

## Quantification of Trace Volatile Sulfur Compounds in Milk by Solid-Phase Microextraction and Gas Chromatography–Pulsed Flame Photometric Detection

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### ABSTRACT

Volatile sulfur compounds have been reported to be responsible for the sulfurous off-flavors generated during the thermal processing of milk; however, their analysis has been a challenge due to their high reactivity, high volatility, and low sensory threshold. In this study, reactive thiols were stabilized and the volatile sulfur compounds in milk were extracted by headspace solid-phase microextraction, and analyzed by gas chromatography and pulsed-flame photometric detection. Calibration curves for 7 sulfur-containing compounds were constructed in milk by the standard addition technique. Raw, pasteurized, and UHT milk samples with various fat contents were analyzed. Compared with raw and pasteurized samples, UHT milk contained substantially higher concentrations of hydrogen sulfide, methanethiol, carbon disulfide, dimethyl trisulfide, and dimethyl sulfoxide. The high odor activity values calculated for methanethiol and dimethyl trisulfide suggested that these 2 compounds, in addition to dimethyl sulfide reported in a previous study, could be the most important contributors to the sulfurous note in UHT milk.

**Key words:** milk off-flavor, volatile sulfur, solid-phase microextraction, pulsed-flame photometric detection

### INTRODUCTION

Thermal processing of milk can generate off-flavors. Although pasteurization of milk (typically at 72°C for 15 s) can impart a slight cooked, sulfurous note, it is acceptable to most consumers. Ultrahigh temperature sterilization (135 to 150°C for 3 to 5 s) can induce strong sulfurous, cooked, cabbage-like notes in milk (Shipe, 1980), thus limiting its acceptance (Steely, 1994). Researchers have identified that hydrogen sulfide ( $\text{H}_2\text{S}$ ), methanethiol ( $\text{MeSH}$ ), carbon disulfide ( $\text{CS}_2$ ), dimethyl sulfide ( $\text{DMS}$ ), dimethyl disulfide ( $\text{DMDS}$ ), and di-

methyl trisulfide ( $\text{DMTS}$ ) are related to this cooked off-flavor defect (Shipe, 1980; Christensen and Reineccius, 1992; Simon and Hansen, 2001; Datta et al., 2002). Several other sulfur compounds including benzothiazole, dimethyl sulfoxide ( $\text{DMSO}$ ), dimethyl sulfone ( $\text{Me}_2\text{SO}_2$ ), carbonyl sulfide ( $\text{COS}$ ), sulfur dioxide, butanethiol, and thiophene have been found in heated milk, but their roles in milk flavor have not been well elucidated (Scanlan et al., 1968; Shibamoto et al., 1980; Shipe, 1980; Moio et al., 1994).

Most sulfur compounds have distinct aroma characteristics; thus, analysis of volatile sulfur compounds is particularly important. However, reliable quantification of sulfur compounds is challenging due to their high reactivity and low concentration in foods (Chin and Lindsay, 1994; Haberhauer-Troyer et al., 2000; Murray, 2001; Nielsen and Jonsson, 2002). Numerous extraction methods, including static headspace, purge and trap, and solvent-assisted flavor evaporation (Mussinan and Keelan, 1994; Steely, 1994; Contarini et al., 1997; Bendall, 2001; Toso et al., 2002) have been evaluated to analyze sulfur compounds. These methods are either time consuming, require an exhaustive concentration procedure that often leads to the loss of the more volatile compounds such as  $\text{H}_2\text{S}$ ,  $\text{MeSH}$ , and  $\text{DMS}$ , or cause  $\text{MeSH}$  oxidation and other artifact formation (Steffen and Pawliszyn, 1996). Headspace solid-phase microextraction ( $\text{HS-SPME}$ ) can substantially shorten analysis time, reduce sample manipulation, and minimize artifact formation (Wercinski and Pawliszyn, 1999).

The widely used flame-ionization detector is not sufficiently sensitive for the detection of most sulfur compounds present in milk (Scanlan et al., 1968; Rerkrai et al., 1987; Contarini et al., 1997; Vazquez-Landaverde et al., 2005). Although the flame photometric detector has been often utilized for detection of sulfur compounds, its application poses difficulties due to reduced response with coeluting hydrocarbons (quenching) besides its inconsistent selectivity and sensitivity (Burmeister et al., 1992; Aguerre et al., 2001; Simon and Hansen, 2001). The pulsed-flame photometric detector ( $\text{PFPD}$ ) can overcome these limitations and has rapidly found application as a sulfur-specific detector (Jing and

