PRODUCING A CONSUMER ACCEPTABLE PRODUCT FROM OFF-FLAVOR CATFISH

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FORMAT OF THESIS

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CHAPTER I

INTRODUCTION

Channel catfish producers lose up to $60 million annually from off-flavors (Schrader and others, 2003). The off-flavor renders the flesh unacceptable for human consumption. Off-flavor abatement is a priority because aquaculture is one of the fastest growing sectors in agriculture. More than half of this industry is represented by channel catfish (*Ictalurus punctatus*). The catfish industry is the largest segment of freshwater aquaculture production in the United States with more than 295 million Kg processed and sold annually. Further growth of channel catfish aquaculture requires a high quality, consumer acceptable product without a muddy/earthy flavor and/or odor. Such off-flavors and odors occur in approximately 80% of harvestable fish each year, with maximum incidence occurring during later summer months (July-September) when water temperature is at its highest (van der Ploeg and others, 2001). Catfish producers are forced to delay harvest until catfish are deemed on-flavor, which decreases profitability and limits supply (Martin and others, 1988). Flavor and odor issues in aquatic environments are caused by the growth of microbial metabolites with odors described as "musty", "earthy", and "muddy". Two off-flavor compounds, geosmin [trans-1,10,-dimethly-*trans*-(9)-decalol] and 2-methlyisoborneol [*exo*-1,2,7,7-tetramethyl-(2.2.1)heptan-2-ol] also known as MIB are secondary metabolites of actinomycetes and cyanobacteria (Dionigi, 1993). The "musty" off-flavor/odor is normally associated with

MIB, while geosmin is usually associated with having an "earthy" off-flavor/odor. MIB and geosmin have been identified as major flavor contributors to off-flavors/odors in aquatic environments in which catfish live. Microbial flavor-producing species of cyanobacteria and/or actinomycetes release the compounds in pond water where catfish then absorb these compounds into their flesh. Catfish containing these off-flavors must be kept in cultured ponds or purging facilites until off-flavors have been removed, delaying harvest up to several days or even months depending on lipid content of the fish, water temperature, and concentration of off-flavors in the production pond (Schrader and others, 2003).

The invention of a safe, practical and reliable methods for controlling and removing off-flavors found in cultured catfish would be highly beneficial to the catfish industry. Several pre-harvest management practices have been implemented to decrease the occurrences of off-flavor in catfish including the use of algicides such as copper sulfate, chelated copper, and Diuron (a substituted phenylurea herbicide), to prevent and kill the cyanobacteria. Unfortunately these chemical compounds are toxic to phytoplankton communities and may lead to water quality deterioration and to death of the catfish (King and Dew, 2003).

Post-harvest methods for removal of catfish off-flavor may be more successful than pre-harvest methods. Post-harvest off-flavor removal research includes methods to mask the flavors, and technologies to physically or chemically alter the flesh to remove off-flavor compounds.

The objectives of this research were to determine if catfish fillets further processed using the acidic protein solubilization method to remove muddy off-flavors caused by 2-methylisoborneol (MIB) and geosmin could be further used to produce a consumer acceptable product.

CHAPTER II

REVIEW OF LITERATURE

Catfish Production, Annual Consumption, Rank, Land Area and Products

Channel catfish is one of the most important farm-raised species cultured in the United States, and is also the most abundant species in aquaculture. Catfish growers within the United States reported total sales of 445 million dollars during 2007, with 94% of sales being accredited to four major states; Mississippi, Arkansas, Alabama, and Louisiana (USDA, 2008). According to the National Fisheries Institute (NFI; 2006), the U.S. estimated 2004 per capita consumption of catfish was 0.5 Kg, placing catfish fifth in popularity behind pollock, salmon, canned tuna, and shrimp. In addition, NFI also reported the total round weight of farm-raised catfish processed during 2007 was an estimated 210 million Kg. This production volume comes from nearly 1,062 catfish operations within the U.S. which cover a total of 62,577 hectares of water surface (USDA, 2008). The state of Oklahoma has approximately 324 hectares of water surface in channel catfish production (Agriculture Statistics Board, 1994).

On average, farm-raised catfish ponds produce 4,000 Kg of catfish per hectare of water. Channel catfish take an estimated 15-18 months to reach market size which ranges from 0.34-0.68 Kg (0.75-1.5 lbs). Commercial catfish and producers typically receive between $1.40 to $1.70 per Kg live weight (Chapman, 2008).

Sylvia and Dean (2001) reported on the different forms of processed catfish. They indicated the traditional form is considered as the whole dressed fish which consists of the beheaded, eviscerated, and skinned carcass, with the tail remaining. This form of catfish can be further processed into more products like fillets (belly flap tissue present), shank fillets (without belly flap), strips, nuggets (belly flap), and steaks. Furthermore, all such forms are marketed fresh or frozen. Nuggets are considered a less valued product because it contains mesenteric tissue, lipids, and hemoglobin, making it dark colored meat. In the late 1980's, specialty products became a popular item in the market place. Examples of these products are fillets and whole dressed catfish with various rubs, coatings, and marinades applied. The most popular flavors for catfish were cajun, lemon-butter, and mesquite. They also indicated that some unique products that were being tested but not yet in the market included catfish "corn-dogs", catfish sausage, salmon, and sardine style canned catfish products and catfish roe. Finally, they reported on other non-food products that can be made from less valuable parts of catfish. These include gelatin made from collagen, belts made from fish hides and the conversion of offal.

Off-Flavors in Channel Catfish

Aquaculture is considered the production of aquatic organisms under controlled conditions. Such conditions may be controlled to some degree but complete control over water quality is not feasible (King and Dew, 2003).

The number one reason why consumers reject a food product is due to its undesirable flavor (Mottram, 1998). Off-flavors/odors in foods, such as catfish, can occur from many different factors including the environment, various pollutants, growth of microorganisms, enzymatic decomposition, or oxidation of lipids. It is imperative to know where these off-flavors are stemming from in order to minimize economic losses, and the loss of consumer support.

Numerous studies have found that the two main pre-harvest off-flavor/odor compounds in catfish are geosmin and 2-methylisoborneol (MIB) (Tucker, 2000). These chemicals are microbial metabolites, with odors referred to as “earthy”, “muddy”, “moldy” and/or “musty”, and arise in the water in which catfish live (Kelly and others 2006). Mamba and others (2007) reported that aquatic fresh water environments that catfish live in are generally populated by algae which grow in populations of prokaryotic and eukaryotic microorganisms. They also indicated that geosmin and MIB are produced by cyanobacteria (blue-green algae) and actinomycetes (bacteria). Different species of cyanobacteria such as *Oscillatoria, Nostoc, Aphanizomenon* and *Anabaena* grow in significant amounts in ponds during summer months coupled with high temperatures (Mamba and others, 2007). Actinomycetes such as *Streptomyces* and *Nocardia* are commonly found growing in soil and can enter pond with storm runoff (Tortora and others, 1998). Blue-green algae are prokaryotic organisms that release oxygen in the water as a by-product of photosynthesis. Besides the beneficial effects of these microorganisms, their by-products are the main cause of off-flavor/odor problems associated with channel catfish.

Geosmin (*trans*-1, 10,-dimethyl –*trans*-(9)-decalol) is a bicyclical tertiary alcohol that when diluted in an aqueous solution has an earthy-muddy odor (Figure 1). Geosmin is naturally found in beets and root crops and is extremely potent in both fish and water. The compound 2-methylisoborneol (*exo*-1, 2, 7, 7-tetramethy-heptan-2-ol) or MIB is also a bicyclical tertiary alcohol and is a natural element in soil and fresh water (Figure 1). It is also associated with a very musty odor when in a concentrated solution. Both compounds are terpenes which make them volatile. They also contain hydroxyl groups that give dual solubility in water and lipids therefore geosmin is associated with an "earthy" flavor, while MIB is described as "musty" (King and Dew, 2003).

Geosmin and MIB can enter the fish in many different ways but the most common way fish become off-flavored is by absorption of these compounds through the gills during respiration (Dionigi, 1993). Fish can also absorb the compounds through their skin or by ingesting food items containing the off-flavor compounds. Geosmin and MIB contain dual solubility, mentioned earlier, which enables them to accumulate in the lipid rich tissues of catfish. Catfish that contain greater body mass index (fat) absorb and store much greater concentrations of off-flavor/odors than catfish containing a lesser body mass index. To be more specific catfish containing 2.5% or more muscle fat absorb more than three times as much Geosmin and MIB, than leaner fish (Johnsen and Lloyd, 1992). The presence and concentration of off-flavor/odor is dependant on season and is associated with algal blooms that tend to arise in warmer summer months.

It takes less than 24 hours of exposure for a fish to be considered off-flavored. Yamprayoon and Noomhorm (2000) studied the concentration of geosmin in fresh Nile tilapia by applying high-vacuum distillation, extraction, and gas chromatography techniques. Analysis concluded that when geosmin was added to fish flesh at concentrations of 2.8 to 390 µg/Kg flesh, the recovery rate was 51 to 89%. Muddy off-flavor was detected at 7.55 to 9.85 µg/Kg flesh. After 2 hours in water with 5 to 50 µg/L geosmin, tilapia absorbed 17.6 and 42.2 µg/Kg geosmin in the flesh. Not only does this fact impact economic profitability and production efficiency, it also delays harvest time which burdens the catfish farmer with more time and money encumbered. Even though off-flavor/odor occurs more often in the hot summer months when microbial growth is at its highest (Tucker and others, 2001), catfish producers must find ways to address and minimize this occurrence year round. For example, processors require catfish producers to test their ponds for off-flavor/odor several times during the growing season, especially prior to harvest. Some producers send 2 to 4 fish per week to facilities that test for off-flavors. Fish that are harvested and proved to be free from any off-flavor compounds may become off-flavored in a matter of one hour just from traveling in the same truck, bin, or container with fish that is considered off-flavored. It is the catfish producers and not the processors that pay for sampling and transportation, and delayed harvest cost (Hanson, 2003). Thus, expenses incurred in trying to control off-flavors/odors in ponds go beyond those normally associated with proper pond management.

Economic Impact of Off-flavors/Odors

Economic loss in the catfish industry due to off-flavors was estimated to range around $60 million annually (Schrader and others, 2003). The aquaculture industry faces the unpredictability of potential off-flavors when trying to produce a high quality, consumer acceptable product. Catfish farmers are burdened with chronic pond management problems that result in the development of off-flavor/odors in catfish that in turn makes the catfish unacceptable for purchase and consumption. King and Dew (2003) indicated that approximately 80% of harvestable fish each year can and will be considered off-flavored. Processors will not accept off-flavor/odor catfish, thus producers are forced to delay harvest until off-flavor/odor is removed or absent. In addition, increased feeding may cause catfish to grow larger than the optimal size and incur a docking fee for oversized fish (Hanson, 2003).

Not only does delayed harvest cause an increase in feeding cost, it affects profit margins as well. For example, if a producer was unable to remove off-flavor/odor from their catfish they would be fortunate if their fish were bought for less valuable by-products, such as fishmeal, or pet food.

