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Instrumental versus sensory detection of off-flavors in farm-raised channel catfish

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Abstract

Results from an instrumental method for determining the presence of the earthy, muddy or blue-green off-flavor in catfish were compared with those of four professional flavor checkers. Solid phase microextraction and gas chromatography/mass spectrometry were used to detect the off-flavor compounds, 2-methylisoborneol and geosmin. The odor threshold for an average flavor checker was determined to lie between 0.1 and 0.2 μg/kg for 2-methylisoborneol and estimated at 0.25 to 0.5 μg/kg for geosmin. The odor threshold for geosmin in channel catfish appears to be only slightly greater than that of MIB. The lower end of the sensory limits served as the instrumental cutoffs for grading fish off-flavor. Comparison of the instrumental method versus the flavor checkers resulted in a high correlation (R=0.9).
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Keywords: Catfish; 2-methylisoborneol; Geosmin; Solid phase microextraction; Gas chromatography/mass spectrometry; Odor thresholds

1. Introduction

The farm-raised catfish industry takes pride in delivering a consistent high quality product to consumers. While there are many off-flavors that can result in a fish being objectionable, fish are primarily rejected for possessing woody, grassy, rotten, diesel, and the earthy, muddy aroma also known as blue-green aroma. Approximately 80% of the rejected fish have the earthy, muddy aroma. This earthy, muddy or blue-green off-flavor is

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primarily caused by the presence of either MIB (1-*R*-exo-1,2,7,7-tetramethyl bicyclo-[2-2-1]-heptan-2-ol) or geosmin (*trans*-1,10-dimethyl-*trans*-9-decalol). Frequently a mixture of both compounds is present. These compounds cause chronic problems in aquaculture and are ubiquitous in nature, with deleterious impacts in such diverse commodities as drinking water, cereal, sugar, whiskey, and paper tissue products. MIB and geosmin are produced by cyanobacteria, actinomycetes, and certain fungi (Lovell and Sackey, 1973; Jensen et al., 1994; Dionigi et al., 1991). Whereas MIB is generally associated with a muddy odor, geosmin has more of a musty or old-book like odor; however, it is nearly impossible to distinguish between them in off-flavor catfish.

The odor threshold in humans for MIB and geosmin has been determined by several groups to range up to 35 and 40 parts per trillion (Wood and Snoeyink, 1977; Persson, 1980; Martin et al., 1987). A recent review (Howgate, in press) places the odor threshold values for both MIB and geosmin at a concentration level around 15 ng/kg in pure water. These compounds are nontoxic at concentrations routinely found in nature (Dionigi et al., 1993). The presence of MIB and geosmin can render farm-raised catfish off-flavor year round, but are especially troublesome in late summer and early fall. It has been estimated that as many as 80% of the ponds have off-flavor catfish in September which can severely hamper processing schedules (Dionigi et al., 1998).

Numerous investigations are underway to understand the reason these compounds are produced and to prevent them from occurring. Until recently, research has been hindered by the methods to accurately measure these compounds in sufficiently large numbers to offer statistically validity. Although the human nose can readily detect MIB and geosmin at the sub parts per billion range (<1 μg/kg), only semi-quantitative data are provided and humans readily succumb to sensory overload. Biological approaches using antibodies hold promise of a simple, inexpensive, technique, but researchers still must overcome the basic problems of sensitivity and selectivity (Chung et al., 1990). Electronic noses employing specialized sensors have received a lot of interest, but practical assessment of their capabilities using MIB and geosmin standards in water suggests that the sensitivity and selectivity required for analyzing trace level compounds in complex matrices (such as fish tissue) are not currently accessible.

Current practice for determination of off-flavor in catfish is by sensory analyses made by a professional flavor checker. Prior to harvest, a flavor checker inspects representative fish from each pond for the presence of any objectionable flavors. Fish are sampled several times prior to pond harvesting and again on arrival at a processing facility to ensure good-flavor quality. The flavor checkers are highly sensitive to these off-flavors, and fish are rejected if any hint of MIB or geosmin is present. Sample loads may well exceed 100 samples per day, particularly in the early part of the week.

