

Volatile Flavor Compounds in Yogurt: A Review

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Considerable knowledge has been accumulated on the volatile compounds contributing to the aroma and flavor of yogurt. This review outlines the production of the major flavor compounds in yogurt fermentation and the analysis techniques, both instrumental and sensory, for quantifying the volatile compounds in yogurt. The volatile compounds that have been identified in plain yogurt are summarized, with the few key aroma compounds described in detail. Most flavor compounds in yogurt are produced from lipolysis of milkfat and microbiological transformations of lactose and citrate. More than 100 volatiles, including carbonyl compounds, alcohols, acids, esters, hydrocarbons, aromatic compounds, sulfur-containing compounds, and heterocyclic compounds, are found in yogurt at low to trace concentrations. Besides lactic acid, acetaldehyde, diacetyl, acetoin, acetone, and 2-butanone contribute most to the typical aroma and flavor of yogurt. Extended storage of yogurt causes off-flavor development, which is mainly attributed to the production of undesired aldehydes and fatty acids during lipid oxidation. Further work on studying the volatile flavor compounds-matrix interactions, flavor release mechanisms, and the synergistic effect of flavor compounds, and on correlating the sensory properties of yogurt with the compositions of volatile flavor compounds are needed to fully elucidate yogurt aroma and flavor.

Keywords yogurt, volatile flavor compound, analysis, aroma, carbonyl compound, off-flavor

INTRODUCTION

Yogurt is a cultured dairy product that is widely consumed as a healthy and nutritious food and for its sensory properties. Chemically, yogurt is a complex gel system incorporating protein, polysaccharide, and lipids in its structure. It is produced from milk by fermentation, one of the oldest methods practiced by human beings for the transformation of milk into products with an extended shelf life (Tamime and Robinson, 1999). Yogurt was likely first discovered by the nomadic people living in the Middle East, and it has been consumed for thousands of years by different civilizations. Today, yogurt is commonly made by fermenting cow's milk using a symbiotic culture of the bacteria *Lactobacillus bulgaricus* and *Streptococcus thermophilus* under controlled temperatures and environmental conditions, especially in industrial production (Marshall, 1993; Tamime and Robinson, 1999). Fermentation of the milk sugar (lactose) produces lactic acid, which denatures milk protein to give yogurt its texture (through formation of the coagulated gel) and its char-

acteristic tang. Small amounts of by-products are also produced during this process, which impart the specific aroma and flavor.

The naturalness and agreeable taste of yogurt make it an attractive food ingredient. The main quality characteristics of yogurt include texture, taste, aroma, and flavor (Panagiotidis and Tzia, 2001; Pourahmad and Assadi, 2005; Sodini et al., 2004). Yogurt is typically characterized as a smooth, viscous gel with a characteristic taste of sharp acid and a green apple flavor (Bodyfelt et al., 1988). Some yogurts exhibit a heavy consistency that closely resembles custard or milk pudding, while others are soft-bodied and essentially drinkable. Yogurt is rich in vitamins and minerals and it provides an excellent source of calcium and protein. Besides the obvious nutritional benefits, consumption of yogurt also shows promising health benefits for certain gastrointestinal conditions, such as lactose intolerance, constipation, and diarrheal diseases (Adam et al., 2004; Adolfsson et al., 2004; Kolars et al., 1984; LaBell, 1989). With the increasing consumer demand for low-fat food products, low- and non-fat yogurts with improved compositional and nutritional properties have also been developed (Rash, 1990). To offset its natural sourness, yogurt can be sweetened or flavored by adding fruit or fruit jam (e.g., strawberry, peach, cherry). Studies have shown that the quality of yogurt is affected by factors such as the types of milk used (Georgala et al., 1995; Kaminarides et al.,

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Table 1 List of volatile compounds that have been identified in plain yogurt

<i>Carbonyl compounds</i>	<i>Alcohols</i>	<i>Sulfur compounds</i>	<i>Heterocyclic compounds</i>
Acetaldehyde	Methanol	Dimethyl sulfide	Furan
Acetone	Ethanol	Dimethyl disulfide	Furfural
Propanal	1-Propanol	Dimethyl trisulfide	2-Methylfuran
2-Propanone	2-Propanol	S-methyl thioacetate	2-Pentylfuran
Butanal	1-Butanol	Methional	2-Furanmethanol
2-Butanone	2-Butanol	Tetramethyl thiourea	Pyrazine
Diacetyl	2-Methyl-1-propanol	<i>Nitrogen compounds</i>	Methylpyrazine
Acetoin	Cyclobutanol	N,N-dimethylformamide	Pyrrole
Pentanal	1-Pentanol	Lactamide	1-Methylpyrrole
2-Methylbutanal	3-Pentanol	N-ethyl-benzenamine	2-Methyl tetrahydrofuran-3-one
3-Methylbutanal	1-Penten-3-ol	<i>Hydrocarbons</i>	2-Methylthiophene
3-Methyl-2-butenal	3-Methyl-2-butenol	Heptane	2-Methyltetrahydrothiophen-3-one
2-Pentanone	3-Methylbutanol	Methylcyclohexane	Benzothiazole
3-Penten-2-one	Pentan-2-one-4-ol	Nonane	Methyl 2-piperidine
2-Hydroxy-3-pentanone	3-Methyl 3-cyclohexenol	Undecane	Furfuralcohol
2,3-Pentanedione	2-Ethyl hexanol	<i>Aromatic compounds</i>	1,2-Dihydro-2,2,4-Trimethylquinoline
Hexanal	2-Buthyl octanol	Benzene	2,3-dihydro-1,3,3-trimethyl 1H-Indole
2-Hexanone	Guaiacol	Toluene	<i>Terpene</i>
3-Hexanone	<i>Acids</i>	Ethylbenzene	L-limonene
Heptanal	Acetic acid	1,3-Dimethylbenzene	<i>Others—solvent contamination?</i>
2-Heptanone	Propionic acid	1,4-Dimethylbenzene	Acetonitrile
3-Heptanone	Butyric acid	1,2-Dimethylbenzene	Dichloromethane
Octanal	2-Methylpropanoic acid	Ethenylbenzene	Trichloromethane
3-Octanone	Pentanoic acid	Propylbenzene	
1-Octen-3-one	Isovaleric acid	Trimethylbenzene	
1-Nonen-3-one	Hexanoic acid	1-Methyl ethenylbenzene	
Nonanal	Heptanoic acid	1-Ethyl-4-methylbenzene	
(E)-2-Nonenal	Octanoic acid		
2-Nonanone	Nonanoic acid		
Decanal	Decanoic acid		
Undecanal	Benzoic acid		
2-Undecanone	<i>Esters</i>		
2-Dodecanone	Methyl formate		
2-Pentadecanone	Methyl acetate		
γ -Dodecalactone	Ethyl acetate		
δ -Dodecalactone	Butyl acetate		
Benzaldehyde	Diethyl phthalate		
Phenylacetaldehyde			

Sources: Beshkova et al. 1998, Guler 2007, Imhof and Bosset, 1994b, Laye et al., 1993, and Ott et al., 1997.

