ACID SOLUBILIZATION ISOELECTRIC PRECIPITATION TO REMOVE OFF ODORS AND FLAVORS ASSOCIATED WITH FARM RAISED CHANNEL CATFISH

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Bachelor of Science

Oklahoma State University

Stillwater, Oklahoma

2002

Submitted to the Faculty
of the Graduate College of
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
July, 2005

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PRECIPITATION TO REMOVE OFF

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CATFISH

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ACKNOWLEDGMENTS

There are many acknowledgments that should be made in regard to the people that have strengthened my ability to succeed as a graduate student and the space required to do so would be many times greater than the length of this thesis. Therefore, for conservations sake, I will limit most of my thanks to those who have contributed to the work of this thesis.

I would first like to thank Dr. Christina Mireles DeWitt for accepting me as a graduate student. Her good character has made it a pleasure for me to have her as an Advisor. The opportunity she has given me has allowed me to gain more knowledge and has resulted in many professional and personal achievements. Thanks.

A special thanks is extended to my wife Kimberly. The many hours and late nights she spent helping with this project are greatly appreciated. Without her help, this project would have taken much longer to complete. She has been a reassuring and loving presence that has been invaluable throughout my graduate career.

Thank you to Dr.Conrad Kleinholz of Langston University for serving on my committee. This project would have not been possible without the help and support of him and his staff.

The author wishes to thank the following for their help with performing this project: Stanley Thomas, Dustin Vann, Chris Bilby, Jill Leslie, and employees of the Food and Ag Products Research and Technology Center. In addition, I would like to

thank Linda Guenther and Kris Novotny for helping with proximate analysis and for the many lunches you provided. My mother would be glad you helped keep me fed. Thanks to my Canadian friends Catherine Gelinas and Josee Amyot for all the help with data entry and analysis.

Thank you to committee member Dr. Danielle Bellmer for the help with texture analysis and to Dr. Stanley Gilliland for taking the time to serve on my committee. A special acknowledgment is extended to Dr. Stephen Kelleher and his staff for their help, support, and equipment use for the acid solubilization isoelectric precipitation process. This project was supported under HATCH project OKLO2347.

Thank you to all my family and friends for your time and support.

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FORMAT OF THESIS

This thesis is presented in the Journal of Food Science style format, as outlined by the Oklahoma State University graduate college thesis handbook. The use of this format allows for independent chapters to be suitably prepared for submission to scientific journals.

Chapter I

INTRODUCTION

Channel Catfish (*Ictalurus punctatus*) is the most abundant aquaculture species produced in the United States with over 650 million pounds being processed in the year 2003. Growth in this sector of aquaculture, however, has not seen its full potential due to the uncertainty of producing a high quality, consumer acceptable product. Many catfish producers have been burdened with this uncertainty due to chronic management problems, resulting in off-odor/flavor catfish. Off-odor and off-flavor catfish results from two compounds that can reside in the water in which catfish are raised. The compounds responsible for these off-odors and flavors are produced by blue-green algae and actinomycetes, which are naturally occurring in many aquaculture ponds. The off-odor/flavor compounds are metabolites of these microorganisms and are known as geosmin [*trans*-1,10,-dimethyl-*trans*-(9)-decalol] and 2-methylisoborneol (*exo*-1,2,7,7-tetramethyl-[2.2.1]heptan-2-ol) which have been characterized as having earthy and musty odors or flavors (Schrader and Rimando 2003). The resulting off-odor/flavors decrease profit margins for producers because processing must be delayed until the off-odor/flavor is absent, or the off-odor/flavor product is processed as a less valuable by-product such as fish meal. Delayed processing leads to increased costs because the producer must continue to feed the fish on a maintenance diet until the off-odor/flavor subsides; therefore there are increased feeding costs, higher mortality rates, and fish can

grow beyond the optimal size for processing. Certain pre-harvest methods have been utilized to eliminate the off-odor/flavor compounds in catfish with varying degrees of success. Purging the live fish in continuously flowing fresh water has been shown to be an effective means of off-odor/flavor reduction. This practice, however, is not used commercially due to the excessive labor and water costs incurred when moving the fish to fresh water, as well as increased fish loss due to handling stress and disease. In addition to these disadvantages the time required for successful purging can be 3-5 days for fish containing MIB and up to 4 weeks for fish containing geosmin (King and Dew 2003).

Another pre-harvest method that has been used employs the use of algicides to kill the microorganisms that produce the off-odor/flavor compounds. Although this method is effective, it has not been used extensively due to the fact that these algicides destroy other microbial species that are beneficial to aquaculture ponds. Furthermore, as explained by King and Dew (2003), when algae die and decompose oxygen is lost from the pond water and can result in suffocation and death of fish. King and Dew (2003) also indicate that some algicides, such as copper-based products may be toxic to fish if applied incorrectly, and that the algicides can create an environmental concern if they are too persistent.

The inability of these pre-harvest methods to provide an efficient, safe, and economical solution to the off-odor/flavor problem has led researchers in search of an effective post-harvest solution. One post-harvest method that has been studied involves the use of food-grade acid to chemically dehydrate the off-odor/flavor compounds geosmin and MIB into non-odiferous products. Forrester and others (2002) found that a

2% citric-acid treatment applied to catfish fillets while vacuum tumbling could reduce MIB concentrations by up to 36.8%.

The research outlined in this thesis stems from a patented process for extracting myofibrillar proteins from collagen and fat using an acid solubilization technique that separates these fleshy components based on their solubility differences (Hultin and Kelleher 1999). Kelleher and Hultin (2000) showed that the myofibrillar proteins of light and dark chicken meat could be processed and recovered while producing a product with a significantly reduced fat content and good gel strength

By applying this method to the off-odor/flavor catfish fillets there is a resulting increase in the tissue surface area, thus creating a more favorable condition for the acid to come in contact with the off-odor/flavor compounds. This condition should allow for a more efficient dehydration of the off off-odor/flavor compounds as compared to the method of vacuum tumbling. In addition, it was hypothesized that reduction or removal of the fillet lipid components would decrease the amount of off-odor/flavor compounds present by physically removing them from the fillets. Geosmin and MIB, which have been shown to be lipophilic in nature, are found primarily in the fatty tissues of catfish (van der Ploeg and others 2001). Therefore it was hypothesized that a process which removes fatty components from the catfish fillet could be beneficial in eliminating or reducing off odors and flavors from catfish fillets.

The purpose of this research was to apply an acid solubilization process as a post-harvest processing technique and evaluate its effectiveness at eliminating or reducing the off odors and flavors associated with catfish fillets.

LITERATURE CITED

Forrester PN, Prinyawiwatkul W, Godber JS, Phlak LC. 2002. Treatment of catfish with citric acid causes reduction of 2-methylisoborneol, but not musty flavor. J Food Sci 67(7):2615-2618

Hultin HO, Kelleher SD. 1999. Process for isolating a protein composition from a muscle source and protein composition. Patent # 6, 005, 073 (Dec 21).

Kelleher SD, Hultin HO. 2000. Functional chicken muscle protein isolates prepared using low ionic strength, acid solubilization/precipitaion. In: 53rd Annual Reciprocal Meat Conference: Meat Science in the New Millennium 2000. Savoy, IL: American Meat Science Association. p 76-81.

King J, Dew T. 2003. Catfish off-flavors and their elimination. In: Schrader KK, Rimando AM. 2003. Off-Flavors in aquaculture. Washington, D.C.: American Chemical Society. p 31-44.

Schrader KK, Rimando AM. 2003. Off-Flavors in aquaculture. Washington, D.C.: American Chemical Society. p 1-12.

van der Ploeg M, Tucker C, Steeby J, Weirich C. 2001. Management plan for blue-green off-flavors in Mississippi pond raised catfish. Mississippi State University extension service. Publication # 2001.

CHAPTER II

REVIEW OF LITERATURE

Catfish Production

Channel catfish is the most abundant aquaculture species produced in the United States, with over 285 million kilograms valued at $366 million being produced in the year 2003 (Harvey 2004). With the U.S. per capita consumption of catfish estimated at 0.5 kilograms in 2003, it is the fifth most popular seafood item consumed in the U.S, behind pollock, salmon, canned tuna, and shrimp (National Fisheries Institute 2004). In 2003 there were approximately 74,800 hectares of catfish production operated by 1,161 producers in more than 16 states (Lange 2004)). In 1999 the National Agricultural Statistics Service (USDA 2000) reported that there were approximately 13 operations producing catfish in Oklahoma.

Commercial catfish production ponds are stocked with up to 30,000 fish per hectare, taking approximately 18 months to produce a market size catfish. A typical farm-raised market size catfish will weigh between 0.68 and 0.91 kilograms (or 1.5-2.0 lbs), which is the optimal size for processing equipment. If the fish grow beyond this optimal size, producers will receive less money upon marketing the fish to processors (Hanson 2003).

Catfish products are sold in a variety of different forms, such as whole dressed fish, fillets, steaks and nuggets (belly flap section removed from fillet) as well as many

other forms (Sylvia and Dean 2001). One of the most common products sold however is the fillet, which is sold either with the belly flap tissue present or without the belly flap, with the latter form being called a shank fillet. The fillet is denuded of the skin, bones, and cartilage and can vary in size and weight.

The basic composition of raw channel catfish fillets is:

Table 1. Composition of Raw Channel Catfish Fillet [1, 2]

MOISTURE	FAT	PROTEIN	ASH	CARBOHYDRATE[3]
75.38 %	7.59 %	15.55 %	1.00 %	0.00 %

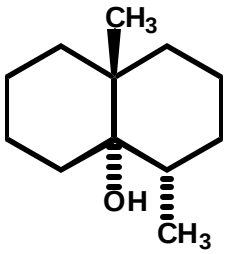
[1] USDA (2004).
[2] Values reported as per 100 g of edible portion.
[3] Carbohydrate determined by difference.

Off-odor/flavor Catfish

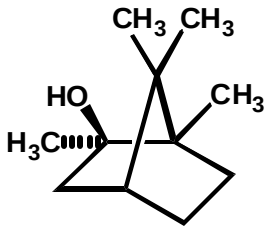
Farm raised channel catfish are grown in aquatic environments normally occupied by large populations of prokaryotic and eukaryotic microorganisms. These naturally occurring microorganisms can benefit the ecosystem of an aquaculture pond in many ways. Cyanobacteria, which are prokaryotic microorganisms often referred to as blue-green algae, produce oxygen as a beneficial byproduct of photosynthesis. Actinomycetes are filamentous bacteria that are common inhabitants of the soil, and are capable of metabolizing many organic materials that can be found in soils and aquatic environments (Tortora and others 1998). In contrast to the benefits these microorganisms provide, the metabolic byproducts of these microorganisms are the source of the off-odor and off-flavor problems associated with farm raised catfish. Juttner (1995) has stated that blue-green algae are the main source of the musty and muddy off-flavors found in aquaculture ponds. The genera of blue-green algae that have been associated with earthy/musty off-flavors include *Anabaena, Aphanizomenon, Nostoc,* and *Oscillatoria* (King and Dew

2003). Actinomycetes, specifically from the genera *Streptomyces* and *Nocardia*, have been found to produce compounds responsible for giving soil and aquaculture ponds an earthy-muddy off-odor (Tortora and others 1998). The two compounds produced by these microorganisms that are predominately associated with causing off-odor/flavor catfish are geosmin [*trans*-1,10,-dimethyl-*trans*-(9)-decalol] and 2-Methylisoborneol (*exo*-1,2,7,7-tetramethyl-[2.2.1]heptan-2-ol, otherwise known as MIB). King and Dew (2003) indicate that these compounds have hydroxyl groups which provide dual solubility characteristics, and they are terpene compounds, which imparts their volatile characteristics. Both compounds are also classified as bicyclic tertiary compounds (Diongi 1993) and provided in figure 1 below is their chemical structure.

Figure 1. Structures of Geosmin and MIB



Geosmin



2-Methylisoborneol

The off-odor/flavor compounds can enter fish in various ways. The compounds can be absorbed through the skin, or by ingesting the off-odor/flavor microorganisms. However, most absorption occurs as the compounds pass by the gill filaments of catfish during respiration (King and Dew 2003). Although these compounds possess dual solubility characteristics, in live catfish they tend to accumulate in the lipid-containing tissues. Research has indicated that catfish with higher fat contents absorb and store higher concentrations of off-odor/flavor compounds than their leaner counterparts. In a study conducted by Johnsen and Lloyd (1992) it was found that catfish with muscle fat

contents of 2.5% or more absorbed nearly three times more MIB than fish with fat contents of 2% or less.

The human sensory threshold concentration at which these off-odor/flavor compounds can be detected in fish varies for both geosmin and MIB. As cited by Forrester and others (2002), the sensory threshold concentration for geosmin in fish ranges from 0.6 to 8 ppb, whereas the threshold values for MIB range from 0.09 to 200 ppb. The actual consumer threshold value of MIB has been reported to be much lower than the upper limit of 200 ppb and King and Dew (2003) have reported it to be 0.7 ppb.

Economic Impact

Research has indicated that up to 80 % of harvestable fish can be off-flavor during any one year (King and Dew 2003) These off-odor/flavor catfish can create an economic burden that substantially decreases profit margins for producers. There are many factors that affect profitability for catfish producers, but production efficiency is one of vital importance. Hanson (2003) explained that efficiency in catfish production requires quick production and turnaround during the stocking, growing, harvesting, and restocking phases. Processors will not accept off-odor/flavor catfish; therefore if catfish producers find their fish to be off-odor/flavor, harvesting must be delayed until the it subsides. This delay in harvesting decreases production efficiency and profits in several ways. While waiting for the fish to become acceptable, producers must continue to feed the fish on a maintenance diet to keep them from growing beyond the optimal size for processing equipment. If the fish do grow beyond the optimal processing size, producers may receive a lower price, in addition to the added feed and labor costs incurred during the extended grow-out period. During an extended grow-out period, fish mortality rates can

increase as a result of disease, poor water quality, and predation. The duration of off-odor/flavor episodes varies greatly and occurs more frequently in the warmer months of the year when microbial growth in aquaculture ponds is at its peak. Tucker and others (2001) found that off-odor/flavor episodes resulted in more than nine months of additional grow-out days.

Off-odor/flavor determination is another scenario that producers must address. Most processors require catfish ponds be tested for on-flavor status several times before harvest and delivery to the processing facility. Producers will obtain 2-3 fish a week before harvest and transport them to the processing facility for on-flavor approval. The fish are then tested the day before, and the day of harvest, for reassurance that the fish have not developed any off-odors or flavors. Human sensory evaluation is used to determine if catfish contain any off-odor/flavors. Experienced checkers can often detect MIB in catfish samples at concentrations as low as 0.2 ppb (King and Dew 2003). The fish sample is generally cooked in a microwave oven and immediately evaluated using olfactory senses, followed by tasting of the sample if the olfactory evaluation could not detect any odor. If the catfish are found to be off-odor/flavor, the processor will not accept the fish and harvest must be delayed until the fish are determined to be on-flavor. Direct expenses for fish sampling and repeated trips to the processing plant are paid by the producer not the processor (Hanson 2003). Hanson (2003) stated that the cost of off-flavor to the catfish industry was estimated to be between $15 and $23 million annually between 1997 and 1999. Hanson (2003) also indicated that various independent studies estimated additional costs of production due to off-flavor delays to be between $0.01 and

$0.25 per kilogram of catfish produced, which could constitute 3-17% of total production costs.

Pre-harvest Elimination of Off-Odors And Flavors

One pre-harvest method used to remove geosmin and MIB from live catfish has been to purge the fish in clean water. This method involves transferring the catfish to a continuously flowing body of water which is free of the off-odor/flavor compounds. Although this method is efficient at removing off-odor/flavor compounds, it is impractical for a production type setting, due to added labor costs and increased fish loss from handling stress and disease. Purging fish can also require varying lengths of time, which are dependent upon such factors as water quality, fat content of fish, size of fish, water temperature, and off-odor/flavor compound concentration (van der Ploeg and others 2001). In general, increased fat content and fish size, as well as lower water temperature, will increase the amount of time required to successfully purge fish. As cited by King and Dew (2003), MIB can usually be purged within 3-5 days, but geosmin is more difficult to purge and can take up to 3-4 weeks to be reduced below sensory levels.

Another method that has been used to eliminate off-odor/flavors in catfish involves the use of chemical algicides, such as copper based products, to inhibit the growth of off-odor/flavor producing cyanobacteria in aquaculture ponds. One positive attribute of this method is that labor and chemical costs are low in comparison to other pre-harvest methods. In contrast to these low costs, many algicides are not selective just towards off-odor/flavor producing cyanobacteria. Therefore, many beneficial algae are killed along with the unwanted microorganisms. In addition, when algae die and

decompose, oxygen is lost from pond water and can result in suffocation and death of fish (King and Dew 2003). Another issue concerning copper based algicides is that there is a small margin of difference between the amounts that kill cyanobacteria and the amount that is toxic to fish. Therefore, if applied incorrectly, large amounts of fish can be lost due to poisoning. Furthermore, Tucker and van der Ploeg (1999) state that treatments such as copper sulfate cause poor water quality and more aeration is required in copper treated ponds than in untreated ponds. One chemical that has shown promise is Diuron, which is a chemical that is known to have algicidal properties at low concentrations, has a wide margin of safety between algicidal concentrations and those that are toxic to fish, and is decomposed by microbial activity and thus should not accumulate in pond bottom soils (Zimba and others 2001). This product, however, is only legal to use when permission is granted on an annual basis by the United States Environmental Protection Agency.