Pre-Harvest Elimination of Off-Flavors

Pre-harvest methods to prevent or eliminate off-flavors are used to treat catfish while they are still alive in the ponds. Proper pond management is the most effective way to help prevent off-flavor from occurring. The use of chemical algicides, such as copper sulfate, has been found to help prevent the growth of cyanobacteria in ponds. This method is labor friendly and is cheaper in contrast with other methods of removing off-flavors/odor. However, using copper to help control the growth of cyanobacteria tends to kill other beneficial algae and other microorganisms that grow with in a pond environment, causing oxygen to be lost due to decomposition with possible suffocation and death of fish (King and Dew, 2003). Copper sulfate treatments also cause poor water quality requiring more aeration in copper treated ponds compared to untreated ponds. Tucker and van der Ploeg (1999) also commented that copper products become toxic to fish and algae as water hardness and alkalinity decreases, making it difficult to obtain a safe and effective treatment. Once a stock of catfish is deemed as off-flavor, few techniques are available to help eliminate and remove the off-flavors. Purging catfish in clean flowing water is one method used to remove MIB and geosmin. The catfish are transferred into large tanks where a continuous flow of clean water, completely free of any off-flavor/odor, flushes out the off-flavor/odor. This method of purging is quite effective at removing off-flavor/odor compounds, but is high in labor costs and results in high mortality rates due to stress and disease from handling. Another downfall to purging is that it is very time consuming depending on fish factors such as fat content, size of fish, water temperature and quality, and concentration of off-flavor/odor that is present. Fish that have greater fat content and tend to be larger in size, along with lower water temperatures, will require more time to purge the fish of off-flavor/odor (van der Ploeg and others, 2001). Additionally, purging may take 3 to 5 days to remove MIB; however, it may take 2-4 weeks for geosmin (King and Dew, 2003).

Post-Harvest Elimination of Off-Flavors

Inconsistent and expensive pre-harvest elimination of off-flavors has prompted researchers to investigate post-harvest techniques as measures of controlling or removing off-flavors in catfish. An effective post-harvest method needs to be capable of reducing off-flavors below the level of human detection while producing a consumer acceptable product.

Marshall and Kim (1996) studied the influence of acetic acid and/or lactic acid dips for 30-60 seconds on microbial numbers, pH, and sensory scores of catfish fillets. Results showed that treatments with acetic acid and lactic acid prolonged microbial shelf life to 12 days. Acetic acid treated fillets contained greater antimicrobial activity than those treated with lactic acid. Fillets treated with acetic acid alone or combinations of both acids were less preferred by sensory evaluations than controls, due to acid odor and flesh discoloration. Forrester and others (2002) compared catfish fillets containing 2-methylisoborneol that were vacuum-tumbled in 0%, 0.5% and 2% citric acid solutions to reduce off-flavors. Solid-phase microextraction and gas chromatography analysis (GC) indicated that MIB was significantly decreased from 4.43 to 2.80 µg/Kg as a result of vacuum-tumbling with 2% citric acid. Moisture content, expressible moisture, and color lightness of samples were all increased as a result of acid conditions.

Tertiary alcohols, such as MIB and geosmin, are susceptible to dehydration. MIB will dehydrate to 2-methylenebornane and 2-methyl-2-bornene; geosmin will dehydrate to argosmin (Forrester and others, 2002). Changing the chemical structures of the alcohols may affect their ability to bind with lipids, or change their taste/odor. In addition, Schumann and Pendleton (1997) evaluated the dehydration of MIB under acidic conditions. MIB was treated with sulphuric acid to give a 1:1 mixture of 2-methylenebornane and 1-methylcamphene, containing only a trace of 2-methyl-2-bornene. They stated that these conditions most closely resemble conditions under which MIB undergo dehydration in a natural water environment, where acid catalysis may lead to dehydration of acid-labile MIB. Their results conclude that under acidic conditions, 2-methylenebornane and 1-methylcamphene would be the major products of MIB dehydration. In addition, Mills and others (1993) stated that MIB and its two dehydration products 2-methylenebornane and 2-methyl-2-bornene do not contribute to musty/earthy off-flavors found in channel catfish. Their study concluded that gas chromatography/mass spectrometry (GC/MS) analysis showed that volatile fractions of catfish covering a range of flavor quality were all found to contain the two dehydration products of MIB. Gas chromatographic effluent sniffing indicated the only off-flavor odor present was that of 2-methylisoborneol and geosmin. It was concluded that the two dehydration products of MIB do not have discernible odors and do not contribute to the muddy off-flavor.

Other researchers investigated protein solubilization as a means to recover protein and remove off-flavors from fish. Protein solubilization is a process that was developed to produce surimi from difficult to process fish (Kristinsson and others, 2005). Kristinsson and others (2005) stated that the traditional surimi process used to make surimi does not always produce a product with good gelation properties, and there can be problems with color and lipid oxidation depending on the fish species being utilized. They also stated surimi involves several washing steps, making it a time consuming process. In addition, the separation of insoluble constituents such as skin, bone, scales, and fat caused great difficulty.

Protein solubilization using an acid and/or alkaline process was originally developed by Hultin and Kelleher (2000) in order to manage problems dealing with small species, dark muscle or by-products for surimi processing. The acid protein solubilization process (Hultin and Kelleher, 1999) involves reducing the pH of a diluted 1:9 (protein: water) homogenized muscle mixture between pH 2 to 2.5. Under these conditions the myofibrillar protein is solubilized. When protein is solubilized, interactions involved in protein aggregation, including hydrogen bonds, van der Waal forces, ionic interactions, disulfide bonds and hydrophobic interactions are disrupted (Rabilloud, 1996). Insoluble material such as skin, fat, bone and scales is removed by centrifugation. The soluble proteins are then precipitated by isoelectric precipitation, and recovered by centrifugation. The resulting product is high in protein concentration and functionality, with improved whiteness, and very little fat. In addition, a significant portion of the membrane lipids and provides a high yield of protein (Hultin and others, 2000b).

Kristinsson and others (2005) stated that solubilization of proteins and the disruption of the muscle cell both cause a large decrease in viscosity of the solution, enough that cellular membranes can be separated from soluble proteins by high-speed centrifugation. They also specified that the resulting isolate has use for various purposes, such as the use of a functional food ingredient or simply to formulate a value-added fish product.

The process has shown remarkable results for some cold water species such as cod and pacific whiting along with temperate and warm water species such as channel catfish (Yongsawatdigul and Park 2003). A study completed by Kristinsson and Hultin (2003) on cod muscle protein stated that the use of alkaline solubilization improved gelation and emulsification properties of cod myofibril and myosin protein. This development was linked to a particular unfolded structure of cod myosin after the alkaline process. However, a different response to the solubilization process can be seen for warm water species because their proteins are more heat stable as a result of their environment. For example, Theodore and others (2003) showed that the alkaline process used on catfish produced greater strength of protein gels compared to the surimi process or acid processes over different ionic strengths (0-600 mM NaCl) and pH 6-8. Additionally, results from Davenport and others (2003) pointed out that significant change in myosin from channel catfish added to the improved gel strength after an alkaline process.

DeWitt and others (2007a) compared the effectiveness of 4 different protein solubilization conditions, (alkaline, acetic, citric, and phosphoric acids) in removing off-flavors from catfish. Furthermore, the effect of centrifugation on off-flavor removal (3,000 x g and 10,000 x g) was also investigated. They concluded that the use of phosphoric acid was better than the other two acid conditions considered for MIB and geosmin removal. It was also better with respect to geosmin, than the alkaline solubilization process. Centrifugation force did not significantly effect off-flavor removal, but it did significantly lower fat content. In part because the solubilization/precipitation process washes away fat and water soluble components and because acidic conditions lead to the conversion of MIB and geosmin to its dehydration products, it was hypothesized by Dewitt and others (2007a) that the protein solubilization process might successfully remove chemical compounds that contribute off-flavors odors in fish. Furthermore, they found that pilot-scale processing of channel catfish fillets using acid and alkaline solubilization procedures; resulted in a low-fat protein with cook yield, color, and gel strength similar to that of non treated catfish.

The idea of better utilization of muscle by-products has become an interest for a number of small to medium size fish processors. Nolsoe and others (2007) investigated the likelihood of utilizing the alkaline solubilization process for fish processors. Interestingly, they used an alternative method to the standard centrifugation such as the use of a filtrate sieve to separate functional properties from excess water. Replacing the first centrifugation with a sieve improve protein yield, yet poor gel quality was displayed. Replacing the second centrifugation with a filtrate sieve had no effect on either yield or quality of protein isolate.

Detection of Off-Flavor's using Human Sensory Evaluation (HSE)

Currently human sensory evaluation (HSE) is a method to detect off-flavors/odorous components in catfish. This process is done prior to pond harvest, and repeated upon arrival to the processing facility. A micro waved cooked sample of fish is evaluated fish by smelling, followed by tasting the sample for off-flavors/odors. Detection thresholds for sensory of both MIB and geosmin vary considerably. Forrester and others (2002) reported that the MIB of sensory threshold detection levels range from 0.09 to 200 ppb whereas geosmin ranges from 0.6 to 8 ppb. It was reported by King and Dew (2003) that MIB detection thresholds are significantly lower than that of geosmin.

Research Objectives

A strong post-harvest off-flavor removal procedure has yet to be implemented in the catfish industry. The catfish industry has come a long way and continues to move forward. Research pertaining to detecting and controlling off-flavor/odors also continues to move forward and will be a success in a matter of time.

The overall objective of this research was to evaluate conditions that employ the acid solubilization process as a post-harvest technique and assess its effectiveness in reducing or eliminating off-flavors associated with catfish. In addition, the purpose of this research was to determine if protein recovered from this process can be further processed into a consumer acceptable product.

CHAPTER III

PRODUCING A CONSUMER ACCEPTABLE PRODUCT FROM OFF-FLAVOR CATFISH

T.M. BROWN, C.A. MIRELES DEWITT AND C.W. KLEINHOLZ

ABSTRACT

Farm-raised catfish weighing two pounds were placed into three tanks each containing 1000 L of water. Treatments were grouped by week: week 1: control (Con), week 2: 1 ppb geosmin and week 3: 1 ppb 2-methylisoborneol (MIB). Treated catfish were sorted into three replicates containing equal male: female ratios. Fillets from each replicate were ground. A portion was kept and labeled as "non-processed". The remaining portion was solubilized to pH 2.5 and filtered through a sieve. The filtrate was collected and labeled "retentate". Processed and non-processed protein from each treatment were evaluated for geosmin and MIB analysis. A consumer acceptance panel was conducted on catfish samples from each treatment (n = 6). Processing decreased % moisture, fat, ash, and collagen content of catfish protein. Flavor rated significantly higher for control samples than geosmin and MIB. There was a slight trend in flavor but not enough to be statistical, that consumers preferred processed samples compared to non-processed. Texture of catfish samples from processed protein was preferred to non-processed samples. Control samples ranked significantly higher for overall liking than other treatments except for geosmin processed. Also there was a slight preference, not enough to be statistical, for overall acceptability of processed sample over non-processed.