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Amirav, 1997; Aguerre et al., 2001; Lestremou et al., 2003). Whereas flame photometric detection uses a continuous flame, PFPD uses a pulsed flame that ignites 2 to 4 times per second to burn compounds eluting from the gas chromatography (GC) column. By setting a timed "gate delay" for the response, the luminescence emission of hydrocarbons can be eliminated from that of sulfur compounds because their emission times are different. Thus, the PFPD produces cleaner chromatograms than the flame photometric detector, making it possible to detect sulfur compounds at low concentrations (Amirav and Jing, 1995). Solid-phase microextraction coupled to GC/PFPD has been successfully used for the analysis of volatile sulfur compounds in several foods and beverages (Pelusio et al., 1995; Hill and Smith, 2000; Fan et al., 2002; Dreher et al., 2003; Burbank and Qian, 2005b; Fang and Qian, 2005). However, few studies reported the accurate quantification of volatile sulfur compounds in foods (Fang and Qian, 2005). Although DMS in milk can be easily quantified by SPME extraction and gas chromatography-flame photometric detection (Vazquez-Landaverde et al., 2005), other trace volatile sulfur compounds cannot be quantified due to the poor sensitivity of this detector. Therefore, the objective of this study was to develop a rapid HS-SPME/GC-PFPD quantification technique for trace volatile sulfur compounds considered important to the flavor of commercially processed milk.

## MATERIALS AND METHODS

### Chemical Standards

Dimethyl disulfide, DMTS, DMSO, Me<sub>2</sub>SO<sub>2</sub>, and isopropyl disulfide (IPDS) were purchased from Aldrich Chemical Co. Inc. (Milwaukee, WI); DMS was purchased from TCI America Inc. (Portland, OR); CS<sub>2</sub> was from EMD Chemicals Inc. (Gibbstown, NJ); and ethyl methyl sulfide (EtMeS) was from TCI-EP Ltd. (Tokyo, Japan).

A cylinder of gaseous MeSH was purchased from Aldrich Chemical Co. Inc. (St Louis, MO) and used to prepare a 34.1 g/kg stock solution by bubbling MeSH gas into cold methanol. The solution was kept at -17°C and used within 2 wk. All glassware used for the MeSH solution was deactivated by soaking it in a 5% dimethyl-dichlorosilane solution in toluene, and then rinsing it with toluene, methanol, and finally water.

A 1.08 g/kg stock solution of H<sub>2</sub>S was prepared by dissolving 0.077 g of sodium sulfide (Na<sub>2</sub>S·9H<sub>2</sub>O, Sigma) in 10 g of a 50 mM phosphate buffer (pH = 8.5). The solution was then diluted with the same buffer to achieve an H<sub>2</sub>S concentration of 15.9 mg/kg and kept at 4°C before use (no more than 1 wk).

Carbonyl sulfide was prepared according to a published method (Metrohm, 2001) with some modification. Concentrated sulfuric acid was added dropwise to potassium thiocyanate salt (both from Mallinckrodt/J. T. Baker Inc., Phillipsburg, KY) in a stoppered Erlenmeyer flask. The generated COS gas was trapped by bubbling into a separate flask containing cold distilled water. The resulting solution was stored at 4°C, and used no later than 1 d after preparation.

### Milk Samples

Raw homogenized milk samples with 1 and 3.25% fat were obtained locally (Lochmead Farms, Junction City, OR). Sodium azide (0.02%) was added and the samples were stored at -17°C until analyzed (no more than 3 d). Pasteurized milk samples with 0, 1, 2, and 3.25% fat content from 2 different commercial brands (A and B) were purchased locally, stored at 4°C, and analyzed before their expiration date. Ultra-high temperature milks with 1 and 3.25% fat were purchased in Mexico (Leche Araceli, Grupo Fomento Queretano, Embotelladora la Victoria, Queretaro, Mexico), stored at 4°C, and analyzed before their expiration date.

### SPME

A Stableflex 85-μm carboxen-polydimethylsiloxane fiber (Supelco, Bellefonte, PA) was used. Before use, the fiber was conditioned in a split/splitless GC injector port at 300°C for 90 min. The fiber was then placed in the SPME adapter of a CombiPAL autosampler (CTC Analytics AG, Zwingen, Switzerland) fitted with a vial heater. The same fiber was used to complete all experimental measurements. The samples were preequilibrated for 5 min at 30°C under agitation at 500 rpm. The extraction was performed at 30°C for 15 min under agitation at 250 rpm.

### GC-PFPD

A Varian CP-3800 gas chromatograph (Varian Inc., Walnut Creek, CA) equipped with a pulsed-flame photometric detector was used. Volatile compounds extracted with the SPME fiber were desorbed at 300°C for 6 min in the injector. The inlet liner was previously deactivated with 15% N,O-bis(trimethylsilyl)trifluoroacetamide in hexane. The injector was maintained in splitless mode for 4 min. Separation of analytes was achieved using a fused silica capillary column (DB-FFAP, 30 m × 0.32 mm, 1.0-μm film; Agilent Technologies, Inc., Palo Alto, CA). Nitrogen was used as the carrier gas at 2 mL/min. The oven temperature was kept at 35°C for 5 min, increased to 150°C at 10°C/min, held for 1 min, and