The use of biochemical methods to inhibit the synthesis of geosmin and MIB by aquatic microorganisms has been studied. One type of biochemical method that has been suggested involves the use of a chemical which enhances biotransformation of off-odor/flavor compounds in fish. However, as indicated King and Dew (2003), these methods are experimental only and are not currently being used in the aquaculture industry.

Post-Harvest Elimination of Off-Odors and Flavors

The lack of effectiveness, along with other disadvantages associated with pre-harvest elimination of off-odor/flavors, has led researchers in search of a post-harvest remediation to the off-odor/flavor issue. An effective post harvest solution should

eliminate or reduce off-odor/flavor compounds below human detection levels while producing a useful and desired end product.

Due to the fact that geosmin and MIB are bicyclic tertiary alcohols, researchers have found that these compounds can be chemically dehydrated to non-odiferous products. Acid catalyzed dehydration of tertiary alcohols occurs through the mechanism of E1 elimination. This mechanism involves the formation of a carbocation intermediate and the loss of H_2O to form an alkene (Brown 2000). Forrester and others (2002) indicate that MIB will dehydrate to 2-methylenebornane and 1-methylcamphene, whereas geosmin will dehydrate to a compound named argosmin.

Forrester and others (2002) performed a study to determine if MIB in catfish fillets could be degraded using the concept of acid dehydration. The authors used food-grade citric acid at concentrations of 0.5% and 2.5%, coupled with vacuum tumbling for 10 min in an attempt to achieve their objective. The treatments were applied to catfish fillets containing MIB levels of 1 to 8 µg/kg. Gas chromatography/mass spectometry data indicated that MIB decreased from 4.43 to 2.80 µg/kg (36.8% decrease) as a result of vacuum tumbling the fillets in a 2% citric acid solution. The authors also indicated that the reduction of MIB may have been a direct result of chemical dehydration, or as an indirect result of the leaching of fat and protein, which was greatest for the acid treated fish. Leaching or removing fat from catfish fillets could be instrumental in off-odor/flavor reduction due to the fact that the compounds associate with the fatty tissues in catfish.

Surimi

Surimi is the term used to describe stabilized myofibrillar proteins obtained from fish flesh that has been mechanically deboned, washed with water, and blended with cryoprotectants (Park and Morrissey 2000). This modern definition of surimi is slightly different than surimi that was produced prior to the 1960s. Surimi is a term that was originally coined by the Japanese to describe ground fish paste. The paste was derived from minced fish and was a pre-cursor to the Japanese fish product known as kamaboko. The name kamaboko is used to describe a number of different processed fish products consumed by the Japanese. Primarily, a half-cylinder fish item, mounted on a wooden plate and heated, is specifically termed "kamaboko," whereas other kamaboko products would include broiled and deep-fat fried products (Okadu 1992). Pre-1960 surimi was produced in low volume establishments that were required to use the refrigerated product within a few days of production due to quality issues. This early form of surimi could not be kept in frozen storage because protein denaturation would occur, thus yielding a product with poor protein functionality and gelling capabilities. However, in 1959 Nishyia and others developed a processing technique that prevented the denaturation of muscle proteins in surimi during frozen storage (Okadu 1992). This processing technique involved adding low-molecular weight carbohydrates, such as sucrose and sorbitol, along with polyphosphates to maintain protein functionality during frozen storage.

The development of stable frozen surimi, along with an increased demand of it for fish sausage manufacture, resulted in increased surimi production throughout the 1960s and early 1970's in Japan. In the 1970's new processing techniques allowed for the production of surimi seafood items, such as imitation crabmeat and other seafood

analogs. Due to the popularity of products like imitation crabmeat, surimi production expanded to the United States, Korea, and Europe, with production reaching 7500 metric tons for the U.S. in 1996 (Park and Morrissey 2000)

Alaska pollock is the primary fish species used for surimi production. Park and Morrissey (2000) state that the most suitable species for surimi processing are those with white flesh and low fat content, which include species such as Pacific whiting (*Merluccius productus*), hoki (*Macruronus novaezelanliae*), and yellow sole (*Solea* lutea) to name a few. Surimi production occurs aboard processing ships or land-based facilities, which are strategically located near shore. Fish are processed in less than 12 hours to obtain optimal surimi quality, but can be stored up to 48 hours before product quality declines rapidly. The fish are first sorted by species and size followed by a washing step. If the fish are being processed as a skin on item, the scales must be removed to prevent clogging of deboning machines. The viscera, head, and backbone are then removed followed by mechanical deboning. Deboning separates the fish tissue from bones, cartilage, and skin. The resulting minced product is then leached extensively with fresh water to remove lipids, sarcoplasmic proteins, and other water-soluble matter. By removing these constituents from the fish mince there is a resulting improvement in color, taste, and gel forming ability. A refining step is performed to remove any residual connective tissue, bones, skin and scales. The refined mince is then dewatered using a screw press to achieve a final moisture content of 82-85%, followed by the addition of cryoprotectants. A blend of 4% sucrose along with 4% sorbitol and 0.2-0.3%-tripolyphosphate are usually the desired concentrations and types of cryoprotectants that are used. The resulting surimi is then frozen to an internal temperature of $-25^{o}C$.

Kim and others (1996) conducted research to determine the efficacy of using fillet frames from channel catfish for surimi production. One of their objectives was to determine the gel-forming behavior of surimi made from washed or un-washed deboned catfish mince. Gel-forming ability data indicated that surimi prepared from washed catfish mince was comparable to that of commercial Alaskan pollack surimi gels. The authors also indicated that catfish surimi produced from fillet frames has functional properties with potential use for commercial production of shellfish analogs and other fabricated products.

Acid Solubilization Isoelectric Precipitaion (Acid-SIP)

The surimi process described above involves the concentration of functional myofibrillar proteins, while decreasing lipids, sarcoplasmic proteins, and other water-soluble constituents. Various processes have been studied and developed to isolate and recover myofibrillar proteins from other muscle components based on differences in their solubility. One process, patented by Hultin and Kelleher (1999) involves the extraction of myofibrillar proteins from collagen, fat, and bone by using an acid solubilization technique that can separate these fleshy components based on their solubility differences. Kelleher and Hultin (2000) showed that by applying this process to light and dark chicken meat, the myofibrillar proteins could be solubilized and recovered while producing a product with a significantly reduced fat content and good gel strength. The authors also demonstrated that this process could be used to separate fish proteins from bone-in fish tissues by utilizing low pH and low ionic strength solubilization, coupled with high force centrifugation (Hultin and Kelleher, 1999).

The generalized acid solubilization isoelectric precipitation (Acid-SIP) process is performed by homogenizing muscle tissue in cold water at a ratio of 1:9, respectively. Protein solubilization is achieved by lowering the pH of the homogenate to approximately 2.8 with some form of acid. The homogenate is then centrifuged using high g force centrifugation (greater than 10,000 x g) to separate the solubilized proteins from other tissue components such as neutral and polar lipids. The proteins are then precipitated by raising the pH of the solution to approximately 5.8, followed by a dewatering step.

Objective and Scope of Research

The research presented in this thesis was performed by applying the concept of acid solubilization isoelectric precipitation (Acid-SIP) to off-odor/flavor catfish fillets. By applying the modified Acid-SIP processing method to the catfish fillets there is a resulting increase in tissue surface area. The increased surface area should create a more favorable condition for the acid to come in contact with the off-odor/flavor compounds as compared to the aforementioned method of vacuum tumbling in citric acid. Therefore, chemical dehydration of the off-odor/flavor compounds should be enhanced. In addition, by reducing or removing fillet lipid components, it was hypothesized that there would be a resulting decrease in the amount of off-odor/flavor compounds present in the processed tissue This hypothesis is supported by the fact that the geosmin and MIB are lipophillic and tend to associate with the lipid components of catfish tissues. The high speed centrifugation used with the Acid-SIP process removes polar lipids, such as membrane lipids, and may aid in the removal of tightly bound off-odor/flavor compounds. It was also thought that the washing effect of the process would also decrease the amount of off-

odor/flavor compounds present as well. Accordingly, the objective of this research was to apply an acid solubilization process as a post-harvest processing technique and evaluate its effectiveness at reducing or eliminating the odors and flavors associated with catfish fillets.

LITERATURE CITED

Brown WH. 2000. Introduction to organic chemistry. 2nd ed. Orlando, FL. Saunders College Publishing. 634 p.

Diongi CP. 1993. Physiological Strategies to control geosmin synthesis in channel catfish aquaculture systems. In: Spanier AM, Okai H, Tamura M.. Food flavor and Safety. ACS symposium series; 528. pub. Washington D.C. p 323-327.

Forrester PN, Prinyawiwatkul W, Godber JS, Phlak LC. 2002. Treatment of catfish with citric acid causes reduction of 2-methylisoborneol, but not musty flavor. J Food Sci 67(7):2615-2618.

Hanson TR. 2003. Economic impact of off-flavor to the U.S. catfish industry. In: Schrader KK, Rimando AM. 2003. Off-Flavors in aquaculture. Washington, D.C.: American Chemical Society. p 13-30.

Harvey DJ. March 2004. Aquaculture outlook. USDA. Washington, D.C.: U.S. Department of Agriculture. Available from: www.ers.usda.gov .

Hultin HO, Kelleher SD. 1999. Process for isolating a protein composition from a muscle source and protein composition. Patent # 6, 005, 073 (Dec 21).

Johnsen PB, Lloyd SW. 1992. Influence of fat content and depuration of the off-flavor 2-methylisoborneol by channel catfish (*Ictalurus punctatus*). Can J Fish Aquat Sci. 49:1-6.

Jutner F. 1995. Physiology and Biochemistry of odorous compounds from freshwater cyanobacteria and algae. Water Sci Technol 31:69-78.

Kelleher SD, Hultin HO. 2000. Functional chicken muscle protein isolates prepared using low ionic strength, acid solubilization/precipitaion. In: 53rd Annual Reciprocal Meat

Conference: Meat Science in the New Millennium 2000. Savoy, IL: American Meat Science Association. p 76-81.

Kim JN, Liu CH, Eun JB. Park JW, Oshimi R, Hayashi K, Ott B, Aramaki T, Sekine M, Horikita Y, Fujimoto K, Aikawa T, Welch L, Long R. 1996. Surimi from fillet frames of channel catfish. J Food Sci 61(2)429-432.

King J, Dew T. 2003. Catfish off-flavors and their elimination. In: Schrader KK, Rimando AM. 2003. Off-Flavors in aquaculture. Washington, DC: American Chemical Society. p 31-44.

Lange J. February 2004. Catfish Production. Washington D.C.: U.S. Dept. of Agriculture. National Agricultural Statistics Service. Available from: www.usda.gov . Accessed April 2005.

National Fisheries Institute. 2004 Top Ten Seafoods. McLean, VA. National Fisheries Insitute Available From: www.nfi.org Accessed Mar 15.

Okadu M. 1992. History of surimi technology in japan. In: Lanier TC, Chong ML. Surimi Technology. New York NY. Marcel Dekker. 3-22.

Park JW, Morrissey MT. 2000. Manufacturing of surimi from light muscle fish. In: Park JW. Surimi and surimi seafood. New York, NY. Marcel Dekker. p 23-58.

Sylvia JL, Dean S. 2001. Processed catfish: Product forms, packaging, yields, and product mix. Southern Regional Aquaculture Center. Publication # 184.

Tortora GJ, Funke BR, Case CE. 1998. Microbiology: An introduction. 6th ed. Menlo Park, CA. Benjamin/Cummins Publishing Company. 832 p.

Tucker CS, Hanson TR, Kingsburry SK. 2001. Management of off-flavors in pond-cultured channel catfish with weekly applications of copper sulfate. North Amer J of Aqua. 63:118-130.

Tucker CS, van der Ploeg M. 1999. Managing off-flavor problems in pond-raised catfish. Southern Regional Aquaculture Center. Publication # 192

U.S. Department of Agriculture, Agricutural Research Service. 2004. USDA National Nutrient Database for Standard Reference, Release 17. Available from: http://www.nal.usda.gov/fnic/foodcomp .

USDA National Agricutural Statistics Service. 2000. Catfish and Trout Production. Washington D.C.: U.S. Dept. of Agriculture. Available from: www.usda.gov . Accessed April 15, 2005

van der Ploeg M, Tucker C, Steeby J, Weirich C. 2001. Management plan for blue-green off-flavors in Mississippi pond raised catfish. Mississippi State University extension service. Publication # 2001.

Zimba PV, Tucker CS, Mischke CC. 2001. Short-term effects of diuron on catfish pond ecology. North Amer J of Aquacult 64(1):16-23

Chapter III

COMPOSITION, OFF-ODOR/FLAVOR CONCENTRATION, AND SENSORY EVALUATION OF FARM RAISED CHANNEL CATFISH FILLETS WHEN TREATED BY ACID SOLUBILIZATION ISOELECTRIC PRECIPITATION

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ABSTRACT

Catfish were placed for 24 hrs in tanks spiked with either 1 ppb geosmin, 1 ppb MIB, or non-spiked. Fillets were removed and further processed with or without acid-solubilization isoelectric precipitation (Acid-SIP). Composition and concentration of off-odors/flavors were evaluated in batters and gels. Sensory analysis was performed on gels. GC/MS data indicates that there were no statistically significant differences in geosmin and MIB concentrations between Acid-SIP samples and non Acid-SIP samples. However, the data does show a trend in that Acid-SIP samples have lower concentrations of the off-odor/flavor compound with the exception of cooked geosmin samples. In regard to composition, protein content was higher in gels vs. batters and no statistical difference was found between Acid-SIP vs. non Acid-SIP samples. The Acid-SIP process significantly reduced fat content in gel batters and there was no difference ($P<0.05$) found for ash. Data indicates that the Acid-SIP process produces a low fat, high protein product with the ability to lower off-odor/flavor compounds.

INTRODUCTION

Many catfish producers are burdened with the chronic management problem of producing off-odor/flavor catfish. Catfish derive off-odor/flavors from naturally occurring compounds that can reside in the aquaculture ponds in which fish are grown. The compounds are produced by various aquatic microorganisms, and can cause o-/of catfish for extended periods of time. The costs associated with off-odor/flavor episodes have been estimated to cost up to $23 million annually for the catfish industry (Hanson 2003).

Research has been conducted in an attempt to create a pre-harvest method capable of preventing off-odor/flavor catfish. Various pre-harvest methods have been developed with varying degrees of success, although most have proven impractical or too costly to implement in a production type setting (King and Dew 2003, Tucker and van der Ploeg 1999, van der Ploeg and others 2001, Zimba and others 2001). The lack of a successful pre-harvest method has led researchers in search of a post-harvest remedy to the off-odor/flavor catfish issue.

Research has indicated that the off-odor/flavor compounds can be chemically dehydrated to non-odiferous products under acidic conditions (Forrester and others 2002**)**. The compounds have also been shown to be lipophilic and therefore associate with the lipid components of catfish tissues (Johnsen and Lloyd 1992). The acid solubilization procedure used to recover myofibrillar proteins reduces the lipid content of muscle while maintaining protein functionality (Kelleher and Hultin 2000). It was hypothesized that by applying the acid solubilization process to catfish fillets there would be an increase in off-odor/flavor compound dehydration, and the compounds could be

physically removed by decreasing/removing the lipid components of the fillets. The objective of this study was to apply an acid solubilization process to catfish fillets containing known amounts of gesomin and MIB and determine its effect on composition and off-odor/flavor compound reduction.

MATERIALS AND METHODS

Treatment of fish

Approximately 136 kg of live channel catfish (*Ictalurus punctatus*) were acquired from a local producer. The catfish were divided equally among three 1050 L high density polyethylene plastic tanks (Polytank Inc, Litchfield, MN, USA) filled with aerated municipal 20^{o}C water. The municipal water was de-chlorinated using sodium thiosulfate and filtered in a re-circulating system using a floating bead filter. The fish were allowed to purge/depurate in the filtered water for 24 h. Following purging, the tanks were drained and refilled with fresh municipal water treated at the same parameters mentioned above except the re-circulation filter was not used. Each of the three tanks were assigned one of the following three treatments: **1**). Control (no spike) **2**). Geosmin **3**). 2-Methylisoborneol (MIB). Geosmin and MIB (Sigma-Aldrich, St. Louis, MO) were added to the assigned tanks with a target concentration of 1 ppb. However, final analysis of water samples indicated that geosmin and MIB concentration were slightly off target with an average concentration of 1.36 ppb for MIB and 0.81 ppb for geosmin (Appendix B). The fish were held in the tanks for 24 h after spiking to allow absorption of the off-odor/flavor compounds. Following absorption, the fish were removed from the tanks, placed in separate ice chests corresponding to each treatment, and covered with ice in preparation for transport to the processing facility. The fish were immediately

transported to a refrigerated (4°C) abattoir room and processed within 3 h of harvest. The fish were put to death with a blunt force to the head. Fillets were removed, absent of belly flesh and skin, and blast frozen (-28°C) in vacuum-sealed Cryovac (Sealed Air Corp, Saddle Brooks, NJ) bags. Frozen fillets were shipped to Proteus Industries (Gloucester, MA) within 2 wk of freezing for further processing.

