Quantitative Determination of Thermally Derived Off-Flavor Compounds in Milk Using Solid-Phase Microextraction and Gas Chromatography

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ABSTRACT

Many volatile compounds generated during the thermal processing of milk have been associated with cooked, stale, and sulfurous notes in milk and are considered as off-flavor by most consumers. A headspace solid-phase microextraction (HS-SPME)/gas chromatographic technique for the quantitative analysis of thermally derived off-flavor compounds was developed in this study. The extraction temperature, time, and sample amount were optimized using a randomized 2^3 central composite rotatable design with 2 central replicates and 2 replicates in each factorial point along with response surface methodology. Calibration curves were constructed in milk using the standard addition technique, and then used to quantify 20 off-flavor compounds in raw, pasteurized, and UHT milk samples with various fat contents. The concentrations of these volatiles in raw and pasteurized milk samples were not significantly different. However, dimethyl sulfide, 2-hexanone, 2-heptanone, 2-nonanone, 2-undecanone, 2-methylpropanal, 3-methylbutanal, heptanal, and decanal were found at higher concentrations in UHT milk as compared with raw and pasteurized milk samples. In addition, the concentration of methyl ketones was greater in UHT milk with higher fat content. The calculated odor activity values suggested that 2,3-butanedione, 2-heptanone, 2-nonanone, 2-methylpropanal, 3-methylbutanal, nonanal, decanal, and dimethyl sulfide could be important contributors to the off-flavor of UHT milk. The HS-SPME technique developed in this study is accurate and relatively simple, and can be used for the quantification of thermally derived off-flavor compounds in milk.
(**Key words:** milk, solid-phase microextraction, off-flavor, quantification)

Abbreviation key: GC = gas chromatography, **HS-SPME** = headspace solid-phase microextraction, **OAV** = odor activity value, **QL** = quantification limit.

INTRODUCTION

Volatile compounds in milk have been extensively studied with many extraction techniques including static headspace, purge and trap, and solvent-assisted flavor evaporation (Contarini et al., 1997; Bendall, 2001; Simon et al., 2001; Toso et al., 2002). These methods are time consuming or require exhaustive concentration steps, and often cause artifact formation. More recently, headspace solid-phase microextraction (**HS-SPME**) has been developed, which can substantially reduce analysis time and sample manipulation steps, and minimize artifact formation (Wercinski and Pawliszyn, 1999). This technique has been used widely to extract volatile components from dairy foods such as cheese, milk powder, milk chocolate, infant formulas, and fluid processed milk (Stevenson and Chen, 1996; Marsili, 1999b, 2000; Nakai et al., 1999; Aardt et al., 2001; Fenaille et al., 2003; Gonzalez-Cordova and Vallejo-Cordoba, 2003; Kim et al., 2003; Pinho et al., 2003; Das et al., 2004; Lachenmeier et al., 2004).

Fresh bovine milk has a distinctive yet subtle and delicate flavor, which can be overshadowed by off-flavor compounds. These off-flavors are directly responsible for product rejection by the consumer; therefore, their quantitative measurement has attracted much interest (Rerkrai et al., 1987; Gaafar, 1991; Parliment and McGorrin, 2000; Karagul-Yuceer et al., 2001). Heat treatments, particularly UHT processing, can promote the development of thermally derived off-flavor compounds such as aldehydes, methyl ketones, and various sulfur compounds (Scanlan et al., 1968; Jeon et al., 1978; Moio et al., 1994; Contarini et al., 1997; Contarini and Povolo, 2002). Contarini and Povolo (2002) studied the effect of heat treatments on volatile compounds in commercially processed milk samples using HS-SPME and gas chromatography (**GC**). They identified 11 compounds, 5 of which (2-pentanone, 2-heptanone, 2-nonanone, benzaldehyde, and 2-undecanone) exhibited a correlation with the severity of the heat treatment.

The aim of the present work was to develop a reliable, fast, and effective technique for the quantification of thermally derived volatile compounds in milk using standard addition and multiple internal standard techniques. Extraction parameters for HS-SPME were optimized to minimize artifact formation and increase the sensitivity. The methodology developed was then used to study off-flavor compounds in commercial milk samples subjected to different thermal treatments.

MATERIALS AND METHODS

Chemical Standards

3-Methylbutanal, 2-methylpropanal (isobutyraldehyde), ethyl acetate, 3-methylbutanol, 2-furaldehyde (furfural), heptanal, octanal, nonanal, decanal, trans-2-hexenal, 2-heptanone, 2-nonanone, 2-undecanone, 3-heptanone, 3-octanone, and 4-decanone were purchased from Aldrich Chemical Co. Inc. (Milwaukee, WI); 2,3-butanedione (diacetyl) and hexanal were purchased from Sigma (St. Louis, MO); 2-octanone was from Fluka Chemical Corp. (Milwaukee, WI); 2-pentanone, 2-hexanone, 2-decanone, and trans-2-nonenal were from K&K Laboratories (Jamaica, NY); 2-methylbutanal was from Polyscience Inc. (Niles, IL); and dimethyl sulfide was from TCI America (Portland, OR).

