lotions or perfumes, no eating or drinking except water one hour before

This instrument is a gas chromatograph (GC). It separates aroma compounds that have been extracted from a food sample in a certain order at different times. This instrument helps us to quantify compounds in a sample. Coupling a GC with a mass spectrometer allows us to identify and quantify each aroma compound in a food. This GC is set up with a sniffing port. As each compound exits the column of the GC, part of it goes to a detector which tells us how much of the compound is present and the rest goes to the sniffing port where you will smell it. This is the sniffing port (show). You will sit with your nose in the nosepiece and breathe in through your nose. This run will run for 15 minutes but runs with samples will be one hour. Any time you smell something, fill out the questionnaire by selecting the odor name and its intensity. (instructions on the questionnaire.) If you cannot name the compound, describe the odor that you smell and the time that you smell the odor. The odors you will be sniffing today were not part of the compounds that you trained on but please select a compound at this time to replicate the GC-O method you will be completing soon.

APPENDIX 2. The even-chain free fatty acid (ppm) and fat (%) content for each enzyme modified cheese (EMC)1

	EMC								
Parameter	A	B	C	D	E	F	G	H	I
Free fatty acid (ppm)[2]	24191	28738	52298	113650	5960	26003	1229	980	1040
Fat (%)	24.63	25.2	25.46	40.73	35.71	27.74	35.69	34.2	34.99

[1]analysis and table courtesy of LOL

APPENDIX 3. A flow chart of the methods explored during the method development of the volatile extraction of cheese powders

Appendix 3.1 Method development for cheese powder experiment A

stir 1 hr – 31% yield

/

DCM + cheese powder at 40% solids

\

Stir 24 hr – 36% yield

Appendix 3.2 Method development for cheese powder experiment B

20 min extraction with UAE – 20% yield

/

DCM + cheese powder at 40% solids stir 15 min

\

2 x 20 min extraction with UAE – 20% yield

Appendix 3.3 Method development for cheese powder experiment C

too gummy

/

Water added to cheese powder until smooth paste

\

Frozen overnight; too gummy

Appendix 3.4 Method development for cheese powder experiment D

1:1 ratio of water to DCM added to cheese powder until smooth paste – emulsion formed and did not break overnight

Appendix 3.5 Method development for cheese powder experiment E

brought to pH 4.6 - DCM added in 1:1 ratio to - a gel was
with 1 N HCl water used and stirred 1 hr formed
/
water + cheese powder
at 20% solids
\ a gel was
brought to pH 5.2 - DCM added in 1:1 ratio to - formed and
with 1 N HCl water used and stirred 1 hr did not break

Appendix 3.6 Method development for cheese powder experiment F

water + cheese brought to DCM added in 1:1 centrifuge
powder at - pH 4.6 with - ratio to water used - 2000 x g - 0% yield
20% solids 1 N HCl and stirred 1 hr for 4 hr

Appendix 3.7 Method development for cheese powder experiment G

physical disruption with glass rod
centrifugation
| /
1 M NaCl + 5% - pH brought to 4.6 – emulsion formed - all attempts failed to
cheese solids with 1 N HCl | \ break the emulsion
solvent addition
overnight filtration

Appendix 3.8 Method development for cheese powder experiment H

5, 10, 20
and 30% - Addition of - rotational – DCM removed – re-addition – rotational
cheese DCM by plate 20 hr by pipette of DCM plate 15 hr
solids pipette
in water

Appendix 3.9 Method development for cheese powder experiment I

5% cheese powder solids in water – liquid-liquid extraction with DEE for 2 hr – emulsion formation – 0% yield

Appendix 3.10 Method development for cheese powder experiment J

maintained native pH of 5.36 – addition of DEE in 1:2 with 1 M HCl – stir 1 hr – centrifuge – 46% yield

/

two solutions of 23 % solids in 1 M NaCl

\

pH brought to 4.2 with 1 M HCl – addition of DEE in 1:2 with 1 M HCl – stir 1 hr – centrifuge – 22% yield

APPENDIX 4. Additional summary tables of the odor-contributing volatiles of each enzyme modified cheese (EMC)

Appendix 4.1 EMC A

Appendix 5.1.1 The peak area, standard deviation, CV (%), intensity and number of panelists who smelled each odorant in enzyme modified cheese A, sorted by quantity (%).

Compound	Panelists	Intensity	Peak area	Standard Deviation	CV (%)	Quantity (%)
butyric acid	6	8.9	21494342	5866711	27	63.953
caproic acid	6	8.2	8276540	2324617	28	24.626
caprylic acid	6	6.0	1717267	492900	29	5.109
capric acid	6	6.7	1049793	308474	29	3.124
lauric acid	5	7.2	270820	80483	30	0.806
valeric acid	5	6.5	230943	65905	29	0.687
9-decenoic acid	3	9.1	106120	34794	33	0.316
myristic acid	4	5.1	85724	36732	43	0.255
benzoic acid	2	7.5	63238	30640	48	0.188
heptanoic acid	3	5.1	62590	19612	31	0.186
acetic acid	6	5.8	34016	13423	39	0.101
propanoic acid	5	3.1	27460	8626	31	0.082
nonanoic acid	3	5.6	25896	9376	36	0.077
ethyl caprate	4	4.6	25538	743	3	0.076
palmitic acid	2	2.8	24256	10646	44	0.072
ethyl caprylate	4	5.8	22434	322	1	0.067
acetoin	2	3.5	10450	557	5	0.031
ethyl caproate	6	4.6	10426	47	0	0.031
ethyl laurate	5	5.9	7709	347	4	0.023
undecanoic acid	3	4.7	7016	3477	50	0.021
δ-decalactone	6	5.1	6717	271	4	0.020
2-heptanone	2	2.8	6310	24	0	0.019
δ-tetradecalactone	2	2.5	5448	1132	21	0.016
ethyl butyrate	6	4.5	4314	25	1	0.013
2-nonanone	3	3.3	4260	23	1	0.013
2-undecanone	6	3.5	4048	79	2	0.012
δ-dodecalactone	5	5.0	3507	185	5	0.010
ethyl 9-decenoate	3	4.9	3073	190	6	0.009
2-hexanone	4	1.8	2637	14	1	0.008
acetol	4	5.1	2460	733	30	0.007
tridecanoic acid	3	5.4	2246	1010	45	0.007

ethyl myristate	3	4.3	2229	155	7	0.007
2-tridecanone	4	4.6	1949	88	4	0.006
δ-octalactone	4	4.5	1175	46	4	0.003
δ-nonalactone	4	3.9	1158	168	15	0.003
γ-dodecalactone	4	5.3	883	50	6	0.003
ethyl palmitate	2	8.0	557	58	10	0.002
ethyl nonanoate	4	4.9	538	41	8	0.002
ethyl valerate	4	4.2	504	38	8	0.001
2-pentadecanone	3	5.4	476	52	11	0.001
ethyl undecanoate	3	4.7	475	60	13	0.001
Benzene ethanol	4	5.4	429	92	21	0.001
hexanal	2	4.7	400	19	5	0.001
indole	4	5.2	357	43	12	0.001
isophorone	2	7.5	300	42	14	0.001
δ-hexalactone	3	4.7	209	69	33	0.001
γ-decalactone	4	3.5	184	63	34	0.001
2-octanone	3	4.1	76	33	0	0.000
1,2,3,4-tetra-methyl benzene	6	6.3				0.000
unknown 11	4	5.2				0.000
unknown 41	2	1.4				

Appendix 4.1.2 The peak area, standard deviation, CV (%), quantity (%), intensity and number of panelists who smelled each odorant in enzyme modified cheese A shown by chemical class and chain length

Compound	Panelists	Intensity	Peak area	Standard Deviation	CV (%)	Quantity (%)
Acid						
acetic acid	6	5.8	34016	13423	39	0.101
propanoic acid	5	3.1	27460	8626	31	0.082
butyric acid	6	8.9	21494342	5866711	27	63.953
valeric acid	5	6.5	230943	65905	29	0.687
caproic acid	6	8.2	8276540	2324617	28	24.626
heptanoic acid	3	5.1	62590	19612	31	0.186
caprylic acid	6	6.0	1717267	492900	29	5.109
nonanoic acid	3	5.6	25896	9376	36	0.077
capric acid	6	6.7	1049793	308474	29	3.124
9-decenoic acid	3	9.1	106120	34794	33	0.316
undecanoic acid	3	4.7	7016	3477	50	0.021
lauric acid	5	7.2	270820	80483	30	0.806
tridecanoic acid	3	5.4	2246	1010	45	0.007
myristic acid	4	5.1	85724	36732	43	0.255
palmitic acid	2	2.8	24256	10646	44	0.072
benzoic acid	2	7.5	63238	30640	48	0.188
Ketones						
2-hexanone	4	1.8	2637	14	1	0.008
2-heptanone	2	2.8	6310	24	0	0.019
2-octanone	3	4.1	76	33	0	0.000
2-nonanone	3	3.3	4260	23	1	0.013
2-pentadecanone	3	5.4	476	52	11	0.001
2-undecanone	6	3.5	4048	79	2	0.012
2-tridecanone	4	4.6	1949	88	4	0.006
Aldehydes						
hexanal	2	4.7	400	19	5	0.001
Lactones						
δ-hexalactone	3	4.7	209	69	33	0.001
δ-octalactone	4	4.5	1175	46	4	0.003
δ-nonalactone	4	3.9	1158	168	15	0.003
δ-decalactone	6	5.1	6717	271	4	0.020
δ-dodecalactone	5	5.0	3507	185	5	0.010
δ-tetradecalactone	2	2.5	5448	1132	21	0.016
δ-hexalactone	3	4.7	209	69	33	0.001
δ-octalactone	4	4.5	1175	46	4	0.003
δ-nonalactone	4	3.9	1158	168	15	0.003
γ-decalactone	4	3.5	184	63	34	0.001

