Analysis of 2-Methylisoborneol and Geosmin in Catfish by Microwave Distillation−Solid-Phase Microextraction

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The semivolatile cyclic alcohols 2-methylisoborneol (MIB) and geosmin (GSM) impart muddy or musty flavors to water and food products. A rapid quantitative analytical technique has been developed whereby microwave distillation is used to remove the volatile organic compounds from a lipophilic matrix into an aqueous matrix. Solid-phase microextraction (MD-SPME) is then used to extract and concentrate the analytes, which are then desorbed in the injection port of a gas chromatograph/mass spectrometer (GC/MS) for analysis. Limits of detection are 0.01 μg/kg and limits of quantification are 0.1 μg/kg. MD-SPME is comparable in precision, requires no solvents, and is faster than current methods of analysis. This methodology allows detection of MIB and GSM at concentrations below human sensory thresholds in fish tissue.

Keywords: *2-Methylisoborneol; catfish; geosmin; microwave distillation; off-flavor; solid-phase microextraction*

INTRODUCTION

Planktonic and benthic algae, fungi, bacteria, and actinomycetes are known to produce geosmin (GSM) and 2-methylisoborneol (MIB). These semivolatile, lipophilic compounds have a muddy, musty odor perceived as disagreeable to consumers. Both compounds are rapidly absorbed from water into the lipid tissue of fish and other aquatic organisms. When either compound is present in tissue at concentrations exceeding 0.7 μg/kg, they render fish unfit for retail sale (Persson, 1980). At present, there are no economically feasible means available to remove these compounds from aquatic organisms (Widrig et al., 1996).

Current methods for quantifying the concentrations of MIB and GSM in catfish include purge and trap−solvent elution (P&T-SE) (Johnsen et al., 1996), microwave distillation−solvent extraction (MD-SE) (Martin et al., 1987), and microwave distillation−solid-phase extraction (MD-SPE) (Conte et al., 1996). These methods are time-consuming (P&T-SE) and labor intensive (P&T-SE, MD-SE) and require the use of small quantities of flammable and/or toxic solvents (all) or the use of expensive microwave equipment (MD-SPE). A faster and less expensive method could find broad application in catfish flavor research, the catfish processing industry, and other aquaculture industries plagued by this problem.

In this report, we combine microwave distillation (MD) with solid-phase micro-extraction (SPME) to develop a rapid method of quantifying off-flavor concentrations in catfish tissue. MD has been used by Conte et al. (1996) in combination with solid-phase extraction and by Martin et al. (1987) in conjunction with solvent extraction. It is a simple, rapid alternative to the use of mantles or hot plates for heating samples. MD transfers lipophilic volatile analytes from the lipid rich matrix of catfish tissue into an aqueous matrix. SPME is then used to extract and concentrate the volatile organic compounds from the aqueous solution.

SPME is a simple and inexpensive method for the quantitative analysis of volatile and semi-volatile compounds occurring in a wide variety of food, water, and environmental matrices (Belardi and Pawliszyn, 1989; Eisert and Levsen, 1996). A fused silica fiber is coated with a suitable adsorbent phase and bound to the tip of a syringe plunger. The plunger is retracted into the needle which serves to protect the delicate fiber. The needle is used to pierce the septum of a sealed vial containing the sample, and the SPME fiber is then extended (see Figure 1). The fiber can be directly immersed into a liquid sample or placed in the headspace above a liquid or solid sample. Analyte molecules are absorbed into the coating. After equilibration, the fiber is retracted into the needle, inserted into the heated injection port of a gas chromatograph, and extended. The analytes are thermally desorbed and transferred onto the head of a capillary GC column for subsequent separation and detection.

Previous work (Lloyd et al., 1998) described the use of SPME for analyzing off-flavors in water with limits of detection in the 10 nanogram per liter range. Due to their lipophilic nature, MIB and GSM partition from fish tissue into the headspace in such low concentrations that direct SPME is ineffective. Combining MD with SPME yields a rapid, extremely sensitive technique for the analysis of thermally stable volatile and semi-volatile compounds in complex matrixes.

