Effects of Alkali and Acid Solubilization on Gelation Characteristics of Rockfish Muscle Proteins

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ABSTRACT: Solubility of rockfish whole muscle and actomyosin was minimum at pH 5 and gradually increased as the pH was shifted to acidic or alkaline pH. Acidic and alkaline solubilization was followed by isoelectric precipitation induced degradation of myosin heavy chain, resulting in a protein band of about 120 kDa. Both myofibrillar and sarcoplasmic proteins underwent denaturation after acidic and alkaline treatment, exhibiting minimal solubility and absence of endothermic peaks. Acid- and alkali-treated muscle proteins readily aggregated upon heating, showing different dynamic rheological patterns compared with whole muscle and washed mince. Disulfide linkages occurred at a greater extent in gel prepared by alkaline solubilization, resulting in higher breaking force and deformation.

Keywords: alkali/acid solubilization, fish proteins, actomyosin, solubility

Introduction

Surimi production is aimed toward concentrating myofibrillar proteins by removing sarcoplasmic proteins through continuous washing of the fish mince. The process consumes a large amount of water and typically results in a yield of as low as 20% to 30%. Recently, Hultin and Kelleher (1999) patented the acid solubilization process based on solubilization of both myofibrillar and sarcoplasmic proteins at acidic conditions (pH 2.5) and subsequent precipitation of all soluble proteins at their isoelectric point. Lipids and phospholipids were also removed, and the recovered proteins exhibited a high yield of 35% to 40%. Gels of recovered proteins from chicken breast and thigh exhibited relatively high shear stress and strain (Kelleher and Hultin 2000). However, Choi and Park (2002) found that the gel produced by the acid solubilization process of Pacific whiting mince yielded a lower breaking force, which resulted from activity of cathepsin L retained in the recovered proteins.

At a pH above the pI, proteins become negatively charged, resulting in increased solubilization and solubility. Lin and Park (1998) showed that solubility of salmon myosin was highest at pH 2 and 8 to 10. Therefore, a process in which a higher yield of surimi can be produced might not be restricted to only acid solubilization but also alkaline treatment. Hultin and Kelleher (2000) indicated that protein gels prepared from alkali solubilization of cod muscle exhibited good gel quality. Therefore, both acid and alkali solubilization processes could be alternatives. However, knowledge of rheological and physicochemical changes of fish muscle proteins prepared under alkali and acid solubilization is limited. Thus, our objective was to investigate the physicochemical characteristics of fish muscle proteins prepared by acidic and alkaline solubilization processes, using rockfish as a model.

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Materials and Methods

Actomyosin preparation

Rockfish (*Sebastes flavidus*) was obtained from Pacific Coast Seafood (Warrenton, Oreg., U.S.A.). The selection of rockfish was not made due to its abundance or economic values, but due to the availability of fresh fish at the time of study. The fish, approximately 24 h after catch, were immediately transported in ice to the Oregon State Univ. Seafood Laboratory. Actomyosin was extracted from the dorsal muscle according to the method of Ogawa and others (1999) with slight modification as follows. Fish muscle (50 g) was mixed in 5 volumes of cold phosphate buffer (pH 7.0) containing 50 m*M* NaCl, 20 m*M* sodium phosphate, and 0.05 m*M* phenylmehtanesulfonyl fluoride (PMSF). Homogenization was conducted in a Polytron (Brinkmann Instruments, Westbury, N.Y., U.S.A.) for 2 min. The homogenate was centrifuged at 5000 × *g* for 10 min at 4 °C. The precipitate was homogenized with the same buffer and centrifuged once more. The resultant residue was homogenized in 500 mL (0.6 *M* NaCl and 20 m*M* potassium phosphate) (pH 7.0) and then centrifuged at 10000 × *g* for 5 min at 4 °C. The supernatant was collected and diluted with 3 volumes of cold distilled water (4 °C). The precipitate was collected by centrifugation at 10000 × *g* for 10 min at 4 °C.

Solubility

To study the effect of pH, 2.5 g of fish muscle and actomyosin was mixed with 20 mL buffer (30 m*M* citric acid, 30 m*M* potassium diphosphate, and 40 m*M* boric acid) at pH 2 to 12 (Lin and Park 1998) and homogenized. The final volume of homogenates were brought up to 25 mL and stirred for 30 min at 4 °C. The homogenates were centrifuged at 20000 × *g* at 4 °C for 20 min, and the protein concentration of the supernatant was measured as described by Lowry and others (1951). Total protein of fish muscle and actomyosin were determined by solubilizing the samples in 5% sodium dodecyl sulfate (SDS) and heated at 95 °C for 15 min. Protein content was determined using Lowry method (Lowry and others 1951). Protein solubility was defined as the fraction of protein remaining soluble after centrifugation.

