Dehydration Products of 2-Methylisoborneol Are Not Responsible for Off-Flavor in the Channel Catfish

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Gas chromatography/mass spectrometry (GC/MS) analyses indicated that volatile fractions of channel catfish (*Ictalurus punctatus*) covering a range of flavor quality were all found to contain 2-methylenebornane and 2-methyl-2-bornene, dehydration products of 2-methylisoborneol. Gas chromatographic effluent sniffing indicated that the only "earthy/musty" odor compounds present were identified as 2-methylisoborneol and geosmin. It was concluded that 2-methylenebornane and 2-methyl-2-bornene, which do not have discernible odors, do not contribute to off-flavor, contrary to an earlier study.

INTRODUCTION

Two "earthy/musty" off-flavors often found in catfish have been identified as 2-methylisoborneol (MIB) (Martin et al., 1987) and geosmin (Lovell et al., 1986). These compounds are produced by cyanobacteria and actinomycetes in the water and are assimilated by the fish, rendering them temporarily unmarketable (Johnsen, 1989). It has been suggested that two dehydration products of MIB, 2-methylenebornane (2MB) and 2-methyl-2-bornene (2M2B), also impart a musty odor and cause off-flavor in catfish (Martin et al., 1988a,b). During investigations in this laboratory, it has been observed that significant levels of 2MB and 2M2B are often present in catfish free of earth/musty off-flavors. In eight samples of acceptably flavored catfish, purchased from a local retail supplier over a period of 4 months, six contained 2M2B. It was, therefore, considered important to reinvestigate the contribution of these compounds to off-flavor.

To test the null hypothesis that 2MB and 2M2B do not contribute to the earth/musty off-flavor observed in catfish, the technique of gas chromatographic effluent sniffing was used to characterize the odor of these compounds. The two dehydration compounds, found naturally in catfish and synthetically synthesized, were characterized by instrumental and human odor assessment methods. No attempt was made to survey the frequency of occurrence at which these compounds might be found in catfish.

MATERIALS AND METHODS

Synthesis of MIB and Its Dehydration Products. MIB was synthesized according to the method of Wood and Snoeyink (1977), and the dehydration products, 2MB and 2M2B, were prepared, with some modification, according to the method of Taber et al. (1987). In the preparation of dehydration products, a mixture of MIB (5 g) and pyridine (90 mL) was cooled with ice in a three-neck flushed with nitrogen gas. Thionyl chloride (4 mL) was added rapidly dropwise. The mixture was stirred for 1 h and then poured into ice. The final reaction mixture was extracted with diethyl ether (3 × 60 mL). The combined ether extracts were washed sequentially with 10% HCl, saturated copper sulfate solution, saturated sodium bicarbonate solution, and saturated sodium chloride solution. These steps served to complete the reaction, neutralize the solution, and salt out remaining organics. The organic layer was dried over anhydrous sodium sulfate and concentrated by distillation. The crude concentrate was allowed to stand at 4 °C for several days. The oily residue that settled on the bottom of the flask then was removed. The resulting concentrate was stored at −20 °C.

Table I. Relative Concentrations of 2-Methylenebornane (2MB), 2-Methyl-2-bornene (2M2B), and 2-Methylisoborneol (MIB) in Catfish Samples: Comparison with Sensory Evaluation and Olfactory Analysis

sample	relative concn,[a] arbitrary units 2MB	2M2B	MIB	earthy/musty flavor[b]	MIB and geosmin odor[c]
A	265	603	nd[d]	0.4	+
B	258	358	35	0.9	+
C	362	232	1065	2.8	+

[a] See text for procedure. [b] See Johnsen and Kelly (1990) for intensity reference scale. [c] + indicated that during olfactory analysis MIB and geosmin odors were present at the retention time of the authentic compounds. [d] nd, not detectable by MS.

Instrumental Analysis. Gas chromatography/mass spectrometry (GC/MS) was carried out with a Hewlett-Packard system (Hewlett-Packard Co., Palo Alto, CA) consisting of an HP 5988A mass spectrometer, an HP 5890 Series II gas chromatograph (GC), and an HP 5987C data system. The GC was fitted with a 50 m × 0.2 mm i.d. Hewlett-Packard Ultra 2 column (cross-linked 5% PhMe silicone) with 0.33-μm phase thickness. The GC was temperature programmed from 35 to 250 °C at 4 °C/min. The mass spectrometer was scanned from 30 to 210 m/z, and mass spectra were generated at 70 eV. The geosmin standard was purchased from Givaudan Corp. (Clifton, NJ).

