FISHERIES SCIENCE 2003; **69**: 1231–1239

New approaches for the effective recovery of fish proteins and their physicochemical characteristics

YOUNG SIN KIM,[1] JAE WON PARK[1]* AND YEUNG JOON CHOI[2]

[1]*Seafood Laboratory and Department of Food Science & Technology, Oregon State University, Astoria, Oregon 97103, USA and* [2]*Division of Marine Bioscience, Gyeongsang National University, Gyeongnam, South Korea*

ABSTRACT: Pacific whiting protein solubility was significantly affected as the pH shifted away from the isoelectric point (pH 5.5). The highest breaking force of gels was measured for fish proteins treated at pH 11, while high deformation values were obtained at pH 2 and 11. Sodium dodecylsulfate-polyacrylamide gel electrophoresis revealed that fish proteins were highly degraded by acid or alkali treatment. High activity of cathepsin B-like enzyme was detected from acid-aided fish proteins. Strong cathepsin L-like activity was found in fish proteins treated at pH 10.5, corresponding well to the lower breaking force and deformation. Disulfide bonds were thought to contribute to the high texture value of fish proteins treated at pH 11.

KEY WORDS: **acid-aided processing, alkali-aided processing, fish proteins, pH.**

INTRODUCTION

Conventional surimi processing from white flesh fish, such as Pacific whiting (PW) and Alaska pollock, uses typically 25–28% of the body of the fish. Conventional surimi is refined myofibrillar proteins processed by removing unnecessary materials, such as fat, pigments, skin and water-soluble sarcoplasmic proteins. A new patented process using acid solubilization and recovery can provide extremely high yields (35–45%).[1] This process consists of isolating the protein component of fish muscle tissue by acid and separating by centrifugation. Before separation, mixing a particulate form of the tissue with acidic liquid at pH < 3.0 produces a protein-rich solution. Then,the protein-rich solution is treated to effect protein precipitation at the isoelectric point of the muscle proteins, followed by protein recovery.

This new technology, using protein charges and isolation, has shown significant potential as a new method for maximal protein recovery and results in commercially acceptable gel characteristics. Unlike the conventional method of surimi manufacturing, no washing or dewatering steps are continuously involved, which significantly reduces waste and water consumption. A significant difference between the conventional and acid-aided surimi processes, in relation to yield, is that in the acid-aided process, sarcoplasmic proteins (hemoglobulin, myoglobulin and proteolytic enzymes) and other proteinous materials are not removed.

The acid-aided process was also applied to Pacific whiting and activation of cathepsin L enzymes, which are responsible for gel softening, was found.[2] Our additional preliminary study, using rockfish, indicated that gels prepared from solubilized proteins at alkaline pH (10–11) exhibited better gel quality than those prepared from the acid-aided or conventional process. Following up our previous study,[2] the application of alkaline pH for the solubilization of Pacific whiting muscle proteins was, therefore, needed. As fish proteins treated at extremely low and high pH for acid- or alkali-aided applications, respectively, are likely to be denatured, the present study focused on characterizing the biochemical and physical properties of fish proteins and compared them to the conventional process.

MATERIALS AND METHODS

Materials

Pacific whiting *Merluccius productus* was obtained from Pacific Surimi JV (Warrenton, OR, USA). Fresh fillets were soaked in 70% sorbitol solution and stored in the freezer (– 80°C). These samples were

*Corresponding author: Tel: 1-503-325-4531
Fax: 1-503-325-2753. Email: Jae.Park@oregonstate.edu
Received 15 October 2002. Accepted 26 June 2003.

thawed, rinsed with refrigerated deionized water twice, and used for the various experiments.

Protein solubility

Protein solubility was measured by mixing 6 g minced meat and 300 mL refrigerated, deionized, distilled water in a homogenizer at setting 3 for 1 min (Power General 700, GLH 115; Fisher Scientific Inc., Pittsburgh, PA, USA). The homogenate (30 mL) was placed in centrifuge tubes and then the pH was adjusted ranging from pH 1.5 to 12.0 using 0.2 N and 1 N HCl or NaOH solution. The amount of HCl/NaOH solution used was recorded. The sample solutions were centrifuged at 27 000 ×*g* at 4–6°C for 20 min. The middle liquid layer was saved for protein analysis. Protein solubility was calculated as protein concentration (mg/g) in the supernatant after pH adjustment. The Bradford dye-binding method was used for protein determination.[3] Total standing time at specific pH was approximately 40 min, including centrifugation.

Sample preparation

Based on the solubility study at various pH, rinsed fillets were subjected to acid-aided (pH 2 and 3) or alkali-aided (pH 10.5, 11 and 12) processing methods. After homogenizing the fillets with distilled water in a 1 : 9 ratio, 2 N HCl or 2 N NaOH was used for the acid- or alkali-aided method, respectively, to first solubilize the fish proteins. Acidic or alkaline homogenates were centrifuged at 8000 ×*g* for 25 min and neutral and membrane lipids, skin and bone were removed using cheesecloth. The pH was adjusted to the isoelectric point (pH 5.5) and then dewatering was done using centrifugation (4000 ×*g*, 20 min). Fish proteins were then mixed with cryoprotectants (5% sucrose, 4% sorbitol and 0.3% sodium tripolyphosphate) and the pH was adjusted to approximately 7.0 using 2 N NaOH. Temperature was maintained below 5°C. Fish protein samples were vacuum packed and stored at – 30°C until used.

