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Influence of food-grade ingredients on offlavor compounds in catfish fillets

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INFLUENCE OF FOOD-GRADE INGREDIENTS ON OFF-FLAVOR

COMPOUNDS IN CATFISH FILLETS

By

Sovann Kin

A Thesis
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INFLUENCE OF FOOD-GRADE INGREDIENTS ON OFF-FLAVOR

COMPOUNDS IN CATFISH FILLETS

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The purpose of this study was to screen the effect of some food-grade ingredients on the off-odors caused by geosmin and 2-methylisoborneol (MIB) compounds in catfish fillets by sensory evaluation. The study revealed that geosmin and MIB odor intensity were reduced to different degrees when fillets were dipped in lime flavor (94% and 67%, respectively), 0.5% acetic acid (AA) (70% and 16%, respectively), hardwood liquid smoke (98% and 86%, respectively), or hickory liquid smoke (98% and 100% respectively) in cooked products. A 0.5% AA proved to be effective in decreasing odor intensity of geosmin (70%) in cooked products, whereas lime flavor (94%), hardwood liquid smoke (98%), and hickory liquid smoke (98%) were very effective in decreasing odor intensity of geosmin in cooked products. These agents added desirable flavors as well, except for AA by panelists' comments. These flavors could be added to a marinade or incorporated in an injection/tumbling solution when catfish fillets are processed.

DEDICATION

I would like to dedicate my research to my parents, Sarin and Tiem, my brother, Socheat, my sisters, Sary, Sarun, and Saroeun, and my beloved niece, Chankesey. Their love and support are my motivation and inspiration. I wish they could have seen me graduate.

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Finally, I am very grateful to my parents, Sarin and Tiem, my brother, Socheat, and my sisters, Sary, Sarun, and Saroeun, for supporting, encouraging and believing in me. I am also very appreciative of everyone in the Department of Food Science, Nutrition, and Health Promotion for intellectually stimulating and enlightening conversations and the sharing of ideas.

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CHAPTER I

INTRODUCTION

Off-flavor problems in aquaculture animals have been extensively studied for many years, but scientific evidence is still limited. Off-flavor is the development of unpleasant flavors caused by odorous compounds absorbed by fish from the water. This makes fish unmarketable, causing a significant problem for some industries like the catfish industry. Its costs, associated with delay in harvesting and processing, increased operational costs, and lower prices of larger size fish, are estimated to be 5 to 20 % of the cost of production (van der Ploeg and others 2001). Lazur (2004) reported that in 2002 about 53 % of the catfish ponds have delayed harvesting due to off-flavors, and that off-flavor problems increased costs to the industry by 15 to 23 million dollars annually. Consumption of off-flavored catfish also causes loss of consumer confidence and demand (Tucker 2000).

A report by van der Ploeg (1991) described off-flavors in many ways, depending on the source of odorous compounds in fish ponds, including algae, microorganisms that decompose vegetation, fish waste products and pollutants such as diesel fuel or pesticides. Some of the more commonly encountered off-flavors are "earthy," "musty," "woody," "rancid," "rotten," and "petroleum" (Schrader and Rimando 2003). Many of the specific compounds that cause these undesirable flavors in fish have not been

identified. However, Killian (1977) stated that two specific compounds, geosmin (1,10-*trans*-dimethyl-*trans*-9-decaol) and 2-methylisoborneol (1,2,7,7-tetramethly-*exo*-bicyclo-[2,2,1]-heptan-2-ol) (MIB), constitute greater than 80% of the off-flavor problems in farm-raised channel catfish. These two compounds are produced by secondary metabolites of some species of blue-green algae and actinomycetes bacteria (Lloyd and others 1998). Both compounds are rapidly absorbed from water into the lipid tissue of fish and other aquatic organisms (Lloyd and others 1998). Geosmin causes an "earthy" sensation, and MIB causes a "musty" sensation (van der Ploeg 1991; Schrader and Rimando 2003). The MIB flavor is often confused with the geosmin flavor but when smelled side by side, the distinction is obvious and the intense MIB flavors are reminiscent of camphor or an "old rag" (van der Ploeg 1991).

These compounds can be detected by humans at concentration as low as 0.6 ppb to 6 ppb for geosmin and 0.08 ppb to 0.6 ppb for MIB, depending on the species of fish (Yurkowski and Tabacheck 1974; Persson 1980). However, the rejection levels as suggested by USDA are 0.8 ppb for MIB and 8.0 ppb for geosmin in channel catfish (Conte and others 1996).

Many instrumental methods for analyzing geosmin and MIB have been developed. Development of an objective evaluation alone would be believable, but there are still problems. Firstly, the taste threshold of these compounds is very low and the sensitivity of an instrumental analytical method would be extremely high (Lovell 1983). Secondly, the extraction of these compounds from fish flesh was more difficult than in a homogenous substance such as water which then makes quantitative identification

difficult (Lovell 1983). Thirdly, extraction and analysis of these compounds are slow and require expensive equipment (Lovell 1983). The traditional extraction methods for these two compounds that have been used include closed-loop stripping (McGuire and others 1981), liquid-liquid extraction (Johnsen and Kuan 1987), steam distillation (Bartels and others, 1989), and purge and trap (Johnsen and Lloyd 1992). These extraction techniques are effective but expensive, time-consuming and labor intensive (Grimm and Zimba 2003). Others techniques include membrane-based extraction (Zander and Pingert 1997) that can detect analytes in part per trillion concentration ranges, solid phase extraction (Conte and others 1996) that is rapid, inexpensive and can detect concentrations at part per billion levels, and solid phase micro-extraction (SPME) (Belardi and Pawliszyn 1989), a simple and inexpensive method for the analysis of these off-flavor compounds. However, Grimm and others (2000) stated that SPME is not so effective for the analysis of the samples composed of a complex matrix such as soil and muscle tissue. For such complex matrices, microwave distillation (MD) should be used to steam-distilled analytes from the sample matrix. This technique effectively removes the analytes from the less optimal matrix and places them in an aqueous matrix for SPME to absorb these compounds for gas chromatography-mass spectrometry (GC-MS). A SPME method, requiring a small sample size and a very efficient extraction process, was developed successfully for the detection of MIB in treated catfish fillets (Kaewplang and others 2006).

Various pre-harvest and post-harvest methods have been studied and used in an attempt to eliminate off-flavor compounds from catfish. Silva and others (2002) reported

that off-flavor control prior to harvest has to be done through proper management and treatment of affected ponds. Pre-harvest methods include purging and raceway use, algaecide use, and biochemical methods (King and Dew 2003). Control of off-flavor fish entering the plant is done by sampling and tasting pond fish periodically prior to harvest (Silva and others 2002). The same is repeated the day of harvest. However, differences between tasters and inadequate sampling of a mixed off-flavor pond may lead to a false result, i.e., accepting fish when some are off-flavored (Silva and others 2002). Therefore, pre-harvest treatment of catfish is sometimes ineffective and costly, and the catfish industry needs more reliable methods for evaluating off-flavor in the fish before the pond is harvested (Silva and others 2002). On the other hand, post-harvest methods have also been studied. Several post-harvest treatments have been speculated as possible measures to decrease or inhibit off-flavor in catfish during processing (King and Dew 2003). Post-harvest methods include chemical treatments such as acids, salt, sodium carbonate, ozone, hydrogen peroxide, and seasonings in conjunction with physical treatments such as injection, tumbling, dipping, vacuum infusion, marinating, deep skinning, smoking, frying, baking, and microwave heating to mask or destroy off-flavors (Silva and others 2002).

However, few studies have been made toward post-harvest off-flavor intervention in catfish and there is no conclusive scientific evidence that any of these methods adequately assure the flavor quality of catfish, and that economical means are not available to remove these compounds from fish (King and Dew 2003). Moreover, sensory evaluation by trained panelists is still the most reliable method to discern the

effectiveness of additives on suppression of off-flavor in fish flesh. This is because sometimes the off-flavor may be physically masked but yet detected at similar levels to the control (Kaewplang and others 2006).

The objective of the present study was to determine if food-grade compounds could decrease odor perception of geosmin and MIB compounds in catfish fillets.

CHAPTER II

LITERATURE REVIEW

Catfish industry in the United States

As the United States and world seafood demand increases, based on the growth of population and consumers' awareness of the health benefits of fish and shellfish, aquaculture of commercial fish and other seafood products have become a significant factor of agriculture and food production in the United States (Nagle and others 2003). Channel catfish (*Ictalurus punctatus*) is the most important species of aquatic animals commercially cultured in the United States, and is being consumed to some extent in all regions (Wellborn 1988). In the early 1900's, the first efforts at growing catfish were made at several federal and state fish hatcheries (Wellborn 1988). In the 1950's, commercial channel catfish farming first started in Kansas and Arkansas, and was then introduced throughout the country (Wellborn 1988).

A majority of the catfish industry in the United States is located in the southern states where the growing season is longer, and water is warmer that are contributing to the optimum production (APHIS 1995). According to a report by the National Agricultural Statistical Services (NASS 2006), catfish production was concentrated in Alabama, Arkansas, Louisiana, and Mississippi, which accounted for 95 % of the total national

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catfish sales in 2005. About 55% of all U. S. catfish production came from Mississippi (Hanson 2006).

The 2002 Census of Agriculture (NASS 2004a) estimated that the value of fish and other aquaculture products sold at approximately 1.1 billion dollars, but combined catfish and trout sold accounted for 78.4 % of the total pounds sold. Particularly, the total value of catfish sales in 11 southern states for 2004 was 480 million dollars, up 13 % from the 2003 total of 425 million dollars (NASS 2005), and 482 million dollars during 2005, up slightly from the previous year (NASS 2006). The average price paid to producers also increased. It cost 66.8 cents per pound for January 2004, 72.5 cents per pounds for January 2005, and 72.6 cents per pound for the same month in 2006 (NASS 2004b/05/06).

Product forms of processed catfish usually include fillets, shank fillets, fillet strips, nuggets, and steaks. All these forms are marketed fresh and frozen, and many are now sold breaded (Anonymous 2002). Processors also sell whole dressed catfish or round-eviscerated catfish with the head still on. Whole dressed catfish and fillets can also be marketed by coating or marinating with flavors and spices such as lemon-butter, cajun and mesquite (Anonymous 2002). In addition, offal, the by-product of catfish processing, is further processed into fish meal and fish oil or as an ingredient for canned pet food (Anonymous 2002).

However, as the farm-raised channel catfish industry continues to grow, catfish producers have some challenges such as off-flavor control, water quality control, disease control, bird predation, harvesting difficulty, and breeding a better catfish (Masser and

others 1997), but the most critical problem is undesirable off-flavor that continues to threaten the prosperity of this business (Tucker 2000).

Off-flavor developments

Flavor is a main attribute of quality for catfish, and its marketability relies largely on flavor quality (Johnsen and Dupree 1991). Understanding the factors which influence flavor quality is critical for the continued success of this rapidly growing industry (Johnsen and Dupree 1991). If off-flavored catfish are marketed, new consumers might make the inaccurate assumption that all catfish tastes this way, and then forgo future consumption (Tucker and Van der Plog 1999). In addition, consumption of aquatic products is elective in most developed countries, and undesirable flavors in the products results in consumer dissatisfaction which may adversely affect market demand (Turker and Van der Plog 1999).

Off-flavor problems

Off-flavor is a complicated problem and producers must understand the possible causes, possible cures, and most importantly, how to check the fish before they are marketed (Masser and others 1997). It is reported that an estimated 10 % of any fish harvested, even the highest quality, is contaminated with "off-flavors". In the United States, more than 75 % of all production ponds may contain fish that are not marketable due to off-flavor problems at certain times of the year (Kinnucan and others 1988; Heikes 1993). Martin and others (1988) reported that up to 80 % of harvestable fish can be off-

flavored during any one year. In 2002, 69.6 % of catfish operations and 53.3 % of catfish ponds experienced delayed harvests due to off-flavors (APHIS 2003).

Off-flavor development varies depending on the season of the year but the problem is predominant in the summer months. Killian (1977) reported that during July, August, and September, approximately 50-70 % of all harvestable sized channel catfish are found to be off-flavored at any given time and rejected by processors.

Catfish farmers consider off-flavors to be an economically important problem for the catfish industry since it can lead to delayed fish harvests (Killian 1977). Catfish farmers are most affected by off-flavors because they are unable to sell their fish when it is economically desirable (Killian 1977). The off-flavors delay harvesting which may cause economic losses by forcing farmers to keep fish in ponds longer, creating an increased risk of loss due to disease problems by occasional oxygen deprivation, loss of sales at processing plants, reduced feed efficiency, and delay in stocking the next crop of catfish (Killian 1977; Johnsen and Lloyd 1992; APHIS 2003). Keenum and Waldrop (1988) estimated that off-flavor may increase production costs by as much as US$ 0.26/kg by delayed harvesting, and Tucker and Martin (1991) reported that increased production costs have been estimated to be from $5.8 to $12.0 million annually. Hanson (2001) reported that off-flavor problems increased production costs by as much as 47 million dollars in the United States catfish industry in 1999, and in general increase the cost to the industry by 15 to 23 million dollars every year (APHIS 2003; Lazur 2004). Mississippi catfish farmers and processors also lose an estimated 16 million dollars in annual sales since fish develop off-flavors, (Hanson 2001).

Sources of off-flavors

Off-flavors develop whenever odorous chemicals accumulate in the fish flesh, and may develop during grow-out or after harvest. During grow-out, off-flavors can be related to the diet of the cultured fish, and/or can be derived from pollution or microbes in a pond (Tucker and van der Ploeg 1999). After harvest, off-flavors can be caused by inadequate post-harvest management strategies (Tucker and van der Ploeg 1999).

There are many types of off-flavors that can be encountered in fish, but some of the more commonly encountered off-flavors are "earthy," "musty," "woody," "fishy," "rancid," "rotten," and "petroleum" (Tucker 2000).

A report by Silva and van der Ploeg (1992) indicated that lipid oxidation during prolonged or improper storage causes "rancid" and "stale" off-flavors. Trimethylamine and other amines are produced by microbial decomposition processes, and produce a distinct "fishy" flavor (Silva and van der Ploeg 1992). However, these off-flavor problems are not of a direct concern to fish producers because processors and retailers are able to prevent spoilage by adherence to accepted processing and storage procedures (Silva and van der Ploeg 1992).

The type of feed/diet may also affect the flavor quality of the fish (Tucker and van der Ploeg 1999). Manufactured feeds containing high levels of marine fish oil can result in a "fishy" flavor in fish (Boyd and Tucker 1998; Morris and others 1995). The diet-related off-flavors are rare in farm-raised catfish because the ingredients used in high-quality commercial feeds do not cause flavor problems (Tucker and van der Ploeg 1999). Fish may ingest certain types of microorganisms that contain off-flavor compounds while

they are consuming feed or other sources of food (Tucker and van der Ploeg 1999). Fish that develop a "decay" or "rotten" off-flavor may have consumed decaying organic matter as they forage for natural foods, especially during winter when many catfish farmers do not routinely feed their fish. (Tucker and van der Ploeg 1999).

Most off-flavors in pond-raised catfish are caused by odorous compounds absorbed from the water (Tucker and van der Ploeg 1999). In general, waterborne odorous compounds can be derived either from pollution or natural sources (Tucker and van der Ploeg 1999). For example, a "petroleum" off-flavor occasionally develops in pond-raised catfish when water is contaminated by accidental spills of diesel fuel or gasoline from boats, well-pump engines or farm equipment (Tucker and van der Ploeg 1999). Off-flavors related to the discharge of chemicals from pulp mills can also occur, and the flavors associated with the pulp mill effluents have been described as "sewage" and "phenolic" or "sulfide" off-flavors (Shumway and Chadwick 1971).

However, the off-flavor problems mentioned above are not a big concern. The most critical concern is the off-flavors caused by naturally occurring organic compounds produced by aquatic bacteria or algae (Tucker and van der Ploeg 1999). Although the aquatic organisms can be beneficial to the pond ecosystem by providing oxygen and helping to remove certain types of metabolic wastes, they can also be a factor in making it undesirable for fish production (King and Dew 2003). Objectionable off-flavors produced by these organisms are described as "earthy/musty", "grassy", or "septic", depending on species, algal density, and whether the algae are alive or dying (Suffet and

others 1999). It is also reported that higher nutrient concentration and temperature could be major factors that cause these off-flavor problems.

Tucker and Boyd (1985) explained that due to high fish stocking densities, aquaculture ponds receive large nutrient inputs from fish and also from fish excretions, and sediment mineralization/re-suspension. Because the pond systems are static and high amounts of nutrients are added daily, algal blooms and bacteria are encouraged to grow and proliferate (Lutz and others 1992), and form near the surface of the water restricting light penetration (Johnsen and Dionigi 1994). Algal blooms mainly consist of blue-green algae (cyanobacteria). The blue-green algae are the main sources of "musty" off-flavors found in aquaculture ponds (Jüttner 1995). Genera of these blue-green algae that have been associated with "musty" off-flavors include *Anabaena*, *Aphanizomenon*, *Nostoc* and *Oscillatoria* (Jüttner 1995). Other microbes that can produce "earthy" off-flavors are actinomycetes (Johnsen and Dionigi 1994). The actinomycetes are associated with the soil and examples of genera that typically produce the "earthy" off-flavors are *Streptomycetes* and *Nocardia* (Johnsen and Dionigi 1994).

However, two specific compounds produced by both blue-green algae and actinomycetes have been specifically identified as the producers of off-flavors (Martin and others 1987). Geosmin [*trans*-1,10-dimethyl-*trans*-(9)-decalol] is responsible for an "earthy" off-flavor (Lovell and others 1986) and 2-methylisoborneol (*exo*-1,2,7,7-tetramethyl-[2,2,1]heptan-2-ol) (MIB) is responsible for a "musty" off-flavor (Lovell and others 1986).

Physicochemical properties of geosmin and MIB

Geosmin was first characterized as a product of actinomycete culture in 1965 by Gerber and Lechevalier (1965). MIB was first identified in 1968 by Medsker and others (1968). The chemical structures and chemical/physical characteristics of these two compounds are summarized in Table 2.1.

Geosmin has a molecular formula of $C_{12}H_{22}O$, and is identified as *trans*-1,10-dimethyl-*trans*-(9)-decalol (Gerber and Lechevalier 1965). It is a volatile metabolite compound and has been shown to be a dimethyl substituted, saturated, two-ring tertiary alcohol with the hydroxyl group that is very sterically hindered (Gerber and Lechevalier 1965). It is a colorless, viscous liquid and resistant odorous compound with an approximate boiling point of 270°C and contains 79% carbon and 12% hydrogen (Gerber and Lechevalier 1965, Medsker and others 1968). Geosmin is hydrophobic and soluble in alcohol but its solubility in water is less than 1 ppm (Grimm and Zimba 2003).

Odor of geosmin is associated with dry must environments like attics and also associated with freshly turned garden soil (Silva and van der Ploeg 1992). Geosmin is responsible for the earthy odor in water and aquaculture products (Rosen and others 1970). The earthy odor is not necessarily related to the presence of a bicyclic system, not even to cyclic compounds (Napolitano and others 1996). Instead, the steric hindrance in the proximity of the alcoholic group seems a more reliable parameter, together with size requirements of 9-12 major atoms, for predicting whether the odor of an alcohol is expected to be earthy (Napolitano and others 1996). The two enantiomeric forms of geosmin do not differ in odor quality but only in intensity (Marshall and Hochstetler

1968). The cis/trans and trans/trans isomers have an overpowering pungent musty/earthy property while the trans/cis and cis/cis forms have only a background earthy aroma but were primarily reminiscent of camphor and cedar (Marshall and Hochstetler 1968). When pure, or present in highly concentrated solution, it possesses a strong, camphorish odor which becomes earthy on dilution (Marshall and Hochstetler 1968). The human nose is exquisitely sensitive to geosmin and is able to detect it at concentrations at as small as part per trillion levels in water (Marshall and Hochstetler 1968). Odor thresholds of geosmin are given in Table 2.2.

Table 2.1 Chemical/physical characteristics of geosmin and 2-methylisoborneol (MIB)
(adapted from Pirbazari and others, 1992)
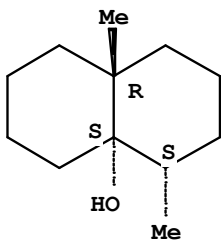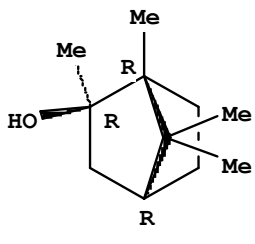
Parameter	Geosmin	MIB
Index name	4a(2H)-Naphthalenol, octahydro-4,8a-dimethyl-, (4S,4aS,8aR)- (9CI)	Bicyclo[2.2.1]heptan-2-ol, 1,2,7,7-tetramethyl-, (1R,2R,4R)-rel- (9CI)
Structure		
Molecular formula	$C_{12}H_{22}O$	$C_{11}H_{20}O$
Molecular weight	182.30 g/mol	168.28 g/mol
Boiling point	252.4±8.0 °C	208.7±8.0 °C
Enthalpy of vap.	56.90±6.0 kJ/mol	51.76±6.0 kJ/mol
Aqueous solubility	150.2 mg/L	194.5 mg/L
Henry's law constant	6.66×10^{-5} atm m^3/mole	5.76×10^{-5} atm m^3/mole
Odor Description	Freshly plowed soil, earthy	Wet, musty environments like cellar or swamps
Appearance	Colorless/yellow oil	White solid

Table 2.2 Reported odor thresholds for musty/earthy compounds

Compound	Medium	Threshold	References.
Geosmin	Water	50 ppt	Medsker and others (1968)
	Water	200 ppt	Safferman and others (1967)
	Water	4-20 ppt	Persson (1980); Watson and others (2000)
	Water	130 ppt	Lovell (1983)
	Water	30-40 ppt	Grimm and others (1996)
	Water	100-200 ppt	Jenkins (1973)
	Water	94-360 ppt	Sano (1988)
	Beet juice	6.0 ppb	Tyler and others (1979)
	Trout	5.8 ppb	Yurkowski and Tabacheck (1974)
	Fish	0.6-6.0 ppb	Yurkowski and Tabacheck (1974); Persson (1980)
	Fish	8.4 ppb	Lovell (1983)
	Catfish	10.0 ppb	Bazemore (2002)
2-Methylisoborneol	Water	100 ppt	Medsker and others (1968); Gerber (1979)
	Water	4-20 ppt	Persson (1980); Watson and others (2000)
	Water	30-40 ppt	Grimm and others (1996)
	Water	100-200 ppt	Jenkins (1973)
	Water	29.0 ppt	Persson (1979)
	Water	9-42 ppt	Krasner and other (1983); Mallevialle and Suffet (1987); Persson (1983); Young and others (1996)
	Fish	0.08-0.6 ppb	Yurkowski and Tabacheck (1974); Persson (1980)
	Catfish	0.7 ppb	Johnsen and Kelly (1990)
	Trout	0.6 ppb	Persson (1980)
	Catfish	30 ppb	Bazemore (2002)

Chemical properties of geosmin have not been well known. It is reported that geosmin darkens very slightly after long storage (Gerber 1979). Under acidic conditions, geosmin decomposes into argosmin (odorless compound), which is composed of 86% carbon and 12% hydrogen (Gerber and Lechevalier 1965). Geosmin is a tertiary alcohol which is can be oxidized by powerful oxidizers but is most susceptible to dehydration (Carey and Sundberg 1984). The behavior of geosmin in a strong acid solution suggests that acid catalyzed dehydration forms a series of isomeric hydrocarbons accompanied by some substitution of Cl^- at the hydroxyl group (Medsker and others 1968). Hensarling and Waage (1990) showed that geosmin reacts with bromine in the presence of formic acid to produce a blue complex with maximum absorbance at 650 nm, but the sensitivity of the reaction is less than organoleptic thresholds which is not proposed as a direct method for determination of geosmin in water or fish. Geosmin can be decomposed effectively by hydroxyl radicals that are produced by the radiolysis of water during gamma-ray treatment (Irie and others 1976).

