Using Microwave Distillation-Solid-Phase Microextraction–Gas Chromatography–Mass Spectrometry for Analyzing Fish Tissue

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Abstract

A technique for the analysis of the volatile compounds from fish tissue employing microwave distillation-solid-phase microextraction–gas chromatography–mass spectrometry is described. A qualitative listing of 174 compounds observed in the headspace is given, and a quantitative method for the determination of the off-flavor contaminants (2-methylisoborneol and geosmin) is presented. Borneol and decahydro-1-naphthol are used as the surrogate and internal standards, respectively. A linear calibration curve is obtained for 0.1 to 5 ppb with a recovery level of 60% at 2.5 ppb. Comparison of the instrumental method with a human flavor checker showed good agreement.

Table I. Peak Areas Generated from the Target Ion

	Molecular weight	Target ion (*m/z*)	Q1* (*m/z*)	Q2* (*m/z*)
Borneol	156	139	110	95
2-Methylisoborneol	168	135	168	95
Decahydro-1-naphthol	154	136	94	67
Geosmin	182	112	182	126

* The qualifier ions ensure selectivity.

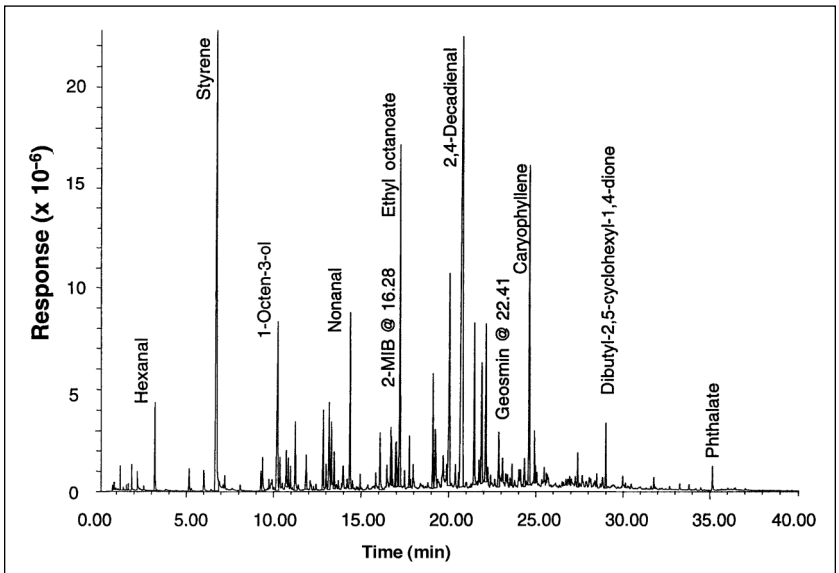

Figure 1. Total ion chromatogram of the headspace from 20 g of catfish fillet using MD-SPME–GC–MS. Geosmin and 2-MIB, although present, are not readily observed in full-scan mode.

Introduction

Currently, crop flavor quality assessment in the farm-raised catfish industry is made by human flavor checkers. Instrument-based quality determinations are potentially faster, less subjective, and more accurate. Much progress has been made in the rapid instrumental determination of the muddy/musty off-flavors, geosmin and 2-methylisoborneol (2-MIB), which constitute greater than 80% of the off-flavor problem in farm-raised catfish (1–6). Readily recognized as the smell of fresh dirt, they are ubiquitous in nature and are produced by actinomycetes and blue-green algae. Their presence adds a noticable taint to the aroma of drinking water and food supplies. Humans are remarkably sensitive to these compounds and can detect them at extremely low levels (on the order of low parts-per-trillion concentrations) in water (7).

Solid-phase microextraction–gas chromatography–mass spectrometry (SPME–GC–MS) has been successfully employed for the qualitative analysis of volatile and semivolatile compounds occurring in the headspace of a wide variety of samples (8,9). The SPME methodology augments direct headspace and purge-and-trap techniques for rapid qualitative and quantitative analyses.

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Table II. Compounds Observed in the Headspace of Cooked Catfish