2007), starter cultures (Beshkova et al., 1998; Imhof and Bosset, 1994a; Kneifel et al., 1992; Pourahmad and Assadi, 2005), and sweeteners (Lubbers et al., 2004; McGregor and White, 1987).

Flavor is one of the most important properties of food products, and is an important factor determining its acceptability and preference. The sensory properties of dairy products depend largely on the relative balance of flavor compounds derived from fat, protein, or carbohydrate in the milk. The distinct flavor of yogurt is contributed by lactic acid and a complex mixture of aroma compounds, which include the volatiles already present in the milk and specific compounds produced from milk fermentation (Imhof et al., 1994; Ott et al., 1997; Tamime and Deeth, 1980). More than 90 different volatiles have been identified in yogurt, including carbohydrates, alcohols, aldehydes, ketones, acids, esters, lactones, sulfur-containing compounds, pyrazines, and furan derivatives (Marshall, 1982; Ott et al., 1997).

During storage, the volatile constituents in yogurt may change depending on the culture, mix formulation, and the storage conditions (Brauss et al., 1999; Tamime and Deeth, 1980;

Tamime and Robinson, 1985; Tamime and Robinson, 1999; Ulberth and Kneifel, 1992). Like many other milk products, yogurt is prone to deterioration, especially at an ambient temperature, within a matter of days. Various microbial, enzymatic, or chemical reactions occurring within yogurt during storage may alter its physical, chemical, and microbiological structure, causing deterioration or spoilage. Generation of volatile by-products leads to off-flavors and makes the product unsatisfactory for the tastes of consumers. The evolution of volatile compounds can often determine the storage and shelf life of yogurt.

This review summarizes current knowledge on the volatile flavor compounds in yogurt, including their production, characterization, and the roles of the key aroma compounds, as well as off-flavor development and correlation of aroma compounds with sensory properties of yogurt. The progress made in characterizing the volatile constituents of yogurt can lead to a better understanding of the complexity of its flavor. Knowing the key flavor compounds and their origins will enable manufacturers to produce more uniform dairy products with greater consumer

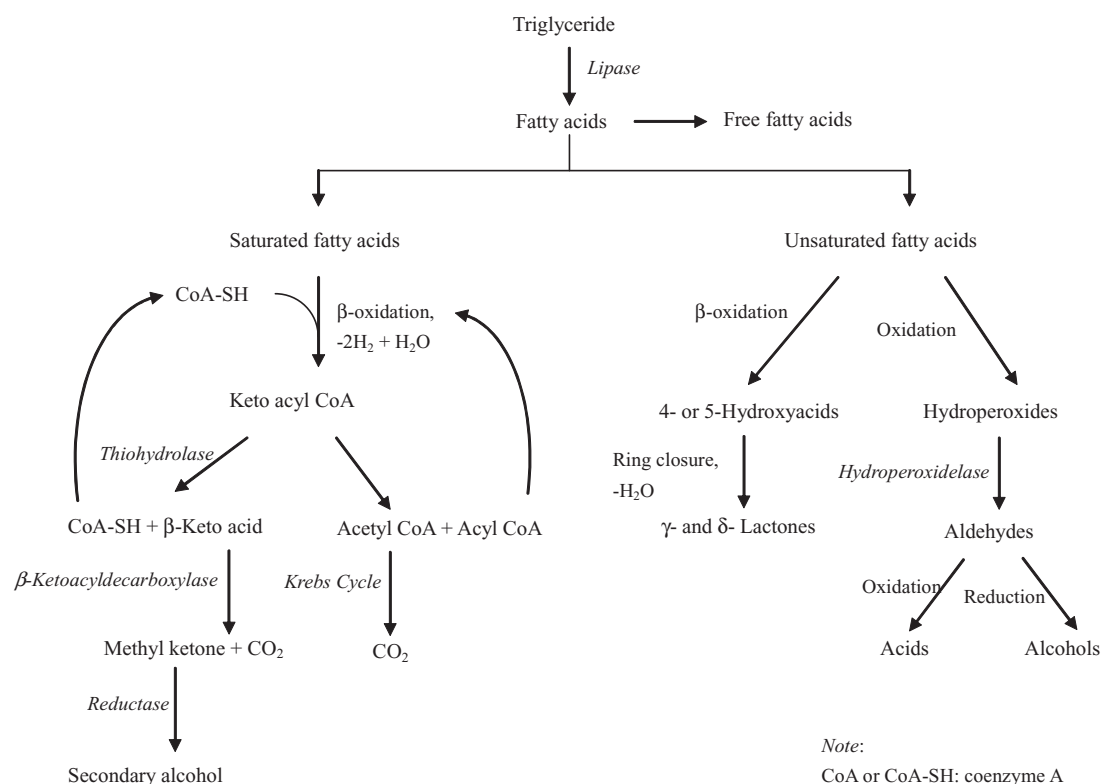


Figure 1 General degradation pathways of milkfat during milk fermentation (modified from Fox and McSweeney, 1998 and McSweeney and Sousa, 2000).

acceptance. A quantitative study of aroma compounds may also help in the creation of more suitable and stable flavors for yogurt. Routine analysis of the key aroma compounds can be used for quality monitoring during yogurt production; the profile of volatile compounds in yogurt can be used as a parameter to provide consumers with better quality and safer food.

PRODUCTION OF VOLATILE FLAVOR COMPOUNDS IN YOGURT FERMENTATION

Table 1 lists the volatile compounds that have been identified in plain yogurt. The underlying flavor of dairy products arises principally from the native volatile constituents in cow's milk, influenced by pasteurization, fermentation, processing, and storage. A large number of the volatile organic compounds found in yogurt are not produced by the starter bacteria but originate from the milk (Imhof et al., 1994; Tamime and Deeth, 1980). Milk is an extremely complicated entity which is comprised of lipids, proteins, carbohydrates, and minerals, and over 400 volatile compounds have been identified in milk products (Walstra and Jenness, 1984).

The differences in the flavor-active compounds of yogurt versus milk are most likely generated by the metabolism of the acid-producing bacteria. These culture-generated aroma compounds result from microbial, enzymatic, or chemical transformations of lactose, lipids, citric acid, and proteins/amino acids

present in milk (Boelrijk et al., 2003; McGorin, 2001; Tamime and Robinson, 1999). The flavor compounds present in yogurt can be divided into four main categories:

- (i) non-volatile acids (e.g., lactic, pyruvic, oxalic, and succinic);
- (ii) volatile acids (e.g., acetic, propionic, and butyric);
- (iii) carbonyl compounds (e.g., acetaldehyde, acetone, acetoin, and diacetyl); and
- (iv) miscellaneous compounds (e.g., certain amino acids and/or constituents formed by thermal degradation of protein, fat, and lactose) (Kang et al., 1988; Tamime and Robinson, 1999).