The instrumental sensitivity required to detect low concentrations of these compounds and high sample throughput has rendered the standard methods of trace levels of analysis impractical due to the high equipment costs and the need for technically trained operators. However, over the last decade, sample preparation techniques and analytical instrumentation for the analysis of trace level compounds have greatly improved. The advent of solid-phase microextraction technology (SPME) has greatly advanced the analysis of aromas from foods (Belardi and Pawliszyn, 1989; Pawliszyn, 1999). Using SPME coupled with gas chromatography/mass spectrometry (GC/MS), the off-flavor odorants, MIB and

geosmin can be readily detected in water at concentrations less than 10 ng/kg (Lloyd et al., 1998; Watson et al., 2000). Determining MIB and geosmin concentrations in water is now readily accomplished on large data sets (Zimba and Grimm, 2003), whereas analysis of fish tissue requires more complicated procedures (Lloyd and Grimm, 1999; Grimm et al., 2000).

SPME is not as effective at extracting compounds from complex sample matrices, such as soil and muscle tissue. Physical bonds allow the prospective analytes to adhere to the sample matrix. This problem can be overcome by using microwave distillation (Conte et al., 1996; Lloyd and Grimm, 1999). The analytes are essentially steam distilled away from the matrix and the steam effluent is collected in a chilled water bath. The technique effectively places the analytes in an aqueous phase, which is readily amenable to SPME analysis. Total analysis time from start to finish requires 25 min. However, the GC/MS analysis is automated, permitting subsequent sampling every 15 min. Typically, a single operator can analyze 50 to 70 fish per day. Detection levels are less than 30 parts per trillion of MIB and less than 20 parts per trillion of geosmin (Grimm et al., 2000).

To evaluate this technology, a comparative study was made between flavor checkers and the instrumental method. Our goal was to assess detection limits for flavor checkers and to determine the similarity of results from each method.

2. Materials and methods

Geosmin and 2-methylisoborneol were obtained from Supelco (Bellefonte, PA) as a 100 μg/ml solution in ethanol. Subsequent dilutions were made at 0.1, 0.5, 1.0, 3.0, and 5.0 μg/kg using high purity water (Waters, Milliford, MA). Off-flavor catfish (*Ictalurus punctatus*) were obtained from four different processing facilities and were of commercial size, averaging ~1 kg. A total of 219 fish were analyzed by both professional flavor checkers and by instrumental analysis. Paired subsamples were taken from fish with one set being presented to flavor checkers for off-flavor assessment. The second set was tagged and frozen prior to instrumental analysis, with one exception where fish were analyzed concurrently at the processing facility by both flavor checker and the instrumental method. In this case, a Finnigan GCQ (Palo Alto, Ca) was temporarily installed at the processing facility. Otherwise, fish were immediately frozen (-20 °C) and shipped to the Southern Regional Research Center for instrumental analysis.

2.1. Sensory analysis

Sensory evaluation methods employed varied from one flavor checker to another, with some preferring to check the muscle tissue nearest the tails, while others preferred the muscle tissue nearest the gut. Off-flavor assessment was performed by microwaving selected portions of the fish fillet. Upon removal form the microwave, the samples were first sniffed to detect grossly off-flavor fish. If no off-flavor was detected in the fish, they were then tasted. Fish determined to be off-flavor by the flavor checkers were classified as to the specific offensive odor and a score (0–5) denoting the relative degree of off-flavor,

with a zero representing an on-flavor fish. A value of '1' means that the fish is slightly off flavor and may be rechecked in a week, whereas '2' denotes a more noticeable off-flavor and should not be rechecked for 2 weeks. One flavor checker employed a reverse scheme with '5' being rated as an on-flavor fish and '0' a severely off-flavor fish.