Protein recovery

Protein recovery (% yield) was calculated based on the weight of recovered protein divided by the weight of the PW fish fillet (82% moisture). After acidic/alkaline processing, the weight of recovered protein was recorded and its moisture content was also measured. For the calculation of protein recovery, the moisture content of recovered protein was equally adjusted to 82%.

$$\text{Protein recovery (\% yield)} = [(\text{Weight of recovered fish protein at 82\% moisture})/(\text{Weight of fillet})] \times 100$$

Surface hydrophobicity

Surface hydrophobicity of samples was determined using hydrophobic fluorescent probes, 1-anilino-8-naphthalene-sulfonate (ANS; 8 mM). Each fish protein sample (3 g) was homogenized with 27 mL of 20 mM Tris-HCl (pH 7.0) containing 0.6 M KCl. Homogenates were centrifuged at 10 000 ×*g* for 30 min at 4°C. The supernatant used as soluble actmyosin was serially diluted with the same buffer to a final volume of 4 mL with a protein concentration ranging from 0.1 to 1 mg/mL. After stabilizing at 20°C, ANS solution (20 μL) was added to the sample solution. The relative fluorescence intensity (RFI) of ANS protein was measured with a spectrophotofluorometer (LS50B; Perkin Elmer, Norwalk, CT, USA), using a 1-cm cell, 374 and 485 nm as the excitation and emission wavelengths, respectively, and 5 nm width of both excitation and emission slits. The net RFI was obtained by subtracting the RFI of each sample measured without a probe from that with a probe. The initial slope (So) of the RFI against protein concentration (%), calculated by linear regression analysis, was used as an index of protein surface hydrophobicity.

Sulfhydryl content

The sulfhydryl (SH) group exposed on the surface of the actomyosin protein molecule was measured as the reactive SH group (R-SH).[4] An aliquot (50 μL) of Ellman's reagent (10 mM 5,5′-dithiobis (2-nitrobenzoic acid) was added to 2.75 mL actomyosin solution. The mixture was then set in a cold room (5°C) for 1 h. The amount of R-SH was measured at 420 nm using a molar extinction coefficient of 13 600/mol per cm. The total SH content (T-SH) was determined by the method of Choi and Park[2] with slight modifications. Actomyosin solution (0.25 mL) was mixed with 2.5 mL of 8 M urea, 2% sodium dodecylsulfate (SDS) and 10 mM EDTA in 0.2 M Tris-HCl buffer (pH 7.0). The sample solution was then mixed with 50 μL Ellman's reagent and subsequently set in a water bath (40°C) for 15 min. The T-SH was also spectrophotometrically determined at 420 nm (Beckman DU640; Beckman Instruments Inc., Washington, DC, USA).

Cathepsin activities

Frozen fish protein samples, which were adjusted to neutral pH after being treated at the various pH conditions, were homogenized with 2 volumes of distilled water to measure the activity of the residual enzymes. Activities of cathepsin L-like, B-like and H-like enzymes were analyzed using synthetic substrates, Z-Phe-Arg-NMec, Z-Arg-Arg-NMec, and Arg-NMec, respectively. Activity measurement was conducted at pH 5.5 for cathepsin L-like, pH 6.0 for cathepsin B-like, and pH 6.8 for cathepsin H-like enzymes.[5] After centrifugation (10 000 ×*g*, 30 min), the supernatant (400 μL) was diluted to 2 mL with 0.1% (w/v) Brij 35 and preincubated in 1 mL assay buffer at 30°C for 1 min. To the sample mixture, 1 mL of 20 μM substrate solution was added to initiate the reaction. The reaction was terminated precisely after 10 min by adding 500 μL of 5 mM iodoacetic acid. Methylcoumarine released was determined at an excitation wavelength of 370 nm and an emission wavelength of 460 nm using a spectrophotoflurometer. One unit (U) of activity was expressed as the amount of enzyme to release 1 μmol aminomethylcoumarine (AMC) within 1 min.

Gel preparation and analysis

Frozen fish proteins were partially thawed, comminuted with 1.5% beef plasma protein as an enzyme inhibitor, 2% NaCl, and enough water to maintain 78% moisture content. The comminution was conducted at 5°C for 6 min (with vacuum for the final 3 min). A small amount (~50 g) of paste was saved for the oscillatory dynamic test. The remaining paste was then stuffed into plastic tubes (3.2 cm i.d.) and cooked at 90°C for 15 min. Gels were refrigerated overnight. Texture properties, after gels were equilibrated to room temperature, were measured by the punch test using a Sintech machine (Sintech 1/G; MTS, Cary, NC, USA). The movement of a spherical probe (5 mm diameter) at the measurement was 6.0 cm/min. Breaking force (*g*) and deformation (mm) were recorded. A CIE Laboratory color scale was used to measure the degree of lightness (L*), redness or greenness (± a*), and yellowness or blueness (± b*) of gels using a colorimeter (Model CR-300; Minolta, Osaka, Japan). Whiteness was calculated as whiteness index, L* – 3b*.[6]

Oscillatory dynamic test

Non-destructive gelation properties of fish protein samples were evaluated using a cone and plate attached to a mechanical dynamic tester (CS-50 Rheometer; Bohlin Instruments, Inc., East Brunswick, NJ, USA). Storage modulus (G′) was measured at the test temperature ranges (10–90°C) at a heating rate of 1°C/min. A solvent trap was used to prevent moisture evaporation during measurement. Based on our preliminary calibration to determine the linear viscoelastic region, 1 Pa torque value and 0.1 Hz frequency were selected.