One major pathway for the production of flavor constituents in yogurt is through lipolysis or oxidation of fatty acids in milkfat, as shown in Fig. 1. Considerable quantities of short-chain fatty acids that are strong flavor contributors to cultured products are produced from saturated fatty acids. Unsaturated fatty acids are oxidized in the presence of free radicals to form hydroperoxides, which rapidly decompose to form hexanal or unsaturated aldehydes (McGorin, 2001). Unsaturated fatty acids also lead to the formation of 4- or 5-hydroxyacids, which readily cyclizes to γ - or δ -lactones, and odd-carbon methyl ketones by decarboxylation of β -keto acids. Another major pathway is microbiological transformations of lactose (and the lactate

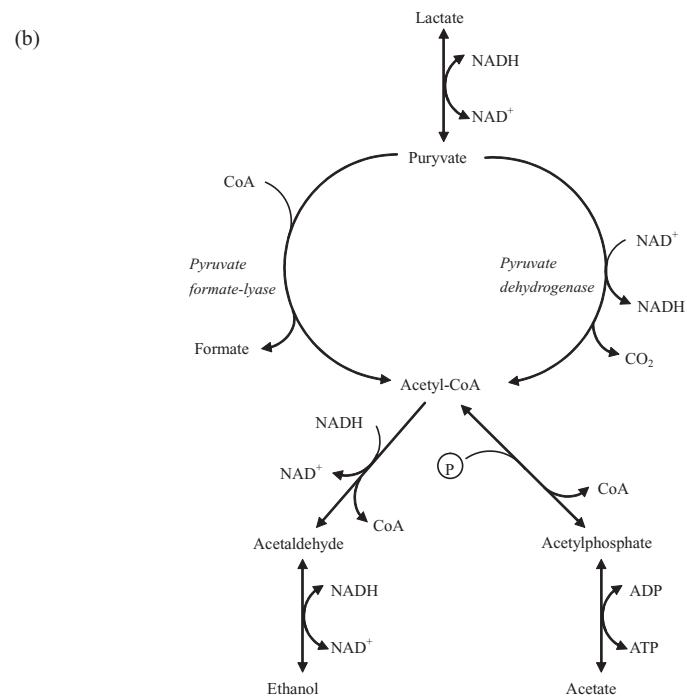
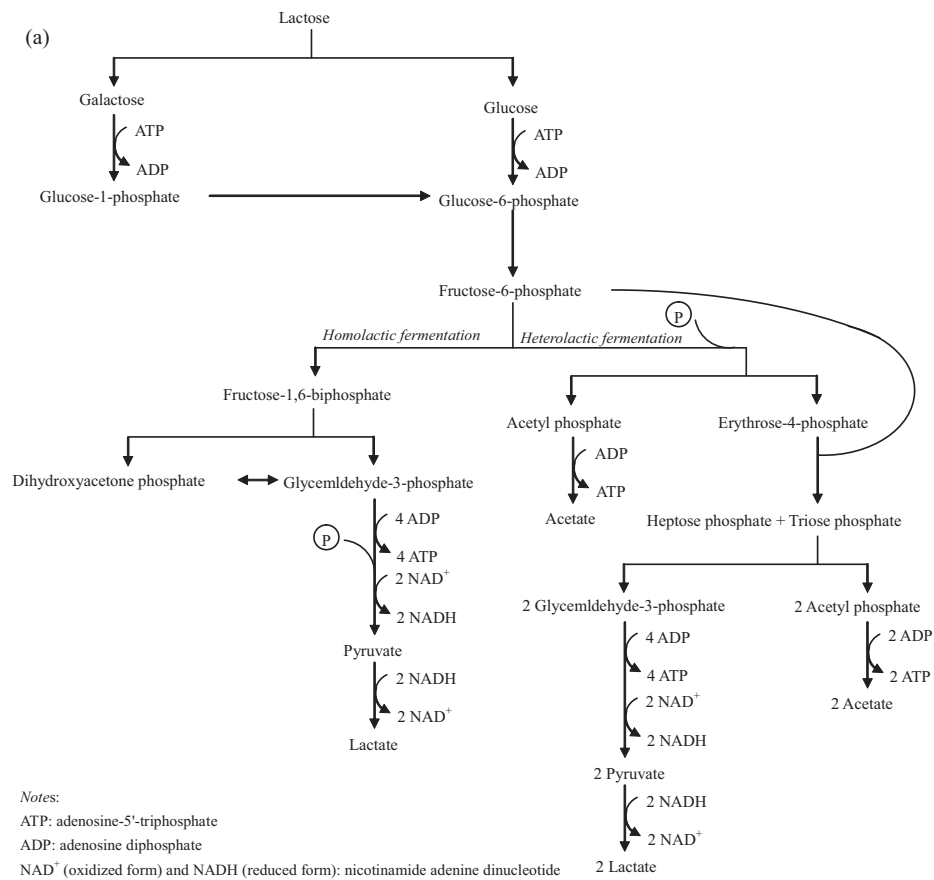


Figure 2 General degradation pathways of lactose and lactate during milk fermentation: (a) homolactic and heterolactic fermentation of lactose (modified from Fox and McSweeney, 1998 and Tamime and Robinson, 1999), and (b) metabolic pathways of lactate (modified from McSweeney and Sousa, 2000).

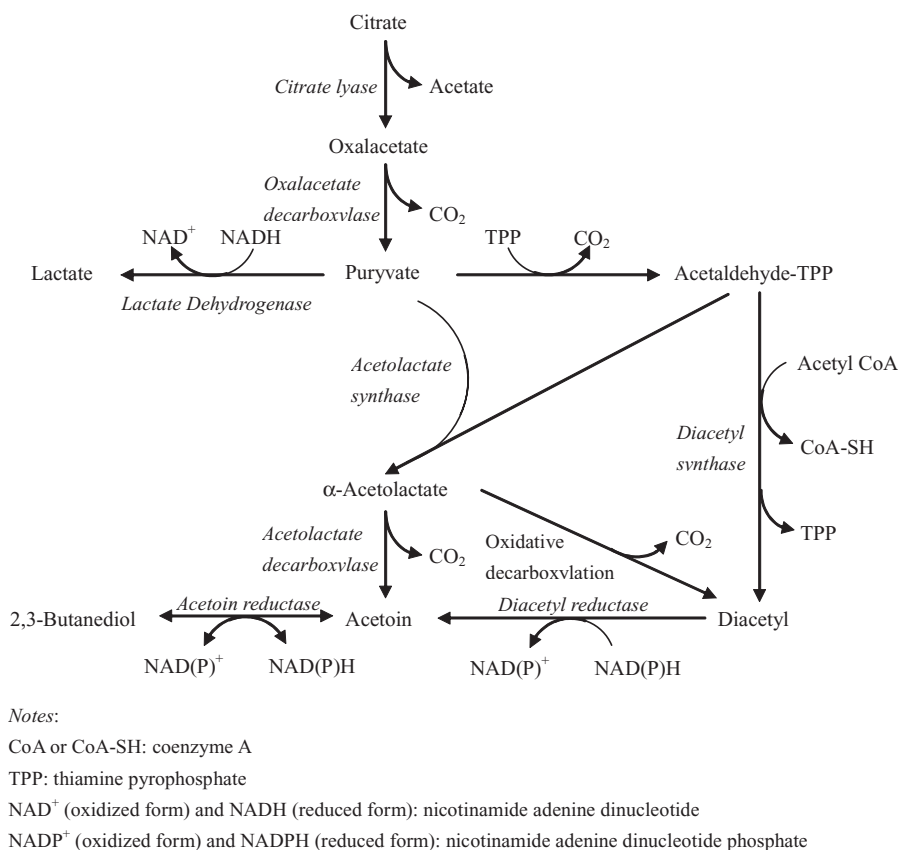


Figure 3 General metabolic pathways of citrate during milk fermentation (modified from Fox and McSweeney, 1998 and McSweeney and Sousa, 2000).

