Chapter

Volatile Aromatic Flavor Compounds in Yogurt: A Review

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Abstract

Lactic acid bacteria are of great importance in the production of yogurt worldwide, yet very little is still known about the mechanisms of aroma formation in foods subjected to lactic acid fermentation. However, advances in the Adolfsson development of instrument methods have made it possible to avoid some of the difficulties in extracting flavoring substances from the otherwise complex matrix of lactic acid products. In this chapter, we present recent developments related to the impact of yogurt starter cultures on the production of the aromatic components in yogurts. In addition, we examine and characterize the aromatic compounds based on the chemical structures and discuss modern analytical techniques for yogurt analysis. As described in this chapter, a large number of flavoring substances can be studied, isolated, and identified with the help of advanced instrument analysis such as synthetic fibers for solid-phase extraction (SPME) and gas chromatography combined with mass spectrometry (GC-MS). These techniques can help us reach a more advanced level of understanding of the importance of specific strains for obtaining the desired sensory qualities of fermented, lactic acid products. At a more advanced stage, these analyses could allow scientists to develop rapid methods for determining the quality and authenticity of lactic acid products based on the aromatic-metabolic profile of starter cultures in the final product.

Keywords: yogurt, volatile, aromatic flavor, *Lactobacillus bulgaricus*, *acetaldehyde*

1. Introduction

Yogurt is defined as the product of fermented milk by *L. bulgaricus* and *Streptococcus thermophilus*. Yogurt was first discovered in the Middle East and has been a part of the human diet for thousands of years. However, it was not until the twentieth century that scientists started to provide scientific evidence for the health benefits associated with yogurt consumption. In 1905, a Bulgarian scientist, Stamen Grigorov, was the first to report on *Bacillus bulgaricus* (now *L. bulgaricus*), a lactic acid bacterial strain found in Bulgarian yogurt. Then, in 1908, the Russian scientist Elie Metchnikoff theorized that one's health could be improved, and senility delayed by colonizing the gut with the host-friendly bacteria found in yogurt. The popularity of yogurt is attributed to its various health benefits as well as the flavors and sensory characteristics [1]. Yogurt is described as having a smooth, viscous gel-like texture with Sharpe acid associated with green apple aroma. These characteristics play a significant role in the consumer acceptability to yogurt products (**Table 1**). The traditional yogurt flavor is a combination of aroma and taste that are typically produced during lactic acid fermentation by the yogurt starter cultures. During fermentation, the yogurt starter culture coverts lactose and other nutrients in milk to several chemicals that lead to the production of various flavor and aromatic compounds. More than 100 different volatile compounds have been reported and produced by various yogurt cultures. Several advanced techniques and instrumentations have been applied to determine the volatile compounds in yogurt products. **Table 2** lists some of the common analytical instrument used for flavor analysis with advantages and limitations of each instrument.

The selection of suitable strain combinations in yogurt starter culture is important for achieving the best technological performance and desirable sensory characteristics. However, the use of genetically modified lactic acid bacteria with encoded and targeted flavoring [7] is not an acceptable solution primarily due to the lack of consumer acceptance of this technology, and restricted regulations for the use of such bacterial strains in food products; this is especially true for the European market [7]. In this regard,

Volatile compounds	Odor descriptor	Volatile compounds	Odor descriptor
Acetic acid	Vinegar like	Pentan-2-one-4-ol	Cucumber, lettuce
Propanoic acid	Cheesy	Ethanol	Alcohol
Methional	Cooked vegetables	2,3-Butanediol	Creamy
Methyl benzoate	Vanilla-like	Butyl acrylate	Tropical fruity
Benzaldehyde	Almond-like	1-Octen-3-one	Mushroom earthy
Nonanoic acid	Earthy	2-Pentanone	Wine-like
Ethyl nitrite	Sweet	Acetone	Fruity
Hexanoic acid	Sweaty	Pentanoic acid	Disgusted
Acetaldehyde	Fresh, green apple-like	Hexanal	Grass-like
Butanal	Cocoa-like	Ethyl acetate	Mild
Octanoic acid	Cheesy	Decanal	Floral
1-Hexanol	Greasy	Isobutyric acid	Buttery
Decanoic acid	Rot-like	1-Nonen-3-ol	Mushroom-like
2-Furanmethanol	Toast-bread like	Diethyl disulfide	Cooked onions-like
Heptanoic acid	Sour	Acetophenone	Sweet almond
2,3-Pentanedione	Sweet	Ethyl 2-methylbutyrate	Pear-like
2-Phenylacelaldehyde	Flowery	Tetradecanoic acid	Coconut-like
3-Methyl-2-butenal	Cherry-like	Pentanal	Fermented-like
Dimethyl sulfide	Lactone-like, sweet	Isopropyl alcohol	Musty
2,3-Butanedione	Butter, diacetyl vanilla	Nonanal	Rosy
Butyl acrylate	Tropical fruity	2-Methyl-1-propanol	Wine-like

Sources: [2–5].

Table 1.
Some identified volatile compounds in yogurt with their description of odors.

Technique	Dairy products	Analytical principle	Pros	Cons
High-vacuum distillation	Yogurt, cheese, sour cream	Involves the use of organic solvents for extracting minute volumes of concentrated aqueous volatiles	Thermal decomposition of compounds is prevented due to process operating at ambient or sub-ambient temperature	Labor and time-intensive process and requires high sample volumes
Simultaneous (steam) distillation extraction (SDE)	Yogurt, milk fat, skim milk powder	Involves a continuous process utilizing organic solvents with extremely low boiling solvents for concentrating volatile compounds	High yield due to extraction rate	Heat-labile volatiles risk breakdown if extraction is not controlled at low pressure
Dialysis	Yogurt, milk	Separation process is based on the diffusivity potential of volatiles through a membrane resulting in a concentration gradient	Yields high concentration gradients	Labor and time-intensive process
Molecular distillation	Butter, cheese	Similar process to the high-vacuum distillation procedure but only requires transfer of volatiles from a matrix to a chilled and condensed system	Ideal for heat-labile volatile compounds	Volatile requires short distance between the condensation system and the food sample under a high-vacuum environment
Dynamic headspace/purge trap	Yogurt, milk, ice cream, hard cheese	Restricted to the number of bases	Minimal use of sample, rapid process and minimized use of thermal artifacts	Time-sensitive and involves use of expensive equipment
Solvent-assisted vapor evaporation (SAFE)	Butter, milk, whey protein	Requires mixing sample in a selected organic solvent and liquid is centrifuged or evaporated	High yields of volatile compounds devoid of thermal process	Limited to only unique glassware use
Stir-bar sorptive extraction	Human milk, cheese	Volatiles are concentrated without using solvents	Highly immiscible in fluids	Labor-intensive
Solid-phase microextraction (SPME)	Yogurt, milk, ice cream	Employs a fiber system that absorbs volatiles and desorbs into a gas chromatograph injection port	Highly sensitive, and requires small sample volumes	Volatiles could be altered during extended thermal application process
Mass spectrometry	Yogurt, milk, cheese	Detects the mass-to-charge ratio of volatiles	Volatiles are detected based on reference spectra	Extremely expensive
Flame ionization	Yogurt, milk, cheese	Detects volatiles in a stream of gas	Broad spectrum for detecting volatiles	Requires reference standards for comparison

Sources: [3, 4, 6].