Milk Samples

Raw homogenized milk samples with 1 and 3% fat were obtained locally (Lochmead Farms, Junction City, OR). Sodium azide (0.02%) was added and the samples were stored at −17°C (for no more than 3 d) until analyzed. Pasteurized milk samples of 2 commercial brands (A and B) with 0, 1, 2, and 3% fat content were purchased from a local store, stored at 4°C, and analyzed before their expiration date (2 wk from manufacturing date). Ultrahigh temperature milk samples with 1 and 3% fat content were purchased in Mexico (Leche Araceli, Grupo Fomento Queretano, Embotelladora La Victoria, Queretaro, Mexico), stored at room temperature, and analyzed before the expiration date (6 mo from manufacturing date).

Optimization of SPME Parameters

A randomized 2^3 central composite rotatable design along with response surface methodology (Kuehl, 2000) was used to study temperature (5 to 35°C), time (10 to 180 min), and sample size (5 to 30 g) effects on the amount of volatile compounds adsorbed by the SPME fiber from pasteurized milk with 3% fat. The response

recorded was the total GC peak area of the compounds of interest (Table 1).

A 2-cm 50/30 μm divinylbenzene/carboxen/polydimethylsiloxane SPME fiber (Supelco Co., Bellefonte, PA) was used for the extraction of volatile compounds. The fiber was conditioned in a GC injection port at 270°C for 1 h before use. Milk samples were weighed in 40-mL amber glass vials (I-Chem, New Castle, DE) with polytetrafluoroethylene septum caps. Samples were equilibrated (5 min) in a thermostatic water bath (same temperature as extraction) with constant stirring. After equilibration, the SPME fiber was exposed to the headspace for volatile extraction, and then desorbed in the GC injection port at 250°C for 8 min. All samples were analyzed in triplicate with the same fiber for the entire experiment.

GC/Flame-Ionization Detection

The analysis of volatile compounds was carried out using an HP 5890 series II gas chromatograph (Hewlett Packard, Wilmington, DE) equipped with a flame-ionization detector and an HP-5 capillary column (50 m × 0.32 mm i.d., 0.52-μm film thickness, Hewlett Packard). The oven temperature was maintained at 35°C for 8 min, increased to 150°C at a rate of 4°C/min, then increased to 230°C at a rate of 20°C/min, and finally held at 230°C for 20 min. The injector and detector temperatures were 250 and 270°C, respectively. Nitrogen was used as carrier gas at 1 mL/min and the injection port was in splitless mode for 5 min.

Volatile Identification

The mass spectra of milk volatiles were obtained using an Agilent 6890 gas chromatograph equipped with a 5973 quadrupole mass analyzer detector (Agilent Technologies, Inc., Wilmington, DE). The SPME fiber was exposed to the headspace of 20 g of 3% UHT milk in a 40-mL amber glass vial for 3 h at 35°C and then inserted in the GC-mass spectroscopy injection port for 5 min under splitless conditions. A DB-5 capillary column (30 m × 0.32 mm i.d., 1-μm film thickness; J&W Scientific, Folsom, CA) was used to achieve chromatographic separation. The oven temperature program was the same as for the flame-ionization detection analysis. Helium was used as the carrier gas at 2.5 mL/min. The injector, detector transfer line, and ion source temperatures were 250, 280, and 230°C, respectively. Electron impact ionization was used at a voltage of 70 eV and m/z range of 35 to 350 was collected at 4.51 scans/s. The instrument control and data analysis were performed using enhanced ChemStation software (Agilent Technologies, Inc.). The volatile compounds in milk were

Table 1. Regression equations for milk flavor compounds spiked in 3% fat raw milk.

Compound		Internal standard	Regression equation[1]	R^2	Calibration limit[2] (μg/kg)
1	Dimethyl sulfide	2-Hexenal	$y = 0.673x$	0.994	0.031
2	2-Methylpropanal		$y = 0.332x$	0.997	0.062
3	2,3-Butanedione		$y = 0.132x$	0.999	0.160
4	Ethyl acetate		$y = 1.048x$	0.998	0.019
5	3-Methylbutanal		$y = 1.072x$	0.996	0.019
6	2-Methylbutanal		$y = 1.614x$	0.998	0.012
7	2-Pentanone		$y = 3.420x$	0.998	0.049
8	3-Methylbutanol		$y = 3.721x$	0.999	0.008
9	2-Hexanone		$y = 3.821x$	0.999	0.004
10	Hexanal		$y = 4.328x$	0.998	0.004
11	2-Furaldehyde		$y = 0.163x$	0.999	0.131
12	2-Heptanone	3-Heptanone	$y = 1.366x$	0.999	0.004
13	Heptanal		$y = 0.921x$	0.996	0.007
14	2-Octanone	3-Octanone	$y = 1.447x$	0.995	0.010
15	Octanal		$y = 0.823x$	0.998	0.017
16	2-Nonanone	2-Nonenal	$y = 2.846x$	0.994	0.045
17	Nonanal		$y = 1.750x$	0.992	0.074
18	2-Decanone	4-Decanone	$y = 0.554x$	0.989	0.194
19	Decanal		$y = 0.143x$	0.992	0.750
20	2-Undecanone		$y = 0.870x$	0.995	0.123

[1]y = Area compound/area internal standard; x = [compound]/[internal standard].