**Table 1.** Calibration curves for trace volatile sulfur compounds<sup>1</sup> in milk

Compound	Internal standard	Calibration concentrations	Regression equation <sup>2</sup>	R <sup>2</sup>	Quantification limit <sup>3</sup>
H <sub>2</sub> S	EtMeS	2.6, 5.3, 7.9, 10.6, 13.3, and 15.9 μg/kg	$y = 0.084x$	0.955	0.005 μg/kg
MeSH		3.4, 6.8, 10.2, 13.6, 17, and 20.4 μg/kg	$y = 0.101x$	0.993	0.004 μg/kg
CS <sub>2</sub>	IPDS	10, 20, 30, 40, 50, and 60 ng/kg	$y = 11.383x$	0.974	0.033 ng/kg
DMDS		2, 4, 6, 8, 10, and 12 ng/kg	$y = 48.544x$	0.995	0.021 ng/kg
DMTS		3.3, 6.6, 10, 13.3, 16.6, and 20 ng/kg	$y = 11.655x$	0.947	0.088 ng/kg
DMSO		100, 200, 300, 400, 500, and 600 μg/kg	$y = 0.0009x$	0.976	1.145 μg/kg
Me <sub>2</sub> SO <sub>2</sub>		0.6, 1, 1.4, 1.8, 2.2, and 2.6 mg/kg	$y = 0.0003x$	0.966	0.003 mg/kg

<sup>1</sup>H<sub>2</sub>S = Hydrogen sulfide; EtMeS = ethyl methyl sulfide; MeSH = methanethiol; CS<sub>2</sub> = carbon disulfide; DMDS = dimethyl disulfide; IPDS = isopropyl disulfide; DMTS = dimethyl trisulfide; DMSO = dimethyl sulfoxide; and Me<sub>2</sub>SO<sub>4</sub> = dimethyl sulfone.

<sup>2</sup>y = Squared root (compound area/internal standard area); x = (compound)/(internal standard).

<sup>3</sup>Quantification limit calculated as the concentration that gives a signal 10 times that of noise.

then increased to 220°C at 20°C/min, with a final holding time of 5 min. The PFPD was operated in the sulfur mode at 300°C and 500 V with air 1 at 17 mL/min, air 2 at 10 mL/min, and hydrogen at 14 mL/min.

### Quantification

A standard stock solution containing 20.4 mg/kg of MeSH, 60 μg/kg of CS<sub>2</sub>, 12 μg/kg of DMDS, 20 μg/kg of DMTS, and 600 mg/kg of DMSO was prepared in methanol. The standard stock solution was then diluted with methanol to 1:2, 1:3, 1:4, 1:5, and 1:6 strengths. The H<sub>2</sub>S stock solution (15.9 mg/kg) was diluted with 50 mM phosphate buffer (pH = 8.5) to 1:2, 1:3, 1:4, 1:5, and 1:6 strength. A separate aqueous solution containing 2.6 g/kg of Me<sub>2</sub>SO<sub>2</sub> was prepared using distilled water and then diluted to 2.2, 1.8, 1.4, 1, and 0.6 g/kg concentrations. To construct the calibration curves, 10-g aliquots of raw milk (3.25% fat) adjusted to pH 2.6 with tartaric acid were spiked with 0.01 g of each of the solutions to give the targeted final added concentration for each compound (Table 1). Finally, 0.01 g of the internal standard solution containing 1.63 mg/kg of EtMeS and 0.61 mg/kg of IPDS in methanol was added to the spiked milk samples. The volatile sulfur compounds were extracted by HS-SPME and analyzed using GC-PFPD. Triplicate analysis at each concentration level and linear regression plots were used to construct calibration curves of the concentration ratio [(compound concentration)/(internal standard concentration)] vs. the response ratio [squared root of the (area of compound)/(area of internal standard)].

Ten grams of milk samples (raw with 1 and 3.25% fat; pasteurized brands A and B with 0, 1, 2, and 3.25% fat; and UHT with 1 and 3.25% fat) were adjusted to pH 2.6 with tartaric acid. Internal standard solution (0.02 g) diluted to half strength (0.81 mg/kg of EtMeS and 0.30 mg/kg of IPDS) was added and the volatile sulfur compounds were analyzed using the HS-SPME/

GC-PFPD procedure described previously. The concentration of volatile compounds was calculated based on the squared root of the peak area ratio of the compound to the internal standard. All samples were analyzed in triplicate.

