A Comparative Study between Acid- and Alkali-aided Processing and Surimi Processing for the Recovery of Proteins from Channel Catfish Muscle

HORDUR G. KRISTINSSON, ANN E. THEODORE, NECLA DEMIR, AND BERGROS INGADOTTIR

ABSTRACT: Channel catfish muscle was subjected to 2 novel protein extraction and precipitation techniques using acid (pH 2.5) or alkaline (pH 11) pH and compared with surimi processing (3 wash cycles). Solubility of catfish proteins was found to be highest at pH 2.5 and 11, and at these pH levels, viscosity was found to be low enough to cause separation of proteins from insoluble materials via centrifugation. Both the acid-aided and alkali-aided processes led to higher recoveries ($P < 0.05$) of protein and larger reduction ($P < 0.05$) in lipids compared with surimi processing. The protein recovery could be increased even more with a modified version of the acid-aided and alkali-aided processes. There was no hydrolytic breakdown detected during low and high pH. The acid-aided process recovered more protein types than the alkali-aided process during isolelectric precipitation (pH 5.5), which indicated that it led to more protein denaturation and thus more aggregation at pH 5.5. The alkali-aided process had more soluble proteins (including heme proteins) at isoelectric precipitation than the acid-aided process, and the soluble proteins were of the same type as the soluble proteins for non–pH-treated catfish muscle at pH 5.5. This suggested the alkali-aided process led to less denaturation than the acid-aided process. Both acid-aided and alkali-aided processes recovered proteins of higher ($P < 0.05$) whiteness scores than surimi. The alkali-aided process recovered proteins of higher whiteness ($P < 0.05$) than the acid-aided process. The acid-aided process led to higher yellowness ($P < 0.05$) than the other 2 processes. All processes led to minimal levels of lipid oxidation as assessed by secondary oxidation products.

Keywords: catfish, protein isolate, acid-aided processing, alkali-aided processing, surimi

Introduction

At the current time, the demand for fish protein has exceeded the supply of traditional raw materials, leading to great pressure on fish stocks (Hultin and Kelleher 2000). Using conventional technologies to process fish and creating value added fish products generally leads to limited utilization of the animal; also, much protein and many lipid-rich byproduct materials are lost and not recovered for human use (Kristinsson and Rasco 2002). Although better use of these materials has always been of great interest, it has been hampered with very limited success in developing functional and acceptable products for the end consumers. A process that has been met with some success in recovering fish proteins is the production of surimi; however, yields are low because the process involves several washing steps. Surimi processing has been unsuccessful on unconventional raw materials, such as pelagic species, in part because of the abundance of oxidatively unstable lipids and many pro-oxidants (especially heme proteins), which result in color and oxidation problems (Hultin 2002). Most attempts with surimi and materials rich in dark muscle have resulted in products with poor gelation properties and considerable problems with color and lipid oxidation (Okada 1980; Hultin and Kelleher 2000). Separation of undesirable constituent of these materials such as bones, scales, skin, and fat from the desirable muscle proteins has also been met with numerous difficulties (Hultin and Kelleher 2000).

To address the problem of utilization of unconventional raw materials, a process was developed to economically produce functional protein isolates (PIs) from fish sources of low value. This process uses the pH-dependent solubility properties of fish muscle proteins for their separation and recovery from other components of muscle not desirable in a final product. The process involves subjecting a diluted (1:9) slurry of homogenized muscle tissue to a low (pH 2 to 3.5) or high (pH 10.5 to 11.5) pH. These pH values allow the muscle proteins to be solubilized and disrupt the cellular membrane encasing the myofibrillar proteins. The acid and alkaline conditions used in the process are far enough from the muscle protein's isoelectric points (approximate pH of 5 to 6) that the protein side chains gain a net positive (at acidic pH) or negative (at alkaline pH) charge, causing the proteins to repel each other and solubilize. The disruption of the muscle cell and solubilization of the proteins causes a great decrease in solution viscosity, enough to enable cellular membranes to be separated from the soluble proteins by high-force centrifugation (Hultin and Kelleher 2000), at the same time removing solids such as bones and scales and neutral fat. The essentially membrane-free and lipid-free soluble proteins are then recovered by isoelectric precipitation by raising the pH to about 5.5, and the resulting isolate can be used for various purposes, for example, as a functional food ingredient or directly to produce value-added fish products such as surimi.

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This process offers a new technology to recover and use protein from the more than 50 million tons of underused aquatic species and byproducts used to make animal feed for human consumption. Furthermore, because the process has the potential to recover most of the muscle proteins from any raw material, yields can be substantially increased on conventional surimi species such as Alaska pollock and whiting. The reduction in lipids is a key step in this process because the raw material going into the process may be rich in triacylglycerols and membrane phospholipids. The membrane phospholipids are known to be the main substrate for oxidative reactions in fish muscle (Hultin 1994; Gandemer 1999) and their removal is expected to greatly enhance the stability of the final product. The increase in yield is also a key advantage because the raw material can be more responsively used. In addition, studies have shown that the proteins recovered by this process have good functionality and in some cases better gelation properties than proteins recovered with conventional surimi processing (Hultin and Kelleher 2000; Undeland and others 2002; Kristinsson and Hultin 2003b; Kristinsson and Demir 2003; Theodore and Kristinsson 2003).

Most work on the acid-aided and alkali-aided processes has centered on species from colder water, and it is of interest to evaluate their use on warm-water species. Channel catfish, a temperate/warm water species, is the major aquacultured species in the United States, with about 680 million pounds harvested in 2002 and having a major economic effect in the southern United States (Mayo 2003). Fillet yields for catfish are at maximum 45% of the weight of catfish (Kim and others 1996), leaving much protein and many lipid-rich byproducts. Currently, these byproducts are primarily used for animal feed or pet food. There is a great potential to utilize these products for human consumption, which could significantly benefit processors economically. Moreover, protein isolates could be further processed into formulated value-added products. The objective of this study was therefore to investigate the use of the novel acid-aided and alkali-aided processes on the recovery and quality of proteins from catfish muscle compared with conventional surimi methods.