SDS-PAGE electrophoresis

Gel samples (3 g) were homogenized with hot 5% (w/v) SDS solution (85°C) at a speed setting of 3 for 1 min. The final volume was adjusted to 30 mL. The homogenates were incubated in an 85°C water bath for 30 min and centrifuged at 3000 ×*g* for 15 min. The protein concentration of the supernatant was determined by the Lowry method.[7] SDS-PAGE was performed using a 5% polyacrylamide stacking gel and 12% polyacrylamide running gel. Gels were stained in Coomassie Brilliant Blue and destained in a mixture solution (v/v) of 50% methanol and 7% acetic acid. A high molecular weight standard mixture containing myosin of rabbit muscle (205 kDa), β-galactosidase of *Escherichia coli* (116 kDa), phosphorylase b of rabbit muscle (97 kDa), fructose-6-phosphate kinase of rabbit muscle (84 kDa), albumin of bovine (66 kDa), glutamic dehydrogenase of bovine liver (55 kDa), albumin of egg (45 kDa) and glyceraldehyde-3-phosphate dehydrogenase of rabbit muscle (36 kDa) was used.

Statistical analysis

All experiments were repeated at least three times. Data were analyzed for the degree of variation and significance of difference based on the analysis of variance (ANOVA) with Tukey's pair-wise comparison test to determine differences ($P = 0.05$) between treatment means. This was done using S-PLUS 2000 PROFESSIONAL RELEASE 3 (MathSoft Inc., Seattle, WA, USA).

RESULTS AND DISCUSSION

Effect of pH on protein solubility

Protein solubility in aqueous solution is dependent on pH. In order to obtain a higher recovery of fish proteins affected by pH, protein solubility at various pH conditions was investigated. Solubility of PW protein was the lowest at pH 5.5 (Fig. 1). A dra-

matic increase in solubility was observed when the pH was shifted from 9 to 12 or from 5 to 3. Maximum solubility was observed at pH 12.

In the present study, approximately pH 5.5 was the isoelectric point where proteins have zero net charge in solution, resulting in minimum solubility and precipitation.[8] Around pH 5.5, a large number of precipitates after centrifugation were observed, due to low electrostatic repulsion between protein molecules. Once the pH shifted away to either direction from the isoelectric point, electrostatic repulsion between molecules increased, resulting in high protein solubility.

The effect of ionic strength on protein solubility was also very significant. The addition of HCl or NaOH solutions in order to change the pH values increased ionic strength. Between the certain points of ionic strength, 0.3–1.0 M sodium chloride[9] or 0.1–0.9 M salt,[10] the salting-in effect was observed. As shown in Fig. 1, the pH shift from 5 to 3, as well as from 9 to 12 using HCl or NaOH resulted in a significant increase of protein solubility. This change was thought to be due to increased electrostatic repulsion and the salting-in effect. However, below pH 3, the protein solubility remained unchanged. Although electrostatic repulsive force could still have increased, the salting-out effect, due to increased ionic strength, might have stopped the further increase of the solubility.

Adjusting the pH to 2 required about threefold more 2 N HCl solution than that of 2 N NaOH required to adjust the pH to 12. The buffering capacity of the fish muscle under acidic conditions was higher than that of the fish muscle under alkaline conditions. A majority of food proteins are acidic proteins; that is, the sum of aspartic acid and glutamic acid residues is greater than the sum of lysine, arginine and histidine residues.[11] Interestingly, we did not observe a dramatic increase of solubility between pH 5.5 and 9.0. On the contrary, as the pH shifted from 5.5 towards the acidic direction, the solubility increased rapidly. This was also probably due to the different buffering capacities of the amino acids in the protein molecules. The most abundant amino acid in fish proteins was glutamic acid, followed by aspartic acid, lysine, leucine and arginine.[12] The pK values of those amino acids correlated with the protein solubility curve (Fig. 1).

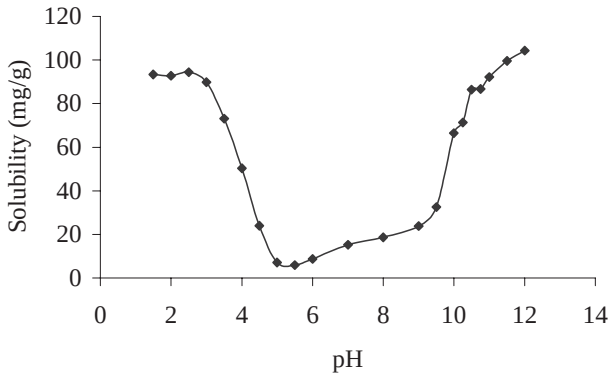

Fig. 1 Effect of pH on the protein solubility of Pacific whiting muscle. Solubility is expressed protein concentration (mg/g) in the supernatant.

