Headspace Solid-Phase Microextraction for the Analysis of Dimethyl Sulfide in Beer

Keywords: *Solid-phase microextraction, SPME; dimethyl sulfide; beer; gas chromatography; headspace analysis; flame photometric detector, FPD*

INTRODUCTION

Dimethyl sulfide (DMS) is the most abundant sulfur compound in beer with typical values of 14−144 μg/L, depending on style, and a sensory threshold of 30−45 μg/L (Meilgaard, 1982). At low levels DMS contributes to palate fullness and overall beer aroma. However, DMS has a powerful off-odor when present at high levels, giving beer a cooked vegetable or cabbage-like odor. Therefore, rapid, reliable, and inexpensive methods are needed for the analysis of DMS to ensure uniform beer quality.

Static and dynamic headspace samplings of beer volatiles coupled with gas chromatography (GC) have commonly been used for DMS analysis (Peppard, 1985, 1988; Lee and Siebert, 1986; Mundy, 1991; Burmeister et al., 1992). However, static and dynamic headspace sampling techniques can be problematic. Static headspace sampling using gastight syringes can present problems of sampling gas volumes reproducibly. Static headspace sampling can also be adversely affected by the carbonation in beer; therefore, sodium hydroxide is often used to remove CO_2 before analysis (Mundy, 1991). Purge and trap methods require special apparatus and are relatively time-consuming.

Solid-phase microextraction (SPME) is a rapid, inexpensive, solvent-free extraction technique that requires no modification of the GC inlet other than possible use of a narrower bore injection liner than otherwise might be employed. The SPME fiber is made of fused silica coated with a polymeric adsorbent material. Volatile compounds adsorb to the SPME fiber when it is introduced into the headspace above a sample in a sealed vial. The extraction times are short (3−15 min) due to the narrow diameter of the fiber and are often chosen to coincide with the GC run time. The fiber is retracted into the SPME housing after the extraction step. Then it is inserted and exposed in the heated GC inlet, and the compounds rapidly desorb. SPME has been used for the analysis of volatile sulfur compounds in wine, truffles, and onions (Mestres et al., 1998; Pelusio et al., 1995; Jarvenpaa et al., 1998). SPME has been used for the analysis of other volatile compounds in beer, wine, vodka, orange juice, coffee, cheese, bananas, and fruit-flavored malt beverages (Jelen et al., 1998; Evans et al., 1997; Hayasaka and Bartowsky, 1999; Vas et al., 1998; Garcia et al., 1997; Ng et al., 1996; Steffen and Pawliszyn, 1996; Agelopoulos and Pickett, 1998; Yang and Peppard, 1994; Constant and Collier, 1997; Chin et al., 1996). This paper describes a rapid, sensitive, and selective method for the quantitative analysis of DMS in beer using headspace solid-phase microextraction (HS-SPME) with GC analysis and flame photometric detection.

METHODS AND MATERIALS

Instrumentation. A Hewlett-Packard 5890 Series II GC (Avondale, PA) with an HP 19256A flame photometric detector (FPD) was used to detect sulfur compounds. Carboxen-polydimethyl siloxane (carboxen-PDMS) SPME fibers were purchased from Supelco (Bellefonte, PA). The inlet was set for splitless injection, and the temperature was 250 °C. The purge valve was opened at 3 min and closed at 13 min after injection. The inlet was fitted with a straight glass liner (0.75 mm i.d., Supelco). The initial oven temperature was set for 40 °C and held for 2 min, then increased by 20 °C/min to 200 °C, and was finally held at 200 °C for 4 min. The GC was fitted with a DB-1 (dimethyl polysiloxane) column, 30 m × 0.53 mm i.d., 1.0 μm film thickness, to which 3 m of DB-Wax column (J&W Scientific, Folsom, CA) was attached (Park, 1993). The carrier gas was helium set at 4 mL/min. The detector temperature was set at 225 °C. Gas flow rates through the detector were 74 mL/min hydrogen, 110 mL/min air, and 33 mL/min helium makeup gas.