Class	Chemical name	i.d.	CAS number	Molecular weight	Formula	R.T.
Alcohol						
	3-Methyl butanol	MS	123-51-3	86	$C_5H_{10}O$	2.82
	2-Methyl butanol	MS	137-32-6	86	$C_5H_{10}O$	2.84
	1-Octen-3-ol	MS	3391-86-4	128	$C_8H_{16}O$	4.99
	1-Hexanol	MS	111-27-3	102	$C_6H_{14}O$	6.65
	2-Butoxy-ethanol	MS	111-76-2	118	$C_6H_{14}O_2$	7.78
	1-Heptanol	STD*	111-70-6	116	$C_7H_{16}O$	9.93
	1-Octen-3-ol	STD	3391-86-4	128	$C_8H_{16}O$	10.19
	2-Ethyl-1-hexanol	STD	104-76-7	130	$C_8H_{18}O$	11.75
	3,7-Dimethyl-1,6-octadien-3-ol	MS	106-25-2	154	$C_{10}H_{18}O$	14.00
	2-Hexyloxy-ethanol	MS	112-25-4	146	$C_8H_{18}O_2$	14.18
	1-Nonanol	STD	143-08-8	144	$C_9H_{20}O$	16.08
	(*E*)-2-decen-1-ol	STD	18409-81-2	156	$C_{10}H_{20}O$	18.63
	1-Octanol	STD	111-87-5	130	$C_8H_{18}O$	13.15
	(*Z*,*Z*)-6,9-pentadecadien-1-ol	MS	77899-11-7	224	$C_{15}H_{28}O$	18.84
	1-Dodecen-3-ol	MS	4048-42-4	184	$C_{12}H_{24}O$	19.19
Aldehyde						
	Hexanal	STD	066-25-1	100	$C_6H_{12}O$	4.52
	Heptanal	STD	111-71-7	114	$C_7H_{14}O$	7.61
	Octanal	STD	124-13-0	128	$C_8H_{16}O$	10.93
	Nonanal	STD	124-19-6	142	$C_9H_{18}O$	14.06
	Decanal	STD	112-31-2	156	$C_{10}H_{20}O$	17.06
	Undecanal	MS	112-44-7	170	$C_{11}H_{22}O$	19.88
	Hexadecanal	MS	629-80-1	240	$C_{16}H_{32}O$	31.89
	Octadecanal	MS	638-66-4	268	$C_{18}H_{36}O$	22.54
Alkane						
	Decane	MS	124-18-5	142	$C_{10}H_{22}$	10.77
	Undecane	STD	1120-21-4	156	$C_{11}H_{24}$	13.92
	Dodecane	MS	112-40-3	170	$C_{12}H_{26}$	16.89
	2,7,7-Trimethyl-decane	MS		184		18.18
	Tridecane	STD	629-50-5	184	$C_{13}H_{28}$	19.67
	Tetradecane	MS	629-59-4	198	$C_{14}H_{30}$	13.50
	Pentadecane	MS	629-62-9	212	$C_{15}H_{32}$	24.78
	2-Methyl-pentadecane	MS	1560-93-6	226	$C_{16}H_{34}$	26.33
	5-Propyl-tridecane	MS	55045-11-9	226	$C_{16}H_{34}$	30.30
	Hexadecane	MS	544-76-3	226	$C_{16}H_{34}$	27.12
	2-Methyl-hexadecane	MS	1560-92-5	240	$C_{17}H_{36}$	28.57
	3-Methyl-hexadecane	MS	6418-43-5	240	$C_{17}H_{36}$	30.50
	Heptadecane	MS	629-78-7	240	$C_{17}H_{36}$	29.39
	2,6,10,14-Tetramethyl-pentadecane	MS	1921-70-6	268	$C_{19}H_{40}$	29.50
	Octadecane	MS	593-45-3	254	$C_{18}H_{38}$	31.48
	Nonadecane	MS	629-92-5	268	$C_{19}H_{40}$	33.52
Alkene						
	(*E*)-4,4-dimethyl-2-pentene	MS	26232-98-4	98	C_7H_{14}	5.13
	2,4-Dimethyl-1-decene	MS	55170-80-4	168	$C_{12}H_{24}$	11.98
	(*Z*)-2,2,5,5-tetramethyl-3-hexene	MS	692-47-7	140	$C_{10}H_{20}$	12.62
	3-Methyl-1,6-heptadiene	MS	50871-05-1	110	C_8H_{14}	15.60
	1-Pentadecene	MS	13360-61-7	210	$C_{15}H_{30}$	24.61
	1-Heptadecene	MS	6765-39-5	238	$C_{17}H_{34}$	29.21
	1-Octadecene	MS	112-88-9	252	$C_{18}H_{36}$	27.97
Amine						
	Trimethylamine	MS	75-50-3	59	C_3H_9N	0.90

* STD, steam distillate.

Applications have been successfully developed for the headspace analysis of foods such as fruits and vegetables and environmental samples such as soil and water (10). The relatively low cost, ease of use, and extensive capabilities of SPME have resulted in a wide range of applications, especially in the area of food analysis (11).

Headspace analysis of an aqueous solution employing SPME is straightforward when compared with other headspace techniques such as purge and trap. The aqueous sample is readily amenable to the use of NaCl and heating in order to drive the analytes from the liquid phase into the gas phase. SPME is not as effective for the analysis of samples composed of a complex matrix, such as soil and muscle tissue. In complex matrices, physical bonds allow the prospective analytes to adhere to the sample matrix. For complex matrices, this problem can be overcome using microwave distillation (MD) (3,5,6). The analytes are essentially steam-distilled from the sample matrix, and the steam effluent carrying the analytes is collected in a flask placed in a chilled water bath. The technique effectively removes the analytes from the less optimal matrix and places them in an aqueous matrix.

Methodology employing SPME has been developed for analyzing these compounds in water at the parts-per-trillion range (4). This report provides qualitative information on the compounds found in the headspace of cooked fish and details for the quantitation of 2-MIB and geosmin. These compounds can be detected by humans at concentrations as low as 20 ppt in water and 0.7 ppb in fish (12). Researchers and water quality control managers need to measure the concentrations of 2-MIB and geosmin in both fish and water at very low levels.