MATERIALS AND METHODS

Reagents and Equipment. 2-Methylisoborneol was synthesized by a modification (Johnsen and Lloyd, 1992) of the method described by Wood and Snoeyink (1977). Geosmin was obtained from Givaudan Corporation (Clifton, NJ). Deuterium labeled 2-methylisoborneol (MIB-d_3) and geosmin (GSM-d_3)

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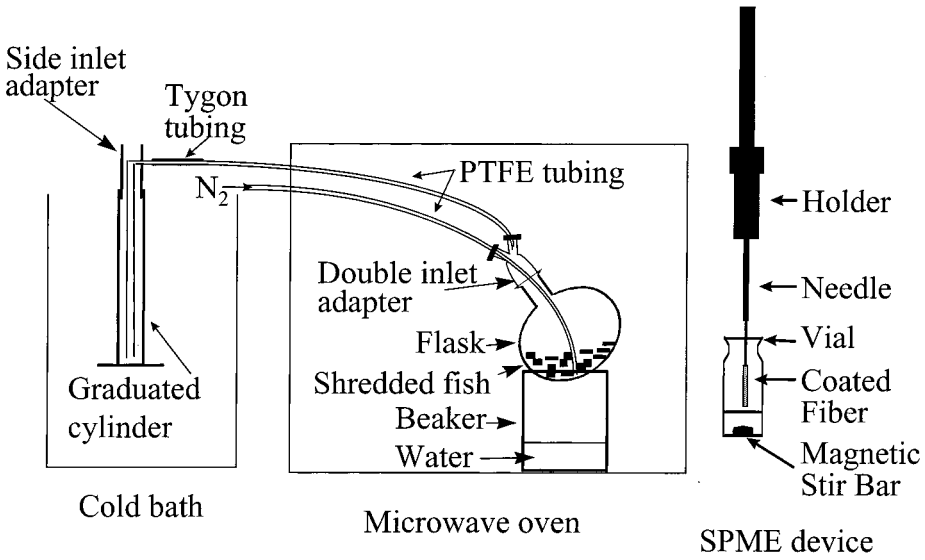

Figure 1. Diagram of microwave distillation apparatus and SPME device with fiber extended into the headspace of a sample vial.

were obtained from CSIRO (Division of Water Resources, Griffith, NSW, Australia). For experiments utilizing spiked fish, stock solutions containing both MIB and GSM in ethanol were mixed with shredded fish. Reagent grade hexane, ethanol, sodium chloride, and anhydrous sodium sulfate were obtained from J. T. Baker Inc. (Phillipsburg, NJ). A 650 W microwave oven (Kenmore Model 721, Sears, Roebuck and Co., Chicago, IL) was modified by drilling two holes in the side (next to the light) to allow passage of gas tubes. A screen was placed over the holes in order to prevent release of microwave radiation. Microwave output power in watts was correlated with power level using a chart in the use and care manual. Shredded fish was placed in a 100-mL round-bottom flask equipped with a 29/42 ground glass joint and a double offset inlet adapter (Ace Glass, Inc., Vineland, NJ). The adapter is equipped with polytriflouroethylene (PTFE) tubes of 5 mm inside diameter and 45 cm in length (Cole-Parmer, Vernon Hills, IL). This assembly is placed in the oven, and the tubes are passed through holes in the oven side. One tube is connected to a flow regulated source of nitrogen, the other to a gas inlet adapter (Ace Glass, Inc., Vineland, NJ) placed in a 50-mL distillation receiving cylinder (Ace Glass, Inc., Vineland, NJ), which is placed in a temperature controlled water bath (Model RM 6, Brinkman Instrument Co., Westbury, NY), and allowed to equilibrate for 2 min before sample heating begins. A diagram of the apparatus appears in Figure 1. Water acts as a target for receiving microwave energy, hence a minimum amount is necessary for correct operation of the microwave oven. Therefore, 10 mL of water is placed in a 250-mL beaker in the oven beneath the sample. Viton septa crimp top caps, SPME holders, and fibers were obtained from Supelco, Inc. (Bellefonte, PA). The viton septa were baked at 100 °C for 12 h to reduce interferences. A Finnigan GCQ ion trap mass spectrometer (Thermoquest Corporation, San Jose, CA) was equipped with a capillary column 30 m long with an internal diameter of 0.25 mm and a 0.25 μm thick (5%-phenyl)-methyl polysiloxane phase (J&W Scientific, Inc., Folsom, CA). An HP 6890 GC equipped with a 5973 mass selective detector (MSD) (Hewlett-Packard, Palo Alto, CA) equipped with an identical column was also used for some experiments. Catfish (*Ictalurus punctatus*), obtained from local supermarkets and Louisiana State University, were filleted, and the fillets shredded in a food processor.