produced, as shown in Figs. 2a and 2b) and citrate (Fig. 3) by acid-producing bacteria (*lactococci* and *lactobacilli*), which produce flavor compounds such as lactic acid, acetaldehyde, diacetyl, acetoin, and ethanol. Alcohols in yogurt can subsequently 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 protein and amino acids, and sulfur compounds can derive from organosulfur compounds (Tamime and Robinson, 1999; McGorin, 2001). Further details on the biochemical processes in the production of flavor compounds in yogurt and other cultured dairy products can be found in recent reviews (Fox and McSweeney, 1998; McGorin, 2001; McSweeney and Sousa, 2000; Singh et al., 2007; Zourari et al., 1992).

ANALYSIS OF THE VOLATILE COMPOUNDS

Despite the extreme importance of aroma as an indicator of quality and product conformity, the chemical analysis of flavor compounds in dairy products is complicated by the heterogeneous nature of the matrices. The relatively high levels of lipids, proteins, and carbohydrates in dairy products make it difficult to separate the flavor-active chemicals based on general properties such as polarity or volatility. Because most volatile organic compounds are only present in small or even trace

concentrations ($\mu\text{g/kg}$ to mg/kg range) in yogurt (Carrillo-Carrion et al., 2007; Friedrich and Acree, 1998; Mariaca and Bosset, 1997; McGorin, 2001), isolation of volatiles from complex matrix and enrichment are often necessary for their analyses. Unfortunately, the extraction and concentration of volatile flavor components from dairy products, prior to their analysis, poses a major analytical challenge. The main problems encountered during this process are:

- (i) tendency of the compounds to degrade or to form artifacts in the presence of heat and/or oxygen;
- (ii) potential formation of secondary volatiles via enzymatic reactions; and
- (iii) incomplete recovery of the polar/semi-volatile flavor constituents (Mariaca and Bosset, 1997; McGorin, 2001).

Classical preconcentration and enrichment techniques for volatiles, such as steam distillation (Alm 1982; Beshkova et al., 1998), direct extraction (Fernandez-Garcia and McGregor, 1994; de Jong et al., 1994), simultaneous steam distillation and solvent extraction (Georgala et al., 1995; Harasawa et al., 1998; Kang et al., 1988), static headspace (Alonso and Fraga, 2001; Carrillo-Carrion et al., 2007; Guler, 2007; Ott et al., 1999; Ulberth, 1991), and dynamic headspace/purge and trap (Beshkova et al., 1998; Harasawa et al., 1998; Imhof and

Table 2 Comparison of different preconcentration and enrichment techniques used for analyzing volatile compounds in yogurt and other cultured dairy products

Technique	Principle	Advantages	Limitations
Static headspace concentration	The volatiles are allowed to equilibrate between the gas phase and the condensed phase, then the gas phase is sampled to analyze the volatiles released	Can be performed rapidly; easy to use; solvent-free; not prone to generate thermal artifacts	Limited to the most volatile components; depends on the concentrations and vapor pressures of the volatiles, and the temperature and matrix of the food product
Dynamic headspace concentration/purge and trap	The volatile components of the gas phase are continuously removed and concentrated in a cold trap or adsorbed onto an inert support, and finally recovered either by thermal desorption or by elution with a suitable solvent	Suitable for compounds of high to medium volatility; moderately sensitive; can be performed rapidly; solvent-free; not prone to generate thermal artifacts	Time-consuming, especially in cleaning equipment and glassware; involves relatively expensive equipment; liquid samples may have foaming problem
Simultaneous steam distillation/solvent extraction	Solvents with low boiling points are used for the concentration of the aroma volatiles, with the condensing water vapor extracted by the condensing solvent vapor	Provides nearly quantitative recoveries of volatiles	Unstable compounds may decompose thermally if not carried out under reduced pressure; thermal artifacts can also form
Steam distillation	The aqueous distillate is concentrated by liquid/liquid partitioning or by cryoconcentration	Suitable for compounds of low to medium volatility	Highly volatile components may have low recoveries and/or may be masked by the solvent peak; unstable compounds may decompose thermally if not carried out under reduced pressure
High-vacuum distillation	Produces small volumes of concentrated aqueous extracts, which are in turn extracted with organic solvents	Prevents thermal degradation by working at ambient or even sub-ambient temperature	Usually require large sample amounts; the operations are very time-consuming; often produces discrimination effects, i.e., highly volatile substances tend to be lost, while those with low volatility are enriched in the final isolate
Molecular distillation	Very similar to high-vacuum distillation and involves the direct transfer of volatile compounds from the matrix to a cold condenser	Well suited for separation and purification of thermally sensitive materials	Requires very short distances between the sample and condenser; requires high-vacuum pumping system
Direct extraction	An extract is obtained by liquid/liquid or liquid/solid partitioning	Generally rapid and efficient	Can only be applied to samples with a very low fat content to minimize simultaneous fat extraction; usually requires a cleanup step
Dialysis	Volatile compounds are separated according to their ability to diffuse through a membrane	High degree of concentration can be obtained	Sample preparation and dialysis are very time-consuming
Solid phase microextraction	A polymer-coated silica fiber is exposed to the headspace above the liquid sample to extract and concentrate the volatile compounds, which is then thermally desorbed in the injection of port of a GC	Solvent-free; rapid; relatively low cost; easy to use; requires small sample amounts; generally more sensitive than other conventional methods	Quantification is complicated by the two equilibrium systems involved: one between the sample and air, and one between the stationary phase and air; prolonged heating of samples during isolation may alter the composition of labile components; low analyte recovery; low determination precision

Bosset, 1994b; Imhof et al., 1995; Kang et al., 1988; Laye et al., 1993; Ott et al., 1997), have been applied for the extraction and concentration of the volatile flavor compounds in yogurt. In recent years, methods based on the newly developed solid-phase microextraction (SPME) have also been applied to analyze yogurt flavors (Lubbers et al., 2004; Lubbers et al., 2007). Unlike conventional steam extraction and purge-and-trap sampling techniques, in which a practically quantitative recovery is often achieved, SPME is more sensitive to experimental conditions. Any change of experimental parameters, which affect the

distribution coefficient and adsorption rate, will also influence the amount adsorbed on the SPME fiber and the corresponding reproducibility (Pillonel et al., 2002; Yang and Peppard, 1999). Table 2 compares the advantages and limitations of different extraction and concentration techniques in analyzing the volatiles in yogurt and other cultured dairy products.