The 2-methylisoborneol (MIB) has a molecular formula of $C_{11}H_{20}O$, and is identified as *exo*-1,2,7,7-tetramethyl-[2,2,1]heptan-2-ol (Maga 1987). The odor of MIB is associated with wet musty environments like cellars or swamps (Silva and van der Ploeg 1992) and is responsible for off-flavors in water and food (Suffet and others 1999; Maga 1987), canned mushroom (Whitfield and others 1983), musty wheat grains (Wascowicz and others 1988), and fish (Lloyd and Grimm 1999). It is a semi-volatile, saturated, cyclic tertiary alcoholic compound (Forrester and others 2002) that is most likely to be produced in sediments (Slater and Blok 1983).This compound has hydroxyl groups which

provide dual solubility characteristics, and it is also a terpene compound which imparts its volatile characteristics with an approximate boiling point of 208°C (King and Dew 2003).

From the literature it would appear that MIB in its pure state has a camphor-like odor, but that at concentrations normally found in foods, its odor is more characteristically earthy/musty (Persson 1980). Even at concentrations of 1 ppm in water the compound is still camphoraceous (Tyler and others 1978). Thus, the odor properties of the compound can be dependent upon its concentration (Polak and others 1978). Odor thresholds of MIB are given in Table 2.2. The threshold odor concentration of MIB and geosmin is higher in fish flesh than in water; and the threshold odor concentration of geosmin is higher than that of MIB in fish (Persson 1980). Apparently, at concentrations below 10 ppb, the compound indeed smells musty (Persson 1980). MIB darkens very slightly after long storage (Carey and Sundberg 1984). It is a tertiary alcohol which is most susceptible to dehydration (Carey and Sundberg 1984). It can dehydrate to 2-methylenebornane and 1-methylcamphene (Forrester and others 2002).

<u>Absorption and distribution of geosmin and MIB into fish</u>

Normally, fish can absorb geosmin and MIB compounds through ingestion of cyanobacterial cells, through skin, and the gills of fish, and then tend to accumulate in the fatty tissue of the fish (Martin and others 1988; Johnsen and Lloyd 1992; Dionigi and others 1998). The lipid-rich tissues include skin and visceral fat, and concentrations in the visceral fat are almost 100 times greater than in water (Tucker 200). Avault (1996) reported that channel catfish and other cultured species are contaminated with off-flavor

compounds by ingesting small amounts of algae, but mostly by absorption across the gill membranes. Similarly, Lovell and Sackey (1973) revealed that the main route of absorption by fish is across the gills and/or skin. Ingestion of the compounds during the consumption of food and water provides another possible route of uptake of off-flavor compounds since these compounds can be absorbed across the lining of the gastrointestinal tract (Lovell and Sackey 1973; Fröm and Horlyck 1984). However, Thaysen and Pentelow (1936) found that Altantic salmon (*Salmo salar*) absorbs the off-flavors primarily through the gills into the blood stream and the blood carries them to the muscles. They showed no evidence of absorption through skin but suggested that absorption might occur in the stomach from ingested water. Yamprayoon and Noomhorm (2003) also concluded that the compounds are distributed in the body through the circulation system. Absorption of odorous compounds is independent of the digestive tract and probably occurs through the gills into the blood stream (Yamprayoon and Noomhorm 2003).

Absorption is affected by a number of factors such as compound concentration, exposure time, synergistic effects between compounds, and species of fish, water temperature, and their physiological state (Persson 1984; Yamprayoon and Noomhorm 2003). Higher water temperatures result in a more rapid uptake of MIB by catfish (Johnsen and others 1996). When catfish were exposed to 1 ppb of geosmin at 20°C, geosmin was rapidly absorbed (Arganosa and others 1992). Off-flavor in catfish develops within hours when exposed to high concentrations of MIB in water, but it takes days to weeks for catfish to develop a detectable off-flavor in water when they are exposed to

low MIB concentrations (van der Ploeg and others 2001). In addition, the transport of the off-flavors through water and blood is increased due to the off-flavor compound structure and function (Tucker 2000). These off-flavor molecules are fat-soluble and likely to deposit themselves under the skin and/or fatty tissues (van der Ploeg and others 2001). Yamprayoon and Noomhorm (2003) found that among various tissues of tilapia, the intestines contained the highest geosmin concentration and appeared in descending order in the abdomen, skin and muscle tissues. However, Fröm and Horlyck (1984) showed that the most rapid absorption of geosmin by rainbow trout occurred in the gills followed by the skin, small intestine, and stomach, and Dionigi and others (1998) found that fish with more adipose tissue were observed to absorb more MIB than leaner fish.

Another phenomenon is the depuration of the off-flavor compounds. These compounds are lipophilic and do not readily diffuse from fish tissue into the atmosphere. The rate at which the off-flavors disappear is related primarily to water temperature and the size and fat content of the fish (Tucker and van der Ploeg 1999). The depuration of these two compounds occurs in 3-14 days, and depuration presumably occurs by gill excretion (Persson 1984; Martin and others 1990). The off-flavors can also be removed (deep skinning) from the fish when the skin mucousa is removed during the processing of the fish (Persson 1984).

Some solutions to off-flavor problems

Common pre-harvest and post-harvest technologies that have been used to assure the flavor quality of catfish have not been completely adequate. However, several approaches have been used to deal with off-flavor problems (Tucker 2000). The

approaches of both pre-harvest and post-harvest methods include managing off-flavor problems, preventing off-flavor problems, removing off-flavors from fish once they have been developed, and/or processing off-flavor fish by masking or chemically degrading off-flavor compounds after harvest (Tucker 2000).

<p align="center">Pre-harvest methods</p>

Managing off-flavor problems

Usually, off-flavor episodes do not occur in all ponds at the same time (Tucker and Martin 1999). Ponds that contain a sufficient quantity of market-sized fish should be checked frequently for off-flavors and sold promptly if the fish are suitable (Tucker and Martin 1999). Market constraints may impact the practicability of this procedure, but it is better to harvest and market fish free of off-flavors (Tucker and Martin 1999).

Preventing off-flavor problems

The incidence and severity of off-flavors can be decreased by lowering fish stocking rates and adding less feed to ponds (Brown and Boyd 1982). However, this approach may not be feasible for pond-raised channel catfish because stocking and feeding rates would have to be lower than those currently considered profitable for this procedure to have a significant effect (Keenum and Waldrop 1988; Tucker and Martin 1991).

Another approach is the use of algicides. Some chemicals have been used as algicides to prevent and manage the growth of algae in catfish ponds (Hou and Clancy 1997; APHIS 2003). The chemicals may include copper sulfate, potassium ricinleate,

hydrogen peroxide, diuron®, dyes, ozone, granular activated carbon, chlorine, chlorine dioxide, potassium permanganate, etc. (Glaze and others 1990; Hutchings 1998; Tucker and van der Ploeg 1999; Coblentz 2001). The advantages of using algicides are that they are easy to apply to ponds, they are relatively inexpensive, and it is easy to observe if they are effective in reducing blue-green algae blooms (King and Dew 2003). However, killing these algal blooms may not improve the situation and could potentially make the problem worse in the near future (King and Dew 2003).

Another approach is polyculture of catfish with others. Torrens and Lowell (1987) suggested there was a decrease in the incidence of off-flavor in channel catfish polycultured with planktonivorous fish as a biological control agent such as blue tilapia (*Oreochrommis aurea*) and silver carp (*Hypothalmichthys molotrix*). The feeding habits of tilapia or silver carp are likely to change the environment in a manner that does not favor the growth of odor-producing microorganisms.

Another approach is a biochemical method. Some biochemical methods can be used to either inhibit off-flavor synthesis by algae or to enhance biotransformation of the off-flavors in the fish. Dionigi and others (1990) suggested that N-octyl bicycloheptene dicarboximide may inhibit cytochrome P450 mixed-function oxidases which may potentially catalyze the hydroxylation of a terpenoid precursor to form geosmin. Schlenk (1994) indicated that the kidney and liver isoforms of P450 may play a role in the biotransformation of MIB to more water-soluble metabolites that enhance the elimination of MIB.

Eliminating off-flavors from fish

One practice is to purge off-flavors out of fish. Purging is achieved by holding fish in a smaller pond and continuously flushing them with fresh water until the off-flavor is gone (Heikes 1993). The MIB can usually be purged within 3-5 days. Geosmin is more difficult to purge and can take up to 3-4 weeks to be reduced below detectable levels (Heikes 1993). Studies have shown that the rate of purging is affected by water quality, holding conditions, water temperatures, concentration of odorous compounds in fish, and fat content of fish (van der Ploeg 1991). This method is relatively simple and does not create an environmental issue, but it can be costly and time-consuming.

Another practice is to use a raceway system with cages (Masser 1995). In this system, the fish are contained in cages in one area of the pond which has raceways for water flow (Masser 1995). The water is constantly aerated mechanically in the area of the cages to maintain adequate oxygen levels (Masser 1995). There are several advantages to this type of system (Masser 1995). It is easier to prevent animals such as birds from preying on the fish in the cages; there are reduced labor costs for harvesting and for disease prevention; and water can be conserved.

<u>Post-harvest methods</u>

Limited research has been conducted to develop post-harvest methods to mask/ eliminate off-flavors. However, several methods can be considered to help maintain the flavor quality of fish before they reach consumers. Yamprayoon and Noomhorm (2003) suggested that off-flavor tilapia and its products can be successfully marketed by applying certain preservation and processing techniques: salting and drying, smoking

with pre-treatment, deep frying, soaking in acetic acid environment, and by lactic acid
fermentation.

Masking off-flavored fish

The purpose of this method is to cover-up the existing off-flavors in the catfish
fillets in a manner that the off-flavors are undetectable or much less objectionable to the
consumers (King and Dew 2003). Some food-grade spices can be used to mask off-
flavors in catfish fillets, but there may not be a large market for the spiced products since
consumers have variability in taste preferences (King and Dew 2003). In addition, the
level of spice needed to mask the off-flavors may be excessive resulting in an
unacceptable product (King and Dew 2003). Although commercial seasonings may be
added to catfish fillets to mask or interact with geosmin/MIB compounds, little
information is available concerning the sensory interaction of seasonings and these two
off-flavor compounds (Bett and others 2000).

Chlorine had a masking effect on both geosmin and MIB in water according to
their taste-and-odor (Worsley and others 2003). However, Oestman and others (2004)
concluded that the presence of chlorine (0.5-20 mg/L) and chloranmines (3-24 mg/L)
confused the panelists, but did not necessarily mask geosmin or MIB in water.

Lemon-pepper and "cajun-spice" seasoning blend can be used to mask MIB-off-
flavored catfish (Bett and others 2000). Sensory evaluation indicated a more frequent
acceptance of "lemon-pepper"-treated fish than either untreated fish or those treated with
a "canjun-spice" seasoning blend (Bett and others 2000). Kaewplang (2005) found that

Seven-up® and Sprite® were able to mask MIB in catfish by adding a pleasant sweet flavor, and suggests that lime flavor may decrease the perception of off-flavors.

Smoking could be used to mask off-flavor. A study by Iredale and Shaykewich (1973) observed that muddy off-flavor in trout could be minimized by smoking the fish. This product was acceptable to consumers, but the muddy flavor was still evident to a trained panel. Waagbo and others (1993) found that smoking of salmon could also mask off-flavors. Kaewplang (2005) found that two cooking methods decreased the perception of off-flavors by GC-MS (frying and marinating prior to baking methods). This researcher also stated that there was no difference in diffusion methods (dipping was not different from tumbling or injection). Certain canning methods were able to significantly reduce the intensity of muddy flavors in trout (Iredale and Shaykewich 1973). It was suggested that steam precooking fillet stripes with subsequent addition of either vegetable oil or smoke-flavored oil resulted in a highly acceptable canned product. However, canned and smoked products constitute a negligible proportion of processed channel catfish, and it is unlikely that these methods will ever be used to any extent to process off-flavor catfish.

In Thailand, a traditional fish preservation technique, called *som fak*, has been found to be very effective in masking muddy flavor in tilapia (Yamprayoon and Noomhorm 2000).

Degrading off-flavor compounds

Limited research has been conducted to destroy off-flavors. However, application of food additives such as oxidizers, acids, antimicrobial agents, antioxidants, and cryoprotectants may be used to destroy off-flavor compounds in fish fillets.

Yamprayoon and Noomhorm (2000) applied salt to tilapia for four hours and found that the geosmin content in the salted and dried fish was reduced about 11%. Waterman (1976) reported that the salt uptake by fish depends on fat content of the fish, thickness of the fish, and concentration of the salt. The use of high temperatures could result in poor quality fish (Yamprayoon and Noomhorm 2000).

Marinades that are a mixture of acetic acid and salt may be used to decrease geosmin content. Geosmin content was found to decrease from an initial value of 21 µg/kg to 15.4 µg/kg and 8 µg/kg in cooked and fried marinades, respectively (Yamprayoon and Noomhorm, 2000). A 2% citric acid treatment that was applied with vacuum tumbling resulted in a 36.8% loss of MIB, but consumers could not detect a difference in musty/earthy flavor when compared to untreated off-flavor controls (Forrester and others 2002). The failure to detect a difference was attributed to potential masking effects of the batter used to coat the fillets (Forrester and others 2002). There was no difference in texture detected instrumentally or by panelists, but the panelists detected sourness and preferred the control to the 2% treated-citric acid samples. In addition, Kaewplang (2005) found that both 1 and 2% acetic acid treated catfish fillets reduced MIB off-flavor odor, but they also induced a sour odor/flavor.

A research by Xi and King (2001) showed that geosmin and MIB were degraded below the consumer threshold when catfish fillets spiked with geosmin or MIB at 5 ppb level were treated with ozone for 10 min, and that at 100 ppb spiked level, after 10 min treatment, MIB was reduced by 35% and geosmin by more than half. It is suggested that ozone may be a way to solve the problems of catfish off-flavors. However, Kaewplang (2005) reported that MIB off-flavor catfish fillets exposed to 3 ppm ozone for 10, 20 and 30 min were not different from the off-flavor control when evaluated by a trained sensory panel.

Characteristics of some masking and destroying agents

<u>Masking agents</u>

Commercial lemon-pepper seasoning, available in the spice section of most markets, is enormously popular as a seasoning for chicken and other foods because it has a sharp smell (Anonymous 2006a). Its ingredients include lemon and black pepper (Anonymous 2006a). The main chemical components of lemon aroma are α-pinene, camphene, β-pinene, sabinene, myrcene, α-terpinene, linalool, β-bisabolene, limonene, trans-α-bergamotene, nerol, and neral (Anonymous 2006a) while black pepper is composed of similar components including α-thujone, α-pinene, camphene, sabinene, β-pinene, α-phellandrene, myrcene, limonene, caryophyllene, β-farnesene, β-bisabolene, linalool and terpinen-4-ol (Anonymous 2006a).

Vanillin (4-hydroxy-3-methoxylbenzaldehyde) can be used in ice-cream, bakery and confectionary products as well as for masking the odors of some other manufactured

products (Atkins 1987). Vanillin is a unique and highly prized flavor compound used in the flavoring of many foods that is detectable in extremely low concentrations (Atkins 1987). However, the strength of its perception does not increase greatly as its concentration increases (Atkins 1987).

Ginger has been used with steamed fish to remove fishy flavor and/or other flavors from fish. Since the ginger smell is strong, it can be used to enhance flavor of food products and overpower weaker-smelling compounds (Anonymous 1999b). It is composed of various chemical constituents including α-pinene, camphene, β-pinene, 1,8-cineole, linalool, borneol, γ-terpineol, nerol, neral, geraniol, geranial, geranyl acetate, β-bisabolene, and zingiberene (Anonymous 1999b).

Lemongrass can be added to soups to provide flavor (Anonymous 1999c). It is frequently used in curries, seafood soups, and tea (Anonymous 1999c). It is a very pungent herb with a lemon, sweet smell and is normally used in small amounts (Anonymous 1999c). The main chemical components of lemongrass are myrcene, citronellal, geranyl acetate, nerol, geraniol, neral and traces of limonene and citral (Anonymous 1999c).

Lime flavor has been used to flavor ginger ale and cola drinks (Anonymous 1999a). Lime has a sharp, citrus smell (Anonymous 1999a). The main components of lime flavor is α-pinene, β-pinene, myrcene, limonene, terpinolene, 1,8-ceneole, linalool, borneol, citral and traces of neral acetated, and geranyl acetate (Anonymous 1999a).

Smoke flavor has been used for thousands of years to enhance and modify the flavor of foods as well as to preserve meat (Anonymous 1985). Important smoke

constituents include many phenolic flavor compounds derived from the pyrolisis of lignin in the wood, as well as items such as maltol and various cyclopentenolones derived from cellulose pyrolysis (Anonymous 1985). The lignin derived constituents such as syringol are the heart of the smoke flavor while the cyclopentenolones provide a "burnt sugar" like note (Anonymous 1985). In smoked meats, the phenolics act as preservatives which help to prevent spoilage (Anonymous 1985).

Destroying agents

Several powerful oxidizers such as chlorine, permanganate, ozone, chlorine dioxide, and hydrogen peroxide have been reported to be effective in eliminating taste and odor-causing compounds in water (Dalecky and Sweet 1997).

Ozone oxidation is efficient for aromatic compounds and for substances that contain amino groups or double bonds (Anonymous 2005). Sulfide groups are also quickly oxidized by ozone (Anonymous 2005). Electron-retreating groups ($-Cl$, $-NO_2$, $-COOH$) cause a decrease in reaction speed, whereas electron-donating groups ($-NH_3$, $-OH$, $-O$, $-OCH_3$) cause an increase in reaction speed (Anonymous 2005). The direct oxidation of organic matters by ozone is a selective reaction mechanism, during which ozone reacts quickly with organic mater that contain double bonds, activated aromatic groups or amines (Anonymous 2005). The indirect reactions in an ozone oxidation process can be very complex and selective, and take place according to 3 steps: initiation, radical chain-reaction, and termination (Anonymous 2005). Ozone is very powerful in oxidizing geosmin and MIB. Xi and King (2001) reported that the use of ozone might reduce off-flavors (geosmin/MIB) of catfish fillets. Most protein (amino)

groups react with ozone very slowly (Anonymous 2005). However, since ozone is a strong oxidizer, other compounds in the fish such as lipids and nutrients may also be oxidized (Anonymous 2005).

Another strong oxidizer is the OH-radical that is generated from Advanced Oxidation Processes (AOP), a type of chemical oxidation which uses ozone combined with either hydrogen peroxide or UV-light or hydrogen peroxide and UV-light, sometimes called peroxone (Anonymous 2005). During the AOP, oxidation is largely brought about by OH-radicals (Anonymous 2005). These radicals are very reactive compounds or atoms that have a very short half-life (Anonymous 2005). This causes an OH-radical to react non-selectively and directly with dissolved solids (Anonymous 2005). The OH-radical compounds contain a very high electronic potential, which makes it one of the strongest oxidizers (Anonymous 2005). The activation of OH-radicals is a very complex process, which can take place according to a variety of different reaction mechanisms (Anonymous 2005). This process is used for ozone-resistant compounds such as pesticides, aromatic compounds and chlorinated solvents (Anonymous 2005). The reaction speed of OH-radicals is much higher than that of ozone (Anonymous 2005). Ferguson and others (1990) found that the peroxone process requires a significantly lower applied ozone dosage to oxidize geosmin and MIB in water as compared with ozone alone. However, ozone was not effective in removing MIB off-flavor in catfish fillets (Kaewplang 2005).

Acid is another option for destroying off-flavor compounds. Acids can be defined as substances that increase the concentration of hydronium ion (H_3O^+) when dissolved in

water, a proton donor, or an electron-pair acceptor (Anonymous 2006b). Acids generally are sour when dissolved in water, produce a stinging feeling, and particularly strong acids react aggressively with or corrode most substances (Anonymous 2006b). It is reported that under acidic environments such as acetic acid (Gerber and Lechevalier 1965), lactic acid (Yamprayoon and Noomhorm 2000), and citric acid (Forrester and others 2002), geosmin may be transformed to argosmin which is an odorless substance (Gerber and Lechevalier 1965).