Procedure. For each experiment, 20 ± 0.5 g of fish are weighed into the flask. Where appropriate, several shredded fish fillets were pooled to give homogeneous samples. Fish tissue is purged with nitrogen while being heated. Martin et al. (1987) suggested that the oven power be pulsed; however, this model of oven pulses the power automatically at all but the maximum power setting. During heating, steam and volatiles are carried to the cold trap by nitrogen flow where they condense.

The condensate is diluted to 8 mL with deionized water, saturated with 2.5 g of sodium chloride (NaCl) and poured into a 12-mL capacity vial containing a magnetic stir bar. The vial is capped with a viton septum. A SPME fiber with a 100 μm polydimethyl siloxane (PDMS) coating is exposed to the headspace, while the sample is stirred in a 40 °C water bath for 15 min. The fiber is then desorbed in the GC injection port for 1 min at 270 °C with the purge valve off (splitless mode). After desorption, the fiber is baked in a separate injection port at 270 °C for an additional 10 min to eliminate the possibility of analyte carryover between samples. The GC oven temperature program begins when the fiber is inserted and holds at 40 °C for 1 min, ramps to 80 °C at 40 °C per min, then to 115 °C at 15 °C/min, and then to 250 °C at 40 °C/min, and holds for 3 min. When the Hewlett-Packard 6890 was used, the initial conditions are identical except that a surge pressure of 25 psi is applied for 1 min. Column temperature is held at 40 °C for 1 m, ramps at 20 °C/m to 100 °C, then at 5 °C/m to 145 °C, and then at 120 °C/m to 250 °C, and holds for 3 min.

The mass spectrometers are run in selected ion monitoring mode. Ions at *m*/*z* 95, 135, and 168 are monitored for MIB, and 112, 126, and 182 are monitored for GSM. When the deuterated forms are analyzed, ions at *m*/*z* 139 and 171 are monitored for MIB-d_3 and 115, 129, and 185 are monitored for GSM-d_3. The extracted ion currents for each ion are integrated. Three ions for each compound are compared in terms of retention time and ion ratios. The area count values for the base peaks at *m*/*z* 95 and 112 are used for quantification.

RESULTS AND DISCUSSION

Microwave distillation, steam trapping, and volatile analyte extraction are dynamic processes. Analyte recovery therefore depends on several interrelated variables: microwave power, purge flow rate, heating/purge time, sample size, sample moisture content, and cold trap temperature. Additional parameters, such as the exposure time of the SPME fiber to the trapped condensate, temperature, and the composition of the SPME fiber effect total recovery. The focus of this research was to develop a method that can detect the presence of GSM and MIB at levels below human perception in catfish. This threshold has been placed at ~0.7 μg/kg in catfish (Persson, 1980). The experimental parameters have been partially optimized to

Table 1. Effect of Microwave Power Level on the Area Counts of 2-Methylisoborneol (m/z = 95) and Geosmin (m/z = 112) from the Analysis of Catfish Spiked with 10 μg/kg of Each Analyte[a]

power (W)	area MIB	coefficient of variation (%)	area GSM	coefficient of variation (%)
200	2 642	52.20	564	82.04
370	135 834	11.54	83 518	7.05
480	125 976	16.93	95 640	7.82
650	118 480	21.23	85 204	18.39

[a] Each number represents five replicate analyses. The percent coefficient of variation = (standard deviation/mean) × 100 (%CV).

provide reliable quantification down to 0.1 μg/kg and a limit of detection of 0.01 μg/kg. Additional experimentation to optimize the procedure may result in even lower limits of detection.

