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Fish Protein Recovered Using pH Shifting Method and Its Physicochemical Properties

Choi, J. Yeung*, and Kim, Jinx-Soo

Department of Food Science, Gyeongsang National University, Tongyeong 650-160, *Korea*

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Abstract The solubility of meat protein of croaker and jack mackerel was significantly affected by pH shifting. The protein yield of alkali-aided processing is higher than those of conventional and acid-aided processing. The addition of sarcoplasmic protein increases the breaking force and deformation value. The breaking force of recovered protein gel from alkali-aided processing is decreased by the addition of NaCl. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) revealed that fish protein of alkali is similar to that at pH 7.0. Alkali-aided processing for recovering fish protein is a valuable method for increasing the utilization of frozen and pelagic fishes and for making kamaboko products.

Key Words fish protein; recovery; pH shifting; property

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1 Introduction

Conventional surimi processing of white flesh fish, such as pacific whiting and alaska pollock, uses typically less than 25% of total body (Toyoda *et al.*, 1992; Park *et al.*, 1997). Conventional surimi is refined myofibrillar proteins processed by removing unnecessary materials, such as fat, pigment, skin and water-soluble sarcoplasmic proteins. New-patented processing protocol, method, and technique, using acid solubilization and recovery, can provide extremely high yields (35%–45%), which demonstrates the excellent gel-forming ability of cod and mackerel protein (Hultin and Kelleher, 1999). This process consists in isolating the protein component of fish muscle using acid. 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 in order to effectively precipitate proteins at the isoelectric point of the muscle proteins, followed by protein recovery. Moreover, the alkaline treatment, instead of an acidic one, provided more yield and gel-forming ability and less degradation of protein (Kim *et al.*, 2003; Prerez-Mateos *et al.*, 2004). This new technology, using pH shifting method, has shown its significant potential for maximal protein recovery and development of commercially acceptable gel characteristics. In alkaline processing, no washing or dewatering steps are continual involved, which significantly reduces waste and cost. One distinguishing attribute of these processes is that the sarcoplasmic protein of fish muscle is retained. The functions of sarcoplasmic proteins in regards to the formation of myofibrillar protein gels are not clear as to whether they work positively or negatively (Shimizu and Nishioka, 1974; Morioka and Shimizu, 1990). The gelling mechanism of protein recovered from acidic and alkaline processes is quite different from that in conventional surimi production because the recovered protein is denatured at extremely low and high pH values.

The present study was conducted to determine the yield and the property of the heat-induced gel of recovered proteins from frozen croaker and flesh jack mackerel.

2 Materials and Methods

2.1 Materials

Frozen croceine croaker (*Pseudosciaena crocea*), black spotted croaker (*P. diacanthus*), croaker (*P. argentata*), small croaker (*Kangchi, P. argentata*), fresh mackerel (*Scomber japonicus*) and jack mackerel (*Trachurus japonicus*) were purchased at the fish market in Tongyeong, Gyeongnam. The thawed and fresh fish were filleted and thoroughly washed.

2.2 Protein Solubility

Protein solubility was ensured by mixing 2 g minced meat and 18 mL of distilled water in a homogenizer at

* Corresponding author. E-mail: yjchoi@nongae.gsnu.ac.kr; choiyj@gsnu.ac.kr

8 000 r min^{-1} for 30 s (IKA-25 basic, IKA Works, Wilmington, NC, USA). The pH of the homogenate was adjusted to a range from 2.0 to 11.0 using 0.2 mol L^{-1} HCl or NaOH solutions, and centrifuged at 2 500 g for 20 min (4−6 ℃). The amounts of HCl and NaOH solutions used were recorded for adjusting protein concentration. The middle liquid layer was saved for protein analysis. The Biuret method was used for protein determination (Umemoto, 1966). Protein solubility was calculated as protein concentration (mg mL^{-1}) in the supernatant after pH adjustment.