INTRODUCTION

Catfish farmers often encounter flavor issues with pond raised channel catfish, which leads to significant economic loss. The farmers are burdened with chronic management problems resulting from off-flavor/odors caused by chemical compounds that occur in ponds which catfish dwell. These chemical compounds are metabolites of various bacterial microorganisms and algae, and are responsible for producing both positive and negative effects on the environment as well as in the catfish. Economic loss in the catfish industry due to off-flavors may be $60 million annually (Schrader and others, 2003).

Preventative pre-harvest methods to reduce or remove off-flavors/odors in catfish have been attempted. The majority fail to provide safe, time effective and economic solutions in a typical production type setting (Tucker and van der Ploeg, 1999). The search for an effective post-harvest remedy has challenged many researchers.

A process known as acid or alkaline protein solubilization is used to extract and recover myofibrillar proteins from fish. This process originated as an alternative to the surimi process used to make fish protein concentrates.

The overall objectives of the current study was to evaluate conditions that employ the acid solubilization process as a post-harvest technique and assess its effectiveness in reducing or eliminating off-flavors associate with catfish. In addition, the purpose of this research was to determine if protein recovered from this process can then be further processed into a consumer acceptable product.

MATERIALS AND METHODS

Treatment of Fish

As outlined in Figure 2, channel catfish (*Ictalurus punctatus*) were collected from cultured ponds at Langston University during 3 separate weeks. At each collection time, approximately 48 Kg of live pond raised catfish were obtained from Langston University. Catfish were fasted for a period of one week prior to collection to allow food and bile to be excreted. Fish were randomly distributed among three 1050 L high density polyethylene plastic tanks (Polytank Inc, Litchfield, MN, USA). Each tank contained 1000 L of aerated municipal water at 20ºC, with 18 fish per tank. The tanks were interconnected and filtered in a re-circulating system using a floating bead filter to allow equal distribution of flowing water. Fish were permitted to depurate/purge and acclimate in the filtered tank water for a period of 24 h. Following purging, tanks were drained and refilled with 945 L of fresh municipal water. Treatments were grouped by week: wk 1 = control (no treatment); wk 2 = 2-methylisoborneol (MIB), and wk 3 = geosmin. Control fish (wk 1) received no spike and were held in tanks for an additional 24 h prior to harvest. Tanks containing fish on wk 2 were treated by spiking tank water to a final concentration of 1 ppb MIB (98% purity; 10 mg/ml solution in methanol; Sigma-Aldrich Inc, St. Louis, MS). Tanks containing fish on wk 3 were treated similarly with 1 ppb geosmin (98% purity; 2mg/ml solution in methanol; Sigma-Aldrich Inc. St. Louis MS). Fish in spiked tanks were held for an additional 24 h prior to harvest to allow uptake of MIB or geosmin by the fish. For all 3 treatments, water samples from each tank each week were collected at 0 and 30 min, and 24 h after spiking. Water samples were collected in 20 mL clear glass vials without headspace to prevent air bubbles, packed on ice, and shipped via overnight delivery to the Thad Cochran Research Center (University, MS) for solid-phase microextraction and gas chromatography analysis of MIB and geosmin.

Harvesting of Fish

Fish were removed from tanks after treatment and immediately placed into coolers containing a crushed ice and rock salt slurry. Fish were transported to the Robert M. Kerr Food & Agriculture Products Center (FAPC, Oklahoma State University) for further processing. Processing occurred in a 4ºC refrigerated room to prevent protein degradation and maintain experimental consistency. Length and weight were collected from each fish (Table 1). Fish were randomly selected, eviscerated, and filleted. Fillets were weighed and distributed into 3 replicates. Each replicate contained fillets from 18 fish with a 1:1 ratio of males to females. Fillets were then reduced to approximately 2.54 cm^3 pieces prior to grinding (Hobart©, Model 8185, Troy, Ohio).

Fish Processing

Approximately 4.7 Kg of chopped fillets (control, MIB, or geosmin) were placed in a bowl grinder (Hobart©, Model 8185, Troy, Ohio) and ground at 3450 rpm for 2 min. As outlined in Figure 3, approximately 2 Kg of sample was collected after grinding and labeled as "non-processed", meaning that ground fillets were not acid solubilized. The remaining ground fillet (2.7 Kg) was mixed with 2.7 Kg ice and 21.8 Kg water for 30 sec using a hand-held immersion blender (Waring WSB 70, Torrington, CT). The mixture was acidified to pH 2.5 using 4.4 N H_3PO_4 (85% FFC diluted 1:10). The insoluble material from solubilization (retentate) was removed by filtering the acidified mixture through a Sweco sieve shaker with a 14 mesh TBC screen (Tensile Bolting Cloth) with a

0.15 cm mesh opening, 0.02 cm wire diameter, and 75% open area (SWECO VIBRO-ENERGY SEPARTOR, SWECO, Florence, KY, USA). Material retained by the sieve was collected and labeled as “retentate”. Filtrate from the mixture was collected and the pH was increased to the isoelectric point of 5.5 using 2 N NaOH. Precipitated protein from the mixture was collected using the Sweco sieve shaker. Protein collected from the sieve was further de-watered by filtration through the use of cheese cloth and labeled as “processed”, meaning that the protein was acid solubilized. Water from the precipitated protein was collected and labeled as “waste water”.

Waste water samples were collected in clear, glass, 30 ml vials without head space. Vials were packed in ice and shipped via overnight delivery to the Thad Cochran Research Center (University, MS) for MIB or geosmin analysis. Waste water samples were also collected in Whirl-Pak® bags and blast frozen -28ºC for further compositional analysis. Retentate, non-processed, and processed protein samples were placed in vacuum bags (Cryovac, Model 9E278, Bolingbrook, Il.), blast frozen, and stored at -28ºC for further compositional analysis.

Sample Preparation for Sensory Analysis

Approximately 400 g of either control-processed (CON-P), control non-processed (CON-NP), MIB processed (MIB-P), MIB non-processed (MIB-NP), geosmin processed (GEO-P), or geosmin non-processed (GEO-NP) protein samples, were partially thawed to a temperature of -2ºC and placed into a food processor (Cuisinart® Pro Classic™, Model 86279, East Windsor, NJ). Ice was added as needed to adjust moisture content to 80%. In addition, 1% NaCl and 0.05% $NaHCO_3$, were added based on moisture adjusted weight of protein. Samples were then mixed at 1000 rpm (medium speed) for 2 min. Following fish protein preparation, sample mixture was formed into a 6 g ball and coated with Uncle Bucks Light N Krispy™ fish batter (coating) (Bass Pro Shops, Cat. No. 090232, Richardson, TX). The coated portions were placed on a cookie sheet and blast frozen -28ºC for 15 min. The resultant samples were removed from the blast freezer and placed in vacuum-sealed bags (Cryovac, Model 9E278, Bolingbrook, Il.), labeled, and stored in a freezer, -15ºC, until the day of the sensory panel evaluation. Protein samples prepared for sensory analysis were deep fried at 177ºC for 2 minutes until internal temperature reached 49ºC using a dual basket Pro Fry™ (Presto, Model 0546607, Eau Claire, WI) fryers. Each fryer was designated to only one treatment. Oil was allowed to drain from samples for at least 1 min. Samples were then placed in serving cups (Dart Container Corporation, Mason, MI.) labeled with a randomly generated 3-digit number. Lids were placed on cup and cups were held in a FWE® (Food Warming Equipment Co. Inc. Model PS-1220-15, Crystal Lake, Il.) food warmer (76ºC) for approximately 10 min or until serving began.

Analysis of Treatments

Triplicate samples were powdered by dipping samples into liquid nitrogen then blended using a previously frozen Waring blender. Blending was conducted in a cold room 4ºC. Powdered samples were analyzed for moisture content and crude ash content (AOAC, 2000). Total fat was obtained using the Soxtec (Tecator Soxtec System HT: 1043 & 1046, FOSS Analytical, Denmark) and crude protein was determined by Kjeldahl (Tecator 2300 Kjeltec Analyzer Unit, FOSS Analytical, Denmark) both following AOAC (2000) methods.

Analyses for MIB and Geosmin in tank water, fish protein, retentate protein and waste water were performed by the USDA Thad Cochran Research Center (University, MS) using solid-phase microextraction and gas chromatography as noted by Schrader and others (2005) and Grimm and others (2004).

Hydroxyproline Determination

Concentration of hydroxyproline was measured to approximate collagen content. Collagen connective tissue on average contains 12.5% hydroxyproline when protein content is calculated using a 6.25 protein factor (Kolar, 1990). Hydroxyproline content of catfish samples was determined as described by Kolar (1990). Samples were thawed to room temperature 21ºC and triplicate 4 g samples of collagen were obtained for analysis. Approximately 30 mL 7 N sulfuric acid was added to each sample. The samples were dried at 105ºC for 16 h. The solution was then filtered and diluted; duplicated samples of the filtrate were then oxidized using a chloramine-T solution. In addition, 4-dimethylaminobenzaldehyde was added to samples which were then placed into 60ºC water bath where a magenta color developed. Samples were measured at 560 nm using a spectrophotometer (Beckman Coulter®, DU 7500 #200776, Houston, TX.). Determination of hydroxyproline content (h) from spectrophotometer data was calculated using the following formula:

h, g/100 g = (h X 2.5) / (m X V), where h = hydroxyproline, (µg/2 ml filtrate) read from calibration curve; m = weight of sample, g; V = volume, (ml) of filtrate taken for dilution to 100 ml of hydrolysis step. Calculation resulted in an arithmetic mean of the absorbance readings taken from duplicates.

Sensory Evaluation Design

Sensory analysis was conducted using consumer preference testing by an untrained panel (n = 120). Panels were not screened based on likeness of catfish or how often they consumed it. They were primarily students and employees of the University that volunteered to taste catfish samples. Each panelist was given a general questionnaire prior to the serving of samples (Appendix A). Panelists were asked to evaluate one sample from each treatment. Each sample from a treatment was randomly distributed and evaluated by 40 panelists. Each time a sample was served, a ballot (Appendix B) was given to the panelists. Panelists were asked to evaluate a sample before the next sample was presented, collecting each ballot after every sample. Panelist evaluated attributes in the following order: overall acceptance, overall flavor, and overall texture. All three categories contained a 9 point hedonic scale ranging from 9 = like extremely to 1 = dislike extremely. The sensory ballot consisted of two pages. First page asked the panelist for their overall liking of the product as a whole. The goal was to ask for their first impression of the catfish sample. The second page consisted of two questions; overall flavor and overall texture of the sample. Here the goal was to ask specifically if they liked the flavor, which would tell if the panelist were able to detect any off-flavors in the sample. Texture would tell if the panelist like the fish protein and if the acid processing actually improved or worsened the texture of the sample.

