Quantification of volatile compounds in goat milk Jack cheese using static headspace gas chromatography

R. Attaie¹

Cooperative Agricultural Research Center, Prairie View A&M University, Prairie View, TX 77446

ABSTRACT

Goat milk Jack cheeses were manufactured with different levels of proteolytic endo- and exopeptidases from lysed bacterial cultures and aged for 30 wk. The aroma compounds that are potentially important in contributing the typical flavor of goat milk Jack cheese were quantified using static headspace gas chromatography. The concentrations of volatile compounds were evaluated every 6 wk throughout the aging period. Odor activity values of volatile compounds were calculated using the sensory threshold values reported in literature and their concentrations in Jack cheeses. Odor activity values of identified compounds were used to assess their potential contribution to the aroma of goat milk Jack cheeses. The odor activity values indicated that the ketones 2-hexanone, 2-heptanone, 2-nonanone, and 2,3-butanedione (diacetyl) were important odor-active compounds. The major odor-active acids found in this semi-hard goat milk cheese were butanoic, 2-methyl butanoic, pentanoic, hexanoic, and octanoic acids. Among the aldehydes, propanal and pentanal had high odor activity values and likely contributed to the aroma of this cheese. The concentrations of butanoic, pentanoic, hexanoic, heptanoic, octanoic, and nonanoic acids increased significantly in goat milk Jack cheese throughout aging. The extracted enzymes from lysed bacterial cultures that were added to the cheeses during manufacturing caused considerable increases in the concentrations of butanoic and hexanoic acids compared with the control. However, the lower concentration of peptidases resulted in an increased concentration of butanal, whereas more peptidases resulted in a lower concentration of 2-nonanone in goat milk Jack cheeses. **Key words:** goat cheese, aroma active compound, headspace gas chromatography

INTRODUCTION

Numerous efforts have been made to characterize the flavor profiles of bovine cheese varieties; however,

few reports exist concerning the flavor profile of hard and semi-hard goat milk cheeses. In general, much less work has been conducted in determining the flavor and odor compounds as well as the physical and chemical characteristics of goat milk cheeses. To our knowledge no report has been published concerning the aromatic and volatile flavor compounds in goat milk Jack cheese. Among the hard and semi-hard goat milk cheeses, Jack cheese is produced more than other cheese varieties in the United States and is a popular cheese (Park and Jin, 1998). Because of the economic importance and recent popularity of goat milk cheeses, this research was focused on the identification of volatile compounds that contribute to the flavor and aroma of goat milk Jack cheese. Additionally, the effect of aging on overall aroma and flavor of this cheese was considered. Enzymatic changes occur during aging that affect both texture and flavor. Most flavor compounds vary in concentrations during aging and some flavor compounds are formed or disappear while aging progresses (Sable and Cotteceau, 1999; Attaie et al., 2005).

Aroma compounds in foods are a critical factor in food quality. Aroma and flavor are the perception of volatile compounds released from foods during eating and are important in both product quality evaluation and for consumer preference. Likewise, cheese aroma is complex and consists of a mixture of compounds that are developed due to several biochemical and chemical reactions of cheese components during processing and aging (Qian and Reineccius, 2002; Carunchia Whetstone et al., 2003; Callon et al., 2005). Volatility and affinity of compounds in food matrices control the effectiveness of the aroma. The volatility of aroma compounds depends on the vapor-liquid partitioning of volatile compounds, which determines the affinity of volatile molecules for the fat and aqueous phases of cheese (Jo and Ahn, 1999; Yackinous and Guinard, 2000). Aroma and flavor to a large extent determine cheese quality and consumer acceptance; therefore, their quantitative measurement is of prime importance.

Most volatile flavor components of goat cheeses are present in trace amounts and require sample extraction, isolation, and concentration before GC analysis. Among sample preparation techniques and GC analysis, static

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¹Corresponding author: raattaie@pvamu.edu

headspace GC is sensitive, least destructive to compounds, and provides a volatile profile similar to the aroma perceived by the nose (Chin et al., 1996). Static headspace GC reflects the true equilibrium concentration of aroma substances in the sample headspace gas and limits the risks of artifacts related to the use of solvents (Yang and Peppard, 1994; Chin et al., 1996). This technique has been used widely to evaluate volatile compounds and various classes of major odorants in food products such as cheese, fluid and powdered milk, infant formula, and yogurt (Lin and Jeon, 1985; Vazquez-Landaverde et al., 2005).

Therefore, it is important to calculate the odor activity values of volatile compounds and understand the potent compounds that contribute to the particular aroma and flavor of this semi-hard goat milk cheese. The objectives of this investigation were 1) to identify the volatile compounds that are in the headspace gases of goat milk Jack cheese, 2) to determine the primary odorant compounds from the headspace gas analysis of this cheese and their potential aroma contributions that are important in imparting typical flavor to this cheese using odor activity values, and 3) to compare the changes in the concentration and composition of volatile components of this cheese during aging when differential levels of proteolytic enzymes are used in the manufacturing of this cheese.