2.3 Sample Preparation

Three different protocols of mincing muscle were applied: (1) 3-cycle washing; (2) acid-aided processing; and (3) alkali-aided processing. Three-cycle washing was done twice using a 1:2 ratio of mince to cold water and using a cold NaCl solution (0.1%) for the final wash cycle. For dewatering, the mixture was centrifuged at 10 000 g for 25 min, and the supernatant, containing water-soluble protein, was discarded. Based on the solubility study, rinsed fillets were subjected to acid-aided (pH 2.5) or alkali-aided (pH 10.5) processing methods. After homogenizing, the fillets with distilled water at a 1:9 ratio, 2 mol L^{-1} HCl or 2 mol L^{-1} NaOH was used for the acid- or alkali-aided method, respectively, to first solubilize the fish proteins. Acidic or alkaline homogenates were centrifuged at 10 000 g for 25 min. The pH of the middle layer was adjusted to the isoelectric point (pH 5.5) and then dewatering was done using centrifugation (5 000 g, 25 min). Fish proteins were adjusted to pH 7.0 using 2 mol L^{-1} NaOH, and mixed with cryoprotectants (5% sorbitol, 4% sucrose and 0.3% sodium tripolyphospahte).

2.4 Preparation of Sarcoplasmic Protein

Minced muscle was homogenized with 2 vol of distilled water, and was centrifuged (10 000 g, 25 min). The pellet was homogenized and precipitated. The supernatant was collected and adjusted to pH 5.5 using 1 mol L^{-1} HCl in order to precipitate the sarcoplasmic protein. After 30 min, the precipitated protein was collected by centrifugation (10 000 g, 25 min).

2.5 Protein Recovery

Protein recovery was calculated based on the weight of recovered protein divided by the weight of the fish fillet. After acidic/alkaline processing, the weight of recovered protein was recorded and its moisture content was also measured.

Yield of protein recovery (%) = (solid weight of recovered protein/solid weight of fillet) × 100

2.6 Gel Preparation and Analysis

The recovered protein was comminuted with and without 2% NaCl, and an appropriate amount of iced water was used to maintain 78% moisture content. To evaluate the effect of sarcoplasmic protein on heated-induced gel, the sarcoplasmic protein from 3-cycle washing was added to surimi during comminuting. The paste was then stuffed into plastic tubes (2.0 cm i.d.) and cooked at 90 ℃ for 15 min. Gels were refrigerated overnight. After gels were equilibrating to room temperature, texture properties were measured by the punch test using a Rheometer (Model CR-100D, Sun Scientific Co., Tokyo, Japan). The movement speed of a spherical probe (5 mm diameter) in measurement was 60 mm min^{-1}. 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 ($\pm a^*$), and yellowness or blueness ($\pm b^*$) of gels using a colorimeter (ZE-2000, Nippon Denshoku, Tokyo, Japan). Whiteness was calculated as whiteness index, L^*-3b^* (Park, 1994).

2.7 SDS-PAGE

The washed mince or recovered protein (3 g) was homogenized with 5% sodium dodecyl sulphate (SDS) solution to a final volume of 30 mL for 30 s using a homogenizer. The homogenates was incubated in an 80 ℃ water bath for 30 min to dissolve all sarcoplamic and myofibrillar proteins. Samples were then centrifuged (3 000 g, 15 min) to remove undissolved debris. The protein concentration was determined by Lowry's method (Lowry *et al.*, 1951). SDS-PAGE was performed in stacking (5%) and separating (7.5%) polyacrylamide gels using the method of Laemmli (1970). Gel was stained in Coomassie brilliant blue and destained in a solution of methanol (50%) and acetic acid (7%). A high molecular-weight standard (SDS-6H, Sigma Chemical Co., St. Louis, Mo., U.S.A) was used to estimate the molecular weight of protein bands.

3 Results and Discussion

3.1 Effect of pH on Protein Solubility

Protein solubility in an aqueous solution is dependent on pH. In order to obtain a higher recovery of fish proteins by acidic/alkaline processing, protein solubility at various pH conditions was investigated. Solubility of croaker and Jack mackerel proteins was the lowest at pH 4.5 and 5.5, respectively (Fig.1). A dramatic increase in solubility was observed when the pH was shifted from 5 to 2.5 or from 10 to 11.5. The maximum solubility was observed around pH 2.0 and 11.0.

In the present study, it was found that an approximate pH 5.5 is the isoelectric point where proteins have zero net charge in solution, resulting in minimum solubility and maximum precipitation. Around pH 5.5, a large amount of precipitates after centrifugation were observed due to the low electrostatic repulsion between protein molecules. Once the pH shifted away to either acid or alkaline direction from the isoelectric point, electrostatic repulsion between molecules increased, resulting in high protein solubility.