Statistical Analysis

All results were analyzed using the general linear models procedure (SAS Inst. Inc., Cary, NC) procedure. Data for sensory evaluation was evaluated as a 3 x 2 factorial in a randomized block design trial with block being panelist number, factorial A being treatment, and factorial B being the type of process. Data for compositional analysis was evaluated as a 3 x 2 factorial in a randomized design trail. Mean separation was achieved using Tukey's Studentized Range (HSD) for multiple comparison of means. Test were conducted at significant level of $\alpha = 0.05$.

RESULTS AND DISCUSSION

Off-Flavor Uptake and Removal

Treatments were grouped by week with each week containing three holding tanks. Tank water samples were obtained at 0 min, 30 min, and 24 hours. Results by solid-phase microextraction and gas chromatography (GC) indicated that both tanks contained about 0.5 ppb after 30 min of their respective spikes, geosmin (Figure 4) and MIB (Figure 5). Targeted spike levels were nearly 1 ppb for MIB or geosmin. Control treated fillets contained insignificant amounts of geosmin and MIB. Only residual amounts of geosmin in MIB spiked tanks or MIB in geosmin spiked tanks were measured. Unfortunately, uptake of geosmin and MIB by catfish was much higher than targeted (1 ppb; Figure 6 and 7). Uptake of geosmin and MIB could be due to water vapor absorption as well as variations in water temperature from pond verses tank. Also, variations in catfish size and fat content highly contribute to differences in geosmin and MIB uptake. Johnsen and Lloyd (1992) concluded that catfish containing greater fat contents absorb and store greater amounts of MIB than leaner catfish. The fat content in both geosmin and MIB was similar to USDA suggested values (Table 2). Its is likely that increased geosmin and MIB uptake was influenced more by the temperature changes experienced by the catfish when they were transferred form outdoor ponds (November and December) to indoor tanks than by the fat content. Moving from a cold to warmer environment likely increased their metabolism and therefore compound uptake.

Although levels of geosmin and MIB uptake were much higher than targeted, results still demonstrate that the process utilized to remove off-flavor compounds was still fairly effective (Figure 6 and 7).

Calculated on a wet basis, geosmin catfish contained 4.04 ± 0.67 ppb geosmin prior to processing. After processing the level was reduced to 2.72 ± 0.91 ppb. Calculated on a dry basis (Figure 6) that was equivalent to a 42% reduction in geosmin as a result of processing. Similar results were reported for MIB spiked catfish. Prior to processing, there was 5.38 ± 0.83 ppb MIB in MIB spiked catfish. After processing, the level was reduced to 2.97 ± 0.86 ppb. Calculated on a dry basis (Figure 7) this was equivalent to a 48% reduction in MIB as a result of processing.

It is also important to note, however, that final concentrations of both MIB and geosmin in processed catfish were still above the sensory threshold values reported by Forrester and others (2002). This will have implications with regard to the sensory evaluation part of the study that is reported and discussed later in the text.

The removal of off-flavor compounds reported by this study is higher than the 36% reduction of MIB reported by Forrester and others (2002) when they exposed fillets to a vacuum tumbling in 2% citric acid. They are also higher than the 35% reduction of geosmin reported by Yamprayoon and Noomhorm (2000) for smoked tilapia placed in a 7% acetic acid brine for 2 weeks. However, neither MIB nor geosmin reduction was as effective as reported by DeWitt and others (2007a). This study used a process similar to what is reported in the current study. However, the process utilized by DeWitt and others removed the fraction that is identified as retentate (Figure 3) by centrifugation at either 3,000 or 10,000 xg. They also recovered the protein (identified as "processed"; Figure 3) utilizing centrifugation. Geosmin removal was 85.2 and 96.0%, respectively, for 3,000 and 10,000 xg. MIB removal was 86.8 and 96.1% respectively, for 3,000 and 10,000 xg.

The current study employed a sieve for collection of "retentate" and "processed". It was selected for this study because a large scale centrifuge was not available and the lab scale models were not sufficient to produce the desired amount of product needed for the sensory evaluation phase of the project. In addition, the sieve would be the most economical option for processors. As can be seen by the results, however, although cost effective, processing with a sieve is less efficient in terms of off-flavor removal when compared to a centrifuge.

Compositional Analysis of Samples

Evaluation of raw, farm-raised, processed, and non-processed catfish fillets is reported in Table 2. Fillets from control catfish were leaner than values reported by the USDA (2004) for channel catfish. Processing decreased ($P < 0.05$) the moisture content in both geosmin and MIB treated samples, but not in the control. Moisture was significantly higher ($P < 0.05$) in control processed (CON-P) and control non-processed (CON-NP) than in geosmin and MIB catfish. There was no significant difference in percent moisture between GEO-NP and MIB-NP treated catfish. The variability in moisture content in protein collected after processing is likely a result of inconsistent processing techniques for de-watering of protein using a sieve. Final removal of water from protein was achieved manually with the aid of cheesecloth. The control samples were run the first week. Inexperience with this technique for de-watering resulted in a final processed protein product with significantly more water in the sample than occurred with subsequent processing on wk 2 and 3 (geosmin and MIB, respectively). Therefore, moisture content is only a reflection of processing technique. Ash was lowered ($P < 0.05$) as a result of processing for all treatments because the removal of bone, collagen, and skin (retentate) were separated from the solubilized myofibrillar protein when filtering the acidified mixture through the Sweco sieve shake occurred. In addition, the solubilization process washes away water soluble ash from the solubilized myofibrillar protein that is later recovered by precipitation. Statistically, there was no difference among treatments for protein content. Fat content in control catfish was significantly lower than MIB and geosmin treated catfish. Since treatments were grouped by day, this indicates the catfish collected for control treatment were leaner as a group than geosmin and MIB. This may be due to the fact that the control fish were collected from a different pond than the MIB and geosmin fish. Although not statistically significant, there was a trend for processing to result in a leaner product. The reduction in lipid content reported by DeWitt and others (2007b), however, was significantly more (74% less) than the results from the current study (26% less; reductions were calculated on a dry basis). This can likely be explained by differences in processing techniques utilized by the two studies. In the former study, protein was recovered by centrifugation allowing for better separation of lipid from protein. In the current study, sieve separation was chosen for protein recovery for two reasons. First, it was the most economical option for processors. Second, since a pilot-scale evaluation with a centrifuge had already been conducted, a comparison of the techniques for removal of geosmin and MIB could be made. As reported earlier when describing how the use of the sieve impacts off-flavor reduction, if processors opt to use a sieve based processing technique as opposed to a centrifugation based technique they will be given up efficiencies in terms of lipid reduction.

Waste Water and Retentate Analysis

No significant difference in moisture and protein content of waste water samples were observed (Table 3). Moisture content for retentate samples was significantly higher in the control group when compared to geosmin and MIB treated retentate (Table 4). On a total solids basis for waste water samples, percent protein was 43%, 53%, and 37% for the control, geosmin and MIB treatments, respectively. This large variation in protein content speaks to the difficulty in maintaining consistent processing conditions from day to day. The retentate protein showed no significant difference among the three treatments when calculated on a wet basis. On a total solids basis for retentate samples, percent protein was 4.0, 4.4, and 5.5 for the control, geosmin and MIB treatments, respectively.

Hydroxyproline Determination

Catfish protein (processed and non-processed), retentate and waste water were all analyzed for hydroxyproline content (Table 5). High hydroxyproline content reflects increased amounts of collagen since hydroxyproline is one of the principle amino acids found in collagen. One of the aims of processing, aside from reducing muddy off-flavors in catfish, was to separate functional myofibrillar protein from insoluble protein such as collagen, which hinders gel formation. Theoretically, at pH 2.5, the majority of insoluble material should be just collagen. On a total solids basis, the recovered retentate material contained significant amount ($P < 0.05$) of hydroxyproline when compared to both processed and non-processed catfish protein. In general, there were lower amounts of hydroxyproline as a result of processing.

Sensory Evaluation Design

Panelists were given a simple questionnaire asking 5 demographic questions pertaining to themselves and their liking of catfish. Little less than half the panelists were 18-25 yrs of age (Table 6). The majority of panelists were female. Two-thirds of the panelist where from the following states in the United States, (South: Oklahoma, Texas, Georgia, Arkansas, Alabama; Mid-West: Kansas, Iowa, Michigan, Indiana; Northeast: New York, Maryland; West: California, Wyoming, New Mexico, and Arizona). Of those from the United States, 50 were from Oklahoma and 13 from Texas. The majority of panelist reported they ate catfish once every 6 months or less. The majority of panelists also reported they consumed catfish in restaurants and the preferred cooking method for catfish was fried.

Panelists evaluated fried catfish samples for three different sensory attributes: overall acceptability, flavor, and texture (Table 7). For texture, processed treatments were preferred to their non-processed counterparts. The mean texture rating for all three processed treatments was 6.1 “like slightly”. The three non-processed treatments, on average, were rated as a 4.6, which corresponds to “dislike slightly”. Based on statistical observations, processing enhanced consumer preference for the texture of the fried catfish samples. A few of the positive descriptors used to describe the samples served to panelist were crunchy, juicy, perfect, great texture, and flavorful. Negative descriptors included salty, fishy, soil flavor, dirt taste, oily, soapy, and chewy.

Flavor values were rated significantly higher for the control samples when compared to MIB and geosmin spiked samples (Table 7). Although there was a slight trend in preference for the flavor of fried catfish samples that were made from processed protein, there was not a statistical significance between processed and non-processed protein. The slight preference in flavor for catfish samples made from P protein may be correlated with the fact that panelists preferred the texture of processed protein and correlated the preference of texture to the preference in flavor. In addition, GC analysis indicates there was a substantial decrease in measured geosmin and MIB in processed samples. However, the decrease in geosmin and MIB was not sufficient to reduce the level of off-flavors compounds below typical sensory threshold levels (Forrester and others, 2002). The slight preference may therefore indicate that the panelist could discern a difference but its removal was not sufficient enough to consider the sample as acceptable as the controls.

For overall acceptability, control samples were rated significantly higher ($P < 0.05$) than all other treatments. Control samples were rated as a 5.6 (6 = like slightly). As with previous evaluations, samples made from processed protein had an average "overall acceptability" rating that was higher than non-processed protein. The rating, however, was only significant (within treatment type) for the control samples. Interestingly, the combined improvements in flavor and texture improved the processed geosmin rating for overall acceptability high enough to make it similar to samples made from CON-NP protein.

CONCLUSION

Results from this study indicate that a sufficient amount of MIB or geosmin was absorbed into the catfish causing them to become off-flavored. In addition, the acid solubilization process was successful in reducing the concentrations of geosmin and MIB compounds in processed catfish samples compared to non-processed samples. However, due to the fact that the off-flavor compounds were above the reported threshold, quantity of off-flavors removed, was less than expected. Data also concludes that the acid solubilization process produced a lower moisture, fat, ash, and collagen catfish product. Texture of processed catfish samples was improved by the acid solubilization processes. In addition, there was a slight non-statistical trend, in that the flavor and overall acceptability of processed samples was preferred in comparison to non-processed samples. Future research should be implemented to utilize better processing methods for the use of the acid solubilization process.

Table 1: Measurement of length (cm), body weight (Kg), and final fillet weight (Kg) of fish prior to processing stratified by treatment.