Table 2.
Analytical techniques for the determination of volatile compounds in fermented dairy products.

knowledge of the aromatic-metabolic profile of the starter cultures used and the influence of the profile on the sensory characteristics of the fermented products is essential, both for the individual consumer and for the food industry. It should be noted that an important consideration in the selection of starter cultures for the production of yogurt and other dairy products is the ability of the starter culture strains to produce metabolites that shape the sensory qualities of the product. Thus, in the selection of strains included in the composition of starter cultures used for the production of dairy products, the metabolic profile of each strain is examined, particularly since some metabolic products involved in the formation of the aroma have antimicrobial activity.

In this chapter, we present a comprehensive review of the general aromatic components that are present in yogurt products. First, we introduce the role of lactic acid bacteria with regard to yogurt flavor. Next, we present the aromatic compounds and group them based on the chemical structure into carbonyl compounds, organic acids, alcohols, and esters as major compounds. We then go on to discuss advanced instrument techniques for yogurt analysis. It is these techniques that could help us to reach a more advanced level of understanding of the impact of specific yogurt strains for obtaining the desired sensory qualities of yogurt products and other fermented lactic acid products.

2. The role of lactic acid bacteria in yogurt flavor

Yogurt is one of the most popular fermented dairy products worldwide nowadays. Moreover, consumption of yogurt has been increasing globally as a result of its pleasing sensory qualities, including texture, color, and flavor. Being one of the key food preservation methods, fermentation has significantly increased the nutritional value, shelf life, and sensory qualities of foods. This process involves a variety of microorganisms that break down the biochemical components of the food's basic materials (carbohydrates, proteins, and lipids), improving catabolism (digestion), taste, and enhancing the pharmacological and nutritional benefits of the food [8]. Most of the flavor compounds found in yogurt are a result of the activity of microbes in starter cultures, lactic acid bacteria (LAB). Microbes found in this starter culture carry out three key biochemical tasks during fermentation, which include the breaking down of milkfat into free fatty acids (lipolysis), caseins into peptides and free amino acids (proteolysis), and carbohydrates into lactic acid or other metabolites (glycolysis) [7]. Flavor is very important in food; consumers consider flavor to be one of the most significant aspects of food since it affects how well a particular product is liked and its overall acceptability.

2.1 Metabolic pathways of flavor compounds formation in yogurt

During fermentation, lactic acid bacteria processes create flavor precursors that are then transformed into flavor compounds. Enzymes hydrolyze several dietary components, including carbohydrates, proteins, and lipids. Carbohydrate metabolism (glycolysis), amino acid metabolism (proteolysis), and fatty acid metabolism (lipolysis) are the three main metabolic processes of LAB that lead to the formation of volatile compounds [9].

2.1.1 Flavor compounds from LAB carbohydrate metabolism

Lactic acid bacteria use the sugar lactose that is present in milk as their primary source of energy and carbon [10]. In fact, the distinctive acidic flavor of yogurt can be attributed to the conversion of lactose to lactic acid by LAB. The two distinct carbohydrate fermentation pathways in LAB—homo-fermentation and hetero-fermentation result in various metabolic end products, depending on the LAB species, substrate, and environmental factors. Homofermentative LAB, which include *Pediococcus*, *Lactococcus*, *Streptococcus*, etc., use the Embden-Meyerhof-Parnas (EMP) pathway to produce lactic acid as the main by-product. However, heterofermentative LAB such as *Leuconostoc*, *Oenococcus*, *Lactobacillus*, etc., use the phosphoketolase pathway (PKP), which also produces other end products, such as ethanol, carbon dioxide, acetic acid [9]. Homofermentative metabolism could also switch to a mixed-acid metabolism with a variety of molecules under specific situations such as carbon limitation, carbon excess of slowly metabolized sugars, aerobic conditions. The metabolic by-products of this metabolism would include multiple flavor compounds such as acetaldehyde, ethanol, and diacetyl. Acetaldehyde, for example, dominates the flavor of yogurt in its normal form and helps in its distinctive flavor. Pyruvate is a crucial metabolic precursor that is usually catalyzed by aldehyde dehydrogenase or α-carboxylase to produce acetaldehyde. The characteristic flavor of yogurt is produced in fermented dairy products by a variety of C4 molecules such as diacetyl, acetoin, and 2, 3-butanediol [7]. These molecules may be produced by the citrate or glycolysis metabolism of certain LAB (**Figures 1**–**3**). Diacetyl is the predominant significant flavor compound among these C4 chemicals, and both *S. thermophilus* and *L. bulgaricus* are capable of producing it. Acetoin, which is diacetyl's reduced form, is important for decreasing the sharpness of diacetyl and also adds to the pleasant, creamy flavor of yogurt.