MATERIALS AND METHODS

Preparation of Lysed Bacterial Cells

Lactococcus lactis ssp. *lactis* culture (Marschall Superstart Concentrated Cultures, M34; Rhodia, Madison, WI) was grown at 31°C for 6 h in 1-L batches of tryptic soy broth, and cells were harvested by centrifugation at $6,500 \times g$ for 10 min at 4°C. The harvested cells were washed with 100 mL of double deionized and distilled water and centrifuged a second time. Harvested cells were mixed with water at 10% (g/mL) and disrupted at room temperature by using a sonicator (model 300, Fisher Sonic Dismembrator, Fisher Scientific, Houston, TX) at speed 90 for 10 min. The probe of the sonicator was cooled by insertion into crushed ice after each 5 min of use. The lysed bacterial cells and extract were added to cheese curd at 0.14 and 0.27 g/100 g of curd.

Cheese Manufacture

Milk from morning and evening milkings was collected by milking machine from alpine goats at the International Goat Research Center at Prairie View A&M University (Prairie View, TX). Milk was stored at 4°C, pasteurized (200 L; 63°C, 30 min), and processed into

Jack cheese. The 3 cheese replicates were manufactured according to a method described previously (Attaie, 2005). Curd from each batch of cheese was split into a control and 2 treatments. The sonicated cells and extract were thoroughly mixed with curd at 0.14 and 0.27% in plastic bags to facilitate the incorporation of cellular enzymes into cheese curd of treatments 1 and 2, respectively. The control did not contain added cellular extract. The curd for each treatment and the control was then placed in molds and gradually pressed to 69 kPa, where it was maintained for 14 to 16 h at 25°C. The control and treated cheeses were vacuum-packed (Multivac, Koch Supplies, Kansas City, MO) and stored at 4 to 5°C for 30 wk to investigate the effect of aroma and flavor development in goat milk Jack cheese.

Samples

Before sampling, a thin layer (0.2 cm) of cheese was removed and discarded from each surface of the cheese blocks to avoid any sampling of volatile compounds that might have transmigrated from packaging materials. Approximately 5 g of the representative sample of cheese was cut as a triangular slab from outside toward the center of each block and was finely grated to increase the surface area. Triplicate 0.5-g representative samples of grated goat milk Jack cheese were used for analyses. The headspace volatile compounds from goat milk cheeses were determined in the first week after manufacturing and then at 6-wk intervals for 30 wk. The cheeses for this study were vacuum packed (Multivac, Koch Supplies) in polyethylene in 0.4-kg packages and stored at 4°C for sampling and aging. All chemicals and authentic flavor standards were purchased from Aldrich Chemical Co. (Milwaukee, WI) and were of the highest purity available.

GC Analysis

Grated sample (0.5 g) was transferred into 20-mL vials of the headspace analyzer (HS 40XL, Perkin Elmer, Norwalk, CT). The vials were sealed air-tight with a Teflon septum and aluminum caps (Perkin Elmer). A 10- μ g aliquot of 2-ethylnonanoic acid (Narchem Corp., Chicago, IL) as internal standard was added to each vial before capping and heating. Prepared samples were heated at 95°C for 60 min with the oven of headspace analyzer in the rotary mode. Ulberth (1991) determined that equilibrium in the headspace of standard milk samples was reached for all the volatile compounds within 60 min. The needle temperature of the headspace analyzer was kept at 120°C and the transfer line was set at 150°C. The samples were pressurized for 0.5 min before injection and the injection time was

0.1 min. The analyses were performed using a bonded polyethylene glycol fused-silica capillary column (Supelcowax-10, 60 m $\times$ 0.32 mm i.d., 0.25- μ m coating thickness; Supelco Inc., Bellefonte, PA). Gas chromatography was performed with a model HP 5890 Series II (Hewlett-Packard, Avondale, PA) equipped with a flame-ionization detector. Ultra-high-purity helium at 1.5 mL/min and 30 mL/min was used as carrier and makeup gas, respectively. Ultra-high-purity hydrogen (30 mL/min) and high-purity air (400 mL/min) were used for flame-ionization detector. Injector temperature was adjusted to 150°C. Detector temperature was at 250°C, whereas the column oven temperature was increased from 40 to 220°C at 3°C/min after an initial hold at 40°C for 2 min. The peak areas of compounds from gas chromatographic responses were measured quantitatively by a model HP 3396 Series II integrator (Hewlett-Packard). Both the integrator attenuation and threshold were set at 2.