Protein recovery

Protein recovery (% yield) is shown in Fig. 2. At pH 12, the highest protein recovery was obtained as a result of the highest protein solubility. According to our preliminary study, the protein recovery at pH 10 was very low (about 50% based on fish fillet).

Even the solubility at pH 10.5 was similar to that at pH 11 (Fig. 1), the protein recovery was the lowest among all treatments. The discrepancy at pH 10.5 could be partially due to the protein extractability being affected by the mixing ratio. Fish proteins were highly soluble with a 50-fold dilution rate at pH 10.5 (Fig. 1). However, the extracting solvent used in the alkali-aided sample (pH 10.5) might have not been sufficient enough because the sample was prepared using only a 10-fold dilution factor. Dagher *et al.*[10] reported that at pH 9.2, maximal protein solubility was seen at dilutions of 36-fold or greater. Solubility at these dilutions was 10 times the amount of solubilization that was observed at a dilution of 1 : 12. In contrast,

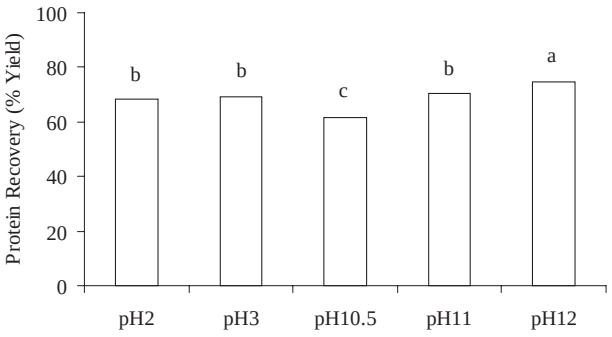

Fig. 2 Protein recovery (% yield) by various treatments. Yield was calculated based on the recovered meat (82% moisture) weight divided by fish fillet weight. pH 2 and pH 3 represent acid-treated fish proteins. pH 10.5, 11 and 12 represent alkali-treated fish proteins. Different alphabetical letters indicate a significant difference ($P = 0.05$).

they observed that at pH 8.5, dilution factors did not significantly affect solubility. The solubility, depending on pH, is therefore affected dramatically by dilution factors.

Hydrophobicity

In order to investigate the degree of denaturation of fish proteins, surface hydrophobicity was studied. Similar hydrophobicity was found among fish protein samples, except for the pH 2 and pH 12 treatments (Fig. 3). For fish proteins treated at pH 2, the hydrophobic group in the molecular interior was highly exposed by strong acid treatment, resulting in the highest hydrophobicity. Alizadeh-Pasdar and Li-Chan[13] also confirmed that hydrophobicity values are higher at acidic pH compared to neutral or alkaline pH. Das and Kinsella[14] reported that the highest hydrophobicity of α-lactoglobulin was measured at pH 2.8 and the hydrophobicity drastically decreased as pH increased. The high surface hydrophobicity at very low acidic pH was attributable to non-covalent monomer-dimer transitions rather than to substantial changes in the protein secondary structures.[15]

When fish proteins were treated at pH 12, the hydrophobicity was lowest among all treatments. A possible explanation is that extremely high pH treatment destroyed the protein conformation and resulted in the formation of aggregates during pH treatments. The pH increase favors denaturating reactions and SH/SS interchange, thereby facilitating the dissociation and aggregation reaction.[16] According to literature information,[17,18] the formation of aggregates decreased the surface hydrophobicity of proteins.

Sulfhydryl content

Sulfhydryl groups are considered to be the most reactive functional group in proteins. Especially, alkaline treatment might induce oxidation of sulfhydryl groups, resulting in the formation of disulfide groups.

Total and reactive SH concentration gradually decreased as the pH increased from 10.5 to 12 (Fig. 4). This trend was probably due to the conversion of cystine and/or cysteine residues to degraded products, such as H_2S and dehydroalanine.[19] Thiol oxidation reactions might have already occurred in addition to disulfide interchange reactions during alkaline treatment, resulting in reduced SH concentration. Moreover, we also noticed some sulfur-like odor indicating the possible formation of H_2S through strong alkaline process (pH 12).

When fish proteins are treated with acid (pH 2 and 3), perhaps all SH groups are exposed to the surface of the proteins. However, some SH groups of fish proteins prepared using the conventional method and alkali treatment appear to be buried in the interior structure. Extensively disulfide-linked proteins, which cannot be easily unfolded, are less surface active than flexible and randomly structured proteins.[19] Disulfide interchange reactions are favored at high pH. In contrast, SH/S-S interchange reactions are inhibited at low pH.[19,20] Exposure to moderately high pH followed by read-

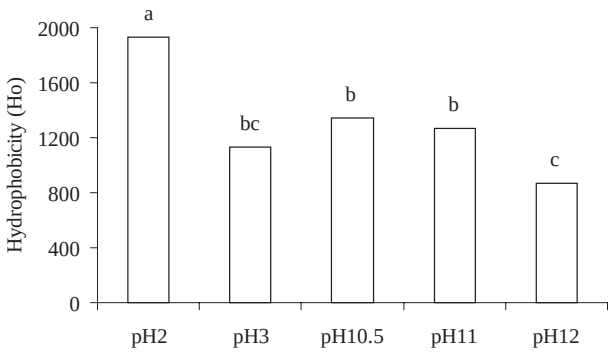

Fig. 3 Hydrophobicity of fish proteins at various treatments. Samples were treated at various pH conditions during protein recovery, and then adjusted to pH 7.0. Hydrophobicity is expressed by initial slopes of relative fluorescence intensity *vs* protein concentration plot in the presence of 1-anilino-8-naphthalenesulfonate (ANS). Different alphabetical letters indicate a significant difference ($P = 0.05$).