Treatment	Length cm	Body Weight Kg	Final Fillet Weight Kg
CON	43.8 ± 2.50 cm	0.84 ± .015 Kg	0.24 ± 0.05 Kg
MIB	47.0 ± 4.70 cm	0.98 ± 0.15 Kg	0.28 ± 0.05 Kg
GEO	46.0 ± 2.50 cm	0.92 ± 0.14 Kg	0.28 ± 0.05 Kg

Data represents means ± standard deviation of the mean
Abbreviations are as follows where:
CON = control; MIB = 2-methylisoborneol; GEO = geosmin

Table 2. Compositional values for catfish during different weeks of the protein solubilization study.

Treatment	Moisture%	Protein%	Fat%	Ash%
CON-NP	79.6 ± 2.30[b]	18.0 ± 1.14[a]	3.0 ± 0.21[a]	1.1 ± 0.09[b]
CON-P	79.3 ± 1.98[b]	18.0 ± 0.97[a]	2.2 ± 0.43[a]	0.7 ± 0.20[a]
GEO-NP	74.7 ± 1.11[a,b]	16.7 ± 2.00[a]	6.6 ± 0.62[b]	1.0 ± 0.04[b]
GEO-P	73.3 ± 1.77[a]	16.3± 3.78[a]	4.9 ± 0.94[b]	0.6 ± 0.03[a]
MIB-NP	75.2 ± 0.78[a,b]	17.5 ± 0.76[a]	5.8 ± 0.80[b]	1.0 ± 0.04[b]
MIB-P	73.7 ± 2.99[a]	19.1 ± 2.62 [a]	4.8 ± 0.46[b]	0.6 ± 0.04[a]
USDA(2004)	75.38	15.55	7.59	1.00

[a,b] Means appearing in the same column with different superscript are significantly different ($P < 0.05$)

Data represents means of replicated samples ± standard deviation of the mean

Abbreviations are as follows where:

CON = control; MIB = 2-methylisoborneol; GEO = geosmin; NP = non-processed; P = processed

Table 3. Analysis values for waste water during the protein solubilization study.

Treatment	Moisture%	Protein%
CON	98.8 ± 0.13[a]	.51 ± 0.11[a]
GEO	99.0 ± 0.21[a]	.53 ± 0.22[a]
MIB	98.6 ± 0.07[a]	.51 ± 0.08[a]

[a] Means appearing in the same column with different superscript are significantly different ($P < 0.05$)
Data represents means ± standard deviation of the mean
Abbreviations are as follows where:
CON = control; MIB = 2-methylisoborneol; GEO = geosmin

Table 4. **Analysis values for retentate during the protein solubilization study.**

Treatment	Moisture%	Protein%
CON	93.5 ± 0.66^{b}	3.78 ± 0.40^{a}
GEO	90.6 ± 0.62^{a}	4.00 ± 0.35^{a}
MIB	89.5 ± 1.19^{a}	4.90 ± 0.82^{a}

^{a,b} Means appearing in the same column with different superscript are significantly different ($P < 0.05$)

Data represents means ± standard deviation of the mean

Abbreviations are as follows where:

CON = control; MIB = 2-methylisoborneol; GEO = geosmin

Table 5. Hydroxyproline analysis of catfish, waste water and retentate during the protein solubilization study.

Treatment	% Hydroxyproline Wet	% Hydroxyproline Solid
CON-NP	1.40 ± 0.04[a]	6.86 ± 0.04[a]
CON-P	1.36 ± 0.20[a]	6.57 ± 0.20[a]
GEO-NP	1.72 ± 0.23[a,b]	6.80 ± 0.23[a]
GEO-P	0.87 ± 0.09[a,d]	3.25 ± 0.09[a]
MIB-NP	2.27 ± 0.10[b]	9.14 ± 0.10[a]
MIB-P	1.35 ± 0.14[a]	5.12 ± 0.14[a]
RET-CON	4.15 ± 0.17[c]	63.82 ± 0.17[e]
RET-GEO	3.52 ± 0.50[c]	37.40 ± 0.50[b,c]
RET-MIB	3.63 ± 0.49[c]	34.53 ± 0.49[b]
WW-CON	0.58 ± 0.01[d]	48.29 ± 0.01[d]
WW-GEO	0.59 ± 0.07[d]	58.91 ± 0.07[e]
WW-MIB	0.64 ± 0.03[d]	45.83 0.03[c,d]

[a-d] Means appearing in the same column with different superscript are significantly different ($P < 0.05$)

Data represents means ± standard deviation

Abbreviations are as follows where:

CON = control; MIB = 2-methylisoborneol; GEO = geosmin; NP = non-processed; P = processed; RET = retentate; WW = waste water

Table 6: Summary of demographic characteristics corresponding to sensory panelists collected during the sensory evaluation of catfish.

Demographics	# Panelist
Age Range	**# Panelist**
18-25	50
25-23	36
35-50	21
50-up	13
Gender	
Female	70
Male	50
Origin	
USA	79
International	41
Frequency Consume Catfish	
1x a week	7
Every two weeks	6
1x a month	30
1x six months	49
1x year	22
Never	6
Location Consume Catfish	
Home	53
Restaurant	80
Other	4
Never	1
Favorite Cooking Method	
Fried	93
Baked	8
Grill	21
Other	7

Table 7. Catfish sensory evaluation data during the sensory evaluation of catfish samples.

Treatment	Overall*	Flavor*	Texture*
CON P	6.5 ± 1.69^{d}	6.5 ± 1.92^{b}	6.4 ± 2.05^{b}
CON NP	5.6 ± 2.16^{c}	6.3 ± 1.75^{b}	4.8 ± 2.38^{a}
GEO P	$5.1 \pm 2.27^{b,c}$	4.7 ± 2.46^{a}	5.9 ± 2.12^{b}
GEO NP	$4.5 \pm 2.33^{a,b}$	4.5 ± 2.31^{a}	4.7 ± 2.33^{a}
MIB P	$4.7 \pm 2.27^{a,b}$	4.4 ± 2.35^{a}	5.8 ± 1.87^{b}
MIB NP	4.2 ± 2.33^{a}	4.1 ± 2.45^{a}	4.6 ± 2.32^{a}

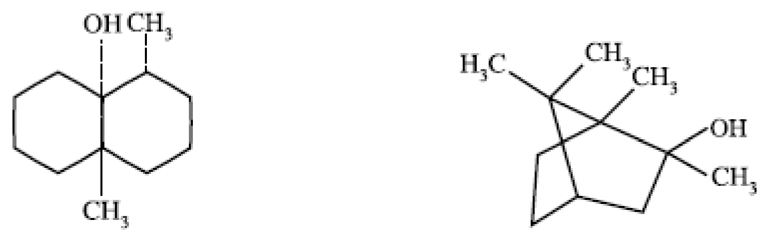
* 9 = like extremely; 8 = like very much; 7 = like moderately; 6 = like slightly; 5 = neither like nor dislike; 4 = dislike slightly; 3 = dislike moderately; 2 = dislike very much; 1 = dislike extremely.

$^{a\text{-}d}$ Means appearing in the same column with different superscript are significantly different ($P < 0.05$)

Data represents means of replicated samples ± standard deviation of the mean

Abbreviations are as follows where:

CON = Control; MIB = 2-methylisoborneol; GEO = geosmin; NP = Non processed; P = Processed



Geosmin 2-Methylisoborneol

Figure 1. Compound names and chemical structure of geosmin and MIB

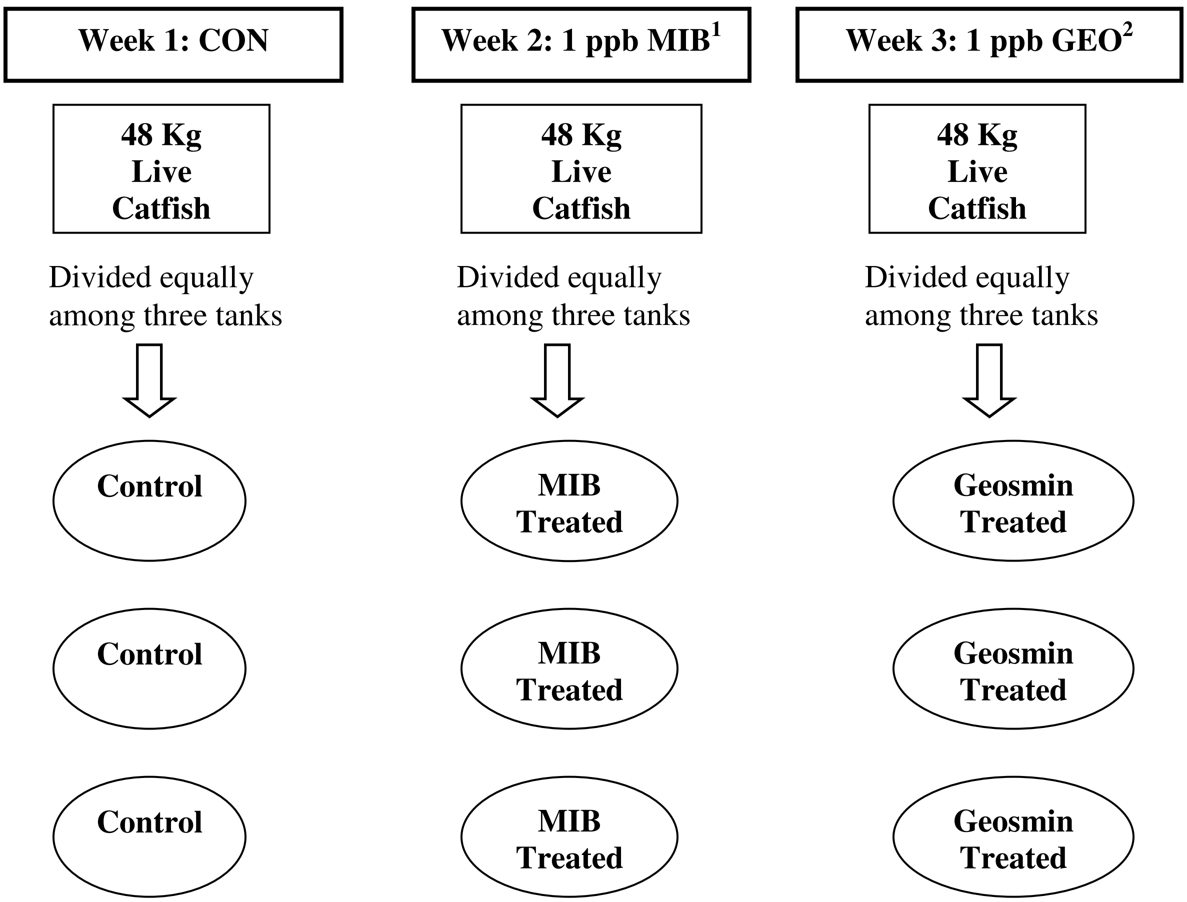


Figure 2: Schematic diagram of fish tanks selected for control and spike treatments to produce a consumer acceptable product from off-flavor catfish.
CON = control; MIB = 2-methylisoborneol; GEO = geosmin;
[1] tanks spiked with 1 ppb MIB (98% purity; 10 mg/ml solution in methanol; Sigma-Aldrich Inc, St. Louis, MS).
[2] tanks spiked with 1 ppb geosmin (98% purity; 2mg/ml solution in methanol; Sigma-Aldrich Inc. St. Louis MS).