Identification of the compounds was based on GC-MS analysis of cheese samples and mass spectra of unknowns were compared with those of authentic standard compounds analyzed under identical conditions. Mass spectrometric identification was further confirmed by comparing GC retention times with those of authentic compounds using headspace GC under identical conditions. Gas chromatography-mass spectrometry of volatile compounds was performed with a quadrupole mass spectrometer model 9000 (Perkin-Elmer), using a Supelcowax-10 capillary column operated with the same temperature programming as reported for GC analysis. Ultra-high-purity helium was used as carrier gas (head pressure, 41.4 kPa). Mass spectrometry was carried out using the electron impact ionization mode. Mass spectral data were collected at electron energy of 70 eV and the ion source temperature was at 200°C. The transfer line temperature was 200°C, whereas the mass analyzer was at 25°C. Mass scan range was m/e 29 to 300 and electron multiplier voltage was 1,050 V. Compounds were identified by comparing mass spectral data with those of the Wiley's mass spectral library (www.wiley.com/WileyCDA/WileyTitle/productCd-0470047879.html).

Response factors of identified compounds were determined by direct addition of known amounts of authentic compounds into vials (0.2 μ L of each, weighed in μ g) and 2-ethylnonanoic acid as internal standard (0.2 μ L, measured in μ g) were mixed and analyzed under identical conditions as cheese samples. The response factor for each compound was calculated from the peak area obtained for the internal standard divided by peak area obtained for each compound of interest multiplied by the fraction of their weights. Response factors were used to correct for the concentration of identified com-

pounds (in μ g/g) in cheese samples according to the following formula:

$$\text{Concentration of comp. } (\mu\text{g/g}) = \frac{(\text{PA of comp.}) (\text{wt. of IS})}{(\text{PA of IS}) (\text{Sample wt.})} \times \text{RF}$$

and

$$\text{RF} = \frac{(\text{PA of IS}) (\text{wt. of comp.})}{(\text{PA of comp.}) (\text{wt. of IS})},$$

where RF = response factor, PA = peak area, IS = internal standard, and comp. = compound of interest. The internal standard and compounds of interest are measured in micrograms and the sample weight is measured in grams.

Statistical Analysis

The quantified data were analyzed by ANOVA using PROC GLM of SAS (version 8.2, SAS Institute, Cary, NC). The least significant difference test of SAS was used to determine significant differences between means at each sampling time at ($P < 0.05$).

RESULTS AND DISCUSSION

A total of 26 volatile compounds were quantified from the goat milk Jack cheese. These included 8 acids, 3 aldehydes, 8 ketones, and 7 alcohols. The concentrations of 10 volatile compounds did not differ between treatments and during aging of goat milk Jack cheese (Table 1). The ketones that did not differ during aging were hydroxyacetone, 3-ethyl-2-pentanone, 2-heptanone, and 2-decanone. Carunchia Whetstine et al. (2003) found that fresh Chevre-style goat cheeses had sweet milky notes because of the presence of methyl ketones that were thermally generated during pasteurization. Contarini and Povolo (2002) studied the effect of heat treatment on commercially processed milk samples using headspace solid-phase microextraction and found that 2-heptanone and 2-nonanone exhibited a correlation with the severity of the heat treatment. It was noticed that 2-heptanone, 2-nonanone, and 2-decanone had very weak aroma intensities in Parmigiano-Reggiano cheese (Qian and Reineccius, 2002).

Alcohols found in the goat milk Jack cheese were methanol, ethanol, pentanol, and 3-methyl-2-pentanol; their concentrations did not differ among treatments or during aging. Most alcohols are formed via oxidation. Lactococci and other lactic acid bacteria are able to

Table 1. Headspace concentration (ppm) of volatile compounds that did not change during aging of goat milk Jack cheese

Compound	Concentration during aging period						Mean concentration	SEM
	0 wk	6 wk	12 wk	18 wk	24 wk	30 wk		
Hydroxyacetone	0.36	0.3	0.36	0.42	0.32	0.34	0.35	0.06
3-Ethyl-2-pentanone	3.10	3.76	4.04	3.51	2.79	2.95	3.36	0.83
2-Heptanone	0.51	0.34	0.47	0.59	0.31	0.30	0.42	0.09
2-Decanone	0.15	0.19	0.16	0.14	0.11	0.07	0.14	0.03
Methanol	0.3	0.27	0.18	0.43	0.39	0.53	0.35	0.09
Ethanol	38.32	34.56	36.75	39.35	35.51	35.77	36.71	4.08
Pentanol	1.45	1.57	1.46	1.66	1.54	1.44	1.52	0.31
3-Methyl-2-pentanol	1.34	1.26	1.04	1.57	1.20	1.06	1.24	0.26
Propanal	5.60	5.01	6.24	10.06	7.23	4.12	6.38	1.3
2-Methyl butanoic acid	2.17	2.72	2.52	3.05	2.41	2.85	2.62	0.36