Method Parameter Determination. Experiments were performed utilizing different conditions and configurations in order to maximize recovery and lower the limit of detection. To determine the optimum microwave power level, catfish fillets spiked with MIB and GSM at a concentration of 10 μg/kg were heated for 3 min at 200 (power level 2), 370 (5), 480 (7), and 650 (10 = full power) W of output power while being purged with N_2 at 80 mL/min. The cold trap was kept at −15 °C. Five replicate analyses were performed for each determination. As can be seen in Table 1, the lowest setting at 200 W resulted in minimal recoveries with the greatest error. However, at higher power settings, steam is produced so rapidly that volatiles are lost from the cold trap before condensation occurs. Also, at the higher power levels, so much water is boiled away from the sample and the added water that there is not enough present to receive the microwave energy and the oven occasionally stops. The 370 W power level results in higher recovery for MIB and the 480 W power level results in higher recovery level for GSM. The lowest coefficients of variation (CVs) are observed at the 370 W power level for both GSM and MIB. As the difference in recovery level for GSM varies only slightly (∼13%) between the 370 and 480 W power settings, the 370 W setting was selected for use in future experiments. The data in Table 1 suggests that the optimum power setting for analyzing both samples concurrently lies somewhere between 370 and 480 W settings. The expected gain from further experimentation did not warrant further experiments at intervening power settings.

To maximize recovery as a function of flow rate, five replicate samples of spiked fish fillets were heated for 2.5 min at a power setting of 370 W. Experiments were conducted using purging flow rates of 40, 80, and 150 mL/min. The data are presented in Figure 2. No significant difference was observed between the recoveries for GSM as a function of flow rate ($p < 0.05$). Maximum area counts for MIB were observed using a N_2 flow rate of 80 mL/min. At the higher flow rate the more volatile MIB is passed through the trap without condensing, thus reducing recovery. The 80 mL/min flow yielded the greatest recovery, so this rate was used for subsequent experiments.

To determine the effect of purging time on recovery, five replicate samples of spiked fillets were heated at 370 W and purged with 80 mL/min for time periods ranging from 2 to 5 min. The data are plotted in Figure 3. Between 2 and 3.5 min, longer cooking time results in higher recovery. After 3.5 min, most or all MIB and GSM have been stripped from the fish and condensed in the trap. Steam and nitrogen continue to pass through the cold trap and strip volatile compounds from the condensate, thereby reducing recovery. Within experimental error, there was no significant difference between the area counts obtained at 2.5, 3.0, and 3.5 min for either MIB or GSM, suggesting that over this time range, the dynamic processes of the analytes condensing and evaporating in the cold bath have reached equilibrium. A purge time of 3 min was selected for use in further experimentation.

Cold trap temperature was varied by utilizing liquid nitrogen, dry ice, and a refrigerated water bath. Five replicate samples of fillets from a fish containing naturally occurring off-flavors were heated for 3 min at 370 W and purged with N_2 at 80 mL/min. The purged products were trapped at −60, −15, 0, and 10 °C. Cold trap temperature had no significant effect on the recovery of GSM ($p > 0.05$). For MIB, no significant difference was observed in the means for −60, −15, and 0 °C, while the mean at 10 °C is significantly lower ($p < 0.05$). To reduce the possibility of frozen condensate blocking the tube, 0 °C was used in subsequent experiments.