[2]Calculated as the concentration that gives a signal-to-noise ratio of 10.

identified by comparing mass spectra and retention times with those of authentic compounds.

Quantitative Analysis

A standard stock solution was prepared in methanol containing 10 g/kg each of 2-methylpropanal, ethyl acetate, 3-methyl-1-butanol, 2-furaldehyde, 2-pentanone, 2-heptanone, heptanal, octanal, 2-nonanone, nonanal, decanal, 2-undecanone, 2,3-butanedione, hexanal, 2-octanone, 2-hexanone, 2-decanone, 3-methylbutanal, 2-methylbutanal, and dimethyl sulfide. The standard stock solution was then diluted with volatile-free distilled water (boiled for 30 min) to final concentrations of 0.02, 0.1, 0.2, 1, 2, 6, and 10 mg/kg. Aliquots (0.1 g) of the diluted standard stock solutions were used to spike 20 g of raw milk (3% fat) to final added concentrations of 0.1, 0.5, 1, 5, 10, 30, and 50 μg/kg. An aqueous internal standard solution containing 2 mg/kg each of trans-2-hexenal, 3-heptanone, 3-octanone, trans-2-nonenal, and 4-decanone was prepared by diluting a 10-g/kg internal standard stock solution. An aliquot (0.2 g) of the diluted internal standard solution was then added to yield a final concentration of 20 μg/kg. The sample was equilibrated at 35°C for 5 min and extracted at the same temperature for 1 h.

Calibration curves for the volatile compounds were constructed based on the standard addition technique (Penton, 1999) and applying linear regression analysis on the concentration ratio (μg/kg of compound per μg/

kg of internal standard) and peak area ratio (area of compound/area of internal standard). Triplicate analysis was performed at each concentration level.

For quantification, 0.2 g of internal standard was added to 20-g milk samples (raw 1 and 3% fat; pasteurized 0, 1, 2, and 3% fat from brands A and B; UHT 1 and 3% fat) and the volatiles were analyzed following the procedure described previously. The concentrations were calculated based on the peak area ratio of the compound to the internal standard.

Statistical Analyses

Statistical evaluations including ANOVA, response surface regression, linear regression, and Tukey honest significant difference (α = 0.05) were conducted using Statgraphics Plus 5.0 (Manugistics Inc., Rockville, MD).