γ-dodecalactone	4	5.3	883	50	6	0.003
Esters						
ethyl butyrate	6	4.5	4314	25	1	0.013
ethyl valerate	4	4.2	504	38	8	0.001
ethyl caproate	6	4.6	10426	47	0	0.031
ethyl caprylate	4	5.8	22434	322	1	0.067
ethyl nonanoate	4	4.9	538	41	8	0.002
ethyl caprate	4	4.6	25538	743	3	0.076
ethyl 9-decenoate	3	4.9	3073	190	6	0.009
ethyl undecanoate	3	4.7	475	60	13	0.001
ethyl laurate	5	5.9	7709	347	4	0.023
ethyl myristate	3	4.3	2229	155	7	0.007
ethyl palmitate	2	8.0	557	58	10	0.002
Other						
acetoin	2	3.5	10450	557	5	0.031
acetol	4	5.1	2460	733	30	0.007
Benzene ethanol	4	5.4	429	92	21	0.001
indole	4	5.2	357	43	12	0.001
isophorone	2	7.5	300	42	14	0.001
1,2,3,4-tetra-methyl benzene	6	6.3				0.000
unknown 11	4	5.2				0.000
unknown 41	2	1.4				

Appendix 4.2 EMC B

Appendix 5.2.1 The peak area, standard deviation, CV (%), intensity and number of panelists who smelled each odorant in enzyme modified cheese B, sorted by quantity (%).

Compound	Panelists	Intensity	Peak area	Standard Deviation	CV (%)	Quantity (%)
butyric acid	6	9.2	26663152	2583955	10	55.918
caproic acid	6	7.7	12482197	1732334	14	26.178
caprylic acid	5	6.5	3674539	1146680	31	7.706
capric acid	6	7.1	2426840	1226873	51	5.090
lauric acid	4	5.3	259574	129207	50	1.432
benzoic acid	3	4.5	372629	189256	51	0.781
valeric acid	6	5.3	313918	30230	10	0.658
myristic acid	4	2.8	292111	223133	76	0.613
9-decenoic acid	4	5.3	682992	456716	67	0.544
heptanoic acid	3	6.3	142613	28002	20	0.299
acetic acid	6	4.7	78795	8035	10	0.165
nonanoic acid	3	6.6	73558	28662	39	0.154
propionic acid	4	5.3	40725	6430	16	0.085
undecanoic acid	3	4.3	30690	16909	55	0.064
acetoin	2	6.7	29532	545	2	0.062
ethyl caprate	4	5.1	22736	383	2	0.048
tridecanoic acid	3	5.7	17687	11882	67	0.037
ethyl caprylate	2	3.7	16741	60	0	0.035
ethyl caproate	6	4.0	10414	74	1	0.022
δ-decalactone	2	5.7	8632	700	8	0.018
ethyl laurate	2	4.9	7028	364	5	0.015
ethyl butyrate	5	5.0	6149	152	2	0.013
2-undecanone	3	2.4	5801	173	3	0.012
2-nonanone	5	3.4	5555	48	1	0.012
δ-dodecalactone	2	5.6	4660	686	15	0.010
ethyl 9-decenoate	3	2.2	2877	58	2	0.006
ethyl myristate	2	5.3	128	43	34	0.004
γ-dodecalactone	4	5.5	1852	181	10	0.004
δ-octalactone	5	5.2	1526	64	4	0.003
acetol	2	5.2	1124	143	13	0.002
ethyl palmitate	2	5.5	635	102	16	0.001
ethyl nonanoate	2	4.5	498	12	2	0.001
indole	4	6.3	476	120	25	0.001
unknown 38	3	6.4	439	81	19	0.001
ethyl undecanoate	2	2.4	387	15	4	0.001
ethyl heptanoate	2	8.0	373	42	11	0.001
2-decanone	3	4.4	354	15	4	0.001

δ-undecalactone	2	6.5	210	25	12	0.000
ethyl valerate	4	3.7	183	31	17	0.000
γ-decalactone	5	7.2	164	30	18	0.000
1,2,3,4-tetra-methylbenzene			149	64	43	0.000
δ-nonalactone	4	5.3	2129	209	10	0.000
1,3-di-tert-butylbenzene			89	14	16	0.000
unknown 14	4	5.9				

Appendix 4.2.2 The peak area, standard deviation, CV (%), quantity (%), intensity and number of panelists who smelled each odorant in enzyme modified cheese B shown by chemical class and chain length

Compound	Panelists	Intensity	Peak area	Standard Deviation	CV (%)	Quantity (%)
Acids						
acetic acid	6	4.7	78795	8035	10	0.165
propionic acid	4	5.3	40725	6430	16	0.085
butyric acid	6	9.2	26663152	2583955	10	55.918
valeric acid	6	5.3	313918	30230	10	0.658
caproic acid	6	7.7	12482197	1732334	14	26.178
heptanoic acid	3	6.3	142613	28002	20	0.299
caprylic acid	5	6.5	3674539	1146680	31	7.706
nonanoic acid	3	6.6	73558	28662	39	0.154
capric acid	6	7.1	2426840	1226873	51	5.090
9-decenoic acid	4	5.3	682992	456716	67	0.544
undecanoic acid	3	4.3	30690	16909	55	0.064
lauric acid	4	5.3	259574	129207	50	1.432
tridecanoic acid	3	5.7	17687	11882	67	0.037
myristic acid	4	2.8	292111	223133	76	0.613
benzoic acid	3	4.5	372629	189256	51	0.781
Ketones						
2-nonanone	5	3.4	5555	48	1	0.012
2-decanone	3	4.4	354	15	4	0.001
2-undecanone	3	2.4	5801	173	3	0.012
Lactones						
δ-octalactone	5	5.2	1526	64	4	0.003
δ-nonalactone	4	5.3	2129	209	10	0.000
δ-decalactone	2	5.7	8632	700	8	0.018
δ-undecalactone	2	6.5	210	25	12	0.000
δ-dodecalactone	2	5.6	4660	686	15	0.010
γ-decalactone	5	7.2	164	30	18	0.000
γ-dodecalactone	4	5.5	1852	181	10	0.004
Esters						
ethyl butyrate	5	5.0	6149	152	2	0.013
ethyl valerate	4	3.7	183	31	17	0.000
ethyl caproate	6	4.0	10414	74	1	0.022
ethyl heptanoate	2	8.0	373	42	11	0.001
ethyl caprylate	2	3.7	16741	60	0	0.035
ethyl nonanoate	2	4.5	498	12	2	0.001
ethyl caprate	4	5.1	22736	383	2	0.048
ethyl 9-decenoate	3	2.2	2877	58	2	0.006
ethyl undecanoate	2	2.4	387	15	4	0.001

ethyl laurate	2	4.9	7028	364	5	0.015
ethyl myristate	2	5.3	128	43	34	0.004
ethyl palmitate	2	5.5	635	102	16	0.001
Other						
1,3-di-tert-butylbenzene			89	14	16	0.000
1,2,3,4-tetra-methylbenzene			149	64	43	0.000
acetoin	2	6.7	29532	545	2	0.062
indole	4	6.3	476	120	25	0.001
acetol	2	5.2	1124	143	13	0.002
unknown 14	4	5.9				
unknown 38	3	6.4	439	81	19	0.001

Appendix 4.3 EMC C

Appendix 5.3.1 The peak area, standard deviation, CV (%), intensity and number of panelists who smelled each odorant in enzyme modified cheese C, sorted by quantity (%).

