Post-Harvest Remediation of Off-Odor and Flavor Catfish (*Ictalurus punctatus*) Fillets Using Acid Solubilization Isoelectric Precipitation

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Story in Brief

Many catfish producers are burdened with the chronic management problem of producing off-odor/flavor catfish. Off-odor/flavor catfish results from two compounds, 2-methylisoborneol (MIB) and geosmin, that can reside in the water in which catfish are raised. The resulting off-odors/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. The objective of this study was to apply a post-harvest process to catfish fillets that solubilizes and then recovers protein using low acid conditions (Acid-SIP) and evaluate its effectiveness at eliminating or reducing the off-odors and flavors associated with catfish fillets. Data indicated there was no statistically significant reduction ($P>0.05$) in geosmin and MIB concentrations between Acid-SIP samples and non Acid-SIP samples. However, data does indicate that geosmin and MIB spiked catfish that were subsequently processed using Acid-SIP tended to have lower concentrations of the off-odor/flavor compounds, with the only exception to this trend being cooked samples of the geosmin spiked catfish. Fat content was significantly reduced in all raw Acid-SIP samples while protein and ash contents did not differ. Data indicated the Acid-SIP process produced a low fat protein product with strong gel characteristics. Results showed the process has the capability to lower off-odor/flavor compounds, although further research is needed to refine the process and prove its efficacy.

Key Words: Off Flavor Catfish, Acid Protein Solubilization, geosmin, 2-methylisoborneol.

Introduction

Off-odor and off-flavor catfish results from two compounds (geosmin and 2-methylisoborneol) that can reside in the water in which catfish are raised. 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. Certain pre-harvest methods such as purging live fish to remove off-odor/flavor compounds and using algicides has been attempted. 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 and vacuum tumbling to chemically dehydrate the off-odor/flavor compounds geosmin and MIB into non-odiferous products (Forrester et al ., 2002). The research outlined in this report 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 ., 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.

Materials and Methods

Three hundred pounds of live Channel Catfish (*Ictalurus punctatus*) (average weight 3.5 lb/fish) were acquired from a local producer and were divided equally among three plastic aquariums containing fresh municipal water. The fish were allowed to purge for 24 h, the tanks were then flushed and filled with fresh water followed by the treatment of two of the tanks with either geosmin or MIB with a target concentration of 1 ppb. However, final analysis indicated that the MIB and geosmin concentrations were slightly off target with an average concentration of 1.4 ppb for MIB and 0.81 ppb for geosmin. The fish were then held for an additional 24 h to allow absorption of the off-odor/flavor compounds and were then removed for processing. Processing involved the removal of the fillet, absent of belly flesh and skin, followed by vacuum sealing and freezing at a temperature of –28^{o}C.

Acid Solubilization was conducted under the guidance of Dr Stephen Kelleher at Proteus Industries (Gloucester, MA). Approximately 25 lb of catfish fillets (control, geosmin, or MIB) were thawed to a temperature of 4^{o}C and chopped at 1750 rpm in a Hobart bowl chopper (Troy, OH) for 30 s. Two pounds of chopped fillet were removed for non Acid-SIP analysis and mixed with cryoprotectants in a Stephan brand vacuum chopper (UMC 5 electronic, Stephan Machinery Corp., Columbus, OH) for 1 min and 30 s at 16,000 rpm. Cryoprotectant levels were as follows: sucrose 4%, sorbital 4%, tri-poly phosphate 0.3%, on a total weight basis. Following the addition of cryoprotectants the samples were vacuum sealed and frozen at –28^{o}C until further analysis. The remaining 23 lb of chopped fillets were mixed with ice water (9:1 ice water/fillet, weight basis) and chopped with a Stephan MCH15 emulsifier for 10 s. This homogenate was transferred to a Waukesha 134 pump (Delevan, WI) and the pH of the homogenate was adjusted to 2.8 with phosphoric acid. The homogenate was then centrifuged using an Alfa Laval centrifugal separator LAPX 404 (Tumba, Sweden) at the following parameters: 9,500 rpm, 200-240 m/hr flow rate. The centrifuged homogenate was subsequently pumped into plastic containers and the pH was increased to 5.8 using sodium hydroxide to precipitate the protein. The protein precipitate was de-watered using a Sweco vibration cage (Florence, Kentucky) and cheese cloth. Cryoprotectants were then added to the protein at the concentrations listed above using a Cuisinart food processor (Waring Commercial, Torrington, CT). Cryoprotectants were also chopped and blended with fillets from catfish that had not been acid solubilized. The cryoprotected protein was then vacuum sealed and frozen at –28^{o}C until further analysis.