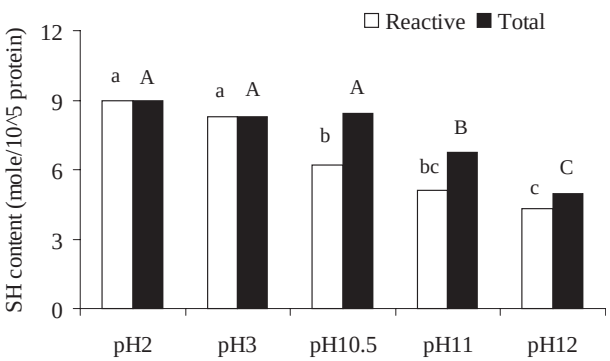

Fig. 4 Total and reactive sulfhydryl (SH) content of fish proteins at various treatments. Samples were treated at various pH conditions during protein recovery, and then adjusted to pH 7.0. Different alphabetical letters indicate a significant difference ($P = 0.05$). Small letters are for reactive SH activity, whereas capital letters are for total SH activity.

justment to neutral pH often activates the protein molecules, thereby improving their functional properties. This could be related to the unfolding of the protein and/or activation of buried sulfhydryl groups.[21] Presumably, alkali-treated fish proteins contained more intermolecular disulfide bonds than acid-treated fish proteins.

Cathepsin activities

Cathepsins are very reactive enzymes causing gel softening in many types of fish species, including Pacific whiting. The control of cathepsin activities is a key factor to obtain high-quality gels. A pH shift was thought to be as one of the effective ways to inactivate cathepsins. Cathepsin L-like activities were found in all samples (Fig. 5). An *et al.*[22] reported that during surimi processing, washing could remove cathepsins B and H, but not cathepsin L.

Fish proteins treated at pH 10.5 showed the highest activities of cathepsin L-like enzymes when the activities were measured using the pH 5.5 buffer, which is known as the optimum pH for L-like enzymes.[23] Dramatic reduction in activity was distinctively observed at pH 11 and 12. A possible explanation for this sudden change is that cathepsin L-like enzymes might be largely inactivated at pH 11 or higher. In general, sarcoplasmic proteins, including proteolytic enzymes, are quite stable under mild alkaline conditions. Presumably, pH 10.5 was not high enough to maintain stable cathepsin L-like activities and the enzyme could be reactivated when the pH was readjusted to 7.0 and further activated during testing at pH 5.5.

Cathepsin B-like enzymes appeared to be highly activated at acid treatment (Fig. 6). However, the alkaline process removed them dramatically. Especially at pH 12, no activities were detected. Most lysosomal proteinases are active at acidic pH.[24] Cathepsin B has maximal activity at pH 6.0 and is unstable above pH 7.0.[25] Cathepsin B-like enzyme could therefore not tolerate alkaline conditions.

Cathepsin H-like activities were not detected in any samples (no data reported). This enzyme was likely damaged by acid or alkali treatment.

Gel properties

The best textural properties were obtained from fish proteins treated at pH 11 and pH 2 (Fig. 7). Fish proteins treated at pH 12 demonstrated the worst texture followed by pH 10.5. Gels at pH 12 did not

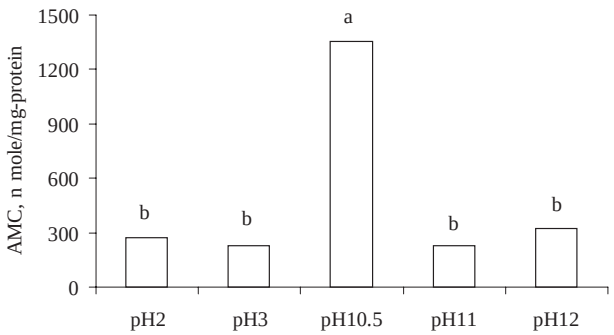

Fig. 5 Cathepsin L-like activities of fish proteins at various treatments. Samples were treated at various pH conditions during protein recovery, and then adjusted to pH 7.0. The measurement was conducted at pH 5.5. Different alphabetical letters indicate a significant difference ($P = 0.05$).

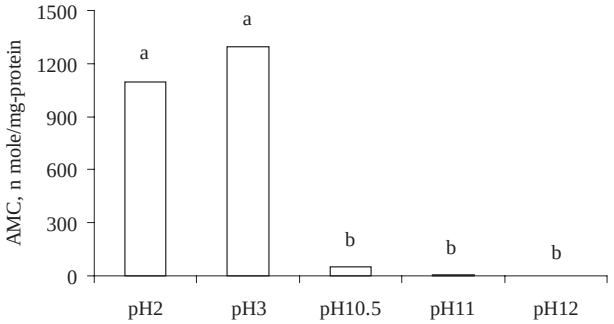

Fig. 6 Cathepsin B-like activities of fish proteins at various treatments. Samples were treated at various pH conditions during protein recovery, and then adjusted to pH 7.0. The measurement was conducted at pH 6.0. Different alphabetical letters indicate a significant difference ($P = 0.05$).