RESULTS AND DISCUSSION

Extraction Time, Temperature, and Sample Size Effects on the Sensitivity

The SPME parameters were evaluated to achieve high sensitivity without artifact formation. Time was the most significant parameter (P < 0.001) affecting milk volatile extraction by the SPME fiber. Increasing extraction time (up to 3 h) improved the sensitivity (Figure 1 A and B). All compounds reached concentrations higher than the quantification limit (**QL**) within
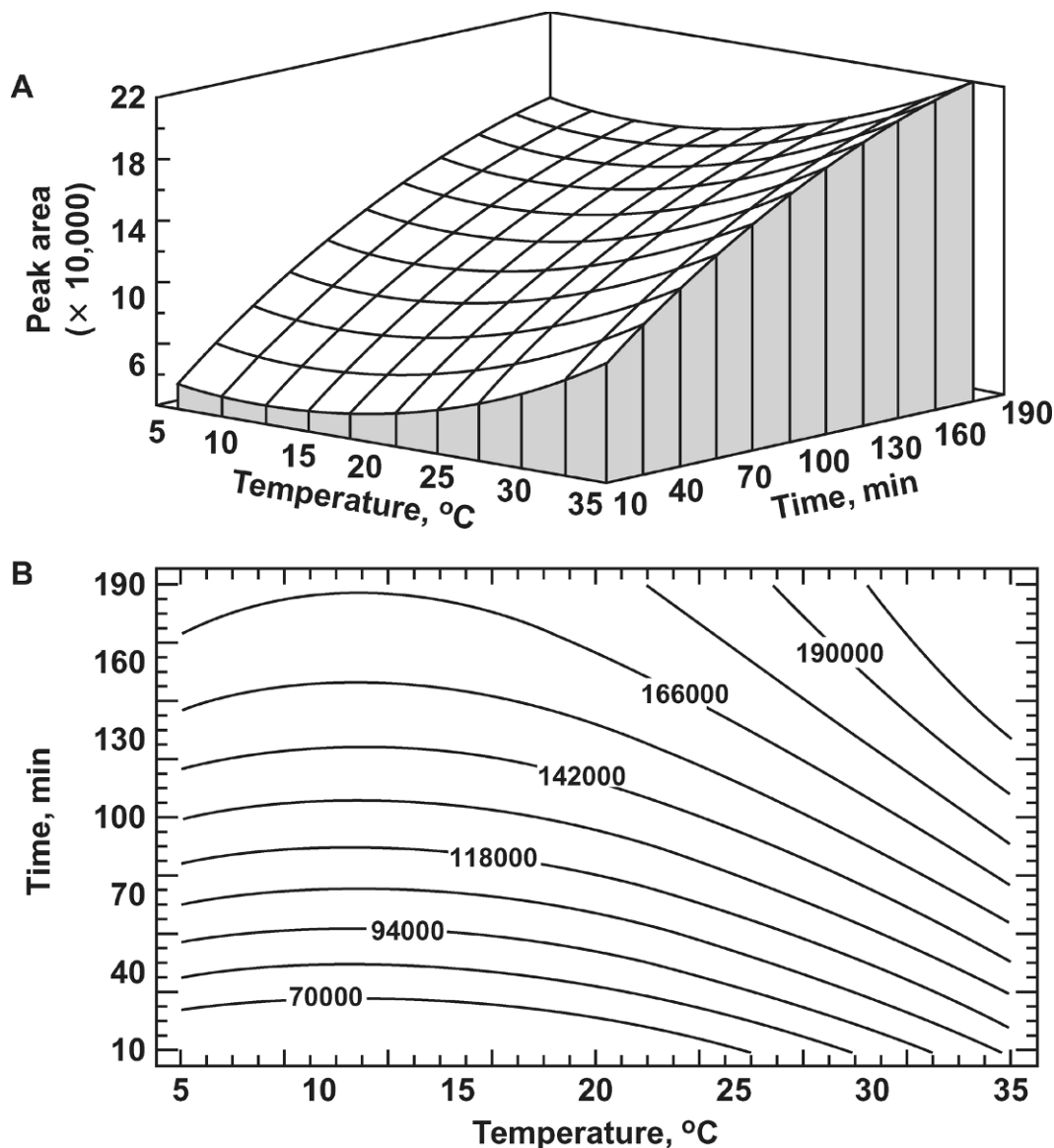
Figure 1. Effect of time and temperature on the solid-phase microextraction sensitivity to milk volatiles. Sample size fixed at 20 g. A) Response surface; B) contour plot.

1 h (QL = signal equal to 10 times the noise). The exceptions were 2-decanone (QL = 0.19 μg/kg) and 2-furaldehyde (QL = 0.13 μg/kg), which remained below QL even after a 3-h extraction. Considering analysis productivity limitations, the extraction time selected was 1 h.

Temperature also had a significant effect ($P = 0.007$) on the total peak area (Figure 1 A and B), reaching maximum at the highest temperature tested (35°C). Although extractions at 45 to 75°C have been used in previous studies of milk volatiles (Simon et al., 2001; Contarini and Povolo, 2002; Toso et al., 2002), temperatures in this range could cause artifact formation. An-

other consideration about temperature regarding the trapping of volatile compounds on SPME fibers is that increasing temperature during headspace extraction may selectively concentrate certain volatiles on the fibers, with the simultaneous displacement of others (Wercinski and Pawliszyn, 1999; Dufour et al., 2000; Pinho et al., 2002). This could lead to the progressive exclusion of some important lower molecular weight analytes in milk, such as dimethyl sulfide (Burbank and Qian, 2005) due to an SPME film capacity effect.

It has been reported that the sample size will not change the extraction efficiency of polar compounds, whereas the opposite has been observed for nonpolar

compounds (Penton, 1999). In this study, sample size did not have a significant effect ($P = 0.197$) on the extraction of volatiles; therefore, a 20-g sample amount was arbitrarily chosen. Finally, none of the quadratic and interaction terms showed a significant effect on the extraction of volatiles ($P > 0.05$) in the range of conditions analyzed.

Standard Calibration Curves

The standard addition technique allows for backward extrapolation to calculate the analyte quantity originally present in the sample. Standard curves for 20 thermally derived compounds were constructed and high linear correlation coefficients ($R^2 > 0.99$) were obtained for all compounds (Table 1). The QL for these compounds were at the parts per trillion levels. Despite the fact that 2-propanone and 2-butanone are 2 of the most abundant volatile compounds in fresh milk, their calibration curves were not constructed because these compounds have been found to be unrelated to the heat treatment of milk (Moio et al., 1994; Contarini et al., 1997; Contarini and Povolo, 2002).

Five internal standards were chosen with properties similar to the corresponding compounds of interest (Table 1). Although one internal standard has been widely used for quantification, this is insufficient when the sample matrix differed from the one used for the standard curve. Fat content affects the volatility of compounds differently, therefore, a standard curve built based on one internal standard cannot be used to accurately quantify compounds in samples at different fat levels. The use of multiple internal standards has been suggested for more accurate quantification (Qian and Reineccius, 2003).