produce ethanol from lactose in addition to the alcohols produced from amino acid catabolism (Centeno et al., 2002). Methylalcohols are mostly derived from the branched-chain amino acids leucine, isoleucine, and valine. In some cheese varieties methylalcohols are recognized as key flavor-contributing compounds. The weak aroma intensities of most alcohols indicated that they contributed very weak alcoholic fruity notes in Parmigiano-Reggiano cheese (Qian and Reineccius, 2002). Esters are formed in cheese by enzymatic or chemical reactions of short- to medium-chain fatty acids with alcohols (Centeno et al., 2002). Propanal and 2-methylbutanoic acid were the only aldehyde and acid that did not differ in concentrations during aging. It has been suggested that 2-methylbutanoic acid is formed through transamination and decarboxylation of leucine (Ha and Lindsay, 1990). Brennand et al. (1989) noticed that 2-methylbutanoic acid was associated with fruity, sweaty aroma notes such as Swiss cheese-like aroma.

The headspace concentrations of one aldehyde, several ketones, alcohols, and acids that did not differ among treatments but differed during aging of goat milk Jack cheeses are shown in Table 2. The concentrations of pentanal and diacetyl (2,3-butanedione) increased ($P < 0.05$) until 18 wk of aging and then gradually decreased upon further aging. However, the concentrations of these compounds at the end of aging were not lower than during the first week after manufacturing. It was suggested that pentanal contributed malty, green aroma notes to Parmigiano-Reggiano cheese (Qian and Reineccius, 2003a). Generally, diacetyl is an important flavor compound in most dairy products and has buttery and cheesy notes. The formation of 2,3-butanedione is thought to be thermally generated and is commonly found in dairy products. It can also be produced during nonenzymatic browning (Scanlan et al., 1968). Diacetyl may originate from the unstable precursor α -acetolactate during citrate metabolism and is one of the major aromatic compounds in fermented milk and

fresh cheese (Garde et al., 2003). Moreover, diacetyl production in cheeses has been associated with *Leuconostoc mesenteroides* and *Lactobacillus casei* (Menendez et al., 2000; Hemme and Foucaud-Scheunemann, 2004). The concentration of acetoin (3-hydroxy-2-butanone) was significantly greater when assessed in the headspace of goat milk Jack cheese at the first week of manufacturing and then decreased ($P < 0.05$) at 6 wk and remained at that level throughout the aging period. Acetoin is produced in dairy products by the same mechanisms as the diacetyl and has a buttery aroma. Acetoin is generated from reduction of diacetyl (Garde et al., 2003). Both acetoin and diacetyl contributed to fruity odor in yogurt and other fermented products using a probiotic culture (Gallardo-Escamilla et al., 2005).

Relatively lower concentrations of propanol, 2,3-butanediol, and 2-hexanone were assessed during the first week after manufacturing goat milk Jack cheese; however, all these compounds significantly increased at 6 wk of aging and remained stable until the end of 30 wk of aging. The initial concentration of butanol was higher in goat milk Jack cheese during the first week of aging and then decreased ($P < 0.05$) at 12 wk of aging. This trend continued until butanol was not detectable after 18 wk of aging.

The concentration of acetic acid was below the level of detection during the first week of manufacturing and increased ($P < 0.05$) at 6, 12, and 18 wk of aging. However, after the peak at 18 wk, the concentration of this acid decreased ($P < 0.05$) toward the end of aging. Acetic acid and branched-chain fatty acids such as methylbutanoic acid may be derived from oxidative deamination of amino acids (Centeno et al., 2002). Acetic acid contributed a strong, pungent, vinegary note in Parmigiano-Reggiano cheese (Qian and Reineccius, 2002).

Concentrations of pentanoic and octanoic acids increased ($P < 0.05$) between 1 and 12 wk of aging and continued to increase toward the end of aging. Similarly,

Table 2. Headspace concentration (ppm) of volatile compounds that differed in goat milk Jack cheese during aging