Sensory methods for detection

Consistently reliable pond treatment methods for control of off-flavors in catfish are not available (Lovell 1983). Johnsen (1996) reported that trained panelists were unable to recognize and evaluate, with precision, the intensity of geosmin and MIB. The catfish industry needs more reliable methods for evaluating off-flavors in the fish prior to harvesting a pond (Lovell 1983).

Sensory analysis

Fish must be routinely screened for flavor quality before harvesting in order to avoid marketing off-flavor fish (van der Ploeg 1991). Persson (1980) indicated that it may be possible to quantify muddy odor compounds in water and fish by sensory methods, and it is the only applicable method for routine evaluation of fish flavor quality (van der Ploeg 1991). It is known that the sensitivity of the human nose exceeds the sensitivity of chemical analysis methods currently used for the analysis of muddy odor

compounds (Persson 1980). Experienced flavor checkers are able to detect from 0.1 ppb to 0.2 ppb MIB in catfish (King and Dew 2003).

Catfish plants contain employees entitled "off-flavor checkers" that smell and/or taste fish samples to ensure delivery of catfish products with desirable flavor quality (Johnsen and others 1992). A sample size of 25 fish per lot of 10,000 fish is recommended for processors who desire near-zero tolerance (Bett and Dionigi 1997). Plants will test one fish weekly until ready to harvest or more than one fish near harvest time. Bett and Johnsen (1996) suggested that fish should be tested more often just prior to planned harvesting. Testing has involved cooking several fish or fillets either by baking or broiling at 425°F for 20 min, or a piece of the fish, normally the tail fin area, in a microwave oven for 3 min (Boggess and others 1971; Lovell 1983; Silva and Ammerman 1984, Jensen 1997). However, the sensitivity of "off-flavor checkers" may also be influenced by illness, weather, food consumed, and so on. All of these factors can affect sensory evaluation results (Bett and Johnsen 1996). Bett and Johnsen (1996) found that descriptive analysis panelists, who are trained to evaluate flavor intensity, also have some difficulties evaluating catfish samples that exhibit geosmin or MIB off-flavor compounds. Individual panelists perceive flavor intensity differently, because of variations in detection thresholds, adaptation, fatigue, carry-over and enhancement or suppression.

<div align="center">Flavor perception</div>

Flavor is a food attribute and is experienced both in the mouth and in the nose (van der Ploeg 1991). The four basic tastes (salty, sweet, sour and bitter) are perceived by taste

buds on the tongue (van der Ploeg 1991). Volatile odorous compounds reach the olfactory area through the nose when sniffing or through the pharyngeal passage when tasting as shown in Figure 2.1 (van der Ploeg 1991). Amoore (1986) indicated that to be smelled, a substance must be volatile, be able to reach the olfactory nerve, and be able to reach nerve receptors in the uppermost recesses of the nasal cavities. This typically occurs when air is sniffed through the nostrils but can also take place when air is exhaled through the nose just after a food or beverage has been swallowed. Depending on the volatility of the odorous compounds, a flavor may be experienced directly after a sample is taken into the mouth or after chewing (van der Ploeg 1991). However, the olfactory nerves in the nose are more sensitive to many chemicals than are the gustatory nerves on the tongue (Amoore 1986). The strength of the odor impression is partly governed by the volatility of the molecules from the samples (Amoore and Buttery 1978).

Age significantly affects the threshold, with an approximate halving of the sensitivity for each 22 years of age after age 20 (Venstrom and Amoore 1968). Women are not more sensitive to odors than men nor are moderate smokers less sensitive than nonsmokers, provided they have not smoked during the 15 minutes before the test (Venstrom and Amoore 1968). Adaptation to flavors is a problem with trained sensory panels (Meilgaard and others 1991). Williams and Arnold (1992) found that panelists rated the intensities of the first sample differently from the second sample. Fatigue causes the sensory system to become less responsive to stimuli over continuous stimulation or repeated evaluations, and requires a lengthy recovery for accurate evaluation of another sample (Burgard and Kuznick 1990). Amerine and others (1965) found that mental

fatigue causes panelists to be less sensitive, and judges differ in their susceptibility to mental fatigue. Carry-over seems to be a problem with some flavors as indicated by many references to cleansing the palate as a standard practice in sensory methods (Meilgaard and others 1991). Another phenomenon is enhancement or suppression. This is the effect on intensity of various descriptors by the presence of another substance and can occur when certain flavors are present (Meilgaard and others 1991). All of these influences can affect sensory evaluation results and must be considered when designing experiments.

<div align="center">Flavor intensity</div>

Flavor intensity is the quantitative aspect of flavor quality and estimates the concentration of the flavor compound of interest (van der Ploeg 1991). Fish samples have been assigned scores ranging from two, for extreme off-flavor, to 10 for no off-flavor as shown in Fig 2.2 (Johnsen and others 1987). Flavor intensity can also be determined by cooking the fish in a microwave and evaluating the flavor by tasting and assigning grades based on a standard scale of 0 to 3 where '0' is on flavor, '1' is slightly off, '2' is distinctly off and 3 is strongly off (Lazur 2004). Another scale (Fig 2.3) rates the intensity of a flavor from 0 to 3 but in 0.5 point increments (van der Ploeg 1991). Extremely strong off-flavors may be indicated with the number 4 (van der Ploeg 1991). When off-flavor is strong enough to warrant a score of 4, it can be smelled and it is not necessary to taste the fish sample (van der Ploeg 1991). Another scoring system, which ranges from threshold (0), very slight (0.5), slight (1), slight to moderate (1.5), moderate (2), moderate to strong (2.5), and strong (3) was proposed by Krasner (1988). Kaewplang

(2005) utilized a 5-point scale (Fig 2.4) for determination of off-flavor strength in catfish fillets.
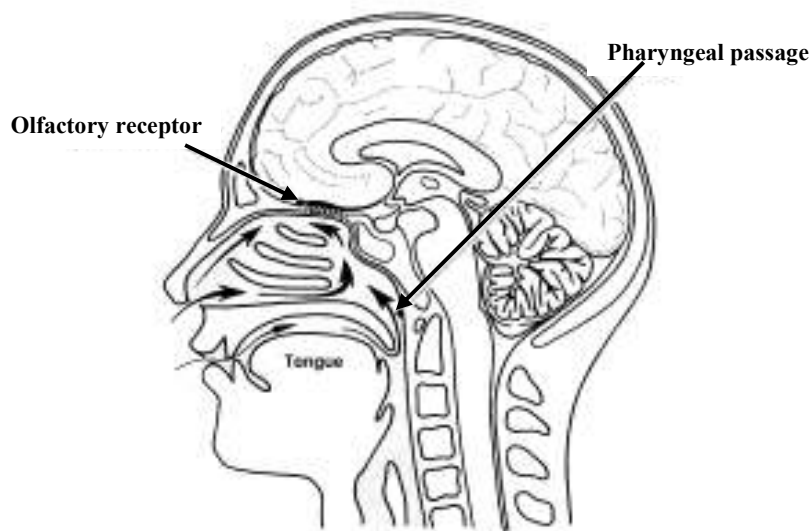
Figure 2.1 Section through the human head showing the olfactory area (van der Ploeg 1991)

No off-flavor	(10)
	(9)
Slight off-flavor	(8)
	(7)
Distinct off-flavor	(6)
	(5)
Intense off-flavor	(4)
	(3)
Extreme off-flavor	(2)
	(1)

Figure 2.2 Fish flavor intensity scale (Lovell 1983)

Verbal description	Intensity scale
No off-flavors	0
Threshold	Threshold
Very slight	0.5
Slight	1
Slight to distinct	1.5
Distinct	2
Distinct to strong	2.5
Strong	3

Figure 2.3 Fish flavor intensity scale (van der Ploeg 1991)

```
None     _____1
Slight _____2
Moderate _____3
Much _____4
Extreme _____5
```

Figure 2.4 Flavor intensity scale (Kaewplang 2005)

The labeled magnitude scale (LMS) as shown in Figure 2.5 can also be used as a flavor intensity scale (Green and others 1996). The LMS is a semantic scale of perceptual intensity characterized by a quasi-logarithmic spacing of its verbal labels (barely detectable, weak, moderate, strong, very strong and strongest imaginable). The verbal descriptors are placed on the scale according to their associated geometric means (i.e. the antilogs of the log means). The positions of the verbal labels on the LMS, as percentage of full scale length, are: barely detectable, 1.4; weak, 6.1; moderate, 17.2; strong, 35.4; very strong, 53.3; strongest imaginable, 100 (Green and others 1993). It is a valid alternative to magnitude estimation (ME) as a tool for measuring the perceived intensity of gustatory, olfactory and chemesthetic sensations within the broadly defined perceptual domains of taste and smell, but it should be modified for use in scaling specific taste and odor qualities (Green and others 1996). The most direct way to study perceptual differences between subjects and to obtain data on the absolute strength of sensations is to use a category scale, but subjects are typically instructed to assign sensations to categories than correspond to constant perceptual intervals rather than to constant perceptual ratios and because category scales often have no true zero, it can not be assumed that the resulting data lie on a ratio continuum (Stevens 1951, 1956). The ME is limited in the information it can provide about two important aspects of perception: the 'absolute' intensity of sensations and individual differences. The ME provides no information about the intensity of sensations in any absolute sense, i.e. whether they are weak, moderate or strong. The LMS encompasses a wider numerical range between its lowest ('barely detectable') and highest ('strongest imaginable') verbal descriptors. It

permits interpretation of perceived intensity only in terms of 'oral sensation'. This limitation prohibits conclusions about the intensity of gustatory or olfactory stimuli within the perceptual domains of taste and smell. Because intense tastes and smells rarely reach or exceed 'strong' on a scale of all imaginable oral sensations, ratings of gustatory and olfactory stimuli would tend to be confined to the lower portion of the LMS. With the upper bound of the LMS defined as either 'strongest imaginable taste' or the 'strongest imaginable odor', it produces psychophysical functions equivalent to those produced by the ME. In theory, the LMS can be used both to determine the relative intensities of different taste or smells on a ratio scale and to provide semantic information about their 'absolute' intensities within each perceptual domain. It may be appropriate for use in the variety of perceptual domains. The advantage of the LMS is that it does not require the assumption that subjects are equally responsive to some comparison modality, and avoids reliance on the unproven hypothesis that the perceptual range is the same in all sensory modalities (Teghtsoonian 1971, 1973).

Analytical methods for detection

Geosmin and MIB have significant relatively low odor thresholds that are not easily be detected by gas chromatography (Maga 1987). For instance, the foods are sometimes judged to be musty by a panel, but gas chromatography analysis does not reveal the presence of geosmin or MIB (Maga 1987). Thus, it should be known that a musty smell may be present when the compound responsible is not present in sufficient quantity to be instrumentally observed and/or identified (Maga 1987).

Strongest

Very

Strong

Moderate

Weak
Barely
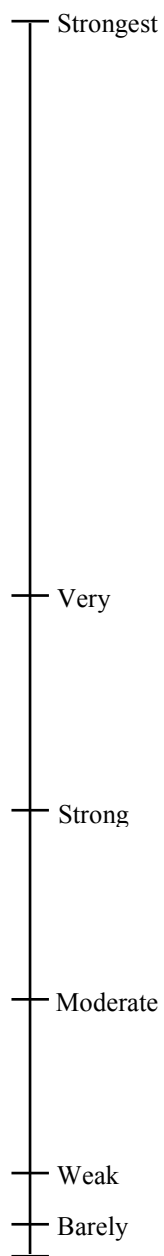
Figure 2.5 Flavor intensity scale (Green and others 1996)

Instrumental analyses used for the identification and quantitative determination of off-flavor compounds depend primarily on gas chromatography mass spectrometry (GC-MS). The sample preparation steps used in extraction techniques for detecting off-flavor compounds in aquaculture products include closed-loop stripping (McGuire and others 1981), liquid-liquid extraction (Johnsen and Kuan 1987), carbon adsorption (Rosen and others 1970), steam distillation (Lloyd and others 1998), and purge and trap (Johnsen and Lloyd 1992). These techniques are effective but expensive, time-consuming and labor intensive (Grimm and Zimba 2003). The problem with the liquid-liquid extraction has been high detection limits (Johnsen and Kuan 1987). The carbon adsorption method has involved the use of vary large sample sizes, and requires a lengthy processing time (Johnsen and Kuan 1987). However, the purge and trap method proved more accepted. Concentrations of geosmin or MIB can be detected down to the 2.5 ppb level (Grimm and others 1996). Improved methodology utilizing the purge and trap method should result in an analysis time approaching 10 min and a sensitivity of less than 0.1 ppb (Grimm and others 1996). The concentration of geosmin or MIB in water can be determined from 30 ppb down to 0.1 ppb using SPME/GC/FID (Grimm and others 1996). Johnsen and Kuan (1987) suggested a simple and rapid method for the extraction and quantification of geosmin and MIB. Using methylene chloride extraction and gas chromatography, the procedure eliminates costly stripping devices (Johnsen and Kuan 1987). Recovery efficiency of the procedure is approximately 65% with sensitivity equal to the human threshold for these two important off-flavor compounds (Johnsen and Kuan 1987).

However, accurate quantitation requires the use of an internal standard. A number of compounds have been used as internal standards (Wood and Snoeyink 1977; Hwang and others 1984) and a mixture of linear chloroalkanes is currently accepted as the best compromise (Lloyd and others 1998). Another option is deuterium labeled standards. The deuterium labeled standards offer many advantages compared with other internal standards used for determination of geosmin and MIB in natural water (Korth and others 1991). They offer high accuracy and precision down to concentrations below the threshold odor concentrations (Korth and others 1991). The new standards (Deuterium labeled geosmin and MIB) gave better precision and accuracy than the chloroalkanes and prevent the underestimation of the concentration of initial analytes which usually results from losses of analyte through adsorption, volatilization, and biodegradation during sample storage (Korth and others 1991).

Therefore, current instrumental methods for detection of geosmin and MIB have been limited to gas chromatographic methods. Most of these are modifications of purge and trap methods utilizing phase separations based on their volatility and/or hydrophobicity (Buttery and Garibaldi 1976; Hwang and others 1984; Jonsen and Kuan 1987). For instance, off-flavor compound analysis in channel catfish tissue is performed by vacuum distillation (Lovell and Sackey 1973) or microwave-cold trap collection (Martin and others 1987) followed by gas chromatography-flame ionization detection (GC-FID) or gas chromatography-mass spectrometry (GC-MS). Other extraction techniques may include Membrane-Based Extraction (Zander and Pingert 1997) that can detect analytes in the parts per trillion. Solid Phase Extraction (Conte and others 1996) is

rapid, inexpensive and can detect concentrations at part per billion levels, and Solid Phase Micro-Extraction (SPME) (Belardi and Pawliszyn 1989).

The SPME has recently proved to be a very sensitive and also low-cost, rapid method. It has been utilized for the analysis of geosmin and MIB mainly in water (Lloyd and others 1998; Watson and others 2000). Geosmin and MIB were isolated by SPME and analyzed by GC-MS. A Carbonxen/PDMS/DVB fiber coating was selected because of its highest extraction efficiency (Jelen and others 2003). Concentrations of geosmin and MIB as low as 0.001µg/kg were detected in the SIM mode using ion trap mass spectrometer (Jelen and others 2003). Although SPME is a simple equilibrium sampling technique, the method requires careful control of sampling conditions for efficient recovery and quantitative analysis of compounds. Such sampling conditions include extraction mode (immersion or headspace), addition of salt, fiber type, temperature, sample agitation, fiber position, sample size, headspace volume, and extraction time (Pawlizyn 1997; Anonymous 1998). Grimm and others (2000) stated that SPME alone is not very effective for the analysis of samples that are composed of a complex matrix such as soil and muscle tissue. For such complex matrices, methods utilizing SPME and GC-MS along with microwave distillation should be used to steam-distill analytes from the sample matrix first.

However, Kaewplang (2006) identified and quantified MIB compounds in treated catfish fillets effectively by using simple SPME-GC/MS. Homogenized 5 g fish samples were added to 10 ml vials (Supelco, Bellefonte, PA, USA) and sealed. The vials were refrigerated at 4°C no longer than 24 h before analysis. For SPME analysis, a fiber sheath

pierces the septum of the vial containing the ground catfish tissue; the fiber (Carboxen/PDMS) was placed over the headspace of tissue and the sample was heated to 70°C for 30 min to achieve equilibrium between fiber and analytes in the headspace. Then, the fiber was withdrawn into the sheath, which was withdrawn from the vial and inserted into a splitless injector held at 200°C. The sample desorbed in the injector for three min. The separation was carried out using gas chromatography (GC) equipped with ZB-5MS column 30 meters × 0.25 mm with a 0.25 micro film thickness. The GC was coupled to a Varian Saturn mass spectrometer (MS) (Varian Inc, Walnut Creek, CA) with wave board technology operated in the electron impact mode. The MS was scanned over a mass range of 60-250 m/z with wave isolation of 112 and 212.

CHAPTER III

MATERIALS AND METHODS

Channel catfish fillets were obtained from a commercial catfish processing plant in Macon, Mississippi, USA prior to chilling. The fillets were kept frozen at -20°C for further experiments. For each experiment, the fillets were thawed overnight in a refrigerator at 4°C.

Some food-grade ingredients were screened for their effect on geosmin or 2-mehtylisoborneol (MIB) compounds spiked in water. These ingredients included ginger powder (Nguan Soon Hand Brand No 1, Bangkok, Thailand), lemongrass powder (Nguan Soon Hand Brand No 1, Bangkok, Thailand), rice cooking wine (Wallong Marketing Inc, Buena Park, CA, USA), sweet cooking wine (Kikkoman corp, Tokyo, Japan), citric acid (Aldrich Chemical Company, Inc, Milwakee, WI, USA), distilled vinegar, 5% acetic acid (Great Value, Wal-Mart Store Inc, Bentonville, AR, USA), hydrogen peroxide (Family News, Miami, FL, USA), pure lime extract (JR Mushrooms & Specialties, Inc., Sunny Isles Beach, FL, USA), pure lemon extract (JR Mushrooms & Specialties, Inc., Sunny Isles Beach, FL, USA), and smoke flavors (Red Arrow Products Company, Manitowoc, WI, USA). After screening, 5% acetic acid, pure lime extract, and smoke flavors were chosen for this research (data not shown). Since other ingredients appeared to be less potential at decreasing odor intensity of geosmin or MIB compounds.

EXPERIMENT I: Threshold determination of panelists

Selection

Seven people (3 women and 4 men, age ranging from 25 to 35 years old), at the Department of Food Science, Nutrition, and Health Promotion of Mississippi State University with prior sensory evaluation experience were screened for their ability to detect geosmin or MIB odor.

Five off-flavor-spiked solutions of concentrations at 0.1 ppb, 0.5 ppb, 2.5 ppb, 12.5 ppb, or 62.5 ppb (v/v) were prepared by diluting chemically synthesized geosmin or MIB (Sigma-Aldrich Co., TX, USA) with deionized water. Vials (8 ml) with caps (Fisher Scientific, Fair Lawn, NJ, USA) coded by three-digit random numbers (Meilgaard and others 1991) were individually filled with 5 ml of each geosmin- or MIB-spiked solutions to allow a headspace in order to allow volatile molecules to escape from the bulk solution so that the panelists could smell geosmin or MIB. The test was replicated three times.

Six sensory sessions were conducted, three sessions with geosmin-spiked solutions and another three sessions with MIB-spiked solutions. Panelists were presented with 5 triangle tests (Fig 3.1) per session per day with one minute break between each test to avoid sensory adaptation/fatigue. All samples were presented in a random order within each session. Panelists were instructed in which order samples were to be tested, and asked to indicate which one was the odd sample (Meilgaard and others 1991).

Name: _____   Date: _____

INSTRUCTIONS

DO NOT DRINK PROVIDED SAMPLES!!!

Sniff the contents of each vial from left to right, using shallow short sniffs. Open each vial only slightly and briefly to reduce contamination of the test room.

After sniffing each vial, select the odd/different sample, the odor that is different from the other two.

If no difference is apparent, you must guess.

Sets of three samples			Which is the odd sample?	Remarks
116	383	415	_____	_____
878	978	421	_____	_____
848	468	543	_____	_____
311	811	286	_____	_____
339	771	835	_____	_____

Notes: If you wish to comment on the reasons for your choice or if you wish to comment on the sample characteristics, you may do so under Remarks.

Thank you very much for your participation!

Figure 3.1 Sensory evaluation sheet used to determine threshold level of geosmin or MIB spiked in water

Data were analyzed to determine the thresholds of the panelists by utilizing the Best Estimate Threshold (BET) method (Meilgaard and others 1991). The individual best estimated threshold (BET) during each sensory session was calculated as the geometric mean of the highest concentration missed and the next concentration (Meilgaard and others 1991). For those panelists who was correct at the lowest geosmin or MIB concentration, their individual BET were estimated as the geometric mean of the lowest concentration (0.1 ppb) tested in this study and the hypothetical next lower geosmin or MIB concentration (0.02 ppb). Similarly, for those panelists who failed to correctly identify the odd water sample at the highest geosmin or MIB concentration, their individual BET were estimated as the geometric mean of the highest geosmin or MIB concentration (62.5 ppb) tested in this study and the hypothetical next higher geosmin or MIB concentration (312.5 ppb). The group BET was the geometric mean of the individual BET of each session (Meilgaard and others 1991). Based on their performance, four panelists (2 women and 2 men) were selected as a 4-member panel for training and further experiments.

<center>Training</center>

A series of training sessions were conducted by allowing a 4-member panel to be familiar with different concentrations of geosmin- or MIB-spiked solutions. At the end of the training, geosmin- or MIB-spiked fish samples of four concentrations (v/w) (1.4 ppb, 7.0 ppb, 35.0 ppb, and 175.0 ppb of geosmin or MIB) were prepared for examining the performance of the panel (Green and others 1996). The treatment was replicated three times.