Compound	Panelists	Intensity	Peak area	Standard Deviation	CV (%)	Quantity (%)
butyric acid	6	9.1	31591090	1731066	5	51.999
caproic acid	6	6.5	18285884	798316	4	30.099
caprylic acid	5	5.4	4839062	209044	4	7.965
capric acid	6	6.5	2738696	146216	5	4.508
benzoic acid	3	6.7	810915	48551	6	1.335
lauric acid	5	5.4	677481	46354	7	1.115
valeric acid	5	4.9	432445	19184	4	0.712
myristic acid	2	3.5	347007	29756	9	0.571
9-decenoic acid	3	3.5	293637	17215	6	0.483
ethyl caprylate	2	2.5	125505	2805	2	0.207
ethyl caprate	5	5.3	113627	5337	5	0.187
nonanoic acid	2	6.4	91337	6612	7	0.150
ethyl caproate	6	5.7	88842	228	0	0.146
propanoic acid	2	6.1	54144	3153	6	0.089
acetic acid	6	6.0	46823	3214	7	0.077
undecanoic acid	3	6.6	33460	3294	10	0.055
2-heptanone	4	4.3	28246	234	1	0.046
ethyl laurate	3	6.1	25762	9881	38	0.042
ethyl butyrate	6	4.6	23710	546	2	0.039
2-nonanone	3	2.7	18291	314	2	0.030
2-undecanone	4	4.4	16381	783	5	0.027
acetoin	4	4.7	15864	443	3	0.026
ethyl 9-decenoate	2	6.9	13127	660	5	0.022
2-tridecanone	2	4.5	9507	725	8	0.016
δ-decalactone	4	6.0	9024	949	11	0.015
δ-nonalactone	3	4.3	4917	537	11	0.008
δ-dodecalactone	2	3.2	4557	1864	41	0.008
2-pentadecanone	3	3.4	3706	411	11	0.006
ethyl nonanoate	6	5.2	2839	89	3	0.005
indole	3	5.5	2082	561	27	0.003
ethyl undecanoate	3	3.0	1857	108	6	0.003
γ-dodecalactone	4	6.4	1433	353	25	0.002
ethyl valerate	4	5.5	878	79	9	0.001
γ-decalactone	3	6.6	418	46	11	0.001
2-decanone	3	5.4	379	29	8	0.001
δ-undecalactone	4	5.6	262	67	25	0.000
δ-octalactone	4	3.9	184	39	21	0.000

heptanal	2	4.6	0	x	x	0.000
1,3-di-tert-butylbenzene	6	5.9	0	0	0	0.000

Appendix 4.3.2 The peak area, standard deviation, CV (%), quantity (%), intensity and number of panelists who smelled each odorant in enzyme modified cheese C shown by chemical class and chain length

Compound	Panelists	Intensity	Peak area	Standard Deviation	CV (%)	Quantity (%)
Acids						
acetic acid	6	6.0	46823	3214	7	0.077
propanoic acid	2	6.1	54144	3153	6	0.089
butyric acid	6	9.1	31591090	1731066	5	51.999
valeric acid	5	4.9	432445	19184	4	0.712
caproic acid	6	6.5	18285884	798316	4	30.099
caprylic acid	5	5.4	4839062	209044	4	7.965
nonanoic acid	2	6.4	91337	6612	7	0.150
capric acid	6	6.5	2738696	146216	5	4.508
9-decenoic acid	3	3.5	293637	17215	6	0.483
undecanoic acid	3	6.6	33460	3294	10	0.055
lauric acid	5	5.4	677481	46354	7	1.115
myristic acid	2	3.5	347007	29756	9	0.571
benzoic acid	3	6.7	810915	48551	6	1.335
Ketones						
2-decanone	3	5.4	379	29	8	0.001
2-undecanone	4	4.4	16381	783	5	0.027
2-tridecanone	2	4.5	9507	725	8	0.016
2-pentadecanone	3	3.4	3706	411	11	0.006
2-heptanone	4	4.3	28246	234	1	0.046
2-nonanone	3	2.7	18291	314	2	0.030
Aldehydes						
heptanal	2	4.6	0	x	x	0.000
Lactones						
δ-octalactone	4	3.9	184	39	21	0.000
δ-nonalactone	3	4.3	4917	537	11	0.008
δ-decalactone	4	6.0	9024	949	11	0.015
δ-undecalactone	4	5.6	262	67	25	0.000
δ-dodecalactone	2	3.2	4557	1864	41	0.008
γ-decalactone	3	6.6	418	46	11	0.001
γ-dodecalactone	4	6.4	1433	353	25	0.002
Esters						
ethyl butyrate	6	4.6	23710	546	2	0.039
ethyl valerate	4	5.5	878	79	9	0.001
ethyl caproate	6	5.7	88842	228	0	0.146
ethyl caprylate	2	2.5	125505	2805	2	0.207
ethyl nonanoate	6	5.2	2839	89	3	0.005
ethyl caprate	5	5.3	113627	5337	5	0.187

ethyl 9-decenoate	2	6.9	13127	660	5	0.022
ethyl undecanoate	3	3.0	1857	108	6	0.003
ethyl laurate	3	6.1	25762	9881	38	0.042
Other						
1,3-di-tert-butylbenzene	6	5.9	0	0	0	0.000
indole	3	5.5	2082	561	27	0.003
acetoin	4	4.7	15864	443	3	0.026

Appendix 4.4 EMC D

Appendix 4.4.1 The peak area, standard deviation, CV (%), intensity and number of panelists who smelled each odorant in enzyme modified cheese D, sorted by quantity (%).

Compound	Panelists	Intensity	Peak area	Standard Deviation	CV (%)	Quantity (%)
butyric acid	6	9.3	42896083	12920761	30	47.811
caproic acid	5	8.2	32303135	9437861	29	36.005
caprylic acid	6	6.6	6500337	1777720	27	7.245
capric acid	6	6.4	3417348	887189	26	3.809
valeric acid	4	5.3	743869	222379	30	0.829
lauric acid	3	4.4	708196	173679	25	0.789
ethyl caproate	3	6.4	548751	5927	1	0.612
ethyl butyrate	4	6.2	537817	25391	5	0.599
myristic acid	2	5.9	402433	94507	23	0.449
9-decenoic acid	4	5.2	344632	89902	26	0.384
ethyl caprylate	2	6.0	311362	8617	3	0.347
ethyl caprate	2	2.7	187758	10481	6	0.209
benzoic acid	2	4.1	167494	48420	29	0.187
nonanoic acid	5	3.7	129499	36954	29	0.144
2-heptanone	4	4.7	128549	45073	35	0.143
propionic acid	3	4.2	118701	36502	31	0.132
2-nonanone	2	7.0	79174	1184	1	0.088
acetic acid	6	4.3	35051	10454	30	0.039
undecanoic acid	3	7.0	31518	9690	31	0.035
2-tridecanone	3	3.6	28696	1940	7	0.032
ethyl laurate	2	4.9	25753	1615	6	0.029
δ-decalactone	2	3.1	12618	1049	8	0.014
ethyl heptanoate	3	4.9	12477	82	1	0.014
ethyl valerate	4	6.1	9865	325	3	0.011
δ-tetradecalactone	3	4.7	8793	4193	48	0.010
tridecanoic acid	6	7.0	7112	1986	28	0.008
δ-dodecalactone	3	5.1	6784	669	10	0.008
2-octanone	2	3.5	2757	786	29	0.003
γ-dodecalactone	3	7.0	2721	261	10	0.003
δ-octalactone	4	5.4	2596	196	8	0.003
3-octanone	2	5.6	1858	387	21	0.002
indole	3	5.6	1546	154	10	0.002
γ-hexalactone	2	2.9	1078	152	14	0.001
γ-decalactone	3	5.4	987	101	10	0.001
octadien-2-one	3	4.0	591	31	5	0.001
propyl caprylate	2	5.3	556	35	6	0.001
δ-nonalactone	3	3.6	427	66	15	0.000

δ-undecalactone	3	5.1	316	33	10	0.000
unknown 14	4	5.6				

Appendix 4.4.2 The peak area, standard deviation, CV (%), quantity (%), intensity and number of panelists who smelled each odorant in enzyme modified cheese D shown by chemical class and chain length

Compound	Panelists	Intensity	Peak area	Standard Deviation	CV (%)	Quantity (%)
Acids						
acetic acid	6	4.3	35051	10454	30	0.039
propionic acid	3	4.2	118701	36502	31	0.132
butyric acid	6	9.3	42896083	12920761	30	47.811
valeric acid	4	5.3	743869	222379	30	0.829
caproic acid	5	8.2	32303135	9437861	29	36.005
caprylic acid	6	6.6	6500337	1777720	27	7.245
nonanoic acid	5	3.7	129499	36954	29	0.144
capric acid	6	6.4	3417348	887189	26	3.809
9-decenoic acid	4	5.2	344632	89902	26	0.384
undecanoic acid	3	7.0	31518	9690	31	0.035
lauric acid	3	4.4	708196	173679	25	0.789
tridecanoic acid	6	7.0	7112	1986	28	0.008
myristic acid	2	5.9	402433	94507	23	0.449
benzoic acid	2	4.1	167494	48420	29	0.187
Ketones						
2-nonanone	2	7.0	79174	1184	1	0.088
2-octanone	2	3.5	2757	786	29	0.003
2-heptanone	4	4.7	128549	45073	35	0.143
2-tridecanone	3	3.6	28696	1940	7	0.032
octadien-2-one	3	4.0	591	31	5	0.001
3-octanone	2	5.6	1858	387	21	0.002
Lactones						
δ-octalactone	4	5.4	2596	196	8	0.003
δ-nonalactone	3	3.6	427	66	15	0.000
δ-decalactone	2	3.1	12618	1049	8	0.014
δ-undecalactone	3	5.1	316	33	10	0.000
δ-dodecalactone	3	5.1	6784	669	10	0.008
δ-tetradecalactone	3	4.7	8793	4193	48	0.010
γ-decalactone	3	5.4	987	101	10	0.001
γ-dodecalactone	3	7.0	2721	261	10	0.003
γ-hexalactone	2	2.9	1078	152	14	0.001
Esters						
ethyl butyrate	4	6.2	537817	25391	5	0.599
ethyl valerate	4	6.1	9865	325	3	0.011
ethyl caproate	3	6.4	548751	5927	1	0.612
ethyl heptanoate	3	4.9	12477	82	1	0.014
ethyl caprylate	2	6.0	311362	8617	3	0.347
propyl caprylate	2	5.3	556	35	6	0.001

ethyl caprate	2	2.7	187758	10481	6	0.209
ethyl laurate	2	4.9	25753	1615	6	0.029
Other						
indole	3	5.6	1546	154	10	0.002
unknown 14	4	5.6				

Appendix 4.5 EMC E

Appendix 4.5.1 The peak area, standard deviation, CV (%), intensity and number of panelists who smelled each odorant in enzyme modified cheese E, sorted by quantity (%).