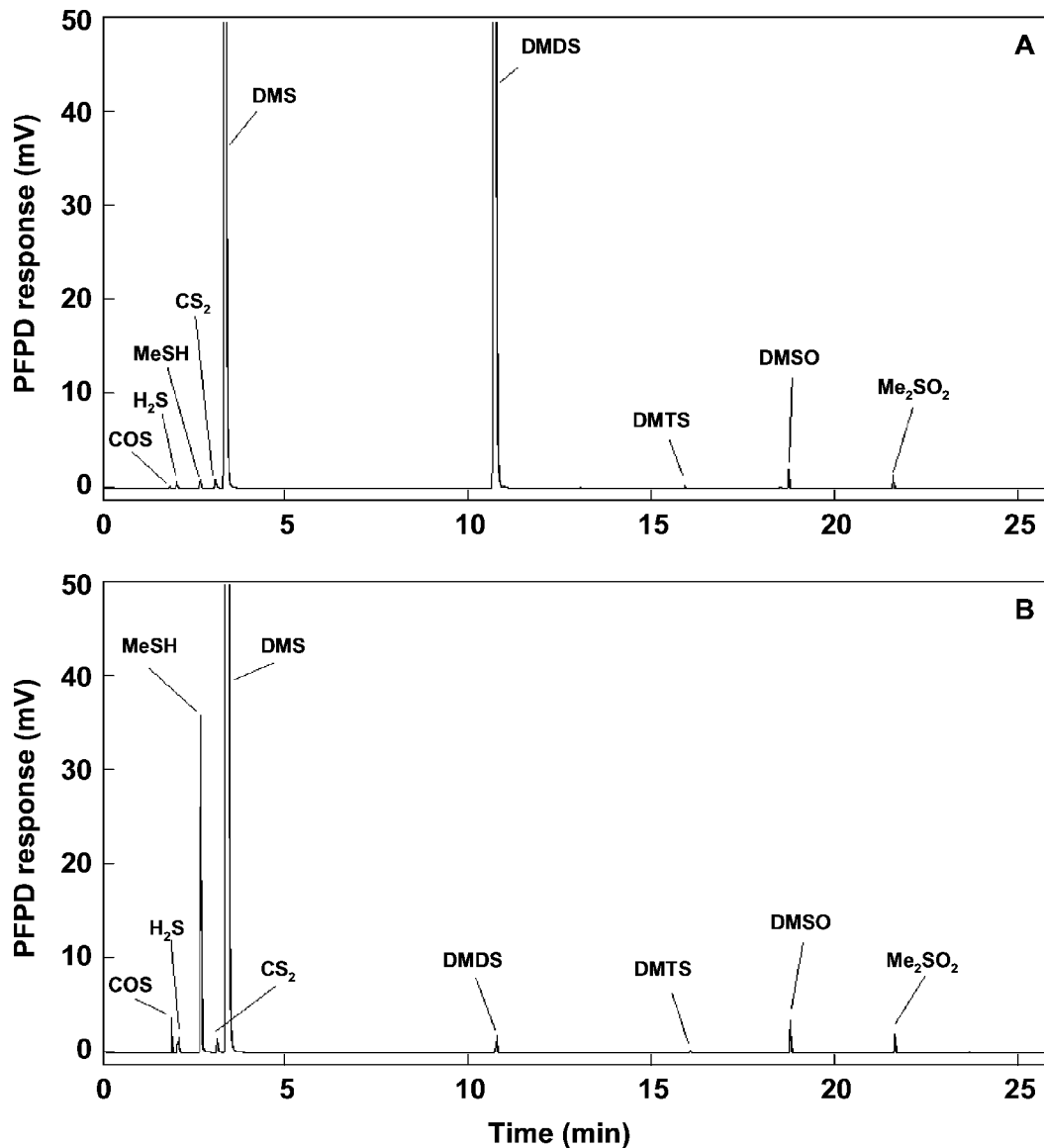
### Statistical Analysis

Statistical evaluations including ANOVA, linear regression, and Tukey's honestly significant difference ( $\alpha = 0.05$ ) were conducted using Statgraphics Plus 5.0 (Manugistics Inc., Rockville, MD).

## RESULTS AND DISCUSSION

### Stabilization of Volatile Sulfur Compounds in Milk Matrix

It has been reported that sulfur compounds are extremely reactive and artifacts can be formed during analysis (Block and Calvey, 1994; Haberhauer-Troyer et al., 2000). In this work, all standards were tested individually for stability and artifact formation during sample extraction and analysis. Most of the compounds were stable and only gave a single chromatographic peak. However, MeSH showed significant DMDS artifact formation (Figure 1A). This observation was consistent with previous reports that MeSH in aqueous solutions can be readily oxidized to form DMDS and DMTS probably caused by the presence of oxygen, transition metals, or free radicals (Chin and Lindsay, 1994; Haberhauer-Troyer et al., 2000). This artifact formation makes the quantitative analysis of volatile sulfur compounds in food unreliable. Recently, methanethiol has been stabilized in cheese and wine matrices (Burbank and Qian, 2005a,b; Fang and Qian, 2005) and quantitative analysis of volatile sulfur compounds in wine was achieved. Similar approaches were attempted in this study to stabilize MeSH in milk. The stability of the MeSH standard was tested by spiking it separately into



**Figure 1.** Methanethiol (MeSH) stability and dimethyl disulfide formation measured by pulsed-flame photometric detector (PFPD) in 3.25% fat raw milk spiked with 7  $\mu\text{g}/\text{kg}$  of MeSH; A) pH = 6.6; B) pH = 2.6. COS = Carbonyl sulfide;  $\text{H}_2\text{S}$  = hydrogen sulfide;  $\text{CS}_2$  = carbon disulfide; DMS = dimethyl sulfide; DMDS = dimethyl disulfide; DMTS = dimethyl trisulfide; DMSO = dimethyl sulfoxide; and  $\text{Me}_2\text{SO}_4$  = dimethyl sulfone.

pure water, ethanol, and a pH 2.6 tartaric acid aqueous solution. Minimum DMDS formation was achieved for the tartaric acid solution, suggesting that acidity could stabilize MeSH in milk. This is consistent with the work reported by Kastner et al. (2003), who found that the oxidation rate of ethanethiol, another highly reactive mercaptan, decreased exponentially when pH was lowered from 5.1 to 3.6. Therefore, an acidified matrix was considered desirable to prevent MeSH oxidation in milk. Decreasing the sample pH from 6.6 to 2.6 minimized the DMDS formation from MeSH (Figure 1B)

allowing the construction of a calibration curve by the standard addition technique. Acidic conditions were also necessary to achieve total conversion of  $\text{Na}_2\text{S}$  standard to  $\text{H}_2\text{S}$ , another important sulfur compound to be quantified in this work.