Experimental

Geosmin (9a,10a-decalol; CAS# 19700-21-1) was obtained from Givaudan Corporation (Clifton, NJ). 2-MIB ([1*R-exo*]-1,2,7,7-tetramethyl-[2,2,1]-bicyclo-heptan-2-ol; CAS# 2371-42-8), borneol ([1*R*]-endo-1,7,7-trimethyl bicyclo[2.2.1]heptan-2-ol; CAS# 464-43-7), and decahydro-1-naphthol (*cis*-decahydro-1-naphthol; CAS# 36159-47-4) (DHNap) (St. Louis, MO) were obtained from Sigma-Aldrich. Standards of 1 ppt were made up in ethanol, with subsequent dilutions in sterile water (Milli-Q, Millipore, Milford, MA). Off-flavor fish were obtained from a commercial processor, and determination of the muddy/musty off-flavor was made by a professional flavor checker.

Table II. (Continued)

Class	Chemical name	i.d.	CAS number	Molecular weight	Formula	R.T.
	Pyridine	STD*	111-86-1	79	C_5H_5N	3.13
	1-Ethyl-1*H*-pyrrole	MS	617-92-5	95	C_6H_9N	4.93
	3-Methyl-1*H*-pyrrole	STD	616-43-3	81	C_5H_7N	6.03
	2,3,4-Trimethylpyrrole	MS	3855-78-5	109	$C_7H_{11}N$	8.78
	2,5-Dimethyl-1*H*-pyrrole	MS	625-84-3	95	C_6H_9N	9.23
	N-butyl-1-butanamine	MS	111-92-2	129	$C_8H_{19}N$	9.54
	2-Ethyl-5-methylpyridine	MS	000-00-0	121	$C_8H_{11}N$	11.58
	4-Methoxy-1,3-benzenediamine	MS	615-05-4	138	$C_7H_{10}N_2O$	13.69
	2,3-Dihydro-1*H*-indole	MS	496-15-1	119	$C_8H_{11}N$	14.31
	3,5-Dimethylanisole	MS	874-63-5	136	$C_9H_{12}O$	14.51
	Benzeneacetonitrile	MS	140-29-4	117	C_8H_7N	15.11
	2,3-Dihydro-5-methyl-1*H*-indene	MS	874-35-1	132	$C_{10}H_{12}$	15.35
	2,3-Dihydro-1-methylindene	MS	27133-93-3	132	$C_{10}H_{12}$	15.36
	2,3-Dihydro-5,6-dimethyl-1*H*-indene	MS	1075-22-5	146	$C_{11}H_{14}$	18.26
	2,3-Dihydro-4,7-dimethyl-1*H*-indene	MS	6682-71-9	146	$C_{11}H_{14}$	18.27
	Indole	STD	120-72-9	117	C_8H_7N	19.52
	N,N-dimethyl-1-dodecanamine	MS	112-18-5	213	$C_{14}H_{31}N$	24.90
	2,3-Dihydro-3,3,5,6-tetramethyl-1*H*-inden-1-one	MS	54789-22-9	188	$C_{13}H_{16}O$	25.29
Aromatic						
	Styrene	STD	100-42-5	104	C_8H_8	7.20
	1,3-Dimethyl-benzene	STD	108-38-3	106	C_8H_{10}	6.53
	1,4-Dimethyl-benzene	STD	106-42-3	106	C_8H_{10}	6.66
	Ethyl-benzene	MS	100-41-4	106	C_8H_{10}	6.29
	Benzaldehyde	STD	100-52-7	106	C_7H_6O	9.41
	Benzeneacetaldehyde	STD	122-78-1	120	C_8H_8O	12.20
	Acetophenone	STD	98-86-2	120	C_8H_8O	12.88
	1-Ethyl-3-methyl-benzene	MS	620-14-4	120	C_9H_{12}	9.50
	1,3,5-Trimethyl-benzene	STD	108-67-8	120	C_9H_{12}	9.70
	1,2,4-Trimethyl-benzene	MS	95-63-6	120	C_9H_{12}	10.51
	1,2,3-Trimethyl-benzene	MS	526-73-8	120	C_9H_{12}	10.51
	Naphthalene	STD	91-20-3	128	$C_{10}H_8$	16.34
	1,2,3,4-Tetramethyl-benzene	MS	488-23-3	134	$C_{10}H_{14}$	7.88
	1,2,4,5-Tetramethyl-benzene	MS	95-93-2	134	$C_{10}H_{14}$	14.15
	1,2,3,4-Tetramethyl-benzene	MS	488-23-3	134	$C_{10}H_{14}$	15.43
	1-(2-Hydroxy-phenyl)-ethanone	MS	118-93-4	136	$C_8H_8O_2$	16.84
	1-Methyl-naphthalene	MS	90-12-0	142	$C_{11}H_{10}$	19.46
	2-Methyl-naphthalene	MS	91-57-6	142	$C_{11}H_{10}$	19.48
	2-(1,1-Dimethylethyl)-phenol	MS	88-18-6	150	$C_{10}H_{14}O$	16.50
	2-Methoxy-1,3,4-trimethyl-benzene	MS	21573-36-4	150	$C_{10}H_{14}O$	17.77
	Methyl salicylate	STD	4670-56-8	152	$C_8H_8O_3$	16.78
	4-(1-Methylethyl)-benzoic acid	MS	536-66-	164	$C_{10}H_{12}O_2$	19.83
	1-(4-Hydroxy-3-methoxyphenyl)-ethanone	MS	498-02-2	166	$C_9H_{10}O_3$	20.39
	6,7-Dimethyl-1-naphthol	MS	31776-14-4	172	$C_{12}H_{12}O$	26.05
	1,2,3,4-Tetrahydro-1,5,8-trimethyl-naphthalene	MS	21693-51-6	174	$C_{13}H_{18}$	21.19
	1,2,3,4-Tetrahydro-1,1,6-trimethyl-naphthalene	MS	475-03-6	174	$C_{13}H_{18}$	21.20
	Benzophenone	MS	119-61-9	182	$C_{13}H_{10}O$	27.81
	4-(1,1,3,3-Tetramethylbutyl)-phenol	MS	140-66-9	206	$C_{13}H_{10}O$	27.298

* STD, steam distillate.