2.1.2 Flavor compounds from amino acid metabolism by LAB

In order for yogurt to have a pleasant taste and aroma (flavor), proteolysis is a crucial biochemical step. Proteolytic abilities in certain LAB allow them to undergo hydrolysis of proteins, which leads to the production amino acids and peptides [9]. Proteolysis and the breakdown of an amino acid (amino acid degradation) make up the first two phases of this process. The enzyme cell-envelope proteinases (CEPs) help to break down the protein into oligopeptides, causing casein to begin to undergo proteolysis by LAB. The second phase then begins and involves the transport of di-, tri-, and oligopeptides into the cell. Peptidases further hydrolyze casein-derived peptides to amino acids after these casein-derived peptides have been absorbed by LAB cells. In a single bacterial genome, peptidases can be encoded in several copies. Free amino acids generated by the breakdown of proteins (proteolysis) may be transformed into a variety of flavoring substances, including those ammonia, amines, aldehydes, phenols, indole, and alcohols, and these compounds all have imparted the flavor of the yogurt. The primary sources of flavor substances obtained from milk protein are mostly branched-chain amino acids such as Val, Leu, Ile, aromatic amino acids such as Phe, Tyr, Trp, and sulfuric amino acids such as Cys, Met [11]. Transamination of amino acids to their respective α-keto acids is the first stage of amino acid breakdown. The α-keto acids then go through several enzymatic processes, such as reduction to produce flavorless α-hydroxy acids and decarboxylation to produce aldehydes that can subsequently be reduced to an alcohol, or oxidative decarboxylation to produce acyl-CoA, and finally, carboxylic acids [7]. After that, esterases or acyltransferases catalyze the formation of esters or thioesters in processes involving alcohols and carboxylic acids [12]. As a member of a different class of lyases, threonine aldolase may convert threonine straight into acetaldehyde.

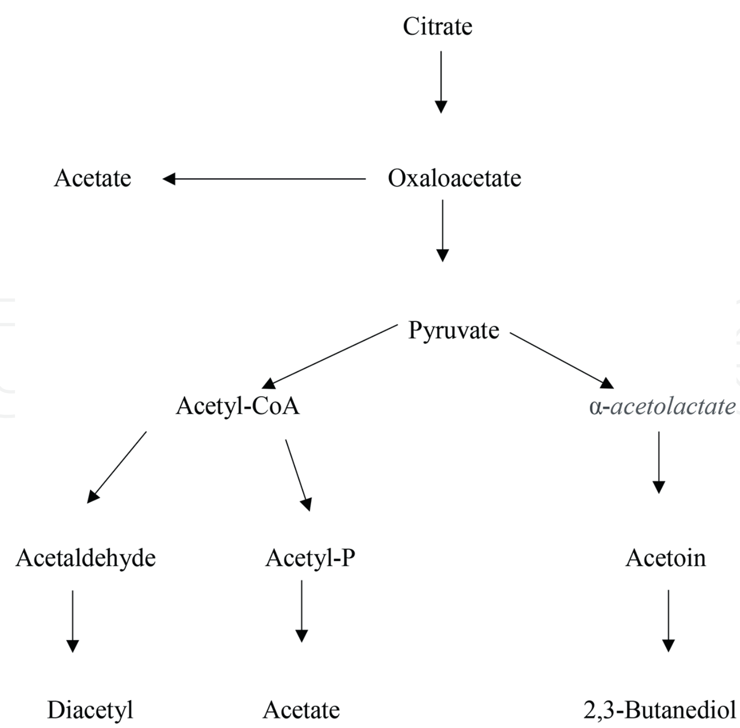

Figure 1.
Pathways of citrate metabolism by lactobacillus [1, 7].

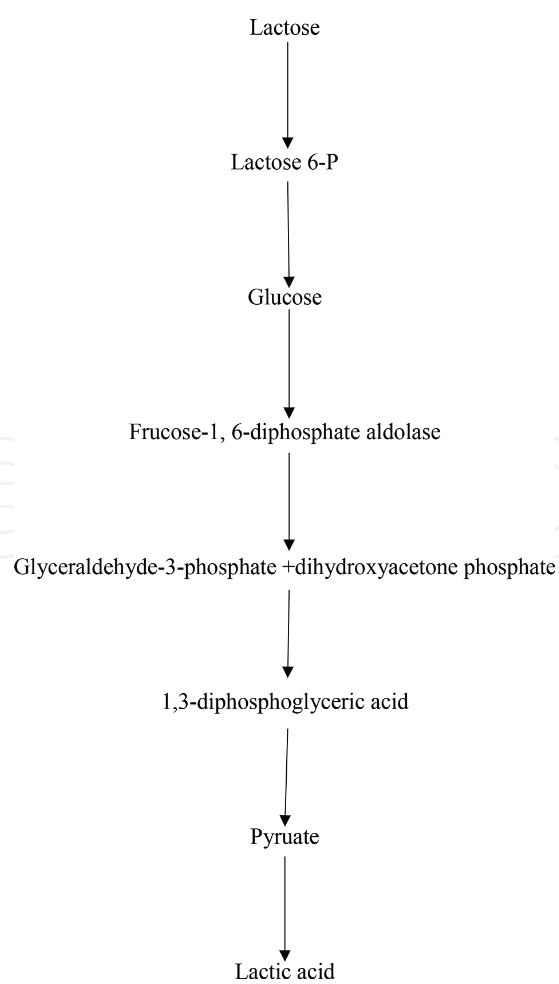

Figure 2.
Pathways of lactic acid production by lactobacillus [1, 7].

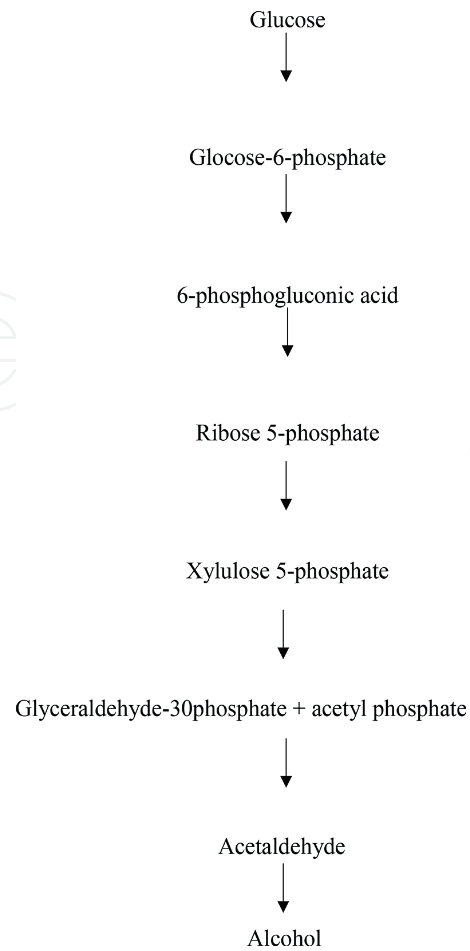

Figure 3.
Pathways of alcohol production by glucose metabolism [1, 6, 7].