Six sensory sessions were conducted. The panel was presented with four random samples per session with one minute break between each sample to avoid sensory adaptation/fatigue. The panel was asked to rate the odor intensity of geosmin or MIB of each sample through descriptive analysis utilizing the Labeled Magnitude Scale (LMS) (Green and others 1996). The LMS consists of a vertical line with verbal labels (barely detectable, weak, moderate, strong, very strong and strongest imaginable) for intensity levels spaced in a quasi-logarithmic fashion (Fig 3.2). Data were logged and analyzed by Linear Regression to examine the performance of the panel (Green and others 1996).

<div align="center">Fillet spiking procedure</div>

Two methods were compared to determine the effectiveness of spiking. The two methods included spiking fish with geosmin or MIB by injection or blending (Kaewplang 2005). Samples for both methods consisted of two blanks (0 ppb), 20 ppb MIB, and 200 ppb geosmin.

For the injection method, 200g of fish fillet were cut into small pieces (20g) and spiked with chemically synthesized geosmin or MIB (Sigma-Aldrich Co., TX, USA) by injection using a 5 ml syringe (Fisher Scientific, Franklin Lakes, NJ, USA) to concentrations (v/w) of 0 ppb, 20 ppb MIB, or 200 ppb geosmin. The samples were kept at a room temperature for 20 min, drained and then evaluation by the sensory panel. The injection was accomplished by injecting a needle into the fish in many spots as shown in Figures 3.3a and 3.3b to allow uniform diffusion throughout the fish. Each treatment was replicated three times.

Name: _____   Date: _____

INSTRUCTIONS

DO NOT TASTE PROVIDED SAMPLES!!!

Sniff the content of each fish sample containing a certain amount of MIB from left to right in a small cup. After sniffing each cup, "**Rate**" the intensity of the MIB odor by making a mark <u>ANYWHERE</u> on a vertical line below which is corresponding to the amount of the perceived MIB aroma.

For 824, 287, 843, 553

Strongest Imaginable

Very Strong

Strong

Moderate

Weak

Barely Detectable

THANK YOU SO MUCH!!!

Figure 3.2 Sensory evaluation sheet used to rate the odor intensity of MIB spiked in water
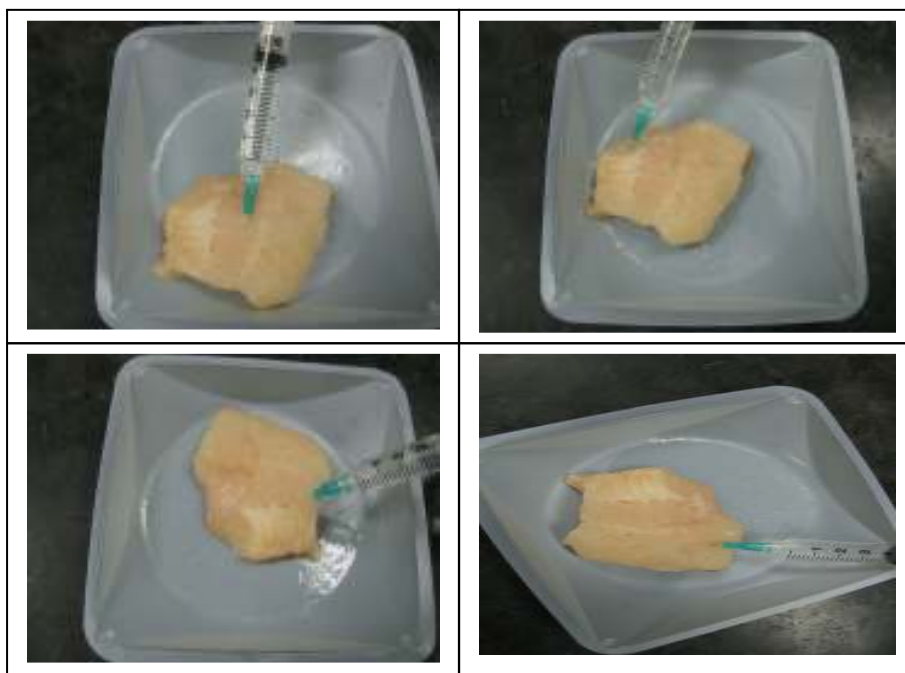
Figure 3.3a Injection method used to spike geosmin or MIB into fish fillets
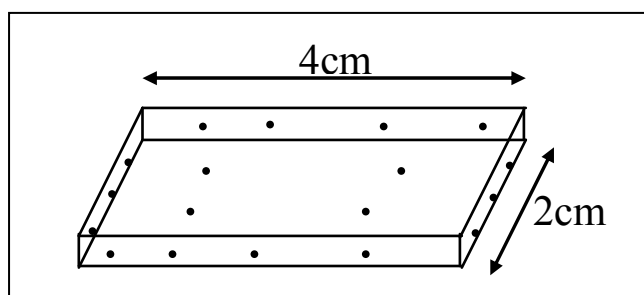
Figure 3.3b   Distribution of spiked geosmin or MIB in injected catfish fillets. Note: 3 ml of geosmin or MIB was injected into a 20 g fish portion in at least fourteen spots in different directions [1:3 (v/w)]

For the blending method, an 80 g fish fillet portion was blended using a blender (BlendMaster®Hamilton Beach/Proctor-Silex, Inc., NC, USA) on high speed for three sec (Kaewplang 2005). The ground fish were divided into 20 g each and placed into four individual plastic cups with lids (ReStockIt.com, Hollywood, FL, USA). Two samples were kept as blanks. One was spiked with MIB by uniformly blending to a theoretical concentration (v/w) of 20 ppb, and another one with geosmin to a concentration (v/w) of 200 ppb. The treatment was replicated three times.

Six sensory sessions were conducted similarly as described in the training section, but the panel was asked to rate the odor intensity of geosmin or MIB in both raw (Fig 3.4) and cooked (Fig 3.5) fish. Cooked samples were prepared by wrapping the same fish samples in an aluminum foil and oven-baking at 420°F for 20 minutes.

A randomized complete block design with three replications (blocks) was utilized to test for differences ($p < 0.05$) among odor intensities (SAS, 2003) of geosmin or MIB. The least significance difference (LSD) test was utilized to separate treatment means ($p < 0.05$) when significant differences occurred among treatments (SAS, 2003).

Threshold determination

After training, fish samples spiked with geosmin or MIB were prepared to determine the threshold of the panel. A 200g fish fillet was cut into small pieces (20g) and spiked with geosmin or MIB (Sigma-Aldrich Co., TX, USA) by an injection method to concentrations (v/w) of 0.4, 0.8, 1.6, 3.2, or 6.4 ppb of MIB, and 4, 8, 16, 32, or 64 ppb of geosmin. Samples were kept at a room temperature for 20 min and then drained prior to sensory evaluation of the raw products. Each treatment was replicated three times.

Name: _____   Date: _____

INSTRUCTIONS
(Raw: 194, 526, 485, 747)
Sniff each of the four fish samples from <u>LEFT to RIGHT</u> by allowing at least **1 min** to lapse between each sample. After sniffing each sample, answer the following questions.

How do you feel about the GEOSMIN/MIB odor?

— Strongest Imaginable

— Very Strong

— Strong

— Moderate

— Weak
— Barely Detectable

Code	MIB/GSM?
194	
526	
485	
747	

THANK YOU VERY MUCH!!!

Figure 3.4 Sensory evaluation sheet used to rate odor intensity of geosmin/MIB in raw fish fillets to screen spiking methods

Name: _____   Date: _____

INSTRUCTIONS
(Cooked: 194, 526, 485, 747)

Sniff each of the four fish samples from <u>LEFT to RIGHT</u> by allowing at least **1 min** to lapse between each sample. After sniffing each sample, answer the following questions.

How do you feel about the GEOSMIN/MIB odor?

— Strongest Imaginable

Code	MIB/GSM?
194	
526	
485	
747	

— Very Strong

— Strong

— Moderate

— Weak
— Barely Detectable   THANK YOU VERY MUCH!!!

Figure 3.5 Sensory evaluation sheet used to rate odor intensity of geosmin/MIB in cooked fish fillets to screen spiking methods

Six sensory sessions were conducted similarly as described in the panelist selection section (p. 44), but this time with raw fish samples (Fig 3.6).

Data were analyzed utilizing the Best Estimate Threshold (BET) method (Meilgaard and others 1991) as described in the panelist selection section (p. 46). Based on the group BET, samples should be spiked with geosmin or MIB to a theoretical concentration (v/w) of 100 ppb geosmin or 10 ppb MIB for further experiments.

EXPERIMENT II: Effect of acetic acid and/or lime flavor on off-flavors

<u>Materials and methods</u>

In this experiment, the effect of acetic acid and liquid lime flavor on fish fillets spiked with geosmin or MIB were investigated. A 200g fish fillet was cut into pieces (50g), and randomly placed into small plastic portion cups with lids (ReStockIt.com, Hollywood, FL, USA). Samples were prepared as spiked controls, and treated-spiked samples. The spiked samples were made as described in the injection method, but the off-flavor concentration for this experiment was 100 ppb geosmin or 10 ppb MIB.

The spiked samples were dipped in 20 ml of 0.5% acetic acid and/or 1:50 lime-flavored solution. Samples were then kept at room temperature for 20 min, and then drained and served to sensory panel. Each treatment was replicated three times. The flowchart diagram for sample preparation is shown in Figure 3.7.

Distilled vinegar (5% acetic acid) was diluted with deionized water to prepare 0.5% (v/v) acetic acid solution.

Name: _____   Date: _____

INSTRUCTIONS

Sniff the odor of each sample from left to right, using shallow short sniffs. Open each cup only slightly and briefly to reduce contamination of the test room.

After sniffing each cup, select the odd/different sample, the odor that is different from the other two.

If no difference is apparent, you **MUST** guess.

Sets of three samples			Which is the odd sample?	Remarks
116	383	415	_____	_____
636	882	394	_____	_____
661	539	591	_____	_____
228	177	448	_____	_____
723	964	257	_____	_____

Notes: If you wish to comment on the reasons for your choice or if you wish to comment on the sample characteristics, you may do so under Remarks.

Thank you very much for your participation!

Figure 3.6 Sensory evaluation sheet used to determine the threshold of geosmin or MIB spiked in fish fillets

Pure lime extract was diluted with deionized water to prepare 1:50 (v/v) lime-flavored solution.

<div align="center">Sensory analysis</div>

Samples were evaluated by a 4-member trained panel, and six sensory sessions were conducted. The panel was presented with four random samples per session with one minute break between each sample to avoid sensory adaptation/fatigue, and were instructed in which order samples were to be tested. The panelists were asked to rate the odor intensity of geosmin or MIB of each sample in both raw and cooked fish (Meilgaard and others 1991). The LMS (Green and others 1996) as described before (Figs 3.8 and 3.9) was used to determine the odor intensity of geosmin or MIB. Cooked samples were prepared by microwaving the same fish samples (after evaluation) on high for 72 sec.

A randomized complete block design with three replications (blocks) was utilized to test for differences (p<0.05) among odor intensities (SAS, 2003) of geosmin or MIB. The least significance difference (LSD) test was utilized to separate treatment means (p<0.05) when significant differences occurred among treatments (SAS, 2003).

<div align="center">Calculation of reduction in off-flavor intensity</div>

Reduction of odor intensity of geosmin or MIB of each treatment was calculated using the formula listed below (Kaewplang 2005). A negative number indicates an increase in the off-flavor intensity by the treatment.

$$\% reduction\ in\ off\ flavor = \frac{(control - treatment)}{control} \times 100$$

```
┌─────────────────────────────┐
│      Catfish Fillets        │
│     (Before chilled)        │
└─────────────────────────────┘
              │
              ▼
┌─────────────────────────────┐
│    Cut into pieces (50g)    │
└─────────────────────────────┘
              │
              ▼
┌─────────────────────────────┐
│  Injected with geosmin or MIB │
│  (100 ppb geosmin, 10ppb MIB) │
└─────────────────────────────┘
           20 min
              ▼
┌─────────────────────────────────────────┐
│ Dipped in acetic acid (0.5%), lime flavor (1:50), or │
│        smoke flavor (1:25) solutions      │
└─────────────────────────────────────────┘
           20 min
              ▼
┌─────────────────────────────┐
│           Drain             │
└─────────────────────────────┘
              │
              ▼
┌─────────────────────────────┐
│        Sensory (raw)        │
└─────────────────────────────┘
              │
              ▼
┌─────────────────────────────┐
│           Cook              │
│      Microwave, 72 sec      │
└─────────────────────────────┘
              │
              ▼
┌─────────────────────────────┐
│       Sensory (cooked)      │
└─────────────────────────────┘
```
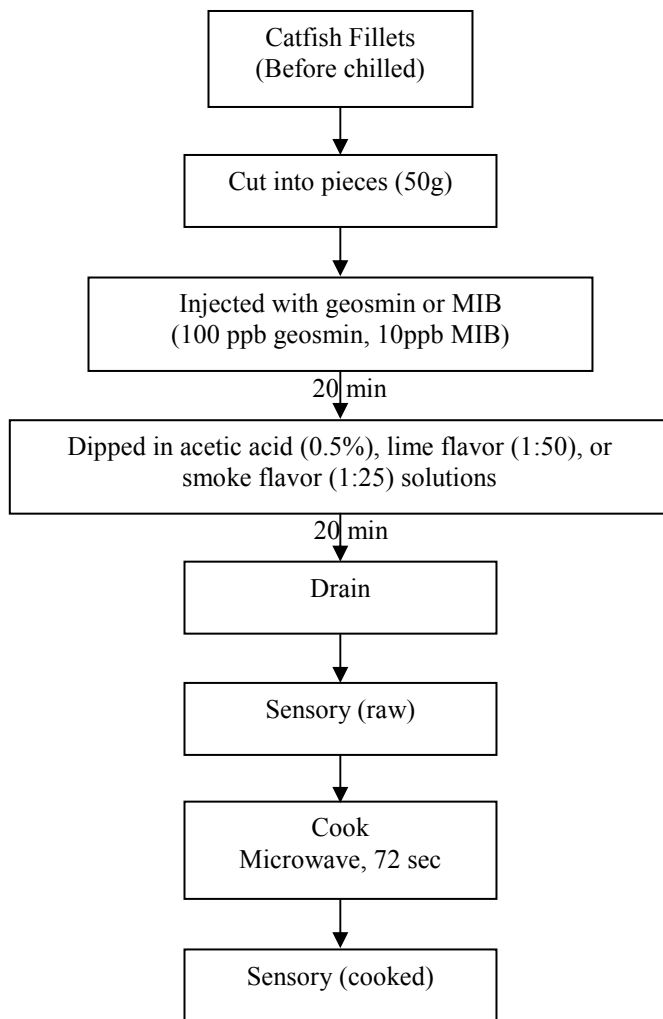
Figure 3.7 Flowchart diagram for preparation of spiked catfish fillets to test the effect of additives on off-flavors

Name: _____   Date: _____

INSTRUCTIONS
(Raw: 183, 481, 662, 776)
Sniff each of the four fish samples from <u>LEFT to RIGHT</u>. After sniffing each sample, answer the following two questions.

1. How do you feel about the GEOSMIN odor?   2. How do you like the overall aroma of this sample?

— Strongest Imaginable

183	481	662	776	Preference
				Like extremely
				Like very much
				Like moderately
				Like slightly
				Neither like nor dislike
				Dislike slightly
				Dislike moderately
				Dislike very much
				Dislike extremely

— Very Strong

Comments:

— Strong

— Moderate

— Weak
— Barely Detectable

THANK YOU VERY MUCH!!!

Figure 3.8 Sensory evaluation sheet used to rate the odor intensity of geosmin spiked in raw fish fillets that were treated with acetic acid and/or lime flavor

Name: _____   Date: _____

INSTRUCTIONS
(Cooked: 183, 481, 662, 776)

Sniff each of the four fish samples from <u>LEFT to RIGHT</u>. After sniffing each sample, answer the following two questions.

1. How do you feel about the MIB odor?   2. How do you like the overall aroma of this sample?

—— Strongest Imaginable

183	481	662	776	Preference
				Like extremely
				Like very much
				Like moderately
				Like slightly
				Neither like nor dislike
				Dislike slightly
				Dislike moderately
				Dislike very much
				Dislike extremely

—— Very Strong

Comments:

—— Strong

—— Moderate

—— Weak

—— Barely Detectable

THANK YOU VERY MUCH!!!

Figure 3.9 Sensory evaluation sheet used to rate the odor intensity of MIB spiked in cooked fish fillets that were treated with acetic acid and/or lime flavor

EXPERIMENT III: Effect of liquid smoke flavors on off-flavors

Materials and methods

In this section, the effect of liquid smoke flavors on fish fillets spiked with geosmin or MIB were investigated. Sample preparation was made similarly to the procedure described in experiment II (p.53), but the off-flavor spiked samples were dipped in 20 ml of 1:25 Charsol®LFBN or Aro-smoke®8068 solution (Red Arrow Products Company, Manitowoc, WI, USA), and kept at room temperature for 20 min (Fig 3.7). Each treatment was replicated three times.

Two types of liquid smoke flavors were diluted with deionized water to obtain a desirable concentration (1:25 v/v). The Charsol®LFBN is a clear, brown liquid with characteristic hardwood smoke aroma, and the Aro-smoke®8068 is a clear, brown liquid with a pungent hickory smoke flavor.

Sensory analysis

Sensory evaluation for this experiment was conducted similarly as described in experiment II (p.55), but the panel was presented with three samples per session (Figs 3.10 and 3.11). In this experiment, the panel was asked to taste cooked samples (Figs 3.12 and 3.13). The same samples after sensory evaluation (raw) were cooked by microwave (high, 72 min). The panelists were asked to chew each of the three fish samples to allow volatiles of the fish tissue to reach their olfactory nerve/receptors.

A randomized complete block design with three replications (blocks) was utilized to test for differences ($p < 0.05$) among odor intensities (SAS, 2003) of geosmin or MIB.

The least significance difference (LSD) test was utilized to separate treatment means (p<0.05) when significant differences occurred among treatments (SAS, 2003).

<u>Calculation of reduction in off-flavor intensity</u>

Reduction of odor intensity of geosmin or MIB of each treatment was calculated using the formula listed below (Kaewplang 2005). A negative number indicates an increase in the off-flavor intensity by the treatment.

$$\%reduction = \frac{(control - treatment)}{control} \times 100$$

Name: _____   Date: _____

INSTRUCTIONS
(Raw: 539, 448, 723)

Sniff each of the three fish samples from <u>LEFT to RIGHT</u> by allowing at least **1 min** to lapse between each sample. After sniffing each sample, answer the following two questions.

1. How do you feel about the GEOSMIN odor?   2. How do you like the overall acceptability of this sample?

— Strongest Imaginable

539	448	723	Preference
			Like extremely
			Like very much
			Like moderately
			Like slightly
			Neither like nor dislike
			Dislike slightly
			Dislike moderately
			Dislike very much
			Dislike extremely

Comments:

— Very Strong

— Strong

— Moderate

— Weak

— Barely Detectable

THANK YOU VERY MUCH!!!

Figure 3.10 Sensory evaluation sheet used to rate the odor intensity of geosmin spiked in raw fish fillets that were treated with smoke flavor

Name: _____   Date: _____

INSTRUCTIONS
(Cooked: 539, 448, 723)

Sniff each of the three fish samples from <u>LEFT to RIGHT</u> by allowing at least **1 min** to lapse between each sample. After sniffing each sample, answer the following two questions.

1. How do you feel about the MIB odor?   2. How do you like the overall acceptability of this sample?

— Strongest Imaginable

539	448	723	Preference
			Like extremely
			Like very much
			Like moderately
			Like slightly
			Neither like nor dislike
			Dislike slightly
			Dislike moderately
			Dislike very much
			Dislike extremely

— Very Strong

Comments:

— Strong

— Moderate

— Weak

— Barely Detectable

THANK YOU VERY MUCH!!!

Figure 3.11 Sensory evaluation sheet used to rate the odor intensity of MIB spiked in cooked fish fillets that were treated with smoke flavor

Name: _____   Date: _____

INSTRUCTIONS
(Cooked: 539, 448, 723)
Chew each of the three fish samples from <u>LEFT to RIGHT</u> to allow volatiles of the fish tissue to reach your olfactory nerve/receptors. **DO NOT SWALLOW IT**. Split it out, rinse your mouth with water, and allow at least **1 min** to lapse between each sample. After chewing each sample, answer the following two questions.

1. How do you feel about the GEOSMIN odor?   2. How do you like the overall acceptability of this sample?

—— Strongest Imaginable

539	448	723	Preference
			Like extremely
			Like very much
			Like moderately
			Like slightly
			Neither like nor dislike
			Dislike slightly
			Dislike moderately
			Dislike very much
			Dislike extremely

Comments:

—— Very Strong

—— Strong

—— Moderate

—— Weak
—— Barely Detectable

THANK YOU VERY MUCH!!!

Figure 3.12 Sensory evaluation sheet used to rate the odor intensity of geosmin spiked in cooked fish fillets that were treated with smoke flavor

Name: _____   Date: _____

INSTRUCTIONS
(Cooked: 539, 448, 723)

Chew each of the three fish samples from <u>LEFT to RIGHT</u> to allow volatiles of the fish tissue to reach your olfactory nerve/receptors. **DO NOT SWALLOW IT**. Split it out, rinse your mouth with water, and allow at least **1 min** to lapse between each sample. After chewing each sample, answer the following two questions.

1. How do you feel about the MIB odor?   2. How do you like the overall acceptability of this sample?

539	448	723	Preference
			Like extremely
			Like very much
			Like moderately
			Like slightly
			Neither like nor dislike
			Dislike slightly
			Dislike moderately
			Dislike very much
			Dislike extremely

- Strongest Imaginable

- Very Strong

- Strong

- Moderate

- Weak
- Barely Detectable

Comments:

THANK YOU VERY MUCH!!!