Reagents used included silicone antifoam emulsion A, (Sigma, St. Louis, MO), dimethyl sulfide (99+%, Fisher, Fair Lawn, NJ), ethyl methyl sulfide [(EMS) 99%, Aldrich, Milwaukee, WI], ethanol (95%, Rossville Gold Shield, Hayward, CA), and sodium chloride (ACS grade, Fisher).

Sampling. Glass volumetric pipets were used to measure all volumes except in cases when an automatic pipet was used (Pipetman, Gilson, France). Microliter syringes (Hamilton, Reno, NV) were used to dispense DMS and EMS and to prepare dilutions of these compounds. Analytical samples were dispensed into 10 mL headspace vials (Fisher) containing 1.5 g of NaCl. Adding excess salt to the samples increased sensitivity by shifting the equilibrium of volatile compounds to the headspace. Vials were sealed with crimp tops and silicone/Teflon-lined septa (Fisher). The vial was immersed in a 30 °C (±0.5 °C) water bath. A clamp was used to support the vial in the bath so that the water level covered all exposed glass below the crimp seal. All SPME sampling was done manually. New SPME fibers were conditioned according to manufacturer's instructions. Throughout the analysis the fiber was either in the injector or the headspace of a vial to minimize analyte loss and contamination of the fiber by ambient air. The SPME fiber was exposed to the headspace of the standard or sample for 15 min. This adsorption time was shown to be optimal for reproducible analyses. The SPME fiber was exposed in the GC inlet for 3 min during the desorption step.

Internal and Reference Standards. Solutions of DMS in 4% ethanol were used as reference standards for calibration. EMS was the internal standard (ISTD). The standard solutions were prepared fresh for each day of analysis. To minimize odor and loss due to evaporation, it was useful to work with small amounts of DMS and EMS, transferring them with microliter syringes into sealed vials of solvent. DMS standard solutions were prepared as follows: A 10.0 mL aliquot of 95% ethanol was pipetted into a 10 mL headspace vial and sealed. A 1.0 g/L solution (solution A, Figure 1) was prepared by injecting 10.0 mg of DMS into the vial using a microliter syringe. The tip of the syringe needle was placed below the surface of the liquid for all injections. All weights were measured on an analytical balance (Mettler, Hightstown, NJ). Solution B was prepared by injecting 5.0 μL of solution A into 5.0 mL of 95% ethanol to make a 1.0 mg/L solution of DMS. Solution C was prepared by injecting 100 μL of solution A into 1.0 mL of 95% ethanol for a final DMS concentration of 91 mg/L. Final solutions of DMS in 4% ethanol were prepared from solutions B and C as in Figure 1. The calibration curve was prepared for DMS concentrations at 9.9, 20, 45, 91, 136, and 163 μg/L.