Catfish Samples. Three pooled samples of catfish fillets were obtained from six fish. Sample A, from a retail supplier, was two fillets weighing approximately 290 g each. Samples B and C were obtained from production ponds. Sample B consisted of two fillets of approximately 175 g each, and sample C consisted of two fillets of approximately 200 g each.

Extraction of Volatiles from Catfish. Catfish fillets were finely shredded in a food processor and placed in centrifuge tubes. The oil layer that was obtained after centrifugation at 14700g for 30 min was carefully removed using a Pasteur pipet. Oil (7.5 g) was placed in a 500-mL three-neck flask and stirred with a magnetic stirring bar. The flask was maintained at 65 °C. The oil was sparged with nitrogen at 20 mL/min for 16 h. Volatiles were trapped on 250 mg of 60/80 mesh Tenax GC (Teklab, Baton Rouge, LA) packed in a glass tube. Volatiles were eluted from the trap with 6 mL of diethyl ether. Internal standard (IS) solution [4 μL, 12 mg of 2-chloropyrazine (Pyrazine Specialities, Atlanta, GA) in 5 mL of methylene chloride] was added and the solvent evaporated to 0.1 mL with a gentle stream of nitrogen.

Olfactory Analysis. The sample of volatiles trapped from oil was injected into a 60 m × 0.75 mm i.d. glass capillary column

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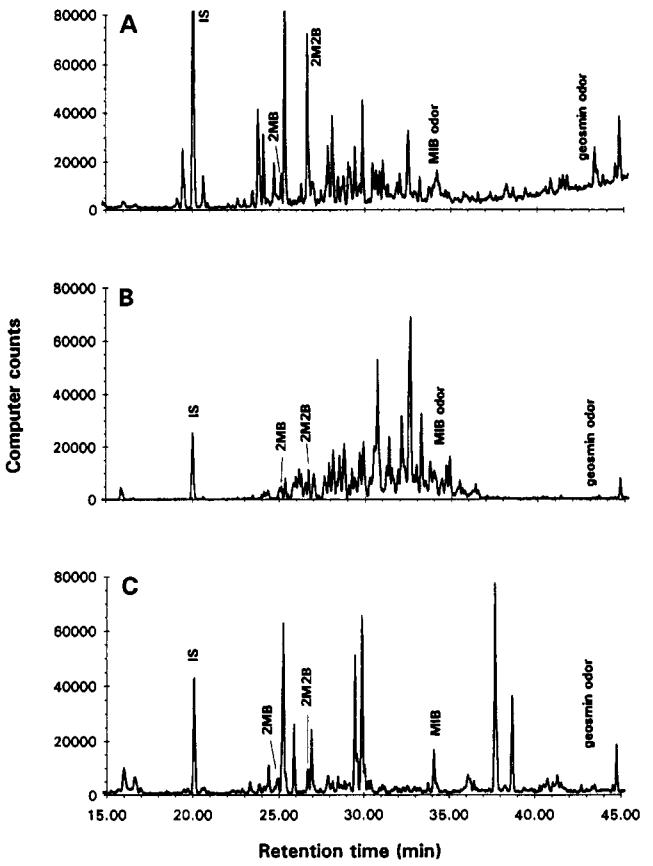

Figure 1. Total ion chromatograms of volatile compounds from catfish oil confirming the presence of 2MB and 2M2B in all three samples and MIB in sample C. Geosmin and MIB odors detected by GC effluent sniffing are shown on the chromatograms, but the concentrations were too low for mass spectral confirmation.

with 1-μm phase thickness. The column (SPB-5, Supelco, Inc., Bellefonte, PA) contained a slightly polar phase (5% diphenyl, 94% dimethyl, 1% vinyl polysiloxane). An HP 5790 gas chromatograph was used with the oven temperature programmed from 35 to 200 °C at 4 °C/min. Helium carrier gas flow rate was 7.5 mL/min. The column effluent was split (1:1) between a flame ionization detector and a sniffer port. The effluent was sniffed continuously, and the retention time of any earthy/musty odor was noted. Each sample was analyzed independently by two trained panelists.