Alkaline and acidic solubilization treatment

The alkaline solubilization treatment was carried out as described by Hultin and Kelleher (2000). Minced muscle was homogenized at a 1:9 (w/v) ratio with cold distilled water. The pH of the homogenates was adjusted to the maximum solubility at alkaline condition determined from the above study using 2 *N* NaOH. Homogenates were centrifuged at 10000 × *g* for 20 min. The alkali-soluble fraction was collected and adjusted to the isoelectric point of muscle proteins obtained from the solubility study using 2 *N* HCl. The precipitate was then filtered through folded cheese cloth and was dewatered by centrifugation at 6000 × *g* for 20 min. The final pH of the sample was adjusted to pH 7.0 using 2 *N* NaOH. The sample was further prepared to gel. All sample preparations were conducted at 4 °C.

Acidic solubilization treatment was prepared using the method of Hultin and Kelleher (1999), which was the same procedure as described previously except that the pH of samples was adjusted to the maximum solubility at acidic condition. Soluble proteins were recovered by isoelectric precipitation and collected. The precipitate was adjusted to pH 7 and used for gel preparation. Three-cycle washing was also conducted at a ratio of mince to cold water at 1:3 (w/v) with the final washing using a cold NaCl solution (0.3%). For dewatering, the mixture was centrifuged at 6000 × *g* for 20 min.

Protein extractability

Protein solubility of all samples at low and high ionic-strength buffers was studied. Each sample (5 g) was homogenized with 40 mL of a low ionic-strength buffer (50 m*M* KCl, 20 m*M* potassium phosphate, pH 7) and a high ionic-strength buffer (0.6 *M* KCl, 20 m*M* potassium phosphate, pH 7). The final volume of homogenate was brought up to 50 mL and stirred for 30 min at 4 °C. The homogenates were centrifuged at 20000 × *g* at 4 °C for 20 min. Due to interference of KCl with the Lowry assay, protein concentration of the supernatant was measured as described by Bradford (1976). Protein extractability was calculated from the amount of protein remaining in the supernatant.

Total sulfhydryl (SH) groups

Total SH groups of samples treated at various treatments were determined according to Monahan and others (1995). Samples (1 g) were homogenized in 9 mL of solubilizing buffer (0.2 *M* Tris-HCl, 2% SDS, 10 m*M* ethylenediaminetetraacetic acid, 8 *M* urea, pH 8.0) (Ultra-Turrax T25; IKA Working Inc., Willington, N.C., U.S.A.). The homogenates were heated at 100 °C for 5 min and centrifuged at 10000 × *g* for 15 min (Eppendorf Model 5415C; Westbury, N.Y., U.S.A.). To 1 mL aliquot of the supernatant was added 0.01 mL Ellman's reagent (10 m*M* 5, 5'-dinitrobis[2-nitrobenzoic acid]). The mixture was incubated at 40 °C for 25 min. The absorbance at 412 nm was measured to calculate the total SH groups using the extinction coefficient of 13600 $M^{-1}cm^{-1}$ (Ellman 1959). The protein content of the solubilized protein was determined by the Lowry method (Lowry and others 1951).

Differential scanning calorimetry

Differential scanning calorimetry (DSC) studies were performed in a DuPont 910 differential scanning calorimeter (DuPont Co., Wilmington, Del., U.S.A.). The temperature calibrations were performed using indium. Samples containing 2% NaCl, 0.3% polyphosphate, and 80% moisture were weighed to 18 to 20 mg in hermetically sealed aluminum pans, ensuring good contact between the sample and the capsule bottom. An empty pan was used as reference. Samples were scanned at 10 °C/min over the range of 5 °C to 95 °C. Helium gas was purged through the purging port at 40 mL/min throughout the heating. Quadruplicate samples with reproducible thermograms were analyzed.

Oscillatory dynamic measurement

Development of gel network was measured as a function of temperature using a CS-50 rheometer (Bohlin Instruments, Inc., Cranbury, N.J., U.S.A.). The pastes (2% NaCl, 0.3% polyphosphate, 80% moisture) were placed between cone and plate (4 °) with a gap of 1 mm. To avoid sample drying during heating, a plastic cover (trapper) with a moistened sponge inside was used. The sample was heated from 10 °C to 80 °C at a heating rate of 1 °C/min. Maximum input strain for dynamic analysis was 0.02 at a frequency of 0.1 Hz, a value found to be in the linear viscoelastic region in this study.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis

Samples (3 g) were homogenized in hot (90 °C) 5% SDS solution and the final volume was adjusted to 30 mL. The homogenate was incubated at 85 °C for 30 min and centrifuged at 5000 × *g* for 10 min. The supernatant was used for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis (Laemmli 1970) using 4% stacking gel and 10% separating gel with the applied protein of 25 μg. Proteins were stained in 0.125% Coomassie blue R-250 and destained in 40% methanol and 10% acetic acid. A wide-range molecular weight standard mixture (Sigma Chemical Co., St. Louis, Mo., U.S.A.) included rabbit myosin (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), bovine serum albumin (66 kDa), glutamic dehydrogenase (55 kDa), chicken egg ovalbumin (45 kDa), glyceraldehyde-3-phosphate dehydrogenase of rabbit muscle (36 kDa), carbonic anhydrase of bovine erythocyte (29 kDa), bovine trypsinogen (24 kDa), soybean trypsin inhibitor (20 kDa), bovine α-lactalbumin (14.2 kDa), and aprotinin of bovine lung (6.5 kDa).