Initial moisture was determined for all samples by oven drying prior to batter preparations (AOAC 1995). Batter preparation involved partially thawing either the Acid-SIP cryoprotected protein or the non Acid-SIP fillet mixture to a temperature of approximately –5°C. All samples were adjusted to pH 7 using 5% NaHCO3. Ice and NaCl were added according to Park and Morrissey (2000). Target moisture and NaCl concentrations were 78% and 2%, respectively. Mixing parameters were followed as outlined by Park (2000) using a Stephan brand vacuum chopper. Batters were immediately transferred to tabletop piston stuffer (12 lb capacity, Friedr. Dick Corp., Farmingdale, NY) equipped with a 12 mm filling tube. Each of the batters were 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 (10x13) and sealed. The links were then cooked in a 90°C water bath for 30 min. Cooked links were chilled on ice for 30 min and refrigerated (4°C) over night.

Proximate composition was determined using accepted AOAC methods. Geosmin and MIB samples were shipped to the Thad Cochran Research Center (USDA., University, MS) and were analyzed using gas chromatography/mass spectrometry as outlined by Grimm et al (2000). Water holding ability and cook yield was determined according to Daum-Thunberg et al (1992). Texture was evaluated with the Stable Micro Systems' Texture Analyzer (Model TA-XT2i, Texture Technologies, Inc., Scarsdale, NY) with three randomly sliced 2 cm long segments per link tempered to room temperature. A macro was programmed into the Texture Analyzer to allow the probe (TA-3 2.5 cm acrylic cylinder probe) to have a double compression into the sample with a 10 s delay between the two 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. The parameters measured were as follows: hardness, cohesiveness, springiness, chewiness, and resilience.

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). 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

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 ($P<0.05$) fat content of all samples in comparison to non Acid-SIP samples. Furthermore, when compared to initial fat contents of raw, unprocessed fillets the Acid-SIP

process decreased fat content by 96-97%. Hultin and Kelleher (2000) reported similar findings, indicating that after protein 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).

Analysis revealed no statistical differences in moisture, protein, fat, and ash of the gels. The analysis did indicate that the non Acid-SIP gels contained a significantly lower ($P<0.05$) fat % than the non Acid-SIP Batters (i.e. starting ingredient of Acid-SIP gels). 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 was higher ($P<0.05$) than the batters. 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.

Initial concentrations of geosmin and MIB in the raw unprocessed fillets are presented in Table 1. Achieved concentrations of geosmin and MIB are above the reported threshold levels for human sensory detection (Forrester et al 2002, King and Dew 2003). This result was desired and necessary to measure the effectiveness of the Acid-SIP process to eliminate 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. All three repetitions were statistically 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 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). Johnsen and Lloyd (1992) found that catfish with higher fat contents absorb and store more MIB than their leaner counterparts.

Table 1. Concentration of Geosmin and MIB in Raw Unprocessed Fillets

	Control	Geosmin	MIB
Geosmin (µg/kg)	$.17 \pm .06^{a}$	3.18 ± 1.45^{b}	$.11 \pm .06^{c}$
MIB (µg/kg)	$.01 \pm .01^{d}$	$.02 \pm .03^{e}$	3.80 ± 1.18^{f}

Data represents µg of geosmin or MIB per kg of sample

^{abcdef}Means within same row or column without common superscript are different ($P<0.05$)

Overall, geosmin and MIB concentrations for batters and gels (Table 2) 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. Although significance was not determined, likely due to the Rep interaction, a definite 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 1). 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 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 did 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 in 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.

Table 2. Concentration of Geosmin or MIB in Batters or Gels

Fish Treatment	Process Type	Geosmin^	MIB^
Spiked	Acid-SIP* Batters	$.308 \pm .147^{ac}$	$.509 \pm .152^{ac}$
	Non Acid-SIP* Batters	$.579 \pm .207^{ac}$	$.939 \pm .337^{ab}$
	Acid-SIP* Gels	$.733 \pm .489^{b}$	$.917 \pm .263^{ab}$
	Non Acid-SIP* Gels	$.557 \pm .149^{abc}$	$1.371 \pm .390b^{b}$
Non Spiked (Control)	Acid-SIP* Batters	$.034 \pm .017^{c}$	$.045 \pm .066^{c}$
	Non Acid-SIP* Batters	$.028 \pm .005^{c}$	$.002 \pm 002^{c}$
	Acid-SIP* Gels	$.025 \pm .009^{c}$	$.017 \pm .02^{c}$
	Non Acid-SIP* Gels	$.052 \pm .053^{c}$	$.012 \pm .014^{c}$

^Data represents µg of geosmin or MIB per kg of sample

*Acid Solubilization Isoelectric Precipitation (Acid-SIP)

[abc]Means within same column without common superscript are different ($P<0.05$)

The cook yield percentage of Acid-SIP samples did not differ ($P>0.05$) from that of the non Acid-SIP samples with both treatment types possessing cook yield percentages of approximately 94 % or greater. The water holding ability 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 + 0.54g/g protein).

There could be many factors that contribute to this finding. 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 (Smith and Culbertson 2000). In contrast, NaCl may not interact effectively with proteins recovered from the Acid-SIP process, resulting in decreased WHA.

Overall, 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

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