Materials and Methods

Raw material

Because this is the 1st published investigation of the acid-aided and alkali-aided processes on catfish, fillets (including fat and dark muscle) were chosen as the raw material for the investigation. Skinless fillets of channel catfish were obtained fresh (never frozen) on ice from a local supplier within 24 h of harvest. On arrival, fish muscle (including both dark and white muscle) was ground at 4 °C in a Scoville grinder (Hamilton Beach, Washington, N.C., U.S.A.) with 6-mm holes, and this material was used for subsequent studies described below. All steps were conducted either on ice or at 4 °C in a cold room, and we ensured that the material did not reach higher than 5 °C in any of the preparations or tests.

Protein solubility

Ground muscle was diluted in deionized water (1:9) and homogenized with a hand held Tissue Tearor (Biospec Products Inc., Bartlettsville, Okla., U.S.A.) on speed 10 for 1 min. The muscle protein homogenate was then separated into 2 fractions. One of the fractions was adjusted down to pH 1.5, whereas the other fraction was adjusted to pH 12. Samples were taken at 0.5 pH intervals for protein solubility and viscosity testing. For the protein solubility tests, the protein homogenate were transferred to 50-mL centrifugation bottles and centrifuged at 10000 × *g* for 20 min (4 °C). The protein content of the middle layer was determined using the Biuret method (Torten and Whitaker 1964), and total protein in the middle layer calculated. Percent soluble protein was determined by dividing the total protein content in the middle layer after centrifuging with the total protein content of the protein homogenate before centrifuging. The effect of centrifugal force (2500 to 15000 × *g*) was also determined at pH 2.5 and 11.

Protein viscosity

Viscosity (η) and phase angle (δ) of the catfish homogenates were determined in 0.5 pH intervals from pH 1.5 to 12.0. Homogenates were prepared individually for each pH by weighing 20 g of ground catfish and 180 g cold deionized water (1:9 dilution) into a 250-mL plastic beaker. The mixture was homogenized with a hand held Tissue Tearor (Biospec Products Inc.) on speed 10 for 1 min. The pH was adjusted to the desired value using either 2 *M* HCl or 2 *M* NaOH. Viscosity and phase angle were determined using a single gap cylinder geometry in an AR2000 Advanced Rheometer (TA Instruments, New Castle, Del., U.S.A.) by adding 15 mL of the homogenate to the cylinder and then lowering a cylindrical head into the cylinder. The sample was allowed to equilibrate for 2 min before measurements were performed using an oscillatory time sweep program with frequency set at 0.1 Hz, oscillatory stress at 0.1809 Pa, temperature at 5 °C, and a run time of 2 min. Graphs were constructed using the equilibrated final readings of viscosity and phase angle for each pH.

Preparation of Protein Isolates

Protein isolates were produced using both the acid-aided and alkali-aided process (Figure 1a). The processes involved homogenizing ground muscle for 30 s in 9 volumes of water at 4 °C in a Waring blender (Waring Products, Torrington, Conn., U.S.A.) with 2 bursts of 30 s at 50% electrical output. The pH of the homogenate was then either lowered to pH 2.5 (for the acid process) or increased to pH 11 (for the alkaline process) by slowly adding 2 *M* HCl or 2 *M* NaOH, respectively, with slow but constant stirring. The homogenate was then transferred into centrifuge bottles and centrifuged at 10000 × *g* for 20 min at 4 °C in a Sorvall RC-5B centrifuge using a GS-3 rotor (Kendro Laboratory Products, Newtown, Conn., U.S.A.). This step separates the soluble proteins from the neutral lipids (that is, storage fat), which remain at the top of the solution, and the membranes (which contain phospholipids) and solid material in the fish (mostly connective tissue and some bone), which settles to the bottom. The middle phase, which contains the solubilized muscle proteins, was then collected by pouring it through a strainer lined with a double layer of cheesecloth. The pH of the recovered middle phase was then adjusted to pH 5.5 to aggregate the proteins. This solution was then centrifuged at 10000 × *g* for 20 min at 4 °C to settle the aggregated proteins. The sediment was the PI. Protein isolate was also made with a modified version of the acid-aided and alkali-aided processes, in which the 1st centrifugation step was omitted and the homogenate readjusted to pH 5.5 after 40 min at low or high pH (the total holding time at low or high pH before readjustment to pH 5.5 for the samples that underwent the 1st centrifugation). The homogenate was centrifuged as described previously, which left a fat layer at the top, supernatant as the middle phase, and a PI as the sediment. Isolates were produced from several different batches of raw material to determine variations in the processes.

Production of surimi (washed catfish muscle)

To produce surimi (washed catfish muscle), a conventional laboratory scale process was used (Figure 1b), except cryoprotectants were not added to the washed fish muscle and the freezing step was

excluded. Ground catfish muscle was gently mixed into 3 volumes of cold (4 °C) water and slowly stirred with a rubber spatula for 15 min, following a 15-min period of settling. The slurry was then dewatered by pouring it into a strainer lined with two layers of cheesecloth followed by squeezing loosely bound water out of the washed material. This process was repeated 2 times, with the last wash including 0.2% NaCl to aid in dewatering. All steps were performed on ice. Surimi was produced from several different batches of raw material (same as that from which the PI was processed) to determine variations in the processes.

Analysis of protease activity and protein breakdown

Potential hydrolysis was investigated at the processing pH's used for surimi and PI. Ground muscle was homogenized and diluted 9-fold with deionized water, and the homogenate was separated into 3 fractions, which were adjusted to pH 2.5 (to represent the acid PI process) and 11 (to represent the alkali PI process), with one being left unadjusted (to represent the surimi process). The pH was between 6.5 and 6.6 in the unadjusted system. Aliquots were taken from the homogenates at selected times and samples prepared for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) electrophoresis and run on pre-cast gels, as described subsequently. Potential hydrolytic activity at low and high pH was also assayed according to the Azocoll assay of Kristinsson and Rasco (2000).