Gel preparation and evaluation

Gels of the whole mince muscle, washed mince, and alkali-treated and acid-treated samples were prepared to contain 2% NaCl, 0.3% polyphosphate, and 80% moisture. All samples were comminuted in a food processor (Hamilton Beach/Proctor-Silex, Inc., Washington, N.C., U.S.A.). The samples were stuffed into a 19-mm-dia stainless-steel tube and heated at 90 °C for 15 min. Gels were removed from the water bath, chilled in iced water, and stored overnight in a refrigerator (5 °C).

Textural properties of various samples were measured by the puncture test using a Sintech machine (Sintech 1/G; MTS, Cary, N.C., U.S.A.). The test was conducted with a spherical plunger (5-mm dia) and the probe speed set at 60 mm/min. Breaking force (g) and deformation (mm) were recorded. Color of gels was measured using a colorimeter (Model CR-300; Minolta, Tokyo, Japan). A CIE Lab color scale was monitored. Whiteness was calculated based on the equation $L^* - 3b^*$ (Park and others 1994).

Statistical analysis

All chemical analyses were performed in duplicate. In gel textural analysis, at least 5 determinations at each treatment were conducted. Analysis of variance and differences between means were determined using Duncan's multiple-range test at $P < 0.05$ (SAS Inst. 1996)

Results and Discussion

Effect of pH on solubility and SDS-PAGE pattern

The solubility of rockfish muscle proteins was lowest at pH 5.0

and increases as pH is shifted away from the isoelectric point (Figure 1). High solubility was attained at pH 2 to 3 and at pH 11 to 12 with a maximum value of approximately 60%. This indicated that fish muscle can be solubilized at either acidic or alkaline pH. Proteins become positively or negatively charged at pH lower or higher than its isolectric point, respectively. This, in turn, increases electrostatic repulsion between protein molecules and hydration of charged residues. As a result, protein solubility increases. Solubility of Pacific whiting mince was similar to that of rockfish, with the maximum solubility at pH 2 and 11, and minimum solubility at pH 5 (Choi and Park 2002).

The effect of pH on solubility of rockfish actomyosin was similar to the whole muscle (Figure 1). Actomyosin was insoluble at pH 5 to 6 and slightly soluble at pH 7 to 9. A sharp increase in solubility occurred at either pH 2 to 4 or pH 10 to 12. Maximum solubility was achieved at pH 12 at about 80% solubility. Therefore, muscle proteins solubilized at alkaline pH (11 to 12) may contain higher actomyosin fraction than those solubilized at an acidic pH (2 to 3). Lin and Park (1998) reported the salmon myosin exhibited the minimum solubility at pH 4 to 5, and maximum solubility at pH 3 and 10.

The concentration of myosin heavy chain (MHC) and actin varied with solubilizing pH (Figure 2). Intensity of MHC decreased as pH moved toward acidic and alkaline conditions. This corresponded to an increase of a protein band of 120 kDa. Therefore, it could be hypothesized that a reduction of MHC was induced by either acid or alkaline hydrolysis during the solubilization process. Choi and Park (2002) reported the degradation of MHC to smaller fragments of 124, 78, 70, and 43 kDa when Pacific whiting mince was solubilized at pH 2.5. Chawla and others (1996) also observed a protein band around 160 kDa from threadfin bream gels prepared using acetic treatment. Although muscle proteins were effectively solubilized at the extreme acidic and alkaline pH values, 2 and 12, these conditions appeared to induce degradation of MHC. Therefore, solubility and integrity of MHC should be taken into consideration in selecting the optimal solubilizing pH values; pH 2.5 and 11 were chosen as the optimal pH values for acidic and alkaline solubilization in this study.