Three different glassware configurations were compared. The standard 50-mL graduated cylinder and gas inlet adapter were replaced with a 25-mL graduated cylinder. The third configuration consisted of a condenser on top of the gas inlet adapter in a 50-mL cylinder. Water/ethylene glycol at 0 °C was circulated through the condenser, which was rinsed with deionized water after the heating interval. Five replicates of fish containing naturally occurring off-flavors were heated for 3 min at 370 W, while being purged with N_2 at 80 mL/min with a 0 °C cold trap. The 50-mL cylinder resulted in significantly better recovery than the 25-mL cylinder ($p < 0.05$). The larger diameter of the 50-mL cylinder causes steam and volatile compounds to move more slowly through the trap, resulting in faster cooling and condensation then in the 25-mL cylinder. Analytes do not pass through to the condenser. For this reason, the use of a condenser did not significantly increase recovery ($p > 0.05$). The 50-mL graduated cylinder was used for subsequent experiments.

Conditions for analysis were chosen to achieve the goal of minimizing cost and time, while maximizing recovery. The rate of condensation of the volatile compounds impacts the recovery of the material and lower temperatures should increase recovery. This trend was not observed at the flow rate (80-mL/min) used in the cold trap experiment. Higher power levels during the heating phase would be expected to decrease analysis time. However, at the conditions used in the power level experiment, better recovery was achieved at 370 W. Other combinations of N_2 flow, power, time, temperature, and sample size may yield better recoveries. Some of the factors considered in determining the conditions used for the method comparison and limit of detection experiments are indirectly related to recovery. For example, cold trap temperatures below the freezing point of water occasionally result in ice blocking the tube inside the cold trap, leading to sample loss when the adapter separates from the sample flask. Subfreezing temperatures also increase the cost and setup time.

The parameters used for subsequent experiments are as follows: The fish sample is heated for 3 min at 370 W while being purged with N_2 at 80 mL/m. The

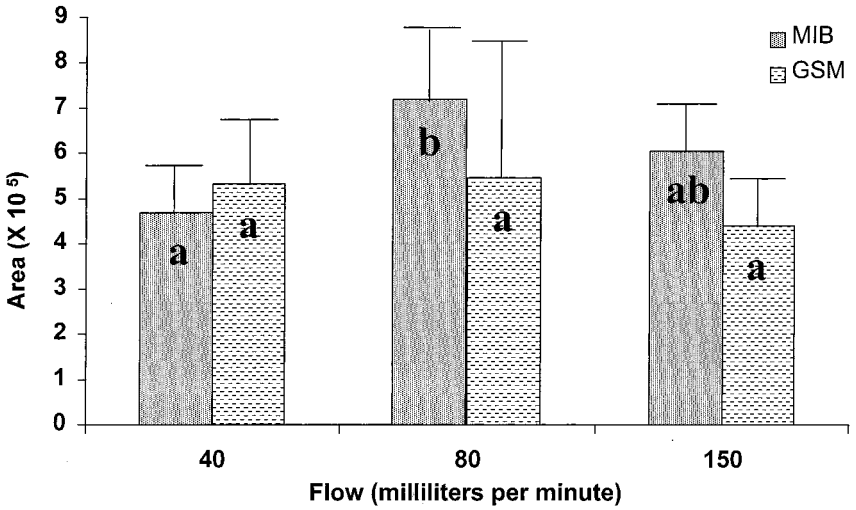

Figure 2. Effect of nitrogen purge flow rate on the area counts of 2-methylisoborneol ($m/z = 95$) and geosmin ($m/z = 112$) from the analysis of catfish spiked with 10 μg/kg of each analyte. Each column represents five replicate analyses. For each analyte, means with different letters are significantly different ($p < 0.05$). Error bars indicate one standard deviation.