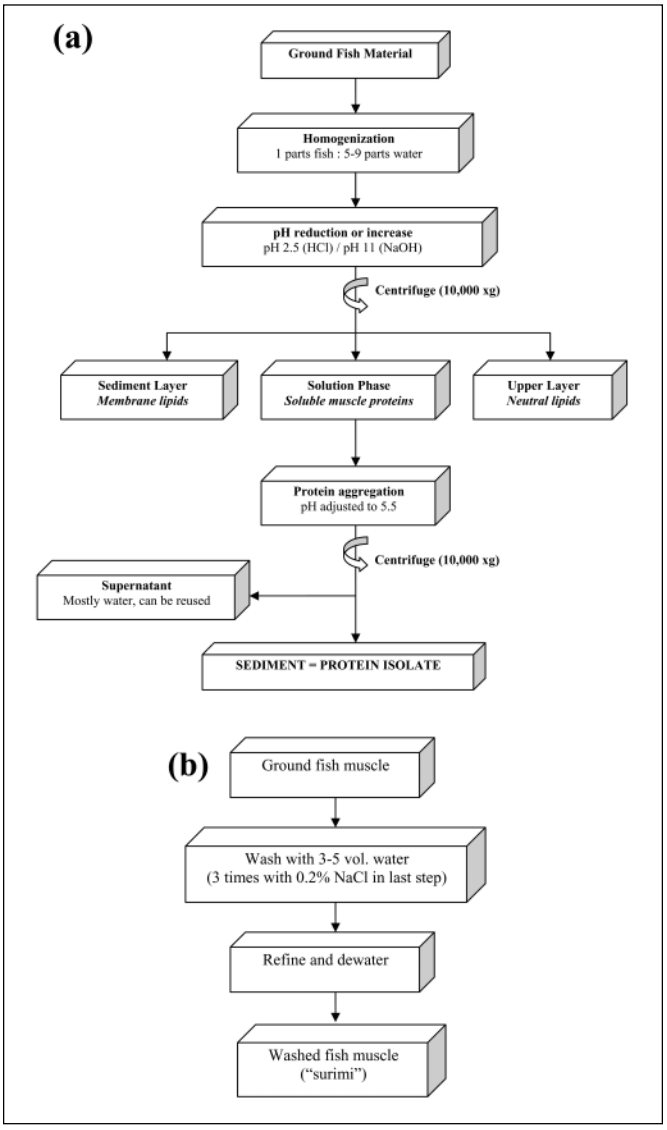

Figure 1—The processes used in the study: (a) the acid-aided and alkali-aided processes; (b) the laboratory scale "surimi" process. The laboratory scale "surimi" made in this study was not frozen and without cryoprotectants.

SDS-PAGE analysis of isolated fractions

The protein composition of the starting ground material, surimi, and the different fractions collected during the different steps of the acid-aided and alkali-aided protein isolation process were analyzed by SDS-PAGE electrophoresis. The protein composition of the precipitated (pH 5.5) isolates and resulting supernatant were also compared with precipitated (pH 5.5) catfish proteins that did not undergo high or low pH treatment and resulting supernatant. Pre-cast 4% to 20% linear gradient SDS-PAGE gels from Bio-Rad (Bio-Rad Laboratories, Hercules, Calif., U.S.A.) were used. The protein sample to be analyzed was prepared in Laemmli buffer (Bio-Rad Laboratories) with β-mercaptoethanol as the reducing agent, to give a 4 mg/mL total protein concentration. A volume of 15 μL was loaded on the gel and the electrophoresis run at 200V in a Mini-PROTEAN 3 Electrophoresis Cell (Bio-Rad Laboratories). Gels were fixed for 1 h in 12% trichloroacetic acid and stained overnight with Sigma brilliant blue perchloric acid (Sigma Chemicals, St. Louis, Mo., U.S.A.) followed by destaining in deionized water. Gels were scanned with an Epson Stylus CX5400 scanner (Epson, Long Beach, Calif., U.S.A.), and images were processed and protein bands quantified using the software Scion Image 4.0.2 (Scion Corp., Frederick, Md., U.S.A.). Muscle protein bands were identified according to Stefansson and Hultin (1994) with a standard curve constructed using a wide range molecular weight SDS-PAGE standard (Sigma Chemicals).

Proximate analysis and calculation of protein recovery and lipid reduction

The ground raw material, surimi, and PI were analyzed for protein content using the Biuret method (Torten and Whitaker 1964). Protein recoveries were calculated from the difference in the total protein in the surimi or PI compared with the starting material. Lipids were extracted and quantified according to the method of Lee and others (1996). Lipid reduction was calculated from the amount of total lipids in the surimi and PI compared with that in the starting material. Moisture content was determined by using a Cenco moisture balance (CSC Scientific Co. Inc., Fairfax, Va., U.S.A.).

Color analysis

Color of the raw material, surimi, and the PI was determined by using a handheld Minolta colorimeter (Minolta Ltd., Osaka, Japan). A white standard plate was used to calibrate the colorimeter. A minimum of 5 readings of Hunter L^*, a^*, and b^* values were taken from each batch of ground raw material, surimi, and PI, and values were averaged. Whiteness was calculated according to the following formula:

$$Whiteness = 100 - \sqrt{(100 - L)^2 + a^2 + b^2}$$

Analysis of lipid oxidation products

A portion of the initial raw material, PIs, and surimi were immediately collected after production and vacuum-packed in bags of very low O_2 permeability (<20 cm^3/in * 24 h) and frozen at –70 °C to arrest oxidation until they were analyzed (7 d). For analysis of oxidation products, the samples were rapidly thawed under cold running water and then analyzed for secondary lipid oxidation products using the modified thiobarbituric acid-reactive substances (TBARS) method described by Lemon (1975).

Statistical analysis

Each experiment and each assay was done in at least triplicate. Reported results represent an average of each experiment and assay. Analysis of variance was used to determine significant differences between samples using Microsoft Excel (Microsoft Corp., Redmond, Wash., U.S.A.)

Results and Discussion

Protein solubility

High solubility and low viscosity is a prerequisite for good extraction of muscle proteins and their separation from undesirable components in the acid-aided or alkali-aided processes (Hultin and Kelleher 1999; Undeland and others 2002). Low solubility, on the other hand, is important in the protein-recovery step of the process in their isoelectric point range. The protein solubility followed a U-shape curve (Figure 2), typical of muscle protein homogenates (Stefansson and Hultin 1994; Choi and Park 2002), where protein solubility is sharply increased below approximately pH 5 and above approximately pH 10 but remains low between pH 5 and 10. Increased solubility at extreme pH's has been attributed to an increased positive and negative charge of the muscle proteins at low and high pH, respectively, leading to electrostatic repulsion between the proteins (Hamm 1994). Solubility was greatest at pH 2.5 (88.3%) in the acid pH range and pH 11 (88.9%) in the alkaline pH range, whereas solubility was the lowest at pH 5.5 (Figure 2), which would correspond to the collective average isoelectric point of the muscle proteins. These pH values, therefore, were chosen to extract and recover the catfish muscle proteins. The effect of centrifugation speed was assessed at the point of highest solubility (pH 2.5 and 11). No clear trend was observed for the effect of centrifugal force on solubility (Figure 3). Solubility, however, was highest at 10000 × *g*, and this centrifugal force was used in the preparation of the PIs.