Samples consisted of 20 g of a single catfish fillet that was finely chopped and placed in a glass container. The sample was then heated for 3 min using a microwave while purging with 80 mL/min of N_2. The effluent was transferred via glass tubing to a receiving vessel (20-mL graduated cylinder) located in a chilled water bath held at 0°C. The collected water was brought up to a total volume of 10 mL using Milli-Q water to rinse the transfer line. The sample was then subdivided into 5-mL aliquots and placed into a 10-mL vial. Three grams of NaCl was added, and the vial was spiked with 5 µL of a 10-ppm solution of the internal standard, DHNap (50 ng). The vial was sealed with a crimp cap fitted with a Viton septum and placed in a CTC SPME autosampler (Leap Technologies, Carrboro, NC). Samples were maintained at room temperature until analyzed.

The sample was then heated to 65°C and exposed to the SPME fiber for a 12-min adsorption period while undergoing vigorous agitation. The autosampler was equipped with a 1-cm-long divinylbenzene–carboxen–polydimethylsiloxane SPME fiber (Supelco, Bellefonte, PA). The fiber was withdrawn from the sample and desorbed at 270°C for 5 min in the injection port of an HP6890 GC equipped with a 5973 mass-selective detector (Hewlett-Packard, Palo Alto, CA). The injection port was operated in splitless mode and fitted with a 0.7-mm-i.d. injection liner. The head pressure was set to 25 psi of helium for the first minute and then to a constant velocity of 40 cm/s for the remainder of the GC run. Two different GC temperature programs were employed: one for qualitative analysis and the second for quantitative analysis. For qualitative analysis, the oven was held at 40°C for 3 min, ramped to 200°C at 5°C/min, and then ramped to 250°C at 50°C/min for a 40-min run. For quantitative analysis using selected-ion monitoring (SIM), the oven was initially held at 80°C for 1 min then ramped to 100°C at 20°C/min, to 152°C at 7.5°C/min, and to 250°C at 65°C/min, and then held to give a total run time of 12.75 min. Cool-down for the GC oven took approximately 4 min.

The quadrupole MS was operated in election ionization mode and was initially scanned from *m/z* 50 to 350 for qualitative analysis. SIM was employed for quantitation of the target and qualifier ions for 2-MIB, geosmin, borneol, and DHNap, as shown in Table I. Q1 and Q2 are the qualifying ions of a given analyte and should give a consistent ratio relative to the target ion. If either ratio falls outside of acceptable limits, then the presence of a coeluting compound is suggested, which may interfere with the

Table II. (Continued)