Compound	Panelists	Intensity	Peak area	Standard Deviation	CV (%)	Quantity (%)
butyric acid	6	7.5	8557735	136748	2	61.447
caproic acid	5	6.6	3325880	141028	4	23.881
caprylic acid	5	5.8	844144	51182	6	6.061
capric acid	4	4.2	634972	47489	7	4.559
lauric acid	4	7.0	150923	13556	9	1.084
acetic acid	5	4.6	111446	3166	3	0.800
valeric acid	4	4.2	87051	3281	4	0.625
9-decenoic acid	3	5.2	62797	5167	8	0.451
nonanoic acid	2	3.5	40923	3058	7	0.294
myristic acid	3	4.9	37464	3545	9	0.269
benzoic acid	3	5.9	18500	1628	9	0.133
palmitic acid	2	6.2	13063	1037	8	0.094
2-methyl-2-butanol	3	4.0	6795	219	3	0.049
δ-dodecalactone	2	5.3	5143	355	7	0.037
δ-decalactone	4	5.1	5046	87	2	0.036
ethyl butyrate	3	3.2	4736	226	5	0.034
acetol	2	3.2	3894	172	4	0.028
undecanoic acid	3	4.4	3326	236	7	0.024
ethyl caproate	5	2.5	2143	18	1	0.015
γ-dodecalactone	5	5.3	1757	102	6	0.013
ethyl caprate	3	4.0	1590	155	10	0.011
2-undecanone	3	3.9	1481	89	6	0.011
δ-octalactone	4	5.1	1418	50	4	0.010
diacetyl	2	3.6	1409	404	29	0.010
2-tridecanone	3	2.6	1203	464	39	0.009
indole	3	6.5	1124	261	23	0.008
ethyl laurate	2	5.4	412	18	4	0.003
γ-decalactone	4	6.2	250	52	21	0.002
decanal	3	3.3	217	73	34	0.002
ethyl undecanoate	4	4.9	143	48	34	0.001
1,3-di-tert-butylbenzene	5	5.5	0			0.000

Appendix 4.5.2 The peak area, standard deviation, CV (%), quantity (%), intensity and number of panelists who smelled each odorant in enzyme modified cheese E shown by chemical class and chain length

Compound	Panelists	Intensity	Peak area	Standard Deviation	CV (%)	Quantity (%)
Acids						
acetic acid	5	4.6	111446	3166	3	0.800
butyric acid	6	7.5	8557735	136748	2	61.447
valeric acid	4	4.2	87051	3281	4	0.625
caproic acid	5	6.6	3325880	141028	4	23.881
caprylic acid	5	5.8	844144	51182	6	6.061
nonanoic acid	2	3.5	40923	3058	7	0.294
capric acid	4	4.2	634972	47489	7	4.559
9-decenoic acid	3	5.2	62797	5167	8	0.451
undecanoic acid	3	4.4	3326	236	7	0.024
lauric acid	4	7.0	150923	13556	9	1.084
myristic acid	3	4.9	37464	3545	9	0.269
palmitic acid	2	6.2	13063	1037	8	0.094
benzoic acid	3	5.9	18500	1628	9	0.133
Ketones						
2-undecanone	3	3.9	1481	89	6	0.011
2-tridecanone	3	2.6	1203	464	39	0.009
Aldehydes						
decanal	3	3.3	217	73	34	0.002
Lactones						
δ-octalactone	4	5.1	1418	50	4	0.010
δ-decalactone	4	5.1	5046	87	2	0.036
δ-dodecalactone	2	5.3	5143	355	7	0.037
γ-decalactone	4	6.2	250	52	21	0.002
γ-dodecalactone	5	5.3	1757	102	6	0.013
Esters						
ethyl butyrate	3	3.2	4736	226	5	0.034
ethyl caproate	5	2.5	2143	18	1	0.015
ethyl caprate	3	4.0	1590	155	10	0.011
ethyl undecanoate	4	4.9	143	48	34	0.001
ethyl laurate	2	5.4	412	18	4	0.003
Other						
indole	3	6.5	1124	261	23	0.008
1,3-di-tert-butylbenzene	5	5.5	0			0.000
2-methyl-2-butanol	3	4.0	6795	219	3	0.049
diacetyl	2	3.6	1409	404	29	0.010
acetol	2	3.2	3894	172	4	0.028

Appendix 4.6 EMC F

Appendix 4.6.1 The peak area, standard deviation, CV (%), intensity and number of panelists who smelled each odorant in enzyme modified cheese F, sorted by quantity (%).

Compound	Panelists	Intensity	Peak area	Standard Deviation	CV (%)	Quantity (%)
butyric acid	6	9.4	59964520	25739103	43	67.931
caproic acid	6	5.8	22559559	10322050	46	25.557
capric acid	3	6.1	3057111	1219965	40	3.463
lauric acid	4	5.0	687472	266273	39	0.779
valeric acid	4	3.7	619000	263786	43	0.701
9-decenoic acid	3	5.4	302927	121422	40	0.343
acetic acid	6	5.8	295677	131083	44	0.335
benzoic acid	2	8.2	198247	84701	43	0.225
heptanoic acid	2	3.6	197469	83307	42	0.224
myristic acid	2	5.9	192281	73788	38	0.218
propionic acid	2	4.0	105364	46243	44	0.119
δ-tetradecalactone	3	4.3	14926	3052	20	0.017
ethyl caprylate	4	5.8	12966	159	1	0.015
ethyl caproate	5	4.5	12615	60	0	0.014
δ-decalactone	4	7.4	10149	854	8	0.011
ethyl butyrate	4	4.5	7760	143	2	0.009
indole	3	6.6	7068	2543	36	0.008
ethyl laurate	3	3.9	6521	1041	16	0.007
tridecanoic acid	2	5.3	6044	2400	40	0.007
δ-dodecalactone	2	8.4	4819	541	11	0.005
undecanoic acid	4	6.4	3064	1157	38	0.003
γ-dodecalactone	4	6.8	2782	277	10	0.003
δ-octalactone	3	5.4	2021	110	5	0.002
δ-undecalactone	2	4.0	894	209	23	0.001
benzaldehyde	2	4.2	356	33	9	0.000
δ-nonalactone	2	4.8	310	212	68	0.000
ethyl heptanoate	3	3.1	278	47	17	0.000
γ-decalactone	3	5.0	187	65	35	0.000
ethyl valerate	3	3.3	161	16	10	0.000
1,2,3,4-tetra-methyl benzene	4	6.5	127	26	20	0.000
benzene ethanol	4	4.3	115	23	20	0.000

Appendix 4.6.2 The peak area, standard deviation, CV (%), quantity (%), intensity and number of panelists who smelled each odorant in enzyme modified cheese F shown by chemical class and chain length

Compound	Panelists	Intensity	Peak area	Standard Deviation	CV (%)	Quantity (%)
Acids						
acetic acid	6	5.8	295677	131083	44	0.335
propionic acid	2	4.0	105364	46243	44	0.119
butyric acid	6	9.4	59964520	25739103	43	67.931
valeric acid	4	3.7	619000	263786	43	0.701
caproic acid	6	5.8	22559559	10322050	46	25.557
heptanoic acid	2	3.6	197469	83307	42	0.224
capric acid	3	6.1	3057111	1219965	40	3.463
9-decenoic acid	3	5.4	302927	121422	40	0.343
undecanoic acid	4	6.4	3064	1157	38	0.003
lauric acid	4	5.0	687472	266273	39	0.779
tridecanoic acid	2	5.3	6044	2400	40	0.007
myristic acid	2	5.9	192281	73788	38	0.218
benzoic acid	2	8.2	198247	84701	43	0.225
Lactones						
δ-octalactone	3	5.4	2021	110	5	0.002
δ-nonalactone	2	4.8	310	212	68	0.000
δ-decalactone	4	7.4	10149	854	8	0.011
δ-undecalactone	2	4.0	894	209	23	0.001
δ-dodecalactone	2	8.4	4819	541	11	0.005
δ-tetradecalactone	3	4.3	14926	3052	20	0.017
γ-decalactone	3	5.0	187	65	35	0.000
γ-dodecalactone	4	6.8	2782	277	10	0.003
Esters						
ethyl butyrate	4	4.5	7760	143	2	0.009
ethyl valerate	3	3.3	161	16	10	0.000
ethyl caproate	5	4.5	12615	60	0	0.014
ethyl heptanoate	3	3.1	278	47	17	0.000
ethyl caprylate	4	5.8	12966	159	1	0.015
ethyl laurate	3	3.9	6521	1041	16	0.007
Other						
indole	3	6.6	7068	2543	36	0.008
1,2,3,4-tetra-methyl benzene	4	6.5	127	26	20	0.000
benzene ethanol	4	4.3	115	23	20	0.000
benzaldehyde	2	4.2	356	33	9	0.000

Appendix 4.7 EMC G

Appendix 4.7.1 The peak area, standard deviation, CV (%), intensity and number of panelists who smelled each odorant in enzyme modified cheese G, sorted by quantity (%).