Protein viscosity

One of the goals of the protein isolation process is to separate proteins from undesirable membrane lipids. It has been reported that a viscosity of 50 mPa or less is desired for membrane lipids to settle on centrifugation while the proteins remain soluble (Hultin 2002). Viscosity of the catfish protein homogenate was highly influenced by pH (Figure 4). Viscosity was notably higher on alkaline pH adjustment compared with acid pH adjustment, peaking at about 8 Pa at pH 9.5 compared with about 2.8 Pa at pH 5. The phase angle of a solution gives useful insight into its viscoelastic properties with a perfectly viscous liquid having a phase angle at 90°, while a perfectly elastic solid has a phase angle of 0°. A viscoelastic material is thus between 0° and 90° (McClements 1999). The phase angle of the protein solution dropped notably in the same range viscosity increased and thus signifies a more solid-like nature of the protein solution. The minimum phase angle and maximum viscosity of the protein solution below pH 6.5 coincided (Figure 3a), whereas the same was not seen above pH 6.5 (Figure 3b). The increase in viscosity seen with increasing and decreasing pH from neutrality (with the exception of pH 6 to 7) is expected to be due to the increased electrostatic repulsion and increased hydrodynamic volume of the muscle proteins. Increased protein charge is known to lead to significant swelling due to repulsive forces between the proteins (Kristinsson and Hultin 2003a). The swelling and thus the accompanying increase in viscosity is believed to be primarily due to the myofibrillar proteins, which occupy most of the space in the muscle cells; and due to their elongate structures and structural assemblies, they have a high hydrodynamic volume, which increases with increasing charge (Kristinsson 2002; Undeland and others 2002). It was interesting to note the substantially higher viscosity at high versus low pH. Work with Tilapia muscle protein homogenates shows the same (Ingadottir and Kristinsson, unpublished). This contradicts findings by Undeland and others (2003) who found slightly higher viscosity at low compared with high pH for herring muscle homogenates. It has been found that beef homogenates, in contrast to whole muscle, take up more water at alkaline pH (max at pH 10) versus low pH (maximum at pH 3) (Hamm 1960). More hydration and a higher hydrodynamic radius could explain the higher viscosity at high pH for the catfish proteins, and it is possible that different species respond differently in terms of pH.

A concurrent drop in viscosity was seen in the same pH range where the solubility increased significantly (Figure 4). This is consistent with findings by Undeland and others (2002). This can be attributed to the disruption of the muscle cell and the myofibrillar protein arrangement on protein solubilization, which would lower the effective hydrodynamic volume of the protein components in the system and thus viscosity. The viscosity measured lower than the desired 50 mPa at pH's at and below 3.5 and at and above 10.5. The drop in viscosity was accompanied by a significant increase in phase angle, that is, leading to a more liquid-like system. Hydrolysis was not the cause of the decrease in viscosity at low and high pH as verified by SDS-PAGE analysis, where no additional protein bands could be seen even after 5.5 h at low or high pH (Figure 5).

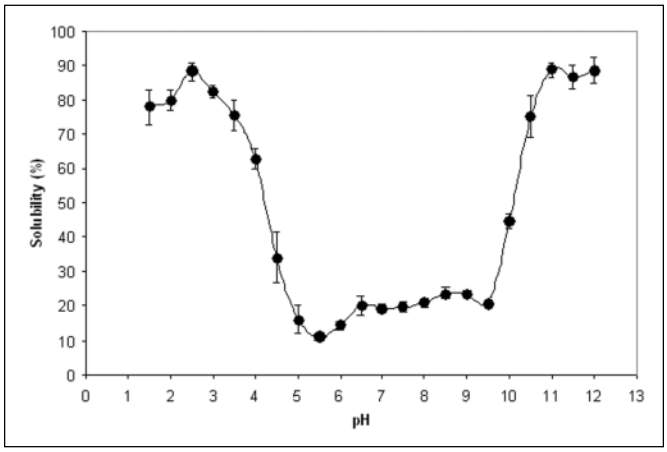

Figure 2—The solubility of catfish muscle proteins as a function of pH.

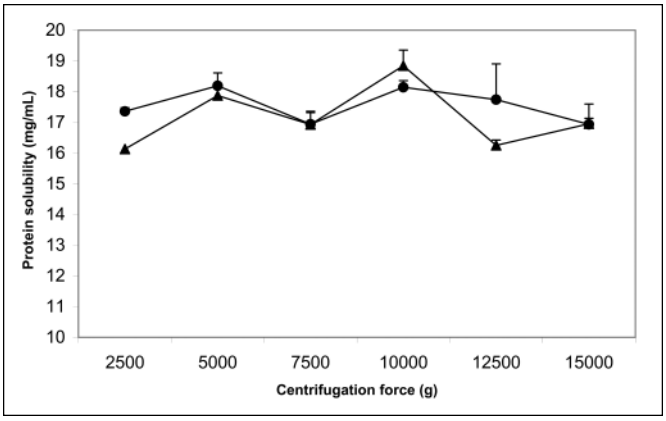

Figure 3—Effect of centrifugation force on the solubility of catfish muscle proteins at pH 2.5 (●) and 11 (▲).

Table 1—Protein recovery and lipid reduction for the surimi and protein isolate (PI) processes[a]

Component	Surimi	Acid PI	Alkali PI	Acid PI (1st centrifugation skipped)	Alkali PI (1st centrifugation skipped)
Protein	62.3% ± 3.1%a	71.5% ± 4.5%b	70.3% ± 2.9%b	85.8% ± 3.6%c	82.1% ± 6.8%c
Lipid	58.3% ± 7.8%b	85.4% ± 2.0%c	88.6% ± 2.8%d	45.4% ± 4.4%a	61.2% ± 6.3%b

[a]Means within 1 row having different letters are significantly different ($P < 0.05$).

The protein bands in the lanes were also quantified, and no difference could be found in the quantity or number of protein bands between the holding times (data not shown). To further verify this, Azocoll assays were conducted and showed no proteolytic activity at any of the pH's tested (data not shown). Hydrolysis is a concern in any protein isolation process because it may lead to adverse effects on the gel-forming properties of the recovered proteins (Choi and Park 2002). The catfish muscle homogenates did not exhibit any hydrolysis even after 24 h at any of the low and high pH's tested (data not shown). Hydrolysis was also not observed for the catfish proteins when they were kept as homogenates for 24 h at their physiological postmortem pH (6.5 to 6.5) (data not shown).