Compound	Concentration during aging period						Mean concentration	SEM
	0 wk	6 wk	12 wk	18 wk	24 wk	30 wk		
3-Hydroxy-2-butanone	0.23 ^a	0.09 ^b	0.09 ^b	0.11 ^b	0.08 ^b	0.08 ^b	0.11	0.02
2,3-Butanedione	5.97 ^b	7.95 ^b	8.88 ^b	13.68 ^a	7.96 ^b	8.29 ^b	8.81	1.30
2-Hexanone	0.37 ^b	2.64 ^a	2.82 ^a	3.23 ^a	3.26 ^a	3.19 ^a	2.58	0.60
Propanol	1.81 ^b	11.46 ^a	11.72 ^a	13.35 ^a	10.37 ^a	14.79 ^a	10.58	2.04
Butanol	0.38 ^a	0.24 ^{ab}	0.14 ^{bc}	0.03 ^c	—	—	0.20	0.06
2,3-Butanediol	5.84 ^b	13.74 ^a	13.9 ^a	15.88 ^a	15.28 ^a	16.98 ^a	13.60	1.95
Pentanal	4.37 ^c	7.14 ^{bc}	7.6 ^{bc}	13.33 ^a	8.28 ^b	7.5 ^{bc}	8.04	1.30
Acetic acid	—	0.79 ^c	1.61 ^b	2.30 ^a	0.72 ^c	0.42 ^c	1.17	0.17
Pentanoic acid	0.49 ^c	1.16 ^{bc}	1.72 ^{ab}	1.90 ^{ab}	2.42 ^a	2.39 ^a	1.68	0.27
Heptanoic acid	1.06 ^c	1.20 ^c	1.23 ^c	2.46 ^b	2.68 ^b	3.60 ^a	2.04	0.25
Octanoic acid	85.29 ^c	129.96 ^{bc}	167.03 ^b	248.39 ^a	280.47 ^a	309.80 ^a	203.49	20.21
Nonanoic acid	2.38 ^c	2.96 ^c	3.03 ^c	6.11 ^b	8.04 ^a	8.80 ^a	5.22	0.42

^{a-c}Means in the same row without common superscripts differ ($P < 0.05$).

the concentrations of heptanoic and nonanoic acids increased ($P < 0.05$) between 1 and 18 wk of aging and continued to increase significantly toward the end of aging. This is in agreement with our previous results obtained for Cheddar-like hard goat cheese (Attaie and Richter, 1996). The trend in increase of concentrations of these compounds with time, particularly of free fatty acids, is indicative of the influence of lipolysis in these high-fat-containing goat cheeses. Pinho et al. (2003) reported significant changes due to lipolysis in the concentrations of these free fatty acids with ripening time in Terrincho ewe cheese. Octanoic acid was described as rancid and pungent. Qian and Reineccius (2002, 2003a) determined that octanoic acid contributed strong sweaty, cheesy, lipolyzed notes, whereas heptanoic and nonanoic acids exhibited weak aromas in Parmigiano-Reggiano cheese. Heptanoic and nonanoic acids contributed soap-like waxy aroma notes (Brennan et al., 1989) to milk fat. Carunchia Whetstine et al. (2003) determined that hexanoic and heptanoic acids contributed not only to volatile aroma of fresh Chevre-style goat milk cheeses but also to the sharp sour taste of these cheeses. They noticed that octanoic acid contributed sweaty/waxy and nonanoic acid dirty/sour flavor to these fresh goat milk cheeses.

The headspace concentrations of volatile compounds that differed with aging and between treatments in goat milk Jack cheeses are shown in Table 3. The concentration of butanal at 12 wk of aging as well as the mean concentration of this compound differed ($P < 0.05$) between control and treatment 1. The concentration of butanal was not different between treatments 1 and 2 throughout the aging period. However, the concentration of butanal in the control changed significantly between 1 and 6 wk of aging and then remained at the same level until the end of the aging period. The addition of endo- and exopeptidases from the extract of lysed

bacterial cells had limited effect on the concentration of this aldehyde. Butanal has been reported to contribute to oaty odor (Gallardo-Escamilla et al., 2005). Most aldehydes are products of lipid autoxidation and the spontaneous decomposition of hydroperoxides and most likely contribute to the overall flavor of goat cheeses.

2-Nonanone, however, was not detected in goat milk Jack cheeses before 12 wk of aging in all treatments, and the mean concentration of this compound was significantly different from 12 to 24 wk in treatment 2 compared with treatment 1 and control. The concentration of this compound was nearly the same between treatment 1 and control. In treatment 1 and control, the concentration of 2-nonanone increased during aging and then decreased ($P < 0.05$) at the end of aging. It appears that the increased concentration of peptidases in treatment 2 reduced ($P < 0.05$) the concentration of 2-nonanone during aging.

The concentration of butanoic acid in the control and treatments increased as aging progressed and were different ($P < 0.05$) between the initial and final weeks of aging. It is evident that the concentration of butanoic acid increased significantly during aging in the control due to lipolysis (Attaie and Richter, 1996; Pinho et al., 2003). However, the concentrations of butanoic acid at 30 wk of aging as well as the mean concentration of butanoic acid were significantly higher in treatments 1 and 2 compared with the control. In addition to lipolysis, the overall effect of added peptidases caused significant increases in the concentrations of this compound in treated cheeses, especially toward the end of aging. Butanoic acid has been found in several unripened and ripened goat milk cheeses and is a major component of cheese flavor (Sable and Cottenceau, 1999). Milo and Reineccius (1997) used headspace olfactometry and determined that diacetyl, acetic acid, and butanoic acid contributed to the aroma profile of mild Cheddar

Table 3. Headspace concentration (ppm) of volatile compounds in goat milk Jack cheeses that differed with aging time and treatment