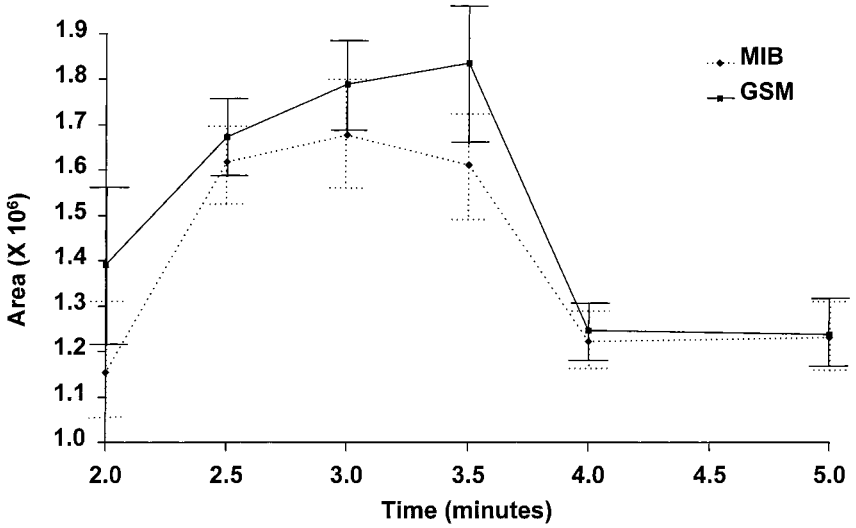

Figure 3. Effect of heating interval on the area counts of 2-methylisoborneol ($m/z = 95$) and geosmin ($m/z = 112$) from the analysis of catfish spiked with 10 μg/kg of each analyte. Each data point represents five replicate analyses. Error bars indicate one standard deviation.

condensate is trapped in a 50-mL distillation receiving cylinder held at 0 °C in a water bath. The condensate is diluted to 8 mL and saturated with salt in a vial sealed with a viton septa. The sample was stirred in a water bath heated to 40 °C for 15 min, while a SPME fiber with a 100 μm PDMS coating was exposed to the headspace.

Linearity, Sensitivity, and Recovery. Several samples of catfish fillets previously determined to contain no off-flavors by a trained taste panel (Daphne Ingram, personal communication) were analyzed. A series of subsamples from one batch was spiked with a solution of MIB and GSM to yield concentrations of 0.01, 0.03, 0.1, 0.3, 1.0, 3.0, 10.0, and 30 μg/kg. The spiked samples were analyzed by MD-SPME to determine the lower limit of detection and the degree of linearity of response. Linear regression showed good linearity over a concentration range from 0.1 to 30 μg/kg ($r = 0.9999$ and 0.9992 for MIB and GSM) and lower linearity for concentrations between 0.01 and 0.1 μg/kg ($r = 0.73$ and 0.79, respectively).

The reconstructed ion chromatograms for m/z 95 of MIB and m/z 112 of GSM are shown in Figure 4 for the spiked fish samples. Because analyses were performed over a one year period, retention times varied and were artificially shifted up to 2 s to align the maxima of the peaks to aid in visual comparison. Peaks are observed even in unspiked fish samples at retention times expected for MIB and GSM for m/z 95 and m/z 112, respectively. The extracted ion currents for the confirming ions showed no peaks at the same retention time, indicating that this could be a coeluting compound or that indigenous MIB and GSM are present in the samples below the limits of detection.

The average peak heights of the MIB and GSM peaks from the analysis of three replicates of unspiked fish samples were subtracted from the heights of peaks from a sample spiked at 0.01 μg/kg. Using these data, the signal-to-noise levels were calculated to be 7 for MIB and 15 for GSM. These data show that these compounds can be detected by MD-SPME at concentrations as low as 0.01 μg/kg. Examination of coefficients of variation from the analysis of samples spiked with various levels of MIB and GSM indicates that quantification can be determined to concentrations as low as 0.1 μg/kg.