Acid Solubilization Isoelectric Precipitation (Acid-SIP)

Acid solubilization was conducted under the guidance of Dr. Stephen Kelleher (Proteus Industries). Approximately 11 kg of treated (control, geosmin, or MIB) frozen catfish fillets were thawed to a temperature of 2°C and treatments were chopped separately at 1750 rpm in a Hobart bowl chopper (Troy, OH) for thirty seconds. Samples were collected at this point for gas chromatography (GC) and proximate analysis. Approximately 0.9 kg of chopped fillet were removed for non Acid-SIP treatment analysis and mixed with cryoprotectants (4% sucrose, 4% sorbitol, and 0.3% sodium tripolyphosphate) according to Kelleher and Hultin (2000). The Non Acid-SIP samples were blast-frozen (-28°C) in vacuum-sealed Cryovac bags until further analysis. The remaining 10.1 kg of chopped fillets were mixed with ice water (9:1 ice water/fillet, weight basis) and emulsified for 10 s using a Stephan MCH15 (Stephan Machinery Corp. Columbus, OH) emulsifier. The emulsified homogenate was transferred to a Waukesha 134 pump (Delevan, WI) and holding tank where the pH was lowered to 2.8 using H_3PO_4 (85% diluted 1 +10 acid/H_20, Fisher Science, Pittsburgh, PA). The homogenate was centrifuged using an Alfa Laval LAPX 404 centrifugal separator (Tumba, Sweden) at the following parameters: 9500 rpm, 11,000 x g, at a 200-240 m/h flow rate. Plastic containers were used to collect the centrifuged homogenate and the pH was raised to 5.8

using 2N NaOH to precipitate and recover the myofibrillar proteins. The protein pellet was de-watered using a Sweco vibration cage (Florence, KY) and cheese cloth. Cryoprotectants were added at the levels and parameters stated above, followed by blast freezing (-28^{o}C) the protein pellet in vacuum sealed Cryovac bags until further analysis.

Batter Preparation

Initial moisture was determined for all samples by oven drying (AOAC 1995) prior to batter preparations. Batter preparation involved partially thawing either the Acid-SIP or the non Acid-SIP (control, geosmin, or MIB) protein mixtures to a temperature of approximately –5^{o}C. All samples were adjusted to approximately pH 7 using food grade $NaHCO_3$ (Arm & Hammer, Princeton, NJ). Ice and NaCl were added according to Park and Morrissey (2000) for the comminution of a surimi gel test specimen. Mixing parameters were followed as outlined by Park and Morrissey(2000) using a vacuum chopper (UMC 5 electronic; Stephan Machinery Corp. Columbus, OH) with the exception that ice alone was used instead of ice/water to obtain 78% moisture. The batters were chopped at 2000 rpm (maximum speed used), with a vacuum of approximately 500-600 mm Hg, for 6 minutes. Following batter preparation, samples were removed for proximate composition, and GC analysis.

Gel Preparation

Treatment batters (n=6) were immediately transferred to a tabletop piston stuffer (12 lb capacity; Friedr Dick Corp. Farmingdale, NY) equipped with a 12 mm filling tube. Each of the batters was stuffed into three-21 mm cellulose casings (Viskase, E-Z Peel® Nojax, Willowbrook, IL) approximately 22 cm in length. The stuffed links were then placed in boilable vacuum bags (size 10x13; Prime Source® pouches, Koch supplies Inc.,

Kansas City, MO) and sealed without pulling a vacuum. The gels were cooked in a 90°C water bath for 30 min, chilled on ice for 30 min, and then refrigerated (4°C) over night.

Analysis of Treatments

Proximate analyses were performed (AOAC 1995) using procedure 960.39 for crude fat in meat with an indirect Soxhlet apparatus, procedure 992.15 for crude protein in meat with a Leco FP-428 (Leco Co., St. Joseph, MI) and procedure 920.153 for crude ash of meat.

Analysis of geosmin and MIB in water and tissue samples was conducted by Kevin Schrader and his research team of the USDA Thad Cochran Research Center (USDA., University, MS) using solid-phase microextraction and gas chromatography-mass spectrometry as outlined by Grim and others (2000) and Schrader and others (2003).

Samples for sensory analysis were acquired during batter preparation for Acid-SIP and non Acid-SIP samples. Approximately 60 g of sample was vacuum-sealed in boilable Kapak pouches (4x6 in,4.5 mil thick; Kapak Corp. Minneapolis, MN). Potential panelists were screened to confirm their ability to detect both geosmin and MIB. The resulting 10 panelists were then trained to detect the off-odor/flavor compounds using prepared dilutions of geosmin and MIB ranging in concentration from 0.5 to 10 ppb. Samples were cooked in a boiling water bath for 10 min and were then tempered at room temperature for 15 min before serving to panelists. Samples were randomly presented to panelists through a turnstile while seated in divided booths. Red filtered incandescent lights were used during sample presentation. Panelists were instructed to open the sample pouch with provided scissors and immediately smell any aromas emitted by the

sample and rank the aroma by the following parameters: "0" to indicate the sample was acceptable, and no off-odor/flavor's were detected, "1" there was an indication of the off-odor/flavor, but its presence was faint, "2" the off-odor/flavor was strong and clearly recognizable. If panelists ranked the sample "0" for smell, they were instructed to taste the sample to determine if geosmin or MIB could be detected and rank the sample by the parameters listed above. Panelists were allowed 5 min in between sample presentations to minimize sensory fatigue. Panelists were also provided with coffee grounds to refresh their olfactory senses along with unsalted crackers and distilled water to cleanse their pallet between samples. Expectorant cups were provided.

Statistical Analysis

The data for Acid-SIP and non Acid-SIP samples were analyzed in a randomized block design (Proc Mixed, SAS Institute, Cary, NC). The model included a split-split plot design with main-units, where blocks were repetition, main-unit treatment factor was chemical treatment (control, geosmin, or MIB), sub-unit treatment factor was process, and sub-sub-unit treatment factor was cook or no cook. Mean separation was accomplished using Tukey's Studentized range for comparisons of means.

Raw unprocessed fillet data were analyzed in a randomized block design (Proc GLM) where block was repetition, and treatment factor was chemical treatment (control, geosmin, or MIB)

Sensory data were analyzed by the Friedman Test to compare treatment means, where blocks were panelists, and sub-unit treatment factor was repetition. The model was prepared in a factorial arrangement for chemical and process factors. Mean separation was accomplished using Tukey's Studentized range for comparisons of means.

Experimental design included three repetitions for all treatments including triplicate analysis for all samples.

RESULTS AND DISCUSSION

Composition

Proximate analysis of the raw unprocessed fillets (Table 2), which were of a composite mixture, indicated that their composition was similar to values reported by the USDA (Table 1, chpt II) for the nutritional content of channel catfish fillets (USDA 2004). Experimental values for moisture were slightly lower than those reported by the USDA, whereas experimental values for protein, fat, and ash were slightly higher. Among the three treatments (control, geosmin, and MIB) there were no statistical differences for moisture, fat, and ash. However, as can be seen in Table 2, fillets from the MIB treatment contained a significantly lower amount of protein in comparison to the other treatments. Research indicates that as fish size increases, there is a resulting increase in protein concentration (Ronsholdt 1995, Ali and others 2004). The variation in size and maturity of live fish (Appendix G) used in this study could have contributed to observed differences in protein content.

Proximate composition data for the batters when processed by Acid-SIP or non Acid-SIP is presented in Table 3. There were no statistical differences found for moisture, protein, or ash between samples processed by Acid-SIP or non Acid-SIP. No difference in moisture % was expected, because all samples were equilibrated to 78% moisture for batter preparations. The Acid-SIP process significantly lowered fat content of all samples in comparison to non Acid-SIP samples. Furthermore, when compared to initial fat contents of raw, unprocessed fillets (Table 2) the Acid-SIP process decreased fat content by 96-97%. Hultin and Kelleher (2000) reported similar findings,

Table 2. Composition of Raw Unprocessed Fillet.

	CONTROL	GEOSMIN	MIB
Moisture %	72.85 ± 1.87^{a}	72.95 ± 1.94^{a}	72.02 ± 2.60^{a}
Protein %	17.70 ± 0.55^{a}	18.18 ± 2.76^{a}	16.09 ± 1.41^{b}
Fat %	9.32 ± 1.39^{a}	8.03 ± 3.28^{a}	8.48 ± 3.17^{a}
Ash %	1.22 ± 0.43^{a}	1.27 ± 0.211^{a}	1.22 ± 0.19^{a}

Means within same row without common superscript are different ($p<0.05$)

Table 3. Composition of Batters Treated by Acid-SIP or Non Acid-SIP.

	CONTROL		GEOSMIN		MIB	
	Acid-SIP*	**Non Acid-SIP***	**Acid-SIP***	**Non Acid-SIP***	**Acid-SIP***	**Non Acid-SIP***
Moisture %	77.28 ± 0.68^{a}	78.00 ± 0.20^{a}	76.99 ± 0.52^{a}	77.19 ± 0.65^{a}	77.66 ± 0.32^{a}	77.96 ± 0.49^{a}
Protein %	9.57 ± 0.56^{a}	9.46 ± 0.27^{a}	10.65 ± 0.38^{a}	9.80 ± 0.63^{a}	9.77 ± 0.18^{a}	9.68 ± 0.26^{a}
Fat %	0.24 ± 0.25^{a}	3.10 ± 1.31^{b}	0.31 ± 0.42^{a}	2.58 ± 1.43^{b}	0.35 ± 0.49^{a}	2.70 ± 1.45^{b}
Ash %	3.03 ± 0.28^{a}	2.83 ± 0.14^{a}	2.94 ± 0.16^{a}	3.05 ± 0.16^{a}	2.74 ± 0.15^{a}	2.97 ± 0.17^{a}

Data represent means $\pm$ standard deviation

Means within same row without common superscript are different ($P<0.05$)

* Acid Solubilization Isoelectric Precipitation (Acid-SIP)

indicating that after acid solubilization in acid solution and centrifugation, 97% of the initial lipid of mackerel muscle was removed. The reduction in fat content of catfish flesh could play a critical role in off-odor/flavor compound reduction. This is supported by the fact that off-odor/flavor compounds are associated with the lipid containing tissues (Johnsen and Lloyd 1992).

Proximate composition data for gels is presented in Table 4. Analysis revealed no statistical differences in moisture, protein, fat, and ash of the gels. Data does indicate that the non Acid-SIP gels (Table 4) contained a significantly lower ($P<0.05$) fat % than the non Acid-SIP Batters (i.e. starting ingredient of Acid-SIP gels, Table 3). Therefore, the cooking process significantly lowered fat content in the non Acid-SIP gels in comparison to their raw counterparts. The protein content of the gels (Table 4) was higher ($P<0.05$) than the Batters (Table 3). The increased protein content in gels was most likely the result of a concentration effect, due to the loss of moisture and fat during the cooking process.

Geosmin & MIB Concentrations

Initial concentrations of geosmin and MIB in the raw unprocessed fillets are presented in Table 5. Achieved concentrations of geosmin and MIB are above the reported threshold levels for human sensory detection (Forrester and others 2002, King and Dew 2003). This result was desired and necessary to measure the effectiveness of the Acid-SIP process to eliminate the geosmin and MIB. Control treated fillets contained insignificant amounts of geosmin and MIB. Residual amounts of geosmin or MIB may have been present in the fish as a result of the off-odor/flavor compounds being present in the aquatic environment of which they were raised. All three repetitions were statistically

Table 4. Composition of Gels Treated by Acid-SIP or Non Acid-SIP.

	CONTROL		GEOSMIN		MIB	
	Acid-SIP*	**Non Acid-SIP***	**Acid-SIP***	**Non Acid-SIP***	**Acid-SIP***	**Non Acid-SIP***
Moisture %	75.42 ± 1.38[a]	76.18 ± 0.96[a]	75.34 ± 1.29[a]	75.27 ± 0.64[a]	75.77 ± 1.23[a]	76.26 ± 1.06[a]
Protein %	11.28 ± 1.35[a]	11.41 ± 1.91[a]	12.00 ± 0.73[a]	10.83 ± 0.58[a]	10.64 ± 1.22[a]	10.58 ± 0.52[a]
Fat %	0.13 ± 0.14[a]	1.34 ± 1.14[a]	0.27 ± 0.58[a]	1.05 ± 0.84[a]	0.05 ± 0.00[a]	0.89 ± 0.56[a]
Ash %	3.08 ± 0.44[a]	2.97 ± 0.19[a]	2.96 ± 0.14[a]	3.20 ± 0.18[a]	2.83 ± 0.06[a]	2.94 ± 0.24[a]

Data represent means ± standard deviation

Means within same row without common superscript are different ($P<0.05$)

* Acid Solubilization Isoelectric Precipitation (Acid-SIP)

Table 5. Concentration of Geosmin and MIB in Raw Unprocessed Fillets.

	CONTROL	GEOSMIN	MIB
Geosmin (µg/kg)	0.17 ± 0.06^{a}	3.18 ± 1.45^{b}	0.11 ± 0.06^{c}
MIB (µg/kg)	0.01 ± 0.01^{d}	0.02 ± 0.03^{e}	3.80 ± 1.18^{f}

Data represents µg of geosmin or MIB per kg of sample
Means within same row or column without common superscript are different ($P<0.05$)

different ($P<0.05$) for initial concentrations of geosmin or MIB in raw unprocessed fillets, indicating that the catfish absorbed off-odor/flavor compounds at different rates for each repetition. Variations in live catfish size (Appendix G) maturity, health, and fat content may have contributed to differences in geosmin and MIB absorption. Research indicates the fat content of live catfish will vary with maturity and size (Ronsholdt 1995, Ali and others 2004). Johnsen and Lloyd (1992) found that catfish with higher fat contents absorb and store more MIB than their leaner counterparts.

Overall, geosmin and MIB concentrations for batters and gels (Table 6) were not significantly reduced by the Acid-SIP process. In addition, mean concentrations of geosmin and MIB were slightly greater in gels compared to batters of the same treatment. This phenomena was probably caused by a concentration effect as a result of cooking, thus increasing the amount of geosmin or MIB per kg of tissue sampled. In contrast to these findings, a trend was observed in that all Acid-SIP samples contained less off-odor/flavor compounds than the non Acid-SIP samples, with the exception of geosmin cooked samples. Furthermore, the concentration of geosmin and MIB in Acid-SIP and non Acid-SIP samples was reduced by approximately 77-90% for geosmin and 64-86% for MIB from the initial concentrations in the raw unprocessed fillets (Table 5). Attempts to explain the reduction of geosmin and MIB in the non Acid-SIP samples when compared to initial concentrations in the raw unprocessed fillets have been unsuccessful. It was originally hypothesized that processing conditions or the addition of batter ingredients could have affected geosmin and MIB concentrations or interfered with their detection. Interference by ingredients on off-odor/flavor compound detection using gas

Table 6. Concentration of Geosmin or MIB in Batters or Gels.

FISH TREATMENT	PROCESS TYPE	GEOSMIN^	MIB^
Spiked	**Acid-SIP* Batters**	$0.308 \pm 0.147^{a\ c}$	$0.509 \pm 0.152^{a\ c}$
	Non Acid-SIP* Batters	$0.579 \pm 0.207^{a\ c}$	$0.939 \pm 0.337^{a\ b}$
	Acid-SIP* Gels	0.733 ± 0.489^{b}	$0.917 \pm 0.263^{a\ b}$
	Non Acid-SIP* Gels	$0.557 \pm 0.149^{a\ b\ c}$	1.371 ± 0.390^{b}
Non Spiked (Control)	**Acid-SIP* Batters**	0.034 ± 0.017^{c}	0.045 ± 0.066^{c}
	Non Acid-SIP* Batters	0.028 ± 0.005^{c}	0.002 ± 0.002^{c}
	Acid-SIP* Gels	0.025 ± 0.009^{c}	0.017 ± 0.021^{c}
	Non Acid-SIP* Gels	0.052 ± 0.053^{c}	0.012 ± 0.014^{c}

^Data represents µg of geosmin or MIB per kg of sample
Means within same column without common superscript are different ($P<0.05$)
* Acid Solubilization Isoelectric Precipitation (Acid-SIP)

chromatography analysis has been evaluated and was determined not to be a factor (DeWitt and Bilby 2005). In addition, dilution of the off-odor/flavor compounds by adding ingredients, and the process of applying a vacuum during batter preparations does not explain the reduction observed. Control samples did not differ ($P>0.05$) from geosmin samples except for geosmin Acid-SIP gels. However, control samples were different ($P<0.05$) than MIB samples, excluding MIB Acid-SIP batters. Variations in initial concentrations of geosmin and MIB of raw unprocessed fillets could have contributed to fewer statistical differences in off-odor/flavor gas chromatography data for Acid-SIP and non Acid-SIP samples

Sensory Characteristics

Data indicates that panelists could detect a difference ($P<0.05$) between MIB and control samples, treated by the Acid-SIP process, in addition to detecting a difference ($P<0.05$) between MIB and control samples processed by non Acid-SIP. In regard to MIB Acid-SIP samples, all panelists indicated that an off-odor/flavor could be detected simply by smelling, therefore tasting the sample was not necessary due to the strong off odor that was emitted. However, this finding is somewhat inconclusive due to the fact that of the 30 control Acid-SIP samples presented to panelists, 16 were ranked as a "1" and 8 were ranked as a "2", with 1 indicating a faint presence of an off odor or flavor and 2 indicating that an off odor or flavor was strong and clearly present. Panelists could not detect a difference ($P<0.05$) between geosmin and control samples treated by the Acid-SIP or non Acid-SIP processes.