Quantification of Volatiles in Commercial Milk

The HS-SPME/GC methodology developed in this study was very sensitive and can be used to quantify thermally derived volatile compounds in milk. The 20 volatile compounds quantified (Table 2) were found in the range 0.01 to 52 μg/kg in milk samples, which were in agreement with previously published results (Contarini et al., 1997; Contarini and Povolo, 2002; Toso et al., 2002). Adequate reproducibility was achieved for most compounds (relative standard deviation < 15%). Although SPME has been reported as being effective in extracting some volatile free fatty acids from dairy products (Pinho et al., 2002), free fatty acids were not quantified in this study.

Concentrations of ketones were not significantly different ($P > 0.05$) in raw and pasteurized milk samples; however, their concentrations were markedly higher in UHT milk (Table 2 and Figure 2A). At the same fat level, UHT milk contained approximately 12 times the amount present in raw and pasteurized milk. The major contributors were 2-heptanone and 2-nonanone, followed by 2,3-butanedione, 2-pentanone, and 2-undecanone. The concentration of 2-heptanone and 2-nonanone in UHT milk were 34 and 52 times higher, respectively, than in raw and pasteurized samples. Because aroma impact is not only dependent on concentration, but also on sensory threshold, the odor activity value (**OAV** = concentration/sensory threshold) was calculated. The OAV for 2-heptanone and 2-nonanone (Table 3) were less than 1 in raw and pasteurized milk, indicating that they were not important aroma contributors. However, their OAV in UHT milk were in the range of 4 to 10 suggesting that these compounds could be very important contributors to the aroma of heated milk. This could be true for other ketones but to a lesser extent because their concentrations were much lower. These observations were consistent with previous work by Contarini et al. (1997) and Contarini and Povolo (2002) who reported that the concentration of 2-pentanone, 2-hexanone, 2-heptanone, 2-nonanone, and 2-undecanone increased in direct proportion to the severity of the heat treatment and were associated with the development of stale-heated flavor in UHT milk. Moio et al. (1994) identified 2-heptanone and 2-nonanone as the most intense volatile flavor compounds in UHT milk. Although methyl ketones are naturally present in raw milk, they can be formed during 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).

In this work, the concentration of 2,3-butanedione in UHT milk samples was higher than in raw milk, but its concentration varied widely in pasteurized milk samples. The OAV was higher than 1 for 3% UHT, 3% pasteurized A, and 0% pasteurized B milk samples, suggesting that 2,3-butanedione is contributing to the aroma of heated milk. 2,3-Butanedione has been reported as a very important flavorant contributing to the rich "heated" note in UHT milk, giving a buttery, pastry-like aroma (Scanlan et al., 1968). Although its formation has been suggested to be heat-induced (Scanlan et al., 1968), it is also attributed to microbial activity in milk (Badis et al., 2004), therefore being an ambiguous indicator for the heat treatment.

Total amount of aldehydes appeared to be affected by heat processing to a lesser extent than ketones (Table 2 and Figure 2B). Overall, UHT milk had higher concentrations of total aldehydes than raw and pasteurized milk samples. Hexanal, octanal, and nonanal concentrations showed a significant ($P < 0.05$) higher concen-

Table 2. Concentration of volatile compounds in commercial milk samples (µg/kg).