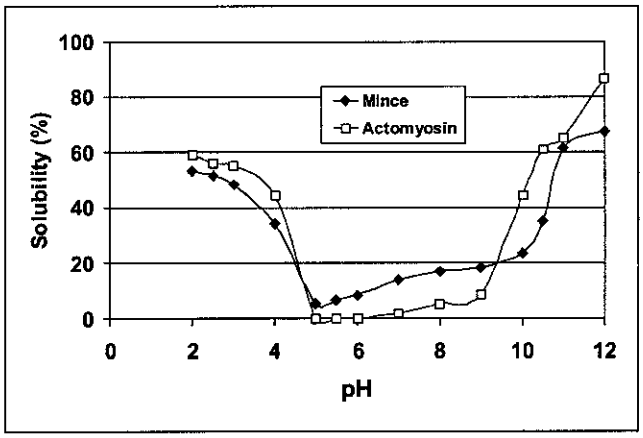

Figure 1—Effect of pH on solubility of rockfish mince and actomyosin

Protein extractability of acid-treated and alkali-treated samples

After acidic and alkaline treatment, the samples exhibited low solubility in both low and high ionic-strength buffers (Figure 3). Low ionic-strength buffer (50 m*M* KCl) was applied to extract sarcoplasmic proteins, whereas myofibrillar protein was solubilized using high ionic-strength buffer (0.6 *M* KCl). Mince sample (M) contained both sarcoplasmic and myofibrillar proteins. Sarcoplasmic protein were leached out during 3 washing cycles, resulting in low sarcoplasmic proteins content in washed mince (WM). Myofibrillar proteins were a main component in the WM. Although sarcoplasmic proteins remained in both alkali (AK)-treated and acid (AC)-treated samples and showed the similar pattern as M as seen on SDS-PAGE (Figure 2), the extraction of these proteins from AK and AC was much lower than that of M. Similarly, myofibrillar proteins of AK and AC were not as soluble as M and WM. Low extractability indicated the denaturation and aggregation of proteins. These results suggested that solubilization of rockfish muscle proteins at either

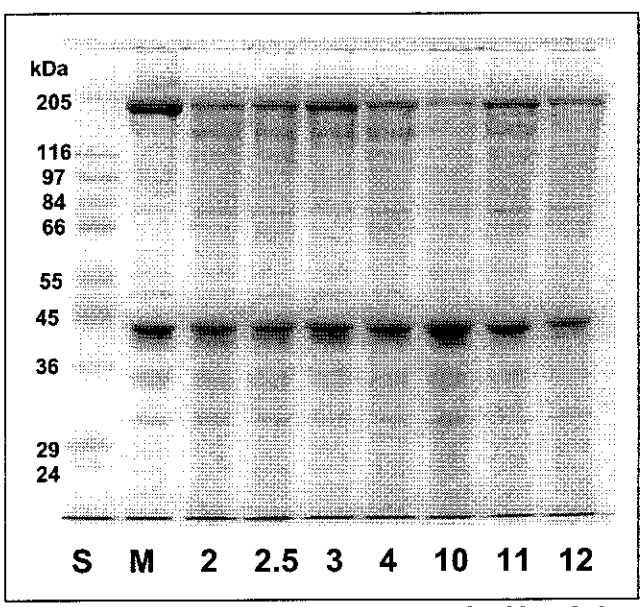

Figure 2—Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) patterns of rockfish mince solubilized at various pH values: M = mince; numbers indicate pH at solubilizing condition; S = standard molecular weight.

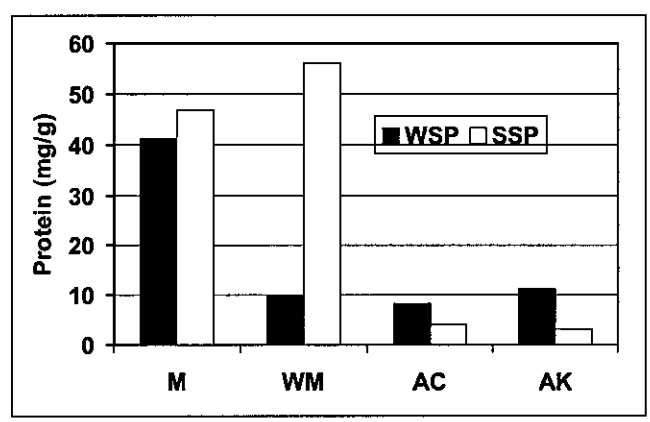

Figure 3—Protein extractability of samples prepared by various treatments: AC = acid solubilizing condition; AK = alkaline solubilizing condition; M = mince; SSP = salt-soluble proteins; WM = washed mince; WSP = water-soluble proteins. Different letters within the same protein (WSP or SSP) are significantly different ($P < 0.05$).

pH 2.5 or pH 11 induced denaturation and aggregation of both sarcoplasmic and myofibrillar proteins.