Although sensory panelists were trained to detect geosmin and MIB, there were many inconsistencies in their sensory responses such as the ranking of control samples as

off odor or flavor. In addition, the concentration of geosmin and MIB in control batters and gels (Table 6) was well below human sensory detection levels. The human sensory detection level for geosmin is 0.6 to 8 ppb and MIB is 0.7 ppb (Forrester and others 2002, Hing and Dew 2003). One explanation for the inconsistent data is that panelists were trained to detect geosmin and MIB from prepared solutions of the compounds, which were placed in test tubes. Although panelists could readily detect geosmin and MIB in test tubes, other aromas emitted from the actual sensory samples may have skewed their responses or diminished their ability to effectively evaluate the samples. Therefore, due to the inconsistency of the sensory data, no valid conclusions can be made to determine the effect of the Acid-SIP process on sensory characteristics of off-odor/flavor catfish fillets.

CONCLUSION

Results indicated the Acid-SIP process significantly lowers ($P<0.05$) fat content in catfish tissue while maintaining protein and ash content. The Acid-SIP process did not significantly reduce the concentration of off-odor/flavor compounds in processed catfish tissue in comparison to non Acid-SIP samples. However, there was a trend in that the Acid-SIP samples contained less off-odor/flavor compound than the non Acid-SIP samples, with the exception of geosmin cooked samples. Further research should be conducted to determine optimal processing parameters for using the Acid-SIP process to eliminate off-odor/flavors. Determining the specific storage site of geosmin and MIB within catfish tissue, such as neutral lipid versus polar lipid storage, could be useful in optimizing processing conditions.

LITERATURE CITED

Ali M, Salam A, Goher S, Tassaduqe K, Latif. 2004. Studies on fillet composition of fresh water Farmed *Labeo rohita* in relation to body size, collected from government fish seed hatchery Mian Channu Pakistan. J Biol Sciences 4(1) 40-46

AOAC. 1995. Official Methods of Analysis, 16th edition. Washington DC. Association of Official Analytical Chemists.

DeWitt Mireles CA, Bilby Chris. 2005. Unpublished data via personal communication. Oklahoma State University. Stilllwater, OK.

Forrester PN, Prinyawiwatkul W, Godber JS, Phlak LC. 2002. Treatment of catfish with citric acid causes reduction of 2-methylisoborneol, but not musty flavor. J Food Sci 67(7):2615-2618

Grimm CC, Lloyd SW, Batista R, Zimba PV. 2000. Using microwave distillation-solid-phase-microextraction-gas chromatography mass spectrometry for analyzing fish tissue. Journal of Chromatographic Science 38:289-296

Hanson TR. 2003. Economic impact of off-flavor to the U.S. catfish industry. In: Schrader KK, Rimando AM. 2003. Off-Flavors in aquaculture. Washington, D.C.: American Chemical Society. p 13-30.

Hultin HO, Kelleher SD. 2000. Manufacturing of surimi from dark muscle fish. In: Park JW. Surimi and surimi seafood. New York, NY. Marcel Dekker. p 59-78

Johnsen PB, Lloyd SW. 1992. Influence of fat content and depuration of the off-flavor 2-methylisoborneol by channel catfish (*Ictalurus punctatus*). Can J Fish Aquat Sci. 49:1-6

Kelleher SD, Hultin HO. 2000. Functional chicken muscle protein isolates prepared using low ionic strength, acid solubilization/precipitaion. In: 53rd Annual Reciprocal Meat Conference: Meat Science in the New Millennium 2000. Savoy, IL: American Meat Science Association. p 76-81.

King J, Dew T. 2003. Catfish off-flavors and their elimination. In: Schrader KK, Rimando AM. 2003. Off-Flavors in aquaculture. Washington, DC: American Chemical Society. p 31-44.

Park JW, Morrissey MT. 2000. Manufacturing of surimi from light muscle fish. In: Park JW. Surimi and surimi seafood. New York, NY. Marcel Dekker. p 23-58.

Ronsholdt B. 1995. Effect of size/age and feed composition on body composition and phosphorus content of rainbow trout, oncorhynchus mykiss. Water Sci and Tech 31(10): 175-183

SAS. 2002. Version 8.0. SAS Institute Inc., Cary, NC, USA.

Schrader KK, Nanayakkara NP, Tucker CS, Rimando AM, Ganzera M, Schaneberg BT. 2003. Novel derivatives of 9,10-Anthraquinone are selective algicides against the musty-odor cyanobacterium *Oscillatoria peronata.* Appl and Envir Microbiology 69(9):5319-5327

Tucker CS, van der Ploeg M. 1999. Managing off-flavor problems in pond-raised catfish. Southern Regional Aquaculture Center. Publication # 192

USDA, Agricutural Research Service. 2004. USDA National Nutrient Database for Standard Reference, Release 17. Available from: http://www.nal.usda.gov/fnic/foodcomp

van der Ploeg M, Tucker C, Steeby J, Weirich C. 2001. Management plan for blue-green off-flavors in Mississippi pond raised catfish. Mississippi State University extension service. Publication # 2001.

Zimba PV, Tucker CS, Mischke CC. 2001. Short-term effects of diuron on catfish pond ecology. North Amer J of Aqua 64(1):16-23

Chapter IV

GEL ATTRIBUTES OF FARM RAISED CHANNEL CATFISH FILLETS WHEN TREATED BY ACID SOLUBILIZATION ISOELECTRIC PRECIPITATION

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ABSTRACT

Gel batters were prepared using catfish fillets processed by acid solubilization-isoelectric precipitation (Acid-SIP) or non Acid-SIP. Moisture of the batters was equilibrated to 78 % and salt was added at 2 %. Samples were stuffed into casings, cooked, and chilled. Cook yield, water holding ability (WHA), color, composition, and Texture Profile Analysis (TPA) were determined. No statistical differences for cook yield were found between Acid-SIP and non Acid-SIP samples. The WHA of non Acid-SIP samples was slightly higher than the Acid-SIP samples. Texture analysis indicated that the gel strength properties of the Acid-SIP samples was maintained and even improved for springiness compared to non Acid-SIP samples. Results indicate the Acid-SIP process produced a low fat protein product with good gel strength properties.

INTRODUCTION

Catfish producers are faced with the management problem of off-odor/flavor catfish. Catfish obtain off-odor/flavors by absorbing the compounds geosmin and MIB, which are naturally occurring microbial metabolites that reside in bodies of water. The

ability to remove these off-odors and flavors from catfish tissue using a post-harvest processing technique would be a valuable asset to the catfish industry.

A post-harvest processing method should produce a functional end product to be a viable technique. One parameter used to evaluate the functionality of protein gels is texture. Kim and Park (2000) indicate that texture is a major component in measuring the functional characteristics of surimi materials.

A process developed by Hultin and Kelleher (1999) involves using acidic conditions to solubilize and collect proteins from muscle tissue, while reducing fat content and maintaining protein functionality. Applying this method to off-odor/flavor catfish fillets could be beneficial in reducing off-odor/flavor compounds and producing a functional protein product.

Chapter III of this thesis evaluates the effectiveness of the Acid-SIP process to eliminate/reduce off-odor/flavor compounds. However, the objective of this study was to apply an acid-solubilization isoelectric precipitation process to catfish fillets and evaluate its effect on composition and gel attributes in comparison to catfish fillets processed using non Acid-SIP.

MATERIALS AND METHODS

Treatment of Fish

Approximately 136 kg of live channel catfish (*Ictalurus punctatus*) were acquired from a local producer. The catfish were divided equally among three 1050 L high density polyethylene plastic tanks (Polytank Inc, Litchfield, MN, USA) filled with aerated municipal 20^{o}C water. The municipal water was de-chlorinated using sodium thiosulfate and filtered in a re-circulating system using a floating bead filter. The fish were allowed

to purge/depurate in the filtered water for 24 h. Following purging, the tanks were drained and refilled with fresh municipal water treated at the same parameters mentioned above except the re-circulation filter was not used. Each of the three tanks were assigned one of the following three treatments: **1**). Control (no spike) **2**). Geosmin **3**). 2-Methylisoborneol (MIB). Geosmin and MIB (Sigma-Aldrich, St. Louis, MO) were added to the assigned tanks with a target concentration of 1 ppb. However, final analysis of water samples indicated that geosmin and MIB concentration were slightly off target with an average concentration of 1.36 ppb for MIB and 0.81 ppb for geosmin (Appendix B). The fish were held in the tanks for 24 h after spiking to allow absorption of the off-odor/flavor compounds. Following absorption, the fish were removed from the tanks, placed in separate ice chests corresponding to each treatment, and covered with ice in preparation for transport to the processing facility. The fish were immediately transported to a refrigerated (4^{o}C) abattoir room and processed within 3 h of harvest. The fish were put to death with a blunt force to the head. Fillets were removed, absent of belly flesh and skin, and blast frozen (-28^{o}C) in vacuum-sealed Cryovac (Sealed Air Corp, Saddle Brooks, NJ) bags. Frozen fillets were shipped to Proteus Industries (Gloucester, MA) within 2 wk of freezing for further processing.

Acid Solubilization Isoelectric Precipitation (Acid-SIP)

Acid solubilization was conducted under the guidance of Dr. Stephen Kelleher (Proteus Industries). Approximately 11 kg of treated (control, geosmin, or MIB) frozen catfish fillets were thawed to a temperature of 2^{o}C and treatments were chopped separately at 1750 rpm in a Hobart bowl chopper (Troy, OH) for thirty seconds. Samples were collected at this point for proximate analysis. Approximately 0.9 kg of chopped

fillet were removed for non Acid-SIP treatment analysis and mixed with cryoprotectants (4% sucrose, 4% sorbitol, and 0.3% sodium tripolyphosphate) according to Kelleher and Hultin (2000). The Non Acid-SIP samples were blast-frozen (-28^{o}C) in vacuum-sealed Cryovac bags until further analysis. The remaining 10.1 kg of chopped fillets were mixed with ice water (9:1 ice water/fillet, weight basis) and emulsified for 10 s using a Stephan MCH15 (Stephan Machinery Corp. Columbus, OH) emulsifier. The emulsified homogenate was transferred to a Waukesha 134 pump (Delevan, WI) and holding tank where the pH was lowered to 2.8 using H_3PO_4 (85% diluted 1 +10 acid/H_20, Fisher Science, Pittsburgh, PA). The homogenate was centrifuged using an Alfa Laval LAPX 404 centrifugal separator (Tumba, Sweden) at the following parameters: 9500 rpm, 11,000 x g, at a 200-240 m/h flow rate. Plastic containers were used to collect the centrifuged homogenate and the pH was raised to 5.8 using 2N NaOH to precipitate and recover the myofibrillar proteins. The protein pellet was de-watered using a Sweco vibration cage (Florence, KY) and cheese cloth. Cryoprotectants were added at the levels and parameters stated above, followed by blast freezing (-28^{o}C) the protein pellet in vacuum sealed Cryovac bags until further analysis.

Batter Preparation

Initial moisture was determined for all samples by oven drying (AOAC 1995) prior to batter preparations. Batter preparation involved partially thawing either the Acid-SIP or the non Acid-SIP (control, geosmin, or MIB) protein mixtures to a temperature of approximately –5^{o}C. All samples were adjusted to approximately pH 7 using food grade $NaHCO_3$ (Arm & Hammer, Princeton, NJ). Ice and NaCl were added according to Park and Morrissey (2000) for the comminution of a surimi gel test

specimen. Mixing parameters were followed as outlined by Park and Morrissey(2000) using a vacuum chopper (UMC 5 electronic; Stephan Machinery Corp. Columbus, OH) with the exception that ice alone was used instead of ice/water to obtain 78% moisture. The batters were chopped at 2000 rpm (maximum speed used), with a vacuum of approximately 500-600 mm Hg, for 6 minutes. Following batter preparation, samples were removed for proximate composition analyses.

Gel Preparation

Treatment batters (n=6) were immediately transferred to a tabletop piston stuffer (12 lb capacity; Friedr Dick Corp. Farmingdale, NY) equipped with a 12 mm filling tube. Each of the batters was stuffed into three-21 mm cellulose casings (Viskase, E-Z Peel® Nojax, Willowbrook, IL) approximately 22 cm in length. The stuffed links were placed in boilable vacuum bags (size 10x13; Prime Source® pouches, Koch supplies Inc., Kansas City, MO) and sealed without a vacuum. The gels were cooked in a 90^{o}C water bath for 30 min, chilled on ice for 30 min, and then refrigerated (4^{o}C) over night.

Analysis of Treatments

Proximate analyses were performed (AOAC 1995) using procedure 960.39 for crude fat in meat with an indirect Soxhlet apparatus, procedure 992.15 for crude protein in meat with a Leco FP-428 (Leco Co., St. Joseph, MI) and procedure 920.153 for crude ash of meat. Color values were obtained using a Minolta Chroma Meter CR-300 (Ramsey, NJ) on 3 slices per link. The results were expressed as L* (lightness) a* (redness) and b* (yellowness).

Cook yield and water holding ability (WHA) were determined according to Daum-Thunberg and others (1992). Cook yield was calculated by the following equation: cook yield % = (wt cooked gel/ wt raw) * 100. To determine WHA, a gel slice

weighing 1.5 ± 0.15 g, was placed on 3 dried, preweighed filter papers and centrifuged at 30,600 x g (Dupont Sorval RC 5C Plus, Rotor 28 SLA-1500, Newton, CT) for 15 min at 4°C. The filter papers were reweighed and calculated as follows: WHA = (total g H_2O- g H_2O lost during centrifugation) / (g protein). Texture was further evaluated with a TA-XT2i Texture Analyzer (Texture Technologies, Inc., Scarsdale, NY, USA/Stable Micro Systems, Godalming, Surrey, UK) with three randomly sliced 2 cm long segments per link tempered to room temperature. A texture profile analysis was conducted. The program allowed the probe (2.5 cm acrylic probe) to have a double compression into the sample with a 10 s delay between the 2 descents. The probe descended into the geometric center of the slice to a distance of 12 mm at a rate of 2 mm per s to measure the tertiary texture attributes. Values were calculated as (www.texturetechnologies.com) follows:

Hardness = Peak Force of the 1st Compression

Cohesiveness = Area 2nd Compression / Area of 1st Compression

Gumminess = Hardness x Cohesiveness

Springiness = Length of 2nd Compression / Length of 1st Compression

Chewiness = Gumminess x Springiness

Resilience = Area Withdrawal of 1st Compression / Area of 1st Compression

All tests were evaluated in at least duplicate for each of three replications.

Statistical Analysis

The composition and color data for Acid-SIP and non Acid-SIP samples were analyzed in a randomized block design (Proc Mixed, SAS Institute, Cary, NC). The model included a split-split plot design with main-units, where blocks were repetition,

main-unit treatment factor was chemical treatment (control, geosmin, or MIB), sub-unit treatment factor was process, and sub-sub-unit treatment factor was cook or no cook.

Cook yield, water holding ability, and Texture profile data were analyzed as a completely randomized block design (Proc Mixed, SAS Institute, Cary, NC). The model included a split-plot design where the main-unit treatment factor was chemical treatment, and sub-unit treatment factor was process (Acid-SIP or non Acid-SIP), with sub-sampling within sub-units. Experimental design included three repetitions for all treatments including triplicate analysis for all samples.

RESULTS AND DISCUSSION

Composition of Gels

Proximate composition data for gels is presented in Table 7. Analysis revealed no statistical differences in moisture, protein, fat, and ash of the Acid-SIP and non Acid-SIP gels. Data does indicate that the non Acid-SIP gels contained a significantly lower fat % than the non Acid-SIP Batters (i.e. starting ingredient of Acid-SIP gels, Table 3, Chapter III). Therefore, the cooking process significantly lowered fat content in the non Acid-SIP gels in comparison to their raw counterparts.

Color

Cooked gel color data (Table 8) indicates there were no statistical differences in L* (lightness) values for samples treated by the Acid-SIP or non Acid-SIP processes. Fish and surimi gel color quality is often determined by whiteness, therefore L* values

Table 7. Composition of Gels Treated by Acid-SIP or Non Acid-SIP.

	CONTROL		GEOSMIN		MIB	
	Acid-SIP*	**Non Acid-SIP***	**Acid-SIP***	**Non Acid-SIP***	**Acid-SIP***	**Non Acid-SIP***
Moisture %	75.42 ± 1.38[a]	76.18 ± 0.96[a]	75.34 ± 1.29[a]	75.27 ± 0.64[a]	75.77 ± 1.23[a]	76.26 ± 1.06[a]
Protein %	11.28 ± 1.35[a]	11.41 ± 1.91[a]	12.00 ± 0.73[a]	10.83 ± 0.58[a]	10.64 ± 1.22[a]	10.58 ± 0.52[a]
Fat %	0.13 ± 0.14[a]	1.34 ± 1.14[a]	0.27 ± 0.58[a]	1.05 ± 0.84[a]	0.05 ± 0.00[a]	0.89 ± 0.56[a]
Ash %	3.08 ± 0.44[a]	2.97 ± 0.19[a]	2.96 ± 0.14[a]	3.20 ± 0.18[a]	2.83 ± 0.06[a]	2.94 ± 0.24[a]

Data represent means ± standard deviation

Means within same row without common superscript are different ($p<0.05$)

* Acid Solubilization Isoelectric Precipitation (Acid-SIP)

Table 8. Color Values of Gels when Treated by Acid-SIP or Non Acid-SIP.