2.2. Instrumental analysis

Fillet samples were finely chopped and placed in a glass container. Each sample was spiked with 5 μl of a 10 mg/l aqueous solution of the internal standard, borneol. The sample was then heated for 3 min in a microwave while purging with 80 ml/min of N_2. The effluent was transferred via glass tubing to a receiving vessel (50 ml graduated cylinder) in a chilled water bath held at 0 °C. The condensed water was normalized to a total volume of 10 ml using deionized water. The sample was then divided into 5-ml aliquots and each placed into a 10-ml vial. NaCl was added in the amount of 1.9 g and the vial spiked with 5 μl of a 10 mg/l solution of a secondary internal standard, decahydro-1-naphthol. The vial was sealed with a crimp cap-fitted with a Viton septum (Supelco) 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). The fiber was withdrawn from the sample and desorbed at 270 °C for 5 min in the injection port of an HP6890 gas chromatograph equipped with a 5973 mass selective detector (Agilent Technologies, Palo Alto, CA). The injection port was operated in pulsed splitless mode and fitted with a 0.7-mm-ID 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, maximum GC resolution was desired and the mass spectrometer was operated in scanning mode. Once target compounds had been identified, GC resolution was traded off to shorten run time and selected ion monitoring was employed on the mass spectrometer for optimum sensitivity.

For qualitative analysis, the oven was held at 40 °C for 3 min, then temperature programmed at 5 °C/min to 200 °C then 50 °C/min to 250 °C 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 temperature programmed at 20 °C/min to 100 °C, 7.5 °C/min to 152 °C, 65 °C/min to 250 °C, where the oven was held to give a total run time of 12.75 min. Cool down for the GC oven took approximately 4 min.

The quadrupole mass spectrometer was operated in electron ionization mode and initially scanned from *m*/*z* 50 to *m*/*z* 350 for qualitative analysis. Selected ion monitoring of the base peak and molecular ion was employed for quantitation of MIB, geosmin, and the two standards. The base peaks and qualifier ions for each of the four compounds monitored were as follows; borenol—*m*/*z* 95, 154; MIB—*m*/*z* 95, 168; decahydronaphthol—*m*/*z* 136, 154, and geosmin—*m*/*z* 112, 182. Quantification was accomplished by

integrating the base peak area at the proper retention time, while the qualifier ions were checked for proper ratios to ensure proper identification and peak purity. To eliminate carryover between samples, glassware was rinsed with a 1 M HCl solution, followed by a water rinse and baked at 200 °C after washing.

Pearson correlations were used to identify significant relationships between analytical measures and sensory scores using SAS V8.5 (SAS, Cary NC). Analytical scores were transformed prior to analyses (Sokal and Rohlf, 1981).

3. Results and discussion

Fig. 1 shows a series of reconstructed ion chromatograms of *m/z* 95, the base peak of MIB. Sample A was blank and contained no MIB, while samples B, C, and D had been spiked with known amounts of MIB. The peak at 9.74 min increased with the MIB concentration level. Integration of the peak areas and subsequent regression against the known concentration levels provides a calibration for the determination of samples with unknown levels of MIB. There are numerous factors that can affect the recovery of MIB from the sample. The instrumental variables, such as recovery, repeatability and sensitivity, have been dealt with previously (Lloyd and Grimm, 1999; Grimm et al., 2000, Watson et al., 2000). Fillet fat content has been previously correlated with increased off-flavor content (Johnsen and Lloyd, 1992). Fat content was not measured for each fish, but as these were market size fish, raised in accordance with standard practices, the range would be expected to vary from 6% to 15% with the average value around 8% (Li et al., 1998).