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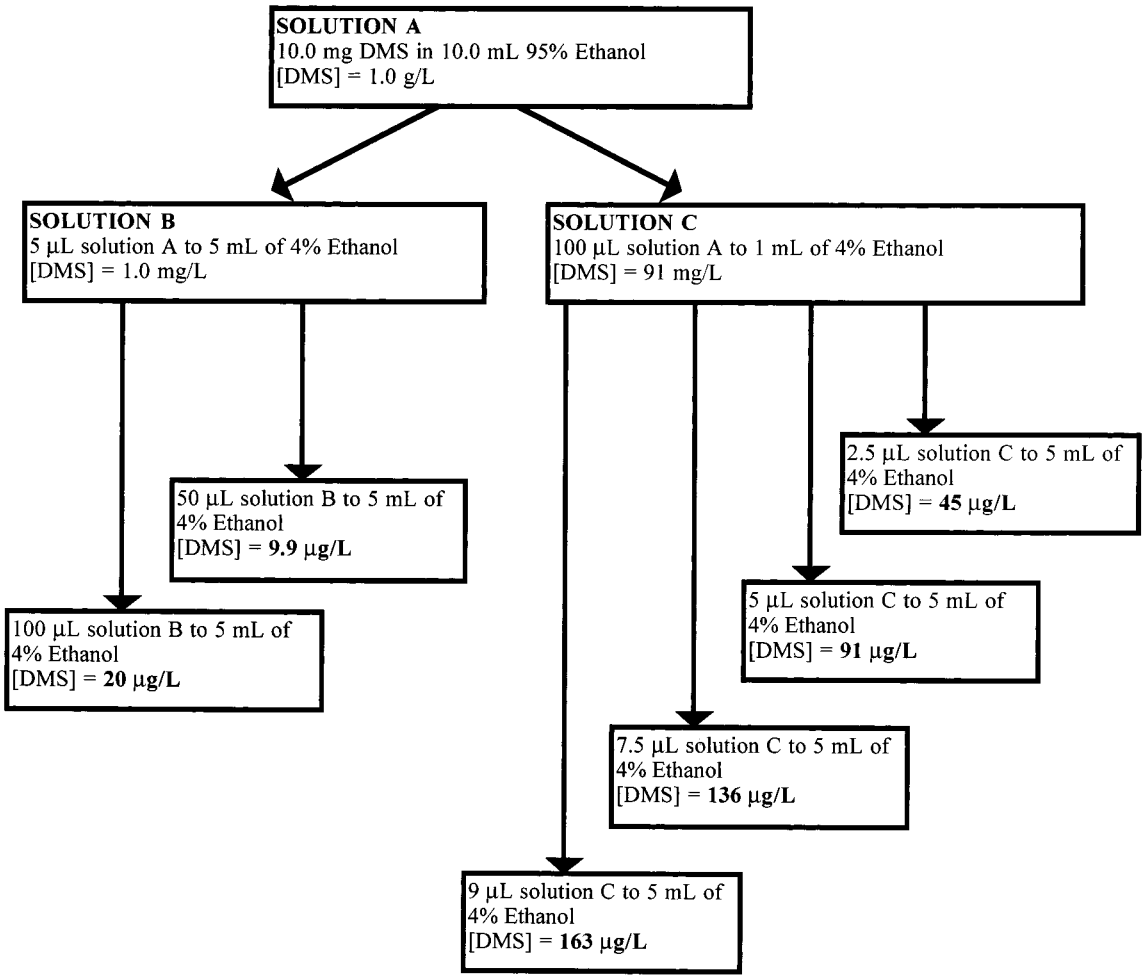

Figure 1. Dilution scheme for the preparation of DMS reference standards.

A 1.0 g/L ISTD stock solution was prepared by weighing 10 mg of EMS into 10 mL of 95% ethanol. A 5.0 μL aliquot of the 1.0 g/L ISTD stock solution was then added to 5.0 mL of 4% aqueous ethanol to yield a concentration of 1.0 mg/L. A 25 μL aliquot of the 1.0 mg/L ISTD solution was injected into each vial after the DMS addition for a final EMS concentration of 5.0 μg/L.

Spiked Beer. DMS was spiked into a commercial lager to determine recovery of the analyte in an actual beer matrix. Beer samples were prepared as follows. A cold (4 °C) 12 oz bottle of beer was poured into a 500 mL Erlenmeyer flask, and 5 drops of antifoam were added. The beer was decarbonated before analysis by stirring it with a stirring bar at moderate speed for 45 min. A portion of the beer was transferred into a 200 mL volumetric flask. A 100 mg/L ISTD stock solution was prepared by weighing 1.0 mg of EMS into 10 mL of 95% ethanol. A microliter syringe (Hamilton) was used to add 10 μL of the 100 mg/L ISTD solution to the 200 mL volumetric flask for a final EMS concentration of 5.0 μg/L. Aliquots (5 mL) of the beer/EMS solution were pipetted into the headspace vials containing 1.5 g of NaCl and sealed. DMS was injected into separate vials (procedure similar to that shown in Figure 1) to make solutions of the following strengths: 9.9, 20, 45, 91, 136, and 163 μg/L.