TREATMENT	L*[x]	a*[y]	b*[z]
Control Acid-SIP^	71.41 ± 1.82[a]	-1.60 ± 0.50[a]	6.01 ± 0.45[a]
Control Non Acid-SIP^	71.60 ± 2.63 [a]	-1.05 ± 0.27[b]	6.72 ± 0.36[b]
Geosmin Acid-SIP^	71.34 ± 0.40[a]	-1.69 ± 0.07[a]	6.91 ± 0.79[a]
Geosmin Non Acid-SIP^	71.43 ± 1.90[a]	-0.92 ± 0.34[b]	7.41 ± 0.48[b]
MIB Acid-SIP^	73.21 ± 1.00[a]	-1.55 ± 0.16[a]	6.83 ± 0.14[a]
MIB Non Acid-SIP^	72.42 ± 1.63[a]	-1.20 ± 0.28[b]	7.14 ± 0.19[b]

^Acid Solubilization Isoelectric Precipitation (Acid-SIP)

[x] L*: 0=Black, 100=White

[y] a*: Negative values = Green, Positive Values = Red

[z] b*: Negative Values = Blue, Positive Values = Yellow

Data represent means ± standard deviation

Means within same column without common superscript are different ($p<0.05$)

are a valuable tool in measuring product quality (Park 2000). Lanier (1992) indicates that most fish surimi gels will have L* values well above 50. This supports our findings for both Acid-SIP and non Acid-SIP samples which exhibited L* values between 71.41 and 73.21. The a* values are an indicator of the redness of a sample. In regard to a* values, all cooked Acid-SIP samples contained significantly lower values than the cooked non Acid-SIP samples.. Lower (or more negative) values indicate that a product is less red. The Acid-SIP samples were expected to have lower a* values in comparison to non Acid-SIP samples, because the Acid-SIP process removes a large portion of the sarcoplasmic proteins, such as hemoglobin and myoglobin, which contribute to the red appearance of meat and fish products. Data indicates that Acid-SIP treatments were significantly different from non Acid-SIP treatments for b* values. Overall, Acid-SIP samples produced slightly lower b* values than non Acid-SIP samples within the same treatment (control, geosmin, MIB). Hultin and Kelleher (2000) reported similar values for Mackrel that was processed using the Acid-SIP process, which resulted in b* values of approximately 7.2.

Cook Yield and Water Holding Ability (WHA)

As shown in Figure 2, the cook yield % of Acid-SIP samples did not differ ($P>0.05$) from that of the non Acid-SIP samples. Both treatment types possessed cook yield percentages of approximately 94 % or greater. Appendix C contains mean values for cook yield.

The water holding ability values (Figure 3) of cooked Acid-SIP gels (1.15 ± 0.49g /g protein) was significantly ($P<0.05$) lower than that of the non Acid-SIP gels ($1.88 \pm$ 0.54g/g protein). There could be many factors that contribute to this finding.

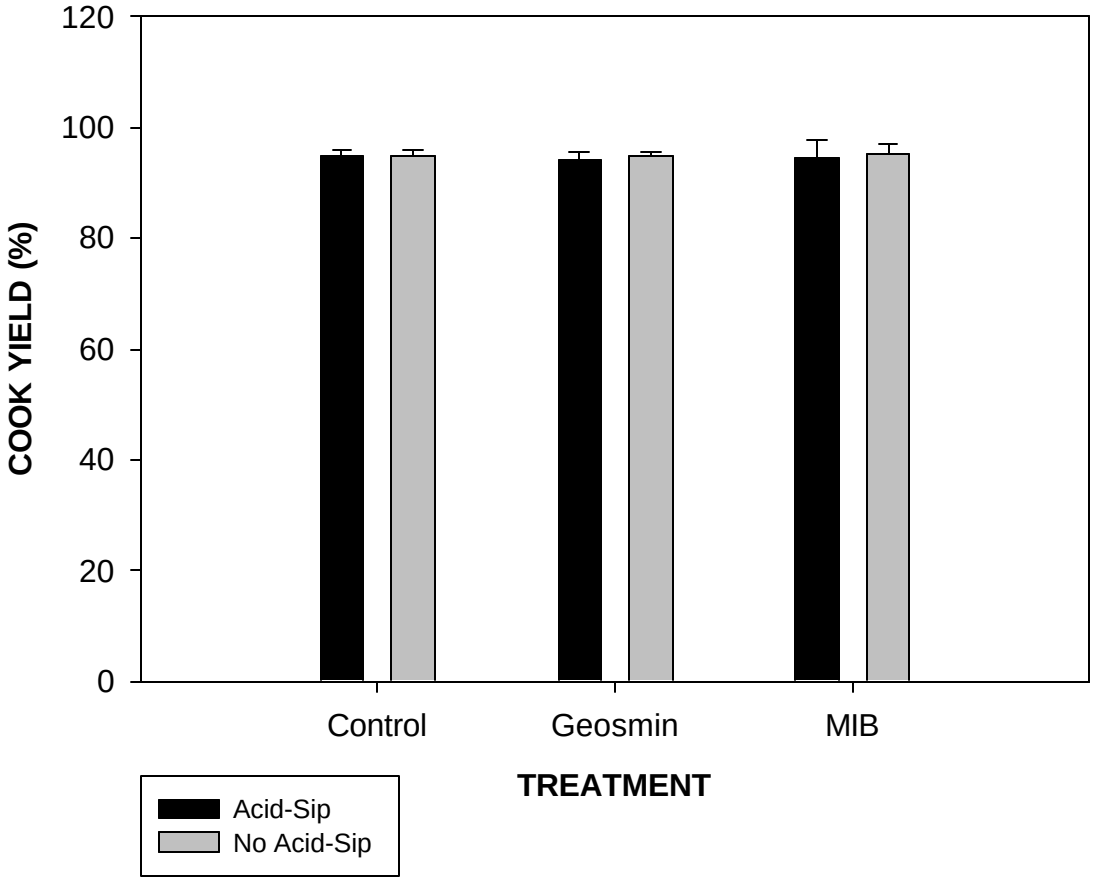


Figure 2. Cook Yield Percentage of Acid-SIP or non Acid-SIP Gels.
Data Represent Means ± Standard Deviation.

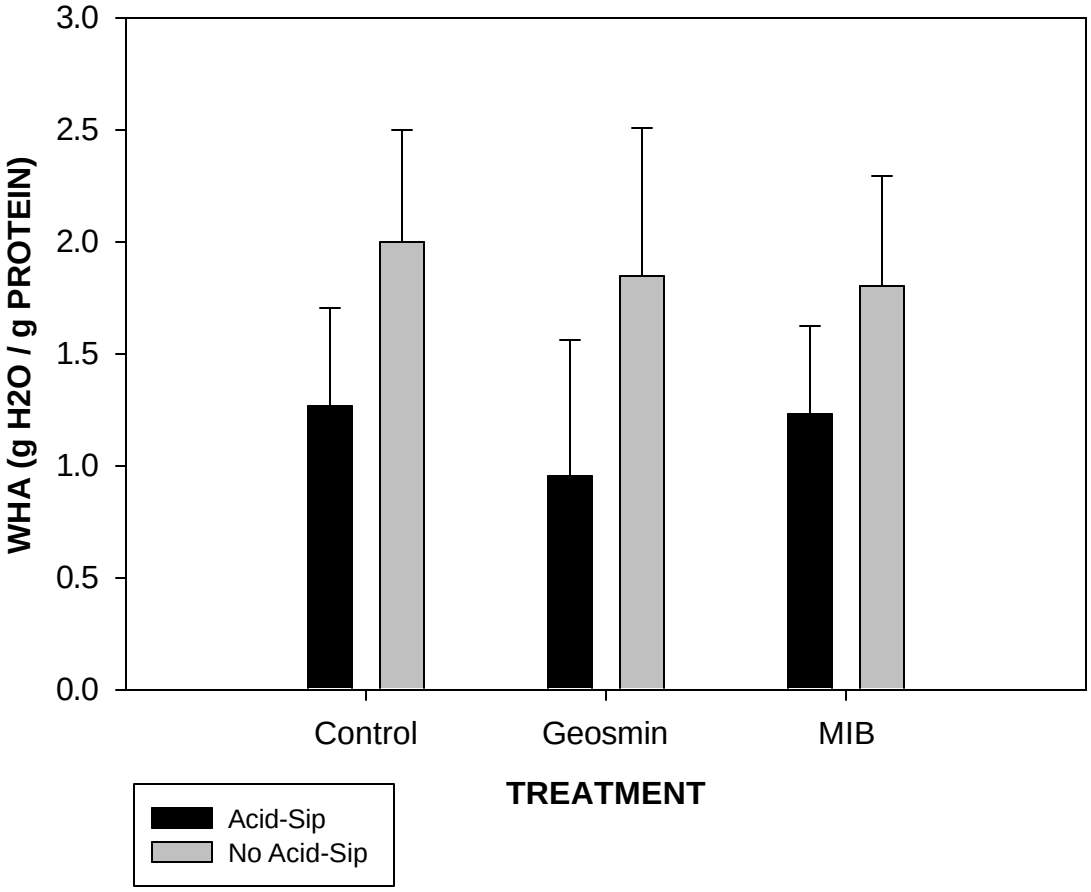


Figure 3. Water Holding Ability (WHA) of Acid-SIP or non Acid-SIP Gels. Data Represent Means $\pm$ Standard Deviation.

Kristinsson (2002) reported that acid and alkali unfolding of myosin appears to lead to different structural and conformational changes in the protein. One hypothesis is that the Acid-SIP process may expose many hydrophobic domains on the solubilized proteins and when the proteins are precipitated and recovered they do not resume their native conformation, resulting in more exposed hydrophobic regions in comparison to the non Acid-SIP proteins. It also is thought that the addition of NaCl to gel batters may have a negative effect on WHA when coupled with a change in the Acid-SIP protein structure. Normally, NaCl enhances the WHA of proteins in their native state by weakening intermolecular interactions between protein fibers. This allows for the binding of more water, because there are fewer protein-protein interactions and more protein-water interactions (Christen and Smith 2000). In contrast, NaCl may not interact effectively with proteins recovered from the Acid-SIP process, resulting in decreased WHA.

Texture Characteristics

Springiness values (Figure 4) for Acid-SIP samples were significantly higher (0.92 $\pm$ 0.02 mm) than non Acid-SIP samples (0.89 $\pm$ 0.02 mm), although the difference was slight. No significant differences ($P>0.05$) were found for the other texture measurements of (Figures 5-9) hardness, cohesiveness, gumminess, chewiness, and resilience, between Acid-SIP and non Acid-SIP samples or within a treatment group. Hardness values for Acid-SIP (1200 $\pm$ 305.59 g) and non Acid-SIP samples (1046.39 $\pm$ 239.09 g) gels were similar to hardness values for washed, cooked catfish mince (1213 g) reported by Wiles and others (2004). Montejano and others (1985) reported similar values for cohesiveness and springiness of fish surimi gels in comparison to the values obtained in this study for Acid-SIP and non Acid-SIP gels.

CONCLUSION

The data presented in this study indicates that the Acid-SIP process produces a low fat protein product with good gel strength, cook yields, and color. Texture data revealed that the Acid-SIP process maintains gel functionality in comparison to non Acid-SIP gels and improved the parameter springiness. The water holding ability was slightly lower in Acid-SIP samples, however all other gel attributes indicate that the Acid-SIP process produces a product that could be used in processed seafood analogs.

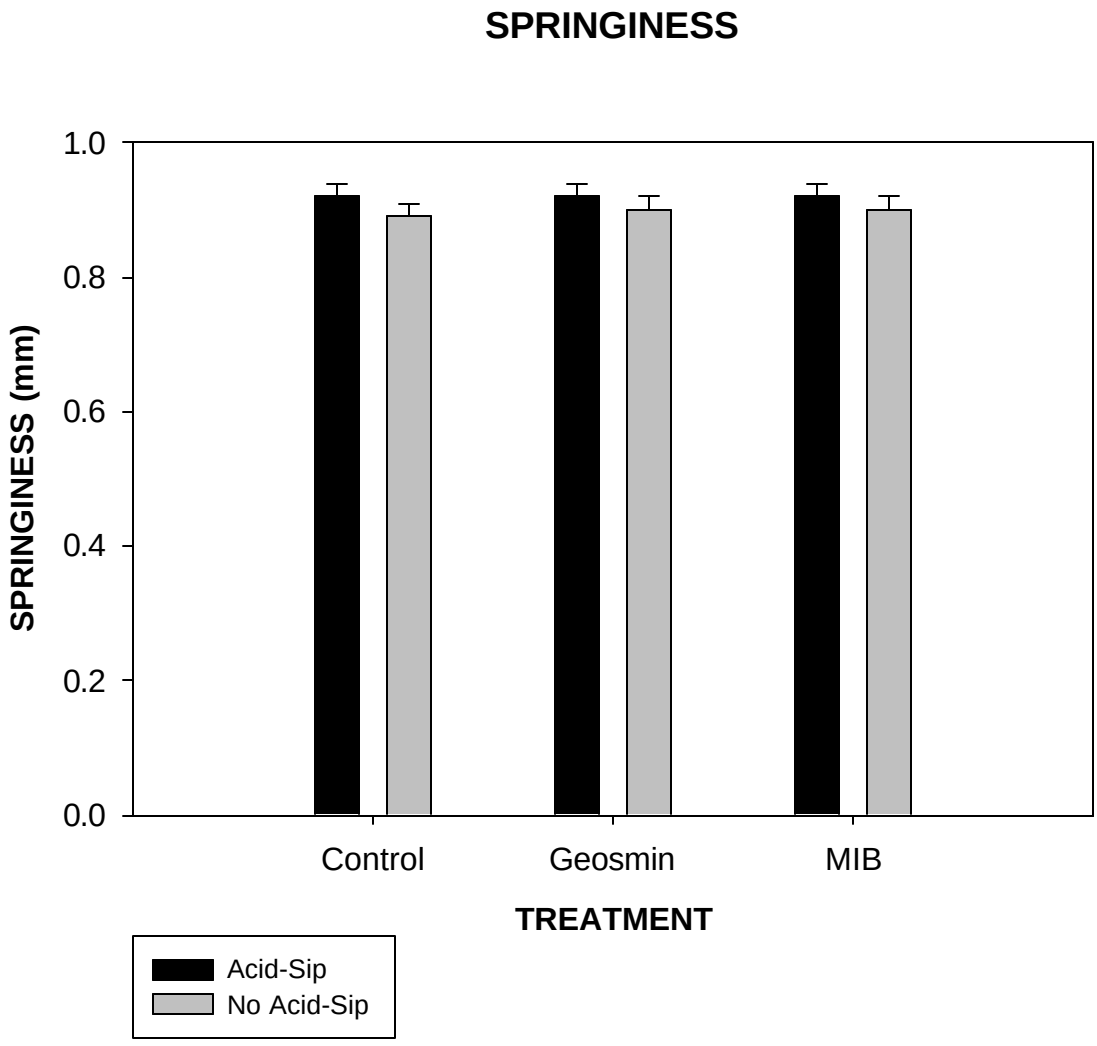


Figure 4. Springiness of Acid-SIP or non Acid-SIP Gels.
Data Represent Means ± Standard deviation

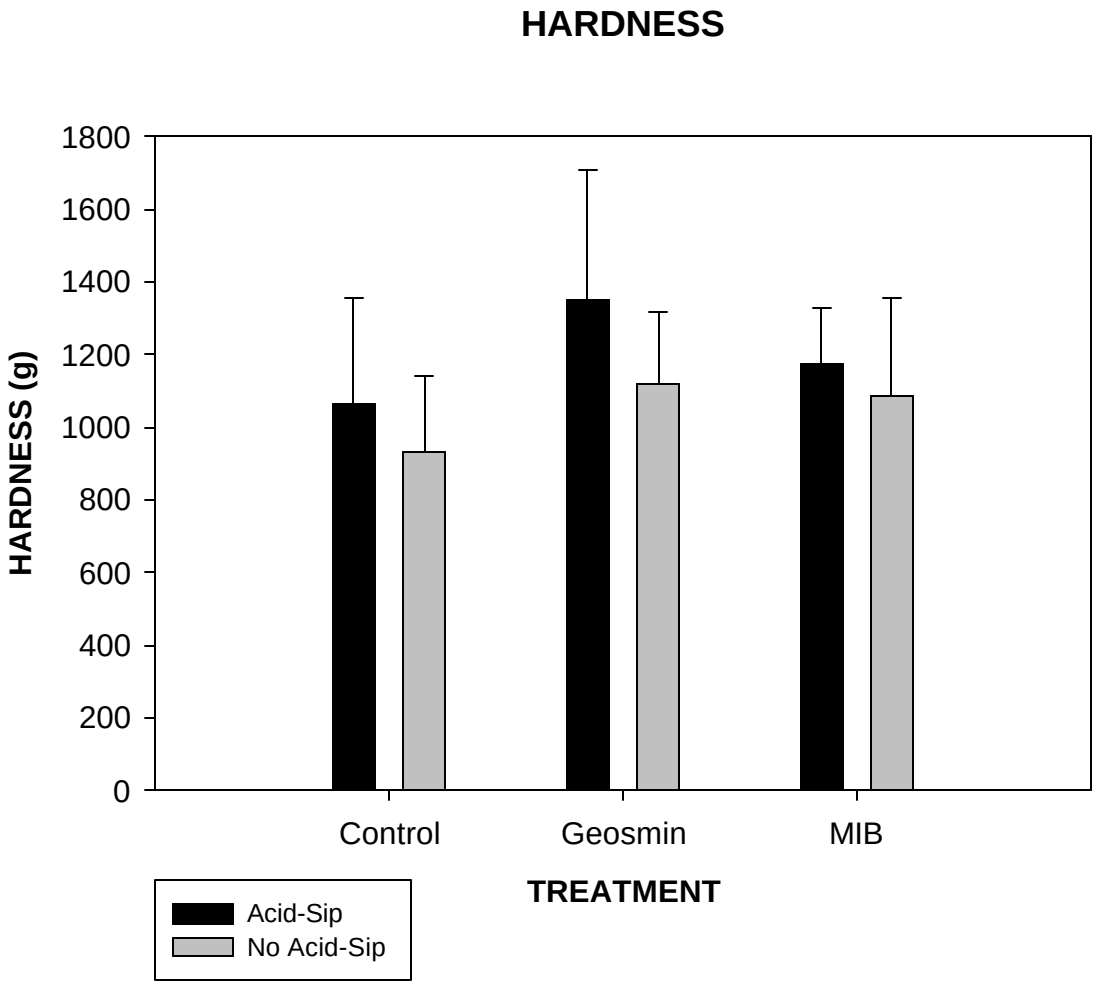


Figure 5. Hardness of Acid-SIP or non Acid-SIP Gels.
Data Represent Means ± Standard Deviation