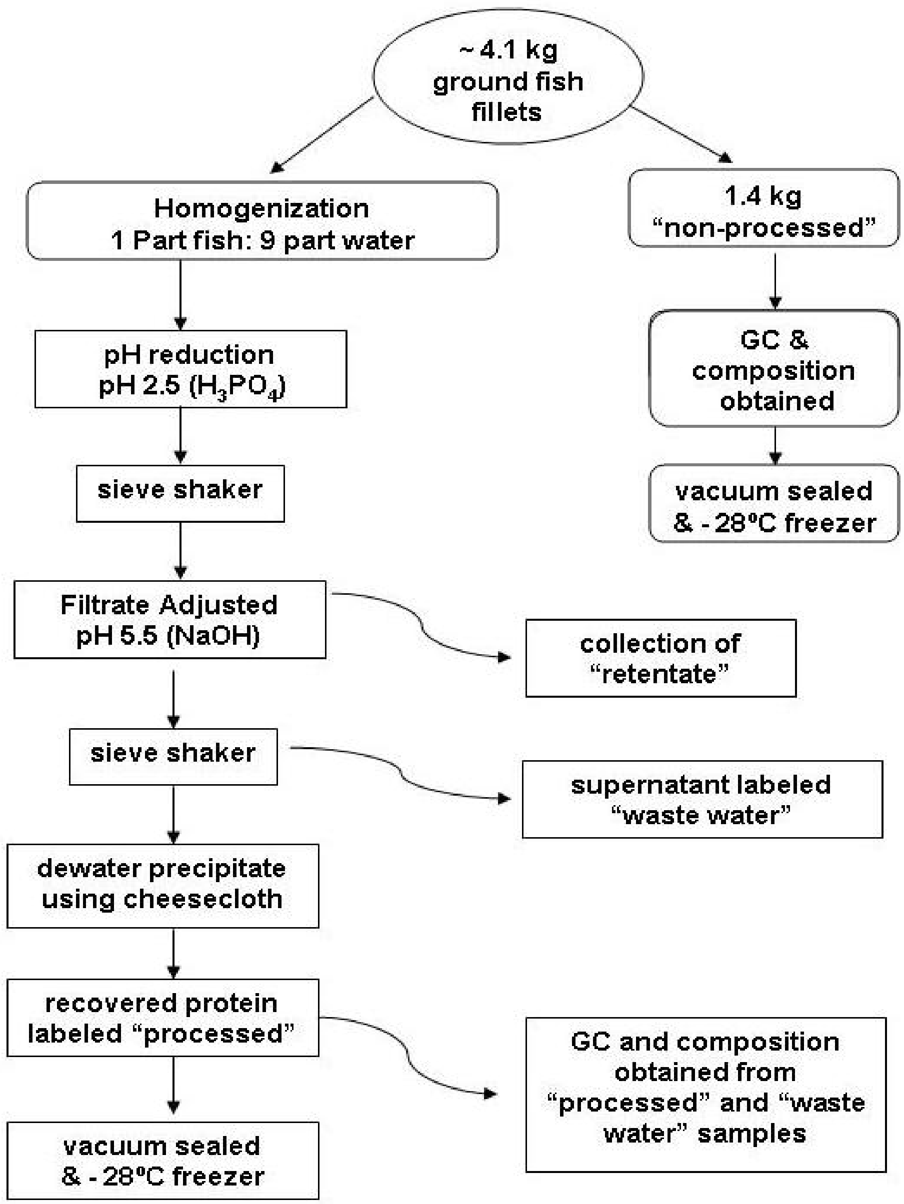


Figure 3: Schematic diagram of ground fillet of catfish distributed for protein solubilization process and non-process to produce a consumer acceptable product from off-flavor catfish.
GC = solid-phase microextraction and gas chromatography analysis

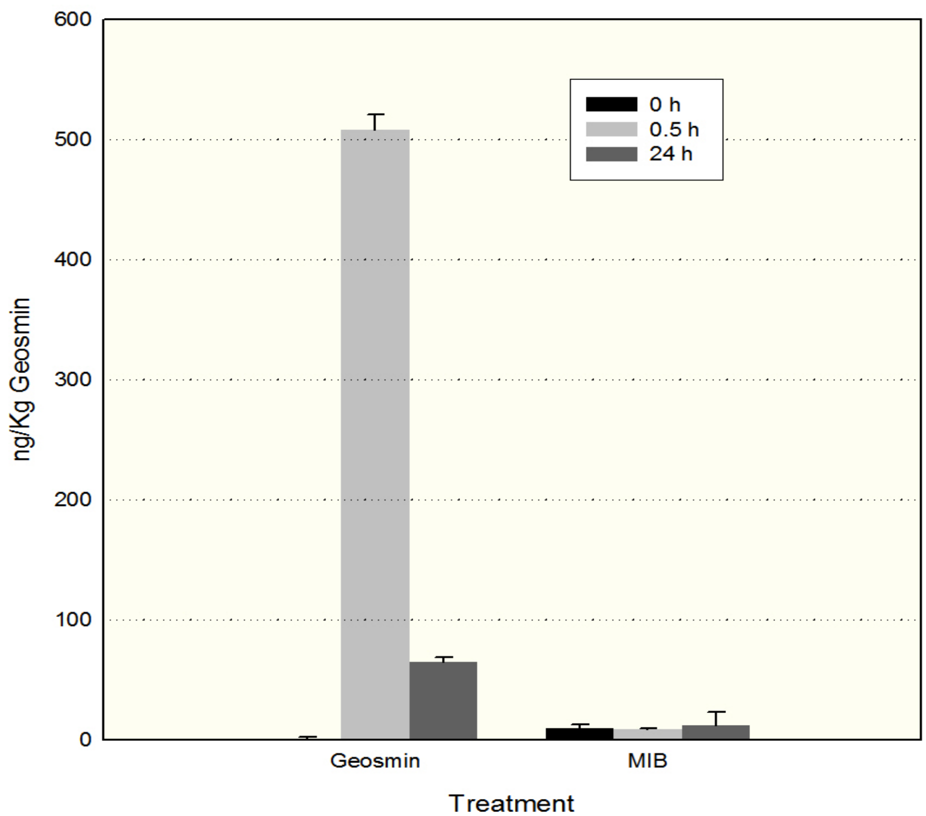


Figure 4: Absorption of geosmin (expressed in ng/Kg) by geosmin and MIB spiked tanks measured with solid-phase microextraction and gas chromatography analysis (GC) at 0 hr, 0.5 hr, and 24 hr.
Data includes standard deviation of the mean

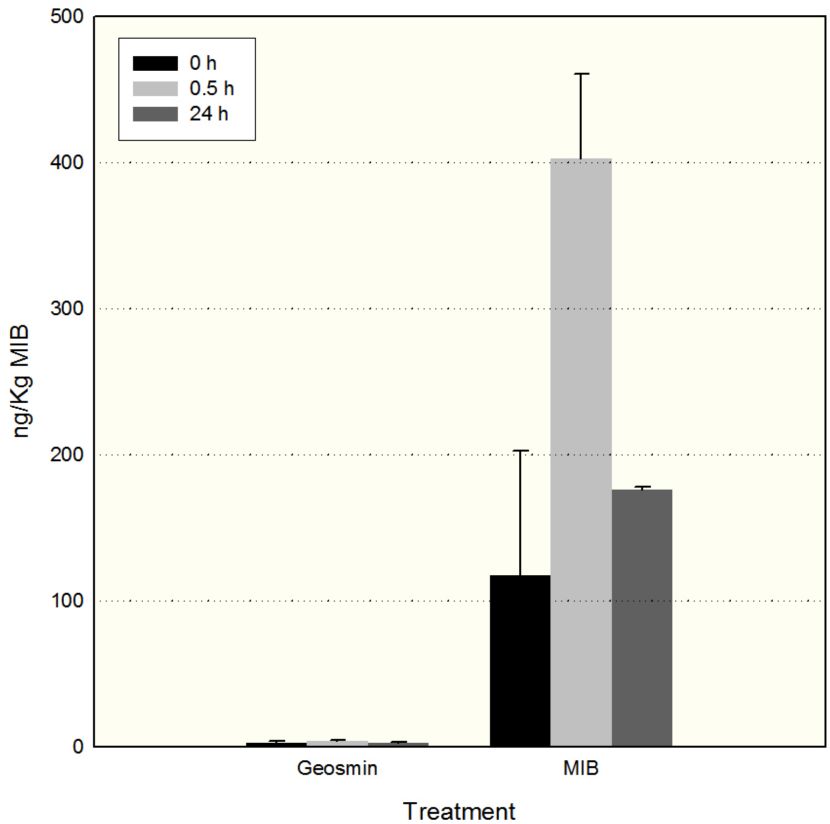


Figure 5: Absorption of MIB (expressed in ng/Kg) by geosmin and MIB spiked tanks measured with solid-phase microextraction and gas chromatography analysis (GC) at 0 hr, 0.5 hr, and 24 hr.
Data includes standard deviation of the mean

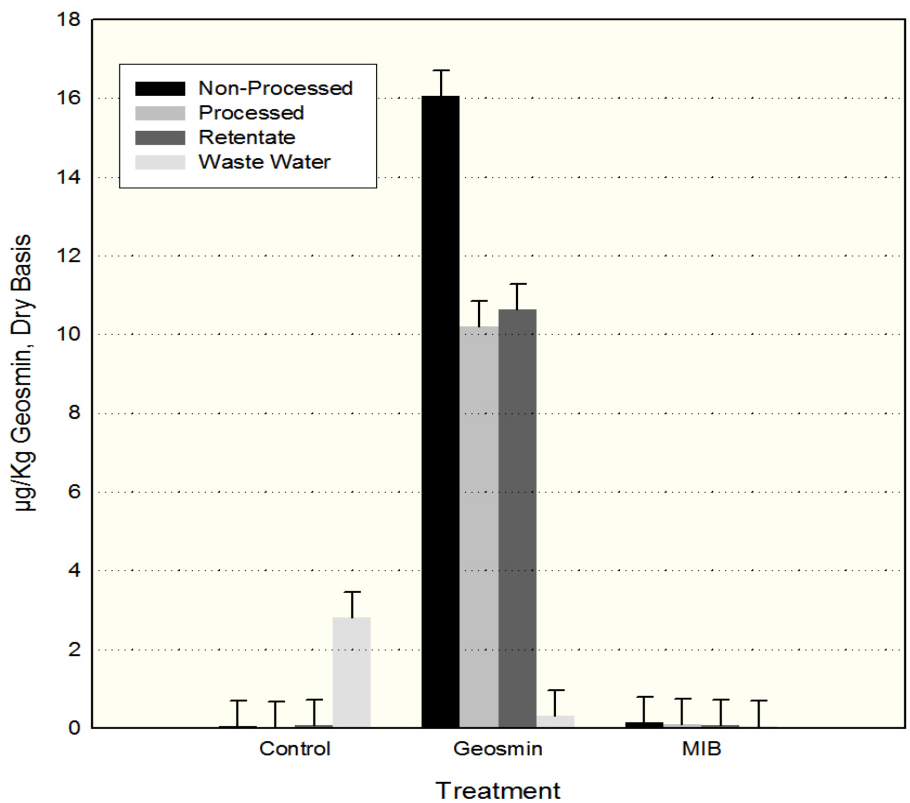


Figure 6: Concentration of geosmin on dried basis (expressed in µg/Kg) in non-processed, processed, retentate, and waste water samples obtained from control, geosmin, and MIB catfish fillet.
Data includes standard error of the mean