#### **Quantification of Sulfur Compounds in Commercial Milk Samples**

The volatile sulfur compounds identified in milk samples were COS,  $\text{H}_2\text{S}$ , MeSH,  $\text{CS}_2$ , DMS, DMDS, DMTS,

**Table 2.** Concentration of volatile sulfur compounds in various milk samples

Compound <sup>2</sup>	Milk samples <sup>1</sup>												CV <sup>3</sup>
	Raw 1%	Raw 3.25%	UHT 1%	UHT 3.25%	A 0%	A 1%	A 2%	A 3.25%	B 0%	B 1%	B 2%	B 3.25%	
H <sub>2</sub> S (μg/kg)	1.78 <sup>b</sup>	1.21 <sup>ab</sup>	3.49 <sup>c</sup>	12.0 <sup>d</sup>	0.63 <sup>a</sup>	0.94 <sup>ab</sup>	0.59 <sup>a</sup>	0.87 <sup>ab</sup>	0.62 <sup>a</sup>	0.51 <sup>a</sup>	0.48 <sup>a</sup>	1.04 <sup>ab</sup>	<13.9
MeSH (μg/kg)	1.95 <sup>abc</sup>	4.80 <sup>d</sup>	16.1 <sup>e</sup>	23.9 <sup>f</sup>	1.65 <sup>ab</sup>	1.92 <sup>abc</sup>	3.56 <sup>bcd</sup>	3.57 <sup>bcd</sup>	5.97 <sup>d</sup>	4.65 <sup>d</sup>	0.41 <sup>a</sup>	4.32 <sup>cd</sup>	<12.6
CS <sub>2</sub> (ng/kg)	17.1 <sup>a</sup>	22.1 <sup>ab</sup>	56.4 <sup>e</sup>	58.9 <sup>e</sup>	16.3 <sup>a</sup>	17.8 <sup>ab</sup>	18.0 <sup>ab</sup>	24.0 <sup>b</sup>	32.3 <sup>c</sup>	40.3 <sup>d</sup>	31.3 <sup>c</sup>	32.8 <sup>c</sup>	<10.5
DMDS (ng/kg)	10.3 <sup>b</sup>	33.4 <sup>ef</sup>	30.3 <sup>de</sup>	32.8 <sup>def</sup>	2.24 <sup>a</sup>	10.21 <sup>b</sup>	17.1 <sup>c</sup>	28.3 <sup>d</sup>	9.37 <sup>b</sup>	35.8 <sup>f</sup>	10.1 <sup>b</sup>	17.2 <sup>c</sup>	<11.7
DMTS (ng/kg)	10.7 <sup>cd</sup>	36.7 <sup>h</sup>	32.9 <sup>h</sup>	47.3 <sup>i</sup>	1.18 <sup>a</sup>	7.27 <sup>bc</sup>	13.8 <sup>def</sup>	16.6 <sup>fg</sup>	3.92 <sup>ab</sup>	12.2 <sup>de</sup>	15.7 <sup>efg</sup>	18.8 <sup>g</sup>	<8.3
DMSO (mg/kg)	0.45 <sup>d</sup>	1.26 <sup>g</sup>	0.70 <sup>e</sup>	1.46 <sup>h</sup>	0.05 <sup>a</sup>	0.22 <sup>b</sup>	0.30 <sup>bc</sup>	0.68 <sup>e</sup>	0.08 <sup>a</sup>	0.41 <sup>cd</sup>	0.82 <sup>f</sup>	0.79 <sup>ef</sup>	<8.9
Me <sub>2</sub> SO <sub>2</sub> (mg/kg)	1.14 <sup>cd</sup>	2.64 <sup>e</sup>	0.78 <sup>c</sup>	1.26 <sup>cd</sup>	0.13 <sup>a</sup>	0.77 <sup>c</sup>	0.74 <sup>bc</sup>	1.39 <sup>d</sup>	0.23 <sup>ab</sup>	1.03 <sup>cd</sup>	4.72 <sup>f</sup>	2.21 <sup>e</sup>	<13.6

<sup>a-i</sup>Different letters for the same compound indicate significant difference between samples (Tukey HSD 95%).

<sup>1</sup>Raw 1% and Raw 3.25% = raw milk with 1 and 3.25% fat, respectively; UHT 1% and UHT 3.25% = UHT processed milk with 1 and 3.25% fat, respectively; A 0%, A 1%, A 2%, and A 3.25% = pasteurized milk of brand A with 0, 1, 2, and 3.25% fat, respectively; B 0%, B 1%, B 2%, and B 3.25% = pasteurized milk of brand B with 0, 1, 2, and 3.25% fat, respectively.