Protein recovery and composition

The acid-aided and alkali-aided processes gave higher protein recoveries ($P < 0.01$) compared with the conventional lab-scale surimi process, 71.5%, 70.3%, and 62.3%, respectively (Table 1). The lower recovery of surimi processing is reportedly due to the removal of water-soluble sarcoplasmic proteins during the washing steps (Xiong 1997) and possibly part of the myofibrillar proteins (Lin and Park 1996). A recent study by Choi and Park (2002) also demonstrated a higher yield using the acid-aided process on Pacific whiting compared with a 3-wash surimi process similar to what was used here. An estimated 60% compared with 40% recovery was obtained for fillets (Choi and Park 2002), which is significantly lower than that obtained for the catfish muscle. Kim and others (1996) reported a 33% recovery from catfish frame mince, which was almost half that reported here. Undeland and others (2002) reported 74% and 68% recovery for the acid-aided and alkali-aided process, respectively, with herring white muscle, which was similar to the catfish protein recovery. The SDS-PAGE analysis of the fractions recovered from the acid-aided and alkali-aided processes of catfish muscle (Figure 6) reveals that muscle proteins (including myofibrillar proteins) were found in the top lipid layer after the 1st centrifugation for both processes. A sizable amount of myofibrillar proteins were also lost in the bottom sediment. Loss of proteins to these 2 phases accounts for the majority of the protein not recovered. A small portion of the protein was lost in the supernatant after the last centrifugation; however, the alkali-aided process led to more protein loss than the acid-aided process. A significant amount of connective tissue is also lost in the 1st sediment, which is in part responsible for the approximate 30% loss of total protein (Davenport and others 2004). Liang and Hultin (2003) pointed out that varying levels of connective tissue in the starting material may have a large influence in the calculated protein yields. Thus, for a raw material with moderate to high levels of connective tissue, an approximate 70% protein recovery would translate to a much higher recovery of myofibrillar and sarcoplamic proteins if connective tissue is excluded form the calculation. The acid-aided and alkali-aided processes have been shown to be very effective in removing the majority of connective tissue (collagen) found in the raw material of beef hearts (Mireles Dewitt and others 2002) and mechanically deboned chicken (Liang and Hultin 2003). The presence of connective tissue may be undesirable for land animal muscle products because they can adversely affect gel formation and strength; however, this is normally not the case for fish muscle products (Lanier 2000). Studies with catfish PIs indicate that the presence of connective tissue may in fact improve their gelling properties (Davenport and others 2004; Kristinsson 2004).

The isoelectric supernatants of the acid-aided and alkali-aided process (lanes 5 in Figure 6) revealed stark differences in the solubility for the 2 processes. Significantly higher amount of heme proteins, actin, troponin, tropomyosin, and desmin remained soluble during the isoelectric precipitation step for the alkali-aided process (Figure 6), suggesting less protein was precipitated compared with the acid process. Undeland and others (2002) recently reported similar findings for herring light muscle processed with the alkali process. These results might initially suggest that the alkali process could solubilize more proteins, which are soluble at pH 5.5, than the acid process, thus leading to more soluble proteins at pH 5.5. However, when the supernatant recovered after the isoelectric precipitation for the alkali-aided process was compared with the super-

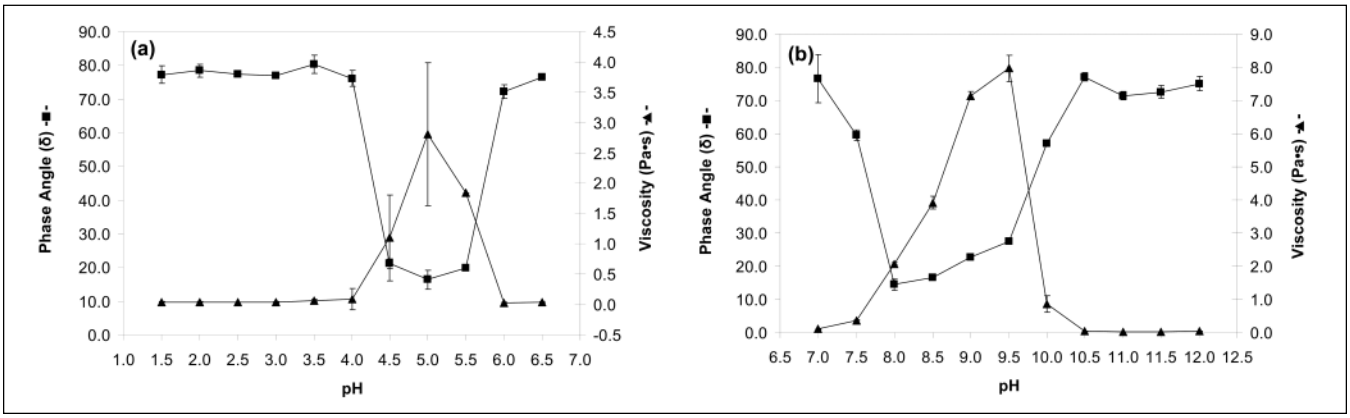

Figure 4—Change in solution consistency of the catfish muscle homogenate as a function of pH. (a) pH 1.5 to 6.5 and (b) pH 7 to 12. Both apparent viscosity (▲) and phase angle (■) are shown.

natant of "native" catfish proteins centrifuged at pH 5.5, it was found that both fractions revealed the same protein bands, except for slightly less actin and hemoglobin/myoglobin in the supernatant for the alkali treatment (compare Figure 6b and Figure 7). This reinforces the hypothesis that more of the sarcoplasmic proteins were co-precipitated with the myofibrillar proteins with the acid treatment rather than were proteins solubilized with the alkali treatment. These data also suggest that minimal denaturation may have occurred at high pH with the proteins found in the supernatant because solubility was largely retained. This is supported by findings by Kristinsson and Hultin (2004a) with trout hemoglobin (a highly water-soluble protein at pH 5.5), which retained its solubility and native structure at pH 5.5 after a 30-min pH treatment at pH 11 followed by adjustment to pH 5.5. Low pH values, on the other hand, irreversibly denatured hemoglobin and led to significantly lower solubility at pH 5.5 (Kristinsson and Hultin 2004a), as seen here with the catfish-soluble proteins. The almost complete loss of actin in the supernatants for the acid process is interesting because low pH's reportedly depolymerize and solubilize actin (Hubbard and Lazarides 1979). This suggests the actin may have been significantly and irreversibly unfolded by the low pH treatment, which in turn would make it more readily aggregate with the other myofibrillar proteins at pH 5.5 via hydrophobic interactions.