2.1.3 Flavor compounds from LAB lipid metabolism

The two major processes that produce flavor components in fermented foods are lipolysis and fatty acid oxidation. Most fermented foods contain free fatty acids as key aroma components produced by the breakdown of lipids (triglycerides, diglycerides, and monoglycerides) [11]. The strains of Lactobacillus exhibit lipases in quite high concentrations, which then produce these free fatty acids [11]. Free fatty acids, particularly saturated and unsaturated fatty acids, serve as catalysts for catabolic processes that result in the oxidation of lipids and the generation of a variety of volatile compounds, including alkanes, methyl ketones, esters, secondary alcohols, and lactones [13]. Unsaturated fatty acids are oxidized by two different pathways, one of which is the formation of hydroxyperoxides via β-oxidation of unsaturated fatty acids in the presence of free radicals. The synthesis of 4-5-hydroxy acids, which are transformed into α-δ-lactones that emit strong fruity aromas, might result from another pathway of unsaturated fatty acid metabolism [14]. In addition, a variety of esterases found in LAB may directly generate flavor ester from glycerides and alcohols through an alcoholysis process. For example, in order to create ethyl butanoate and ethyl hexanoate, LAB can esterify ethanol with butyric and hexanoic acids [11].

2.2 Effect of different lactic acid bacteria on yogurt flavor

The starter cultures, processing conditions, sources of milk, and some other ingredients all have an impact on the flavor of yogurt [15]. However, within these parameters, the development of the flavor components in yogurt is mostly influenced by the starter cultures used. The culture used for yogurt is primarily composed of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. bulgaricus. As a result, some nations only allow the use of the word “yogurt” for products prepared using starters that include bacteria from both of these strains [16]. The symbiotic connection between these two bacteria is referred to in mixed cultures as proto-cooperation and makes them mutually advantageous during fermentation even though each grows well in milk on their own [16]. Due to their associative development and mutual stimulation, the number of flavor constituents in the mixed cultures is significantly higher than that in either of the two individual cultures. It was discovered that the largest percentage of flavor compounds, including acetaldehyde, diacetyl, acetoin, acetone, ethanol, and 2-butanone (**Figure 3**), were produced when mixed cultures were utilized during lactic acid fermentation [7]. Additionally, the levels of methylated sulfides and dimethyl trisulfide were extremely low in *Lactobacillus bulgaricus* and *S. thermophilus* monocultures, suggesting that perhaps the mixed culture’s higher levels were the result of interspecies interaction. The proto-cooperation of the mixed cultures is also significantly influenced by proteolytic activity. Compared to pure cultures or cultures with proteolytic *S. thermophilus*, this combination will generate higher aroma volatiles and nonvolatile metabolites. Thus, the combination of both microorganisms influences the synthesis of volatile and nonvolatile compounds essential to flavor development. The Lactobacillus strains are the most widely used commercial probiotics in yogurt. In addition to imparting yogurt’s flavor and enhancing organoleptic qualities, these strains also provide health benefits. Yogurt flavor development is influenced by a variety of environmental factors, such as the composition of the culture medium, competition for nutrients, and interactions between microorganisms.

2.3 Metabolic engineering application for flavor enhancement

A significant approach for genetically modifying strains in order to increase the production of flavor compounds such as acetaldehyde, diacetyl, and esters is metabolic engineering. The changing of one or more genes or enzymes is a popular method for producing many different flavor molecules [11]. For example, formation of acetaldehyde by yogurt bacteria occurs via a variety of pathways, with threonine aldolase likely being the primary enzyme in this process. Serine hydroxymethyltransferase (SHMT), which is produced by the glyA gene in *S. thermophilus*, also has threonine aldolase activity. According to Chaves et al. [17], overexpression of the glyA gene in *S. thermophilus* increases acetaldehyde synthesis by 80–90%, whereas inhibition of the gene completely abolishes acetaldehyde formation. The main goal of these numerous metabolic engineering solutions for LAB has been to efficiently produce diacetyl for its significance in yogurt flavor. The als or ilvBN genes, ldh gene, and aldB gene that are coded for the enzymes α-acetolactate synthase, lactate dehydrogenase, and α-acetolactate decarboxylase, respectively, are the main enzymes involved in the synthesis of diacetyl.

2.4 Correlation between lactic acid bacteria and flavor compounds in yogurt

In several studies, certain volatile compounds found in yogurt have been linked to LAB species, demonstrating that LAB significantly affect the flavor of many fermented foods, including yogurt. Yogurt and other dairy products are often fermented from milk from various plants and animal sources. The characteristic LAB species found in these fermented dairy products come from the genera Lactobacillus and are naturally prevalent in a variety of environments; however, they are mostly used for fermentation purposes [18–20]. Lactobacillus was found to be the most prevalent species in most samples of fermented yak milk. Microbial analysis, as well as the flavor profile of the product, revealed that these bacteria were significantly correlated with flavor compounds such as ethanol, benzaldehyde, ethyl acetate, 2, 3-pentanedione, and benzaldehyde [21]. Through correlational analysis using bidirectional orthogonal partial least square, it was determined that bacteria contribute more to flavor production than fungus. The majority of studies that compared the relationship between the LAB community and volatile chemicals found a strong correlation between LAB and the development of flavor in yogurt and other fermented foods. In yogurt, the main species, *Lactobacillus*, is predominant and helps create esters, aldehydes, acids, ketones, and alcohols.

3. Volatile and aromatic compounds in yogurt

Flavor is one of the most important properties of food products and is an important factor determining consumer acceptability. With regard to dairy products, their sensory properties largely depend on the relative balance of flavor compounds derived from fat, protein, or carbohydrates in the milk. For example, the distinctive flavor of yogurt is contributed by lactic acid and a complex mixture of flavor compounds that include the volatiles already present in the milk and specific compounds produced during lactic fermentation [22]. More than 100 different volatiles have been identified in yogurt, including carbohydrates, alcohols, aldehydes, ketones, acids, esters, lactones, sulfur-containing compounds, pyrazines, and furan derivatives [3].

Characterization of the volatile compounds allows for examination of the mechanism of formation of the aromatic profile of the product. Knowledge of the primary flavor compounds and their origin will thus support the production of dairy products of consistent quality that will be more readily accepted by consumers. For example, routine analysis of the primary aroma compounds can be used for quality monitoring during yogurt production. In addition, the profile of volatile compounds in yogurt can be used as a parameter to provide consumers with a better quality and safer food [3].