Compound	Treatment ¹	Concentration of compounds during aging period						Mean value	SEM
		0 wk	6 wk	12 wk	18 wk	24 wk	30 wk		
Butanal	1	0.47	0.37	0.44 ^e	0.31	0.34	0.28	0.37 ^e	0.03
	2	0.37	0.21	0.23 ^{ef}	0.23	0.19	0.16	0.23 ^f	0.03
	Control	0.45 ^a	0.19 ^b	0.18 ^{bf}	0.19 ^b	0.14 ^b	0.06 ^b	0.2 ^f	0.03
2-Nonanone	1	—	—	1.98 ^{b,e}	2.65 ^{ab,e}	3.07 ^{a,e}	0.69 ^c	2.10 ^e	0.14
	2	—	—	0.3 ^f	0.33 ^f	0.21 ^f	0.49	0.33 ^f	0.14
	Control	—	—	1.96 ^{b,e}	2.93 ^{a,e}	2.02 ^{b,g}	0.03 ^c	1.73 ^e	0.14
Butanoic acid	1	3.9 ^d	5.56 ^d	7.15 ^{c,d}	12.38 ^{bc}	14.89 ^{ab}	18.2 ^{a,e}	10.34 ^e	0.75
	2	3.6 ^c	6.04 ^c	8.24 ^{b,c}	11.94 ^{ab}	14.38 ^a	16.88 ^{a,e}	10.17 ^e	0.75
	Control	3.81 ^b	4.2 ^b	7.73 ^{a,b}	9.2 ^{ab}	10.49 ^a	11.0 ^{a,f}	7.74 ^f	0.75
Hexanoic acid	1	9.29 ^c	18.19 ^c	41.67 ^{b,e}	56.44 ^{b,e}	53.27 ^b	77.05 ^{a,e}	42.65 ^e	2.12
	2	10.25 ^c	20.69 ^{bc}	34.43 ^{b,ef}	50.90 ^{a,ef}	54.16 ^a	64.73 ^{a,ef}	39.19 ^e	2.12
	Control	11.45 ^c	16.59 ^c	23.36 ^{c,f}	39.46 ^{b,f}	43.99 ^{ab}	55.58 ^{a,f}	31.74 ^f	2.12

^{a-d}Means in the same row without common superscripts differ ($P < 0.05$).

^{e-g}Means in the same column for each compound without common superscripts differ ($P < 0.05$).

¹Treatments 1 and 2 had lysed bacterial cells and extract at concentrations of 0.14 and 0.27% of curd, respectively.

cheese, but among all the compounds, acetic acid and butanoic acid were the most predominant odorants in mild Cheddar cheese. Dacremont and Vickers (1994) noticed that the most Cheddar-like odor was achieved by a combination of diacetyl at 20 ppm, butanoic acid from 16 to 40 ppm, and methional at 0.8 ppm.

Similarly, the mean concentration of hexanoic acid is significantly higher in the treatments compared with the control. The concentration of this compound increased significantly between the initial and final weeks of aging in the control due to lipolysis. However, addition of peptidases increased the overall concentration of this acid during aging in treatments compared with control. Increases in concentrations of hexanoic acid during 24 wk of aging due to lipolysis were also observed in Cheddar-like hard goat cheese (Attaie and Richter, 1996). Salles et al. (2000) identified that the aroma of volatile free fatty acids, particularly hexanoic and octanoic acids, contributed to the cheesy notes in the crude water-soluble extract of goat cheeses. Salles et al. (2002) in their study of goat cheese flavor found that hexanoic and decanoic acids were described as goaty. Qian and Reineccius (2002, 2003a) determined that butanoic and hexanoic acids contributed strong sweaty, cheesy, lipolyzed notes to Parmigiano-Reggiano cheese. Carunchia Whetstine et al. (2003) determined that hexanoic acid contributed not only to volatile aroma of fresh Chevre-style goat milk cheeses but also to the sharp sour taste of these cheeses.

The quantification of potent odorant compounds and the calculation of their odor activity values (OAV) are needed to estimate the contribution of each compound to the flavor of goat milk Jack cheese. The OAV (the concentration/sensory threshold), which gives the aroma impact of volatile compounds, is dependent on