The instrumental analysis of volatile compounds in yogurt is almost exclusively carried out by gas chromatography (GC), although high performance liquid chromatography (HPLC) has also been used in a limited number of cases (e.g.,

Table 3 Odor descriptors of selected volatile compounds found in yogurt

Compound	Descriptor	Reference
Carbonyl compounds		
Acetaldehyde:	Ethereal, fresh, green, pungent	<i>a, b, d</i>
Acetone	Sweet, fruity	<i>c</i>
2-Propanone	Sweet, fruity	<i>c</i>
2-Butanone	Varnish-like, sweet, fruity	<i>c, d</i>
Diacetyl	Buttery, creamy, vanilla	<i>a, b, c, d</i>
Acetoin	Buttery	<i>c, d</i>
3-Methyl-2-butenal	Metallic, aldehydic, herbaceous	<i>b</i>
2-Pentanone	Fruity, acetone	<i>c</i>
3-Methylbutanal	Green, malty, unripe, cocoa, malty	<i>a, d</i>
2,3-pentanedione	Butter, vanilla, mild	<i>b</i>
Hexanal	Green, cut-grass	<i>a, d</i>
2-Hexanone	Floral, fruity	<i>c</i>
Heptanal	Green, sweet	<i>a, d</i>
Nonanal	Sweet, floral, citrus, grass-like	<i>a, d</i>
2-Nonanone	Fruity, musty	<i>c</i>
2-Pentanone	Sweet, fruity, cheesy	<i>d</i>
Heptanone	Fruity, spicy, cinnamon	<i>a, d</i>
3-Octanone	Mushroom, fruity	<i>c</i>
1-Octen-3-one	Mushroom-like, earthy, fruity	<i>a, b, c</i>
Nonanone	Grassy-herbal, green-fruity, floral	<i>a, d</i>
2-Undecanone	Floral, rose-like, herbaceous	<i>a, c</i>
g-Dodecalactone	Fresh fruit, peach, par, plum, coconut, buttery	<i>c</i>
d-Dodecalactone	Peach, coconut, buttery, musky	<i>a, c</i>
2-Phenylacetaldehyde	Flowery	<i>b</i>
Alcohols		
Ethanol	Mild, ether	<i>c</i>
1-Pentanol	Alcoholic, iodoform-like	<i>d</i>
2-Heptanol	Earthy, oily	<i>d</i>
1-Octen-3-ol	Mushroom-like	<i>a</i>
Octan-2-ol	Green	<i>c</i>
Oct-1-en-3-ol	Mushroom	<i>c</i>
Guaiacol	Bacon, phenolic, smoked, spicy	<i>b</i>
Esters		
Ethyl acetate	Solvent-like, fruity, pineapple	<i>c, d</i>
Ethyl butanoate	Fruity, sweet, banana	<i>a, d</i>
Ethyl hexanoate	Fruity, apple, banana	<i>d</i>
Ethyl octanoate	Fruity, banana, apple	<i>a, d</i>
Butyl acetate	Pineapple	<i>c</i>
Acids		
Acetic acid	Vinegar, pungent, acidic	<i>a, b, c</i>
Propionic acid	Vinegar, pungent, sour milk	<i>a, c</i>
Butyric acid	Sharp, cheesy, rancid, sweaty, sour, putrid	<i>a, c</i>
Isobutyric acid	Sweet, mild, rotten apple	<i>c</i>
Isovaleric acid	Rotten fruit, mild, sweaty, rancid, fecal, putrid, flowery	<i>a, b, c</i>
Hexanoic acid	Pungent, rancid, flowery	<i>b, c</i>
Octanoic acid	Wax, soap, goat, musty, rancid, fruity	<i>c</i>
Decanoic acid	Rancid	<i>c</i>
Sulfur compounds		
Dimethyl sulfide	Intense, lactone-like, sulfurous, cabbage	<i>a, b, c</i>
Dimethyl disulfide	Boiled cabbage, cauliflower, garlic	<i>c, d</i>
Dimethyl trisulfide:	Sulfurous, fecal	<i>b</i>
Methional	Cooked cabbage, Boiled potato, sulfury	<i>a, b, c</i>
S-methyl thioacetate	Cooked cauliflower	<i>C</i>

Notes:

*a.*Friedrich and Acree, 1998;*b.*Ott et al., 1997;*c.*Molimard and Spinnler, 1996;*d.*Mariaca et al., 2001.

Fernandez-Garcia and McGregor, 1994; Laye et al., 1993). A variety of detectors, including flame ionization (FID), thermal conductivity (TCD), electron capture (ECD), flame photometric (FPD), photoionization (PID), and mass spectrometry (MS) can be used for detection of the volatiles (Fisher and Scott, 1997; Mariaca and Bosset, 1997). In particular, GC-MS is the most popular technique used in flavor analysis because of its capability in detecting and quantifying known compounds, identifying unknown compounds, and elucidating the chemical properties of molecules. Although the sensitivity of MS depends upon the nature of the analytes and the type of instrumentation used, the detection limits of charged species can usually be down to picogram levels or even less. Besides direct calibration, quantitative determination of the volatile compounds can be accomplished by spiking the matrix with labeled compounds or the standard addition method.

Recently, electronic noses have been introduced to the direct analysis and quality monitoring of yogurt with promising results (Cimander et al., 2002; Navratil et al., 2004). Electronic nose instruments are tools for visually comparing the aromas of samples, usually with chemometric techniques or artificial neural networks incorporated in data interpretation (Ampuero et al., 2002; Cimander et al., 2002). These instruments usually employ an array of chemical sensors based on conductive polymers, metal oxides, or surface acoustic waves utilizing the piezoelectric effect, quartz crystal microbalances, or combinations of these devices (Bartlett et al., 1997; Mariaca and Bosset, 1997). Compared to sensory panels (described in the following paragraph), the main advantage of electronic noses is that once calibrated, they can perform odor assessment on a continuous basis with a fast speed and a low cost. However, the micro-sensor arrays employed in these instruments respond principally to the higher concentration components, and they mimic neither the sensitivity nor the selectivity of a human's olfactory system. Electronic noses do not provide the same type of detailed chemical information as with GC-MS methods, and the technique is probably not sensitive enough for some analytes and some types of matrices.

Besides the instrumental techniques, sensory evaluation through expert panels is also employed to detect aroma and off-flavors in yogurt (Brauss et al., 1999; Guler, 2007; Ott et al., 2000; Panagiotidis and Tzia, 2001; Pourahmad and Assadi, 2005). Sensory evaluation methods are designed to translate qualitative information based on the judgment of panelists into numerical data by applying a statistically valid experimental design and controlled testing conditions. The sensory panel can be compared to an instrument that objectively measures sensory differences or classifies the quality of a product by using a well-defined scale. On the other hand, this method is subjected to error from differences among the individual panelists (Kim and Morr, 1996).