The acid-induced and alkali-induced denaturation of muscle proteins was confirmed by the DSC thermogram (Figure 4). Endothermic transition peaks in M were found at 36.3 °C. The onset temperature of endothermic transitions in WM was also found at 36.4 °C and 65.5 °C, presumably contributing from myosin and actin, respectively. T_{max} of myosin and actin in washed hake mince was at 46 °C to 47 °C and at 71 °C to 73 °C (Beas and others 1991). No endothermic transitions were observed in both AK and AC, suggesting myosin and actin could have undergone alkali-induced and acid-induced denaturation. Therefore, energy required for thermal denaturation of AK and AC was minimal. In addition, it could be interpreted that aggregation, which is an exothermic process, extensively occurred in the acid-treated and alkali-treated samples, and compensated the enthalpy of endothermic denaturation of AK and AC. As a result, no net enthalpy appeared in the DSC thermogram.

Oscillatory dynamic properties

Storage moduli (G') of all samples increased as the temperature increased from 10 °C to 90 °C (Figure 5a). The G' pattern of M and WM was very similar, indicating that actomyosin is the main protein component responsible for rheological changes of mince muscle. G' initially increased at about 34 °C, showed a peak at 39 °C, and declined to the minimum at 46 °C before continuously reaching the maximum after 70 °C. Such rheological characteristics were also observed in myofibrillar proteins (Xiong and Blanchard 1994), and myosin (Wu and others 1991; Yongsawatdigul and Park 1999) of avian, bovine, and fish muscles, respectively. Egelandsdal and others (1986) reported that initial increase of G' was attributed to the cross-linking of myosin, whereas the decline of G' resulted from denaturation of light meromyosin, leading to increased fluidity. The second increase of G' was attributed to formation of permanent myosin filaments. The changes in phase angle of M and WM corresponded to those of G' (Figure 5b). Phase angle increased from 10 °C to 34 °C, suggesting the viscous characteristic of the paste. An elasticity of samples started to develop at 32 °C to 34 °C, corresponding to an initial increase of G'. As muscle proteins at high ionic strength were heated, actomyosin started to unfold and interact with water, consequently exhibiting the viscous nature of the paste. As temperature continuously increased, unfolded actomyosin entangled and formed gel networks, which was evident by an increase of G' at >46 °C.

Storage modulus of AC and AK showed a different pattern. G' increased continuously from 25 °C without a decline starting at 39 °C and a sharp increase starting at 46 °C (Figure 5a). Similar results were also reported in cod myosin treated at acidic and alkaline pH values (Kristinsson and Hultin 2003a). Aggregation at low temperature indicated that conformation of myosin was less stable after treated at alkali and acid conditions (Kristinsson and Hultin 2003a). Because a decline of G' was attributed from denaturation of light meromyosin (Egelandsdal and others 1986), it could also be assumed that light meromyosin might have undergone denaturation during acid and alkali treatment as suggested by DSC results (Figure 4). Kristinsson and Hultin (2003b) also suggested that myosin rod of cod underwent dissociation when solubilized at pH 2.5 and readjusted to pH 7.5. Therefore, viscoelastic properties of AC and AK were mainly contributed from aggregation of denatured muscle protein, which was previously induced by acid and alkali conditions. Phase angle of these samples started to decrease at approximately 20 °C, indicating the formation of an elastic material at relatively low temperature. Recovering proteins using isoelectric precipitation at pH 5.5 could result in a zero net charge and promote protein aggregation. Morita and others (1987) reported that the myosin had a long filamentous structure at pH 5.4 and formed a fine-strand gel structure through myosin head interactions, exhibiting a higher rigidity. Therefore, muscle proteins could readily aggregate to form elastic gel networks after isoelectric precipitation.

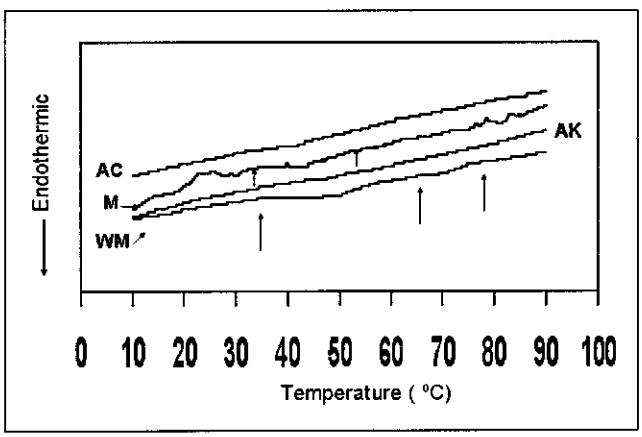

Figure 4—Differential scanning calorimetry (DSC) thermograms of rockfish mince prepared by various treatments. See Figure 3 for abbreviations.