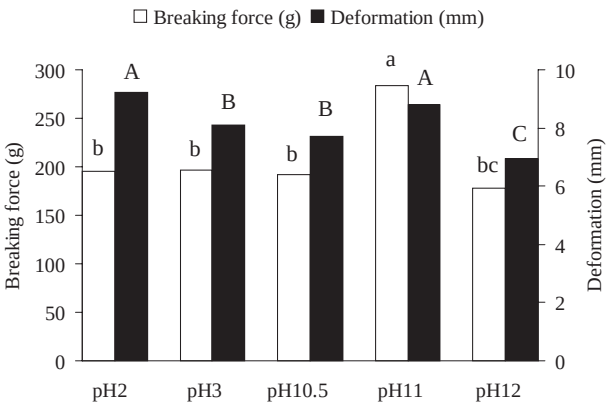

Fig. 7 Textural properties of fish protein gels prepared after various treatments. Samples were treated at various pH conditions during protein recovery, and then adjusted to pH 7.0. Different alphabetical letters indicate a significant difference ($P = 0.05$). Gels were prepared with 1.5% beef plasma protein.

show gel-like appearance. They appeared to be coagulants, as indicated by the low deformation value. Extensive formation of degraded products from cysteine and cystine occurred due to extremely high pH treatment, resulting in thermal coagulation.[19] Presumably, pH 12 was too strong to enable gel formation.

Enzyme activity, surface hydrophobicity and surface SH showed a significant relationship with textural properties. Between two similar acidic pH, 2 and 3, the deformation values were quite different. Generally, hydrophobic bonds dominantly influence gel formation. Hydrophilicity/hydrophobicity ratio on the protein surface is very important for gel network. Probably higher hydrophobicity at pH 2 contributed to higher deformation compared with pH 3. Kim and Park[26] also confirmed that the enhancement of shear strain values for surimi gels at higher temperatures (55–60°C) is closely related to hydrophobic interaction.

Surface SH content was critical for the formation of disulfide bonds. As discussed above, alkali conditions, especially pH 10.5 and pH 11, were favored for disulfide bond formation, as indicated by the reduced surface SH concentration. However, pH 10.5 treatment did not give a high texture value. This was probably due to high cathepsin L-like activities (Fig. 5), which interfered with gel formation. In addition, at pH 11, extensive thiol oxidation and disulfide interchange reactions occurred and more disulfide bonds contributed to strong gel formation.

High lightness values (L*) were obtained from gels treated at pH 10.5 and 11 (Fig. 8). The lowest lightness value (L*) and the highest yellow hue (b*) were obtained from gels treated at pH 12. Consequently, gels with pH 12 had the lowest whiteness value.

Fish protein gels were visually quite different from each other. Gels treated at pH 10.5 and 11 were acceptable, and gels treated at pH 2, 3 and 12 were unacceptable. Based on the fact that acid- or alkali-treated gels contained sarcoplasmic proteins (e.g. hemoglobin and myoglobin), it would be difficult to obtain high-quality color values. In addition, the pH shift to acid or base might have contributed to the enhancement of the Maillard browning reaction when the gels were cooked. However, the color properties of gels treated at pH 10.5 and 11 were good with whiteness values between 61and 62.

Oscillatory dynamic properties

Oscillatory dynamic rheology can reveal the change of protein conformation in regards to thermal denaturation and association. Hence, it is meaningful to compare oscillatory dynamic properties among fish proteins prepared by various methods.

The storage modulus (G′) of paste increased gradually for acid- or alkali-aided fish proteins as the temperature increased from 30 to 90°C (Fig. 9). Storage modulus, G′, is a measurement of energy recovered per cycle of sinusoidal shear deformation.[27] The patterns of slopes on acid- or alkali-aided fish proteins were very similar. Typically, a curve from conventional surimi shows two valleys

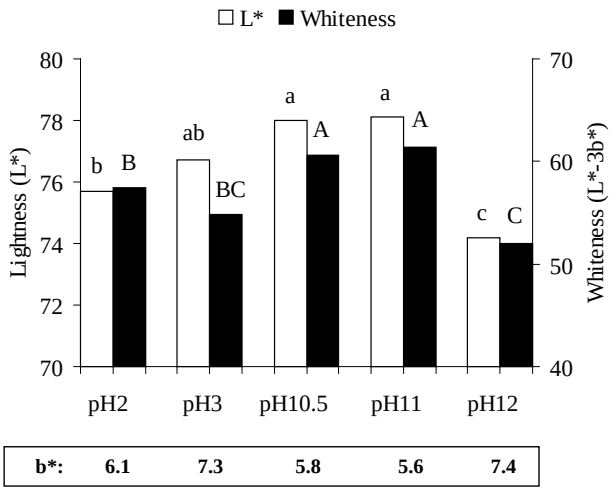

Fig. 8 Comparison of lightness (L*), yellowness (+ b*), and whiteness (L* – 3b*) at various treatments. Samples were treated at various pH conditions during protein recovery and then adjusted to pH 7.0. Different alphabetical letters indicate a significant difference ($P = 0.05$). Gels were prepared with 1.5% beef plasma protein.

