OFF-FLAVOR IN THE CHANNEL CATFISH (ICTALURUS PUNCTATUS) DUE TO 2-METHYLISOBORNEOL AND ITS DEHYDRATION PRODUCTS

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ABSTRACT

Off-flavor in channel catfish is a serious problem in the commercial culture industry affecting 50-80% of growout ponds during the warmer months of the year. A microwave distillation technique has been developed to isolate volatile compounds from affected material. 2-Methylisoborneol (MIB) is the predominant compound isolated from off-flavored catfish during the growing season (April-October) in west Mississippi and is responsible for a musty type of flavor. Geosmin and other as yet unidentified compounds are also important causes of an earthy type off-flavor. 2-Methylenebornane and 2-methyl-2-bornene, dehydration products of MIB, were shown to contribute to the off-flavor problem. The absorption (2 hours) and depuration (48 hours) of MIB in catfish fingerlings were determined. MIB and its dehydration products are concentrated primarily in subepidermal and abdominal fat. Lesser concentration occurs in the liver and muscle tissue of off-flavored fish. Removing fish to clean, flowing water to purge MIB and geosmin could provide a partial means for off-flavor abatement.

KEYWORDS

2-methylisoborneol, 2-methylenebornane, 2-methyl-2-bornene, microwave distillation, off-flavor, bioconcentration, depuration.

INTRODUCTION

Off-flavor in channel catfish (Ictalurus punctatus) is a result of acquisition of tastes and odors which render fish flesh unpalatable. Maximum incidence and severity of off-flavor occurs during late summer (July-September) and affects up to 80 percent of the fish samples submitted at the processing plant for sensory evaluation (Stanley Marshall, Delta Pride Catfish Inc., Indianola, Mississippi USA, pers. comm.). The problem decreases profitability and limits supply of catfish because producers are forced to delay processing of market weight fish until the off-flavor subsides.
The predominant off-flavor encountered by processor taste panels has been described as "musty" or "earthy". The "musty" or "lagoon" flavor apparently is caused by methylisoborneol (MIB), and the "earthy" or "woody" flavor is caused by geosmin or other, as yet unidentified, compounds (Lovell et al., 1986; Martin et al., 1987a). Off-flavor types such as moldy, astringent, rotten, and sewage have been described for which no chemical compounds have been isolated (Armstrong et al., 1986; Johnsen et al., 1987). The off-flavor phenomenon clearly has many chemical etiologies which have yet to be elucidated.

Metabolism of off-flavor compounds in the channel catfish is poorly understood. The uptake and depletion of off-flavor compounds derived from odorous algae cultures in rainbow trout and channel catfish is rapid (less than one hour) and depuration of musty off-flavor occurs in 3-10 days (Lovell et al., 1976; From and Horlyck, 1984). An earlier study has identified and quantitated geosmin in off-flavored fish (Yurkowski and Tabachek, 1974). They determined a purge time for geosmin in rainbow trout of 3-5 days and a taste threshold concentration of 6 μg/kg. The uptake and release of odorous compounds has been reviewed by Persson, 1984. However, in many of the studies the cause of the "muddy" odor was not analytically determined. The alimentary tract was found to be significant in the uptake of MIB in trout (Persson and York, 1978). However, the rate of uptake and depletion of MIB is as yet to be determined in the channel catfish.

MIB and geosmin are secondary metabolites of certain species of cyanobacteria (Izaguirre et al., 1982; Slater and Blok, 1983; Siguiara et al., 1986), and actinomycetes (Sivonen, 1982; Gerber, 1983). The production and occurrence of MIB and geosmin in aquatic environments is correlated with increasing eutrophication (Persson, 1982; Juttner 1984). Warm water temperatures, high fish standing crops and large additions of fish feed all contribute to the high incidence and seasonal severity of off-flavor in catfish culture ponds (Brown and Boyd, 1983; Armstrong et al., 1986). The abatement of the problem will most likely involve methods designed to improve the water quality (such as raceway depuration or carbon filtration) since lower stocking densities and feeding regimes would decrease profitability. Future studies should be designed to determine the efficacy of these methods for reducing the off-flavor problem.