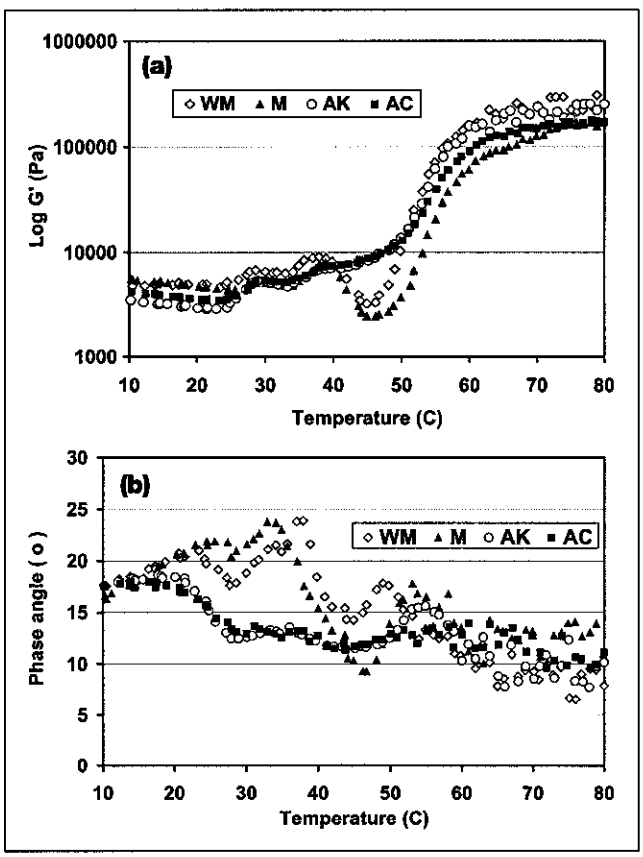

Figure 5—Changes in storage moduli (G') (a) and phase angle (b) of rockfish muscle proteins prepared by various treatments

Total SH groups

The total SH content of pastes varied with treatments (Figure 6). The acid solubilization process reduced a level of SH groups to a greater extent than did alkaline and washing treatments ($P < 0.05$). The M samples contained the highest total SH content ($P < 0.05$), which was contributed from actomyosin and other sarcoplasmic proteins. Washing out sarcoplasmic proteins resulted in lower SH groups of WM. A marked decrease in SH groups was observed in the acid-treated samples. This suggested that oxidation and SH/S-S interchange reactions could occur during acid solubilization. This might also contribute to low protein extractability in low and high ionic-strength buffers (Figure 3).

When gels were thermally prepared, levels of SH decreased as a result of disulfide formation (Figure 6). A drastic decrease of SH groups in the gel samples compared with their paste was noticed in the alkaline-treated sample. Because muscle proteins carried strongly repulsive, negative charges, they underwent extensive unfolding at alkaline pH. Because pKa of SH group of free cysteine is about 8.3, SH groups were likely to deprotonate ($SH \rightarrow S^-$), which were in turn oxidized to disulfide bonds or participated in SH/S-S interchange reactions. Therefore, the lowest SH content was observed in the alkali-treated gels. Shimada and Cheftel (1988) also reported that formation of disulfide bonds was favorable at neutral and alkaline pH values (6.5 to 9.5), contributing to elasticity of whey protein isolate (WPI) gel. SH oxidation and interchange appeared to occur at the greater extent in the paste of acid-treated samples, resulting in a lower total SH content (Figure 6). Therefore, available SH groups were limited for disulfide formation and interchange during gelation of acid-treated sample. Shimada and Cheftel (1988) also found that no additional disulfide bonds were formed in WPI gel at acid pH. Our results indicated that disulfide linkages appeared to play a more important role in gelation of alkaline than acid-treated samples.

SDS-PAGE pattern under reducing and nonreducing conditions

The concentration of MHC in gel samples slightly decreased when compared with that of respective raw samples (Figure 7a). Choi and Park (2002) found significant cathepsin L activity in Pacific whiting muscle proteins treated by the acid solubilization process. It should be noted that both acidic and alkaline solubilization processes did not significantly promote proteolysis of MHC as indicated by a minimal loss of MHC in both samples.

When raw samples were solubilized in a buffer without β-mercaptoethanol (BME), the intensity of MHC and actin bands (Figure 7b) was less than those solubilized in a buffer with BME (Figure 7a). In addition, larger-molecular-weight cross-links that did not enter the 10% polyacrylamide gel were noticed in the samples without BME (Figure 7b). It could be assumed that myosin and actin of all samples interacted via disulfide linkages. Actin bands of AC and AK pastes were less intense than those of M and WM. Actin may favorably interact with MHC of AC and AK samples through disulfide linkages. This assumption was supported by the lower SH content of AC pastes (Figure 6). The alkaline-treated and acid-treated cod myosin were found to have more exposed reactive SH groups,