Figure 3.13 Sensory evaluation sheet used to rate the odor intensity of MIB spiked in cooked fish fillets that were treated with smoke flavor

CHAPTER IV

RESULTS AND DISCUSSION

EXPERIMENT I: Threshold determination of panelists

Selection and training of panelists

Threshold values vary generally from person to person and from group to group. However, thresholds can be used as a means of selecting or testing panelists (Meilgaard and others 1991). In this study, the average individual odor best estimated threshold (BET) of geosmin or MIB spiked in water was calculated (Table 4.1) and used as a tool to select panelists (Table 4.2). Three panelists had much higher individual odor BET of geosmin and MIB (Table 4.1). Thus, only four panelists were selected for further training.

For the selected panelists, individual odor BET values ranged from 0.4 ppb to 9 ppb for geosmin spiked in water, and 0.04 ppb to 47 ppb for MIB spiked in water (Table 4.2). The group BET for odor detection of geosmin or MIB spiked in water was 0.4 ppb and 0.1 ppb, respectively (Table 4.2). This indicates the panelists were more sensitive to MIB than geosmin. However, these threshold values are higher than previous literature records (Lovell 1983; Sano 1988; Grimm and others 1996; Watson and others 2000). This is probably attributed to differences in training and expertise of the panelists.

Table 4.1 Individual odor best estimated threshold (BET) of geosmin and MIB spiked in
water

Panelist	Geosmin (ppb)	MIB (ppb)
1	9	0.04
2	0.4	0.04
3	0.5	0.4
4	3	47
5	93	102
6	58	140
7	102	140

Table 4.2 Selected individual and group odor best estimated threshold (BET) of
geosmin and MIB spiked in water

Panelist	Geosmin (ppb)	MIB (ppb)
1	9	0.04
2	0.4	0.04
3	0.5	0.4
4	3	47
Group	0.4	0.1

Figure 4.1 shows the scatter plot of the log of perceived intensity (y axis) by the panel versus the log of concentration (ppb) of geosmin or MIB spiked in fish fillets (x axis) using the Labeled Magnitude Scale (LMS) procedure (Green and others 1996). The regression line has the slope a = 0.4122 and intercept b = 0.7281 for geosmin and slope a = 0.4072 and intercept b = 0.7039 for MIB. Therefore, it can be concluded that there is a strong association between the perceived odor intensity of the panel and the concentration of geosmin (R^2=0.9519) or MIB (R^2=0.9708).

The above results indicate that the performance of the panel was acceptable for further experiments. Lovell (1983) mentioned that sensory evaluation by humans was the only method for screening for flavor quality of fish in order to avoid marketing off-flavor fish. Chemical analysis can also be applied in identifying and quantifying geosmin or MIB compounds in fish tissue, but geosmin and MIB have a significant characteristic of relatively low odor thresholds that may not be easily detected by gas chromatography (Maga 1987). In addition, some extraction methods such as vacuum distillation (Lovell and Sackey 1973) and microwave-cold trap collection (Martin and others 1987) are time-consuming and can not be routinely used. Though other advanced extraction methods are simpler and faster such as solid phase micro-extraction (SPME), these methods require careful control of sampling conditions for efficient recovery and quantitative analysis of compounds. Finally, if the treatment masks the off-flavor compounds, the chemical analysis will not show any difference between treatment and control samples (Kaewplang 2005). Therefore, sensory evaluation is more applicable at catfish plants and is the method currently used.
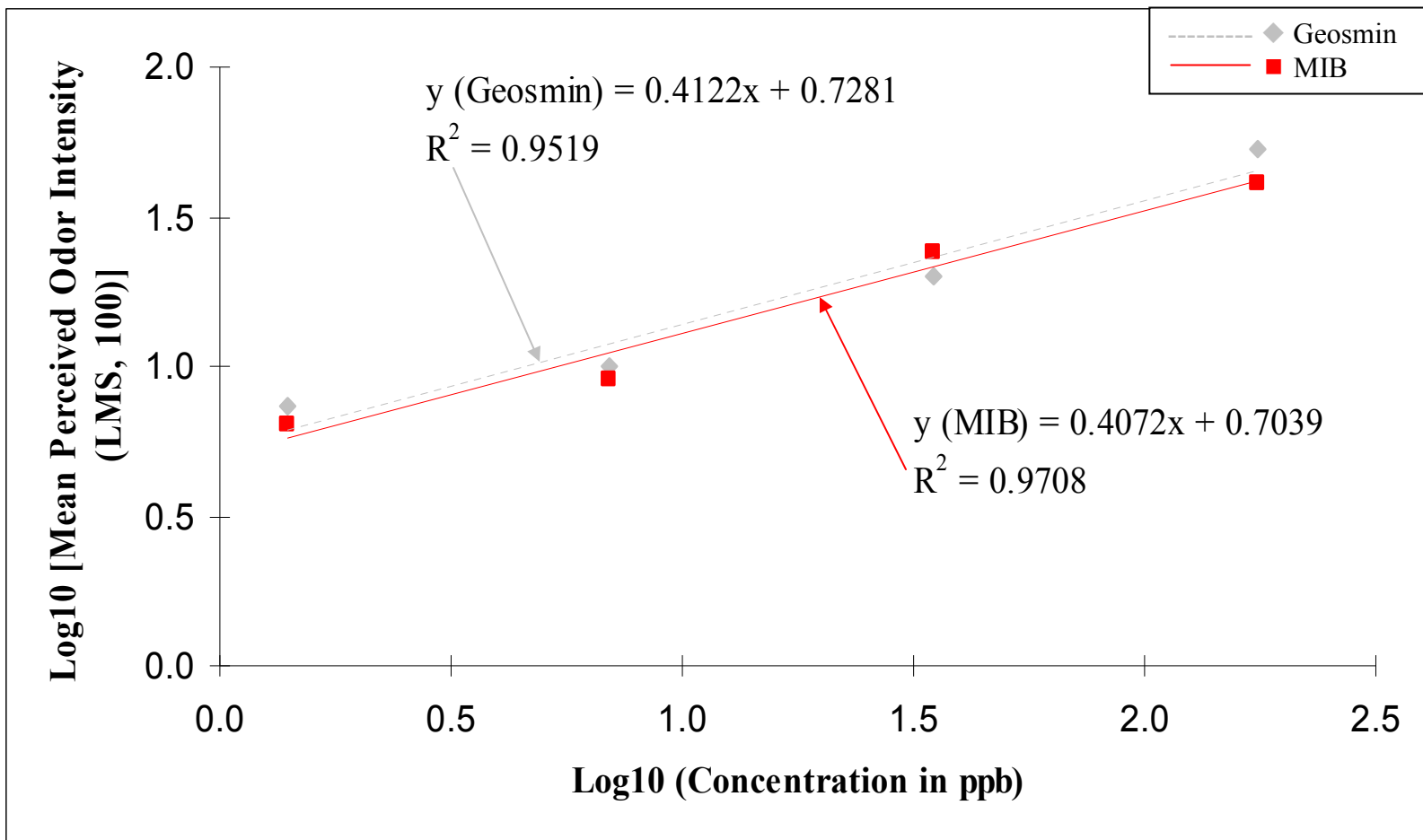
Figure 4.1   Log-log regression between perceived odor intensity and chemical concentration for determination of the performance of sensory panelists for geosmin and 2-methylisoborneol (MIB) spiked in catfish fillets

<u>Fillet spiking procedure</u>

The mean perceived odor intensity rating of two spiking methods by sensory evaluation using the LMS is shown in Figure 4.2 for geosmin and Figure 4.3 for MIB. For the perceived odor intensity, there were no significant differences between both methods ($p>0.05$) in odor intensity for either geosmin or MIB of raw fish samples. For odor intensity of cooked fish samples, there were no significant differences ($p>0.05$) in odor intensity of geosmin but there were significant differences ($p<0.05$) in odor intensity of MIB between methods. For the blending method, there were no significant differences ($p>0.05$) in perceived odor intensity for geosmin or MIB between raw and cooked fish samples. For the injection method, there were significant differences ($p<0.05$) in perceived odor intensity of geosmin or MIB between raw and cooked fish samples. The results indicate that both methods were similar, but the injection method was selected for further experiments because it is more applicable in fish products. McEvoy (2003) reported that injection distributes the functional ingredients evenly throughout the products. McGilberry and others (1989) suggested that "marinated" catfish products such as lemon-butter, hot and spicy, or smoked fillets, can be prepared by injection.

<u>Threshold determination</u>

Table 4.3 shows the individual and group BET for recognition of odor of geosmin and MIB spiked in fish fillets. Individual BET odor detection values ranged from 39 ppb to 75 ppb for geosmin and 4.5 ppb to 7.5 ppb for MIB. The group BET odor detection values for geosmin and MIB were 43 ppb and 6 ppb, respectively. This agrees with the results found by Persson (1980), suggesting that the threshold odor concentration of
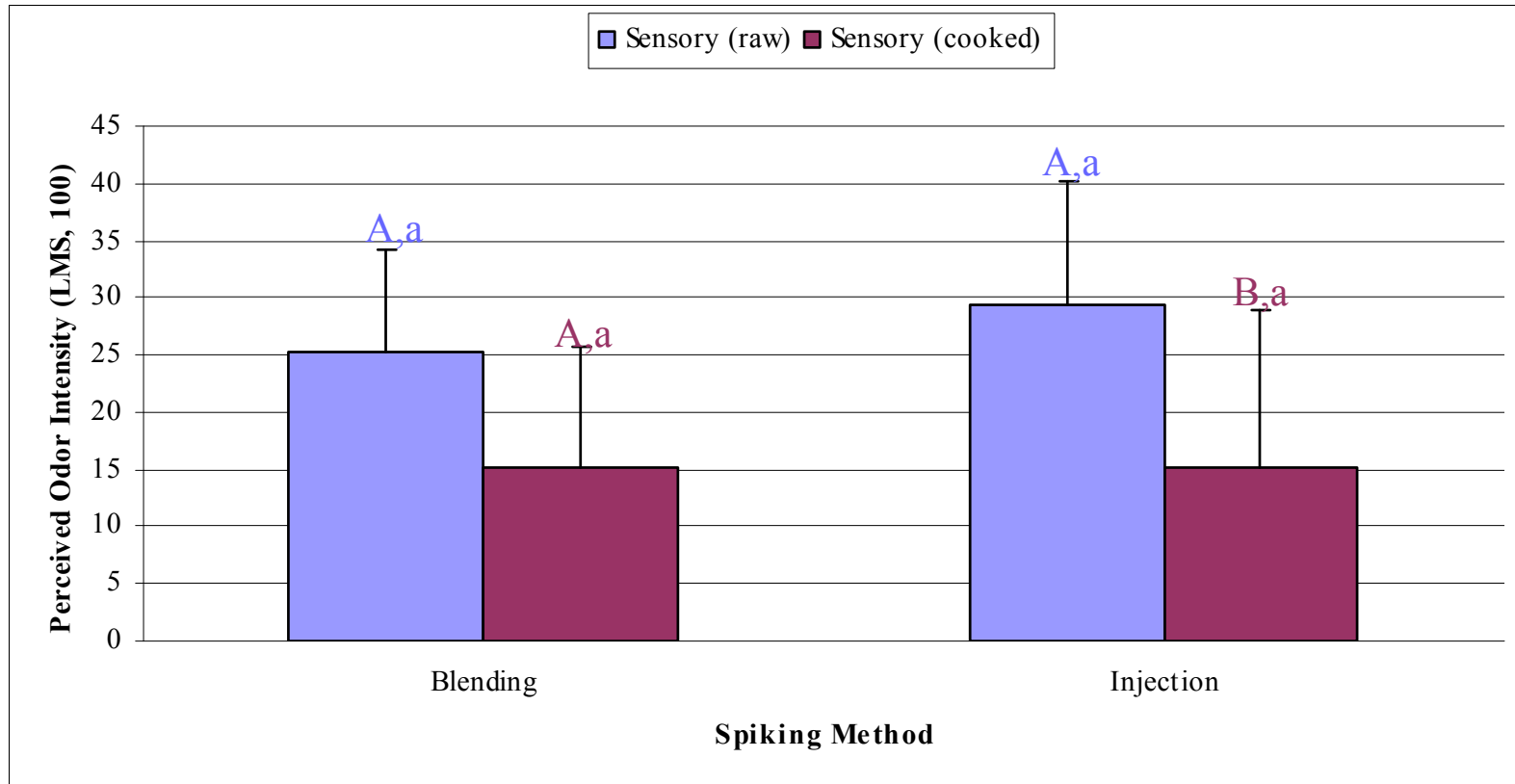
Figure 4.2 Mean perceived geosmin odor intensity of catfish spiked through either blending or injection of 200 ppb by sensory evaluation utilizing LMS scale.

AB – Means between spiking methods differ ($p<0.05$).
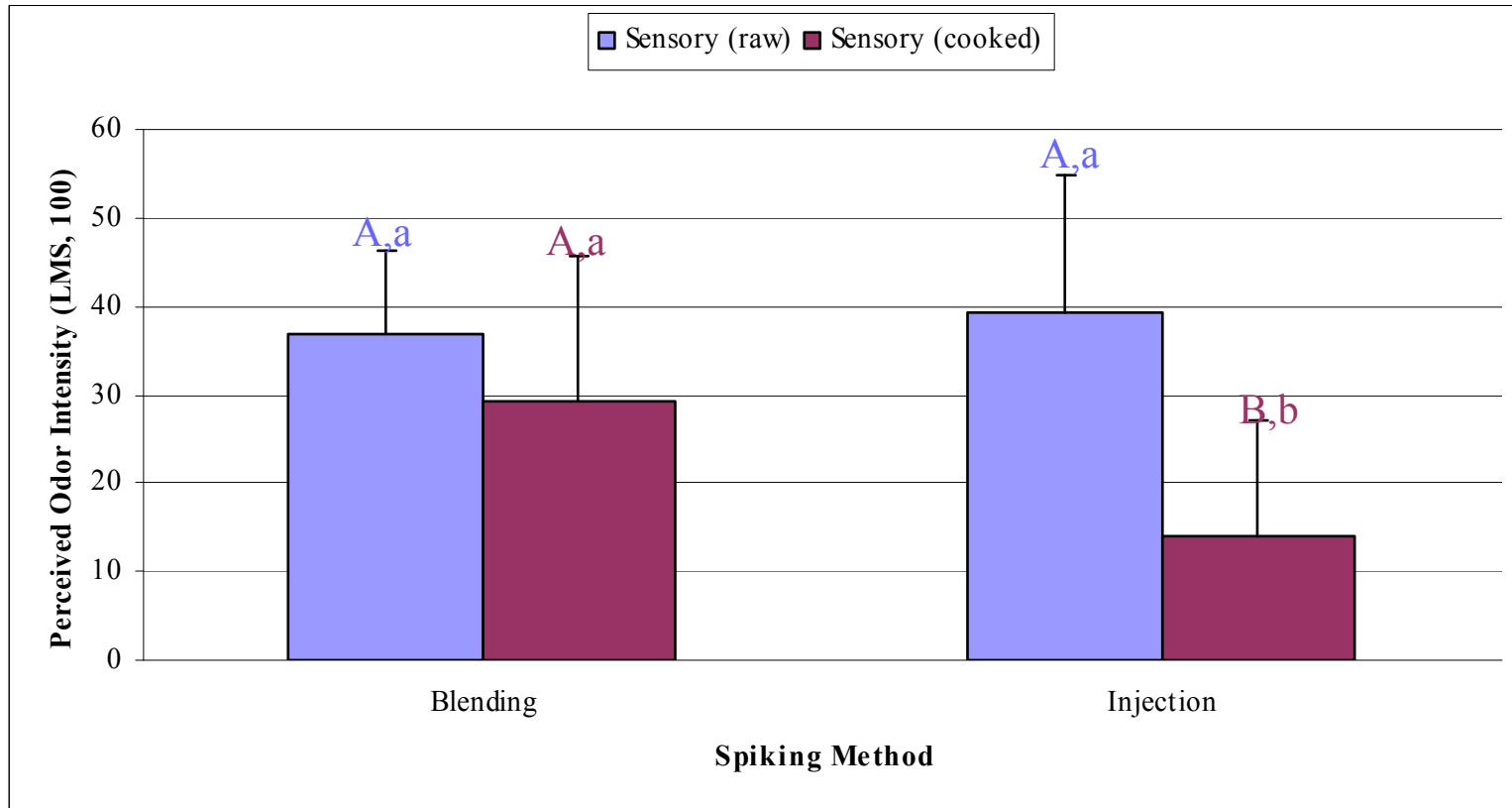ab – Means within spiking methods differ ($p<0.05$).

Figure 4.3 Mean perceived MIB odor intensity of catfish spiked through either blending or injection of 20 ppb by sensory evaluation utilizing LMS scale.

AB – Means between spiking methods differ ($p<0.05$).
ab – Means within spiking methods differ ($p<0.05$).

Table 4.3   Individual and group odor best estimated threshold (BET) of geosmin and
MIB spiked in fish fillets

Panelist	Geosmin (ppb)	MIB (ppb)
1	60	4.5
2	39	6
3	75	7.5
4	53	7.5
Group BET	**43**	**6**

geosmin is higher than that of MIB in fish. These results indicate that the BET odor detection value for geosmin was about 7-fold higher than that for MIB. This means that the panel is more sensitive to MIB than geosmin. However, the results in this study show that the panelists may be less sensitive to lower concentrations of geosmin or MIB than certain flavor checkers (in the industry), who are selected for their acute sensitivity to geosmin or MIB.

Previous studies reported that these two compounds can be detected by humans at concentration as low as 0.6 ppb to 6 ppb for geosmin and 0.08 ppb to 0.6 ppb for MIB, depending on the species of fish (Yurkowski and Tabacheck 1974; Persson 1980), but the rejection levels as suggested by USDA are 8 ppb for geosmin and 0.8 ppb for MIB in channel catfish (Conte and others 1996).

EXPERIMENT II: Effect of acetic acid and/or lime flavor on off-flavors

For off-flavor spiked samples treated with 0.5% acetic acid, there were differences ($p<0.05$) in odor intensity for both geosmin (Fig 4.4) and MIB (Fig 4.5) between the treated samples and off-flavor controls for raw fish products. This was probably the result of the vinegar-like smell, which was able to mask geosmin and MIB odor in raw fish products. For cooked fish products, the treated samples showed no difference ($p>0.05$) in odor intensity for both geosmin (Fig 4.4) and MIB (Fig 4.5) over off-flavor controls. Geosmin or MIB was unlikely dehydrated, when treated with acetic acid, to produce nonodiferous products, since acetic acid is a weak acid (Sessler 2004). Dehydration of alcohols by the E1 elimination mechanism is usually favored under strong acidic conditions (Sessler 2004). However, Kaewplang (2005) found that 2% acetic acid

treated catfish fillets reduced MIB odor of raw and cooked fish, but induced a sour odor/flavor. In this study, the fillets treated with 0.5% acetic acid proved to be marginal in reducing the geosmin odor (Fig 4.4) but inefficient in reducing MIB (Fig 4.5) for cooked products. It was observed that the raw samples presented a 'pale' appearance and the panelists detected a vinegar-like smell in the samples. This is probably caused by the erosive activity of acids on the fish surface or protein denaturation by the acids. In addition, the panelists perceived MIB at a higher intensity level than geosmin in both raw and cooked products, and slightly preferred the treated samples.

For off-flavor spiked samples treated with 1:50 liquid lime flavor, the odor intensity was lower ($p<0.05$) for geosmin (Fig 4.4) and MIB (Fig 4.5) in the treated samples than the off-flavor controls in raw fish products. For cooked products, the treated samples decreased ($p<0.05$) odor intensity for geosmin (Fig 4.4), but not ($p>0.05$) MIB (Fig 4.5).

Kaewplang (2005) concluded that Seven-up® and Sprite® were able to reduce/mask off-flavors in catfish by adding a pleasant sweet flavor, and that lime flavor may influence the off-flavors. In this study, the lime flavors appear to effectively decrease odor perception of geosmin but marginally decrease odor perception of MIB in catfish tissue. In addition, the treated samples were observed to have a 'slightly yellow' appearance and the panelists liked the treated samples better. Kaewplang (2005) also found that panelists preferred the "Soda" treated samples, being lightly sweet and slightly lime flavor.

These results may be caused by masking agents present in the lime flavor. The presence of the stimulus (geosmin or MIB) may still be sensed, but it is less perceived by the panelists due to the interactive effects of the masking agents present (Bett and others 2000). One or more components of liquid lime flavor may interfere with the olfactory neuro-chemical recognition of geosmin or MIB, and/or component(s) of liquid lime flavor may render geosmin or MIB less active (Bett and others 2000). Amoore (1986) indicated that to be smelled, a substance must be volatile and able to reach the olfactory nerve and able to reach the olfactory nerve receptors in the uppermost recesses of the nasal cavities. The strength of the odor impression is partly governed by the volatility of the molecules in the samples (Amoore and Buttery 1978). Moreover, Leffingwell (2002) proposed that odorant binding proteins may bind lipophillically to odorants in the aqueous/liquid mucous increasing the concentration, and then facilitate transport through the mucous layer to the receptors in the olfactory membrane. Therefore, it seems that to reconcile the ability of the panelists to detect many discrete odors, one receptor must be able to interact with several discrete odorants. Conversely, an odor molecule must be capable of interacting with multiple receptors.