In order to characterize an aroma or to trace the source of an odor, it is necessary to isolate and measure those relatively few components that contribute most to the olfactory response. The coupling of the gas chromatography's ability to resolve mixtures

of volatile compounds and the unique selectivity and sensitivity of the human nose is a valuable analytical technique (known as GC-sniff or GC-olfactory detection), which can provide direct insights into the cause of aroma/odor issues. Basically, the effluent from a GC column is split and fed in parallel to a conventional detector (e.g., MS) and a heated sniffing cup, which is manned by an experienced operator acting as olfactory detector. The aroma compounds are detected and identified based on the operator's record of olfactory response and the simultaneous detector data. Besides directly addressing aroma and off-flavor problems, GC-sniff can establish the olfactory importance of different aroma components in yogurt (Friedrich and Acree, 1998; Ott et al., 1997). The main limitation of this technique is that it can over-estimate the importance of the non-volatile compounds which can also be vaporized in the injection port of the GC. In some cases, combinatory effects between two or more volatile components can enhance or reduce the overall aroma impression (Rothe, 1988), but such synergistic phenomena cannot be detected by GC-sniff.

MAJOR VOLATILE FLAVOR COMPOUNDS IN YOGURT

Not all of the volatile components found in foods are of sensory importance. Despite the long list of volatile compounds found in yogurt (Table 1), a few of them had relatively high concentrations in most investigations. Only acetaldehyde, ethanol, acetone, diacetyl, and 2-butanone have a high impact on the desired production flavor (Tamime and Robinson, 1985) and moreover are present in amounts detectable by common laboratory techniques (Ulberth, 1991). The aroma/odor attributes of some commonly found volatile compounds in yogurt are summarized in Table 3. Friedrich and Acree (1998) showed that diacetyl and acetaldehyde are the most potent flavorants in yogurt, which differs from other fermented dairy products and milk. The major volatile compounds commonly reported responsible for imparting desirable flavor to yogurt are the carbonyl compounds—acetaldehyde, diacetyl, acetone, acetoin, and 2-butanone (Badings and Neeter, 1980; Kneifel et al., 1992; Marshall, 1984; Marshall, 1993; Pourahmad and Assadi, 2005; Tamime and Deeth, 1980; Tamime and Robinson, 1985; Ulberth, 1991; Ulberth and Kneifel, 1992). Although produced in only small amounts in yogurt, these compounds are important organoleptically.

The basic volatile components participating in the formation of the flavor of typical Bulgarian yogurt were acetaldehyde, acetone, 2-butanone, diacetyl, ethyl acetate, and ethanol (Gyosheva, 1985; Gyosheva and Rusev, 1979; Kondratenko and Gyosheva, 1978). The major volatile compounds present in Egyptian yogurt were acetaldehyde, diacetyl, acetoin, acetone, 2-butanone, and acetic acid (Gaafar, 1992). Kaminarides et al. (2007) found that acetic acid, acetaldehyde, acetone, diacetyl, 2-butanone, acetoin, and 3-methyl-2-butanone were the main volatile flavor compounds in yogurt made from sheep's milk. The key aroma components in Switzerland yogurt determined by the GC-sniff technique were acetaldehyde, diacetyl,

2,3-pentanedione, methional, 2-methyltetrahydrothiophene-3-one, (E)-2-nonanal, 3-methylbutyric acid, guaiacol, benzothiazole and two unidentified compounds (Ott et al., 1997). Imhof and co-workers (1994; 1995) determined the key aroma components in Switzerland yogurt based on the ratio between the concentration of the volatile in the product and its threshold concentration and identified six volatiles (acetaldehyde, dimethylsulphide, diacetyl, 2,3-pentanedione, L-limonene, and undecanal) as having high impact. However, these results should be interpreted with caution because the odor threshold values of the volatiles are usually determined in water instead of dairy products. The main ingredients (fat, proteins, and carbohydrates) in yogurt can significantly reduce the actual release of volatiles (Brauss et al., 1999; Fares et al., 1998; Roberts and Pollien, 2000).

Carbonyl Compounds

Carbonyl compounds comprise the main aromatic substances in yogurt, among which acetaldehyde is the compound that contributes most to the typical flavor of yogurt (Bottazzi and Dellaglio, 1967; Gaafar, 1992; Law, 1981; Ott et al., 1997; Pourahmad and Assadi, 2005). Pure acetaldehyde possesses a pungent irritating odor but at dilute concentrations it gives a pleasant fruity aroma. Acetaldehyde imparts yogurt its characteristic green apple or nutty flavor, and both *Lactobacillus bulgaricus* and *Streptococcus thermophilus* readily produce it (Bodyfelt et al., 1988). Acetaldehyde is an indispensable aroma compound in yogurt; good flavored yogurt results when proper levels (23–40 mg/kg and at least 8–10 mg/kg) of acetaldehyde are produced (Gaafar, 1992; Georgala et al., 1995; Kang et al., 1988; Kneifel et al., 1992; Rasic and Kurmann, 1978).

Diacetyl is an important aroma compound that gives the buttery flavor and it may improve yogurt flavor quality at elevated concentrations. Diacetyl reportedly contributes to the delicate, full flavor and aroma of yogurt and is especially important for products that contain low acetaldehyde concentrations (Groux, 1973). Diacetyl is a diketone, derived by the fermentation of citrate present in milk and dairy mixes (Vedamuthu, 2006). Small quantities of diacetyl, ranging from traces to 0.90 mg/kg or more contribute to the pleasant and delicate flavor and aroma of yogurt (Rasic and Kurmann, 1978). The typical concentrations of diacetyl in yogurt ranged from 0.2 mg/kg to 3 mg/kg (Georgala et al., 1995; Hernandez et al., 1995; Kang et al., 1988; Marshall, 1984; Pourahmad and Assadi, 2005; Rysstad and Abrahamsen, 1987; Ulberth, 1991). There are differing views on the role of diacetyl in the formation of yogurt flavor—some researchers regard it as a dominating flavor component only when acetaldehyde contents are low (Groux, 1973; Rysstad and Abrahamsen, 1987), while others attribute to it the leading role in flavor formation (Imhof et al., 1994; Kneifel et al., 1992).

Acetoin is a common flavor substance in many cultured dairy products. Acetoin has a mild creamy, slightly sweet, butter-like flavor that is similar to that of diacetyl. Meanwhile, the flavor of acetoin is considerably weaker than that of diacetyl and it tends

to reduce the harshness of diacetyl. Acetoin is readily converted from diacetyl by the enzyme diacetyl reductase (Collins, 1972). Typical acetoin concentrations in yogurt ranged from 1.2 to 28.2 mg/kg (Alonso and Fraga, 2001; Beshkova et al., 1998; Pourahmad and Assadi, 2005). Diacetyl combined with acetoin imparts the mild, pleasant, buttery taste, and they are critical to the rich perception of yogurt.