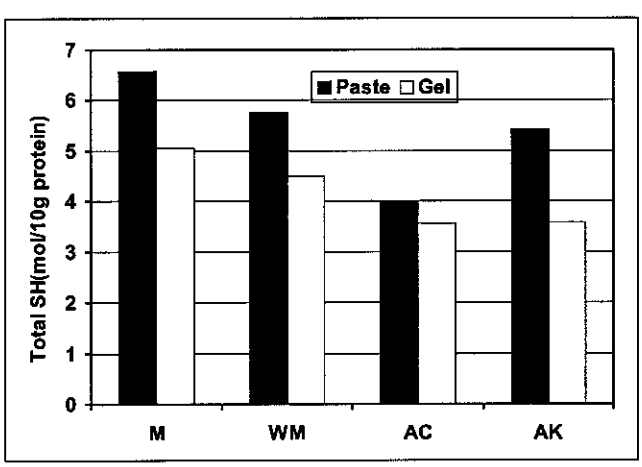

Figure 6—Total sulfhydryl (SH) groups of rockfish muscle proteins prepared by various treatments. Different letters within the same sample (paste or gel) are significantly different ($P < 0.05$).

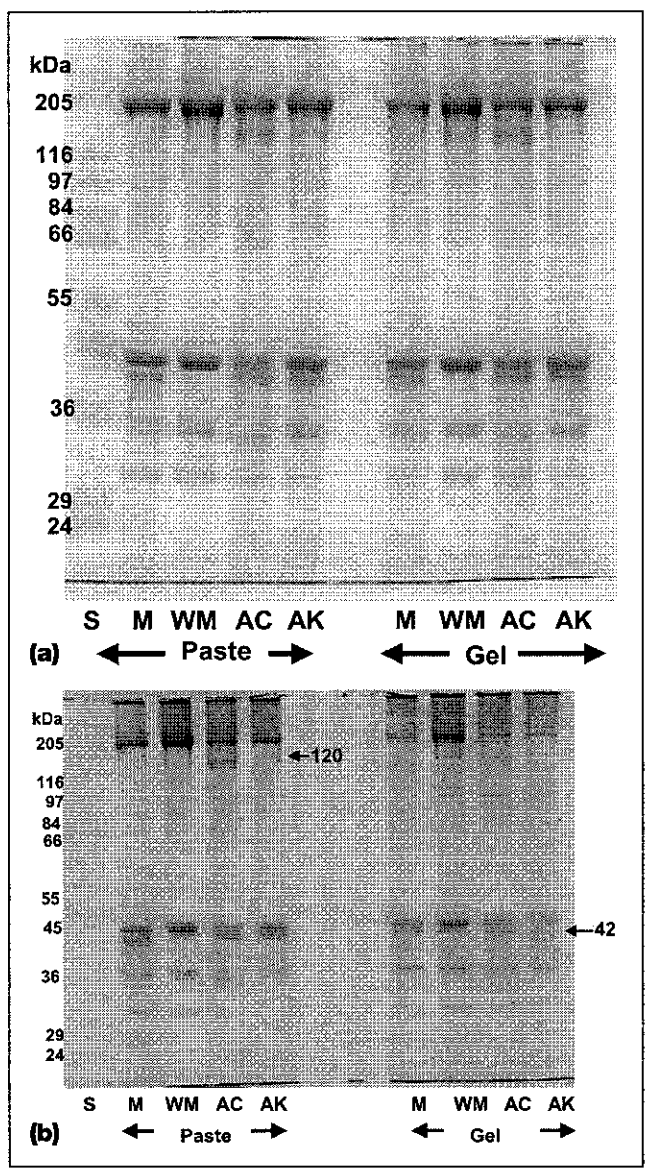

Figure 7—Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) patterns of rockfish muscle proteins prepared by various treatments solubilized in buffer with (a) and without (b) β-mercaptoethanol

which presumably promoted myosin head-to-head aggregation during gelation (Kristinsson and Hultin 2003a, 2003b). Our results indicated that formation of disulfide linkages did not limit only among myosin molecules, but appeared to take a place between MHC and actin.

It should be noted that a distinct protein with MW of 120 kDa was found in AC-treated paste, and its density substantially decreased in AC-treated gel (Figure 7b). This protein band, which presumably resulted from degradation of MHC at acidic condition, appeared to interact with other proteins through disulfide linkages during gelation of acid-treated samples. In AK gel, protein bands with MW of 120 and 42 kDa almost disappeared. Therefore, gelation of AK could have been completed through interaction of several protein components, both myofibrillar proteins (MHC, actin, tropomyosin), and sarcoplasmic proteins, via disulfide linkages. This was also supported by a drastic decrease of total SH content in the AK gels compared with other samples (Figure 6). It should be mentioned that other interactions, such as hydrophobic interactions, have been reported to play an important role in the gelation of fish muscle proteins (Park and others 1994), which could also contribute to the rheological properties of acid-treated and alkali-treated samples. In addition, Kristinsson and Hultin (2003a) reported an increase in surface hydrophobicity of cod myosin treated at acid and alkaline pH values. Therefore, hydrophobic interactions could also play an important role in the gelation of alkali-treated and acid-treated muscle proteins.