One major pathway for the production of flavor compounds in yogurt is through lipolysis or oxidation of the fatty acids in milk fat. Unsaturated fatty acids are oxidized in the presence of free radicals to form hydroperoxides, which rapidly decompose to form hexanal or unsaturated aldehydes. Unsaturated fatty acids also lead to the formation of 4- or 5-hydroxyacids, which readily cyclize to γ- or δ-lactones and odd-carbon methyl ketones by decarboxylation of β-keto acids. Another major pathway would be the microbiological transformation of lactose (and produced lactate) and citrate by acid-producing bacteria into acetaldehyde, diacetyl, acetoin, and ethanol. The alcohols in the yogurt can then combine with the free acids to form esters such as ethyl acetate and butyl acetate. In addition, biogenic amines and nitrogen-containing compounds can be transformed from proteins and amino acids, and sulfur compounds can be derived from organosulfur compounds [23].

However, not all volatile components found in foods are important for the foods' organoleptic properties. For example, in most studies, despite the long list of volatile compounds found in yogurt (**Table 1**), only a few had relatively high concentrations. Only acetaldehyde, ethanol, acetone, diacetyl, and 2-butanone exert a strong influence on the desired aroma and are also present in amounts detectable by common laboratory techniques. The main volatile compounds commonly reported to be responsible for imparting the desired aroma to yogurt are the carbonyl compounds—acetaldehyde, diacetyl, acetone, acetoin, and 2-butanone. Although present in small amounts in yogurt, these compounds are important organoleptic factors.

The primary volatile components involved in the formation of the aroma of typical Bulgarian yogurt are acetaldehyde, acetone, 2-butanone, diacetyl, ethyl acetate, and ethanol. Kaminarides et al. [24] found that acetic acid, acetaldehyde, acetone, diacetyl, 2-butanone, acetoin, and 3-methyl-2-butanone were the primary volatile aroma compounds in yogurt made from sheep's milk. The primary aroma components in Swiss yogurt as determined by GC-sniff technique are acetaldehyde, diacetyl, 2, 3-pentanedione, methional, 2-methyltetrahydrothiophen-3-one, 2-neonal, 3-methylbutyric acid, guaiacol, benzothiazole, and two unidentified compounds [3]. The aromatic compounds in Swiss yogurt were investigated and found that few major compounds that had high-impact yogurt flavor, these compounds are acetaldehyde, dimethylsulfide, Diacetyl, 2, 3-pentanedione, L-limonene, and undecanal. However, other major constituents (fat, protein, and carbohydrates) in yogurt could play a major factor in the release of volatiles compounds. The aromatic components produced by the starter culture can be grouped into separate classes as carbonyl compounds, organic acids, alcohols, and esters, depending on their respective chemical structure.

3.1 Carbonyl compounds

The quality of yogurt is heavily reliant on the relative balance of volatile compounds including carbonyl substances derived from fat, protein, and carbohydrate in the milk base during the fermentation process. Carbonyl compounds are the primary aromatic substances in fermented yogurt where more than 38 of these compounds have been detected [3]. They are composed of aldehydes and ketones. The type and level of compounds derived during fermentation depend on the starter culture, variety of milk, and the conditions of the fermentation process. The metabolism of citric acid and amino acids by lactic acid bacteria—*Lactobacillus acidophilus*—and *Streptococcus thermophiles*, both of which are commonly used in the yogurt industry, produces the flavor compounds characteristic of yogurt products. **Table 3** shows the most common carbonyls compounds in yogurt and typical concentrations in yogurt products [3].

Several carbonyl compounds including diacetyl, acetoin, and butanediol are derived from citrate metabolism while several amino acids are converted into the intermediate metabolite pyruvate and finally acetaldehyde or directly into acetaldehyde.

In citric acid metabolism (**Figure 1**), citrate is converted into acetate and oxaloacetate with the presence of citric acid lyase catalyze. Next, oxaloacetate is decarboxylated and produces pyruvate and carbon dioxide. Subsequently, pyruvate is metabolized by lactic acid bacteria to produce different end products, including diacetyl, acetoin, and butanediol [25].

The crucial role of carbonyl compounds in yogurt can be identified when considering the sensory attributes of yogurt. Although each of these carbonyls is responsible for its characteristic flavor or aroma, the ultimate sensory properties of yogurt are decided by a relative balanced mixture of all flavored substances as well as their dominant properties.

Type	Typical level in yogurt (mg/kg)
Acetaldehyde	23–40
Diacetyl	0.2–3
Acetoin	1.2–28.2
Acetone	0.3–4.0
2-Butanone	0.1–7

Table 3.
List of common carbonyl compounds found in yogurt.

Acetaldehyde is an essential aroma and flavor compound found in fermented yogurt and provides the essential unique green apple or nutty flavor in fermentation by *L. bulgaricus* and Streptococcus thermophiles. However, a proper concentration level of acetaldehyde is required in order to obtain the most desired sensory quality. For example, although acetaldehyde gives a pleasant fruity aroma at diluted concentrations, high levels can result in a pungent irritating odor [3].

Diacetyl, which produces a characteristic buttery flavor in yogurt, is derived by fermentation of the citrate present in milk. It is equally as important as acetaldehyde with regard to the sensory quality of yogurt. The preferred typical yogurt flavor would thus be obtained by a 1:1 mixture of acetaldehyde and diacetyl. However, when the acetaldehyde level in yogurt is low, diacetyl contributes to producing a delicate, full flavor and aroma in the product. At higher concentrations, diacetyl can act as a flavor and quality enhancer as well [3].

Another flavored substance commonly available in yogurt is acetoin, which gives a mild creamy, slightly sweet, butter-like flavor. While acetoin is converted from diacetyl by the diacetyl reductase enzyme [5, 7], its flavor properties are also similar to those of diacetyl. A proper combination of both substances thus results in a typically mild, pleasant, buttery yogurt taste. Moreover, acetoin tends to reduce the harshness of diacetyl.

Acetone and 2-butanone reportedly have similar flavor characteristics with regard to minor but important flavor compounds found in yogurt. Both compounds make a positive contribution to sweet, fruity aroma and flavor qualities. Typical concentrations of acetoin in yogurt range from 1.2 to 28.2 mg/kg [26, 27]. Diacetyl in combination with acetoin is responsible for the soft, pleasant, fatty taste of yogurt that is crucial to yogurt's widespread appeal.