Compound	Raw 1%	Raw 3%	UHT 1%	UHT 3%	Past A 0%	Past A 1%	Past A 2%	Past A 3%	Past B 0%	Past B 1%	Past B 2%	Past B 3%
Total ketones	5.87	10.72	77.59	121.37	8.55	5.66	14.93	11.94	9.25	4.78	5.75	7.72
2,3-Butanedione	0.25[a]	0.48[ab]	3.13[c]	7.39[c]	2.00[b]	0.51[ab]	9.75[d]	0.92[ab]	6.50[c]	1.75[ab]	1.71[ab]	2.07[b]
2-Pentanone	0.21[a]	0.28[a]	5.89[b]	9.53[c]	0.18[a]	0.14[a]	0.19[a]	0.22[a]	0.19[a]	0.13[a]	0.14[a]	0.22[a]
2-Hexanone	0.22[bcd]	0.37[d]	1.46[e]	1.81[f]	0.26[cd]	0.10[ab]	0.09[ab]	0.06[a]	0.34[d]	0.17[abc]	0.17[abc]	0.16[abc]
2-Heptanone	1.03[abc]	0.95[abc]	22.32[d]	34.46[e]	2.06[c]	0.87[abc]	1.79[bc]	1.12[abc]	0.54[ab]	0.45[a]	0.55[ab]	0.72[ab]
2-Octanone	2.11[ab]	3.82[bc]	2.65[abc]	4.51[cd]	2.93[abc]	2.16[ab]	1.89[ab]	6.39[d]	0.91[a]	1.44[a]	2.15[ab]	3.02[abc]
2-Nonanone	0.20[a]	0.24[a]	35.04[b]	52.64[c]	0.79[a]	0.43[a]	0.59[a]	0.53[a]	0.77[a]	0.44[a]	0.33[a]	0.61[a]
2-Decanone	0.28[b]	BQL[2]	0.46[b]	1.33[c]	BQL	BQL	BQL	BQL	BQL	BQL	BQL	BQL
2-Undecanone	1.98[cd]	4.58[e]	6.64[f]	9.70[g]	0.33[a]	1.46[bc]	0.63[ab]	2.70[d]	BQL	0.40[a]	0.70[ab]	0.92[ab]
Total aldehydes	10.08	8.98	14.36	21.15	7.23	4.57	5.98	8.75	8.14	3.80	3.42	3.56
2-Methylpropanal	0.35[a]	0.40[ab]	2.24[cd]	2.52[d]	2.13[c]	0.21[a]	0.74[b]	0.52[ab]	0.27[a]	0.33[a]	0.48[ab]	0.29[a]
3-Methylbutanal	0.03[ab]	BQL	0.85[d]	1.14[e]	0.02[ab]	0.02[ab]	0.17[c]	0.06[ab]	0.03[ab]	0.19[c]	0.17[c]	0.08[b]
2-Methylbutanal	0.46[b]	0.90[c]	0.57[b]	0.91[c]	0.14[a]	0.09[a]	0.14[a]	0.09[a]	0.18[a]	0.13[a]	0.11[a]	0.13[a]
Hexanal	4.77[de]	2.68[bc]	1.58[abc]	12.97[f]	2.80[c]	0.75[a]	1.65[abc]	0.81[ab]	5.21[e]	1.62[abc]	0.82[ab]	0.74[a]
2-Furaldehyde	BQL	0.20[b]	0.52[c]	0.38[c]	BQL	BQL	BQL	BQL	0.14[ab]	BQL	0.13[ab]	BQL
Heptanal	0.22[bc]	0.20[abc]	0.49[d]	1.68[e]	0.03[a]	0.04[ab]	0.12[ab]	0.07[ab]	0.37[cd]	0.11[ab]	0.08[ab]	0.14[ab]
Octanal	0.43[c]	0.52[c]	0.48[c]	0.95[d]	0.08[a]	0.07[a]	0.21[b]	0.12[ab]	0.14[ab]	0.10[a]	0.09[a]	0.15[ab]
Nonanal	1.40[bcd]	1.36[bcd]	1.71[cd]	3.92[f]	1.21[abcd]	0.65[abc]	1.28[abcd]	1.15[abcd]	1.80[e]	0.53[ab]	0.28[a]	0.42[ab]
Decanal	2.42[a]	2.72[a]	5.92[b]	6.68[b]	0.82[a]	2.74[a]	1.67[a]	5.93[b]	BQL	0.79[a]	1.26[a]	1.61[a]
Ester												
Ethyl acetate	0.22[a]	0.26[a]	2.26[e]	2.15[e]	0.28[a]	0.61[ab]	0.14[a]	0.35[ab]	1.22[cd]	0.81[bc]	1.33[d]	0.41[ab]
Sulfur												
Dimethyl sulfide	7.40[a]	8.16[a]	22.39[e]	21.41[e]	8.45[ab]	7.38[ab]	6.61[a]	8.49[a]	11.44[bc]	11.55[bc]	14.18[cd]	16.08[d]
Alcohol												
3-Methyl-1-butanol	0.60[b]	1.09[c]	0.19[a]	0.15[a]	0.07[a]	0.13[a]	0.33[ab]	0.14[a]	0.18[a]	0.17[a]	0.30[ab]	0.13[a]

[a–f]Different letters for the same compound indicate significant difference between sample means (Tukey HSD 95%, from a triplicate).

[1]Past A and Past B = Two brands of commercial pasteurized milk.

[2]BQL = Below quantification limit.

tration in 3% UHT samples whereas 2-methylpropanal, 3-methylbutanal, 2-furaldehyde, heptanal, and decanal concentrations were higher ($P < 0.05$) for both 1% and 3% UHT samples. The total aldehyde concentration was not significantly different ($P > 0.05$) between raw and pasteurized milk samples; however, a significantly ($P < 0.05$) lower 2-methylbutanal and octanal content was observed in pasteurized samples. Based on their OAV (Table 3), nonanal and decanal appeared to be important compounds contributing to the aroma of raw, pasteurized, and UHT milk samples, whereas octanal, hexanal, 2-methylbutanal, 3-methylbutanal, and 2-methylpropanal could be important only for UHT milk aroma. This suggested that, despite their low concentration, aldehydes could contribute very much to the aroma of heated milk.

Rerkrai et al. (1987) stated that the increase of $C_{2-7,9}$ saturated aldehydes concentration is the main cause for the stale flavor in UHT milk, due to their low flavor thresholds. Contarini and Povolo (2002) found 3-methylbutanal to increase with the heat treatment severity whereas hexanal and heptanal did not. Hexanal, heptanal, octanal, nonanal, and decanal result from the autoxidation of unsaturated fatty acids (C18:1 and C18:2) and also the spontaneous decomposition of hydroperoxides promoted by heat (Grosch, 1982). Hexanal can also be transferred to milk from cow's feed (Scanlan et al., 1968) or originate from light-induced lipid oxidation (Marsili, 1999a). The presence of 2-methylpropanal, 2-methylbutanal, and 3-methylbutanal in heated milk is due to the Strecker degradation of amino acids during Maillard reactions (Damodaran, 1996).