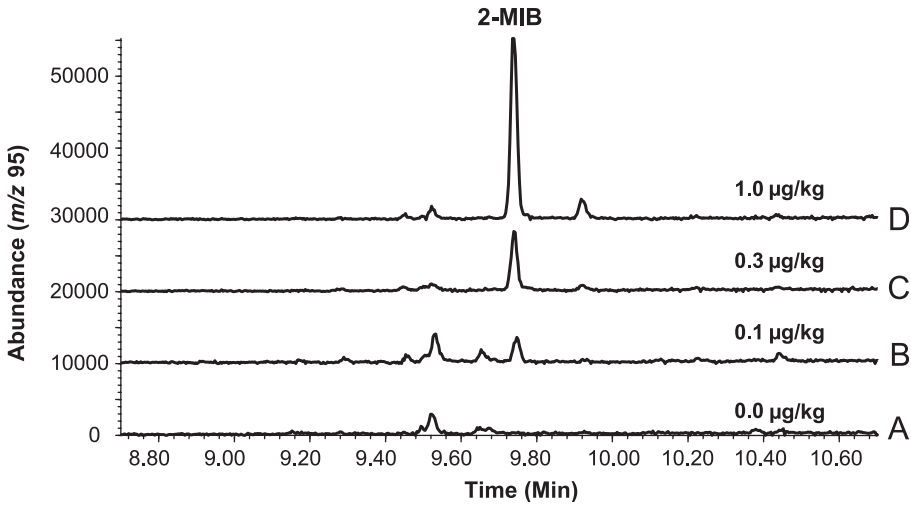

Fig. 1. Reconstructed ion chromatogram of *m/z* 95 for a blank, and spiked solutions of MIB at a 0.1, 0.3, and 1 µg/kg.

3.1. Flavor checkers sensitivity

The average consumer generally considers catfish off-flavor when the concentration level of either geosmin or MIB exceeds 0.7 μg/kg (Johnsen and Kelly, 1990, Bett et al., 2000). However, professional flavor checkers possess more acute detection capabilities than the average consumer. Instrumental results (using the 0.7 μg/kg as the instrumental cutoff to differentiate between on and off-flavor fish) did not adequately match professional flavor checkers, resulting in fewer instrumental samples being considered off-flavor. There was clearly a difference in the odor threshold of the flavor checkers and the 0.7 μg/kg value of the average consumer.

An estimate of the odor threshold for each flavor checker was calculated by examining the samples with instrumental values just above and below fish deemed on and off-flavor. The average of the five fish with the highest levels as measured by the instrumental method and determined to be on-flavor by each flavor checker serves as the lower bracket for the odor threshold. Similarly the five fish with the lowest levels as determined by the instrumental method and called off-flavor by the flavor checkers serves as the upper bracket for the odor threshold. The true odor threshold should lie between these two endpoints (Table 1). The flavor checker from plant four had an insufficient number of on-flavor fish to make the determination for the low end. Averaging the low endpoints and the high endpoints gave the range in which a "theoretical average flavor checker's" odor threshold would lie. That value would lie in a range between 0.1 and 0.2 μg/kg.

Odor threshold is defined as the concentration when the odor is detected 50% of the time. Insufficient samples were obtained to accurately determine the odor threshold for each of the professional flavor checkers. Examining the combined data for all four checkers with instrumental values for MIB concentrations from 0.1 and 0.2 μg/kg results in a 41% detection rate. Examining the combined data from 0.15 to 0.25 μg/kg results in a 57% detection rate. Some flavor checkers may have superior ability and might yet determine a fish to be off-flavor with less than a 0.1 μg/kg off-flavor. Setting the value at 0.1 μg/kg as the instrumental standard for determining off-flavor provides an objective benchmark that is at the lower end of the odor threshold for flavor checkers. This value can be adjusted as more data becomes available, and could possibly be intentionally varied depending upon ultimate crop usage.

Table 1
MIB threshold values for flavor checkers

	Top five on-flavor fish (μg/kg)	Lowest five off-flavor fish (μg/kg)
Plant #1	0.14	0.23
Plant #2	0.11	0.22
Plant #3	0.08	0.25
Plant #4	–	0.17
Average	0.11	0.22

Average concentration of MIB from fish deemed to be on-flavor (column 1) and to be off-flavor (column 2).