Acetone and 2-butanone are two volatile compounds with a minor contribution to aroma in dairy products [1, 24, 27, 28]. For example, acetone has a sweet fruity aroma and is known to affect the flavor and taste of yogurt. Small amounts of acetone typically originate from milk, but certain amounts are produced by bacteria in yogurt and the concentration of acetone in yogurt ranges from 0.3 to 4.0 mg/kg [27]. The taste characteristic of 2-butanone is similar to that of acetone and the concentrations in yogurt range from 0.1 to 7.0 mg/kg [24, 27]. Gallardo-Escamilla et al. [28] reported that 2-butanone is important for the aroma development of yogurt and contributes to its fruity flavor.

However, many of the carbonyl compounds also play a role in the loss of yogurt taste stability by developing off flavor during storage. For example, reactions from carbonyl compounds can generate off-flavor chemicals. Lipid oxidation in milk results in an undesirable stale, oxidized flavor. Moreover, some malodorous compounds such as 2, 4, 5-trimethyloxazole can be generated from diacetyl and acetaldehyde in the presence of ammonia [29]. Due to their off-character and low aroma and taste thresholds, these compounds can lead to serious taste and aroma defects.

3.2 Organic acids

The most perceptible chemical compound in yogurt in flavor detection is carbonyl compound followed by organic acids. Degradation of polysaccharides by lactic acid bacteria during fermentation produces monosaccharaides and acids. Organic acids contribute significantly to the sensory properties in fermented yogurt, especially with regard to acidity. For example, changes in acid concentration lead to the development of a characteristic flavor and aroma along with desirable consistency. Lactic acid is the major organic acid found in fermented yogurt, and it has both positive and negative impacts on taste (**Figure 2**). Approximately 20–40% of lactose present in milk base is metabolized into lactic acid, which increases the acid concentration up to 0.9%.

Lactic acid bacteria utilize lactose and then glucose as the carbon source to produce pyruvate through glycolysis. Lactic acid is produced by lactate dehydrogenase. Taste and mouthfeel of the final product can vary with the concentration of lactic acid regardless of the flavor compound contained [30]. Moreover, formation of acid directly involves the texture development. To obtain desirable consistency, it should reach the optimum pH level. Typical pH level in yogurt is 4.4 [3].

Acetic acid, folic acid, and longer-chain organic acid are generated during yogurt fermentation in addition to lactic acid for example, acetic acid amounts range from 0.5 to 18.8 mg/kg in typical products. However, high levels of acetic acid impart an unacceptable vinegar-like taste [31]. Folic acid is mainly derived by *Streptococcus thermophiles* by amino acid utilization. Accumulation of folic acid stimulates the growth of other lactic acid bacteria including *Lactobacillus acidophilus* in the fermentation medium. Longer-chain acids such as octanoic acid develop a characteristic soap-like flavor [3].

Thus, in order to obtain a yogurt with desirable properties, acid production should be controlled. Extended acidification during fermentation or in storage results in the development of off-flavors. Syneresis, the most common issue associated with the sensory quality of yogurt, is a qualitative defect in the yogurt structure that tends to lower consumer acceptability by weakening the appearance, texture, and consistency of the product. Syneresis develops as a result of post acidification, which causes some leakages of whey proteins.

Post acidification depends on the type of strain, microbial ratio in the yogurt starter culture, the storage temperature, and the storage time. Post acidification manipulation can be done changing the microbial ratio, and it increases the shelf life of the yogurt. Volatile acids are also important from a nutritional and therapeutic point of view in addition to their influence on organoleptic properties of the products.

In addition to lactic acid, other acids are produced during the fermentation of yogurt, both by lipolytic processes and by bacterial fermentation. For instance, acetic acid is an important compound produced by lactic acid starter cultures [26]. Acetic acid has been reported at a concentration of 0.5–18.8 mg/kg in yogurt [26]. High levels of acetic acid impart a vinegar-like taste that may not be accepted by consumers [32]. Longer-chain acids (e.g., octanoic acid) may contribute to the characteristic soap-like aroma [33].

3.3 Alcohols

In addition to carbonyl compound and acid, another volatile compound generated during yogurt fermentation is alcohol. However, the contribution of alcohol compounds in flavor development is comparatively less. A total of eight to nine alcohol compounds associated with fermented yogurt have been detected [34, 35]. The type and concentration of compound primarily depend on the starter culture used (**Figure 3**).

Ethanol is considered to be the principal alcohol derived in lactic acid fermentation. It is produced by breakdown of glucose and catabolism of amino acids. In the ethanol production pathway, glucose breaks down into lactic acid, ethanol, and CO_2 with the presence of ATP. As acetaldehyde degradation occurs during alcohol production, the amount of acetaldehyde in the medium is reduced.

As ethanol has an effective olfactory and trigeminal stimulus, it can act as a flavor enhancer to some extent. In typical yogurt made by cow milk fermentation, ethanol content ranged from 0.2 to 9.9 mg/kg [3] while yogurt made by other milk contained a lower amount. In addition to ethanol production, 1-hexanol and 1-heptanol production was also detected during fermentation. However, production of high levels of alcohol, particularly ethanol, was measured during storage while a reduction in acetaldehyde was reported. The level of ethanol ranged between 8.13 and 10.99% throughout the storage period [34, 35].

3.4 Esters

Esters are another volatile compound found in fermented yogurt and have a similar role to alcohol and acid in flavor development. A total of three to six ester compounds were detected in fermented milk [34, 35] depending on the starter culture. This included ethyl ester, butyl ester, and ethenyl ester. Ethyl ester, which is significant among other esters in flavor development, is derived from the enzymatic or chemical esterification of acids with ethanol. Ethyl ester adds unique fruity and floral aroma and flavor while minimizing the sharpness and bitterness imported by fatty acids and amines [1, 33]. Esters contained in yogurt are in a low amount and are able to withstand extended storage periods.

3.5 Sulfur compounds

Volatile sulfur compounds are significant contributors to the off-flavors of yogurt. As a class, sulfur compounds are typically present in foods at extremely low concentrations and have low sensory detection thresholds. Some of these compounds provide background sensory nuances to the flavor, while others provide unique flavor characterizing identities to the products. Sulfur compounds including methanethiol, dimethyl disulfide, and hydrogen sulfide can be detected in fermented milk products, and their presence contributes to the strong, unacceptable aroma [33–35].