Texture and color properties of gels

Three washing cycles and alkaline solubilization yielded higher breaking force and deformation than other treatments ($P < 0.05$) (Figure 8). Kim and others (2003) also reported that the highest gel breaking force was obtained when Pacific whiting mince was treated at pH 11. Myofibrillar proteins, especially actomyosin, contribute to elasticity of a muscle protein gel. Removal of sarcoplasmic proteins resulted in higher myofibrillar protein concentration and, consequently, increased breaking force of WM. The greater extent of disulfide formation of myofibrillar and sarcoplasmic proteins induced by alkaline solubilization could contribute to higher breaking force and deformation of AK gels. Smyth and others (1998) reported that intermolecular disulfide bonds of myosin subfragment-1 played an important role in gel network formation. It should be noted that the presence of sarcoplasmic proteins in the alkaline solubilization process did not interfere with the textural properties of gels.

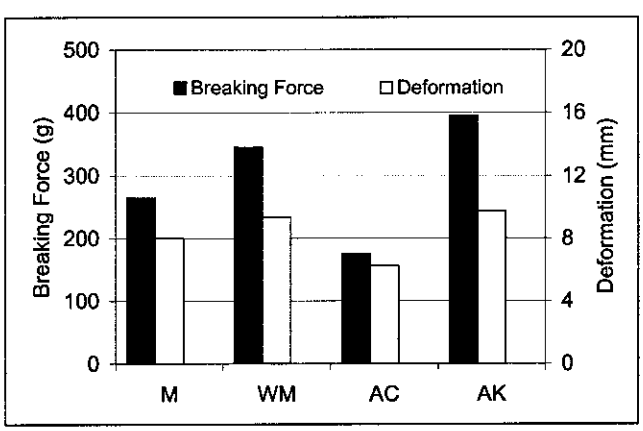

Figure 8—Breaking force and deformation of rockfish muscle proteins prepared by various treatments. Different letters within the same texture parameter (breaking force or deformation) are significantly different ($P < 0.05$).

Table 1—Color values of samples prepared by various treatments[a]

Sample	L^*	a^*	b^*	Whiteness ($L^* - 3b^*$)
M	77.63[c]	2.60[a]	7.19[a]	56.07[d]
WM	81.22[a]	1.69[b]	0.83[d]	78.74[a]
AC	79.06[b]	1.17[c]	4.74[c]	64.84[b]
AK	76.20[d]	−0.04[d]	5.67[b]	59.18[c]

[a]Means with different letters in the same columns are significantly different ($P < 0.05$).

Lower breaking force and deformation was observed in the AC samples ($P < 0.05$) (Figure 8). Although rheological changes of AC resembled those of AK, the extent of disulfide formation was much lower (Figure 6). This might have resulted in lower breaking force and deformation of AC. Choi and Park (2002) also reported a low breaking force and deformation of the gel obtained from the acid solubilization process of Pacific whiting. In contrast, Hultin and Kelleher (1999) showed high shear stress and shear strain of gel prepared from acid solubilization of Atlantic mackerel and cod. Good gel quality with high shear stress and strain was also obtained from chicken breast treated with the acid solubilization process (Kelleher and Hultin 2000).

The L^* value of WM was higher than that of M, AK, and AC ($P < 0.05$) (Table 1). Yellowness of AC and AK was higher than that of WM ($P < 0.05$). WM exhibited the whiter appearance due to the removal of myoglobin during washing. Although whiteness of M and AK was similar, the color of M showed a reddish hue, whereas AK gels were slightly brownish. This was probably because of the red pigment of myoglobin in M and the denaturation of myoglobin at alkaline treatment. Alkaline (pH = 9 to 10) and acid (pH = 4.5) conditions accelerated autoxidation of tuna myoglobin (Chow 1991). Myoglobin of milkfish also underwent oxidation at a higher rate with decreasing pH in a range of 5.5 to 7.0 (Chen 2001). High b^* values were also observed in the acid solubilization process of chicken thigh (Kelleher and Hultin 2000) and Pacific whiting (Choi and Park 2002). It appeared that solubilization of whole muscle at either alkaline or acid condition would accelerate oxidation of myoglobin, resulting in higher b^* value.

Conclusions

Acid solubilization followed by isolectric precipitation induced degradation of rockfish myosin, resulting in low gel quality. In contrast, alkaline solubilization yielded relatively high-quality fish proteins, which are comparable with those obtained from the 3-cycle washing process. Myofibrillar proteins appeared to undergo denaturation after both acidic and alkaline treatments. Alkaline solubilization promoted the formation of disulfide linkages, providing gels with higher breaking force and deformation.

Acknowledgments

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