3.2. Head-to-head results

Paired fillets from 219 samples were analyzed by the instrumental method and by one of four flavor checkers. The flavor checkers determined 106 of the samples to be on-flavor and 113 were determined to be off-flavor. Of the 219 samples, 15 fish were deemed off-flavor by the flavor checkers for odors other than those caused by MIB and geosmin and are eliminated from further analysis. Table 2 presents the data using an instrumental threshold of 0.1μg/kg as the cutoff for off-flavor fish. Of the 204 samples deemed acceptable or off-flavor due to the blue-green odor, the instrumental and flavor checkers were in agreement on 155 of the fish, or 76% of the samples. Instrumental cutoff values of 0.7 and 0.2 μg/kg were also evaluated and gave similar results (75% and 77%, respectively), when compared with the flavor checkers. There are two types of disagreements possible: the first occurs when the instrumental method determines the sample to be off flavor and the checker does not; the second where the checker determines the sample to be off-flavor and the instrumental method does not. Of the 49 samples in disagreement, 15 were deemed off-flavor by the flavor checkers and not by the instrumental method while 34 were deemed off-flavor by the instrumental method and not by the flavor checkers. When employing an instrumental cutoff value of 0.2 μg/kg, only 20 samples were deemed off-flavor by the instrumental method and not by the flavor checkers, while only one sample is in dispute when an instrumental cutoff value of 0.7 is employed. For the first type of disagreement, there is a clear inverse relationship between the number of samples in disagreement and the instrumental cutoff value employed. Dropping the instrumental cutoff value to levels below 0.1 μg/kg would clearly result in an excessive number of fish being determined as off-flavor.

The majority of the 34 samples determined to be off-flavor by the instrument and not deemed to be off-flavor by the flavor checkers have MIB or geosmin levels near the cutoff threshold. Of the 34 fish with MIB above the 0.1 μg/kg instrumental limit and not called off-flavor by the flavor checker, all but 1 fish have levels below 0.5 μg/kg. This type of disagreement is not totally unexpected as we are working near the threshold limit of the flavor checker and the lower end of the scale for the instrumental method. Instrumental

Table 2
Comparison of instrumental determination with professional flavor checkers

	Instrumental analyte considered	Total fish	Agree		Disagree			
					Checker		Instrument	
			#	%	#	%	#	%
All fish	MIB	219	161	73.5	24	11.0	34	15.5
BG only	MIB	204	155	76.0	15	7.4	34	16.7
BG only	(MIB or GSM)	204	149	73.0	4	2.0	51	25.0
BG only	Sum of MIB and GSM	204	135	66.2	4	2.0	65	31.9

The number (#) and percentage (%) of fish in agreement as determined by the instrumental method and the flavor checkers. Values are given when considering all fish and fish only possessing a blue green (BG) odor. The BG odor is further subdivided based upon the analyte being considered, MIB at 0.1 μg/kg, MIB or GSM, and MIB and GSM.

measurements are not exact and routinely express an error of 10–20%, which can become even greater at the lower end of the calibration curve.

From an analytical perspective, the second type of disagreement is of greater concern as the instrumental method is considered to be more sensitive and to provide a greater level of objectivity as well. Fifteen fish were deemed off-flavor by the flavor-checkers, but considered on-flavor by the instrumental method when employing a cutoff value of 0.1 μg/kg for MIB. One possibility for the discrepancy is the presence of geosmin. Although previous reports indicate that the odor threshold for geosmin in catfish is an order of magnitude higher than MIB (Lovell et al., 1986), we believe the odor threshold in catfish to be much closer to that of MIB. It would then be prudent to consider that some of the fish may have been considered off-flavor due to geosmin, which is hard to distinguish from MIB in catfish. Of the 15 fish, 11 are off-flavor if the instrumental cutoff value for geosmin is considered at the MIB level of 0.1 μg/kg, leaving only four fish in disagreement.