For the combined treatment, there was a decrease ($p<0.05$) in the odor intensity for geosmin (Fig 4.4) and MIB (Fig 4.5) when compared to the off-flavor controls for raw catfish. For cooked products, the treated samples decreased ($p<0.05$) the odor intensity for geosmin (Fig 4.4), but not ($p>0.05$) MIB (Fig 4.5). This result was similar to that of the lime flavor alone. Therefore, the lime flavor is better than acetic acid for masking off-flavors in fish.
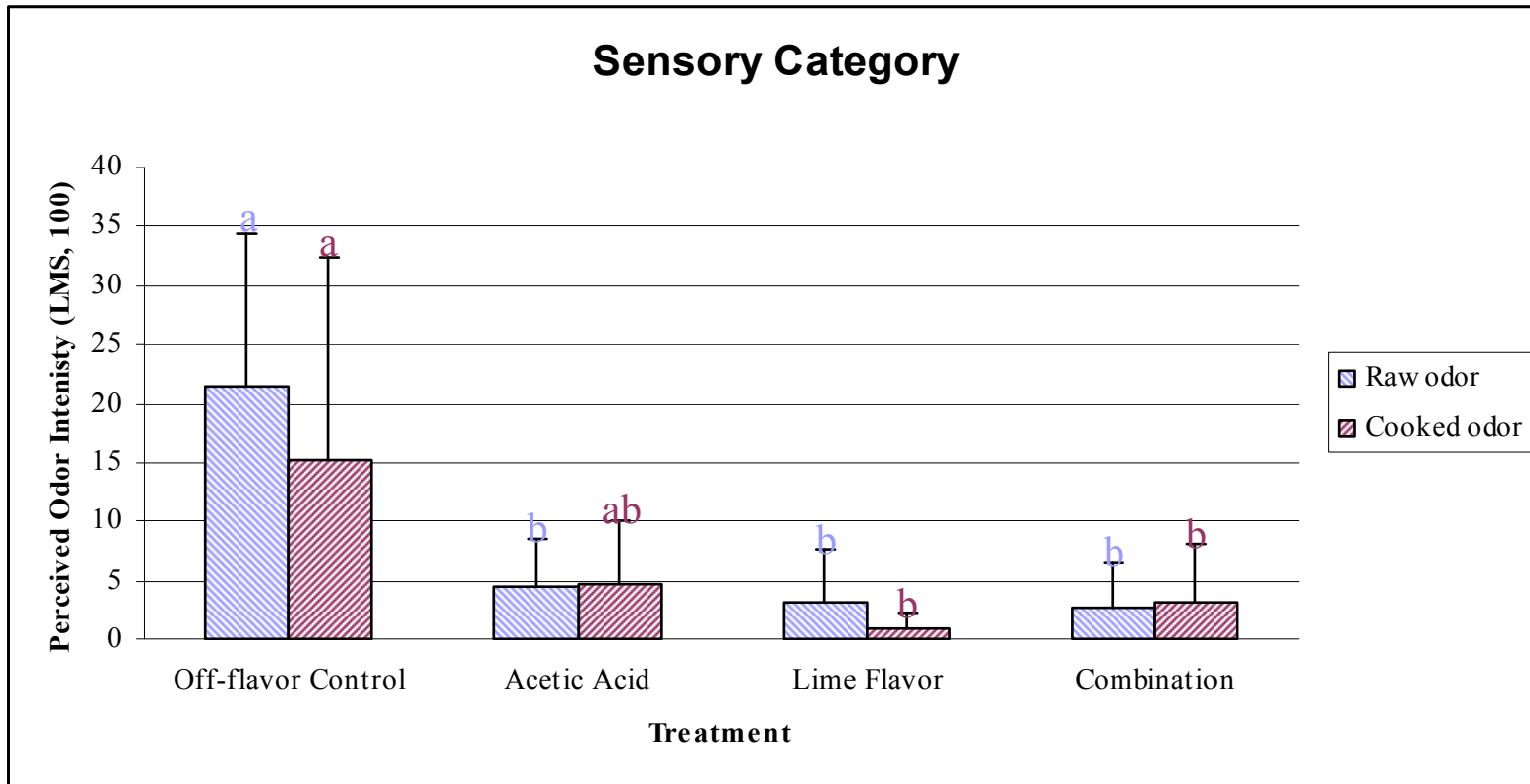
Sensory Category

Figure 4.4 Mean perceived odor intensity of geosmin in raw or cooked catfish fillets treated with lime flavor and/or acetic acid solution as determined by a trained panel (n=4).

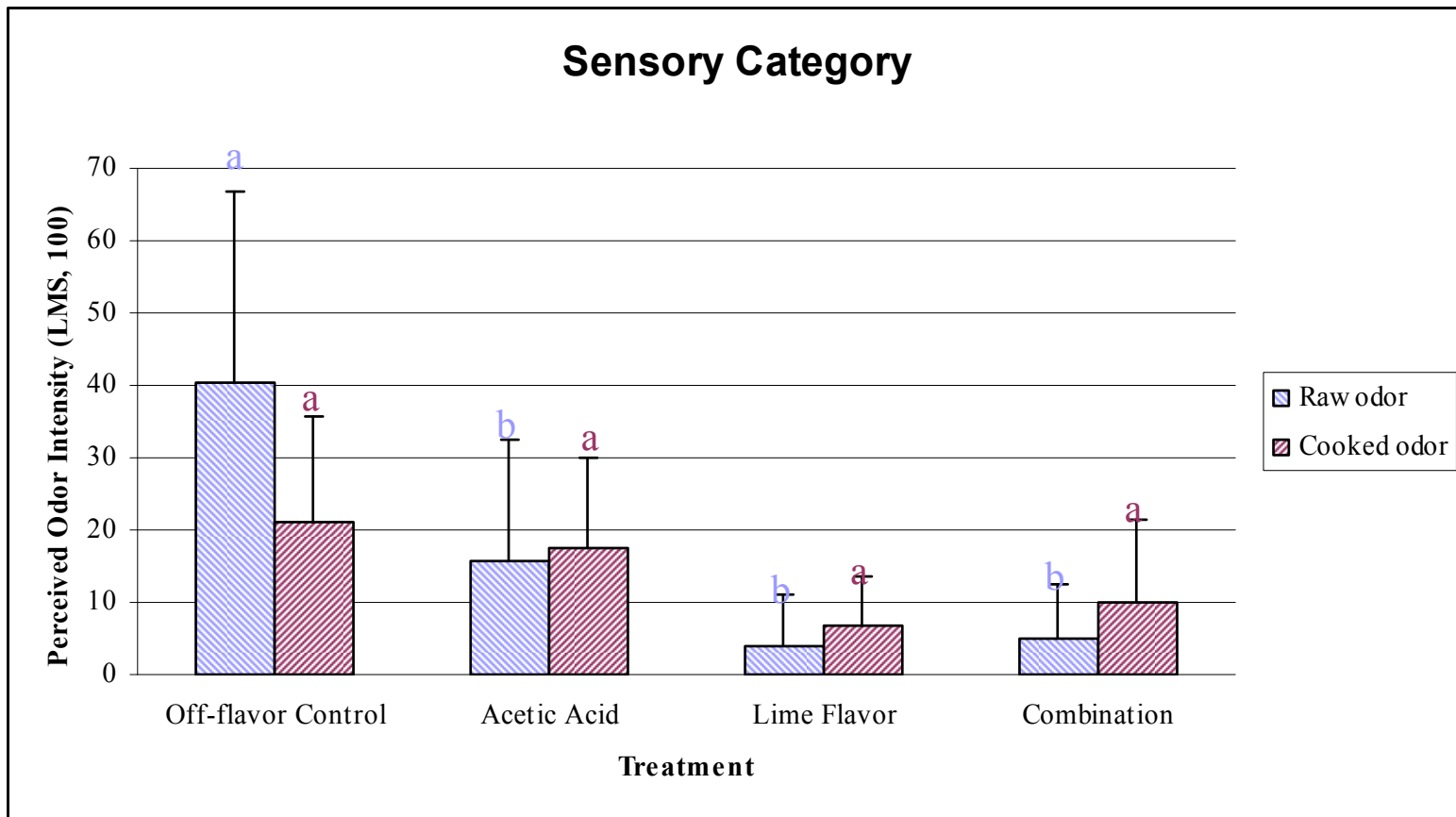ab – Means for each sensory category followed by a different letter differ ($p<0.05$).

Figure 4.5 Mean perceived odor intensity of MIB in raw or cooked catfish fillets treated with lime flavor and/or acetic acid solution as determined by a trained panel (n=4).

ab – Means for each sensory category followed by a different letter differ (p<0.05).

Panelists' interaction plot

The interaction among panelists for perceived odor intensity of geosmin (Fig 4.6) and MIB (Fig 4.7) was plotted for each treatment in raw catfish. The panelist-by-treatment interactions were not significant ($p > 0.05$) for odor intensity of geosmin (Fig 4.6) and MIB (Fig 4.7) in raw catfish products. This indicates that the panelists tend to rate the treatments in similar directions, but the interaction plot shows that there was a slightly different relative degree of intensity. This may be caused by the sensitivity of the panelists and amount of training.

Other interaction plots for perceived odor intensity of geosmin and MIB in raw and cooked catfish products of all treatments are included in the Appendix A (Fig 4.10-4.17). The interaction plots reveal a similar trend in the evaluations of the treatments but a large variation among panelists. Therefore, expertise and training of the panelists should be taken into consideration so that consistency, precision and unbiased panelist performance can be achieved.

Reduction in off-flavor intensity by acetic acid and/or lime flavor

Table 4.4 summarizes percent reduction of perceived odor intensity of geosmin and MIB. For the acetic acid treatment, the reduction of perceived odor intensity for geosmin was 79% for raw fish and 70% for cooked fish. The reduction of perceived odor intensity for MIB was 61% for raw fish and 16% for cooked fish. For the lime flavor treatment, the reduction of perceived odor intensity for geosmin was 86% for raw fish and 94% for cooked fish. The reduction of perceived odor intensity for MIB was 90% for raw fish and 67% for cooked fish. For the combined treatment, the reduction of perceived
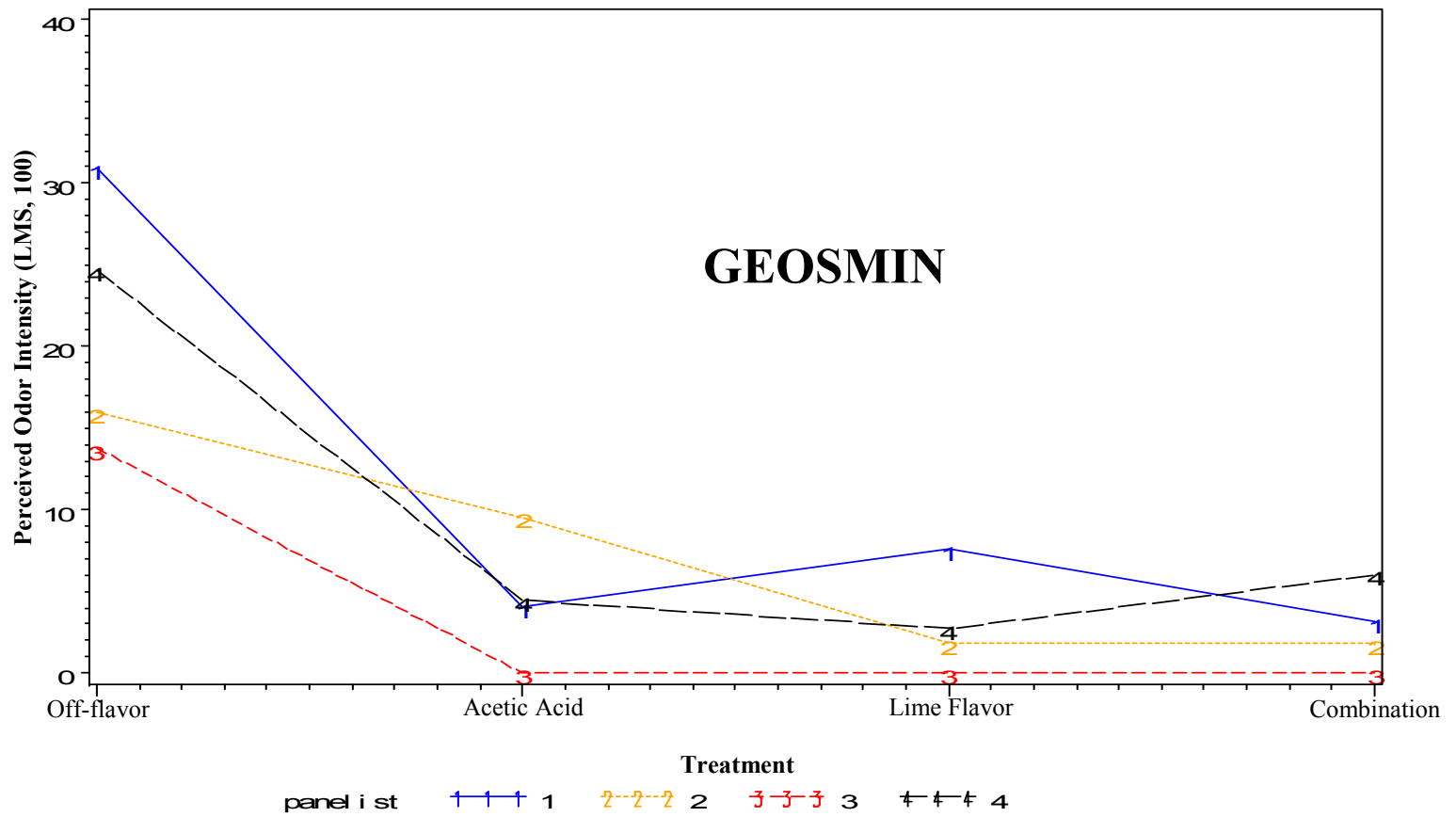
Figure 4.6 Interaction plot between panelist and geosmin odor intensity (smell) of raw catfish products for four trained panelists and treatments (off-flavor control, acetic acid, lime flavor, and combined treatment)
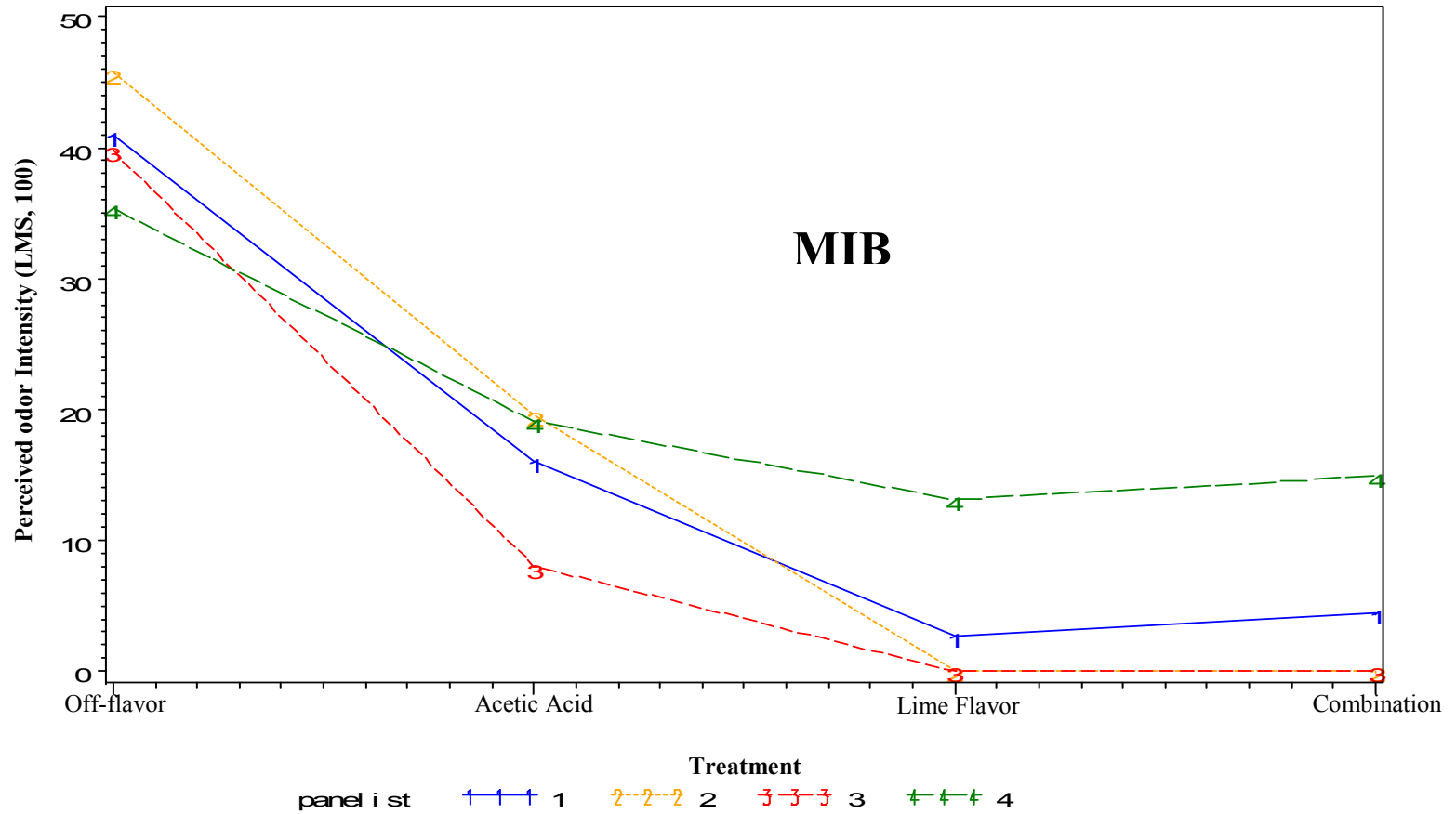
Figure 4.7 Interaction plot between panelist and MIB odor intensity (smell) of raw catfish products for four trained panelists and treatments (off-flavor control, acetic acid, lime flavor, and combined treatment)

Table 4.4   Percent Reduction (range) of off-odor intensity by acetic and/or lime flavor treatment in off-flavor-spiked catfish fillets

Treatment	Geosmin		MIB	
	Raw	**Cooked**	**Raw**	**Cooked**
Acetic acid	79 (17)	70 (11)	61 (25)	16 (3)
Lime flavor	86 (18)	94 (14)	90 (37)	67 (14)
Combination	87 (19)	80 (12)	88 (36)	52 (11)

odor intensity for geosmin was 87% for raw fish and 80% for cooked fish. The reduction of perceived odor intensity for MIB was 88% for raw fish and 52% for cooked fish.

Practically, the acetic acid treatment proved to be quite effective in decreasing odor perception of geosmin (70% reduction) in cooked catfish products, but not effective in decreasing odor perception of MIB (16% reduction), whereas liquid lime flavor was very effective in decreasing odor intensity of geosmin (94% reduction) and MIB (67% reduction) in cooked fish products. The combined treatment was not more effective than the lime flavor treatment alone. Results also indicate that panelists were able to detect higher odor intensities for geosmin and MIB in cooked products than when compared to raw products.

EXPERIMENT III: Effect of liquid smoke flavors on off-flavors

For off-flavor spiked samples treated with 1:25 hardwood smoke flavor, there was a difference ($p<0.05$) in odor intensity for geosmin (Fig. 4.8) and MIB (Fig 4.9) in the treated samples when compared to the off-flavor controls for raw fish products. This may be attributed to chemical components in liquid hardwood smoke flavorings. This hardwood smoke flavor contains 7.0-11.0 % of carbonyls and 3.0-7.0 mg/ml of unknown smoke flavor compounds, but, in general, liquid smoke flavorings consist of syringol, main component of the smoke flavor, cyclopentenolones, which provide a 'burnt-sugar' like note, and phenolics which act as preservatives to help prevent spoilage (Guillen and Ibargoitia 1998; Guillen and others 1995). For cooked fish, the treated samples decreased ($p<0.05$) flavor and odor intensity for geosmin (Fig 4.8) but not ($p>0.05$) MIB (Fig 4.9).

The panelists were able to slightly perceive geosmin odor in cooked fish products, but this was not detectable in the raw treated fish.

For off-flavor spiked samples treated with 1:25 hickory smoke flavor, there was decrease ($p<0.05$) in odor intensity for geosmin (Fig 4.8) and MIB (Fig 4.9) when compared to the off-flavor controls for raw fish products. This hickory smoke flavor contains 30.0-40.0 mg/ml of unknown smoke flavor compounds. For cooked fish, the treated samples decreased ($p<0.05$) the odor and flavor intensity for geosmin (Fig 4.8) but not ($p>0.05$) MIB (Fig 4.9) when compared to off-flavor controls. However, for geosmin, the panelists were able to slightly detect geosmin odor in cooked fish products by smell and taste while geosmin odor was not detectable in raw treated fish. For MIB, the panelists were able to detect MIB in cooked fish products by taste, but not by smell.

Again, this may have been attributed to the interactive effect of the masking agents present in smoke flavorings (Bett and others 2000). Functionality and volatility of compound(s) in smoke flavorings may interfere with odor perception of geosmin and MIB (Kim and others 1974; Radecki and others 1977; Baltes and others 1981; Napolitano and others 1996). To be smelled, a substance must be volatile and able to reach the olfactory nerve and able to reach the olfactory nerve receptors in the uppermost recesses of the nasal cavities Amoore (1986). In addition to this, one receptor must be able to interact with several discrete odorants.