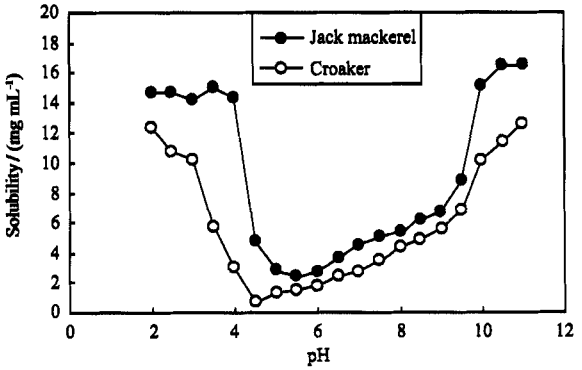

Fig.1 Effect of pH on the protein solubility of croaker and Jack mackerel muscle protein.

3.2 Protein Recovery

Yield of protein recovery (%) is shown in Fig.2. The recovery from acid-and alkali-aided processing was dependent on fish species.

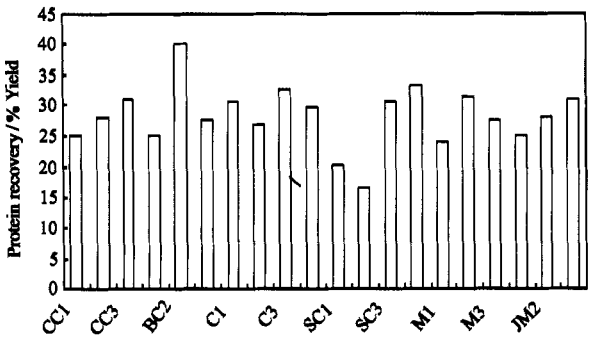

Fig.2 Protein recovery by various treatments. CC. croceine croaker; BC. balckspotted croaker; C. croaker; SC. small croaker (kangchi); M. mackerel; JM. Jack mackerel; 1. 3-cycle washing; 2. acid treatment; 3. alkali treatment.

The yield of conventional processing is about 25%, while that of acid- and alkali-aided processing ranges from 31% to 33%. The yield of conventional surimi reported ranged from 21% to 25% (Toyoda *et al.*, 1992; Park *et al.*, 1997; Roussel and Cheftel, 1990). The increasing yield of acid- and alkali-aided processing was due to recovery of sarcoplasmic protein in muscle.

3.3 Texture and Color Properties

Breaking forces of the recovered protein gel from alkali-aided processing were higher than those from the 3-cycle washing and acid-aided processing of frozen croceine croaker, frozen croaker and jack mackerel, while they were lower than those of surimi gel from 3-cycle washing processing of frozen blackspotted croaker and mackerel (Fig.3). The results showed that breaking force of recovered protein gel from alkaline processing is dependent on fish species. The fish protein was more sensitive to acidic pH than to alkaline pH. The chemical modification involving cross-linking of protein was easier in alkaline condition. The amount of myosin heavy chain and actin was greatly reduced in acidic pH compared with alkaline pH. The decrease of myosin heavy chain contributed to decrease of breaking force of heat-induced gel from acidic processing.

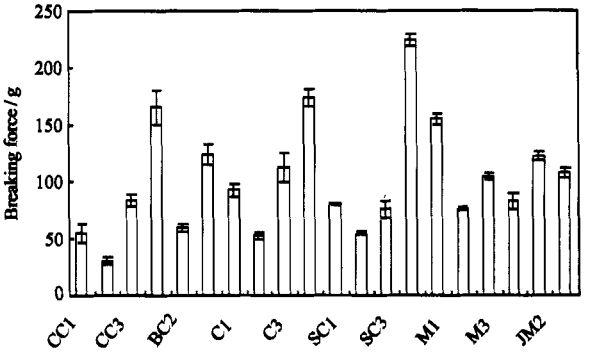

Fig.3 Breaking force by various treatments. The symbols were the same as in Fig.2. Values shown are means (n=3) ± standard deviation.