Samples were also collected for SDS-PAGE analysis from the laboratory scale surimi process (Figure 8). As expected, a significant amount of soluble proteins were removed during the 1st washing process, including a very high amount of actin (along with sizable amounts of other myofibrillar proteins), which had presumably in part dissociated from myosin and likely depolymerized. The washing process dilutes the ground muscle 3-fold; this may be low enough to induce some actin depolymerization, which takes place at low ionic strengths (Hubbard and Lazarides 1979). This would also explain the presence of actin in the supernatant of the 1:9 homogenates from the alkali-treated and untreated catfish muscle (Figure 6 and 7). The following 2 washing steps continued to remove progressively less soluble proteins. All washing steps appeared to also remove a small amount of myosin, thus collectively, a significant amount of myofibrillar proteins were removed in the washing process. No significant differences in protein patterns could be seen for the PIs or the washed muscle (that is, surimi).

To significantly increase protein recovery, a modification of the processes was used by skipping the 1st centrifugation. Recovery was increased from 71.5% to 85.8% for the acid-aided process and from 70.3% to 82.1% for the alkali-aided process (Table 1). Because the 1st centrifugation was skipped, the proteins otherwise lost in the bottom layer (Figure 1b) were recovered, including more connective tissue. The higher recovery for the acid process is again likely due to less protein remaining in the supernatant during the 2nd centrifugation at pH 5.5 (lane 7 in Figure 5). The same proteins were found in the supernatant for the modified version of the process as were found in the normal process (compare lanes 5 and 7 in Figure 5). This variation would result in most of the membrane lip-

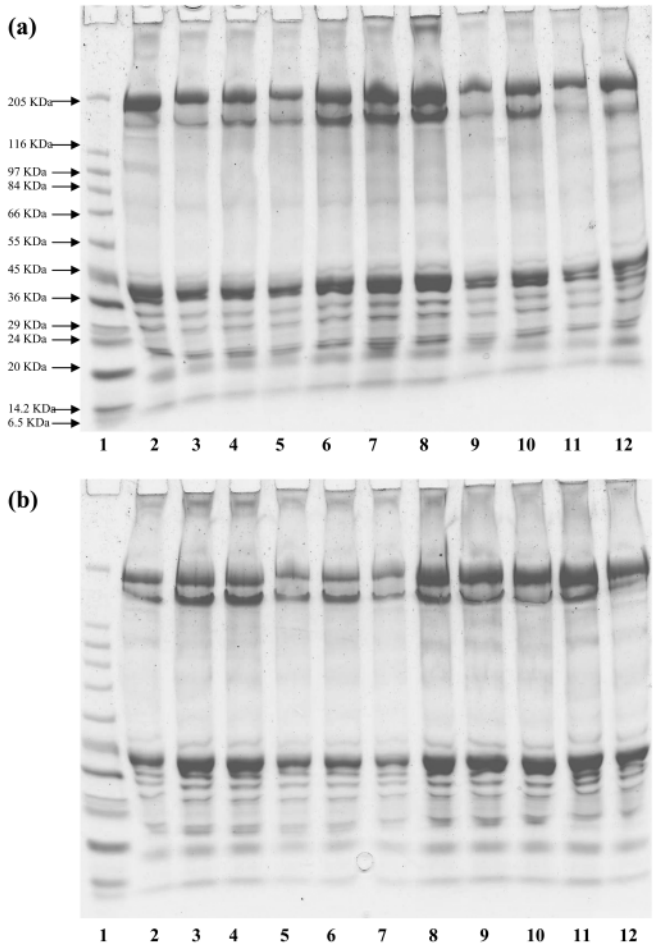

Figure 5—The effect of incubation time at (a) pH 2.5 and (b) pH 11 on the structural integrity of the catfish muscle proteins as assessed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Lane 1 represents molecular weight standards. Lanes 2 to 12 represent time points from 30 min to 5.5 h, each lane at 30-min intervals.

ids and the connective tissue to be recovered with the muscle proteins. The presence of membrane phospholipids may pose a problem for many oxidatively susceptible species (Hultin 1994). However catfish muscle, especially its white muscle, has been found to have reasonably good oxidative stability compared with many other species, especially if the alkaline process is used (Kristinsson and Demir 2003). If oxidation is not a major issue with the raw material, this variation of the process is an excellent way to get increased protein recovery.

Lipid reduction

Research on the acid-aided or alkali-aided processes on mackerel (Hultin and Kelleher 2000; Kristinsson and Demir 2003), herring (Undeland and others 2002), mullet (Kristinsson and Demir 2003), croaker (Kristinsson and Demir 2003), chicken (Hultin and Kelleher 2000; Liang and Hultin 2003), and beef heart (Mireles Dewitt and others 2002) has demonstrated their effectiveness in removing lipids from the starting material. Catfish muscle is relatively high in storage fat (Silva and Chamul 1999). The starting material varied from 4.7% to 9.8% fat, mostly in the form of neutral storage lipids. All processes were effective in lipid removal (Table 1). The acid-aided and alkali-aided processes, however, led to more reduction in lipids ($P < 0.001$) compared with the surimi process, 85.4% and 88.6% versus 58.3%, respectively. A higher reduction in lipids for surimi processing from catfish frame mince was reported by Kim and others (1996). The larger reduction for the acid-aided and alkali-aided processes was expected because at low and high pH, the solubilized proteins are separated from the storage lipids and the membrane phospholipids. On centrifugation, these components separate on the basis of density and solubility differences. Most of the storage fat and thus the more saturated fatty acids would be found in the upper phase, whereas if conditions are favorable, a large portion of the unsaturated membrane phospholipids are expected to settle during the 1st centrifugation (Hultin 2002). Higher lipid content is therefore expected for the upper layer versus the sediment for fatty fish rich in storage lipids. This was the case for the catfish protein isolation (Kristinsson and Theodore, unpublished results) and for herring protein isolation (Undeland and others 2002). The lower lipid reduction for the simplified version of the acid and alkali-aided process was not surprising because membrane lipids are retained and a portion of the storage lipids co-ag-