Acetone and 2-butanone are both contributed by milk and are described as two volatile compounds of minor importance to flavor contribution in milk products (Carcoba et al. 2000). Acetone has a sweet, fruity aroma and is known to influence the aroma and flavor qualities of yogurt. Small quantities of acetone originate usually from milk, but certain quantities are produced by the yogurt bacteria (Georgala et al., 1995). The typical acetone content in yogurt varied from 0.3 to 4.0 mg/kg (Hild, 1979; Ott et al., 1999; Pourahmad and Assadi, 2005; Tamime and Robinson, 1999; Ulberth, 1991; Vescovo, 1970; Yu and Nakanishi, 1975), while 0.8–2.7 mg/kg acetone was found in cow's milk (Hild, 1979; Ott et al., 1999). The flavor characteristic of 2-butanone is similar to that of acetone. 2-butanone concentrations in yogurt ranged from 0.1 to 7 mg/kg (Kaminarides et al., 2007; Ott et al., 1999; Pourahmad and Assadi, 2005; Ulberth, 1991), while its content in milk was up to 0.2 mg/kg (Imhof and Bosset, 1994b; Ott et al., 1999). Gallardo-Escamilla et al. (2005) reported that 2-butanone is significant for eliciting yogurt odor and contributes to the "fruity" flavor.

Although each of these carbonyl compounds constitutes a recognizable aroma alone, yogurt flavor is determined by a balanced mixture of the important volatile compounds. For example, a 1:1 acetaldehyde and diacetyl ratio would give a preferred typical yogurt flavor (Bottazzi and Dellaglio, 1967; Zouari and Desmazeaud, 1991), while too much acetaldehyde compared to diacetyl would lead to a "green" off-flavor. Also, the ratio of acetaldehyde to acetone plays a significant role in the development of yogurt flavor (Panagiotidis and Tzia, 2001), and a ratio of 2.8:1 results in the desired "fullness" flavor (Bottazzi and Vescovo, 1969). There is much discussion about the concentrations of these volatiles that are needed in order to produce an optimal yogurt flavor, but so far no agreement is found in the literature (Boelrijk et al., 2003).

Acids

Lactic acid and other acids also play specific roles in the aroma and flavor properties of yogurt. Although lactic acid exhibits low volatility, it is included in the discussion here because of its flavor importance. Lactic acid is a prime source of flavor and functionality for many fermented food products, and is responsible for the refreshing tart flavor of yogurt (Panagiotidis and Tzia, 2001). In the process of yogurt production, around 20–40% of lactose present in milk is transformed into lactic acid, and the content of lactic acid in yogurt is around 0.9%. Acidity is an important factor in the perception of yogurt flavor, and the pH of yogurt is typically near 4.4. Less acidic

(milder) yogurts, which are preferred by some consumers, are rated as less flavorful (Kneifel, 1992; Kneifel et al., 1992). Ott et al. (2000) showed that there are important flavor differences between traditional acidic and mild, less acidic yogurts but that they are mainly due to the differences in acidity and not due to different concentrations of the three flavor impact compounds (acetaldehyde, diacetyl, and 2,3-pentanedione). Panagiotidis and Tzia (2001) showed that lactic acid and acetaldehyde contribute largely to the typical flavor and aroma of yogurt. Some researchers considered lactic acid as the most important component of yogurt flavor (Ott et al., 2000; Rash, 1990).

Besides lactic acid, other acids are also produced during yogurt fermentation, by both lipolytic processes (C_4 – C_{20}) and bacterial fermentation (C_2 – C_4). Acetic acid is an important compound produced by lactic starter cultures (Alonso and Fraga, 2001; Beshkova et al., 1998; Tamime and Robinson, 1999), and acetic acid in the concentration range of 0.5 to 18.8 mg/kg has been reported in yogurt (Alonso and Fraga, 2001; Beshkova et al., 1998; Suomalainen and Mayra-Makinen, 1999). High levels of acetic acid impart a “vinegary” taste that may not be accepted by consumers (Tamime and Robinson, 1999). Longer chain acids (e.g., octanoic acid) can contribute to the characteristic “soapy” flavor (Guler, 2007; Molimard and Spinnler, 1996).

Alcohols and Esters

Alcohol is another group of volatile compounds found in yogurt, and the principal alcohol in yogurt is ethanol, which is a common terminal end product in the breakdown of glucose and catabolism of amino acids (Guler, 2007; Urbach, 1995). Ethanol content in the range of 0.2 to 9.9 mg/kg was found in cow's milk yogurt (Hild, 1979; Ott et al., 1999; Pourahmad and Assadi, 2005; Rysstad and Abrahamsen, 1987), while its contents in the yogurts made from goat and sheep milk were generally lower (Georgala et al., 1995; Rysstad and Abrahamsen, 1987). Despite the common report of ethanol as a major volatile compound in yogurt, its contribution to the overall aroma and flavor is not clear. It has been reported that ethanol probably provides a complementary flavor (Vedamuthu, 2006), while others believe that small amounts of ethanol produced during lactic acid fermentation are of no practical importance in the flavor of yogurt (Rasic and Kurmann, 1978).

Esters, which correspond to the acids and alcohols present, are also found in the yogurt. Ethyl esters, originated from the enzymatic or chemical esterification of acids with ethanol (Molimard and Spinnler, 1996), possess pleasant sweet and fruity notes and contribute to the aroma of cultured dairy products such as cheese (Mariaca et al., 2001; McSweeney and Sousa, 2000). Esters are typically found at low concentrations in yogurt which has undergone extended storage. Trace levels (<0.03 mg/kg) of ethyl acetate were found in freshly produced yogurt (Imhof et al., 1995), while Guler (2007) observed that ethyl acetate only started to appear in salted yogurt after 30 days of storage and its concentration increased slowly with stor-

age time. Most esters have a fruity and floral flavor and may contribute to the aroma and flavor by minimizing the sharpness and bitterness imparted by fatty acids and amines (Guler, 2007; Molimard and Spinnler, 1996).

OFF-FLAVOR DEVELOPMENT DURING YOGURT STORAGE

Yogurt flavor changes continuously during manufacturing and storage. The changes in concentration of the flavor-related compounds during storage are due largely to reactions that result in their formation or conversion to other compounds by bacterial metabolic enzymes, and their losses due to volatilization. Lipid oxidation is well recognized as a major cause of quality deterioration during processing or storage of lipid-rich dairy products (Kim and Morr, 1996). Besides loss of nutrients, products of lipid oxidation (aldehydes and ketones) give dairy products the stale and “oxidized” flavors (Maarse, 1991; Romeu-Nadal et al., 2004; Valero et al., 2001). Off-flavor development limits the shelf life and stability of dairy products. Knowledge of changes in yogurt composition during storage is of great technological relevance, and it can help differentiate normal-tasting yogurt samples from those containing off-flavors and malodors.