<sup>2</sup>H<sub>2</sub>S = Hydrogen sulfide; MeSH = methanethiol; CS<sub>2</sub> = carbon disulfide; DMDS = dimethyl disulfide; DMTS = dimethyl trisulfide; DMSO = dimethyl sulfoxide; and Me<sub>2</sub>SO<sub>4</sub> = dimethyl sulfone.

<sup>3</sup>CV = % Coefficient of variation.

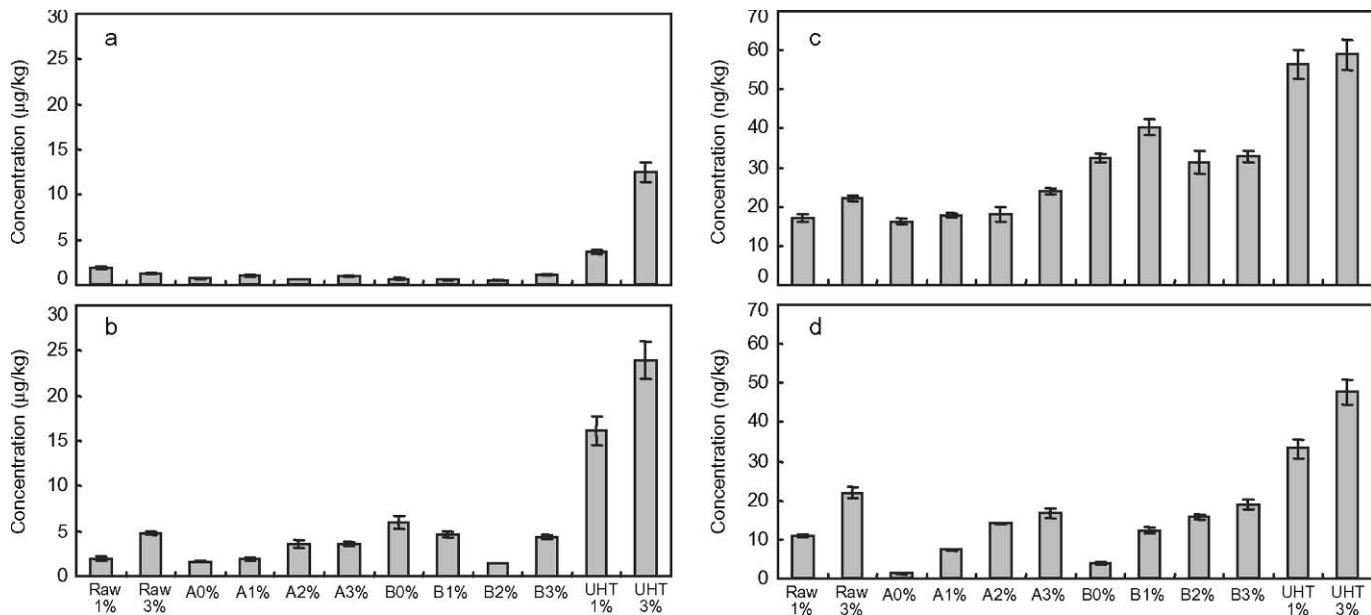
DMSO, and Me<sub>2</sub>SO<sub>2</sub>. When the extraction conditions were optimized for the analysis of other trace sulfur compounds by PFPD, DMS reached the saturation limit of the PFPD and was therefore not quantified in this study. Quantification of DMS by GC and flame-ionization detection was previously reported (Vazquez-Landaverde et al., 2005) because it is the most abundant sulfur compound in milk and its quantification can be easily achieved. Quantification of COS was not performed because it is an odorless sulfur compound and it is not important to the flavor of heated milk (Simon and Hansen, 2001).

Calibration curves with high correlation coefficients ( $R^2 > 0.94$ ) and low quantification limits (signal equal to 10 times the noise) for 7 sulfur compounds were obtained (Table 1). The quantification limits were below their sensory thresholds for all compounds included in this study. The more volatile H<sub>2</sub>S and MeSH could be easily extracted and had low quantification limits whereas the less volatile DMSO and Me<sub>2</sub>SO<sub>2</sub> were harder to extract. Carbon disulfide, DMDS, and DMTS had the highest quantification sensitivity possibly due to their high affinity to the carboxen/polydimethylsiloxane SPME fiber. Adequate repeatability of the measurements (coefficient of variation < 13.6%) was achieved in this study considering the high volatility and reactivity of some of the compounds analyzed.