2-Furaldehyde was quantifiable only in UHT, raw 3%, and pasteurized 0% brand B samples. Its concentration was higher in UHT milk, but the calculated OAV was too low to be considered as an important contributor to milk aroma. However, it is considered a good indicator of the heat treatment because it is the precursor of melanoidins in Maillard reactions between sugars and the free amino group of milk proteins or amino acids (BeMiller and Whistler, 1996).

Ethyl acetate was the only ester quantified in this work. Its concentration was approximately 10 times higher in UHT milk compared with raw samples, whereas its concentration varied greatly between pasteurized samples (Table 2 and Figure 2C). It has been reported that ethyl acetate is formed by esterification of ethanol and acetic acid via the Fischer reaction catalyzed by heat (Hart, 1991). However, its very low OAV
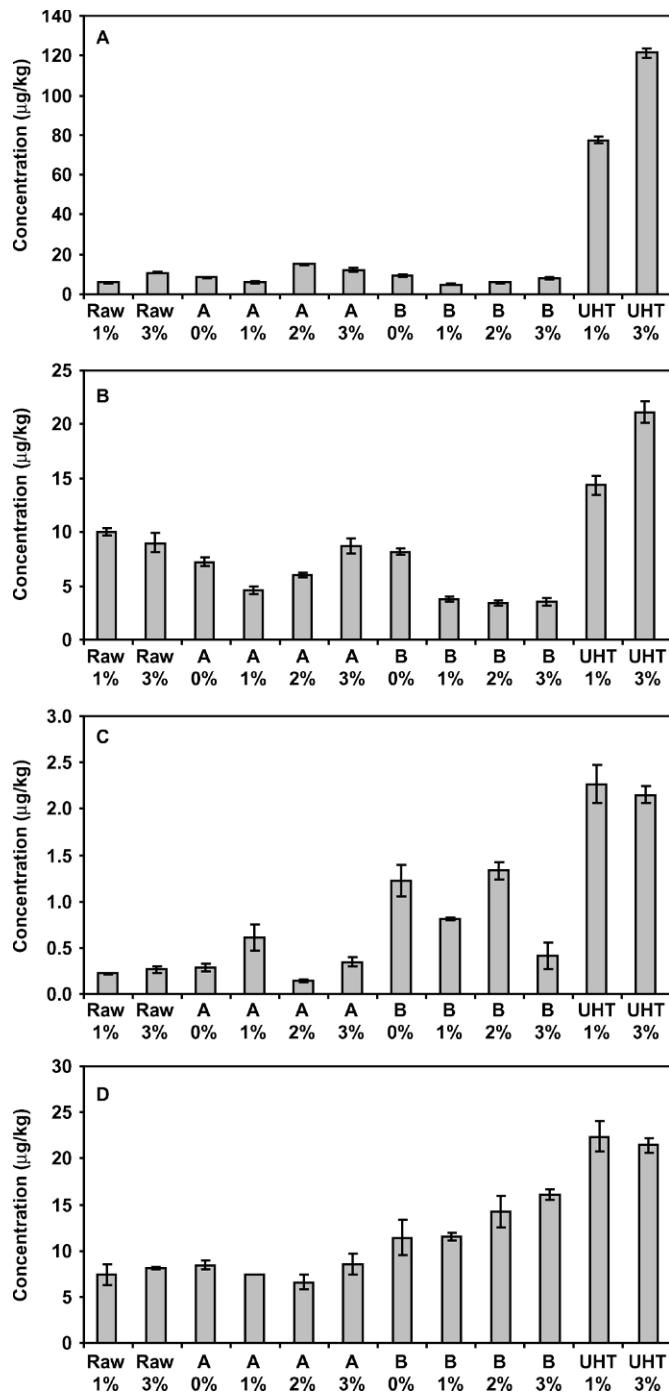
Figure 2. Concentration of volatile compounds in commercial milk samples. A) Total ketones; B) total aldehydes; C) ethyl acetate; and D) dimethyl sulfide. A and B = 2 brands of commercial pasteurized milk.

taining volatiles. Its concentration was almost 3 times higher in UHT than raw milk, whereas pasteurized 2% and 3% brand B had a significantly higher concentration ($P < 0.05$) than raw and pasteurized brand A milk (Table 2 and Figure 2D). This appeared to reflect a difference in the heat treatment or the origin of the milk samples. Although dimethyl sulfide is present naturally in high amounts in raw milk (Toso et al., 2002), it can also be formed from the sulfhydryl group of milk proteins subjected to thermal denaturation (Datta et al., 2002). Its calculated OAV suggested that this compound could be an important contributor to the aroma of both heated and fresh milk.