Sensory Evaluation. Samples for sensory analysis were presented to a trained flavor panel in 10-g portions. Descriptive analysis profiles were prepared using a lexicon of catfish flavor descriptors, while intensities of attributes were judged on an open-ended reference scale of flavor intensities in specific food products (Johnsen and Kelly, 1990). The trained panel judged the intensity of the earthy/musty complex as the combined descriptive terms geosmin and MIB.

RESULTS AND DISCUSSION

The earthy/musty flavor perceived in sample A was given a mean score of 0.4 by the trained flavor panelists. From panel experience, a value of this magnitude is equivalent to the attribute being undetectable to the general public. This result was expected as sample A was obtained from a retail supplier whose product had been screened for the presence of off-flavors prior to its leaving the processing plant. The mean of flavor evaluation scores (0.9) established the earthy/musty flavor in sample B to be identifiable but just above recognition threshold. Sample C score mean was highest (2.8) and, from panel experience, was rated in the midrange of intensity for earthy/musty and would be unacceptably off-flavor to the consumer (Table I). Thus, these three samples represented a range of flavor quality.

When volatiles from each fish sample were subjected to olfactory analysis by sniffing the GC column effluent, only two odor compounds with a musty/earthy odor were found in each sample. These compounds were tentatively identified as MIB and geosmin on the basis of retention times being identical to those of authentic samples (Figure 1). The perceived odor intensity of the presumptive MIB peak increased in order from sample A through sample C (Table I).

Gas chromatography/mass spectrometry (GC/MS) analyses confirmed MIB by a full-scan mass spectrum only in sample C. Either low concentration or coelution prevented mass spectral confirmation in the other two samples. Relative concentrations of MIB in all samples were obtained by determining the area under the 95 m/z ion peak, the base peak for MIB, and proportioning this area to that of the total ion current (TIC) area for the internal standard. Relative concentrations of MIB are shown to

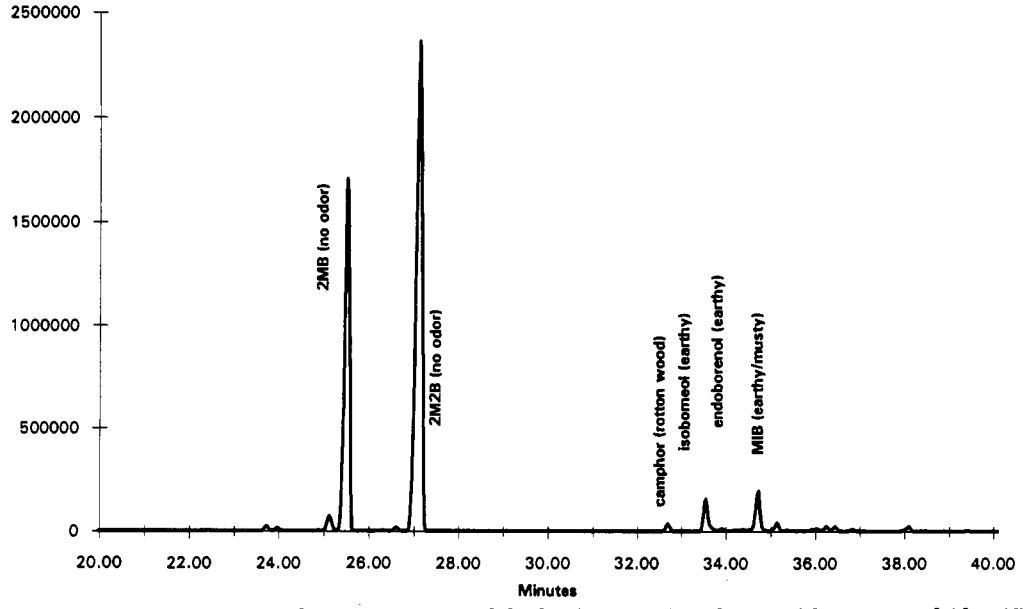

Figure 2. Total ion chromatogram of products of the MIB dehydration reaction shown with compound identification and odor description.

increase with increased earthy/musty flavor (Table I). Geosmin levels were below GC/MS detection levels in all samples.