Compound	Panelists	Intensity	Peak area	Standard Deviation	CV (%)	Quantity (%)
butyric acid	6	5.8	79938	799	1	28.367
acetoin	2	5.0	71737	2356	3	25.457
caproic acid	4	4.9	39577	574	1	14.044
capric acid	2	5.4	32676	878	3	11.595
caprylic acid	4	3.9	22540	1021	5	7.999
acetic acid	3	4.9	11537	314	3	4.094
lauric acid	3	4.5	6462	423	7	2.293
δ-decalactone	2	5.3	3804	102	3	1.350
diacetyl	2	1.6	1637	54	3	0.581
nonanoic acid	2	5.2	1500	349	23	0.532
undecanoic acid	2	2.3	1490	27	2	0.529
δ-octalactone	3	5.3	1415	38	3	0.502
hexanal	3	5.7	1203	12	1	0.427
γ-dodecalactone	4	7.2	922	31	3	0.327
δ-hexalactone	3	4.3	847	117	14	0.301
ethyl caprate	2	6.7	645	37	6	0.229
dimethyl glutarate	3	2.2	636	30	5	0.226
1,3-di-tert-butylbenzene	5	5.8	575	12	2	0.204
naphthalene	3	4.6	486	21	4	0.172
ethyl caproate	3	5.3	367	17	5	0.130
indole	3	6.1	347	59	17	0.123
ethyl laurate	3	7.3	223	20	9	0.079
γ-decalactone	3	4.8	217	8	3	0.077
benzene ethanol	3	4.3	215	13	6	0.076
δ-tetradecalactone	2	4.3	191	15	8	0.068
ethyl butyrate	2	2.9	153	20	13	0.054
isophorone	3	3.8	139	25	18	0.049
ethyl caprylate	3	2.9	129	54	42	0.046
unknown 17	2	2.5	100	7	7	0.036
ethyl myristate	2	2.9	92	22	24	0.033
2-octanone	3	3.5	0			0.000

Appendix 4.7.2 The peak area, standard deviation, CV (%), quantity (%), intensity and number of panelists who smelled each odorant in enzyme modified cheese G shown by chemical class and chain length

Compound	Panelists	Intensity	Peak area	Standard Deviation	CV (%)	Quantity (%)
Acids						
acetic acid	3	4.9	11537	314	3	4.094
butyric acid	6	5.8	79938	799	1	28.367
caproic acid	4	4.9	39577	574	1	14.044
caprylic acid	4	3.9	22540	1021	5	7.999
nonanoic acid	2	5.2	1500	349	23	0.532
capric acid	2	5.4	32676	878	3	11.595
undecanoic acid	2	2.3	1490	27	2	0.529
lauric acid	3	4.5	6462	423	7	2.293
Ketones						
2-octanone	3	3.5	0			0.000
Aldehydes						
hexanal	3	5.7	1203	12	1	0.427
Lactones						
δ-hexalactone	3	4.3	847	117	14	0.301
δ-octalactone	3	5.3	1415	38	3	0.502
δ-decalactone	2	5.3	3804	102	3	1.350
δ-tetradecalactone	2	4.3	191	15	8	0.068
γ-decalactone	3	4.8	217	8	3	0.077
γ-dodecalactone	4	7.2	922	31	3	0.327
Esters						
ethyl butyrate	2	2.9	153	20	13	0.054
ethyl caproate	3	5.3	367	17	5	0.130
ethyl caprylate	3	2.9	129	54	42	0.046
ethyl caprate	2	6.7	645	37	6	0.229
ethyl laurate	3	7.3	223	20	9	0.079
ethyl myristate	2	2.9	92	22	24	0.033
Other						
indole	3	6.1	347	59	17	0.123
1,3-di-tert-butylbenzene	5	5.8	575	12	2	0.204
acetoin	2	5.0	71737	2356	3	25.457
naphthalene	3	4.6	486	21	4	0.172
benzene ethanol	3	4.3	215	13	6	0.076
isophorone	3	3.8	139	25	18	0.049
dimethyl glutarate	3	2.2	636	30	5	0.226
diacetyl	2	1.6	1637	54	3	0.581
unknown 17	2	2.5	100	7	7	0.036

Appendix 4.8 EMC H

Appendix 4.8.1 The peak area, standard deviation, CV (%), intensity and number of panelists who smelled each odorant in enzyme modified cheese H, sorted by quantity (%).

Compound	Panelists	Intensity	Peak area	Standard Deviation	CV (%)	Quantity (%)
acetoin	2	6.5	14138	761	5	41.570
δ-decalactone	3	6.4	4250	307	7	12.495
butyric acid	4	4.3	3311	1475	45	9.735
caproic acid	4	4.9	2459	1266	51	7.230
benzoic acid	2	6.0	2368	465	20	6.961
δ-dodecalactone	3	3.2	1957	199	10	5.755
hexanal	4	5.4	1175	14	1	3.454
δ-octalactone	2	3.6	944	85	9	2.776
styrene	3	5.6	913	35	4	2.684
myristic acid	2	2.2	878	560	64	2.581
naphthalene	2	2.3	284	33	12	0.836
benzyl alcohol	4	5.7	282	36	13	0.829
2-tridecanone	2	2.5	254	123	48	0.746
1,3-di-tert-butylbenzene	2	4.6	201	5	2	0.591
isophorone	5	5.1	172	51	30	0.504
dimethyl glutarate	4	3.8	150	51	34	0.440
γ-dodecalactone	2	6.5	141	58	41	0.415
unknown 17	3	5.6	79	34	43	0.232
limonene	2	2.3	56	36	64	0.165
unknown11	2	4.4	0			0.000
δ-nonalactone	4	0.0	0			0.000

Appendix 4.8.2 The peak area, standard deviation, CV (%), quantity (%), intensity and number of panelists who smelled each odorant in enzyme modified cheese H shown by chemical class and chain length

Compound	Panelists	Intensity	Peak area	Standard Deviation	CV (%)	Quantity (%)
Acids						
butyric acid	4	4.3	3311	1475	45	9.735
caproic acid	4	4.9	2459	1266	51	7.230
myristic acid	2	2.2	878	560	64	2.581
benzoic acid	2	6.0	2368	465	20	6.961
Ketones						
2-tridecanone	2	2.5	254	123	48	0.746
Aldehydes						
hexanal	4	5.4	1175	14	1	3.454
Lactones						
δ-octalactone	2	3.6	944	85	9	2.776
δ-decalactone	3	6.4	4250	307	7	12.495
δ-dodecalactone	3	3.2	1957	199	10	5.755
γ-dodecalactone	2	6.5	141	58	41	0.415
Other						
acetoin	2	6.5	14138	761	5	41.570
benzyl alcohol	4	5.7	282	36	13	0.829
styrene	3	5.6	913	35	4	2.684
unknown 17	3	5.6	79	34	43	0.232
isophorone	5	5.1	172	51	30	0.504
1,3-di-tert-butylbenzene	2	4.6	201	5	2	0.591
unknown 11	2	4.4	0	0	x	0.000
dimethyl glutarate	4	3.8	150	51	34	0.440
limonene	2	2.3	56	36	64	0.165
naphthalene	2	2.3	284	33	12	0.836
δ-nonalactone	4	0.0	0	0	x	0.000

Appendix 4.9 EMC I

Appendix 4.9.1 The peak area, standard deviation, CV (%), intensity and number of panelists who smelled each odorant in enzyme modified cheese I, sorted by quantity (%).

Compound	Panelists	Intensity	Peak area	Standard Deviation	CV (%)	Quantity (%)
butyric acid	5	6.1	31893	4304	13	37.445
acetoin	2	6.8	31721	604	2	37.244
caprylic acid	2	4.6	10285	3375	33	12.075
1,3-di-tert-butylbenzene	4	4.8	2995	8274	276	3.516
2-methyl-2-butanol	2	3.6	2394	259	11	2.811
1-decene	3	4.7	1857	42	2	2.180
δ-undecalactone	5	5.4	853	55	6	1.002
δ-octalactone	3	6.4	771	13	2	0.906
δ-hexalactone	3	3.1	487	148	30	0.572
δ-nonalactone	1+	3.0	217	37	17	0.254
benzaldehyde	3	3.2	163	41	25	0.192
unknown 17	4	6.3	160	23	14	0.188
2-tridecanone	2	3.0	151	35	23	0.177
dimethyl glutarate	3	4.1	149	32	22	0.175
γ-decalactone	2	9.9	146	11	7	0.172
unknown 23	3	4.0	144	25	18	0.169
indole	3	4.4	141	23	16	0.166
isophorone	3	5.4	132	15	12	0.155
limonene	2	4.6	116	14	12	0.136
hexanal	4	4.0	108	35	33	0.127
ethyl laurate	2	4.8	105	23	22	0.123
benzyl alcohol	2	6.4	104	44	42	0.122
unknown 30	3	4				