The sensory properties of yogurt depend largely on the relative balance of chemical compounds derived from carbohydrates, protein, or fat in the milk base. The flavor components of yogurt include the volatile compounds naturally present in the milk and specific compounds produced from milk fermentation. It has been reported that more than 100 different volatile compounds have been identified in yogurt, including carbonyl compounds, alcohols, acids, esters, and sulfur-containing compounds.

The major compounds responsible for imparting the desirable flavor in yogurt are acetaldehyde diacetyl, acetoin, 2-butanone, and lactic acid. Moreover, the optimum flavor of yogurt results when proper levels of these compounds are produced. The desirable concentrations of acetaldehyde in yogurt ranged from 23 to 40 mg/kg, while lesser concentrations resulted in weak flavor and higher acetaldehyde led to an off-flavor. Additionally, as with many other dairy products, yogurt is prone to deterioration, especially under improper storage conditions. The generation of volatile byproducts leads to off-flavors, which make the product unsatisfactory for consumers.

4. Extraction and analysis of volatile aromatic compounds in yogurt

4.1 Solid-phase microextraction (SPME)

The chemical analysis of aroma compounds in yogurt products is typically a complicated process. For example, such analysis requires an extraction stage and, despite the outstanding importance of aroma as an indicator of product quality and conformity, it is still difficult to separate aroma compounds based on common properties such as polarity or volatility. This is particularly true since most volatile organic compounds are present only in small concentrations (μg/kg to mg/kg) in yogurt [36, 37]. As a result, it is often necessary to isolate the volatiles from the complex matrix and concentrate these volatiles for analyses. Unfortunately, the extraction and concentration of volatile aroma components from yogurt products present a major analytical challenge. The most significant problems encountered during this process are:

i. the tendency of the compounds to decompose or transform in the presence of heat and/or oxygen.

ii. potential formation of secondary volatiles by enzymatic reactions.

iii. incomplete recovery of polar/semi-volatile aromatic components.

Classical techniques for preconcentration of volatiles such as steam distillation direct extraction simultaneous steam distillation and extraction with a solvent static headspace [26, 33, 36] and dynamic headspace [30] have been applied to the extraction and concentration of volatile aromatic compounds in yogurt. In recent years, solid-phase microextraction (SPME)-based methods have been used to analyze yogurt flavors [38, 39]. Unlike conventional extraction techniques, SPME is more sensitive to experiment conditions. Any change in experiment parameters that affects the partition coefficient and adsorption rate will also affect the amount adsorbed onto the SPME fiber and the corresponding reproducibility [40].

Solid-phase microextraction (SPME) methods were developed in the 1990s by Arthur and Pawliszyn as a rapid and useful technique for volatile compound analysis. SPME coupled with GC-MS can provide high sensitivity with a small sample volume; consequently, it can be used to analyze the aroma profile of a wide variety of substances. Recently, this technique has been used to study the volatile profiles of fermented camel milk [41], grapes and wine [42], and dried fermented sausage [43].

The amount of analyte extracted on the fiber depends on the polarity and thickness of the stationary phase of the fiber, time and temperature of the extraction, agitation and pH of the sample solution, addition of salt to the sample, and the concentration of the analyte in the sample. These SPME parameters must be optimized for each analyte and matrix. Various fiber coatings are available with thicknesses from 7 to 150 μm. Although fibers coated with thicker films require longer time to reach extraction equilibrium, they can be more sensitive because they can extract larger amounts of analyte [44].

Solid-phase microextraction onto silica fibers externally coated with a suitable stationary phase is used in combination with GC and is also directly coupled to HPLC for the analysis of low-volatile or thermally labile compounds that are not subject to GC analysis. The SPME device consists of a stand with an integrated extraction fiber inside a needle and an assembly holder. Silica fibers (1 or 2 cm long) coated on the outer surface with a thin film of an extraction phase consisting of a liquid polymer and/or a solid sorbent are commercially available. StableFlex fibers consist of a flexible condensed silicon core and are less fragile. Although SPME has maximum sensitivity to the equilibrium distribution, there is a proportional relationship between the amount of analyte adsorbed by the SPME fiber and its initial concentration in the sample prior to partition equilibrium. As a result, complete equilibrium is not necessary for quantitative analysis by SPME [44].

4.2 Gas chromatography with mass spectrometry (GC-MS)

Chromatographic methods are widely used in the identification of various aromatic metabolites. In lactic acid products, these methods include Fourier transform infrared spectroscopy, electron impact ionization-mass spectrometry (EI-MS), electrospray ionization-mass spectrometry (ESI-MS), and nuclear magnetic resonance (NMR) spectroscopy. Mass spectrometers are generally more sensitive and more selective than any other type of detector. Prior to MS analysis, metabolites must be separated, and the separated compounds must be ionized. Ionization techniques can vary, especially for GC-MS and LC-MS [45]. Each of these techniques has advantages and limitations, and no single analytical technique is yet available for the complete study of the metabolome [46].

GC-MS-based metabolome analyses have been developed and applied for metabolic profiling in plants and microorganisms. The aforementioned studies clearly demonstrated the utility of GC-MS for non-target metabolite profiling in a variety of matrices [47].

GC-MS is a combined system where volatile and thermally stable compounds are first separated by GC after which the eluted compounds are detected traditionally by mass spectrometry. In metabolomics, GC-MS has been described as the gold standard [48, 49].

Instrumental analysis of volatile compounds in yogurt is almost exclusively performed by gas chromatography (GC), although high-performance liquid chromatography (HPLC) has also been used in a limited number of cases. Various detectors, including ionization detectors (FID), thermal conductivity detectors (TCD), electron capture detectors (ECD), photoionization detectors (PID), and mass spectrometry (MS) can be used to detect volatiles [3]. In particular, GC-MS is the most popular technique used in the analysis of aromatic volatile components due to its ability to detect and quantify known compounds, identify unknown compounds, and elucidate the chemical properties of molecules. Although the sensitivity of MS depends on the nature of the analytes and the type of equipment used, the detection limits of the charged species can typically range down to picogram levels or even less. In addition to direct calibration, the quantification of volatile compounds can be performed by matrix addition of the labeled compounds or by addition of the so-called internal standard [34, 35].

The application of GC-MS has boosted research on the aroma of yogurt and other products, especially when coupled with SPME as a pretreatment method. The primary advantages of SPME are its simplicity, low cost, ease of automation and in situ sampling. SPME coupled with GC-MS has been widely used to assess the aroma chemical profiles of volatile components derived from a wide variety of matrices, including fermented milk [50], fruit and mango juice [51], grapes and wine [42], dry fermented sausage [43], and alcoholic beverages [52, 53].