Class	Chemical name	i.d.	CAS number	Molecular weight	Formula	R.T.
	2,6-bis(1,1-Dimethylethyl)-4-ethyl-phenol	MS	4130-42-1	234	$C_{13}H_{10}O$	26.43
	2,5-bis(*t*-Butyl)-2,4-cyclohexyl-1,5-dione	MS	4584-63-8	248	$C_{16}H_{24}O_2$	29.01
Contaminant						
	Methylene chloride	MS	75-09-2	84	CH_2Cl_2	1.02
	Chloroform	STD*	67-66-3	118	$CHCl_3$	1.37
	1,2-Dichloro-benzene	MS	95-50-1	146	$C_6H_4Cl_2$	11.07
	Butylated hydroxyanisole	MS	25013-16-5	180	$C_{11}H_{16}O_2$	24.17
	Butylated hydroxytoluene	MS	128-37-0	220	$C_{15}H_{24}O$	24.13
	Butyl, 2-methyl-propyl phthalate	MS	17851-53-5	278	$C_{16}H_{22}O_4$	34.75
	Dibutyl phthalate	MS	84-74-2	278	$C_{16}H_{22}O_4$	34.73
Ester						
	2-Methyl-butanoic acid, ethyl ester	STD	7452-79-1	130	$C_7H_{14}O_2$	6.05
	Hexanoic acid, methyl ester	STD	106-70-7	130	$C_7H_{14}O_2$	8.38
	Hexanoic acid, ethyl ester	MS	123-66-0	144	$C_8H_{16}O_2$	10.81
	Acetic acid, hexyl ester	MS	142-92-7	144	$C_8H_{16}O_2$	11.26
	Octanoic acid, methyl ester	STD	111-11-5	158	$C_9H_{18}O_2$	14.72
	Octanoic acid, ethyl ester	MS	106-32-1	172	$C_{10}H_{20}O_2$	16.89
	Decanoic acid, methyl ester	MS	110-42-9	186	$C_{11}H_{22}O_2$	11.72
	3,7-Dimethyl-1,6-octadien-3-ol, acetate	MS	115-95-7	196	$C_{12}H_{20}O_2$	18.48
	Dodecanoic acid, methyl ester	MS	55554-08-0	214	$C_{13}H_{26}O_2$	25.41
	Tetradecanoic acid, ethyl ester	MS	124-06-1	256	$C_{16}H_{32}O_2$	31.37
	(*Z*)-9-hexadecenoic acid, methyl ester	MS	1120-25-8	268	$C_{17}H_{32}O_2$	33.59
	Hexadecanoic acid, methyl ester	MS	112-39-0	270	$C_{17}H_{34}O_2$	34.02
	Ethyl 9-hexadecenoate	MS	000-00-0	282	$C_{18}H_{34}O_2$	34.93
	Hexadecanoic acid, ethyl ester	MS	628-92-7	284	$C_{18}H_{36}O_2$	35.28
Ketone						
	2-Butanone	MS	78-93-3	72	C_4H_8O	1.23
	2-Pentanone	STD	107-87-9	86	$C_5H_{10}O$	2.14
	3-Hexanone	MS	589-38-8	100	$C_6H_{12}O$	2.72
	Methyl isobutyl ketone	MS	108-10-1	100	$C_6H_{12}O$	2.77
	2-Heptanone	MS	110-43-0	114	$C_7H_{12}O$	7.28
	(*E,E*)-3,5-octadien-2-one	MS	30086-02-3	124	$C_8H_{12}O$	13.81
	4-Methyl-2-heptanone	MS	6137-06-0	128	$C_8H_{16}O$	8.87
	2,4-Dimethyl-3-heptanone	MS	18641-71-9	142	$C_9H_{18}O$	8.45
	2,3-Octanedione	MS	585-25-1	142	$C_8H_{14}O_2$	10.31
	2-Nonanone	STD	821-55-6	142	$C_9H_{18}O$	13.71
	2-Methyl-5-nonanone	MS	22287-02-1	156	$C_{10}H_{20}O$	8.23
	2-Decanone	MS	693-54-9	156	$C_{10}H_{20}O$	8.66
Pyrazine						
	2,5-Dimethyl-pyrazine	STD	123-32-0	108	$C_6H_8N_2$	7.86
	2,6-Dimethyl-pyrazine	STD	108-50-9	108	$C_6H_8N_2$	7.88
	2-Ethyl-6-methyl-pyrazine	STD	13925-03-6	122	$C_7H_{10}N_2$	10.70
	2,3,5-Trimethyl-pyrazine	STD	14667-55-1	122	$C_7H_{10}N_2$	10.82
	2-Methyl-6-propyl-pyrazine	MS	29444-46-0	136	$C_8H_{12}N_2$	13.49
	3-Ethyl-2,5-dimethyl-pyrazine	MS	13360-65-1	136	$C_8H_{12}N_2$	13.31
	2,5-Diethyl-pyrazine	STD	13238-84-1	136	$C_8H_{12}N_2$	13.43
	2,3-Diethyl-5-methyl-pyrazine	MS	18138-04-0	150	$C_9H_{14}N_2$	15.58

* STD, steam distillate.

quantitation. Following the first GC–MS run, subsequent samples were prepared ahead of time so that one sample was run every 18 min. To eliminate carryover between samples after washing, glassware was rinsed with a 1M HCl solution followed by a water rinse and baked at 200°C.

Results and Discussion

The volatile and semivolatile compounds observed in the headspace of the steam distillate from microwave distilled catfish are listed in Table II, and a total ion chromatogram of the compounds is shown in Figure 1. The large number of aldehydes and alcohols are lipid oxidation products and commonly result from the breakdown of all living organisms. The relative amounts of these compounds are dependent upon the initial distributions of the fatty acids present, the mechanistic pathways for decomposition, and the environmental factors during storage and pressing such as moisture, time, and temperature. Although hexanal has little aroma impact until it is present at concentrations approaching several hundred parts-per-million, the concentration of hexanal is often used as a measurement of the state of lipid oxidation (13,14). Tertiary amines and pyridine produce the characteristic odor of old fish. Numerous heterocyclic amines further contribute to the fishy aroma. The pyrazines are associated with nutty-like aromas and are normally found in roasted foods such as peanuts and meats. The straight-chain alkanes from C_{10} to C_{20} were observed using this method. Most probably, there are additional alkanes in the headspace, but they are not observed because of limitations of the method employed.

Of critical importance to the flavor of catfish is the presence of 2-MIB or geosmin or both. When either compound is present at a concentration approaching 1 ppb, they render the fish off-flavor. Trace levels of 2-MIB and geosmin are present in the off-flavor fish presented in Figure 1. However, there is no discernible chromatographic peak, and they are essentially lost in the noise. Their location can be found by using an extracted ion search, and their retention times are noted on the chromatogram. For targeted analysis of these compounds, the MS is operated in SIM mode rather than scan mode.