Appendix 4.9.2 The peak area, standard deviation, CV (%), quantity (%), intensity and number of panelists who smelled each odorant in enzyme modified cheese I shown by chemical class and chain length

Compound	Panelists	Intensity	Peak area	Standard Deviation	CV (%)	Quantity (%)
Acids						
butyric acid	5	6.1	31893	4304	13	37.445
caprylic acid	2	4.6	10285	3375	33	12.075
Ketones						
2-tridecanone	2	3.0	151	35	23	0.177
Aldehydes						
hexanal	4	4.0	108	35	33	0.127
Lactones						
δ-hexalactone	3	3.1	487	148	30	0.572
δ-octalactone	3	6.4	771	13	2	0.906
δ-nonalactone	1+	3.0	217	37	17	0.254
δ-undecalactone	5	5.4	853	55	6	1.002
ethyl laurate	2	4.8	105	23	22	0.123
γ-decalactone	2	9.9	146	11	7	0.172
Other						
acetoin	2	6.8	31721	604	2	37.244
benzyl alcohol	2	6.4	104	44	42	0.122
isophorone	3	5.4	132	15	12	0.155
1,3-di-tert-butylbenzene	4	4.8	2995	8274	276	3.516
1-decene	3	4.7	1857	42	2	2.180
limonene	2	4.6	116	14	12	0.136
indole	3	4.4	141	23	16	0.166
dimethyl glutarate	3	4.1	149	32	22	0.175
2-methyl-2-butanol	2	3.6	2394	259	11	2.811
benzaldehyde	3	3.2	163	41	25	0.192
unknown 17	4	6.3	160	23	14	0.188
unknown 23	3	4.0	144	25	18	0.169
unknown 30	3	4				

APPENDIX 5. A list of standards used in the identification of odor-contributing compounds including Kovats indices, elution time (min) from the GC-MS and GC-O, and their odor descriptors as found in literature and sorted by elution time from the GC-O with the parameters used during experimentation

Name	Kovats[1]	MS[2] (min)	GC[3] (min)	Odor
ethyl propanoate	910-977; 957[4]	-	2.626	sweet, fruity, rummy, juicy, grape, pineapple[5]; sweet, ethereal, rummy, grape, winey, fermented eggnog[5]
2-butanone	866-953; 908	-	-	acetone, ethereal, fruit, camphor[5]
2-methyl-2-butanol	966-1048; 1000	2.218	2.425	camphor, roasted, winey, onion, fruity, fusel, alcoholic, whiskey[5]
diacetyl	935-1020; 980	-	2.744	sweet, creamy, buttery, pungent, with a pungent caramellike nuance[5]
2-pentanone	938-1025; 975	-	2.843	sweet, fruity, ethereal, winey, banana, woody[5]
decane	1000	-	2.888	
ethyl butyrate	990-1081; 1025	2.562	3.426	fruity, juicy, pineapple, cognac[5]
hexanal	1034-1127; 1084	3.243	4.049	fresh, green, fatty, aldehydic, grass, leafy, fruity, sweaty[5]
2-hexanone	1051-1124; 1083	3.210	4.136	fruity, fungal, meaty, buttery[5]
undecane	1100	3.480	4.286	
ethyl valerate	1113-1170; 1134	4.238	5.063	sweet fruity apple pineapple green tropical[5]
limonene	1152-1245; 1203	5.577	5.181	citrus, herbal, terpenic, camphor[5]
heptanal	1148-1222; 1186	5.404	6.122	fresh, aldehydic, fatty green, herbal, cognac, ozone[5]
2-heptanone	1145-1216; 1185	5.308	6.199	fruity, spicy, sweet, herbal, coconut, woody[5]; cheesy, fruity, ketonic, green, banana, creamy[5]
dodecane	1200	5.776	6.517	
1-decene	1020-1061; 1048	6.686	6.990	Pleasant[5]
styrene	1228-1310; 1262	6.673	6.990	sweet, balsamic, floral, plastic, almond[5]
ethyl caproate	1183-1271; 1223	6.758	7.482	sweet, fruity, pineapple, waxy, green banana[5]

3-octanone	1205-1300; 1244	7.236	7.639	fresh, herbal, lavender, sweet, mushroom[5]; musty mushroom ketonic moldy cheesy fermented green vegetable[5]
octanal	1247-1329; 1296	8.440	9.013	aldehydic, waxy, citrus, orange peel, green, fatty[5]
2-octanone	1262-1323; 1278	8.279	9.064	musty, mushroom, ketonic, cheesy, bleu cheese, cheesy, parmesan cheese, earthy, dairy[5]; fresh, herbal, lavender, sweet, mushroom[5]
acetoin	1236-1333; 1278	8.125	9.146	sweet, buttery, creamy, dairy, milky, fatty[5]
tridecane	1300	8.888	9.503	
acetol	1266-1340; 1323, 1295	8.648	9.698	pungent, sweet, caramellike, ethereal[5]
ethyl heptanoate	1310-1375; 1331	9.986	10.561	fruity pineapple sweet ester banana berry cognac and slightly green with a seedy nuance[5]
hexyl ether	1349-1368; 1368	11.193	10.725	Mild[5]
2-nonanone	1342-1420; 1392, 1394	11.862	12.488	fruity, sweet, waxy soapy, cheesy, green, herbal, coconut[5]
nonanal	1348-1441; 1400	12.061	12.458	waxy, aldehydic, rose, fresh, orris, orange peel, fatty, peel; waxy, aldehydic, citrus, fresh, green, lemon peel, cucumber, fatty[5]
tetradecane	1400	12.453	12.905	
1,3-Di-tert-butylbenzene	1420-1454; 1427	13.248	13.292	car grease[7]
1,2,3,4-tetramethyl benzene	1430-1505; 1462	15.141	15.081	gasoline, sweet, plastic[6]
benzyl ethyl ether	1421-1439; 1421	13.527	-	fruity, pineapple, tropical[5]
ethyl caprylate	1392-1477; 1429	13.648	14.046	fruity, winey, sweet, apricot, banana, brandy, pear[5]
acetic acid	1400-1498; 1449	14.186	14.696	sharp pungent sour vinegar[5]
2-decanone	1463-1519;	15.719	16.140	orange, floral, fatty, peach[5]

	1493, 1497			
decanal	1448-1538;1506	15.912	16.119	sweet, aldehydic, waxy, orange peel, citrus, floral[5]
pentadecane	1500	16.107	16.371	
benzaldehyde	1470-1569; 1520	16.422	16.545	sharp, sweet, bitter, almond, cherry[5]; almond, fruity, powdery, nutty, cherry, maraschino cherry[5]
Propyl caprylate	624-13-5	16.762	16.951	coconut, cocoa, cognac, winey, fatty
ethyl nonanoate	1503-1581; 1526	17.260	17.469	fruity, rose, waxy, rum, wine, natural tropical[5]
propanoic acid	1486-1574; 1525	17.231	17.554	pungent, acidic, cheesy, vinegar[5]
octadiene-2-one	1522-1516; 1516	17.832	18.438	fruity, fatty, mushroom[5]
isophorone	1541-1621; 1607	18.266	18.990	camphor, peppermint, sweet, green, woody, fruity, musty, cedarwood, tobacco, leathery[5]
2-undecanone	1564-1628; 1604, 1596	19.027	19.280	waxy fruity creamy fatty orris floral[5]
hexadecane	1600	19.286	19.356	
butyric acid	1576-1670; 1625	19.864	20.056	rancid, cheese, sweat; sharp, dairy, cheesy, buttery, fruity[5]
ethyl caprate	1595-1684; 1647	20.222	20.313	sweet, waxy, fruity, apple, grape, oily, brandy[5]
ethyl 9-decenoate	1664-1712; 1694	21.534	21.552	fruity, fatty[5]
dimethyl glutarate	1667-1699; 1687	21.774	-	ester, floral[5]
heptadecane	1700	21.842	21.810	
2-dodecanone	1674-1711; 1698	21.801	21.936	fruity, citrus, floral, orange[5]
γ-hexalactone	1655-1745; 1694	21.403	22.134	herbal, coconut, sweet, coumarin, tobacco[5]
naphthalene	1694-1792; 1763	22.238	22.005	Unpleasant, moth balls[7]; coal, tar[8]
valeric acid	1685-1780; 1720	22.663	22.739	Sweat, acidic and sharp, cheese-like, sour, milky, tobacco, with fruity nuances[5]