Commercial milk samples processed with various heat treatments were analyzed using this technique and the results showed that the different concentration of volatile sulfur compounds could be related to the difference in heat treatment (Table 2 and Figure 2). At the same fat level, UHT milk contained significantly higher concentrations of H<sub>2</sub>S, MeSH, CS<sub>2</sub>, DMTS, and DMSO than did raw and pasteurized milk. Hydrogen sulfide was the sulfur compound with the highest in-

crease (up to 7-fold) in concentration. Methanethiol increased about 5-fold, and CS<sub>2</sub> almost doubled. There was a significant difference between some pasteurized milk samples when compared with raw milk at the same fat level. Pasteurized sample B 1% fat showed higher concentrations of MeSH, CS<sub>2</sub>, and DMDS compared with raw and pasteurized brand A milk with 1% fat. Contrary to expectations, pasteurized samples A and B with 3.25% fat had lower concentrations of DMDS, DMTS, and DMSO than raw samples with 3.25% fat, whereas other sulfur compounds were not different. The “cooked” note perceived in pasteurized sample B with 3.25% fat was stronger compared with brand A and raw milks with 3.25% fat. In a previous work, we found that B 3.25% had a significantly higher concentration of DMS than raw and pasteurized brand A milk samples (Vazquez-Landaverde et al., 2005), whereas the thermally formed ketones and aldehydes were not significantly different.

Because aroma impact is dependent not only on concentration, but also on sensory threshold, the odor activity value (OAV = concentration/sensory threshold) was calculated (Table 3). In this study, OAV values for MeSH and DMTS in UHT milk samples were much greater than 1, indicating that their concentrations were much higher than their sensory thresholds, thus contributing to the aroma. According to the magnitude of its OAV, MeSH could be the most important sulfur-containing contributor to the aroma of UHT milk, at a concentration 80 to 119 times higher than its reported threshold. The second most important contributor could be DMTS, with a concentration 4 to 6 times higher than its reported threshold. The concentration of H<sub>2</sub>S was slightly higher than its reported threshold only for one UHT sample, indicating that this compound could be a contributor to the sulfurous aroma in heated milk,



**Figure 2.** Concentration of volatile sulfur compounds in commercial milk samples. a) Hydrogen sulfide, b) methanethiol, c) carbon disulfide, and d) dimethyl trisulfide. Raw 1% and Raw 3.25% = raw milk with 1 and 3.25% fat, respectively; UHT 1% and UHT 3.25% = UHT processed milk with 1 and 3.25% fat, respectively; A 0%, A 1%, A 2%, and A 3.25% = pasteurized milk of brand A with 0, 1, 2, and 3.25% fat, respectively; B 0%, B 1%, B 2%, and B 3.25% = pasteurized milk of brand B with 0, 1, 2, and 3.25% fat, respectively.

albeit to a lesser degree than MeSH and DMTS. A previous study (Vazquez-Landaverde et al., 2005) suggested that DMS could be an important contributor to the aroma of both heated and fresh milk with an OAV of 3 to 11. The concentration of DMS was almost 3 times higher in UHT milk than in raw milk, whereas pasteurized brand B milk samples (2 and 3.25% fat) had significantly higher concentrations ( $P < 0.05$ ) of DMS than raw and pasteurized brand A milk. This trend with the severity of the heat treatment was in agreement with the concentration of the trace volatile sulfur compounds observed in this study.

Volatile sulfur compounds may be present originally in milk at low concentrations because of the cow's metabolism (Toso et al., 2002). They can also be generated during the thermal processing of milk (Shibamoto et al., 1980; Steely, 1994). It has been reported that the volatile sulfur compounds formed during the high-temperature heating process are mainly responsible for the development of the cooked flavor defect (Christensen and Reineccius, 1992; Steely, 1994; Simon and Hansen, 2001; Datta et al., 2002). Upon heating, sulfur-containing amino acids from whey proteins, mainly  $\beta$ -LG, as well as proteins associated with the fat globule mem-

**Table 3.** Odor activity values (concentration/reported threshold) of some volatile sulfur compounds in milk samples

Compound <sup>2</sup>	Aroma threshold <sup>3</sup>	Milk sample <sup>1</sup>											
		Raw 1%	Raw 3.25%	UHT 1%	UHT 3.25%	A 0%	A 1%	A 2%	A 3.25%	B 0%	B 1%	B 2%	B 3.25%
H <sub>2</sub> S	10 µg/kg <sup>4</sup>	0.1	0.1	0.3	1.2	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.1
MeSH	0.2 µg/kg <sup>4</sup>	9.7	24	80	119	8.2	9.6	17	17	29	23	2.0	21
CS <sub>2</sub>	5 µg/kg <sup>5</sup>	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
DMDS	0.16 µg/kg <sup>4</sup>	<0.1	0.2	0.1	0.2	<0.1	<0.1	0.1	0.1	<0.1	0.2	<0.1	0.1
DMTS	8 ng/kg <sup>4</sup>	1.3	4.5	4.11	5.9	0.1	0.9	1.7	2.0	0.4	1.5	1.9	2.3

<sup>1</sup>Raw 1% and Raw 3.25% = raw milk with 1 and 3.25% fat, respectively; UHT 1% and UHT 3.25% = UHT processed milk with 1 and 3.25% fat, respectively; A 0%, A 1%, A 2%, and A 3.25% = pasteurized milk of brand A with 0, 1, 2, and 3.25% fat, respectively; B 0%, B 1%, B 2%, and B 3.25% = pasteurized milk of brand B with 0, 1, 2, and 3.25% fat, respectively.

<sup>2</sup>H<sub>2</sub>S = Hydrogen sulfide; MeSH = methanethiol; CS<sub>2</sub> = carbon disulfide, DMDS = dimethyl disulfide; and DMTS = dimethyl trisulfide.