However, if we consider the instrumental cutoff value at 0.1 μg/kg for geosmin to be off-flavor, an additional 17 samples previously considered being on-flavor by both the instrumental method and the flavor checkers would now be off-flavor. Increasing the instrumental cutoff value for geosmin to 0.25 μg/kg would result in six additional samples as off-flavor, while an increase to 0.5 μg/kg would result in only two additional samples being considered off-flavor. Consequently, the number of samples from the 15 samples in dispute, explained by the presence of geosmin, would drop from eleven (0.1μg/kg) to nine (0.25 μg/kg), and to six samples (0.5 μg/kg). Clearly the instrumental cutoff for geosmin of 0.1 μg/kg is too low and a working value would most likely lie in the range between 0.25 and 0.5 μg/kg.

Even using the most sensitive instrumental cutoff value for determining off-flavor due to geosmin (0.1μg/kg), there are four remaining samples that were considered to be strongly off-flavor by the flavor checkers and not detected by the instrumental method. The possible reasons for the disagreement on the four fish are unknown and could result from mislabeling, sample preparation error, and/or instrumental malfunction. We have no definitive explanation for these four fish and consider them to be anomalies.

A high correlation (R=0.9) was observed between the instrumental method and the flavor checkers for the 204 fish with an instrumental cutoff of 0.1 μg/kg for MIB and a cutoff value of 0.25 μg/kg used for geosmin.

4. Odor threshold of geosmin in catfish

Recently the odor threshold for geosmin in trout was determined to be 1.5 μg/kg (Robertson and Lawton, 2003), much lower than the previous values of 6 to 10 μg/kg in fresh water trout; (Yurkowski and Tabachek, 1974). Moreover, odor thresholds for geosmin in water are similar to that for MIB. The odor thresholds for geosmin and MIB in water were initially reported around 35 and 40 ng/l, respectively (Persson, 1980). Subsequent reports place those values below 10 ng/l (Polak and Provasi, 1992; Rashash et al., 1997). Due to the inherent error in such a measurement, one would assume that the odor thresholds for MIB and geosmin in water are not significantly different. Early reports

in the literature suggest that the odor threshold for geosmin is nearly an order of magnitude higher in catfish than MIB (Yurkowski and Tabachek, 1974; Persson, 1980). The data presented here would tend to suggest that the geosmin odor threshold in catfish is only slightly greater than that of MIB. In the data set, 15 fish were considered off-flavor by flavor checkers and did not contain sufficient MIB to be determined off-flavor by the instrumental method (<0.1 μg/kg). Employing a cutoff value of 0.25 μg/kg for geosmin would account for nine fish being off-flavor. The geosmin concentrations for those nine samples ranged from 0.25 to 7.82 μg/kg with an average value of 1.55 μg/kg and a median of 0.86 μg/kg. With only one exception, all of these are well below the literature value of 7 μg/kg for the odor threshold in catfish.

The discrimination by the instrumental method can be further increased not only by lowering the cutoff limit, but also by employing the additive effect of MIB and geosmin. There is no reported information in the literature as to whether the presence of both MIB and geosmin produce an additive effect. The combined results are presented in the bottom row of Table 2. Due to the similar structure and odor of the two compounds, one would expect that the mechanism for sensory stimulation would be similar if not the same and would therefore imply that their concentrations would be additive. On the other hand, anecdotal information indicates that some people can be anosmic to geosmin while being quite sensitive to MIB (Bett, 2003). This suggests that different receptors might be involved. At this time, there is insufficient data to draw a reliable conclusion on the additive effect of MIB and geosmin.

5. Conclusions

An instrumental method for determining the presence and concentration of the compounds responsible for the blue-green off-flavor in catfish was compared with four professional flavor checkers. Geosmin was determined to have an odor threshold in catfish similar to that of MIB, relative to the order of magnitude difference reported in the literature. No conclusion could be drawn on the additive effect due to the presence of both MIB and geosmin in catfish. Odor threshold for a theoretical average flavor checker was determined to lie between 0.1 and 0.2 μg/kg for MIB and between 0.25 and 0.5 μg/kg for geosmin. The lower end of the range (0.1 μg/kg for MIB and 0.25 μg/kg for geosmin) was selected as the instrumental cutoff for determining off-flavor fish.

6. Uncited reference

Lalezary et al., 1984

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