ethyl undecanoate	1719-1775; 1725	22.793	22.786	soapy, waxy, fatty, cognac, coconut[5]
octadecane	1800	24.257	24.079	
2-tridecanone	1771-1845; 1815	24.308	24.337	fatty, waxy, dairy, milky, coconut, nutty, herbal, earthy; fatty, waxy, mushroom, coconut, earthy, chicken, fat, fatty[5]
δ-hexalactone	1751-1830; 1818	23.465	24.344	creamy, fruity, coconut[5]
ethyl laurate	1811-1874; 1850	25.130	25.028	sweet, waxy, floral, soapy, clean[5]
caproic acid	1797-1889; 1848, 1849	25.114	25.091	Sour, fatty, sweaty, cheesy[5]
Δ-heptalactone	1976	25.208	25.963	coconut, oily, green, earthy[5]
benzyl alcohol	1821-1919; 1870	25.669	25.380	floral, rose, phenolic, balsamic[5]; sweet, fruity floral, chemical[5]
nonadecane	1900	26.416	26.129	
benzene ethanol	1856-1955; 1905	26.415	26.371	floral, rose, dried rose, sweet[5]; floral, fresh, bready, rose, honey[5]
dimethyl sulfone	1869-1914; 1912, 1895	26.246	26.947	sulfurous burnt[5]
heptanoic acid	1900-2000; 1950	27.371	27.253	pungent, rancid, cheesy, sour, sweaty, fermented pineapple, fruity[5]
δ-octanolactone	1929-2002; 1988	27.295	27.916	sweet, fatty, coconut, tropical, dairy[5]
eicosane	2000	28.519	28.098	
2-pentadecanone	1995-2056; 2023	28.718	28.551	fresh Jasmin, celery, fatty, oily, waxy, burnt[5]
ethyl myristate	2015-2094; 2065	29.343	29.060	sweet, waxy violet orris[5]
caprylic acid	2011-2106; 2084	29.495	29.284	fatty, waxy, rancid, oily, vegetable, cheesy[5]
heneicosane	2100	30.408	29.905	
δ-nonalactone	2023, 2038	29.553	30.040	creamy, sweet, coconut, fatty and milky with a coumaric and oily nuance[5]
γ-decalactone	2090-2185; 2136, 2131	30.805	31.032	fresh, oily, waxy, peach, coconut, buttery, sweet[5]

nonanoic acid	2119-2211; 2192	31.496	31.198	green, fat; waxy, dirty, cheesy, dairy[5]
docosane	2200	32.243	31.651	
δ-decalactone	2144-2243; 2190	31.683	32.050	coconut, peach[5]
ethyl palmitate	2205-2288; 2246	33.102	32.653	waxy, fruity, creamy, milky, balsamic[5]
capric acid	2227-2329; 2300	33.404	33.021	rancid, sour, fatty, citrus[5]
tricosane	2300	34.012	33.333	
δ-undecanolactone	2264-2356; 2345	33.753	34.002	creamy, coconut, fruity, peach, milky, waxy, lactonic, green, fruity[5]
9-decenoic acid	2305-2369; 2341	34.444	34.116	waxy, green, fatty, soapy with a slight creamy cheese type nuance[5]
undecanoic acid	2359-2421; 2400	35.226	34.764	waxy, creamy, cheesy, fatty, coconut[5]
γ-dodecalactone	2333-2415; 2376	34.910	34.914	fatty, peach, sweet, metallic, fruity[5]
tetracosane	2400	35.680	34.930	
δ-dodecanolactone	2386-2470; 2426	35.718	35.858	fatty sweet creamy dairy fruity peach apricot milky buttery[5]
indole	2398-2478; 2444	36.050	35.517	animal, floral, naphthyl, fecal[5]; pungent, floral, naphthyl, fecal, animal, musty[5]
ethyl stearate	2409-2483; 2458	36.624	36.012	waxy[5]
benzoic acid	2380-2457; 2420	35.935	36.167	balsamic, urine[5]
lauric acid	2436-2538; 2503	36.969	36.433	mild fatty coconut bay oil[5]
pentacosane	2500	37.318	36.489	
δ-tridecalactone	2565	37.696	37.227	creamy milky oily buttery musty[5]
hexacosane	2600	38.895	37.993	
tridecanoic acid	2570-2664; 2617	38.642	38.040	waxy, woody[5]
δ-tetradecalactone	2701, 2675	39.385	39.323	waxy creamy oily fatty sweet milky dairy[5]
heptacosane	2700	40.392	39.429	

myristic acid	2648-2734; 2713	40.259	39.588	waxy fatty soapy coconut[5]
octacosane	2800	41.870	40.843	
pentadecanoic acid	2777-2839.5; 2822	41.824	41.084	waxy[5]
palmitic acid	2871-2954; 2931	43.486	42.541	low heavy waxy, with a creamy, candle waxy nuance[5]
heptadecanoic acid	2968-3036; 2980	45.650	44.229	
stearic acid	3090-3181; 3134	48.370	46.339	mild, fatty, waxy[5]

[1](PubChem 2021); [2]retention time (min) of the odorants as seen during GC-MS run using the run parameters discussed in section 2.3.6; [3]retention time (min) of the odorants as seen during GC-O run using the run parameters discussed in section 2.3.5; [4]the Kovats retention index values are presented in the following format: minimum value-maximum value; most common value; [5](TheGoodScentsCompany 2021);[6]as determined by researchers; no literature reference could be found; [7](ChemicalBook 2017); [8](CameoChemicals 2021)

APPENDIX 6. A summary of the odor descriptors given by panelists for every odor-contributing chemical compared to those found in literature[1]

Name	Odor descriptors[2]	Panelist odor descriptors[3]
diacetyl	sweet, creamy, buttery, pungent, with a pungent caramellike nuance[4]	melted butter or margarine, fruity, butter
2-methyl-2-butanol	camphor, roasted, winey, onion, fruity, fusel, alcoholic, whiskey[4]	sweet fruity
ethyl butyrate	fruity, juicy, pineapple, cognac[4]	aromatic, bubblegum, vanilla, pineapple, light, airy, fresh, sweet but fermented, tropical, blueberry, sweet, fruity, ester
hexanal	fresh, green, fatty, aldehydic, grass, leafy, fruity, sweaty[4]	earthy, organic, melting plastic, mineral, waxy but sweet, rancid, sweet, mixture of hot glue and alcohol, crayon, waxy
2-hexanone	fruity, fungal, meaty, buttery[4]	n/a
ethyl valerate	sweet fruity apple pineapple green tropical[4]	sweet, light, clear, fruity, ester,
limonene	citrus, herbal, terpenic, camphor[4]	grass, sour, fresh cut grass, green, lemon undertones
heptanal	fresh, aldehydic, fatty green, herbal, cognac, ozone[4]	n/a
2-heptanone	fruity, spicy, sweet, herbal, coconut, woody[4]; cheesy, fruity, ketonic, green, banana, creamy[4]	light aldehyde
1-decene	pleasant[5]	slightly fruity
styrene	sweet, balsamic, floral, plastic, almond[4]	fishy, rancid, not musty but earthy, organic
ethyl caproate	sweet fruity pineapple, waxy green banana[4]	sweet, mild licorice, barley, pineapple, fresh, fruity, tangy, heavy, anise, black licorice, pear, apple, acidic, rubber, cracker, earthy, fishy, rancid
3-octanone	fresh, herbal, lavender, sweet, mushroom[4]; musty mushroom ketonic moldy cheesy fermented green vegetable[4]	leaves, mint
2-octanone	musty, mushroom, ketonic, cheesy, bleu cheese, cheesy, parmesan cheese, earthy, dairy[4]; fresh, herbal, lavender, sweet, mushroom[4]	musty, mushroom, earthy, slightly fruity with a musty overtone, wet mushroom/fungi

acetoin	sweet, buttery, creamy, dairy, milky, fatty[4]	crayon, oxidized, mild butter, sweaty, dairy, lactones
acetol	pungent, sweet, caramellike, ethereal[4]	mildly acidic
ethyl heptanoate	fruity pineapple sweet ester banana berry cognac and slightly green with a seedy nuance[4]	corn flakes, unbuttered popcorn, starch, rice, Wheat Thins, cardboard
unknown 11		barn, malt, copper, metal, stale French fries
2-nonanone	fruity, sweet, waxy soapy, cheesy, green, herbal, coconut[4]	rice, formaldehyde and peaches, diary, fruity, grassy
1,3-Di-tert-butylbenzene	car grease[6]	barn, malt, salty, potato chip, cracker, wheat, car grease, fermentation, mashed potatoes without butter, salty like braised pork rice, sweet, oily, carbohydrate, metal, baked goods, earthy, French fries
unknown 14	n/a	new cheap plastic, plastic toy, barn, malt, car grease, machinery grease, iron, mineral, fermentation, potato agar, melted butter, floral, earthy, carrot juice
1,2,3,4-tetramethyl benzene	gasoline, sweet, plastic[6]	earthy, plastic, pet store, bird food pellets, malt, metal, French fry, potato, cracker, butter, wheat, cheese, brain, baking
benzyl ethyl ether	fruity, pineapple, tropica4	n/a
ethyl caprylate	fruity, winey, sweet, apricot, banana, brandy, pear[4]	barn, cheesy, Cheddar, wheat, sweet like potato agar or garlic potato, pet store, pet food, hamster cage, diary, not too sweet, smelly, metal, iron, dry erase marker
acetic acid	sharp pungent sour vinegar[4]	barn, malt, acidic, vinegar, fermented, sweet, cracker, cheese
2-decanone	orange, floral, fatty, peach[4]	fresh, Febreze, rotten but not so fermented
decanal	sweet, aldehydic, waxy, orange peel, citrus, floral[4]	fresh, Febreze; dew on grass, clover, not sharp
benzaldehyde	sharp, sweet, bitter, almond, cherry[4]; almond, fruity, powdery, nutty, cherry, maraschino cherry[4]	grassy, hot sauce, siracha, carrot juice, a mildly sweet plant, fresh, Febreze, light
Propyl caprylate	coconut, cocoa, cognac, winey, fatty	earthy