<sup>3</sup>Values measured in water.

<sup>4</sup>Rychilk et al., 1998.

<sup>5</sup>ETS Laboratories, 2002.

brane (Parry, 1974; de Koning et al., 1990; Chan and Reineccius, 1994; Tressl et al., 1994; Zheng and Ho, 1994) can be released from the proteins and form sulfides, thiols, and other minor sulfur-containing compounds.

Methanethiol is important to the flavor of many foods due to its low detection threshold. It has a strong and unpleasant cabbage, sulfur-like aroma (Rychlik et al., 1998). A correlation between the increase in concentration of this compound and the increase in the "cooked" flavor defect has been reported previously (Badings et al., 1981; Christensen and Reineccius, 1992; Simon and Hansen, 2001). Methanethiol is thought to be generated from methionine during heat treatment by breakdown of the sulfur-bearing side chain (Damodaran, 1996) but the actual pathway has not been elucidated. Despite the importance of MeSH to the flavor of milk and dairy products, its study has been limited due to its high reactivity and volatility. Only few studies have reported reliable quantification techniques for this compound (Burbank and Qian, 2005a; Fang and Qian, 2005).

The formation of DMDS is probably due to the oxidation of MeSH (Ferreti, 1973; Chin and Lindsay, 1994). Jaddou et al. (1978) reported that DMDS concentration increased in UHT milk, but decreased in sterilized samples. Dimethyl disulfide and DMTS have a sulfur- and cabbage-like aroma (Rychlik et al., 1998) with low sensory threshold values.

The concentration of H<sub>2</sub>S in milk increases linearly with heating temperature (Hutton and Patton, 1952; Christensen and Reineccius, 1992). Also, the log concentration of H<sub>2</sub>S has a strong linear relationship with the heated flavor intensity of milk (Badings, 1978). This compound is also indirectly responsible for the formation of other sulfur compounds (Zheng and Ho, 1994). Hydrogen sulfide is produced mainly from sulphydryl groups of sulfur-containing amino acids (cysteine) in  $\beta$ -LG (Badings et al., 1981; Damodaran, 1996). Some authors suggested that this could be the most important contributor to the "cooked" flavor of milk because it is the major sulfur compound formed in heated milk (Badings et al., 1978; Jaddou et al., 1978; Badings et al., 1981; Rerkrai et al., 1987; Christensen and Reineccius, 1992) and it has a characteristic sulfur-like aroma (Rychlik et al., 1998). However, the OAV values calculated in this study indicate that H<sub>2</sub>S could be less important to the aroma of heated milk than previously thought. The OAV suggested that MSH and DMS were the 2 most important sulfur compounds in heated milk.

Although its origin has not been well elucidated, CS<sub>2</sub> has been identified as a breakdown product of other sulfur compounds (Urbach, 1993). However, there are no previous reports of a correlation between CS<sub>2</sub> and heat treatment, but our results showed that this com-

pound could be a good indicator of heat treatment. It has a sweet, ethereal, slightly green, sulfur-like aroma (Rychlik et al., 1998) but its sensory threshold is very high and the calculated OAV was less than 1, so it most likely does not contribute to the aroma.

It has been proposed that Me<sub>2</sub>SO<sub>2</sub> is produced in milk by the heat-induced oxidation of DMS via DMSO as the intermediate (Shibamoto et al., 1980). Under oxidative conditions, methionine is easily oxidized to methionine sulfoxide and methionine sulfone, which eventually breakdown to yield DMSO and Me<sub>2</sub>SO<sub>2</sub>, respectively (Damodaran, 1996). Shibamoto et al. (1980) found that Me<sub>2</sub>SO<sub>2</sub> concentration decreases when milk is subjected to treatments between 60 and 90°C, but starts to increase considerably above 90°C. Moio et al. (1994) found that the Me<sub>2</sub>SO<sub>2</sub> concentration was lower for UHT milk than for raw and pasteurized samples. Dimethyl sulfone has an aroma defined as hot milk, leather, and bovine sweat-like (Rychlik et al., 1998), but its sensory threshold is too high to be considered an important contributor to the aroma of milk.

A general trend was observed that the concentrations of H<sub>2</sub>S, MeSH, and DMTS increased with the fat level in milk (Table 2 and Figure 2). The results suggested that the concentration of sulfur compounds in milk was also affected by fat level. Although it is possible that the fat level may not have the same affect on the volatility of sulfur compounds and internal standard, it was more likely that the effects of heating on denaturation of the fat globule membrane protein were greater for samples with higher fat content, and that membrane proteins contributed to the formation of sulfides (de Koning et al., 1990).

## CONCLUSIONS

The high reactivity and volatility of sulfur compounds makes their detection and quantification in foods a challenge. Proper sample manipulation and protection eliminated artifact formation from highly reactive sulfur compounds, allowing for the accurate quantification of volatile sulfur compounds in milk. The SPME/GC-PFPD technique developed in this study was effective in detecting a wide range of sulfur-containing compounds in milk at low concentrations, and was used successfully to study the sulfur compounds in milk subjected to different thermal processes.

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