5. Aromatic components produced by *Lactobacillus delbrueckii* subsp. *bulgaricus*

Recent work has focused on the isolation and characterization of *L. bulgaricus* with a particular interest in the metabolite profile of these strains. For example, eight strains were examined for the metabolic profiles and found at least 47 different aromatic compounds that were recently identified (**Table 4**) [5]. These aromatic compounds were divided into six main groups based on the chemical structure of each, as follows: 1. organic acid group 2. alcohol group, 3. aldehyde group, 4. ketone group, 5. ester group, and 6. aromatic group. As expected, the primary aromatic component, acetaldehyde, was produced by all of the bacterial strains. Importantly, acetaldehyde is recognized as a major flavor component in yogurt and provides the traditional strong, fruity aroma, sometimes described as green-apple-like that is characteristic of yogurt products. All of the tested strains produced pentanoic acid along with octanoic acids and acetone in a wide range of concentrations. We also observed that using different combinations of yogurt cultures led to the formulation of a wide range of unknown aromatic compounds and higher levels of acetaldehyde. Results showed that the interaction between strains generated a favorable yogurt volatile profile

Volatile compounds chemical formula	
Aldehyde compounds:	
Acetaldehyde	C_2H_4O
Furaldehyde	$C_5H_4O_2$
3-Hydroxybutanal	$C_4H_8O_2$
Benzaldehyde	C_7H_6O
Benzacetaldehyde	C_8H_8O
Ethylbenzaldehyde	$C_9H_{10}O$
2-Octenal	$C_8H_{14}O$
Decanal	$C_{10}H_{20}O$
Ketone compounds:	
2-Pentanone	$C_5H_{10}O$
Acetoin	$C_4H_8O_2$
2,3-Butanedione	$C_4H_6O_2$
2-Acetylfuran	$C_6H_6O_2$
2-Nonanone	$C_9H_{18}O$
2-Heptanone	$C_7H_{14}O$
3-Methyl-2-butanone	$C_5H_{10}O$
2-Undecanone	$C_{11}H_{22}O$

Volatile compounds chemical formula	
Acid compounds:	
Formic acid	CH_2O_2
Butyric acid	$C_4H_8O_2$
Acetic acid	$C_2H_4O_2$
Hexanoic acid	$C_6H_{12}O_2$
Pentanoic acid	$C_5H_{10}O_2$
Benzoic acid	$C_7H_6O_2$
Octanoic acid	$C_8H_{16}O_2$
1,2-Benzenedicarboxylic acid	$C_8H_6O_4$
Alcohol compounds:	
2-Furanmethanol	$C_5H_6O_2$
Ethanol, 2-(octyloxy)-	$C_{10}H_{22}O_2$
3-Methyl-2-butanol	$C_5H_{12}O$
2-Undecanol	$C_{11}H_{24}O$
Ester compounds:	
Propanoic acid, ethenyl ester	$C_5H_8O_2$
2(5H)-Furanone, 5-methyl-	$C_5H_6O_2$
Benzoic acid, 2-ethylhexyl ester	$C_{15}H_{22}O_2$
3-Methyl-2-butenoic acid, tridec-2-ynyl ester	$C_{18}H_{30}O_2$
Ethanone, 1-(2,4-dimethylphenyl)-	$C_{10}H_{12}O$
4-Ethylbenzoic acid, methyl ester	$C_{10}H_{12}O_2$
Aromatic hydrocarbons:	
3-Carene	$C_{10}H_{16}$
Undecane	$C_{11}H_{24}$
Tridecane	$C_{13}H_{28}$
3-Heptene, 2,2,4,6,6-pentamethyl-	$C_{12}H_{24}$
2-Methylundecane	$C_{12}H_{26}$
2-Pentene, 2,4,4-trimethyl	C_8H_{16}
Tetradecane	$C_{14}H_{30}$
2,4,6-Trimethyldecane	$C_{13}H_{28}$
Nonadecane	$C_{19}H_{40}$
Pentadecane	$C_{15}H_{32}$
Hexadecane	$C_{16}H_{34}$
Octadecane, 3-ethyl-5-(2-ethylbutyl)	$C_{26}H_{54}$
Octadecane	$C_{18}H_{38}$

Source: [5]

Table 4.
Aromatic components produced by symbiotic starter cultures of Lactobacillus delbrueckii subsp. bulgaricus and Streptococcus thermophilus.

resulting in the highest numbers of compounds identified as key-aroma compounds that are desirable for an acceptable organoleptic yogurt quality. Moreover, a different starter culture preparation could also lead to the production of different metabolites. Preparation steps would include bulk growth media, media additives, protein sources, and amino acid composition (especially glycine). The use of direct-to-vat culture or a lyophilized starter culture could also impact the aromatic profile of yogurt products. Our work also demonstrated that there are synergistic effects among the aromatic compounds that contributed to the overall yogurt flavor.

6. Conclusion

In this chapter, we reviewed the volatile flavor compounds in yogurt products. In addition, we discussed yogurt starter cultures, major aromatic compounds, and modern instrument analysis of yogurt flavor. Currently, yogurt sales are among the fastest growing in the dairy industry. Consequently, a greater knowledge of enhanced manufacturing procedures and consumer preferences would be highly useful in helping the yogurt industry to improve on its current products and develop additional innovative products. For example, the use of advanced techniques such as synthetic fiber solid-phase extraction and gas chromatography combined with mass spectrometry could help to identify a large number of aromatic compounds in yogurt. This would open up the possibility for more comprehensive exploration of the importance of specific strains in obtaining desired sensory qualities. Moreover, it would support the selection of production strains and the flavor producers. On a more advanced level, such analyses could allow us to develop rapid methodologies for quality control and authenticity of lactic acid products based on the aroma-metabolite profile of the starter cultures in the final product. There remains a lack of sufficient data related to the importance of specific process parameters and strain specificity for aroma formation in lactic acid products. As a result, qualitative and quantitative analysis of volatile aromatic compounds should merely be the first step toward achieving this goal. By determining the relationship between key flavor compounds and the sensory properties of yogurt, we will have a better understanding of how yogurt flavor is affected by the presence of critical flavor compounds. This will enable us to facilitate the production of more uniform yogurt products for enhanced consumer acceptance.

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