ethyl nonanoate	fruity, rose, waxy, rum, wine, natural tropical[4]	carrot juice, carrot, crayon, waxy, earthy
unknown 17	n/a	carrot juice with a little cinnamon or tomato, earthy, organic, waxy, crayon, cardboard, not fruity, grain, floral, carrot juice
propanoic acid	pungent, acidic, cheesy, vinegar[4]	sharp, acidic, rancid cheese
octadien-2-one	fruity, fatty, mushroom[4]	green, watermelon, sweet
isophorone	camphor, peppermint, sweet, green, woody, fruity, musty, cedarwood, tobacco, leathery[4]	cherry candy, sweet, sour, acidic, artificial watermelon, cucumber, rancid, fruity, cucumber
2-undecanone	waxy fruity creamy fatty orris floral[4]	grassy, slight watermelon, mild, fresh green, sour, green but not sour, coconut, weak pear, fruity
butyric acid	rancid, cheese, sweat; sharp, dairy, cheesy, buttery, fruity[4]	rancid dairy, acidic dairy, acidic, rancid cheese, unpleasant, mold, cheese, rancid, sharp, disgusting, expired diary products, stinky cheese
ethyl caprate	sweet, waxy, fruity, apple, grape, oily, brandy[4]	floral, mushy, moldy, grain, chocolate, rancid, honey, organic - not in a good way, earthy, fruity, lavender, hay, straw
ethyl 9-decenoate	fruity, fatty[4]	grain, milk licorice flavor, minty - not peppermint, grocery store bakery, buttercream frosting on cookies, mint
dimethyl glutarate	ester, floral[4]	buttercream frosting with a little black licorice, sugary, grocery store buttercream frosting, slightly sweet with a darker note, sweet onion, fruity, floral, salty, Play-Doh, dairy, acidic sting in nose
γ-hexalactone	herbal, coconut, sweet, coumarin, tobacco[4]	oat, black licorice, anise
naphthalene	Unpleasant, moth balls[5]; coal, tar[8]	light anise, tea, vinegar, rancid
valeric acid	Sweat, acidic and sharp, cheese-like, sour, milky, tobacco, with fruity nuances[4]	mild licorice, light fruity, rancid cheese, sharp but not too strong
ethyl undecanoate	soapy, waxy, fatty, cognac, coconut[4]	licorice, fennel, black licorice, anise, malt, tortilla chip, fried corn and lard, popped popcorn, light

unknown 23	n/a	salty, corn flour, masa, fried tortilla chip; corn, grain; wheat, toast, rancid
2-tridecanone	fatty, waxy, dairy, milky, coconut, nutty, herbal, earthy; fatty, waxy, mushroom, coconut, earthy, chicken, fat, fatty[4]	mild, sweet, fruity, oatmeal, light fermented, tortilla chip with a brightness or fruitiness similar to lime chips, fatty, salty, corn chips, buttery, nutty, earthy, organic, not too sweet
δ-hexalactone	creamy, fruity, coconut[4]	grain, milk licorice flavor, tortilla chips, salty, corn, sour chips like lime tortilla chips, organic, acidic, lactic acid, fermentation
ethyl laurate	sweet, waxy, floral, soapy, clean[4]	wheat but sweet, clean, fruity, oatmeal with a little cinnamon, fruity, coconut, rancid, sweaty, cheese, marker, soapy, oats, bar soap, matcha green tea
caproic acid	Sour, fatty, sweaty, cheesy[4]	sour, chocolate, grain, haystack, cadmium chocolate, rancid, cardboard, old barn, acidic but coconut, oxidized, sweaty armpit, hay, barn, acidic, glycerin, sweet oatmeal, waxy, goat, oat, soapy, Velveeta, cheesy
benzyl alcohol	floral, rose, phenolic, balsamic[4]; sweet, fruity floral, chemical[4]	green tea, matcha, soapy oatmeal, oats straw, hay, fodder, toasted grain, watery
benzene ethanol	floral, rose, dried rose, sweet[2]; floral, fresh, bready, rose, honey[4]	floral, hand lotion, fruity, cinnamon, sweet, coconut, Greek yogurt, sour and dairy
heptanoic acid	pungent, rancid, cheesy, sour, sweaty, fermented pineapple, fruity[4]	light and weak fermented milk
δ-octanolactone	sweet, fatty, coconut, tropical, dairy[4]	coconut, warm snickerdoodle, tropical, almond, sunscreen with coconut oil, pina colada, sweet, fruity, coconut sunscreen, palm tree, artificial coconut, coconut lotion, creamy, anise, dry erase marker, fresh
2-pentadecanone	fresh Jasmin, celery, fatty, oily, waxy, burnt[4]	French fries, salty, oily
ethyl myristate	sweet, waxy violet orris[4]	

caprylic acid	fatty, waxy, rancid, oily, vegetable, cheesy[4]	caramel, burnt sugar, sweet, spit up, urine, sweaty armpits, fermented, sour lemon, roasted caramel apples, fruity, musty, urine, background of strawberry, burnt, BBQ, acidic, coconut
δ-nonalactone	creamy, sweet, coconut, fatty and milky with a coumaric and oily nuance[4]	coconut, tropical, Styrofoam but sweet, pineapple, a little smokey/grassy, dairy, lactonic, wet dog, chlorinated water, creamy, fresh, fruity, sweet, panelist associates with formaldehyde
unknown 30	n/a	cat pee; melted butter; wet dog; earthy, organic, dirt and seaweed or swamp scent; fishy
γ-decalactone	fresh, oily, waxy, peach, coconut, buttery, sweet[4]	bad buttered popcorn flavor, doctor's office, fresh, medical, minty - "Extra brand gum in a pool but not the chlorine smell, just amount of water", light fermented with milk, sweet, wet, slightly rancid ocean breeze, earthy, metal, sweet, syrup, fruity, Febreze, tropical, creamy
nonanoic acid	green, fat; waxy, dirty, cheesy, dairy[4]	butter popcorn flavor, intense butter, sweet, brown sugar, sugar, acidic, rancid, not sweet, industrial, metal, dust, woody, musty, doctor's office, latex
δ-decalactone	coconut, peach[4]	coconut, pineapple, creamy, pina colada, bad fake butter popcorn flavor, light acidic, creamy, sweet, pear, floral, warm buttery, brown sugar, fresh
capric acid	rancid, sour, fatty, citrus[4]	tart watermelon, watermelon Jolly Rancher, vomit, acidic, lightly fermented, soapy, baby formula, pheromones, musty, watery
δ-undecanolactone	creamy, coconut, fruity, peach, milky, waxy, lactonic, green, fruity[4]	fresh, sweet, floral, sugary, cream, vanilla, lactone, dairy, shower gel, fragrant soap, fruity
9-decenoic acid	waxy, green, fatty, soapy with a slight creamy cheese type nuance[4]	potpourri, cucumber, floral, plants, field, watermelon, fruity, sour watermelon, strawberry, fresh, watermelon jolly rancher

undecanoic acid	waxy, creamy, cheesy, fatty, coconut[4]	pepper, acidic, old, stale, milky, butter popcorn flavor, sweet, floral, creamy, soapy, fatty, shower product, clean
γ-dodecalactone	fatty, peach, sweet, metallic, fruity[4]	fruity, sweet, milky, fragrance of hot or steamed milk, lactose, clear, fresh, floral, laundry softener, fermented, dragon fruit Vitamin water, soapy, fermented lactose, fruity shower gel, acidic, floral, lavender, lactonic
δ-dodecanolactone	fatty sweet creamy dairy fruity peach apricot milky buttery[4]	bad buttered popcorn flavor, fresh, floral, lactonic, dairy, milky, not sweet
indole	animal, floral, naphthyl, fecal[4]; pungent, floral, naphthyl, fecal, animal, musty[4]	cow manure, chlorine, butter, moth balls, old lady, light, clear, grandma's cupboard, musty, gross, fruity, soapy, buttery, very unpleasant, grassy, moldy, bad artificial butter popcorn flavor, sanitary, doctor's office, fresh
benzoic acid	balsamic, urine[4]	sharp, astringent, Michael's Craft Store, potpourri, Grandma's house, floral, artificial, bad or fake buttered popcorn flavor, eucalyptus
lauric acid	mild fatty coconut bay oil[4]	floral, coconut, melted butter, grass, milky, cow manure, bad buttered popcorn, musty, office, shredded paper, printer, paste, creamy, acidic, pheromone, musty, moth ball
tridecanoic acid	waxy, woody[4]	grain, chocolate, cat urine, cocoa, hay, straw, rancid, glue
δ-tetradecalactone	waxy creamy oily fatty sweet milky dairy[4]	sweet, paper, office supplies, acidic, rancid, dog urine
myristic acid	waxy fatty soapy coconut[4]	spicy, sweet, soapy, office supplies, paper, envelope, adhesive, acidic
palmitic acid	low heavy waxy, with a creamy, candle waxy nuance[4]	weak, acidic, floral

[1]odorants are sorted by their elution time (min) from the GC-O; [2]as found in publications; [3]repeated descriptors were omitted; [4](TheGoodScentsCompany 2021); [5](ChemicalBook 2017); [6]as determined by researchers - no publication reference could be found; [7](CameoChemicals 2021)