The compounds reported in smoke and smoke flavorings are very numerous and include many functional groups such as acids, alcohols, aldehydes, ketones, esters, furan and pyran derivatives, lactones, phenolic derivatives, hydrocarbons and some

nitrogenated derivatives. In general, phenol derivatives have been considered the primary contributors to smoke aroma, and also have antioxidant and antimicrobial activity (Tóth and Pothast 1984; Maga 1988; Daun 1969; Zaitsev and others 1973; Kim and others 1974; Olsen 1977; Wendorff 1981; Faith and others 1992). Furan and pyran derivatives soften the heavy aromas associated with phenolic compounds (Kim and others 1974; Radecki and others 1977) and carbonyl groups contribute to changes in the texture and color of meats. Aldehydes and ketones influence the development of texture, color, and aroma, and also contribute to antimicrobial activity. Their role in the development of texture and color has been associated with reactions with amino groups from food proteins similar to the Maillard reaction (Kim and others 1974, Baltes and others 1981). In general, the aroma of the compounds of this group has been described as caramel or burnt sugar (Kim and others 1974, Baltes and others 1981). In the same way, lactones, acids, and alcohol derivatives are responsible for sensory properties. Some of these components also contribute to antimicrobial activity (Zaitsev and others 1973; Olsen 1977; Wendorff 1981). Recently, lignans or lignin dimmers and trimmers have been detected in smoke flavorings (Guillen and Ibargoitia 1998; Guillen and Ibargoitia 1999), and compounds of this group have very strong antioxidant abilities (Ayres and others 1990; Lu and Liu 1992; Barclay and others 1997).
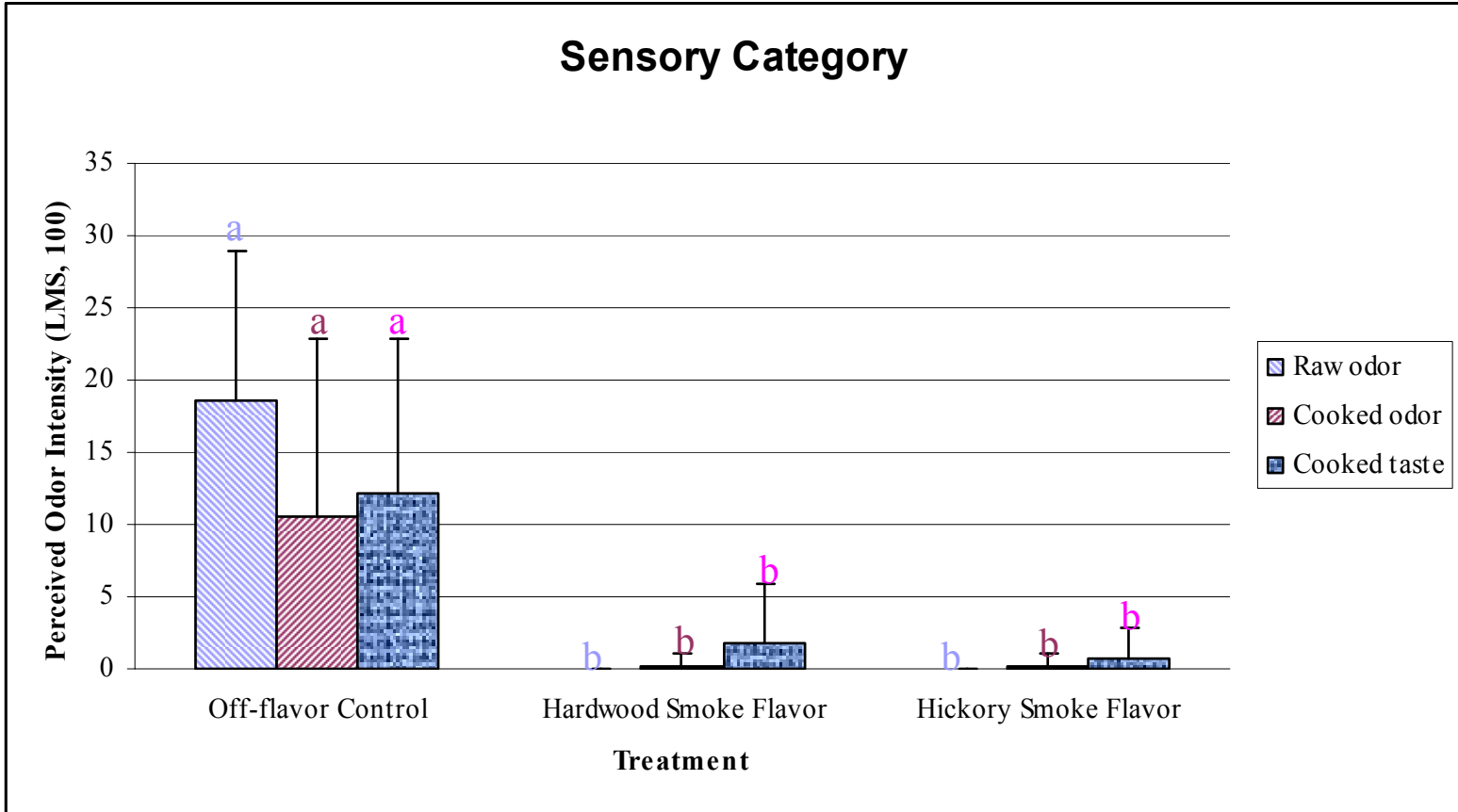
Figure 4.8 Mean perceived odor intensity of geosmin in raw or cooked catfish fillets treated with liquid smoke flavors as determined by a trained panel (n=4).

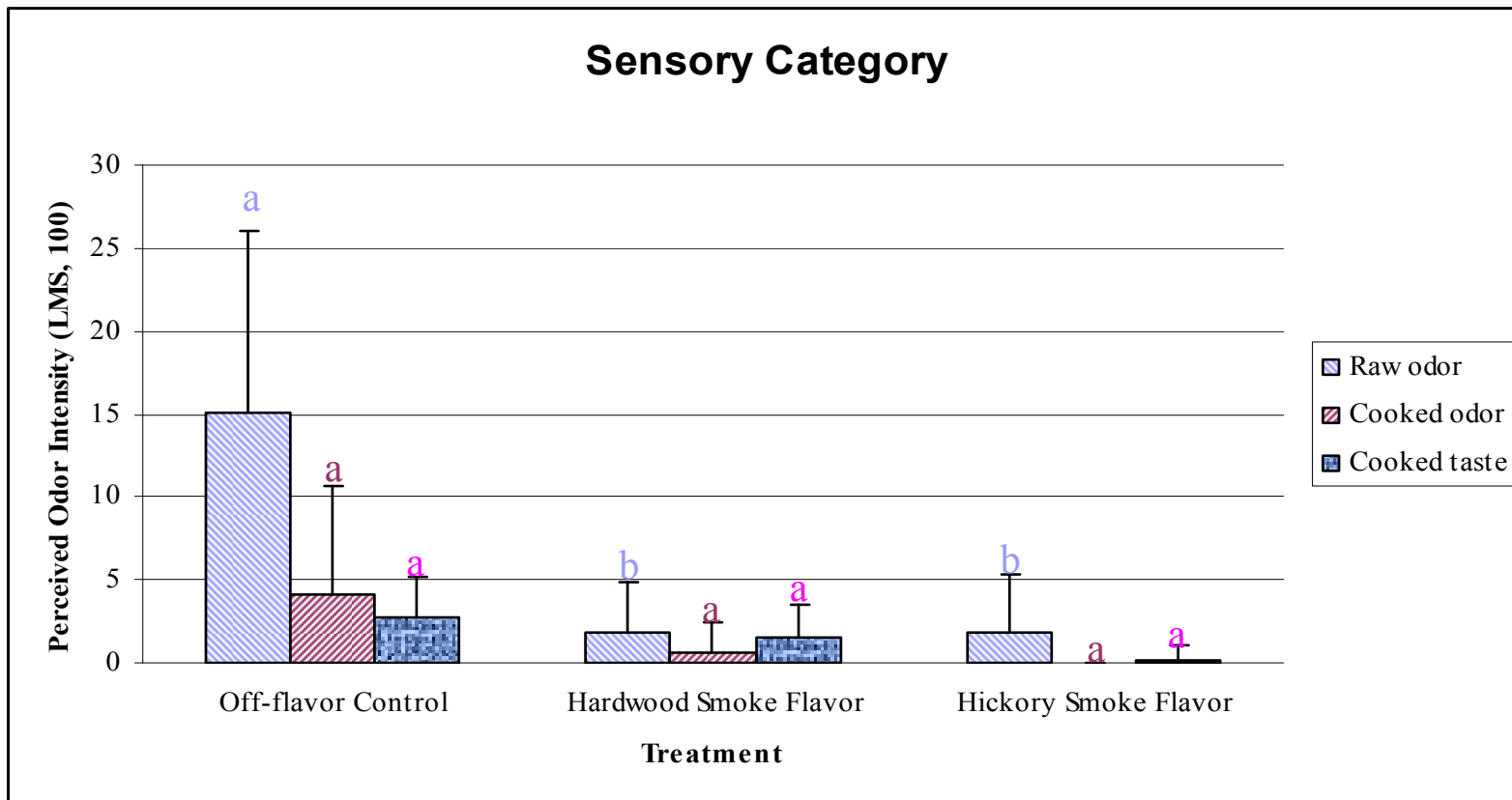ab – Means for each sensory category followed by a different letter differ (p<0.05).

Figure 4.9 Mean perceived odor intensity of MIB in raw or cooked catfish fillets treated with liquid smoke flavors as determined by a trained panel (*n*=4).

ab – Means for each sensory category followed by a different letter differ (*p*<0.05).

Reduction in off-flavor intensity by smoke flavorings

Table 4.5 summarizes percent reduction of perceived odor intensity of geosmin and MIB by odor and taste. For the hardwood smoke flavor treatment, the reduction of perceived odor intensity for geosmin by smell was 100% for raw fish products and 98% for cooked fish products. By taste sensory evaluation, it was 85%. This indicates that hardwood smoke flavor is very effective (>80% reduction) in decreasing odor perception of geosmin in catfish fillets. The reduction of perceived odor intensity for MIB by smell sensory evaluation was 88% for raw fish and 86% for cooked fish. This reduction was 47% when the sample was tasted. These results reveal that hardwood smoke flavor appeared to decrease odor perception of MIB in cooked catfish fillets.

For the hickory smoke flavor treatment, the reduction of perceived odor intensity for geosmin by odor evaluation was 100% for raw fish products and 98% for cooked fish products. By taste sensory evaluation, it was 94% for cooked fish products. This indicates that hardwood smoke flavor is very effective (>90% reduction) in decreasing odor perception of geosmin in raw and cooked catfish products. The reduction of perceived odor intensity for MIB by odor evaluation was 88% for raw fish products and 100% for cooked fish products. This reduction was 92% when the sample was tasted. Therefore, hickory smoke flavor is very effective in masking/degrading not only geosmin but also MIB. Moreover, the results indicate that panelists were able to detect a higher odor intensity of geosmin and MIB in cooked products than in raw products.

Table 4.5   Percent Reduction (range) of off-odor/flavor intensity by liquid smoke treatment in off-flavor-spiked catfish fillets

Treatment	Geosmin			MIB		
	Raw	Smell	Taste	Raw	Smell	Taste
Hardwood smoke flavor	100 (30)	98 (13)	85 (19)	88 (14)	86 (14)	47 (7)
Hickory smoke flavor	100 (30)	98 (13)	94 (20)	88 (14)	100 (14)	92 (8)

CHAPTER VI

SUMMARY AND CONCLUSIONS

Based on the results in this study, the odor BET detection value for geosmin spiked in water appears to be 0.4 ppb, and for MIB spiked in water appears to be 0.1 ppb. These are similar to the numbers reported by others. This confirmed a well selected and trained panel. Based on the results in this study, the odor BET detection value for geosmin spiked in catfish appears to be 43 ppb, and for MIB spiked in catfish appears to be 6 ppb. These results were also compatible with the results found by other researchers.

The fillets treated with 0.5% (v/v) acetic acid proved to be effective (70% reduction) in decreasing odor perception of geosmin but ineffective (16% reduction) in decreasing odor perception of MIB odor in cooked catfish products. In addition, it was observed that the raw samples exhibited a 'pale' appearance and the panelists indicated a vinegar-like smell that was associated with the samples. This caused the panelists to prefer treated samples only slightly. Fillets treated with liquid lime flavor appeared to be very effective (94% reduction) in decreasing odor perception of geosmin but only slightly effective (52% reduction) in decreasing odor perception of MIB in cooked catfish products. In addition, it was observed that the raw samples had a 'slightly yellow' appearance and the panelists like treated samples slightly due to an undesirable chemical-like smell in the samples.

Practically, hardwood and hickory smoke flavors proved to be very effective (>80%) in decreasing odor perception of geosmin in raw and cooked catfish products. However, hardwood smoke flavor proved to be less effective (<50% reduction) in decreasing odor perception of MIB in cooked catfish products, but hickory smoke flavor was very effective (>80%) in decreasing odor perception of MIB in cooked catfish products. It is not easy to know the concrete functionality of each smoke component because synergistic effects can occur. The compounds reported in smoke and in smoke flavorings are very numerous and show different functional groups. They include acids, alcohols, aldehydes, ketones, esters, furan and pyran derivatives, lactones, phenolic derivatives, hydrocarbons and some nitrogenated derivatives. Interactions between the geosmin or MIB compounds with the components of liquid smoke should be investigated further.

In conclusion, it is suggested that lime flavor and/or liquid smoke could be added to a marinade or incorporated in an injection/tumbling solution after catfish fillets are processed. The components of these ingredients that are responsible for off-flavor reduction should be studied further. Main interactive effects of masking agents that render off-flavors less active should also be studied further. However, the effectiveness of masking/degrading off-flavor compounds in channel catfish depends on many factors including initial amount of off-flavor compounds in fish, method of treatment (physical and chemical treatment), treatment time, and method of testing (sensory evaluation and chemical analysis).

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APPENDIX A

ADDITIONAL FIGURES
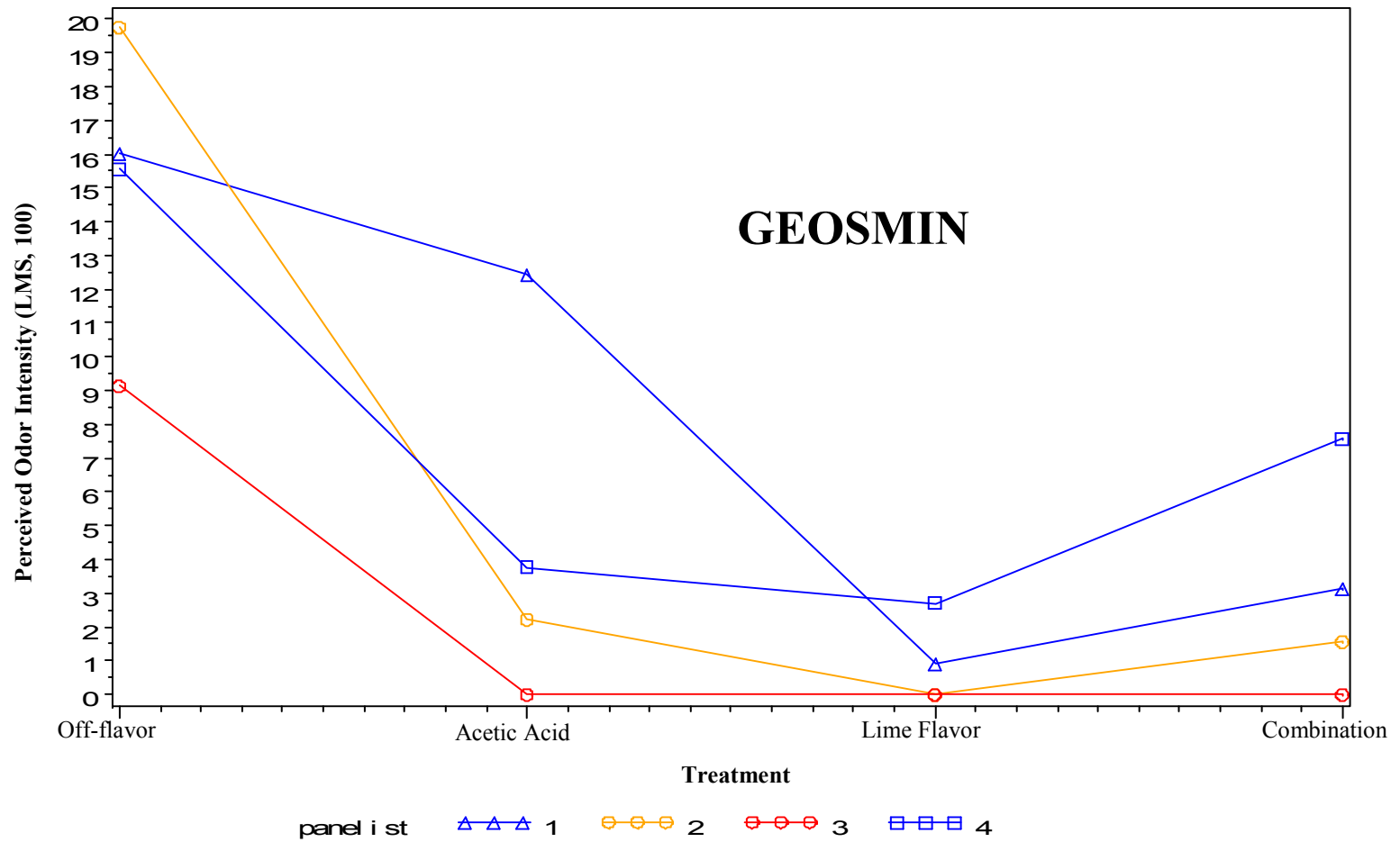
Figure 4.10 Interaction plot between panelist and geosmin odor intensity (smell) of cooked catfish product for four trained panelists and treatments (off-flavor control, acetic acid, lime flavor, and combined treatment)
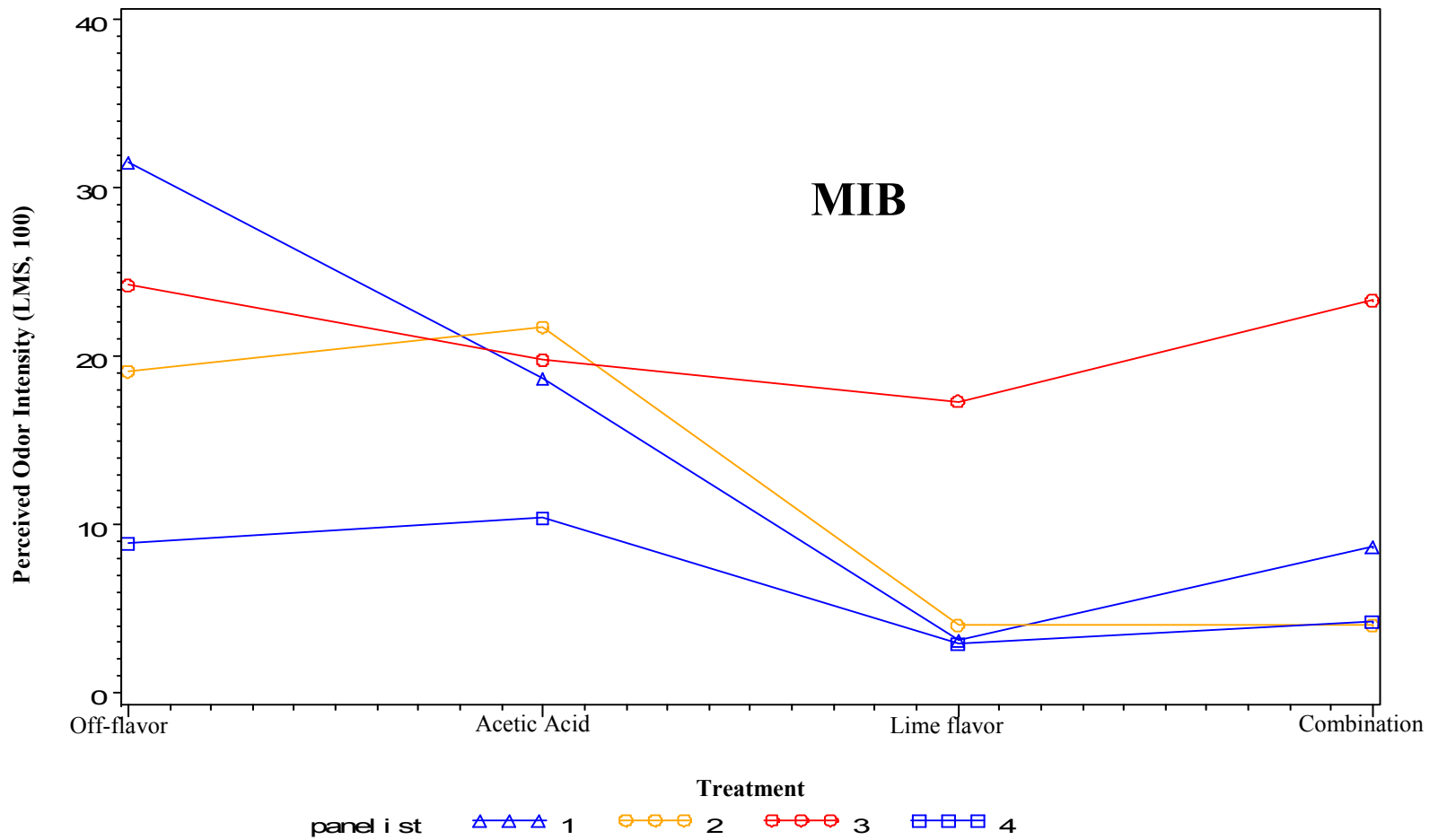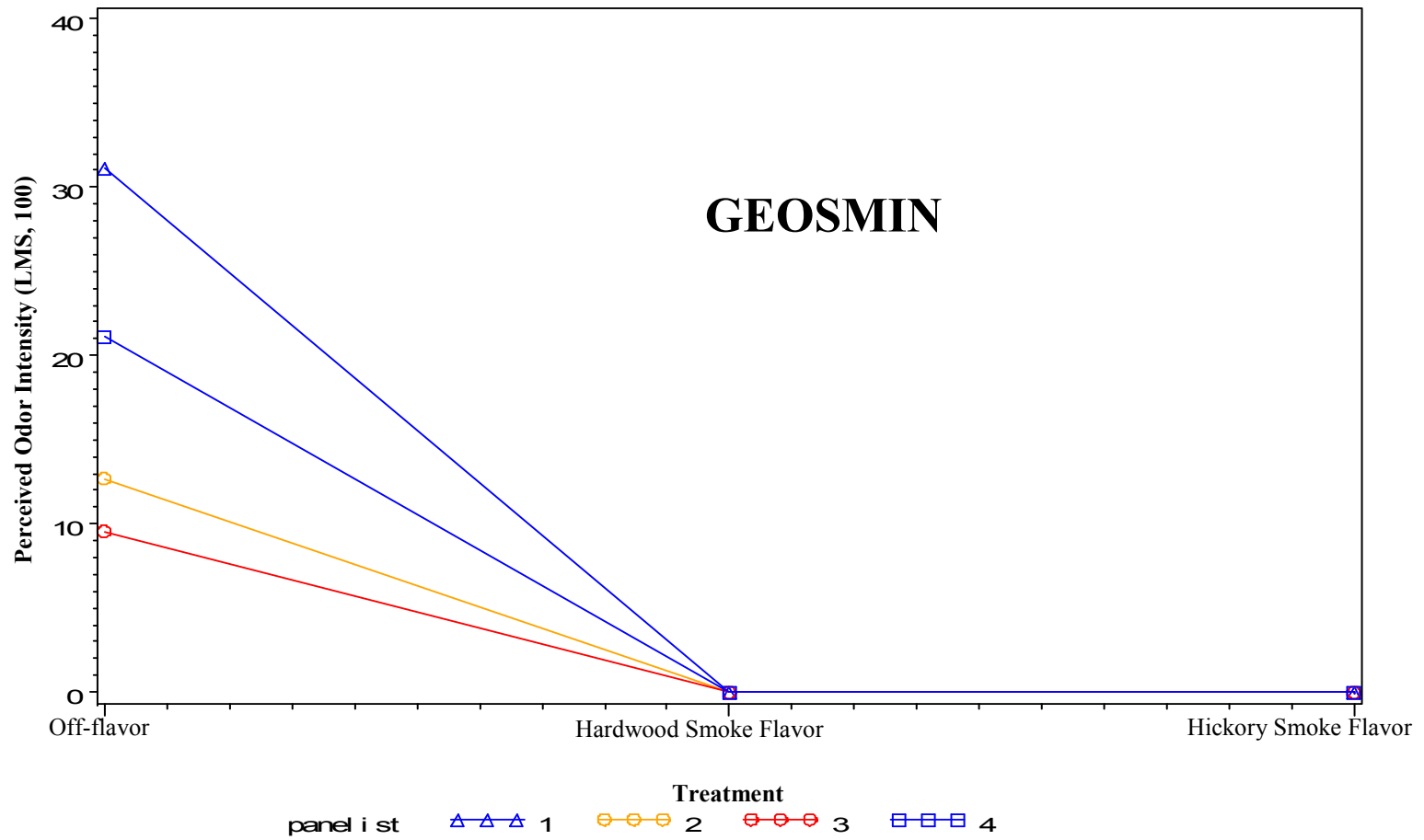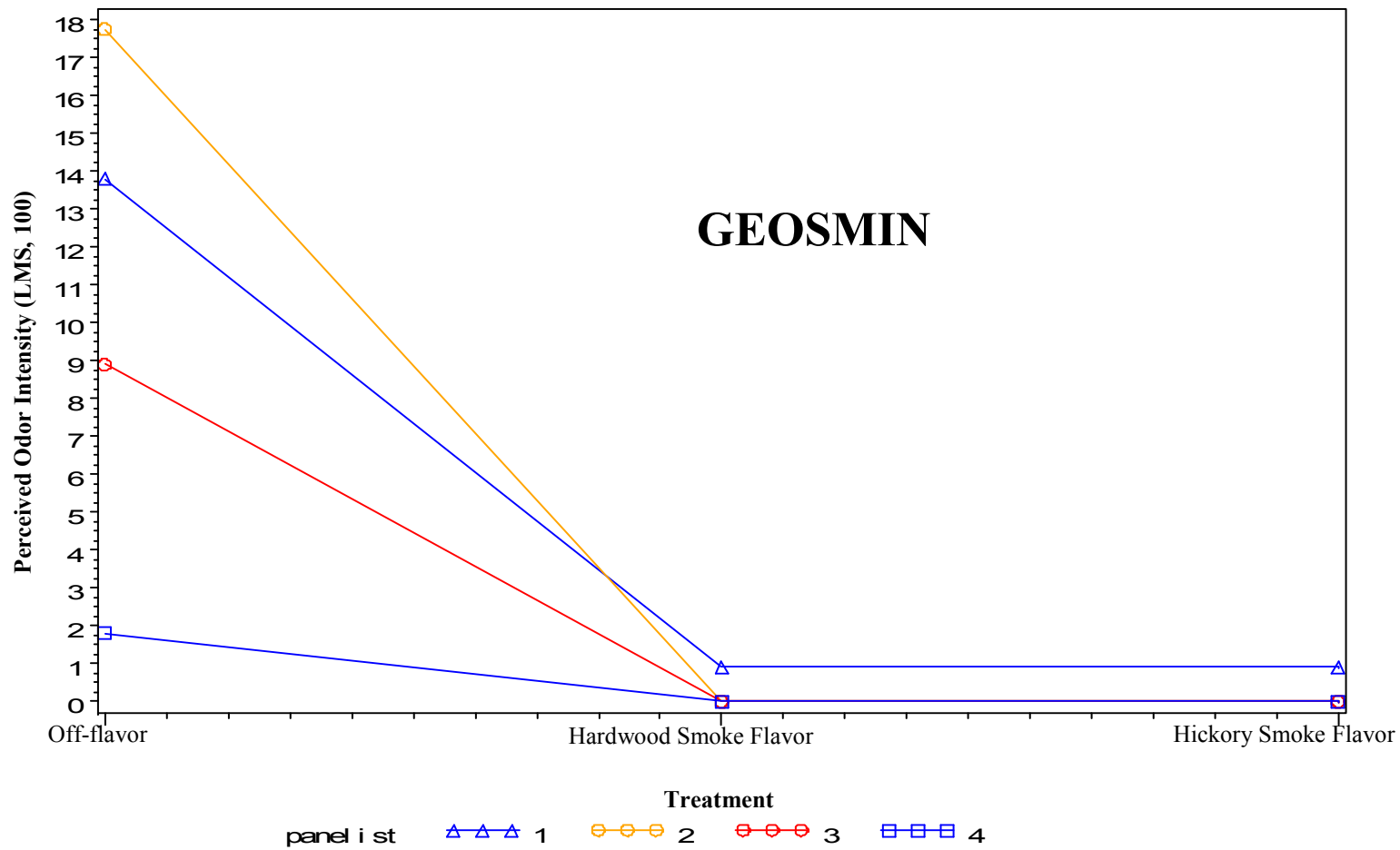
Figure 4.11   Interaction plot between panelist and MIB odor intensity (smell) of cooked catfish products for four trained
panelists and treatments (off-flavor control, acetic acid, lime flavor, and combined treatment)