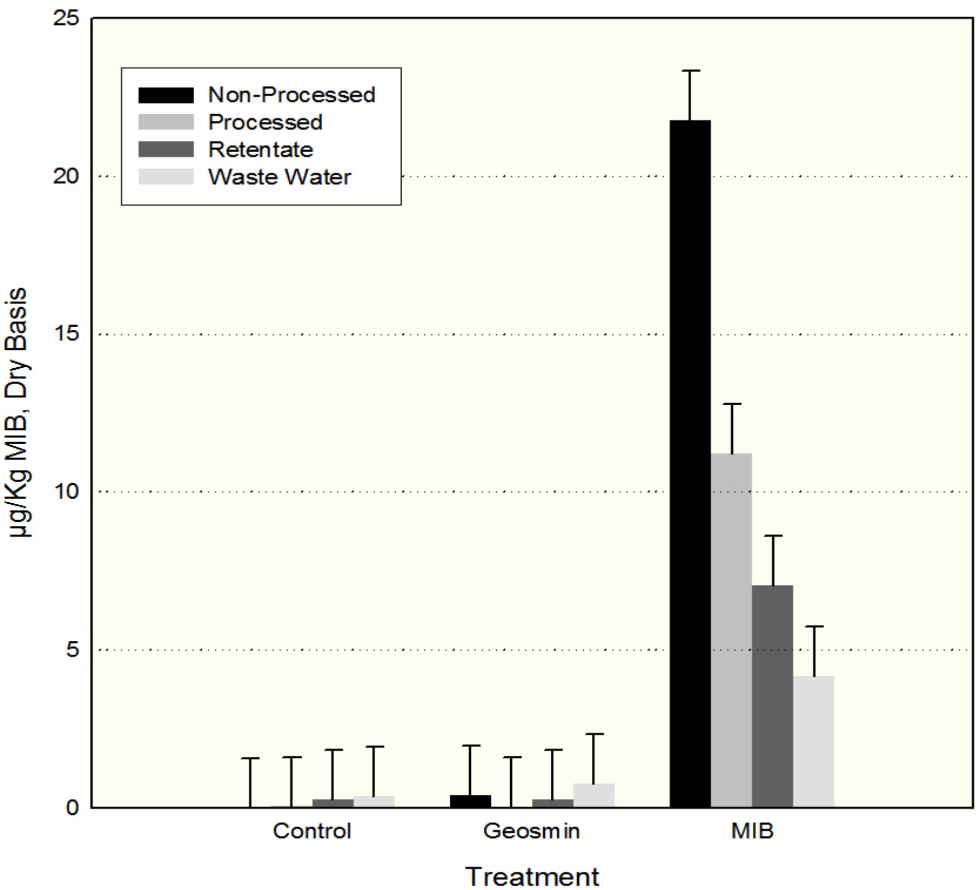


Figure 7: Concentration of MIB on dried basis (expressed in µg/Kg) in non-processed, processed, retentate, and waste water samples obtained from control, geosmin, and MIB catfish fillet.
Data includes standard error of the mean

CHAPTER IV

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CHAPTER V
APPENDIX

Appendix A

APPENDIX A
Oklahoma State University - Food Science
Catfish Sample Questionnaire

Panelist #: ____________________ Date ____________________

Product #: ____________________ Time ____________________

1. What is your Age range ?

18-25 ☐
25-35 ☐
35-50 ☐
50-up ☐

2. What is your Gender ?

Female ☐
Male ☐

3. Where are you from?

Oklahoma ☐
Other ☐ if other, where: ____________________

3. How often do you consume catfish?

once a week ☐ once a month ☐ once a year ☐
every two weeks ☐ once in six months ☐ never ☐

4. Where do you eat catfish?

home ☐ other ☐
restaurant ☐ if other, where: ____________________

5. Which method of cooking is your favorite?

fried ☐ grill ☐ other ☐
baked ☐ if other, how: ____________________

Appendix B

APPENDIX B

Oklahoma State University - Food Science
Catfish Sample Ballot

Panelist #: ______________________ Date ______________________

Product #: ______________________ Time ______________________

Please rinse your mounth before starting
Evaluate the product in front of you by looking at it and tasting it
Considerate **ALL** characteristics (odor, flavor, and texture) indicate
your **OVERALL** opinion by checking one box [X]

- ☐ Like extremely
- ☐ Like very much
- ☐ Like moderately
- ☐ Like slightly
- ☐ Neither like nor dislike
- ☐ Dislike slightly
- ☐ Dislike moderately
- ☐ Dislike very much
- ☐ Dislike extremely

Comments: Please indicate WHAT in particular you liked or disliked about this product.
(USE WORDS NO SENTENCES)

LIKED	**DISLIKED**
______________________	______________________
______________________	______________________
______________________	______________________

Catfish Nugget Liking Questions

Panelist #: ______________________ Date ______________________

Product #: ______________________ Time ______________________

Please indicate how much you much you like or dislike the following. Check the box that represents your response box [X]

Overall Catfish Flavor

☐	Like extremely	**LIKED**
☐	Like very much	______________________
☐	Like moderately	______________________
☐	Like slightly	______________________
☐	Neither like nor dislike	**DISLIKED**
☐	Dislike slightly	______________________
☐	Dislike moderately	______________________
☐	Dislike very much	______________________
☐	Dislike extremely	

Overall Catfish Texture

☐	Like extremely	**LIKED**
☐	Like very much	______________________
☐	Like moderately	______________________
☐	Like slightly	______________________
☐	Neither like nor dislike	**DISLIKED**
☐	Dislike slightly	______________________
☐	Dislike moderately	______________________
☐	Dislike very much	______________________
☐	Dislike extremely	

Appendix C

Producing a consumable acceptable product from off flavor catfish

CONTROL 1

TANK DATA:

Initial Water in Tank	1000 L
Final Water in Tank	1000 L
Total Target Pound	27 lbs
Total Chemical Added	1 ppb MIB or 1 ppb Geosmin
Purity of Chemical	98%

Fish N*	Length Inches	Initial Wt lbs	Sex	Fillet Wt lbs	% Recovery
1	17	1.66	F	0.56	33.73493976
2	16.5	1.52	F	0.48	31.57894737
3	17.5	1.82	M	0.5	27.47252747
4	18	1.86	F	0.62	33.33333333
5	18	1.92	F	0.56	29.16666667
6	17.5	1.9	F	0.54	28.42105263
7	17.5	1.9	F	0.56	29.47368421
8	18	2.02	F	0.66	32.67326733
9	17	1.84	M	0.5	27.17391304
10	16	1.36	M	0.4	29.41176471
11	19	2.48	M	0.68	27.41935484
12	17	1.96	F	0.66	33.67346939
13	17.5	2.02	F	0.5	24.75247525
14	17.5	1.9	M	0.58	30.52631579
15	18.5	2.38	M	0.76	31.93277311
16	17.5	1.92	M	0.58	30.20833333
17	17	1.64	F	0.52	31.70731707

Producing a consumable acceptable product from off flavor catfish

CONTROL 2

TANK DATA:

Initial Water in Tank	1000 L
Final Water in Tank	1000 L
Total Target Pound	27 lbs
Total Chemical Added	1 ppb MIB or 1 ppb Geosmin
Purity of Chemical	98%

Fish N*	Length Inches	Initial Wt lbs	Sex	Fillet Wt lbs	% Recovery
1	17.5	1.74	M	0.48	27.5862069
2	18.5	2.24	F	0.5	22.32142857
3	17	1.92	F	0.54	28.125
4	16	1.44	F	0.34	23.61111111
5	16	1.5	M	0.42	28
6	15.5	1.26	M	0.38	30.15873016
7	17.5	2.02	F	0.58	28.71287129
8	18	2.26	F	0.6	26.54867257
9	19.5	2.74	F	0.8	29.19708029
10	16.5	1.68	F	0.48	28.57142857
11	17.5	1.68	F	0.44	26.19047619
12	17	1.9	M	0.56	29.47368421
13	17	1.82	M	0.42	23.07692308
14	17.5	1.98	F	0.68	34.34343434
15	18.5	2.46	M	0.7	28.45528455
16	18.5	2.56	F	0.7	27.34375
17	17.5	1.82	M	0.52	28.57142857

Producing a consumable acceptable product from off flavor catfish

CONTROL 3

TANK DATA:

Initial Water in Tank	1000 L
Final Water in Tank	1000 L
Total Target Pound	27 lbs
Total Chemical Added	1 ppb MIB or 1 ppb Geosmin
Purity of Chemical	98%

Fish N*	Length Inches	Initial Wt lbs	Sex	Fillet Wt lbs	% Recovery
1	19	2.32	F	0.6	25.86206897
2	15.5	1.44	M	0.36	25
3	18	1.84	M	0.58	31.52173913
4	15.5	1.4	F	0.42	30
5	16	1.56	M	0.42	26.92307692
6	15.2	2.12	F	0.58	27.35849057
7	17	1.58	F	0.44	27.84810127
8	18.5	2.32	F	0.6	25.86206897
9	16.5	1.86	F	0.54	29.03225806
10	16.5	1.6	F	0.42	26.25
11	16.5	1.52	F	0.5	32.89473684
12	17	1.6	M	0.44	27.5
13	16	1.44	M	0.36	25
14	18	2.08	F	0.64	30.76923077
15	17.5	1.94	M	0.56	28.86597938
16	17.5	2.06	F	0.64	31.06796117
17	16.5	1.42	M	0.36	25.35211268

Producing a consumable acceptable product from off flavor catfish

GESOMIN 1

TANK DATA:

Initial Water in Tank 1000 L

Final Water in Tank 1000 L

Total Target Pound 27 lbs

Total Chemical Added 1 ppb MIB or 1 ppb Geosmin

Purity of Chemical 98%

Fish N*	Length Inches	Initial Wt lbs	Sex	Fillets Wt lbs	% Recovery
1	19	2.36	M	0.66	27.96610169
2	17.5	2.32	F	0.7	30.17241379
3	18.25	2.28	M	0.56	24.56140351
4	18	2.06	F	0.64	31.06796117
5	16.5	1.56	M	0.5	32.05128205
6	17.25	1.62	F	0.52	32.09876543
7	18	2.34	F	0.7	29.91452991
8	16.5	1.64	F	0.54	32.92682927
9	18	2.1	M	0.64	30.47619048
10	17	1.7	F	0.52	30.58823529
11	19.5	2.4	M	0.76	31.66666667
12	18	1.92	M	0.64	33.33333333
13	18.5	2.02	F	0.64	31.68316832
14	18.5	2	M	0.62	31
15	19.5	2.58	F	0.74	28.68217054
16	18.5	2.28	F	0.82	35.96491228
17	20	2.32	F	0.62	26.72413793
18	16	1.56	M	0.42	26.92307692