For quantitation using SPME, a calibration curve was constructed and an internal standard employed. Initially, deuterated analogues of 2-MIB and geosmin were employed as internal

Table II. (Continued)

Class	Chemical name	i.d.	CAS number	Molecular weight	Formula	R.T.
	3,5-Diethyl-2-methyl-pyrazine	MS	18138-05-1	150	$C_9H_{14}N_2$	15.65
	2,5-Dimethyl-3-propyl-pyrazine	MS	18433-97-1	150	$C_9H_{14}N_2$	15.74
	2,3,5-Trimethyl-6-propyl-pyrazine	MS	92233-82-4	164	$C_{10}H_{16}N_2$	18.07
Sulfur						
	Dimethyl disulfide	STD*	624-92-0	94	$C_2H_6S_2$	3.16
	3-Methyl-thiophene	MS	616-44-4	3.97	MDS8UX2.D	91
	2,4-Dimethyl-thiophene	MS	638-00-6	6.78	MDS8UX2.D	87
	4,5-Dimethyl-thiazole	STD	3581-91-7	113	C_5H_7NS	8.56
	Dimethyl trisulfide	STD	3658-80-8	126	$C_2H_6S_3$	9.68
	2-Ethyl-4-methylthiazole	STD	15679-12-6	127	C_6H_9NS	9.82
	2-Acetylthiazole	MS	24295-03-2	127	C_5H_5NOS	11.36
	Benzothiophene	MS	11095-43-5	134	C_8H_6S	16.66
	Benzothiazole	STD	95-16-9	135	C_7H_5NS	17.58
	4-(1,1-Dimethylethyl)-benzenethiol	MS	2396-68-1	166	$C_{10}H_{14}S$	18.74
Terpenoid						
	1,3,3-Trimethyl-tricyclo [2.2.1.02,6]heptane	MS	488-97-1	136	$C_{10}H_{16}$	8.59
	3,7,7-Trimethyl-bicyclo [4.1.0]heptane	MS	554-59-6	138	$C_{10}H_{18}$	11.54
	Limonene	STD	138-86-3	136	$C_{10}H_{16}$	11.63
	1,3,3-Trimethyl-bicyclo [2.2.1]heptan-2-ol	MS	1632-73-1	154	$C_{10}H_{18}O$	14.36
	Camphor	MS	76-22-2	152	$C_{10}H_{16}O$	15.23
	Bicyclo[3.3.1]nonane	MS	280-65-9	124	C_9H_{16}	15.59
	MIB	STD	$C_{11}H_{20}O$	168	$C_{11}HO$	16.39
	1,7,7-Trimethyl-bicyclo [2.2.1]hept-2-ene	MS	464-17-5	136	$C_{10}H_{16}$	16.87
	Isobornyl acetate	MS	125-12-2	196	$C_{12}H_{20}O_2$	19.35
	Dihydrojasmone	MS	1128-08-1	166	$C_{11}H_{18}O$	20.06
	trans-1,10-Dimethyl-*trans*-9-decalol	STD	19700-21-1	182	$C_{12}H_{22}O$	22.39
	Caryophyllene	STD	87-44-5	204	$C_{15}H_{24}$	22.87
	a-Caryophyllene	MS	6753-98-6	204	$C_{15}H_{24}$	23.71
Unsaturated aldehydes						
	2-Methyl-2-pentenal	STD	623-36-9	98	$C_6H_{10}O$	5.46
	(*E*)-2-hexenal	MS	6728-26-3	98	$C_6H_{10}O$	6.07
	(*E,E*)-2,4-heptadienal	MS	4313-03-5	110	$C_7H_{10}O$	10.73
	(*E*)-2-octenal	STD	2548-87-0	126	$C_8H_{14}O$	12.66
	(*E,E*)-2,4-octadienal	MS	30361-28-5	124	$C_8H_{12}O$	14.25
	(*E,Z*)-2,6-nonadienal	MS	557-48-2	138	$C_9H_{14}O$	15.59
	(*Z*)-2-nonenal	MS	60781-31-8	140	$C_9H_{16}O$	15.78
	(*E,E*)-2,4-nonadienal	STD	5910-87-2	138	$C_9H_{14}O$	17.31
	(*E*)-2-decenal	STD	3913-81-3	154	$C_{10}H_{18}O$	18.63
	(*E,E*)-2,4-decadienal	STD	25152-84-5	152	$C_{10}H_{16}O$	20.13
	(*E*)-2-dodecenal	MS	4826-62-4	182	$C_{12}H_{22}O$	21.37
	(*E*)-2-undecenal	MS	2463-77-6	168	$C_{11}H_{20}O$	21.38
	(*Z*)-9-octadecenal	MS	2423-10-1	266	$C_{18}H_{34}O$	26.35
	(*Z*)-9,17-octadecadienal	MS	56554-35-9	264	$C_{18}H_{32}O$	35.22

* STD, steam distillate.

standards, but the high cost, inconsistent supply, and low purity (approximately 95%) resulted in the need for more practical standards. DHNap was selected as the internal standard for its chemical and physical similarity to geosmin. A surrogate standard was also employed as a quality check on each sample. Borneol was selected as the surrogate standard because of its physical and chemical similarities with 2-MIB.