The purpose of this study was to further characterize MIB off-flavor in the channel catfish. Experiments were conducted to determine the etiology of MIB type off-flavor in the winter months (September-January), the bioconcentration of MIB in various tissue compartments, and the absorption and depletion of this compound in channel catfish fingerlings.

MATERIALS

Five compounds with a musty odor were used as calibration standards; MIB (Environmental Protection Agency (EPA), Cincinnati, Ohio); geosmin (EPA), 2-isopropyl-3-methoxypyrazine (Aldrich Chemical Co., Milwaukee, Wisconsin); 2-isobutyl-3-methoxypyrazine (Aldrich), and 2-isobutyl-6-methoxypyrazine (Aldrich) formulated in 10mg/l concentrations. Camphor (Aldrich) was used as an internal standard for water and sediment samples. 1-Chlorododecane (Eastman Organic Chemicals) was used as an external standard. MIB was also synthesized using the method of Wood and Snoeyink (1977). Synthesis of authentic standards of 2-methylenebornane and 2-methyl-2-bornene was accomplished using the method of Lapalme et al. (1979) in which MIB was dehydrated using thionyl chloride in dry pyridine.
PROCEDURE

Selection of culture ponds. Six ponds were selected for study based on a chronic history of off-flavor (i.e. at least 4 months duration). Historic records of sensory evaluations by a processor taste panel were used to identify these ponds. The odor and taste of fish from these ponds was described as the muddy "lagoon" MIB type. Water, sediment, and fish were obtained from these ponds every two weeks, from October through January, and analyzed for volatile odor producing compounds. A commercial culture pond with a history of producing "lagoon" type off-flavored catfish during the previous growing season and throughout the winter was sampled during the following growing season for MIB and its dehydration products. Twelve fish were collected from this pond twice during the growing season (July) at two week intervals. Muscle, skin, liver, and abdominal fat were analyzed in duplicate by microwave distillation and capillary gas chromatography. Water samples also were taken when fish were sampled, and analyzed in duplicate as previously described.

Collection and analysis of fish samples. For each sampling six fish were caught by hook and line from commercial culture ponds in western Mississippi. Two of the fish samples were examined for sensory qualities by an industry taste panel. In this method flesh and skin posterior to the anal orifice were placed in a plain paper bag and cooked in a microwave oven for five to seven minutes. Bags were opened and the fish aroma and taste were described by each panelist. The fish sample was judged acceptable or unacceptable by odor and taste, the off-flavor described as severe or not severe, and the flavor was described as musty (MIB), earthy (geosmin-like), or other.

The remaining four fish were subjected to microwave distillation. Briefly, the fish were cooked in silanized glassware in a microwave oven under a constant flow of nitrogen. The distillate was collected at -80°C, and the volatile compounds were extracted with hexane and concentrated to 100μl under a stream of nitrogen. The volatiles were analyzed by capillary gas chromatography (GC) with flame ionization or mass spectroscopic detection (MS) according to methods described by Martin et al., 1987a.

Collection and analysis of water and sediment. Water samples were collected in 1-1 Silanized amber bottles from a single site approximately 10cm below the surface of the water. Sediment samples were obtained by scraping the upper 1-2cm of bottom sediment until 100g of sample was obtained. Prior to analysis, camphor (0.5 ppb concentration) and 40mg mercuric chloride were added as an internal standard and enzyme inhibitor, respectively.

Closed loop stripping was used to isolate volatile organic compounds from both sediment and water. Fifty grams of wet sediment was blended with 550 ml distilled water and subjected to closed loop stripping for two hours. The pond water samples (600 ml) were also stripped for two hours. The absorbed volatiles from water and sediment samples were eluted with 20 μl carbon disulfide and analyzed as described above for the fish extracts. Quantitation was based on the method of Hwang et al., (1984) using camphor as an internal standard and 1-chlorododecane as an external standard/time marker.