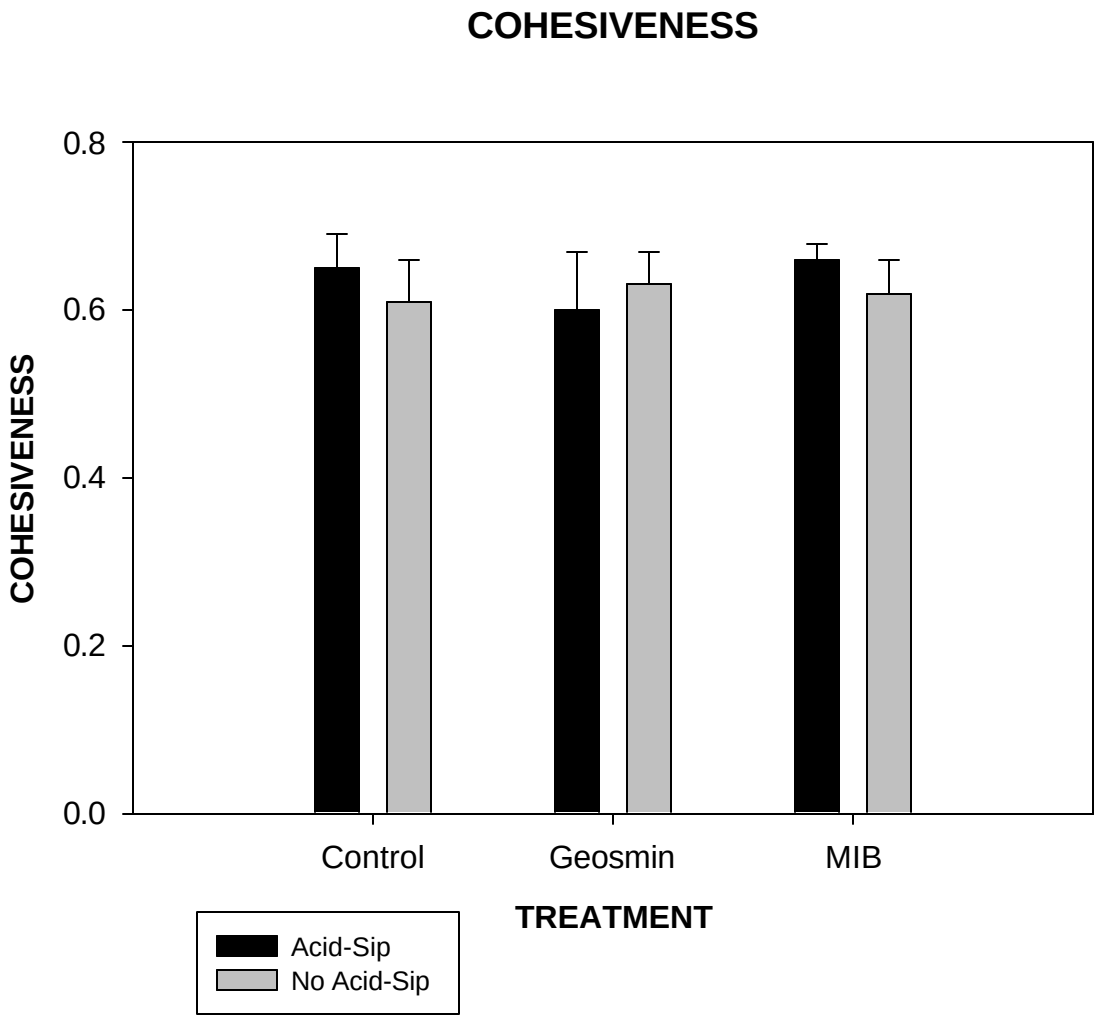


Figure 6. Cohesiveness of Acid-SIP or non Acid-SIP Gels.
Data Represent Means $\pm$ Standard Deviation.

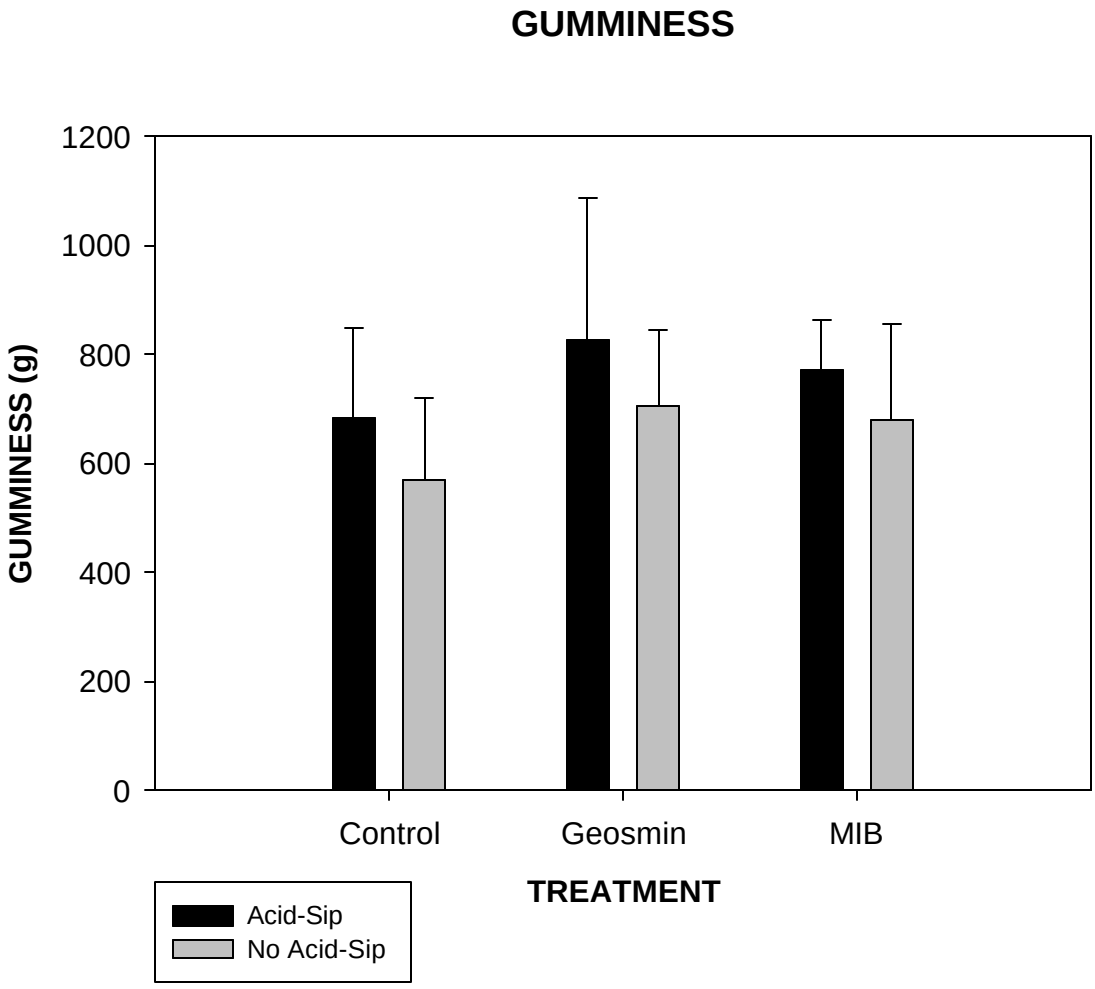


Figure 7. Gumminess of Acid-SIP or non Acid-SIP Gels.
Data Represent Means ± Standard Deviation.

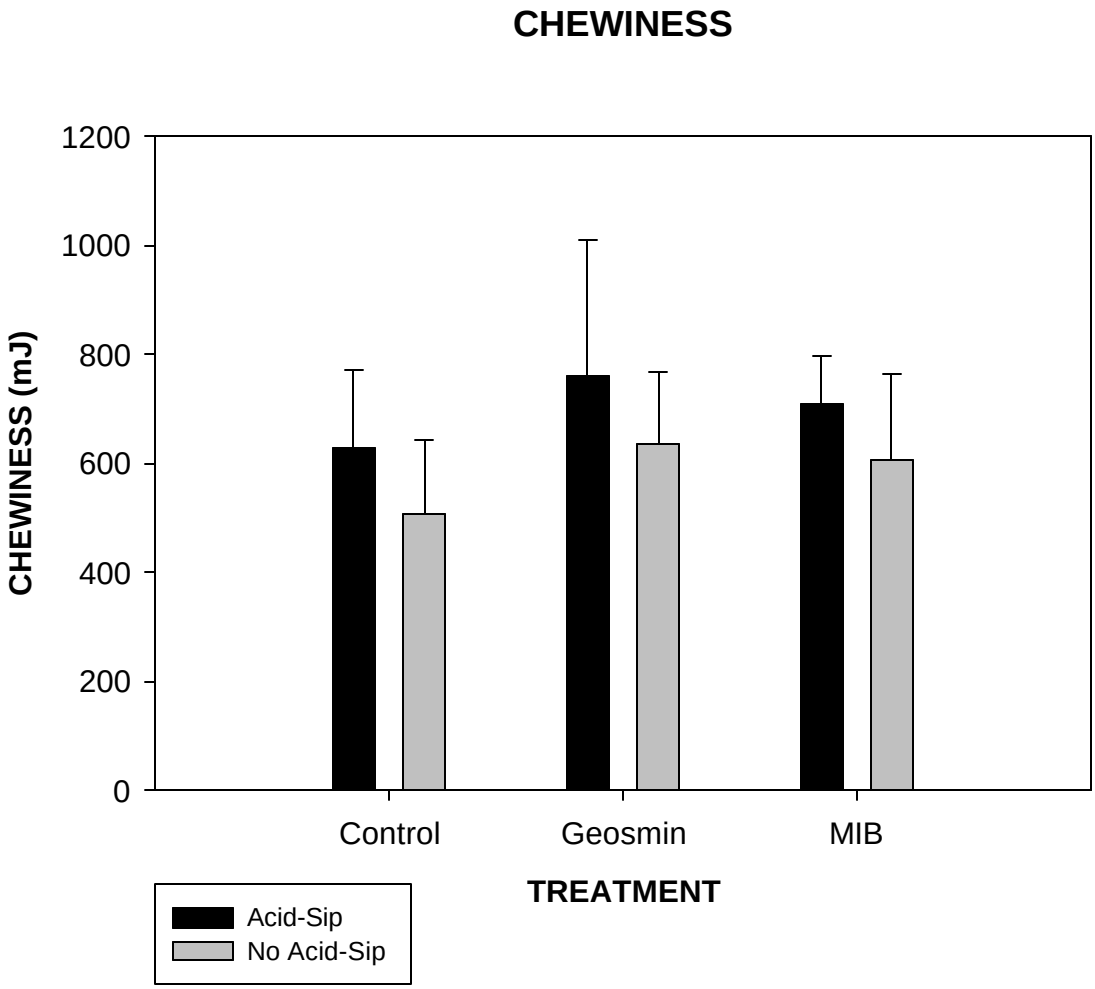


Figure 8. Chewiness of Acid-SIP or non Acid-SIP Gels.
Data Represent Means ± Standard Deviation.

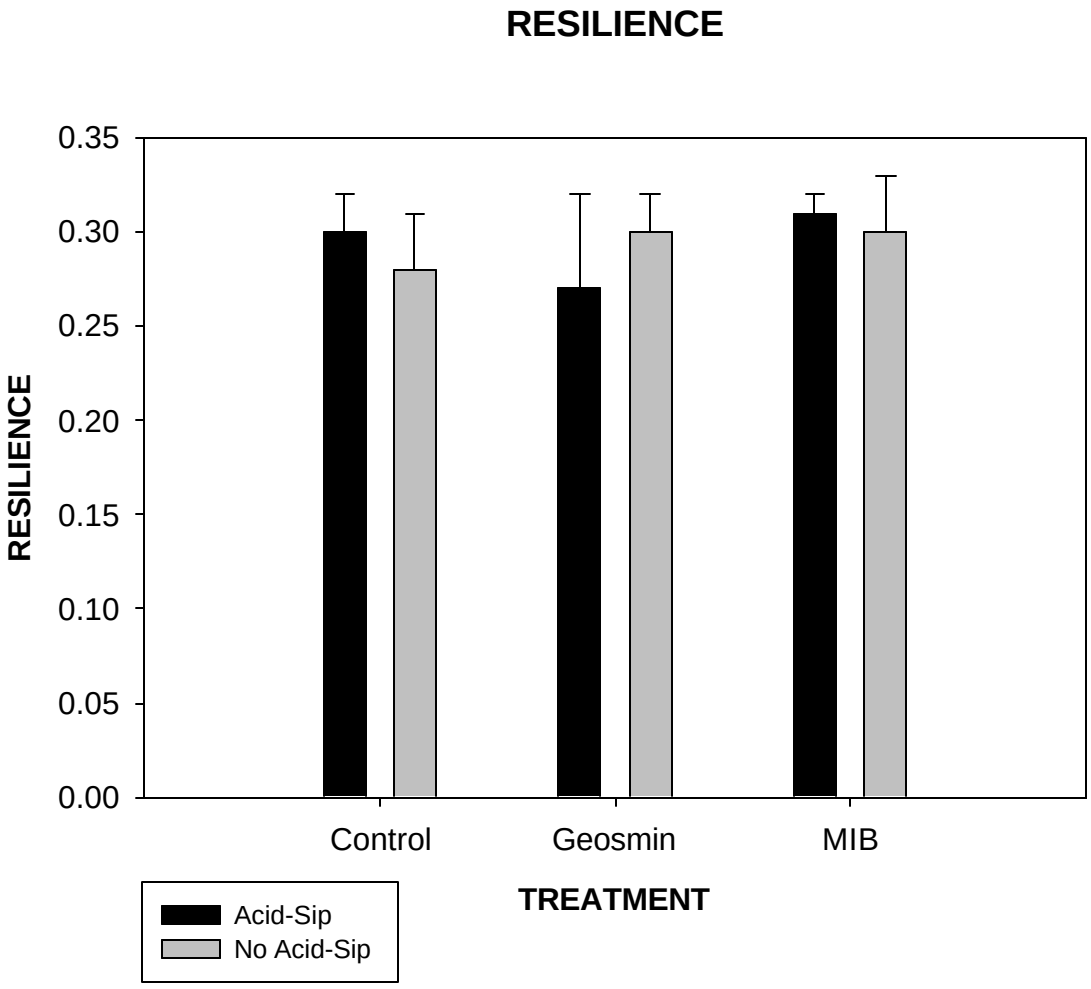


Figure 9. Resilience of Acid-SIP or non Acid-SIP Gels.
Data Represent Means ± Standard Deviation.

LITERATURE CITED

AOAC. 1995. Official Methods of Analysis, 16th edition. Washington DC. Association of Official Analytical Chemists.

Smith DM, Culbertson JD. 2000. Proteins: Functional properties. In: Christen GL, Smith JC. Food Chemistry: Principles and applications. West Sacramento, CA. p 131-148

Daum-Thunberg DL, Foegeding EA, Ball HR. 1992. Rheological and water-holding properties of comminuted turkey breast and thigh: Effects of initial pH. J Food Sci 57:333-337

Forrester PN, Prinyawiwatkul W, Godber JS, Phlak LC. 2002. Treatment of catfish with citric acid causes reduction of 2-methylisoborneol, but not musty flavor. J Food Sci 67(7):2615-2618

Hultin HO, Kelleher SD. 2000. Surimi processing from dark muscle fish. In: Park JW. Surimi and surimi seafood. New York, NY. Marcel Dekker. p 59-77.

Hultin HO, Kelleher SD. 1999. Process for isolating a protein composition from a muscle source and protein composition. Patent # 6, 005, 073 (Dec 21).

Johnsen PB, Lloyd SW. 1992. Influence of fat content and depuration of the off-flavor 2-methylisoborneol by channel catfish (*Ictalurus punctatus*). Can J Fish Aquat Sci 49:1-6

Kelleher SD, Hultin HO. 2000. Functional chicken muscle protein isolates prepared using low ionic strength, acid solubilization/precipitaion. In: 53rd Annual Reciprocal Meat Conference: Meat Science in the New Millennium 2000. Savoy, IL: American Meat Science Association. p 76-81.

Kim BY, Park JW. 2000 Rheology and texture properties of surimi gels. In: Park JW. Surimi and surimi seafood. New York, NY. Marcel Dekker. p 267-324.