Analysis of Commercial Product. Bottled commercial beers (12 oz) were purchased at a local market (Davis, CA) and kept refrigerated (4 °C) until they were analyzed. The bottles were allowed to reach room temperature before analysis. The bottles were carefully opened, and 5 drops of antifoam and 17.5 μL of a 100 mg/L ISTD solution (prepared by weighing 1.0 mg of EMS into 10 mL of 95% ethanol) were added to the beer (355 mL). Then the bottle was resealed by screwing on the original crown or by using a crown sealer with a fresh crown. The bottle was gently inverted 20 times to mix and then was set aside for 5 min to equilibrate. The bottle was opened, and 5 mL of sample was carefully pipetted using an automatic pipet (Pipetman) into a 10 mL glass vial, which was then sealed with a Teflon-lined septum. The pipet was calibrated against deionized water before analysis.

RESULTS AND DISCUSSION

Calibration and Limit of Quantitation. DMS was well resolved from the EMS ISTD peak (retention times of 3.1 and 4.4 min, respectively). DMS in beer samples was identified by comparing the retention time of the pure compound in 4% ethanol and by spiking pure DMS into beer. Integrated peak area ratios (peak area of DMS/peak area of EMS) were calculated and plotted against the concentration of DMS added to each 4% ethanol solution to yield a calibration curve. A power function was fit through the data points [area ratio = $(0.013 \times [\text{DMS}]\ \text{added})^{1.76}$] with a coefficient of determination (r^2) of 0.984. DMS could be reliably detected at 1 μg/L with an estimated signal-to-noise ratio of 7:1.

The results of the curve fitting are similar to those previously reported (Lee and Siebert, 1986). FPD response is nonlinear and should theoretically respond to sulfur compounds proportional to the square of the sulfur compound concentration. Analysis of SO_2 and H_2S may approach exponent values close to 2. However, exponent values of 1.59−1.74 are more typical for DMS analysis (Lee and Siebert, 1986).

Effect of Carbonation. An experiment was done to determine if beer needed to be decarbonated before

Table 1. Comparison of Carbonated and Decarbonated Beer Analyzed by SPME ($n = 7$ for Each Sample)

sample	mean [DMS] (μg/L)	range (μg/L)	SD (μg/L)	%CV
carbonated	33.6	33.0−35.9	1.2	3.6
decarbonated	35.0	32.5−38.4	2.0	5.7

Table 2. Precision Assay for Two Levels of DMS Spiked into 4% Ethanol ($n = 7$ for Each Level)

[DMS] spiked into 4% EtOH (μg/L)	mean measd [DMS] (μg/L)	range (μg/L)	SD (μg/L)	%CV	mean % diff from theor
10	9.4	8.8−10.1	0.5	5.0	6.0
99	92.1	87.5−96.8	4.4	4.8	7.1

Table 3. Recovery of DMS Spiked into a Commercial Lager ($n = 4$ for Each Level)

[DMS] spiked into beer (μg/L)	initial measd [DMS][a] (μg/L)	measd [DMS] after spike addition (μg/L)	recovery of spike (%)
9.9	23.9	32.4	95.9
20	23.9	44.7	102.6
45	23.9	72.4	104.3
91	23.9	117.8	102.6
136	23.9	168.9	105.4
163	23.9	186.4	99.5

[a] The initial measured [DMS] is the mean of six replicates.

analysis. Two beers from the same six pack were used. One was left fully carbonated and the other was decarbonated by stirring (stir plate, moderate rpm, 45 min at 20 °C). Seven replicate vials were prepared for each beer. The results for each beer were nearly identical; carbonation does not seem to influence SPME sampling (Table 1).

Accuracy and Precision. Precision assays were conducted at 10 and 99 μg/L in 4% ethanol ($n = 7$ for each concentration, Table 2). The measured values were within 0.3−13% of the theoretical concentration with a coefficient of variation (%CV) of 4.8−5.0%. Bottles of beer from the same lot were analyzed on two separate days. The between-day mean DMS concentration was 25.3 ± 1.0 (CV = 3.9%; $n = 4$ for each day).