To determine the recovery from an aqueous solution, standards of 2-MIB and geosmin were repeatedly analyzed over the range of 0.1 to 5 ppb. For a newly conditioned fiber, recovery of geosmin and 2-MIB from an aqueous solution showed that approximately 90% of the analytes were recovered on the first sampling. This value varied only slightly from fiber to fiber but dropped as the fiber aged. Fibers can routinely be used for several hundred samplings and are changed when area counts for a 1-ppb standard drops below acceptable levels. To compensate for variation in recovery, a series of geosmin and 2-MIB standards were run before and after each batch of samples, and the calibration curve was generated from these runs. In large sample sets consisting of 20 or more samples, a third or fourth set of calibration standards would be inserted into the autosampler queue after every 10–15 samples.

In analyzing fish tissue, loss in recovery can result from four possible sources: (a) analyte remains in the sample, (b) analyte is destroyed by the MD process, (c) analytes remain in the transfer lines, and (d) material is lost to the atmosphere from the open end of the recovery vessel. To check for incomplete desorption of the analytes, off-flavor fish were microwaved for 3 min to drive off all the moisture, leaving behind a rubbery tissue. The residual material was then weighed, and sufficient water was added to the sample to bring it back to the initial 20-g weight. The sample was then reheated and the distillate collected. Only trace amounts (below quantifiable levels) of 2-MIB, geosmin, or borneol were observed in the distillate from any of the reheated samples. To reduce blow-by, 1 mL of Milli-Q water was placed in the recovery vessel and allowed to cool before starting MD. Residual analyte in the transfer line was recovered by rinsing with Mill-Q water, which was then added to the recovered distillate.

The total recovery consisted of two steps: the SPME analysis of the distillate (discussed previously) and the recovery from the MD step. To obtain values for total recovery, tissue samples consisting of 20 g of shredded fillet were spiked with 5 µL of a 10-ppm solution of borneol,

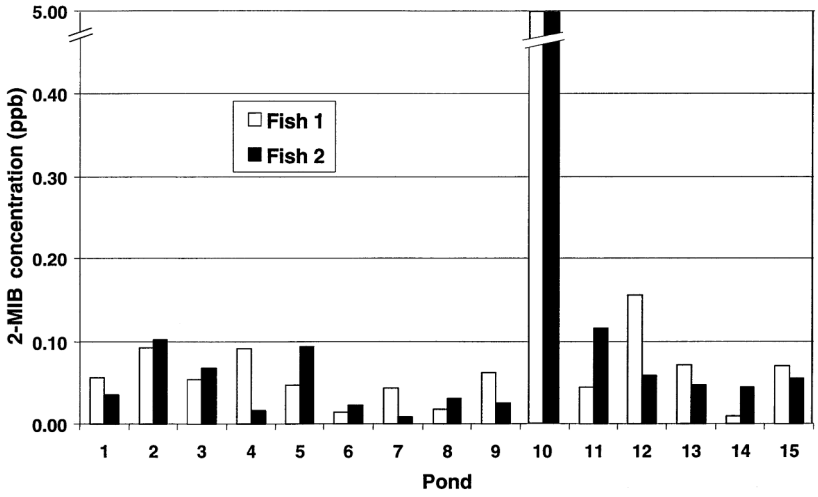

Figure 2. Results of the analysis of 2 fish from 15 ponds using MD-SPME–GC–MS. Pond 10 was determined to be off-flavor by a professional flavor checker; all other ponds were deemed acceptable.

2-MIB, and geosmin, which effectively placed 50 ng of standard in the 20-g sample to give concentrations of 2.5 ppb for each compound. The spiked samples were then microwaved, and the distillate was captured. Aliquots consisting of 5 mL of the recovered distillate were spiked with 5 µL of a 10-ppm solution of DHNap and then analyzed. For comparison, 5-mL samples of water were spiked with 50 ng of borneol, 2-MIB, geosmin, and DHNap.

In Table III, DHNap gave an average area count of 586754 from water, 574042 from fish 1, and 574540 from fish 2. Within experimental error (RSDs of 5.8, 7.2, and 6.6% for water, fish 1, and fish 2, respectively), there was no difference in the recovery of the internal standard spiked into water or fish distillate. Likewise, for the surrogate standard (borneol), RSDs were between 5 and 10% for the individual aliquots. Because the distillate was split between two samples, the peak

Table III. Recovery of 2-MIB and Geosmin from Spiked Fish Samples

Standards in water		DHNap	Borneol		2-MIB		Geosmin	
Rep		Area	Area		Area		Area	
1		623354	985017		247637		3105487	
2		556867	972074		239921		3669151	
3		580042	891010		249964		3711880	
Average		586754	949367		245841		3495506	
Std*		33748	50951		5257		338441	
RSD (%)		5.8	5.4		2.1		9.7	
Spiked fish #1		**DHNap**	**Borneol**		**2-MIB**		**Geosmin**	
Rep	Vial	Area	Area	Vial A+B	Area	Vial A+B	Area	Vial A+B
1	A	631113	402829	736926	93199	167207	838984	1762855
1	B	571365	334097		74008		923871	
2	A	592251	337782	708165	78980	140716	1030007	2009356
2	B	515049	370383		61736		979349	
3	A	593229	399436	742556	79201	120078	900166	1884007
3	B	541245	343120		40877		983841	
Average		574042	364608	729216	71334	142667	942703	1885406
Std		41279	31047	18446	18030	23625	68672	123256
RSD (%)		7.2	8.5	2.5	25.3	16.6	7.3	6.5
Recovery		97.8	38.4	76.8	29.0	58.0	27.0	53.9
Spiked fish #2		**DHNap**	**Borneol**		**2-MIB**		**Geosmin**	
Rep	Vial	Area	Area	Vial A+B	Area	Vial A+B	Area	Vial A+B
1	A	538689	327171	767471	72897	168553	1114268	2040676
1	B	637940	440300		95656		926408	
2	A	567152	424628	783212	77118	141143	1009941	1919157
2	B	547830	358584		64025		909216	
3	A	602294	410805	758559	88747	163197	1187613	2228320
3	B	553333	347754		74450		1040707	
Average		574540	384874	769747	78816	157631	1031359	2062718
Std		38191	46311	12483	11471	14528	107489	155756
RSD (%)		6.6	12.0	1.6	14.6	9.2	10.4	7.6
Recovery		97.9	40.5	81.1	32.1	64.1	29.5	59.0