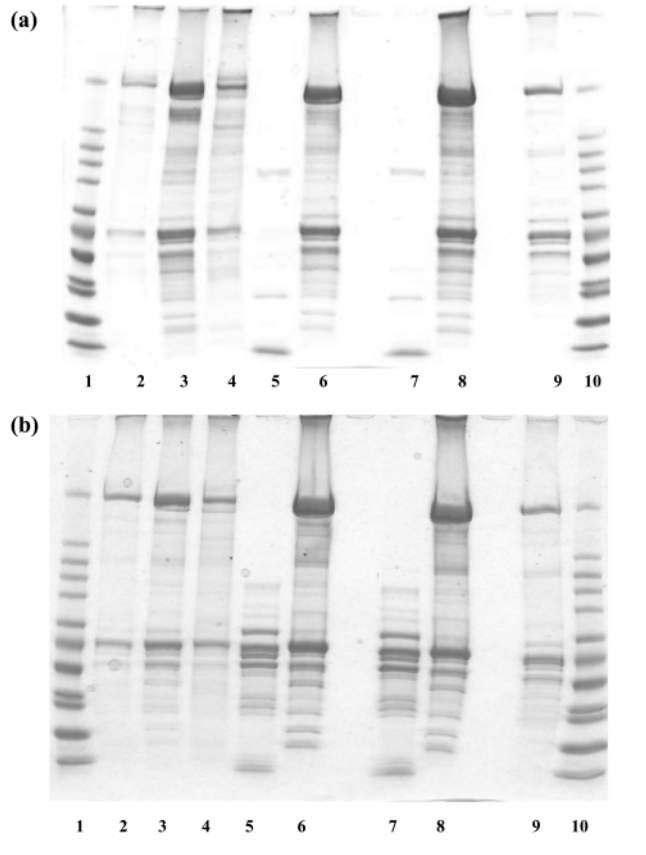

Figure 6—The protein composition of the different fractions obtained by the 2 versions of the (a) acid-aided and (b) alkali-aided protein solubilization/precipitation processes as assessed with sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Lanes 1 and 10: molecular weight standards (same as in Figure 5). Lanes 2 to 6 represent the different fractions obtained by using the full processes with 2 centrifugation steps. Lane 2: upper fat layer after 1st centrifugation. Lane 3: soluble fraction after 1st centrifugation. Lane 4: sediment after 1st centrifugation. Lane 5: supernatant after 2nd centrifugation (isoelectric precipitation). Lane 6: protein isolate (PI) (sediment after 2nd centrifugation). Lanes 7 and 8 represent the modified version of the processes in which the 1st centrifugation is skipped. Lane 7: supernatant after centrifugation (isoelectric precipitation). Lane 8: PI (sediment after centrifugation). Lane 9: ground catfish muscle (starting raw material).

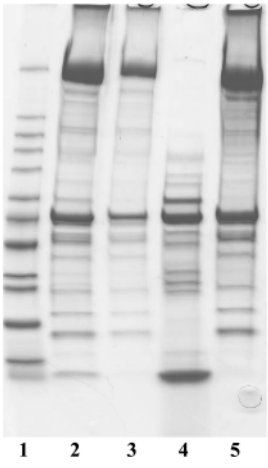

Figure 7—The protein composition of the 3 different fractions obtained during isoelectric precipitation (pH 5.5) of native catfish muscle proteins as assessed with sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Lane 1: molecular weight standards (same as in Figure 5). Lane 2: ground catfish muscle (starting raw material). Lane 3: upper fat layer after centrifugation. Lane 4: supernatant after centrifugation. Lane 5: sediment after centrifugation.

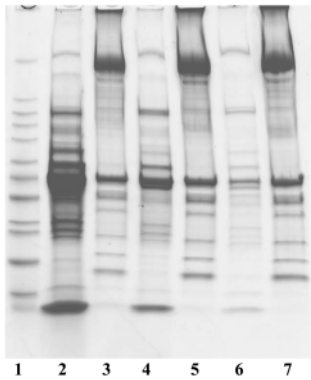

Figure 8—The protein composition of washed ground catfish muscle and wash water as assessed with sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Lane 1: molecular weight standards (same as in Figure 5). Lane 2: wash 1—wash water. Lane 3: wash 1—washed muscle. Lane 4: wash 2—wash water. Lane 5: wash 2—washed muscle. Lane 6: wash 3—wash water. Lane 7: wash 3—washed muscle.

Table 2—Color values of ground raw material, surimi, and protein isolates (PIs)

Sample	*L**	*a**	*b**	Whiteness
Surimi	70.4 ± 1.1a	–0.9 ± 0.2a	0.7 ± 0.4a	70.4a
Acid PI	73.8 ± 0.4b	–3.6 ± 0.2c	5.7 ± 0.3c	72.9b
Acid PI (skip 1st centrifugation)	75.1 ± 0.3c	–2.3 ± 0.2b	7.9 ± 0.5d	73.8c
Alkali PI	75.0 ± 0.7c	–3.0 ± 0.2 c	0.2 ± 0.4a	74.8d
Alkali PI (skip 1st centrifugation)	78.4 ± 0.3d	–2.2 ± 0.1b	3.2 ± 0.0b	78.1e

[a]Means within 1 species having different letters are significantly different (P <0.05).

gregates with the proteins during the isoelectric precipitation step (Davenport and others 2004).

Of the 2 processes, the alkali process was more successful (P < 0.05) in reducing lipids compared with the acid process. The higher lipid reduction correlated to more protein loss in the lipid phase, which would indicate that the high emulsification ability of the proteins at high pH may have contributed to the increased lipid removal, but at the cost of slightly lower protein recovery. Kristinsson and Hultin (2003b) reported that alkali treatment (pH 11 → 7.5) of cod myosin and washed cod muscle significantly improve their emulsifying properties compared with an acid treatment (pH 2.5 → 7.5).