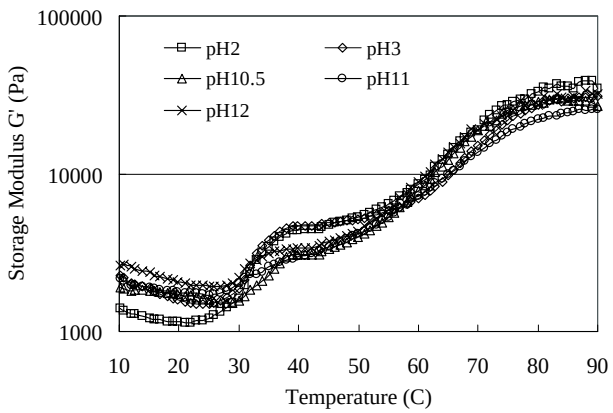

Fig. 9 Changes in storage modulus during linear heating of fish protein pastes. Samples were treated at various pH conditions during protein recovery, and then adjusted to pH 7.0. Pastes were prepared with 1.5% beef plasma protein.

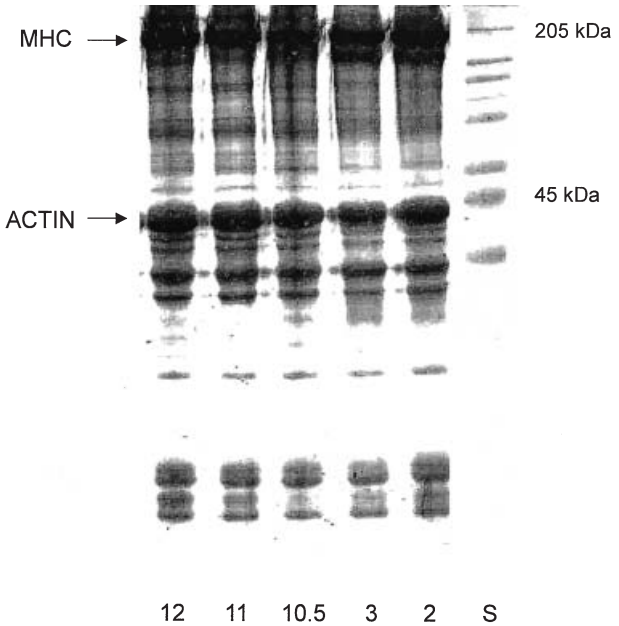

Fig. 10 Sodium dodecylsulfate-polyacrylamide gel electrophoresis patterns of fish proteins after various treatments. Samples were treated at various pH conditions during protein recovery, and then adjusted to pH 7.0. The number indicates pH and S denotes high molecular standard. Gels were prepared with 1.5% beef plasma protein.

(approximately 30°C and 45°C).[27] However, acid- or alkali-treated fish proteins had only one (30°C). A small drop of G′ values starting at 25°C indicated the unfolding of α-helices of myosin molecules in the tail portion.[28] When G′ reached the bottom of the second valley at approximately 45°C, it indicated that the myosin molecules were completely unfolded. Gelation was then completed at 75–80°C as exhibited by no changes in the G′ values.

Unlike fish proteins prepared using conventional methods,[27] fish proteins-treated with acid or alkali showed no changes in G′ around 45°C and then gradual increases of G′ as temperature increased. This dynamic curve indicates that acid or alkali-treated fish proteins were all unfolded (denatured) before thermal treatment. It further denoted that the gelation mechanism between conventional and new pH-driven methods was quite different.

Protein patterns on SDS-PAGE

Different protein patterns were observed among fish proteins prepared using acid- or alkali-aided methods (Fig. 10). A large molecular band was observed right below the myosin heavy chain (MHC) from fish proteins treated at pH 2 and 3. These protein patterns were similar to our previous report.[2] However, it was not clear whether the appearance of the small bands was the result of high cathepsin B-like activities or acid hydrolysis. The proteases from PW hydrolyzed the myosin heavy chain, resulting fragments and/or smaller peptides.[22] The numerous molecules appearing for both acid- and alkali-treated fish proteins could have been contributed by the retained sarcoplasmic proteins and myofiblillar proteins degraded by either acid or alkali.

While the conventional surimi method avoids any denaturation during processing, new approaches induce fish proteins to be denatured through strong acid or alkaline treatments. Surprisingly, some fish proteins denatured by acid or alkali were effectively recovered in terms of gelation properties through adjusting pH to neutral. The gelation mechanism of acid- or alkali-treated fish proteins was quite different from that of fish proteins prepared using the conventional method. The highest protein recovery was obtained for the alkali-aided method at pH 12. Color quality of gels treated at pH 10.5 and 11 was quite acceptable. Neither acid treatment nor alkaline treatment could inactivate cathepsin L-like activities. However, the alkali-aided method at pH 11 demonstrated the best gelation properties of PW fish proteins, probably due to enhanced disulfide bond formation.

ACKNOWLEDGMENTS

This research was partially funded by the National Sea Grant College Program of the US Department of Commerce's National Oceanic and Atmospheric Administration under NOAA grant number NA76RG0476 (project number R/SF-24), and by appropriations made by the Oregon State Legislature. The views expressed herein do not necessarily reflect the views of any of those organizations. Special thanks are made to Professor Herb Hultin and his associates at the University of Massachusetts Marine Station (Gloucester, MA) for their collaborative research advice based on their pioneering works on cod and mackerel.