Although many studies have reported changes in volatile flavor compounds in milks during storage (e.g., Horimoto and Nakai, 1998; Maarse, 1991; Perkins et al., 2005), only limited information exists on this behavior in yogurt. Most studies found that acetaldehyde content decreased in yogurt during storage (Gaafar, 1992; Hruskar et al., 1995; Laye et al., 1993; McGregor and White, 1987), while few investigations reported increasing acetaldehyde concentration during storage (Kang et al., 1988; Kwak, 1995). For yogurt produced by Iranian native starter cultures, significant increases in acetaldehyde content were found after 21 days of refrigerated storage, while the organoleptic characteristics (taste, smell, and texture) showed no changes (Pourahmad and Assadi, 2005). For sheep's milk yogurt, Georgala et al. (1995) found that acetaldehyde level generally increased in the yogurt made by single stains of *Streptococcus thermophilus* or *Lactobacillus delbrueckii subsp bulgaricus*, but mostly decreased when mixed strains were used. Both increase (Hamdan et al., 1971; Hruskar et al., 1995) and decrease (Gaafar, 1992; Kang et al., 1988; Kondratenko and Gyosheva, 1978) in diacetyl concentrations in yogurt during refrigerated storage have been observed, while no change in diacetyl level during the storage was also reported (Kwak, 1995; Laye et al., 1993). Georgala et al. (1995) found that the diacetyl level generally increased in sheep's milk yogurt made by single stains of *Streptococcus thermophilus* or *Lactobacillus delbrueckii subsp bulgaricus*, but it mostly decreased when mixed strains were used. Similarly, decrease, maintenance, and increase in acetone concentration during refrigerated storage have been observed in yogurts made from cow's milk (Gaafar, 1992; Kang et al., 1988; Kondratenko and Gyosheva, 1978; Kwak, 1995) and from sheep's milk (Georgala et al., 1995). Changes in

concentrations of 2-butanone and acetoin during yogurt storage were also reported (Gaafar, 1992; Kwak, 1995; Laye et al., 1993). There is little agreement on the observed changes in the concentrations of these carbonyl compounds in yogurt in literature, probably due to the differences in storage conditions, which affect both their productions and volatilization losses. In contrast, ethanol content was generally observed to increase in yogurt during storage (Georgala et al., 1995; Guler, 2007; Gyosheva, 1985; Hruskar et al., 1995; Kang et al., 1988; Kwak, 1995).

Based on the above results, it can be concluded that the development of off-flavors in yogurt during storage is not likely caused by changes in the contents of the major aroma compounds. Instead, productions of undesired odorants are probably responsible for the deterioration of yogurt flavor. During yogurt storage, the decrease in lactose concentrations was generally accompanied by proportional increases in lactic, acetic, and propionic acid concentrations (Laye et al., 1993). Significant increase in acetic acid content in yogurt has been observed during refrigerated storage (Gaafar, 1992; Guler, 2007; Kang et al., 1988). For yogurt produced by Iranian native starter cultures, acidity increased significantly, while no significant difference in organoleptic characteristics was found after 21 days of refrigerated storage (Pourahmad and Assadi, 2005). Rychlik et al. (2006) observed that the intensity of cheese-like off-flavor increased in parallel with the increase in free fatty acids during storage of pasteurized yogurt and showed that these acids caused the off-flavor by synergistic sensory behavior. Guler (2007) found marked increases in 2-methyl propanol, 3-methyl butanol, and 2-ethyl hexanol contents in salted yogurt between 60 and 90 days of storage, which was closely followed by a flavor decline during this period. A marked increase in pentanal in salted yogurt between 60 and 90 days of storage was also observed (Guler, 2007). Carrillo-Carrion et al. (2007) proposed that the total concentration of volatile aldehydes (C_5 – C_9) can be used as a marker of the degradation of yogurt as a consequence of inadequate storage conditions.

CORRELATIONS BETWEEN YOGURT SENSORY PROPERTIES AND FLAVOR COMPONENTS

Correlating sensory evaluation results with chemical composition data is a valuable methodology in the improvement of flavor and flavor stability. Statistical tools (e.g., multivariate analysis) are often used to analyze the data, which makes it possible to establish the relationship between the sensory quality of yogurt and specific aroma compounds. Only few studies have attempted to correlate the changes in yogurt sensory properties with its chemical compositions during storage. Kwak (1995) found a negative correlation between acetone and yogurt flavor, and a positive correlation between ethanol and off-flavor in yogurt during the storage of 15 days at 10°C. Using quantitative descriptive analysis, Panagiotidis and Tzia (2001) showed that yogurt containing more acetaldehyde, which was obtained with

pectin enrichment, had more pleasant flavor and aroma. Guler (2007) conducted factor analysis on mean sensory scores and some compounds in salted yogurt during storage and determined the individual relationship between sensory terms and chemical compounds using correlation analysis. Results show that the soapy flavor was positively correlated with total free fatty acids, octanoic acid, 9-decenoic acid, and tetradecanoic acid. Fruity flavor was positively correlated with octanol and ethyl acetate, while the bitter flavor was positively correlated with tyrosine. The overall flavor of salted yogurt was found to be negatively correlated with tyrosine, acetic acid, ethyl acetate, ethanol, 2-methyl propanol, and 3-methyl butanol (Guler, 2007). Gallardo-Escamilla et al. (2005) used partial least squares regression to model the complex interactions and relative contribution of chemical compounds important for eliciting odor characteristics of yogurt. Acetaldehyde, 2,3-pentanedione, and several unidentified volatile compounds were found to contribute most to the prediction of yogurt flavor. They also found that the fruity flavor of yogurt was contributed by 1-propanol, ethanol, acetone, acetic acid, 2-butanone, diacetyl, and acetoin (Gallardo-Escamilla et al., 2005).

The results on the correlation between sensory properties and flavor compounds provide the basis for making qualified decisions in producing high quality yogurt. Based on the correlations determined, the quality of yogurt can be checked by measuring the key aroma compounds during production and storage of yogurt. A better understanding of how flavor is affected by the presence of critical aroma compounds can be obtained through determining the correlations of yogurt sensory properties with the volatile compound compositions.

CONCLUSIONS

Advances made on characterizing the volatile aroma compounds in yogurt provide a basis for improving the quality of yogurt and quality control during production and storage. More than 100 volatile compounds have been identified in yogurt, among which acetaldehyde, diacetyl, acetoin, acetone, 2-butanone, play the most important role in imparting the typical aroma and flavor. Formation of undesired aldehydes and fatty acids during lipid oxidation causes off-flavor development during extended storage of yogurt. Much work remains to be done to gain a more complete understanding of the formation of the aroma and flavor of yogurt. Qualitative and quantitative analysis of the volatile flavor compounds is only the first step towards achieving this goal. Quantifying the volatile compounds-matrix interactions, the release mechanisms of volatile compounds, and their synergistic actions are necessary to accurately assess their aromatic contributions. Determining the relationship between key aroma compounds and the sensory properties of yogurt will provide a better understanding on how yogurt flavor is affected by the presence of critical aroma compounds and facilitate the production of more uniform yogurt products with greater consumer acceptance.

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