Kristinsson HG. 2002. Conformational and functional changes of hemoglobin and myosin induced by pH: functional role in fish quality. [DPhil dissertation]. Amherst, MA: U Mass. 241 p. Available from: Proquest Information ans Learning, Ann Arbor, MI>

Lanier TC.1992. Measurement of surimi composition and functional properties. In: Surimi technology. New York, NY. Marcel Dekker. p 123-166.

Montejano JF, Hamann DD, Lanier TC. 1985. Comparison of two instrumental methods with sensory texture of protein gels. J Texture Studies 16:403-424

Park JW, Morrissey MT. 2000. Manufacturing of surimi from light muscle fish. In: Park JW. Surimi and surimi seafood. New York, NY. Marcel Dekker. p 23-58.

SAS. 2002. Version 8.0. SAS Institute Inc., Cary, NC, USA.

Wiles JL, Green BW, Bryant R. 2004. Texture Profile Analysis and composition of a minced catfish product. J Texture Studies 35(3):325-337

CHAPTER V

SUMMARY OF RESULTS AND CONCLUSIONS

Proximate composition data revealed the acid solubilization ioselectric precipitation (Acid-SIP) process significantly reduced fat content of channel catfish batters compared to batters processed using non Acid-SIP. The reduction in fat content could play a critical role in the reduction or elimination of off-odors/flavors in catfish fillets because off-odor/flavor compounds associate predominately with lipid containing tissues. No differences ($P>0.05$) were found between Acid-SIP and non Acid-SIP batters for moisture, protein, or ash. Non Acid-SIP gels contained a significantly higher percentage of protein ($P<0.05$) than their raw counterparts, most likely as a result of decreased moisture and fat from cooking.

Reductions of geosmin and MIB by the Acid-SIP process were found not to be significant in comparison among gels, although a trend was observed in that Acid-SIP samples contained lower amounts of off-odor/flavor compounds except for geosmin cooked samples. In addition, Acid-SIP samples contained 77-90 % less geosmin and 64-86 % less MIB than the initial concentrations in unprocessed fillets. Sensory data was inconclusive due to inconsistent panelists responses.

Acid-SIP gels had a significantly lower water holding ability (WHA) than non Acid-SIP gels. It is thought that the Acid-SIP process alters native protein structure, thus slightly decreasing the proteins ability to hold water. In addition, NaCl may also have an adverse effect on the WHA of Acid-SIP proteins, due to the conformational change in

their structure. No differences ($P>0.05$) in cook yield were found between Acid-SIP and non Acid-SIP gels. Texture profile analysis revealed that Acid-SIP proteins maintained the ability to produce a functional gel, and even improved the parameter springiness.

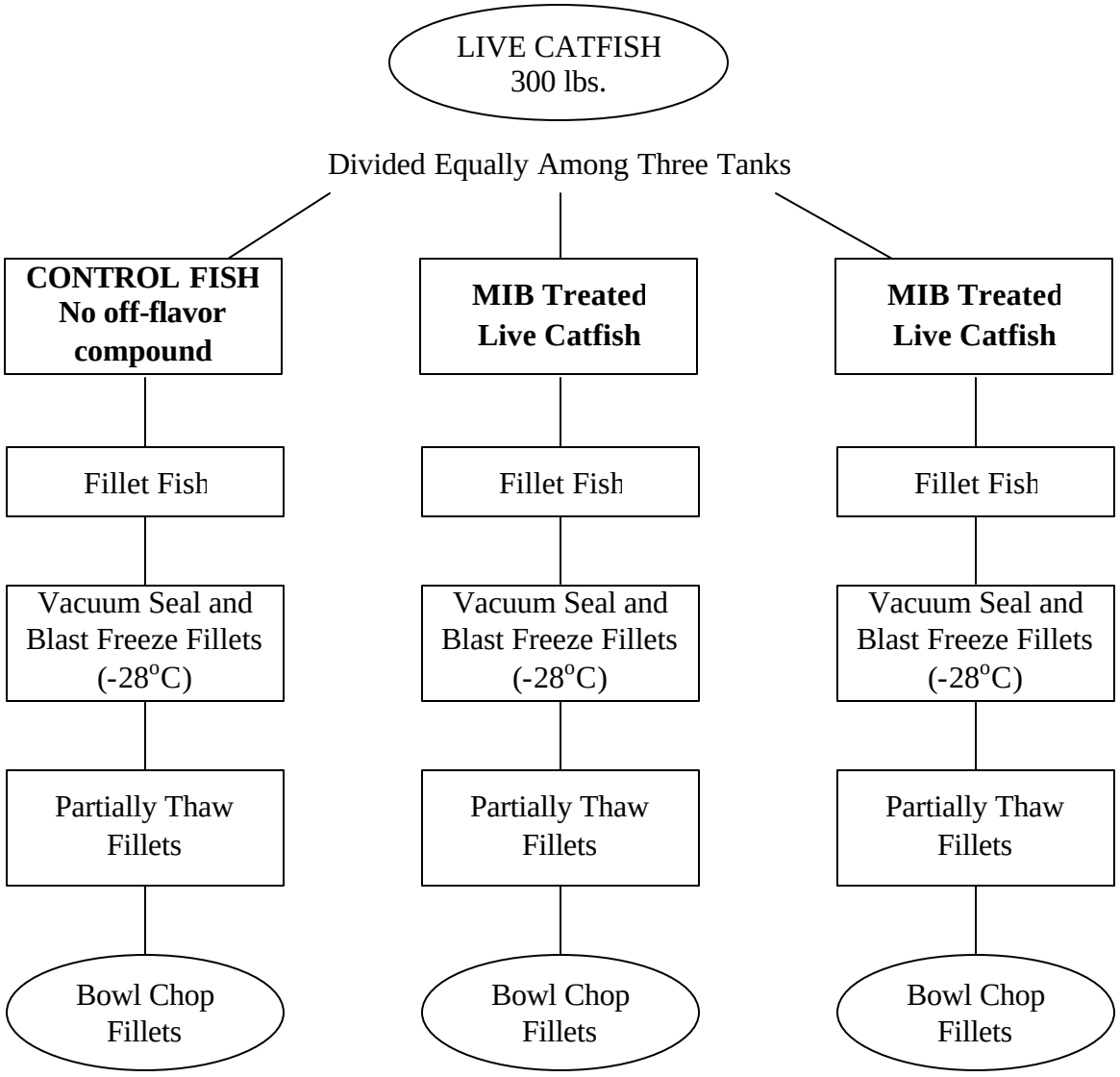
The Acid-SIP process produced a low fat, high protein product with good gel strength attributes. Therefore, the resulting Acid-SIP catfish proteins have the potential to be used in products such as value-added seafood analogs. Furthermore, the Acid-SIP process has the capability to reduce off-odors and flavors in catfish fillets, but further research is needed to develop optimal processing conditions and prove its efficacy.

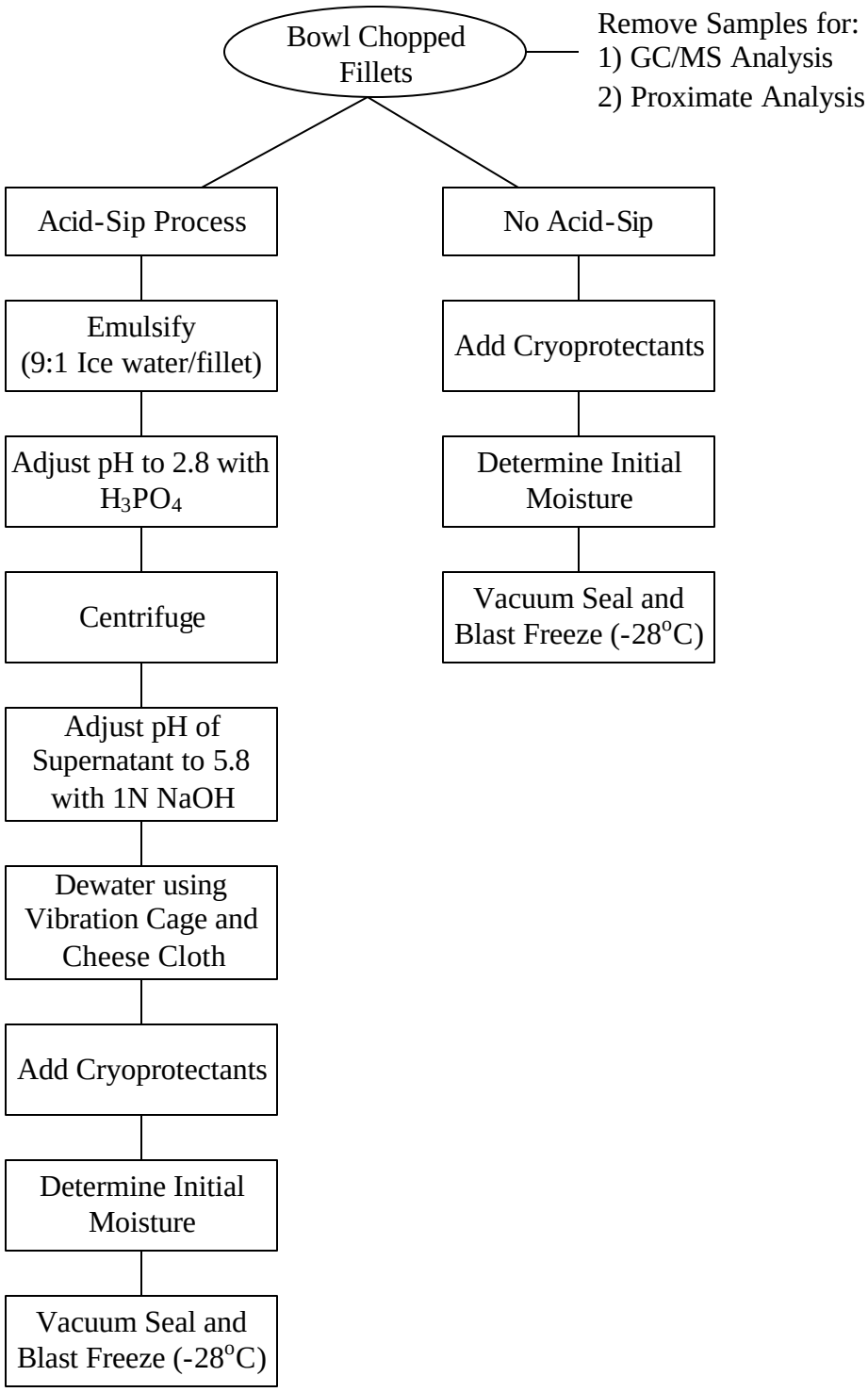
CHAPTER VI

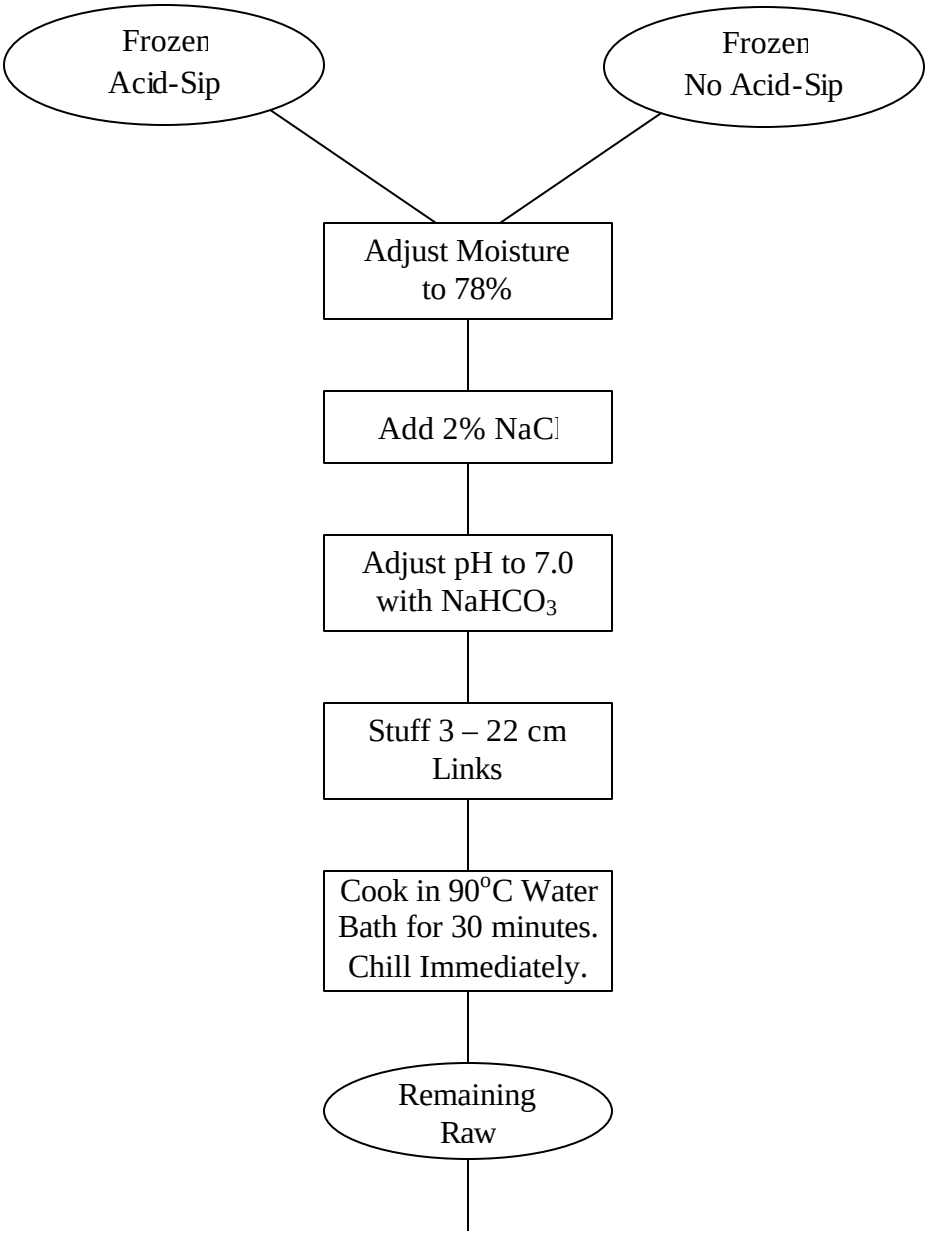
APPENDIX

APPENDIX A

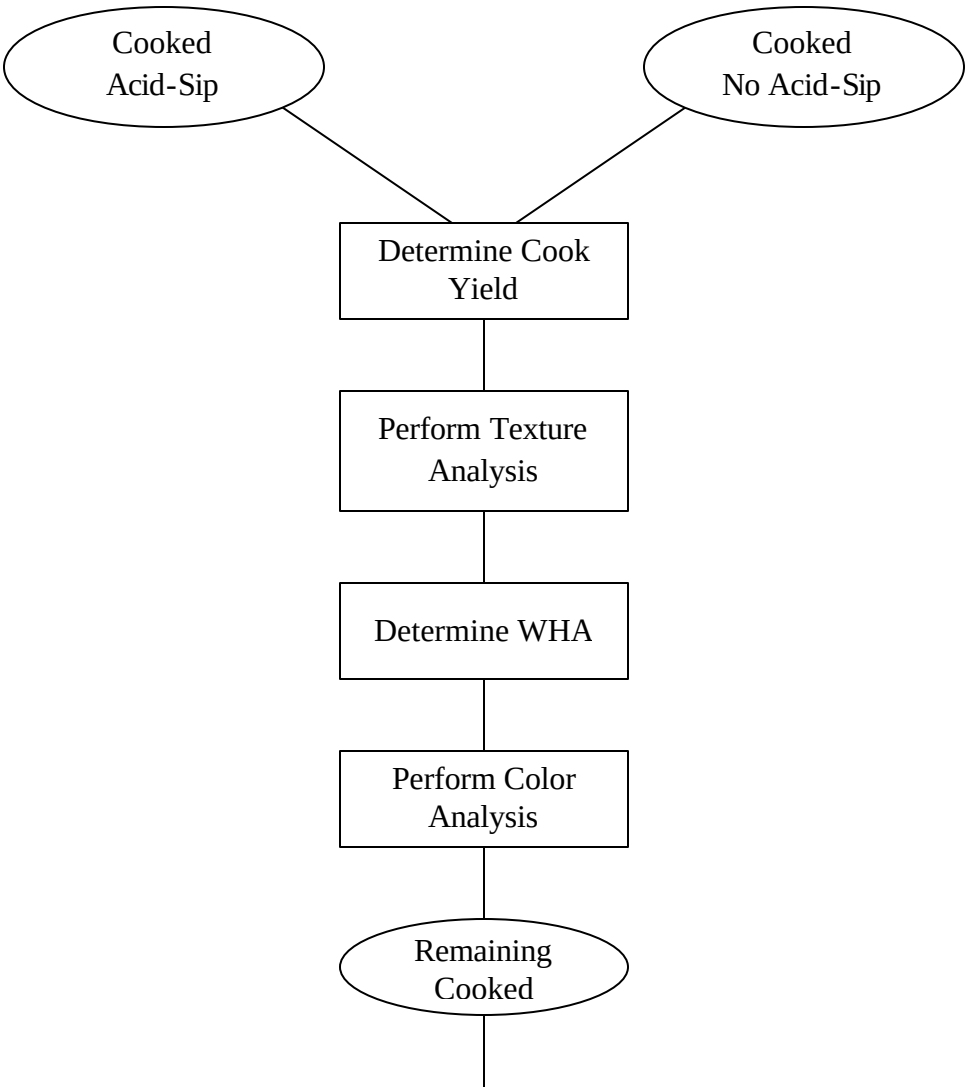
SCHEMATIC OF EXPERIMENTAL DESIGN




Bowl Chopped Fillets
Remove Samples for:
1) GC/MS Analysis
2) Proximate Analysis
Acid-Sip Process
No Acid-Sip
Emulsify (9:1 Ice water/fillet)
Add Cryoprotectants
Adjust pH to 2.8 with H_3PO_4
Determine Initial Moisture
Centrifuge
Vacuum Seal and Blast Freeze (-28°C)
Adjust pH of Supernatant to 5.8 with 1N NaOH
Dewater using Vibration Cage and Cheese Cloth
Add Cryoprotectants
Determine Initial Moisture
Vacuum Seal and Blast Freeze (-28°C)