REFERENCES

1. Hultin HO. Process for isolating a protein composition from a muscle source and protein composition. US patent 6,005,073. 1999.
2. Choi YJ, Park JW. Acid-aided protein recovery from enzyme-rich Pacific whiting. *J. Food Sci.* 2002; **67**: 2962–2967.

3. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 1976; **72**: 248–254.
4. Riddles PW, Blakeley RL, Zerner B. Ellman's reagent: 5,5′-Dithiobis (2-nitrobenzoic acid) – reexamination. *Anal Biochem.* 1979; **94**: 75–81.
5. Barrett AJ, Kirschke H. Cathepsin B, cathepsin H and cathepsin L. In: Colowick SP, Kaplan NO (eds). *Methods in Enzymology 80*. Academic Press, New York. 1981; 535–561.
6. Park JW. Functional protein additives in surimi gels. *J. Food Sci.* 1994; **59**: 525–527.
7. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with Folin phenol reagent. *J. Biol. Chem.* 1951; **193**: 256–275.
8. Christen GL, Smith JS. *Food Chemistry: Principles and Applications*. Science Technology System, West Sacramento, CA. 2000.
9. Hultin HO, Feng Y, Stanley DW. A re-examination of muscle protein solubility. *J. Muscle Foods* 1995; **6**: 91–107.
10. Dagher SM, Hultin HO, Liang Y. Solubility of cod muscle myofibrillar proteins at alkaline pH. *J. Aqua Food Product Technical.* 2000; **9**: 49–59.
11. Fennema OR. *Food Chemistry*, 3rd edn. Marcel Dekker Inc., New York. 1996.
12. Seo HS, Endo Y, Muramoto K, Fujimoto K, Moku M, Kawaguchi K. Amino acid composition of proteins in myctophid fishes in the subarctic and tropical pacific ocean. *Fish Sci.* 1998; **64**: 652–653.
13. Alizadeh-Pasdar N, Li-Chan ECY. Comparison of protein surface hydrophobicity measured at various pH values using three different fluorescent probes. *J. Agric. Food Chem.* 2000; **48**: 328–334.
14. Das KP, Kinsella JE. pH dependent emulsifying properties of α-lactoglobulin. *J. Dispers. Sci. Techn.* 1989; **10**: 77–102.
15. Lin TM, Park JW. Solubility of salmon myosin as affected by conformational changes at various ionic strengths and pH. *J. Food Sci.* 1998; **63**: 215–218.
16. Petruccelli S, Anon MC. Thermal aggregation of soy protein isolates. *J. Agric. Food Chem.* 1995; **43**: 3035–3041.
17. Ishizaki S, Hamada M, Tanaka M, Taguchi T. Conformational changes in actomyosins from fish and pork muscles by ultraviolet irradiation. *Nippon Suisan Gakkaishi* 1993; **59**: 2071–2077.
18. Wagner JR, Sorgentine DA, Anon MC. Relation between solubility and surface hydrophobicity as an indicator of modifications during preparation processes of commercial and laboratory-prepared soy protein isolates. *J. Agric. Food Chem.* 2000; **48**: 3159–3165.
19. Monahan FJ, German JB, Kinsella JE. Effect of pH and temperature on protein unfolding and thiol/disulfide interchange reactions during heat-induced gelation of whey proteins. *J. Agric. Food Chem.* 1995; **43**: 46–52.
20. Errington AD, Foegeding EA. Factors determining fracture stress and strain of fine-stranded whey protein gels. *J. Agric. Food Chem.* 1998; **46**: 2963–2967.
21. Damodoran S, Paraf A. *Food Proteins and Their Applications*. Marcel Dekker Inc., New York. 1997.
22. An H, Weerasinghe V, Seymour TA, Morrissey MT. Cathepsin degradation of Pacific whiting surimi proteins. *J. Food Sci.* 1994; **59**: 1013–1017.
23. Lanier TC. Surimi gelation chemistry. In: Park JW (ed). *Surimi and Surimi Seafood*. Marcel Dekker Inc., New York. 2000; 237–265.
24. Jiang ST, Lee B, Tsao C, Lee J. Mackerel cathepsins B and L effects on thermal degradation of surimi. *J. Food Sci.* 1997; **62**: 310–315.
25. Kang IS, Lanier TC. Heat-induced softening of surimi gels by proteinases. In: Park JW (ed). *Surimi and Surimi Seafood*. Marcel Dekker Inc., New York. 2000; 445–474.
26. Kim BY, Park JW. Rheology and texture properties of surimi gels. In: Park JW (ed). *Surimi and Surimi Seafood*. Marcel Dekker Inc., New York. 2000; 267–341.
27. Yongsawatdigul J, Park JW. Thermal aggregation and dynamic rheological properties of Pacific whiting and cod myosin as affected by heating rate. *J. Food Sci.* 1999; **64**: 679–683.
28. Ogawa M, Kanamaru J, Miyashita H, Tamiya T, Tsuchiya T. Alpha-helical structure of fish actomyosin: changes during setting. *J. Food Sci.* 1995; **60**: 297–299.