Producing a consumable acceptable product from off flavor catfish

GEOSMIN 2

TANK DATA:

Initial Water in Tank	1000 L
Final Water in Tank	1000 L
Total Target Pound	27 lbs
Total Chemical Added	1 ppb MIB or 1 ppb Geosmin
Purity of Chemical	98%

Fish N*	Length Inches	Initial Wt lbs	Sex	Fillet Wt lbs	% Recovery
1	17.25	1.84	M	0.53	28.80434783
2	17	1.72	F	0.5	29.06976744
3	17	1.6	M	0.48	30
4	17.75	2.04	F	0.7	34.31372549
5	17.5	1.84	M	0.54	29.34782609
6	19	2.46	F	0.84	34.14634146
7	17	2.16	F	0.62	28.7037037
8	18	2.24	F	0.72	32.14285714
9	20	2.42	M	0.66	27.27272727
10	18.5	2.12	F	0.72	33.96226415
11	19	2.18	M	0.7	32.11009174
12	18	2.16	M	0.7	32.40740741
13	18	2.02	F	0.62	30.69306931
14	18	1.96	M	0.56	28.57142857
15	16	2.04	F	0.36	17.64705882
16	19	2.4	F	0.66	27.5
17	17	1.82	F	0.58	31.86813187
18	17	1.36	M	0.4	29.41176471

Producing a consumable acceptable product from off flavor catfish

GEOSMIN 3

TANK DATA:

Initial Water in Tank	1000 L
Final Water in Tank	1000 L
Total Target Pound	27 lbs
Total Chemical Added	1 ppb MIB or 1 ppb Geosmin
Purity of Chemical	98%

Fish N*	Length Inches	Initial Wt lbs	Sex	Fillet Wt lbs	% Recovery
1	18	1.94	M	0.54	27.83505155
2	19	2.02	F	0.64	31.68316832
3	18.5	1.98	M	0.6	30.3030303
4	17.5	1.86	F	0.58	31.1827957
5	18	2.1	M	0.64	30.47619048
6	17	1.8	F	0.58	32.22222222
7	19	2.44	F	0.76	31.14754098
8	17.5	2.1	F	0.66	31.42857143
9	17	1.84	F	0.62	33.69565217
10	18	2.06	M	0.6	29.12621359
11	18.5	1.96	M	0.58	29.59183673
12	20.5	2.84	F	0.94	33.09859155
13	18.5	2.48	F	0.68	27.41935484
14	17	1.62	M	0.74	45.67901235
15	19	2.64	M	0.42	15.90909091
16	18	1.86	F	0.72	38.70967742
17	17.5	1.86	F	0.6	32.25806452
18	17	1.74	F	0.54	31.03448276

Producing a consumable acceptable product from off flavor catfish

MIB 1

TANK DATA:

Initial Water in Tank	1000 L
Final Water in Tank	1000 L
Total Target Pound	27 lbs
Total Chemical Added	1 ppb MIB or 1 ppb Geosmin
Purity of Chemical	98%

Fish N*	Length Inches	Initial Wt lbs	Sex	Fillet Wt lbs	% Recovery
1	19.75	2.36	M	0.66	27.96610169
2	17.75	2.06	F	0.6	29.12621359
3	18	2.38	M	0.66	27.73109244
4	19	2.24	F	0.48	21.42857143
5	20	2.88	F	0.9	31.25
6	17.88	1.84	M	0.48	26.08695652
7	18	2	M	0.5	25
8	20.2	2.26	F	0.6	26.54867257
9	17.6	1.84	F	0.62	33.69565217
10	18	1.5	M	0.42	28
11	16.5	1.62	M	0.44	27.16049383
12	19.4	2.58	F	0.81	31.39534884
13	19.2	2.4	F	0.7	29.16666667
14	19.5	2.38	M	0.78	32.77310924
15	22	2.62	F	0.76	29.00763359
16	17.4	1.7	M	0.52	30.58823529
17	19.4	2.32	F	0.62	26.72413793

Producing a consumable acceptable product from off flavor catfish

MIB 2

TANK DATA:

Initial Water in Tank	1000 L
Final Water in Tank	1000 L
Total Target Pound	27 lbs
Total Chemical Added	1 ppb MIB or 1 ppb Geosmin
Purity of Chemical	98%

Fish N*	Length Inches	Initial Wt lbs	Sex	Fillet Wt lbs	% Recovery
1	19.25	2.82	M	0.8	28.36879433
2	17.75	1.92	F	0.52	27.08333333
3	17	1.8	F	0.6	33.33333333
4	17.75	1.9	M	0.52	27.36842105
5	18.5	2.26	M	0.62	27.43362832
6	18.3	1.9	F	0.52	27.36842105
7	19.5	2.16	F	0.62	28.7037037
8	7.8	1.92	M	0.48	25
9	19.4	2.12	F	0.62	29.24528302
10	18.7	2.26	M	0.68	30.08849558
11	18.5	2.16	M	0.54	25
12	20	2.46	F	0.76	30.89430894
13	17.8	1.94	F	0.62	31.95876289
14	20	2.62	M	0.64	24.42748092
15	19.5	2.36	F	0.72	30.50847458
16	19	2.02	M	0.52	25.74257426
17	18.6	2.44	F	0.78	31.96721311

Producing a consumable acceptable product from off flavor catfish

MIB 3

TANK DATA:

Initial Water in Tank	1000 L
Final Water in Tank	1000 L
Total Target Pound	27 lbs
Total Chemical Added	1 ppb MIB or 1 ppb Geosmin
Purity of Chemical	98%

Fish N*	Length Inches	Initial Wt lbs	Sex	Fillet Wt lbs	% Recovery
1	18.5	1.98	M	0.5	25.25252525
2	18.25	2.24	F	0.64	28.57142857
3	18.25	2.26	F	0.72	31.85840708
4	18.25	2.58	M	0.8	31.00775194
5	19.8	2.46	M	0.78	31.70731707
6	20.5	2.86	F	0.78	27.27272727
7	18.5	2.14	F	0.62	28.97196262
8	20.5	2.64	M	0.66	25
9	19.4	2.14	F	0.66	30.8411215
10	17.3	1.68	M	0.44	26.19047619
11	18	2.02	M	0.6	29.7029703
12	19.4	2.6	F	0.7	26.92307692
13	17	1.68	F	0.36	21.42857143
14	18.6	2.04	M	0.6	29.41176471
15	18.2	2.12	F	0.62	29.24528302
16	18.4	2.24	F	0.72	32.14285714
17	17.1	1.82	F	0.54	29.67032967

Appendix D

Oklahoma State University
Institutional Review Board

Date : Friday, January 25, 2008 IRB Application No AG0622

Proposal Title: Producing a Consumer Expectable Product From Off-flavor Catfish

Principal
Investigator(s) :

Christina Mireles DeWitt
104E Animal Science
Stillwater, OK 74078

Teresa Brown
107 Animal Science
Stillwater, OK 74078

Reviewed and
Processed as: Exempt **Continuation**

Approval Status Recommended by Reviewer(s) : Pending Revision

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There are revisions to your application, which must be completed satisfactorily before your continutation will be approved. They are listed on the following page.

Please submit a revised IRB application incorporating and HIGHLIGHTING the changes listed. The revised application does not need to be signed. If any changes are required to your consent form, you must submit a new consent form incorporating the changes.

This material containing your revisions should be returned to the IRB Office at 219 Cordell North, Stillwater, OK 74078. These revisions will be reviewed by the IRB Chair and/or the review committee of the IRB. When all outstanding issues have been addressed satisfactorily, you will receive an approval letter from the Chair of the IRB.

If your current approval has expired, you may not continue this research until these revisions have been made and the IRB has granted final approval to continue the research using human subjects under this protocol. You will be allowed 60 days to respond satisfactorily to the revisions required by the IRB. After that period, your protocol will be CLOSED.

If you have questions or wish to discuss the reviewers' comments, please contact Beth McTernan at 405-744-5700 or via e-mail at beth.mcternan@okstate.edu.

VITA

Teresa Marie Brown

Candidate for the Degree of

Master of Science

Thesis: PRODUCING A CONSUMER ACCEPTABLE PRODUCT FROM OFF-FLAVOR CATFISH

Major Field: Food Science

Biographical:

Personal Data: Born in San Diego California, April 18, 1980

Education: Graduated from Lincoln Professional Academy for Medical Sciences (High School) in 1998, San Diego California
Received Bachelors of Sciences Degree in Animal Science from Oklahoma State University, Stillwater, Oklahoma in May 2005
Completed Master of Science Degree in Food Science at Oklahoma State University, Stillwater, Oklahoma in July 2008

Experience: Graduate Student 2005- Present

Professional Memberships: Institute of Food Technologist

Name: Teresa Marie Brown Date of Degree: May, 2009

Institution: Oklahoma State University Location: Stillwater, Oklahoma

Title of Study: PRODUCING A CONSUMER ACCEPTRABLE PRODUCT FROM OFF-FLAVOR CATFISH

Pages in Study: 66 Candidate for the Degree of Master of Science

Major Field: Food Science

Scope and Method of Study: The objectives of this research were to determine if catfish fillets further processed using the acidic protein solubilization method to remove muddy off-flavors caused by 2-methylisoborneol (MIB) and geosmin could be further used to produce a consumer acceptable product

Findings and Conclusions: Farm-raised catfish weighing two pounds were placed into three tanks each containing 1000 L of water. Treatments were grouped by week: week 1: control (Con), week 2: 1 ppb geosmin and week 3: 1 ppb 2-methylisoborneol (MIB). Treated catfish were sorted into three replicates containing equal male: female ratios. Fillets from each replicate were ground. A portion was kept and labeled as "non-processed". The remaining portion was solubilized to pH 2.5 and filtered through a sieve. The filtrate was collected and labeled "retentate". Processed and non-processed protein from each treatment were evaluated for geosmin and MIB analysis. A consumer acceptance panel was conducted on catfish samples from each treatment (n = 6). Processing decreased % moisture, fat, ash, and collagen content of catfish protein. Flavor rated significantly higher for control samples than geosmin and MIB. There was a slight trend in flavor but not enough to be statistical, that consumers preferred processed samples compared to non-processed. Texture of catfish samples from processed protein was preferred to non-processed samples. Control samples ranked significantly higher for overall liking than other treatments except for geosmin processed. Also there was a slight preference, not enough to be statistical, for overall acceptability of processed sample over non-processed.

ADVISER'S APPROVAL: Christina A. Mireles DeWitt

ADVISER'S APPROVAL: ____