3-Methylbutanol was the only alcohol quantified in this work. Its concentration was significantly higher ($P < 0.05$) in raw milk than in UHT (Table 2). However, its OAV indicated that this compound was not important for the aroma of raw and heated milks. 3-Methylbutanol is naturally present in raw milk and is produced mainly by the microbial reduction of 3-methylbutanal (Toso et al., 2002).

Fat content seemed to have an influence on the concentration of methyl ketones in UHT milk, where their concentration in 3% fat samples was almost double that found in 1% fat samples, with the exception of 2,3-butanedione. For raw milk samples, total ketones amount was higher for the 3% with respect to the 1% fat sample. However, this trend was not noticed for the total methyl ketone concentration in pasteurized milk samples. Fat content did not seem to influence the concentration of aldehydes.

Milk fat contains 10% (wt/wt) of $C_{6,8,10,12}$ fatty acids, which are precursors for odd carbon numbered $C_{5,7,9,11}$ methyl ketones during heat treatment. It also provides 24% and 2% (wt/wt) of the C18:1 and C18:2 fatty acids, respectively, which are required for the formation of the $C_{6,7,8,9,10}$ aldehydes during heat-promoted lipid autoxidation (Jensen et al., 1995). However, methyl ketones can also be formed through direct decarboxylation of β-ketoacids present in raw milk. Milk fat contains approximately 1% lipids in which oxo fatty acids of various chain lengths are esterified to glycerol. These oxo fatty acids can be liberated as β-ketoacids and decarboxylated to C_{6-16} methyl ketones when the fat is heated in the presence of water (Grosch, 1982; Jensen et al., 1995).

CONCLUSIONS

The technique developed in this study allowed for the accurate quantification of a large number of important flavor compounds present in milk. The method is simple, fast, and reproducible and can be used to analyze a large number of samples. The HS-SPME technique

in the samples analyzed suggested that this compound is not an important contributor to the aroma of milk.

Dimethyl sulfide was the only sulfur compound detected in the present work probably due to the poor flame-ionization detector sensitivity to sulfur-con-

Table 3. Odor activity values (OAV)[1] for some volatile compounds in commercial milk samples.

Compound	Aroma threshold[3] (µg/kg)	Raw 1%	Raw 3%	UHT 1%	UHT 3%	Past A 0%	Past A 1%	Past A 2%	Past A 3%	Past B 0%	Past B 1%	Past B 2%	Past B 3%
Ketones													
2,3-Butanedione	5	<0.1	<0.1	0.6	1.4	0.4	0.1	1.9	0.1	1.3	0.3	0.3	0.4
2-Heptanone	5	0.2	0.1	4.4	6.8	0.4	0.1	0.3	0.2	0.1	<0.1	0.1	0.1
2-Nonanone	5	<0.1	<0.1	7.0	10.5	0.1	<0.1	0.1	0.1	0.1	<0.1	<0.1	0.1
Aldehydes													
2-Methylpropanal	0.7	0.5	0.5	3.2	3.6	3.0	0.3	1.0	0.7	0.3	0.4	0.6	0.4
3-Methylbutanal	0.04	0.7	<0.1	21.2	28.5	0.5	0.5	4.2	1.5	0.7	4.7	4.2	0.2
2-Methylbutanal	0.9	0.5	1.0	0.6	1.0	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.1
Hexanal	4.5	1.0	0.5	0.3	2.8	0.6	0.1	0.3	0.1	1.1	0.3	0.1	0.1
2-Furaldehyde	3000	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Heptanal	3	<0.1	<0.1	0.1	0.5	<0.1	<0.1	<0.1	<0.1	0.1	<0.1	<0.1	<0.1
Octanal	0.7	0.6	0.7	0.6	1.3	0.1	0.1	0.3	0.1	0.2	0.1	0.1	0.2
Nonanal	1	1.4	1.3	1.7	3.9	1.2	0.6	1.2	1.1	1.8	0.5	0.2	0.4
Decanal	0.1	24.2	27.2	59.2	66.8	8.2	27.4	16.7	59.3	<0.1	7.9	12.6	16.1
Ester													
Ethyl acetate	5	<0.1	<0.1	0.4	0.4	<0.1	0.1	<0.1	<0.1	0.2	0.1	0.2	<0.1
Sulfur													
Dimethyl sulfide	2	3.7	4.0	11.1	10.7	4.2	3.6	3.3	4.2	5.7	5.7	7.0	8.0
Alcohol													
3-Methylbutanol	250	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1

[1]OAV = Concentration / reported threshold.

[2]Past A and Past B = Two brands of commercial pasteurized milk.

[3]Aroma thresholds measured in water (Rychilk et al., 1998).

is very sensitive and allows for the quantification of low concentrations of off-flavor compounds in milk samples. This technique was used successfully to study the thermally derived off-flavor compounds such as aldehydes, ketones, and dimethyl sulfide in milk subjected to different thermal processes. Due to its accurate determination of the compounds of interest, the simple steps, and short time required for the extraction and analysis, the technique developed in this study has a high potential to achieve rapid quantitative analysis of volatiles in milk when large numbers of samples need to be analyzed.

ACKNOWLEDGMENTS

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