Using GC/MS analysis it was also possible to confirm the presence of 2MB and 2M2B in all three catfish samples. TIC chromatograms for the volatiles from each fish sample are shown in Figure 1. The intensity scales have been normalized to the internal standard peak. Identification of 2MB and 2M2B was made by both retention times, which were the same as those of the two major products of the dehydration of MIB, and the mass spectra, which were the same as those previously reported (Burgstahler et al., 1976; Martin et al., 1988b; Walter et al., 1983). Quantitation was made by determining the relative areas under the 107 m/z ion peak for each compound and proportioning this area to that of the TIC area for the internal standard. This procedure was necessary as coelution of compounds with 2MB or 2M2B occurred in some samples and no pure standards were available for instrument calibration. Relative concentrations of the two compounds are shown in Table I. However, unlike MIB, there is no obvious relationship between concentrations of either dehydration product and the perceived intensity of earthy/musty flavor.

While MIB and geosmin were perceived to have earthy/musty odors, no odor of any description was detected by sniffing the GC effluent at the retention time for either dehydration compound. This was true for all three catfish samples. Confirmation that MIB dehydration products lacked earthy/musty odor was obtained by sniffing the synthetic products of the MIB dehydration reaction as they emerged from the GC column. Figure 2 shows a total ion chromatogram of this solution. On the basis of the TIC response factors, the amounts of synthetic 2MB and 2M2B injected were 63 and 35 times, respectively, that naturally found in sample A. Even at this high concentration, neither dehydration compound had a perceptible odor.

It is concluded that 2-methylenebornane and 2-methyl-2-bornene do not produce earthy/musty off-flavors in channel catfish. While these MIB dehydration products were found by GC/MS in both acceptable and off-flavor catfish, odor evaluation by gas chromatographic effluent sniffing demonstrated that neither natural nor synthetic 2MB or 2M2B produced any discernible odors. This evidence supports the null hypothesis that 2MB and 2M2B do not contribute to the earthy/musty off-flavor observed in catfish. The significance of their presence in the catfish is unknown.

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LITERATURE CITED

Burgstahler, A. W.; Boger, D. L.; Naik, N. C. Synthesis and chiroptical properties of some asymmetric planar cisoid dienes. *Tetrahedron* **1976**, 32, 309–315.

Johnsen, P. B. Factors influencing the flavor quality of farm-raised catfish. *Food Technol.* **1989**, *43*, 94–97.

Johnsen, P. B.; Kelly, C. A. A Technique for the Quantitative sensory evaluation of farm-raised catfish. *J. Sens. Stud.* **1990**, *4*, 189–199.

Lovell, R. T.; Lelana, J. Y.; Boyd, C. E.; Armstrong, M. S. Geosmin and musty muddy flavors in pond raised Channel catfish *Ictalurus punctatus*. *Trans. Am. Fish. Soc.* **1986**, *115*, 485–489.

Martin, J. F.; McCoy, C. P.; Greenleaf, W.; Bennet, L. Analysis of 2-methylisoborneol in water, mud, and Channel catfish (*Ictalurus punctatus*) from commercial culture ponds in Mississippi. *Can. J. Fish. Aquat. Sci.* **1987**, *44*, 909–912.

Martin, J. F.; Bennett, L. W.; Graham, W. H. Off-flavor in the Channel catfish (*Ictalurus punctatus*) due to 2-methylisoborneol and its dehydration products. *Water Sci. Technol.* **1988a**, *20*, 99–105.

Martin, J. F.; Fisher, T. H.; Bennett, L. W. Musty Odor in chronically off-flavored Channel catfish: Isolation of 2-methylenebornane and 2-methyl-2-bornene. *J. Agric. Food Chem.* **1988b**, *36*, 1257–1260.

Taber, D. F.; Raman, K.; Gaul, M. D. Enantioselective ring construction: synthesis of (+)-estrone methyl ether. *J. Org. Chem.* **1987**, 52, 28–34.

Walter, S. R.; Marshall, J. L.; McDaniel, C. R.; Canada, E. D.; Barfield, M. Experimental and theoretical studies of ^{13}C-^{13}C coupling constants. 1. Conformational and substituent dependencies of long range coupling constants ($^{4}J^{13}C-^{13}C$). *J. Am. Chem. Soc.* **1983**, *105*, 4185–4190.

Wood, N. F.; Snoeyink, V. L. 2-Methylisoborneol, improved synthesis and a quantitative gas chromatographic method for trace concentrations producing odor in water. *J. Chromatogr.* **1977**, *132*, 405–420.

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