Absorption and depuration of MIB. Sixty, 5-10g fingerling catfish were placed in each of three 100 l aerated holding tanks and acclimated for two weeks. MIB synthesized by the method of Wood and Snoeyink, 1977 was dissolved in a minimum volume of ethanol and inoculated into the tanks to achieve a concentration of zero (ethanol only), 5.0, and 50 μg/l MIB. Partial (50%) water exchanges were made daily to maintain water quality. MIB was added continuously by a peristaltic metering pump throughout the experiment (Ismatic SA, Cole Parmer, Chicago,
Illinois) to offset evaporation losses. Six fish were removed from each tank upon initial inoculation of MIB and then at 2, 4, 8, 24, 48, 72 and 168 hours after inoculation. After the last sampling, the remaining fish were removed and placed in tanks containing water devoid of MIB. Six fish were removed each at 24, 48 and 72 hours. The fish were subjected to microwave distillation and the MIB was quantitated as described previously.

Analysis of fish, water, and sediments extracts. A Perkin Elmer Sigma 3 (Perkin Elmer Inc., Norwich, Connecticut) GC equipped with a flame ionization detector and capillary column DB-5 (J & W Scientific, Folsom, California) 30 m x .32 mm I.D. fused silica (5% phenyl methyl silicone) was used to analyze volatiles from fish, sediment and water samples. The operating temperatures were as follows: Column 50-200°C, programmed at 8°C/min. with a one minute initial hold, injector, 225°C, detector 325°C. Helium at a flow rate of 1 ml/min. was used as the carrier gas. Injection volume was 3µl split 3:1.

Mass spectroscopic analysis of fish, sediment and water samples was accomplished using a Hewlett Packard 5890 gas chromatograph mass spectrometer system scanning 40-400 AMU, equipped with a commercially available (HPI) 25 m X .25 mm ID fused silica capillary column coated with crosslinked methyl silicone (Hewlett-Packard, Palo Alto, California). The operating temperatures were as follows: Column 50-120°C at 4°C/min. with a one minute initial hold; 120-220°C at 20°C/min. with a two minute initial hold; injector 240°C "splitless" injector. The carrier gas was helium at a flow rate of one ml/min.

Comparison of retention times and mass spectra of authentic samples with fish extracts was used to confirm identification of 2-methylenebornane and 2-methyl-2-bornene.

RESULTS AND DISCUSSION

Winter study of six culture ponds with a chronic MIB type off-flavor. In this study no volatile "musty" standard compounds (MIB, geosmin, pyrazine isomers) were isolated from water, sediment, and fish. However, fish samples consistently contained elevated (>100µg/kg) concentrations of 2-methylene bornane and 2-methyl-2-bornene (Table 1) (Figure 1).

<table>
<thead>
<tr>
<th>Pond</th>
<th>2-methylenebornane µg/kg</th>
<th>2 methyl-2-bornene µg/kg</th>
<th>Duration of off-flavor</th>
</tr>
</thead>
<tbody>
<tr>
<td>T11</td>
<td>3860±810</td>
<td>1050±160</td>
<td>18-20 months</td>
</tr>
<tr>
<td>T21</td>
<td>534±46</td>
<td>91±18</td>
<td>8-9 months</td>
</tr>
<tr>
<td>T22</td>
<td>2620±510</td>
<td>337±46</td>
<td>24 months</td>
</tr>
<tr>
<td>F31</td>
<td>267±31</td>
<td>35±4</td>
<td>7 months</td>
</tr>
<tr>
<td>F22</td>
<td>241±36</td>
<td>25±1</td>
<td>4 months</td>
</tr>
<tr>
<td>F1</td>
<td>155±28</td>
<td>5.4±1.0</td>
<td>5 months</td>
</tr>
</tbody>
</table>

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Off-flavor in the channel catfish