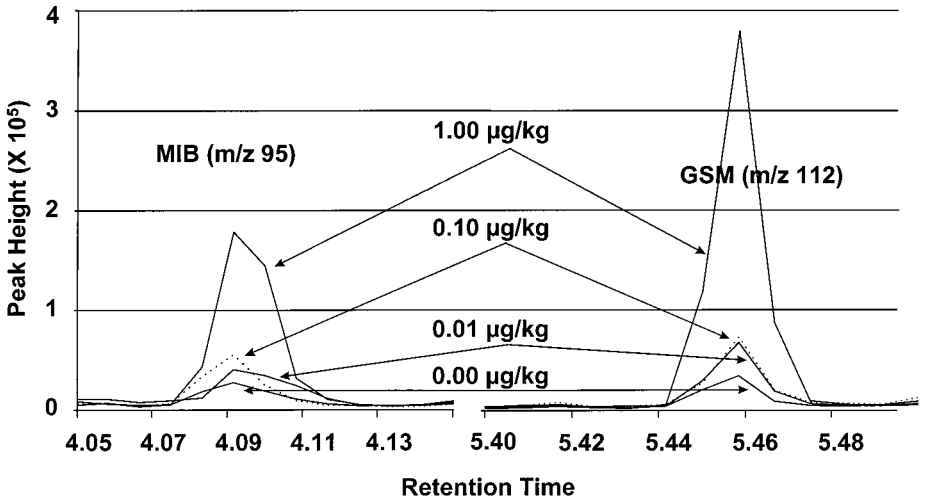

Figure 4. Reconstructed ion chromatogram, of *m/z* 95 (MIB) and *m/z* 112 (geosmin) of a catfish fillet spiked with (a) 1 μg/kg, (b) 0.1 μg/kg, (c) 0.01 μg/kg, and (d) 0 μg/kg.

Table 2. Comparison of Analytical and Taste Panel Results[a]

fillet	panelist score	MIB (μg/kg)	GSM (μg/kg)
1	0.2	0.53	0.10
2	1.0	0.61	0.07
3	1.4	1.46	0.06
4	0.4	0.58	0.20

[a] MIB and GSM values are the result of one determination; panelist score is an average of five panelists. Panelist scores are not μg/kg values. See text for further explanation.

Percent recovery was calculated by comparison with direct injection. A fish sample was spiked with 1 μL of a 1 mg/L solution of MIB and GSM and analyzed by MD-SPME. One microliter of the same solution was analyzed by GC/MS using the same column and temperature ramp. The areas of the *m/z* 95 peak of MIB and the *m/z* 112 peak of GSM were calculated and compared to determine the analyte recovery ratio (the amount of an analyte which arrives at the detector) for this method. Recoveries were 4.4% for MIB and 5.0% for GSM.

Quantification. Catfish fillets purchased from a local supermarket were spiked with 1 μL of a solution containing 5 μg/L each of MIB-d_3 and GSM-d_3. Five subsamples from one fillet were analyzed by MD-SPME using the Hewlett-Packard instrument. Concentrations were calculated by the internal standard method outlined by Korth et al. (1991). The fillet contained 1.00 μg/kg MIB with a coefficient of variation of 8.8% and 0.11 μg/kg GSM with a coefficient of variation of 16.4%. One subsample from each of four other fillets was analyzed by the same procedure, and each fillet was analyzed by a trained taste panel. Each of five panelists rated the fillets as having no off-flavor (score = 0), trace off-flavor (score = 1), or definite off-flavor (score = 2). The numerical scores were averaged. The results are shown in Table 2. The data show good agreement between the panel and the analytical results. It appears that many commercially available catfish contain MIB and GSM at levels below the human taste threshold of 0.7 μg/kg in catfish (Persson, 1980).

Method Comparison. The MD-SPME technique described above was compared to two other methods used to quantitate MIB and GSM in fish tissue: (1) purge and trap followed by solvent elution and concentration (P&T-SE) and (2) microwave distillation followed by solvent extraction and concentration (MD-SE). Fillets from two fish containing naturally occurring off-flavors were shredded. Five subsamples of each fish were analyzed by each of the three methods. MD-SPME was carried out using a 3 min purging interval at 370 W, 80 mL/min nitrogen flow, and a 50-mL cold trap held at 0 °C. P&T-SE was performed by the method of (Johnsen et al., 1996). MD-SE was modified from Martin et al. (1987) as follows. Microwave distillation was carried out as for MD-SPME. The condensate was extracted with 10 mL of hexane. The hexane was dried through a bed of anhydrous sodium sulfate and concentrated under a stream of nitrogen to a final volume of 50 μL. A 1 μL aliquot was injected for analysis by GC/MS.