1. Perform Proximate Analysis
2. Perform Color Analysis
3. GC/MS Analysis


Cooked
Acid-Sip
Cooked
No Acid-Sip
Determine Cook Yield
Perform Texture Analysis
Determine WHA
Perform Color Analysis
Remaining Cooked
1. Perform Proximate Analysis
2. Perform Color Analysis
3. GC/MS Analysis

APPENDIX B

CONCENTRATION OF GEOSMIN OR MIB IN WATER OF TREATMENT TANKS

	CONTROL TANK	GEOSMIN TANK	MIB TANK
Geosmin (ppb)	0.00^{a}	0.81 ± 0.17^{b}	0.00^{c}
MIB (ppb)	0.00^{d}	0.00^{e}	1.36 ± 0.18^{f}

Means within same row or column without common superscript are different ($p<0.05$)

APPENDIX C

COOK YIELD & WATER HOLDING ABILITY OF ACID-SIP AND NON ACID-SIP GELS

TREATMENT	COOK YIELD %**	WHA (g H_20/g PROTEIN)**
Control Acid-SIP*	94.94 ± 0.84[a]	1.27 ± 0.43[a]
Control Non Acid-SIP*	94.87 ± 0.88[a]	2.00 ± 0.50[b]
Geosmin Acid-SIP*	94.25 ± 1.46[a]	0.95 ± 0.61[a]
Geosmin Non Acid-SIP*	94.92 ± 0.53[a]	1.85 ± 0.66[b]
MIB Acid-SIP*	94.58 ± 3.21[a]	1.23 ± 0.39[a]
MIB Non Acid-SIP*	95.29 ± 1.72[a]	1.80 ± 0.49[b]

Data represent mean + standard deviation

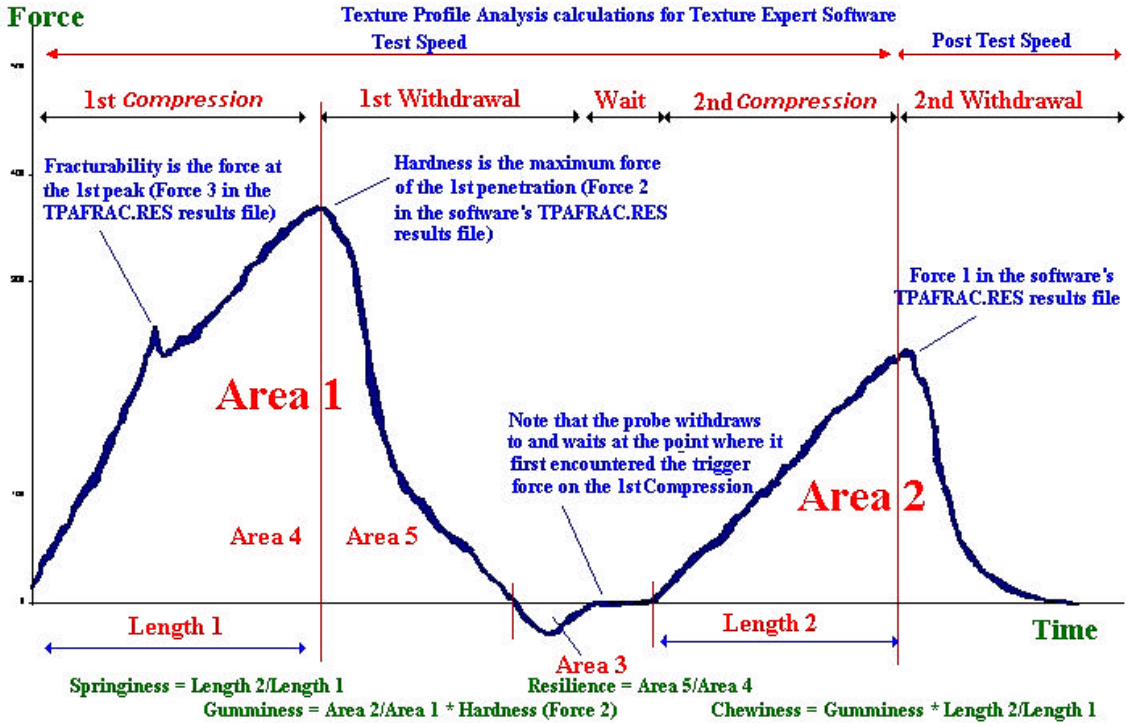
Means within same column without common superscript are different ($p<0.05$)

* Acid Solubilization Isoelectric Precipitation (Acid-SIP)

** Determined according to Daum-Thunberg and others (1992)

APPENDIX D

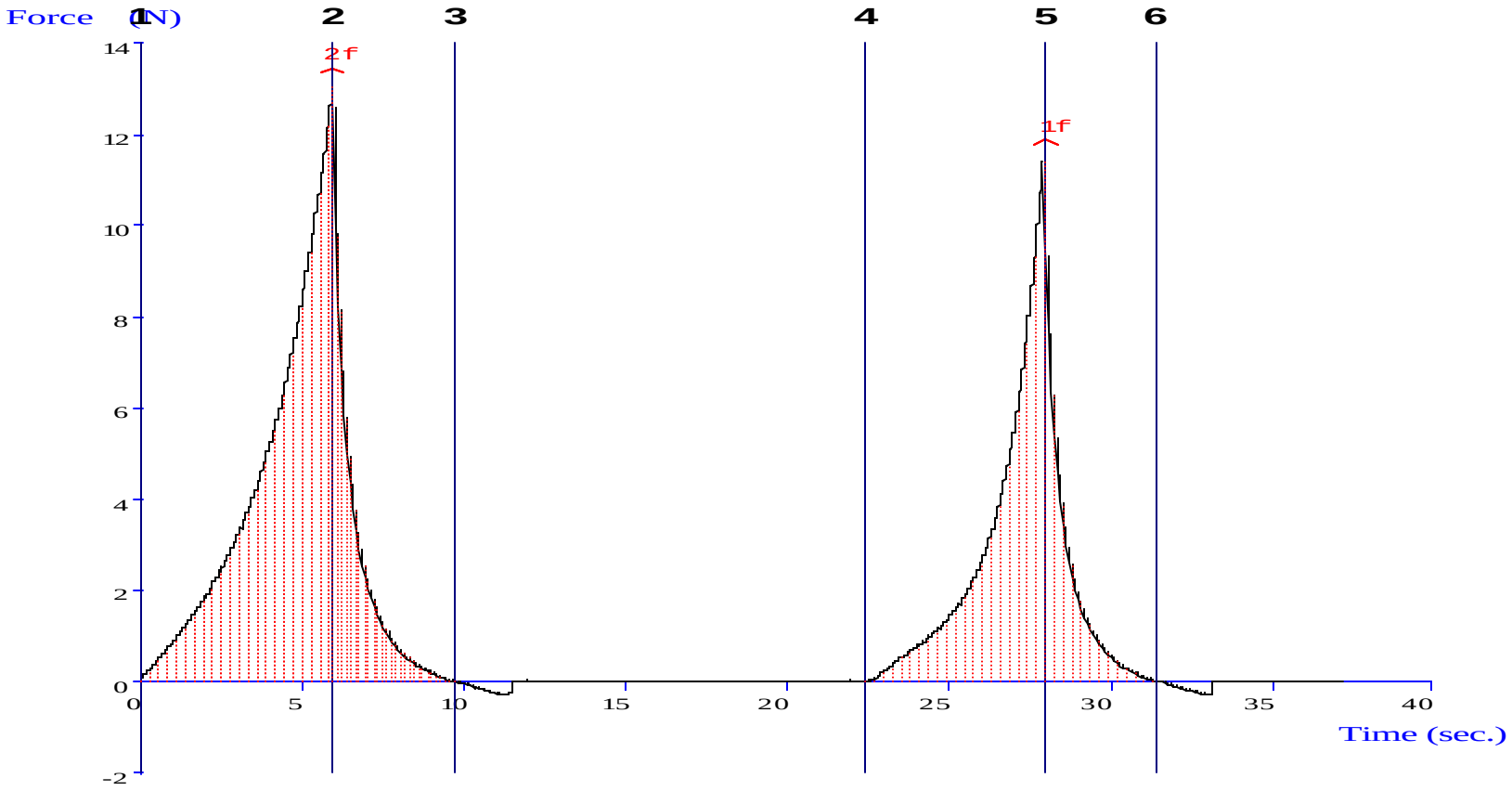
TEXTURE PROPERTIES OF COOKED GELS
Texture Profile Analysis Calculations
www.texturetechnologies.com



APPENDIX E

TEXTURE PROFILE ANALYSIS

Representative TPA of Cooked Acid-SIP or Non Acid-SIP Gels



APPENDIX F

TEXTURE PROFILE ANALYSIS OF ACID-SIP AND NON ACID-SIP GELS.

TREATMENT	HARDNESS (g)	SPRINGINESS (mm)	COHESIVENESS	GUMMINESS (g)	CHEWINESS (mJ)	RESILIENCE
Control Acid-SIP*	1063.33 ± 292.64[a]	0.92 ± 0.02[a]	0.65 ± 0.04[a]	684.88 ± 162.35[a]	628.49 ± 143.48[a]	0.30 ± 0.02[a]
Control Non Acid-SIP*	932.22 ± 210.86[a]	0.89 ± 0.02[b]	0.61 ± 0.05[a]	569.11 ± 153.08[a]	508.39 ± 135.77[a]	0.28 ± 0.03[a]
Geosmin Acid-SIP*	1351.45 ± 354.61[a]	0.92 ± 0.02[a]	0.60 ± 0.07[a]	825.44 ± 262.98[a]	759.64 ± 249.81[a]	0.27 ± 0.05[a]
Geosmin Non Acid-SIP*	1119.04 ± 200.08[a]	0.90 ± 0.02[b]	0.63 ± 0.04[a]	705.31 ± 139.96[a]	636.57 ± 131.33[a]	0.30 ± 0.02[a]
MIB Acid-SIP*	1172.11 ± 157.99[a]	0.92 ± 0.02[a]	0.66 ± 0.02[a]	770.17 ± 94.06[a]	710.20 ± 88.52[a]	0.31 ± 0.01[a]
MIB Non Acid-SIP*	1087.92 ± 265.70[a]	0.90 ± 0.02[b]	0.62 ± 0.04[a]	678.24 ± 177.33[a]	607.20 ± 155.97[a]	0.30 ± 0.03[a]

Data represent mean ± standard deviation

Means within same column without common superscript are different ($p<0.05$)

* Acid Solubilization Isoelectric Precipitation (Acid-SIP)

APPENDIX G
FISH WEIGHTS (REP 1)

CONTROL^		GEOSMIN^		MIB^	
0.86	3.50	1.08	4.56	0.8	4.40
1.26	4.62	1.24	5.20	0.9	4.42
1.38	4.82	1.38	5.30	1.28	4.58
1.40	5.08	1.70	5.48	1.54	4.72
21.52	5.32	1.76	5.76	1.66	4.94
1.54	5.52	1.96	5.84	1.84	5.46
1.72	5.52	2.10	6.08	2.46	5.50
1.74	5.72	2.72	6.30	2.62	5.90
1.76	2.90	2.84	6.38	2.70	5.94
1.94	6.04	2.98	6.60	2.88	7.96
2.36	6.28	3.66	7.1	2.96	
3.32	6.8	4.18	7.24	3.04	
3.44	7.54	4.22	7.36	3.14	
AVG	3.73		4.27		3.55
ST DEV	2.09		2.09		1.89

^Data represents pounds of live fish.

FISH WEIGHTS (REP 2)

CONTROL^		GEOSMIN^		MIB^	
1.86	5.00	2.22	7.02	1.74	3.88
2.4	5.74	2.36	7.38	1.86	4.04
2.58	5.82	2.42	7.42	1.9	4.28
2.64	6.14	2.96	8.2	2.14	4.94
2.66	6.24	3.98	8.9	2.34	6.06
3.26	6.24	5.44	9.38	2.62	6.46
3.32	6.54	5.54		3.08	6.62
4.14	7.22	5.84		3.42	7.06
4.14	7.98	6.24		3.44	7.08
4.22	8.02	6.54		3.52	7.42
4.58		6.78		3.56	7.64
4.66		6.98		3.76	9.74
AVG	4.79		5.87		4.52
ST DEV	1.85		2.24		2.21

^Data represents pounds of live fish.

FISH WEIGHTS (REP 4)

CONTROL^		GEOSMIN^		MIB^	
0.62	2.70	0.54	2.52	0.5	1.68
0.82	3.02	0.94	2.52	0.6	1.70
1.22	3.24	1.04	2.72	0.66	1.76
1.32	3.38	1.14	2.78	0.76	1.80
1.32	3.48	1.32	2.92	0.92	1.80
1.32	3.68	1.36	2.96	1.02	2.02
1.42	3.85	1.38	2.99	1.04	2.05
1.42	3.96	1.40	3.50	1.06	2.16
1.54	4.12	1.40	3.52	1.08	2.30
1.56	4.22	1.42	3.54	1.14	2.40
1.56	4.22	1.44	3.76	1.22	2.48
1.60	4.76	1.46	4.34	1.30	2.48
1.76	5.00	1.48	4.52	1.30	2.98
1.78	5.60	1.52	4.64	1.30	4.30
1.86	7.36	1.54	5.30	1.36	4.46
1.88		1.56	5.80	1.38	5.04
1.94		1.60	6.46	1.40	5.12
2.08		1.68	6.70	1.42	5.22
2.08		1.76		1.46	5.70
2.14		1.76		1.50	5.97
2.14		1.80		1.54	6.28
2.40		1.84		1.62	6.76
2.44		1.92		1.62	8.56
2.52		1.98		1.66	
AVG	2.75		2.54		2.42
ST DEV	1.58		1.54		1.68

^Data represents pounds of live fish

APPENDIX H
IRB FORM

Oklahoma State University Institutional Review Board

Date:	November 3, 2003
IRB Application No:	AG0414
Proposal Title:	Producing a Consumer Acceptable Product from Off-Flavored Catfish (Ictalurus puntatus)
Reviewed and Processed as:	Exempt

Status Recommended by Reviewer(s): Approved Protocol Expires: 11/2/2004

Principal Investigator(s):

Christina A. Mireles DeWitt
104E Animal Science
Stillwater, OK 74078

Russell Nabors
203 FAPC
Stillwater, OK 74078

The IRB application referenced above has been approved. It is the judgment of the reviewers that the rights and welfare of individuals who may be asked to participate in this study will be respected, and that the research will be conducted in a manner consistent with the IRB requirements as outlined in section 45 CFR 46.

As Principal Investigator, it is your responsibility to do the following:

1. Conduct this study exactly as it has been approved. Any modifications to the research protocol must be submitted with the appropriate signatures for IRB approval.
2. Submit a request for continuation if the study extends beyond the approval period of one calendar year. This continuation must receive IRB review and approval before the research can continue.
3. Report any adverse events to the IRB Chair promptly. Adverse events are those which are unanticipated and impact the subjects during the course of this research; and
4. Notify the IRB office in writing when your research project is complete.

Please note that approved protocols are subject to monitoring by the IRB and that the IRB office has the authority to inspect research records associated with this protocol at any time. If you have questions about the IRB procedures or need any assistance from the Board, please contact Beth McTernan in 415 Whitehurst (phone: 405-744-5700, beth.mcternan@okstate.edu).

Sincerely,

Sue C. Jacobs, Chair
Institutional Review Board

VITA

Russell Lee Nabors

Candidate for the Degree

Master of Science

Thesis: Acid Solubilization Isoelectric Precipitation to Remove Off Odors and Flavors Associated with Farm Raised Channel Catfish.

Major Field: Food Science

Biographical:

Personal Data: Born in McAlester, Oklahoma, December 19, 1979, the son of Sam and Vickie Nabors

Education: Graduated from Wynnewood High School, Wynnewood, Oklahoma, May 1998; Received Bachelor of Science Degree in Animal Science from Oklahoma State University, Stillwater, Oklahoma, May 2002; Completed the Requirements for the Master of Science degree with a major in Food Science at Oklahoma State University in July 2005.

Experience: Raised in rural Garvin County, Oklahoma; Employed by the Oklahoma Food and Agricultural Products and Research and Technology Center (FAPC) as an undergraduate, 1999-2002; summer intern at Wright Brand Foods Inc in 2001; graduate research assistant & teaching assistant, 2002-2003; employed by the Oklahoma FAPC as Meat Lab Coordinator/Assistant Meat Plant Manager and a graduate student, 2003-present.

Professional Organizations: American Society of Animal Science.