the concentration of volatile compounds in samples and their sensory thresholds (Qian and Reineccius, 2003b). In this study the OAV of identified compounds were used to assess their potential contributions to the aroma of control cheese. This parameter was calculated for each compound found in goat milk Jack cheese from the threshold values reported in the literature (Table 4). Odor activity values >1 indicate that the concentration of the compound is higher than the sensory thresholds and thus the compound contributes to the aroma of cheese. The calculated OAV of the mean concentration of 2,3-butanedione (diacetyl) was 1,762, suggesting that diacetyl could be a significant contributor to the buttery, nutty aroma of goat milk Jack cheese (Table 4). The calculated OAV of the mean concentration of 2-hexanone was 2.8, indicating that this compound contributed to the aroma of this cheese. The OAV calculated for 2-heptanone was 84, suggesting that this compound could be a very important contributor to the aroma of goat milk Jack cheese. 2-Heptanone was found to be an odor-active compound in Parmigiano-Reggiano cheese (Qian and Reineccius, 2003b) and likely contributed cheesy fruity notes to the aroma of goat milk Jack cheese. The OAV of the mean concentration of 2-nonanone for the control and treatment 1 were in the range of 346 to 420, respectively, whereas the OAV of 2-nonanone for treatment 2 was 66, suggesting that this compound is an important contributor to the aroma of all goat milk Jack cheeses and was more intense in the control and treatment 1 than in treatment 2. Vazquez-Landaverde et al. (2005) found that, according to the magnitude of the OAV, 2-heptanone and 2-nonanone were important contributors in the aroma of UHT milk. The OAV of methyl ketones such as 2-hexanone, 2-heptanone, and 2-nonanone were high, indicating that they are likely to

Table 4. Odor activity values (OAV) of aroma compounds in goat milk Jack cheese

Compound	Mean concentration (ppm)	Sensory threshold (ppm)	Reference	OAV
Hydroxyacetone	0.35	NR ¹	—	—
3-Hydroxy-2-butanone	0.11	NR	—	—
2,3-Butanedione	8.81	0.005	Rychlik et al. (1998)	1,762
3-Ethyl-2-pentanone	3.36	NR	—	—
2-Hexanone	2.58	0.93	Siek et al. (1971)	2.8
2-Heptanone	0.42	0.005	Rychlik et al. (1998)	84
2-Nonanone	1.73	0.005	Rychlik et al. (1998)	346–420; 66
2-Decanone	0.14	0.19	Siek et al. (1971)	0.74
Propanal	6.38	0.43	Honkanen et al. (1964)	14.8
Butanal	0.2	0.19	Honkanen et al. (1964)	1.1
Pentanal	8.04	0.07	Siek et al. (1971)	114.8
Methanol	0.35	NR	—	—
Ethanol	36.71	200	Siek et al. (1971)	0.2
Propanol	10.58	45	Siek et al. (1971)	0.2
Butanol	0.20	7.5	Siek et al. (1971)	0.03
Pentanol	1.52	4.5	Siek et al. (1971)	0.3
3-Methyl-2-pentanol	1.24	NR	—	—
2,3-Butanediol	13.60	NR	—	—
Acetic acid	1.17	36	Rychlik et al. (1998)	0.03
Butanoic acid	7.74	0.86	Rychlik et al. (1998)	9
2-Methyl butanoic acid	2.62	0.6	Amoore et al. (1968)	4.4
Pentanoic acid	1.68	1.37	Amoore et al. (1968)	1.23
Hexanoic acid	31.74	2.8	Rychlik et al. (1998)	11.3
Heptanoic acid	2.04	10.4	Amoore et al. (1968)	0.2
Octanoic acid	203.49	8.7	Baldwin et al. (1973)	23.4
Nonanoic acid	5.22	8.8	Amoore et al. (1968)	0.6

¹NR = not reported.

contribute to the characteristic aroma of goat milk Jack cheese. Only 2-decanone had an OAV of <1 (i.e., below the sensory detection threshold) and was not likely to be important to the aroma of this cheese. Although methyl ketones are naturally present in raw milk, their concentrations are increased due to heat treatment by β -oxidation of saturated fatty acids followed by decarboxylation (Nawar, 1996) or by decarboxylation of β -ketoacids naturally present in milk fat (Grosch, 1982; Jensen et al., 1995). Methyl ketones are also formed from enzymatic conversion of free fatty acids into CO₂ and methyl ketones (Alewijn et al., 2003).

The OAV of aldehydes were either 1 or >1, making this group of compounds important in contributing to the aroma of this cheese. In particular, propanal and pentanal had very high values and likely contributed significantly to the aroma of goat milk Jack cheese.

Most alcohols had higher sensory detection thresholds and their OAV were <1. Thus, alcohols as a group did not appear to have any significant effect on the aroma of this Jack cheese. Qian and Reineccius (2003b) also noticed that alcohols were not important contributors to the aroma of Parmigiano-Reggiano cheese.

Acetic, heptanoic, and nonanoic acids had OAV of <1 and probably had minor contributions to the overall aroma of this cheese. However, butanoic, 2-methyl butanoic, pentanoic, hexanoic, and octanoic acids had higher OAV and more likely were responsible for a major part of the aroma of goat milk Jack cheese.

Most of the compounds that did not have sensory threshold values reported in the literature had low concentrations in goat milk Jack cheese except for 2,3-butanediol. It is assumed that 2,3-butanediol (similar to the other alcohols with their large sensory thresholds and very low OAV) may not have contributed significantly to the aroma of this cheese. Most of the identified and monitored volatiles in this study were previously reported to be present in various concentrations in other cheese varieties.