Figure 4.12   Interaction plot between panelist and geosmin odor intensity (smell) of raw catfish products for four trained panelists and treatments (off-flavor control, hardwood smoke flavor and hickory smoke flavor)

Figure 4.13 Interaction plot between panelist and geosmin odor intensity (smell) of cooked catfish products for four trained panelists and treatments (off-flavor control, hardwood smoke flavor and hickory smoke flavor)
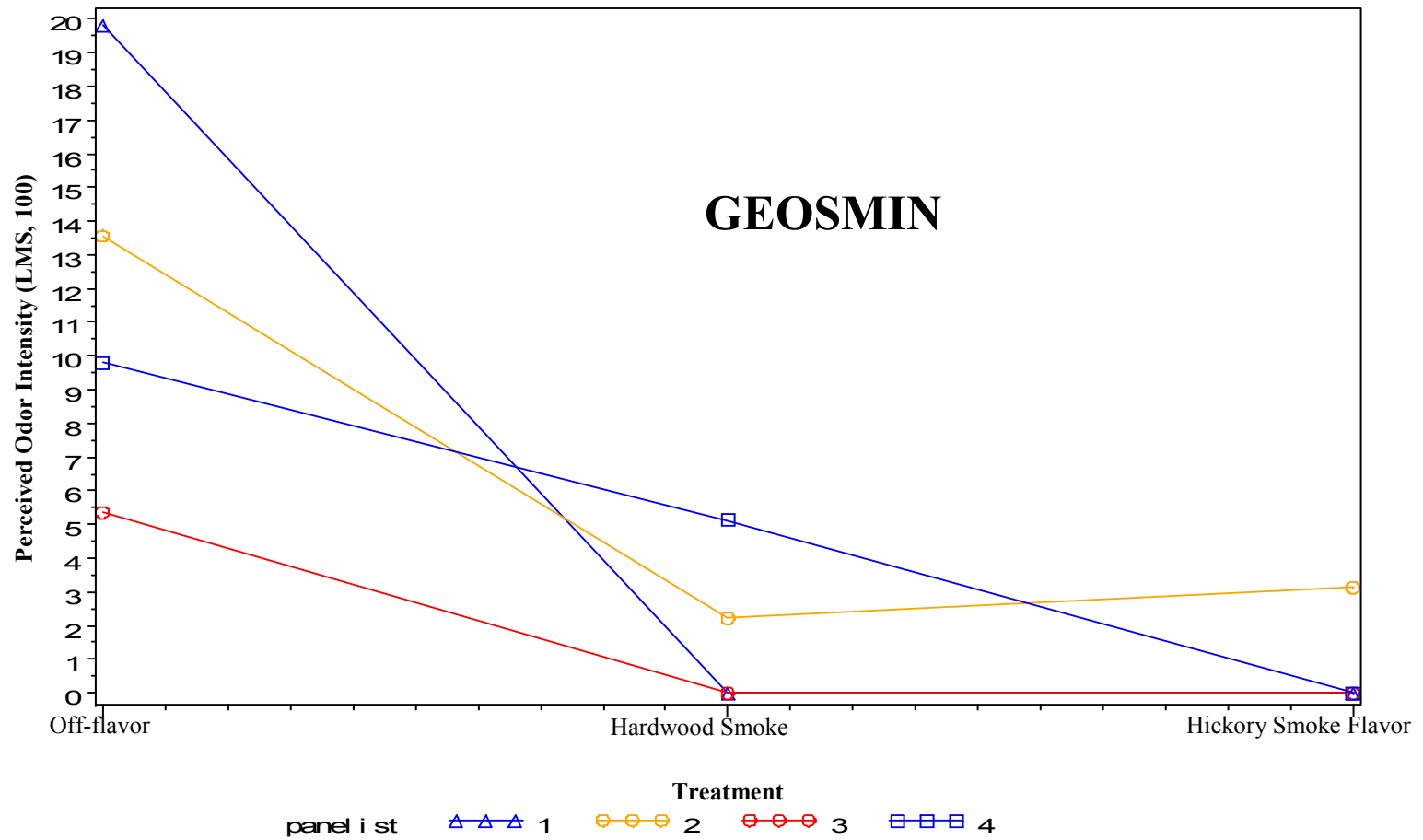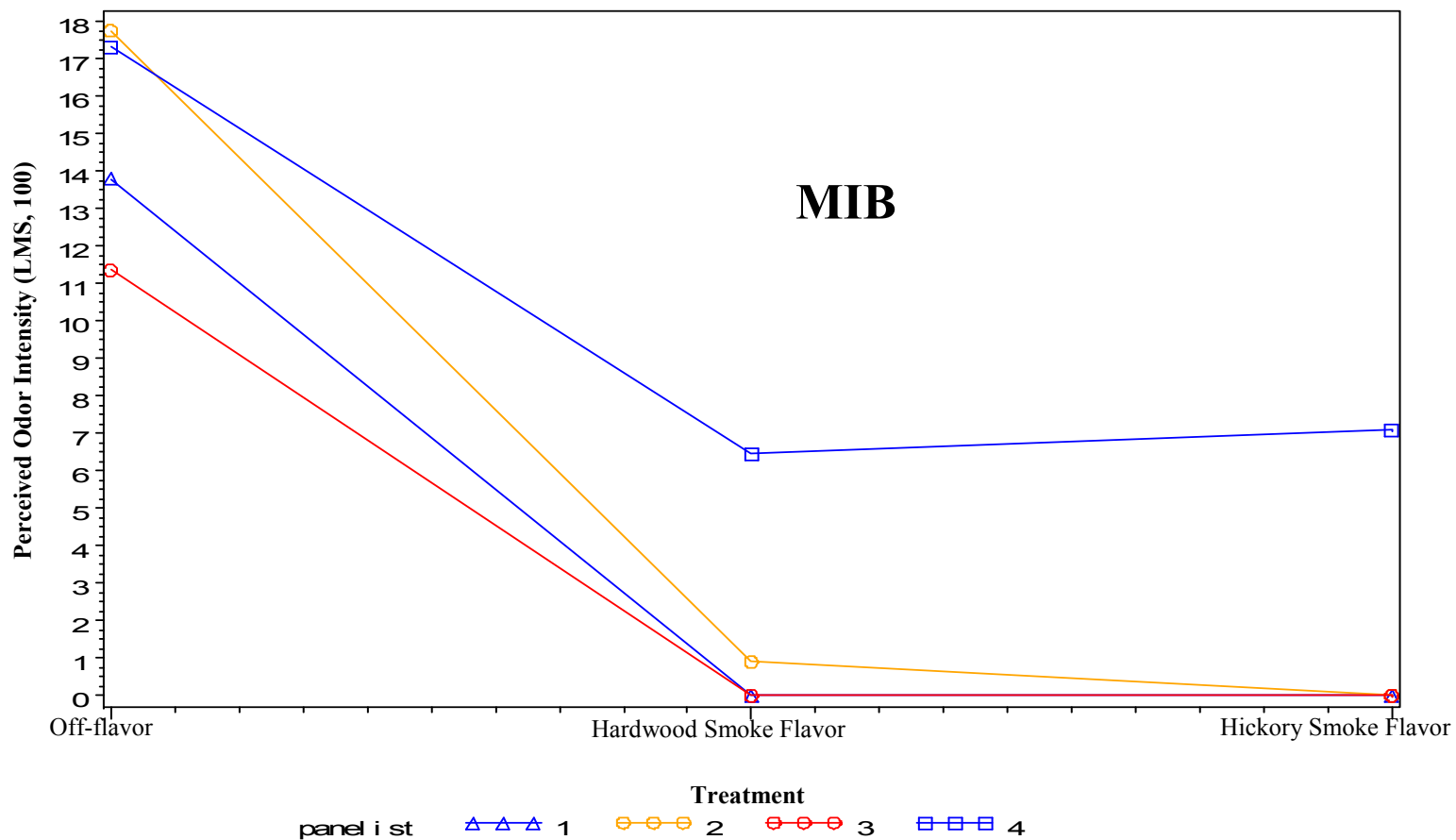
Figure 4.14   Interaction plot between panelist and geosmin odor intensity (taste) of cooked catfish products for four trained panelists and treatments (off-flavor control, hardwood smoke flavor and hickory smoke flavor)

Figure 4.15 Interaction plot between panelist and MIB odor intensity (smell) of raw catfish products for four trained panelists and treatments (off-flavor control, hardwood smoke flavor and hickory smoke flavor)
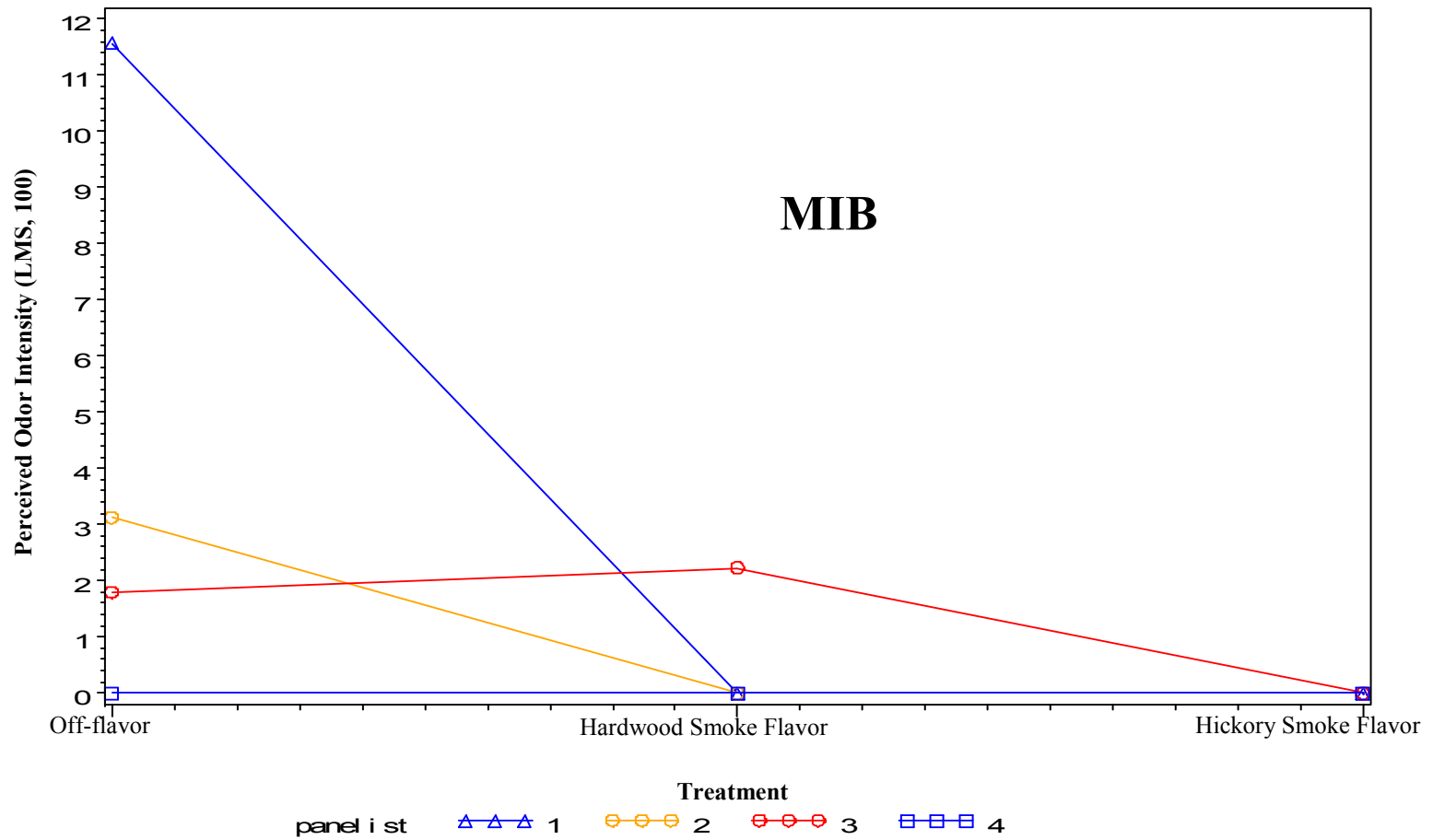
Figure 4.16   Interaction plot between panelist and MIB odor intensity (smell) of cooked catfish products for four trained panelists and treatments (off-flavor control, hardwood smoke flavor and hickory smoke flavor)
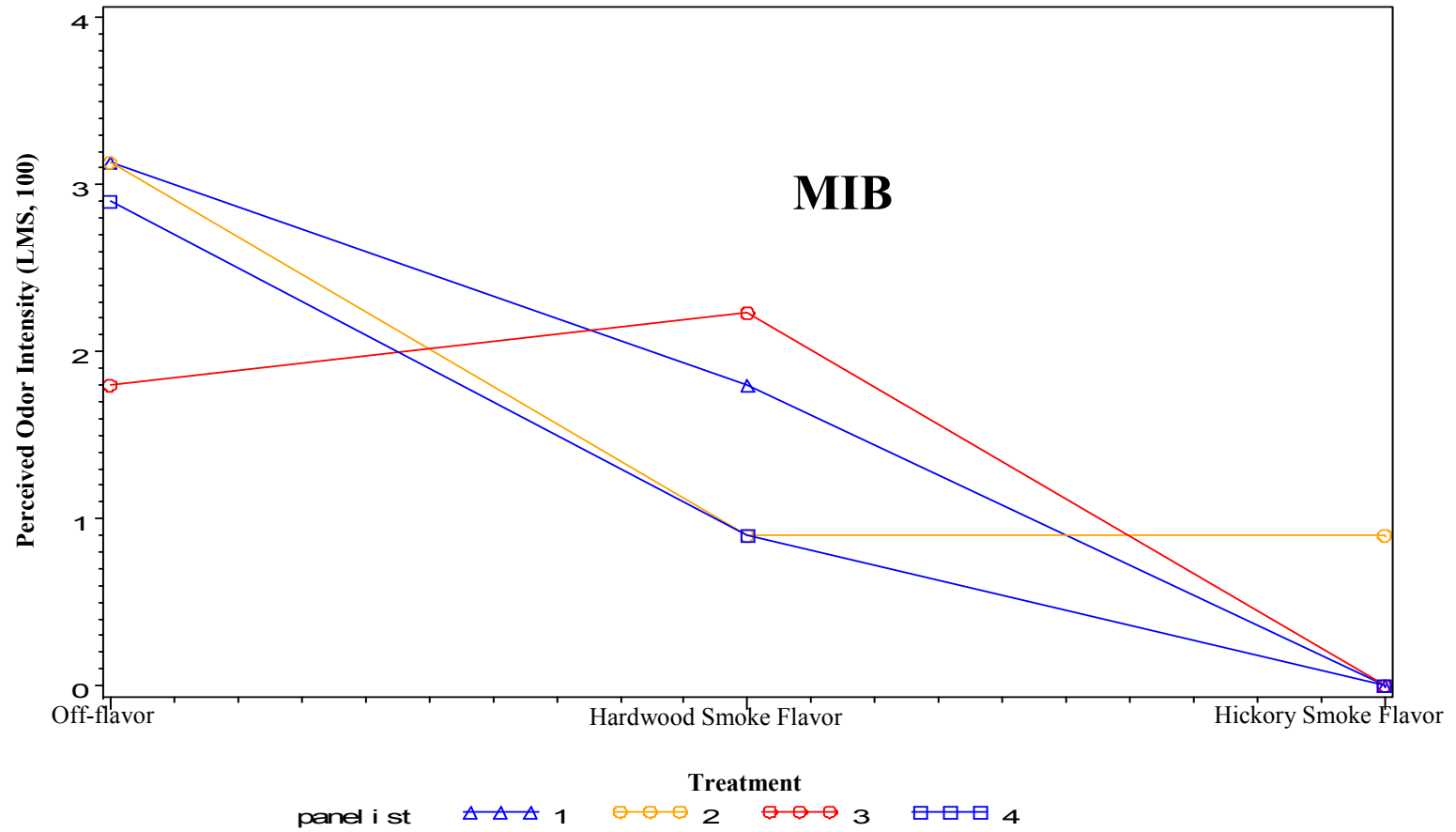
Figure 4.17   Interaction plot between panelist and MIB odor intensity (taste) of cooked catfish products for four trained
panelists and treatments (off-flavor control, hardwood smoke flavor and hickory smoke flavor