Color of the Protein Isolates

The color characteristics differed among the different protein preparations (Table 2). Surimi had a lower L^* value, and thus lower whiteness score, compared with both acid and alkali PIs. This lower whiteness likely stems from more retention of native heme proteins in the final material because redness (a^* value) was higher for surimi compared with the other treatments. Kim and others (1996) reported higher whiteness values for catfish frame mince surimi than those reported here, whereas higher yellowness (b^* value) and redness values were reported in that study. The alkali process led to a whiter PI (P < 0.05) compared with the acid process, as well as substantially less (P < 0.01) yellowness. It has been found that at acid pH (pH 1.5-3), hemoglobin unfolds, oxidizes, and aggregates when it is readjusted to pH 5.5 and thus is easily precipitated with centrifugation (Kristinsson and Hultin 2004a). Denatured, unfolded, and oxidized hemoglobin has a yellow-brownish color associated with it. It is therefore likely that the unfolded heme proteins in catfish muscle co-precipitated with the muscle proteins at pH 5.5, which would explain the higher yellowness and the lower whiteness and L^* value. To verify this, the UV-visible spectrum of the supernatant from the 2nd centrifugation was obtained (Figure 9). It is evident that after the alkali-aided process, a considerable amount of native heme proteins remain soluble at pH 5.5, whereas only a faint spectrum of oxidized heme proteins is seen for the acid process. This shows that a larger fraction of the heme proteins co-precipitated at pH 5.5 after the acid-aided process. It is interesting to note that the simplified version of the process yields higher L^* values and more whiteness, higher a^* values (more redness), and higher b^* values (more yellowness) compared with the original process. The higher L^* value could be attributed to retention of connective tissue. More yellowness could be in part due to more retention of lipids for this process. The slight increase in redness is likely attributed to more co-precipitation of heme proteins.

Lipid oxidation products

It was of interest to investigate whether the different processes led to any increase in lipid oxidation products. Lipid oxidation can lead to undesirable appearance, odor, and taste problems, in addition to possibly being responsible for textural problems via interaction of oxidation products with proteins (Hultin 1994). We used the TBARS assay to determine the level of secondary oxidation products after acid-aided and alkali-aided processing. TBARS data have been found to correlate well with sensory data for oxidation in fish systems (for example, Richard and Hultin 2000; Undeland and others 2004). Kristinsson and Demir (2003) found that acid processing resulted in significantly more lipid oxidation compared with alkali processing for several species. We have also found that the level of TBARS formed after the process has a great effect on subsequent oxidative stability of the isolates during refrigerated storage; the

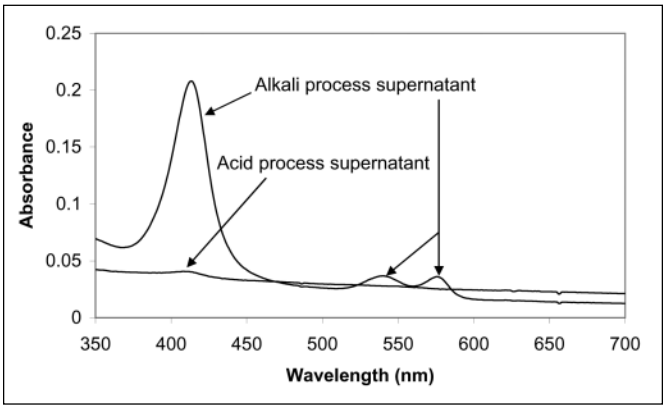

Figure 9—The presence and absence of heme proteins as determined by an UV-visible scan of supernatant collected after isoelectric precipitation of catfish muscle proteins. The peak at 414 nm refers to the heme peak of heme proteins (hemoglobin and myoglobin) and the 2 smaller peaks between 525 and 600 nm refer to oxygen bound to the heme.

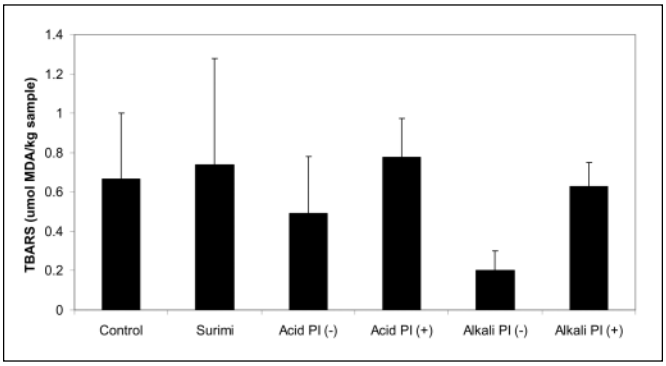

Figure 10—Effect of the different processes on the development of secondary lipid oxidation products (thiobarbituric acid-reactive substances). Acid protein isolate (PI) (–) = acid isolate made by skipping the 1st centrifugation. Acid PI (+) = acid isolate made by including both centrifugations. Alkali PI (–) = alkali isolate made by skipping the 1st centrifugation. Alkali PI (+) = alkali isolate made by including both centrifugations.

higher the TBARS the more rapid and extensive the oxidation (Petty and Kristinsson 2004). Research with a model system consisting of hemoglobin and washed cod demonstrated a rapid and substantial oxidation development at low pH but no oxidation at high pH (Kristinsson and Hultin 2004b). It is evident that none of the processing methods led to significantly higher TBARS values compared with the starting raw material, that is, control (Figure 10). There was no statistically significant difference between the samples ($P > 0.05$), except for the simplified alkali process, which gave very low TBARS values. One might expect the PIs with the membranes present would have more oxidation products. However, the TBARS values for all samples were very low compared with values obtained for other species (for example, Kristinsson and Demir 2003). This good stability toward lipid oxidation is likely due to the more saturated character of catfish lipids due to its warmer water habitat (Silva and Chamul 1999). Similar findings have been found for tilapia (Kristinsson and others, unpublished findings). Heme proteins are known to be a predominant catalyst or mediator of oxidation in fish muscle. There is a possibility that the heme proteins of catfish may be less reactive than heme proteins of fish from colder waters, thus leading to less oxidation. This, however, remains to be investigated using the appropriate model systems. Studies with hemoglobin from another warm water fish, tilapia, have demonstrated that it is less active than hemoglobins from fish from colder habitats, possibly due to better stability toward autoxidation (Kristinsson and others, unpublished findings).

Conclusions

This study demonstrated that acid and alkali processing were more successful than a washing process (i.e. surimi processing) for the recovery of proteins from catfish muscle. Both processes led to higher protein yields adn lower lipid content and had improved whiteness. The functional properties adn shelf life of the isolates compared to surimi is a subject of ongoing investigation.

Acknowledgments

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