Figure 1. MIB and its dehydration products

Authentic samples of these compounds imparted a musty odor in dilute 5 ppb aqueous solution. Further, the authentic samples matched the mass spectra of the fish extracts and the mass spectra reported for these compounds by Burgstahler et al. (1976) and Walter et al. (1983). These dehydration products were associated with chronic off-flavor ponds and had previously been observed during the growing season when MIB was also present. This study indicates that these dehydration products can cause off-flavor problems in the absence of MIB, and that these compounds are related to chronic off-flavor problems. A previous study suggested that MIB type off-flavor was of 2-3 months duration in late summer and occurred in ponds where the principal phytoplankton population was Oscillatoria agardhii (Martin et al., 1987b). This study suggests that in the winter months when dense phytoplankton communities are not usually present the MIB type off-flavor can be due to dehydration products and presumably these compounds contribute to off-flavor in the growing season when MIB is also present.

The biodistribution of MIB in commercially cultured chronically off-flavored fish. MIB was found in the highest concentration in the subepidermal layers and abdominal fat (Table 2). Lower concentrations of MIB were found in the liver and muscle tissue. The highest concentrations of the MIB dehydration products also occurred in the abdominal and subepidermal fat. In protracted cases of off-flavor the dehydration products may be more slowly depurated than the parent compound due to their more lipophilic nature. The concentration of MIB in water also was determined on both sampling occasions (Table 2). There was a wide variation in bioconcentration values, i.e. 3 vs. 16 for muscle tissue obtained for fish on days 1 and 2 respectively. This variation was not unexpected considering the complex bioenvironment of the commercial pond.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Day 1 Mean µg/kg</th>
<th>Subset*</th>
<th>Day 2 Mean µg/kg</th>
<th>Subset*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin</td>
<td>342.3</td>
<td>A</td>
<td>1408.9</td>
<td>A</td>
</tr>
<tr>
<td>Fat</td>
<td>243.2</td>
<td>AB</td>
<td>1334.5</td>
<td>A</td>
</tr>
<tr>
<td>Liver</td>
<td>131.8</td>
<td>BC</td>
<td>368.1</td>
<td>B</td>
</tr>
<tr>
<td>Muscle</td>
<td>77.1</td>
<td>C</td>
<td>260.5</td>
<td>B</td>
</tr>
<tr>
<td>Water</td>
<td>Concentration µg/l 25.1</td>
<td></td>
<td>16.0</td>
<td></td>
</tr>
</tbody>
</table>

* Means in the same column followed by the same letter are not significantly different at the 5% level by the Least Significant Difference Test.
MIB absorption and depletion. Studies of the absorption, bioconcentration, and depuration of MIB in the channel catfish gave more consistent results under laboratory conditions. Catfish fingerlings were found to contain MIB concentrations 10 fold greater than found in water (Table 3). Maximal uptake of MIB occurred in less than two hours with depuration to less than 2µg/kg over a period of 48 hours. Previous studies have reported that the uptake of musty odor compounds from algal cultures is rapid (0.5 hrs) (Lovell et al., 1976; From and Horlyck, 1984) with its depletion occurring in 4-10 days in trout and channel catfish. However, the analytical determination of the musty compounds produced by the algae in these studies was not undertaken and they were not identified.

Our laboratory results obtained for MIB indicate a similar rate of absorption and a rapid rate of depuration. No MIB dehydration products were isolated from these fish supporting the contention that these compounds accumulate during prolonged MIB exposure. The purge time for MIB in catfish fingerlings did not depend on the amount of chemical absorbed as has been reported for other compounds (Maligalig et al., 1975).

CONCLUSION

Analysis by GC and GC/MS has identified MIB and its dehydration products as the principal muddy/lagoon off-flavor producing compounds in western Mississippi. MIB and its dehydration products are bioconcentrated primarily in the skin and intramesenteric fat. The absorption and depuration of MIB is rapid, suggesting that raceway technology may be useful in off-flavor abatement for this compound. The depuration of the MIB dehydration products has not been determined. In addition, chemical etiologies for other types of off-flavor (woody, rancid, sewage, stale) have not been identified. Continued identification, metabolism, and depuration studies of specific off-flavor producing compounds will be necessary to determine the efficacy of raceway depuration as a viable method for off-flavor abatement.

REFERENCES