* Std, standard deviation

areas were combined in order to determine values for a single repetition. The first column under borneol, 2-MIB, or geosmin lists the peak areas for a single analysis, and the second column lists the combined results. For the combined aliquots, RSDs improved for the surrogate standard, 2-MIB, and geosmin. This may be an indication of nonhomogeneous mixing of the analytes in the distillate. Total recoveries for borneol, 2-MIB, and geosmin were approximately 80, 60, and 57%, respectively.

To determine actual amounts of 2-MIB and geosmin in fish tissue, the observed peak areas were adjusted based upon the ratio of the observed peak area to the expected peak area of the internal standard. The expected peak area of DHNap was obtained by taking the average of the peak areas from two or more calibration series (10 values). Corrected peak areas for 2-MIB and geosmin were then converted to mass (nanograms) using the calibration curve generated with each run. Because the two aliquots from the steam distillate were generated with each fish sample, their amounts were added together. In cases where only one of the aliquots was analyzed, the lone value was doubled to give the amount of analyte found in the steam distillate. To obtain the total amount of a given analyte found in 20 g of tissue, the amount found in the distillate was divided by the recovery factor (the recovery factor of 0.6 was used for both geosmin and 2-MIB). The resultant figure was the amount of analyte found in 20 g of tissue. Concentration was obtained by dividing the amount of analyte by 20 g. Borneol was employed as a quality check. If this value fell outside of the range of 80 to 120% of normal, the results were rendered suspect. Additional checks were employed using two qualifier ions (Q1 and Q2) for each analyte.

Typical ranges observed in catfish are from < 0.1 ppb for on-flavor catfish, 0.1–0.7 ppb for marginal flavor fish, and > 0.7 ppb for off-flavor fish (15). The majority of off-flavor fish contain < 5 ppb of either geosmin or 2-MIB. However, some fish have been observed with concentrations in excess of 20 ppb of either 2-MIB or geosmin. When 2-MIB or geosmin or both are present at concentrations > 0.1 ppb, professional flavor checkers describe the fish as having a "blue-green algae" or "muddy" flavor.

To validate the instrumental method, two fish from 15 ponds were obtained from a commercial producer and checked for blue-green off-flavor by a professional flavor checker and analyzed using the MD-SPME–GC–MS technique. The results are shown in Figure 2. Pond 10 was rejected by the flavor checker and given the worst possible acceptance score of 5 (on a scale of 0 to 5). The measured values were well above the upper end of our calibration curve, which puts the concentration of 2-MIB well in excess of 5 ppb. All other ponds were found acceptable. With the exception of ponds 2, 11, and 12, the flavor-checked data was in good agreement with the measured amounts of 2-MIB for fish 1 and 2. Concentrations of 2-MIB in these ponds exceeded the 0.1-ppb cut-off value. The discrepancy is minor and may result either from errors in the instrumental technique, a mistake by the flavor checker, or the acceptance of the value of 0.1 ppb as being absolute. Ponds 2 and 11 are actually within a standard deviation of being on-flavor. The value of 0.1 ppb as being off-flavor for a flavor checker is a subjective value resulting from an average of four flavor checkers and fails to take into consideration other factors that may impact the flavor of fish (15).

It is interesting to note that 2-MIB could be detected in all fish by the instrumental technique. This is generally the case as our detection limits are at approximately 10 pg of 2-MIB or geosmin when placed on the column and employing SIM of the MS. Using the recovery factor of 0.6 for fish to detector, this would translate to concentrations of approximately 2 ppt starting from a 20-g sample of fish tissue. The instrument is sensitive enough to accurately measure these levels, but the poor recovery factor and proportionate loss to glassware may preclude the use of MD-SPME–GC–MS for sub-parts-per-trillion analyses.

Conclusion

This report lists the major compounds found in the headspace of cooked catfish and describes a detailed method for the quantitation of 2-MIB and geosmin using MD-SPME–GC–MS. The instrumental method is more sensitive than a human flavor checker, and presumably more consistent, but takes longer and costs more. Other off-flavors described as woody, sewage, rotten, and diesel can also render fish unacceptable. Before implementing an instrument-based quality determination, the compounds that produce these other flavors must be identified. When a specific compound has been identified as the cause of an off-flavor, rapid methods can be developed to quantitate that compound.

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