Recovery of DMS Spiked into Beer. A DMS spike was added to separate samples of beer from the same lot at six different levels (9.9, 20, 45, 91, 136, and 163 μg/L). The analysis was replicated four times at each level. The recovery of the DMS spike ranged from 96 to 105% for this beer (lager) matrix (Table 3).

Comparison of SPME and Static Headspace Analysis. Three lots of one brand of commercial beer that had known levels of DMS were analyzed. The DMS concentrations had been previously determined by a commercial laboratory using static headspace sampling (SHS). Measured values using HS-SPME were within 3.9−7.8% of the reported values (Table 4). HS-SPME yields similar results to SHS without the variability that may be caused by using gastight syringes or carbonation in beer.

Survey of Commercial Beer. The DMS contents of various commercial beers were tested: one ale, four lagers, and one light lager (Table 5). All of the beers were from domestic breweries and were purchased locally. The overall DMS concentration ranged from 17 to 83 μg/L. Lager A was a premium lager, and light lager A was the "light" version of that product from the same brewery. Often, light beers are brewed to have a higher alcohol content than the regular product. They are then diluted with carbonated water after fermentation. The results confirm that this dilution step can result in a lower concentration of DMS in the light beer. Lager D was chosen to test for variability in DMS concentration because it had a strong cooked vegetable aroma. There was wide variation in DMS concentration between two different lots of this beer (D.1 and D.2), but both lots had a DMS content well above the sensory threshold of 35 μg/L. Several authors have reported that lagers have a higher concentration of DMS than ales (Anderson et al., 1975; Clapperton and Piggott, 1979; Pickett et al., 1976; Meilgaard, 1982). The brief survey conducted for this method development does not allow conclusions regarding differences between lagers and ales. However, it does show that lagers may have a broad range of DMS content that is both above and below the sensory threshold for DMS.

Table 4. Comparison of SPME versus Static Headspace Sampling

lot[a]	mean [DMS] by SPME (μg/L)	[DMS] previously determined by static headspace sampling (μg/L)	% diff
A	30.4 ± 0.6	33	7.8
B	38.5 ± 2.0	40	3.9
C	45.2 ± 3.1	49	7.8

[a] Values for lot A are from four replicate samples from the same bottle. Lots B and C are from two replicate samples from two bottles each.

Table 5. Survey of DMS Concentration in Commercial Beer

sample[a]	mean [DMS] (μg/L)	SD (μg/L)	%CV
ale	41	0.6	1.6
lager A	26	0.6	2.2
light lager A	21	0.7	3.5
lager B	49	2.8	5.8
lager C	17	0.4	2.4
lager D.1	83	1.5	1.8
lager D.2	66	0.6	1.0

[a] Lagers D.1 and D.2 are bottles of the same brand from the same brewery with two different lot numbers. In this case two replicate samples were analyzed from each bottle. For all other beers, two replicate samples were analyzed from two bottles of each beer of the same lot number for a total of four data points per sample.

Conclusion. SPME is a useful sample preparation technique for analyzing DMS in beer. It is rapid, low cost, and solvent free and requires no sample preparation steps (i.e., decarbonation) or modification of the GC. When combined with selective detectors such as the FPD, it is sensitive and selective, providing quantitative results that are comparable to those of existing static headspace methods for DMS analysis. The method can be readily automated for routine quantitative analysis of beer as well as other foods and beverages.

ABBREVIATIONS USED

SPME, solid-phase microextraction; HS-SPME, headspace solid-phase microextraction; SHS, static headspace sampling; PDMS, poly(dimethylsiloxane); GC, gas chromatography; ISTD, internal standard; DMS, dimethyl sulfide; EMS, ethyl methyl sulfide; FPD, flame photometric detector; SCD, sulfur chemiluminescence detector; SD, standard deviation; %CV, coefficient of variation = (mean/SD) × 100.

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