Table 3. Average, Standard Deviations, and % Coefficients of Variation ((Standard Deviation/Mean) × 100) of the Area Counts of 2-Methylisoborneol (*m/z* = 95) and Geosmin (*m/z* = 112) of Catfish Containing Naturally Occurring Off-Flavors Analyzed by Microwave Distillation−Solvent Extraction (MD-SE), Purge and Trap (P&T), and Microwave Distillation−Solid-Phase Microextraction (MD-SPME)[a]

	MIB			GSM		
	average	std dev	c.v. (%)	average	std dev	c.v. (%)
			Fish 1			
MD-SE	15 409	3 922	25.45	16 208	5 535	34.15
P&T	162 639	79 475	48.87	119 406	10 782	9.03
MD-SPME	285 095	35 101	12.31	370 427	44 390	11.98
			Fish 2			
MD-SE	32 064	8 067	25.16	34 210	7 738	22.62
P&T	147 877	30 702	20.76	124 375	17 371	13.97
MD-SPME	217 696	24 433	11.22	414 832	77 881	18.77

[a] Each column represents five replicate analyses.

The advantage of SPME as compared with other extraction techniques is that analytes can be concentrated without the use of solvents. For a single injection, the SPME method results in two to 10 times as much analyte reaching the detector relative to the other two methods (Table 3). The disadvantage is that multiple injections are not possible. The MD-SPME technique is based upon establishing an equilibrium between the sample and the fiber. Subsequent headspace analyses yield less and less material and are not reproducible. With the concentrated solvent methods, as many as 50 injections are theoretically possible. The limit of detection of the MD-SPME method is superior because all analytes captured by the SPME fiber are introduced into

the injection port, whereas only 2% of the extract volume is injected using MD-SE or P&T-SE. These data suggest that the limit of detection in the P&T method could be improved by thermally desorbing the analytes from the trap.

Table 3 contains the mean area counts, standard deviation (SD), and CV for MIB and GSM for the three methods. The larger number of area counts for MD-SPME indicate that it has a lower limit of detection for within lab comparison. The low limit of quantification for MD-SPME (0.1 μg/kg) is better than that reported for MD-SE (Martin et al., 1987) and comparable to that for P&T-SE (Johnsen et al., 1996). There are fewer steps required by this method, thereby reducing errors.

CONCLUSIONS

A rapid, extremely sensitive method using MD-SPME for the analysis of MIB and geosmin in catfish fillets has been described. This technique is capable of detecting GSM and MIB at concentrations below human sensory perception and uses significantly smaller sample sizes (20 g vs 40 g for MD-SE and 50 g for P&T-SE). Total time for analysis of one sample is 30 min, but this can be reduced to 15 min per sample when multiple fish are being tested. In contrast, P&T-SE requires 3.5 h per sample (although this can be reduced considerably by analyzing several samples simultaneously), and MD-SE requires 1 h per sample. The comparison study indicated that MD-SPME results in better precision and higher recovery than either MD-SE or P&T-SE.

This technique may find broad application in analyzing volatile and semi-volatile compounds in complex matrices particularly of a lipophilic nature. Thermally generated volatiles can be determined by this method; however, it cannot be used to determine which recovered substances are thermally generated and which are not. Because many different SPME fiber types are now available and the microwave time and purge flow rates can be varied over a broad range, this method should be applicable to the analysis of thermally stable volatile compounds in a wide variety of food products.

ABBREVIATIONS USED

CV, coefficient of variation = (standard deviation/mean) × 100; GC/MS, gas chromatograph/mass spectrometer; GSM, geosmin; GSM-d_3, deuterated geosmin; MD, microwave distillation; MD-SE, microwave distillation−solvent extraction; MD-SPE, microwave distillation−solid-phase extraction; MD-SPME, microwave distillation−solid-phase microextraction; MIB, 2-methylisoborneol; MIB-d_3, deuterated 2-methylisoborneol; NaCl, sodium chloride; $p < 0.05$, probability is less than 5% that the difference between means is due to chance; P&T-SE, purge and trap−solvent elution; PDMS, polydimethyl siloxane; PTFE, polytrifluoroethylene; SD, standard deviation; SPME, solid-phase microextraction.

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