The threshold value is defined as the lower concentration detected with a probability of 50% by a randomly selected subject. When analyzing the effect of compounds on flavor, pH is an important variable for threshold value measurements and should be considered because it affects the state of compounds in cheese such as free, dissolved, or bound to other components. Flavor compounds have different physical and chemical properties that can affect their release from the microstructure of food matrices. Hydrophobic compounds have a lower vapor pressure and a higher sensory threshold in oil than in water (Relkin et al., 2004). The volatility of flavor compounds would be influenced not only by the polarity and partitioning coefficients of compounds but also by the composition and characteristics of the medium. Flavor and flavor release can become complicated due to several mechanisms that affect component interaction such as mass transfer, matrix structural hindrance, and flavor-matrix interactions (Seuvre et

al., 2000). Caseins of cheese can also have effects on the retention of flavor compounds, because caseins have distinct polar and hydrophobic regions that can act as binding sites for different flavor compounds (Swaigood, 1996; Seuvre et al., 2000). If aroma threshold measurements are performed in a cheese-like medium and at typical pH of cheeses, then the sensory threshold values and hence the OAV will be reliable. The sensory threshold values found in the literature were carried out under different settings and conditions and small differences could be introduced in the calculations of OAV or the aroma contribution of compounds based on their OAV. Because sensory threshold values for different compounds were not available in a cheese matrix, most of the sensory thresholds used in this study were either in milk, milk derivatives, or at pH conditions close to the pH of this cheese, which ranged from 5.11 to 5.05 during 30 wk of aging (Attaie, 2005). Thus, it is expected that the OAV in this study would reflect, relatively accurately, the contribution of these compounds to the overall aroma of goat milk Jack cheese. Although sensory studies are required to validate the findings of this study, it is likely that the compounds identified in this study are responsible for the characteristic aroma of goat milk Jack cheese.

Headspace gas chromatography was performed to determine the contributions of highly volatile odorants to the aroma of goat milk Jack cheese during 30 wk of aging. Although static headspace analysis gives a more reproducible quantification and mostly recovers the highly volatile compounds, it is expected that the heavier molecules have a low recovery using this technique and hence the method yields a relatively smaller number of compounds (Milo and Reineccius, 1997; Alewijn et al., 2003). To reduce the problem of quantifying only those compounds that have higher vapor pressure, a higher incubation temperature and longer times were used in this study to establish headspace equilibrium. This would help in the release of flavoring compounds that had higher boiling and lower vapor pressure. During the preliminary studies, the quantification of volatile compounds in goat milk Jack cheeses was performed by using a standard addition technique. Extraction parameters for static headspace technique were optimized to increase sensitivity and minimize artifact formation. The equilibration time necessary for the optimum analyses of cheese samples was determined by the results of replicates of static headspace GC. Based on those results, equilibrium was reached for all volatiles of the cheese standards in 60 min. Thus, a 1-h equilibration time was chosen as standard procedure in this study.

CONCLUSIONS

Quantitative analysis of volatile aromatic compounds in goat milk Jack cheese was performed by static headspace GC and confirmed by GC/MS. The results of this investigation provided a profile of the compounds with the highest OAV in goat milk Jack cheese. During 30 wk of aging of goat milk Jack cheese, important changes in the profile of aromatic compounds took place. The concentrations of butanoic, pentanoic, hexanoic, heptanoic, octanoic, and nonanoic acids increased significantly in goat milk Jack cheese as aging progressed. Among other compounds, 2-hexanone, propanol, and 2,3-butanediol increased significantly at 6 wk of manufacturing this cheese, whereas butanal, butanol, and 3-hydroxy-2-butanone decreased during aging.

Although the concentrations of most free fatty acids increased significantly between the initial and final weeks of aging, the different levels of proteolytic enzymes that were added to cheeses caused considerable increases in the concentrations of butanoic and hexanoic acids compared with the control. However, only the lower concentration of peptidase enzymes in treatment 1 increased the concentration of butanal significantly, whereas the use of the higher concentration of enzymes significantly reduced the concentration of 2-nonanone in goat milk Jack cheese. The OAV of different compounds that were calculated based on reported sensory thresholds and their concentrations in cheeses indicated that methyl ketones such as 2-hexanone, 2-heptanone, 2-nonanone, and diacetyl were important odor-active compounds in goat milk Jack cheese using static headspace technique. Aldehydes also contributed to the aroma of this cheese, especially propanal and pentanal had high OAV. However, alcohols did not have a major effect on the aroma of this cheese and probably played a minor role. Among the acids, acetic, heptanoic, and nonanoic acids had no significant effect on the aroma of this cheese, whereas butanoic, 2-methyl butanoic, pentanoic, hexanoic, and octanoic acids were odor-active compounds and probably played a significant role in the aroma of goat milk Jack cheese.

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