Deformations of heat-induced recovery protein gel from alkali-aided processing were lower than those from 3-cycle washing processing except for frozen croaker (Fig.4). The results suggested that fish protein is denatured deeply due to the extreme pH in alkali-aided processing. However, the better textural properties were obtained from fish protein treated with alkali compared with that with acid. Alkali conditions, especially at pH 10.5, were favored for disulfide bond formation (Kim *et al.*, 2003). Alkali-aided processing demonstrated better gel-forming ability than either conventional or acid-aided processing (Yongsawatdigul and Park, 2001). Ninomiya *et al.* (1990)

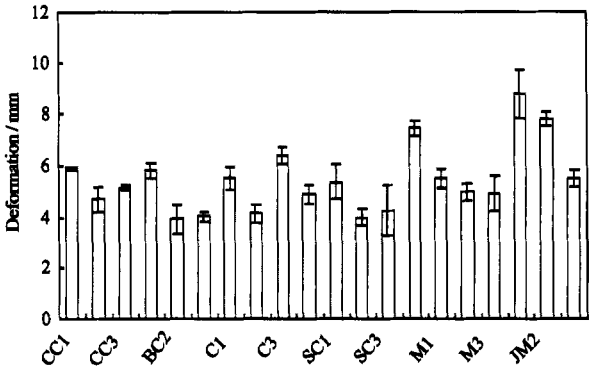

Fig.4 Deformation by various treatments. The symbols were the same as in Fig.2. Values shown are means (n=3) ± standard deviation.

also observed the formation of more preferable gels when treated at pH12 than at pH3 using water-soluble proteins from mackerel.

High whiteness was obtained from gels treated at 3-cycle washing (Fig.5). The lower whiteness of gels treated with acid and alkali was due to the hemoglobin and myoglobin remaining in the recovered protein.

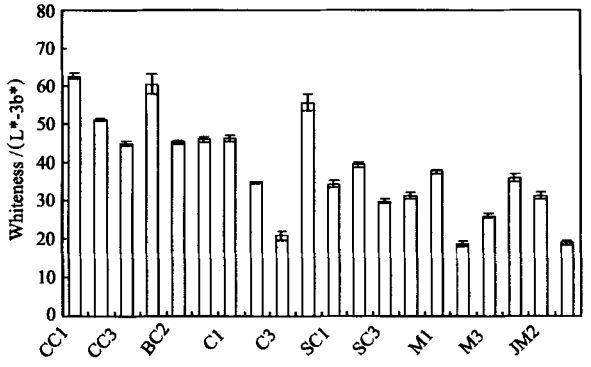

Fig.5 Whiteness by various treatments. The symbols were the same as in Fig.2. Values shown are means (n=3) ± standard deviation.

3.4 Effect of Sarcoplasmic Protein on Gel Texture

Breaking force and deformation values were increased by the addition of sarcoplasmic protein, but were not increased significantly when the addition was less than 9% (Fig.6). The sarcoplasmic protein was needed to be purified for improving the gel-forming ability through the washing step in surimi processing (Shimizu and Nishioka, 1974; Okada, 1964; Hashimoto *et al.*, 1985). However, the breaking strength of mackerel surimi gel was increased by the addition of 10% - 20% sarcoplasmic protein (Morioka *et al.*, 1992). The gel properties depend on the kind of protein with different proportions of sarcoplasmic protein (Morioka and Shimizu, 1993).

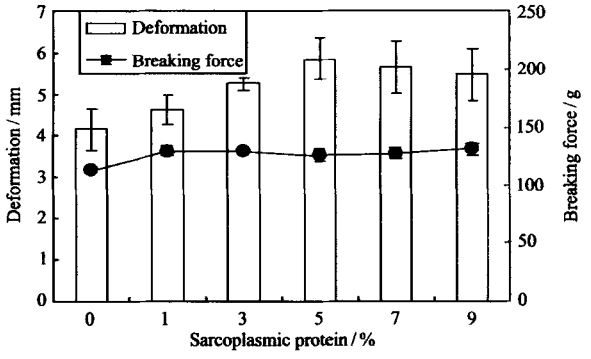

Fig.6 Effect of sarcoplasmic protein on gel texture.

3.5 Effect of NaCl on Gel Texture

The breaking force was significantly decreased, while the deformation was constant with the increasing of NaCl ($P<0.05$) (Figs.7 and 8). The decreased amount of breaking force was higher in jack mackerel than in croaker. Upon comminution of the surimi with salt, the hydrated salt ions are adsorbed to the surface of the myofibrillar proteins and thus increase their affinity for water molecules. Solubilization of the proteins results in the formation of a viscous paste. Salt addition is necessary for the development of an elastic structure in the heat-induced gel. The results suggested that the gelling mechanism was different from that of conventional surimi gel.

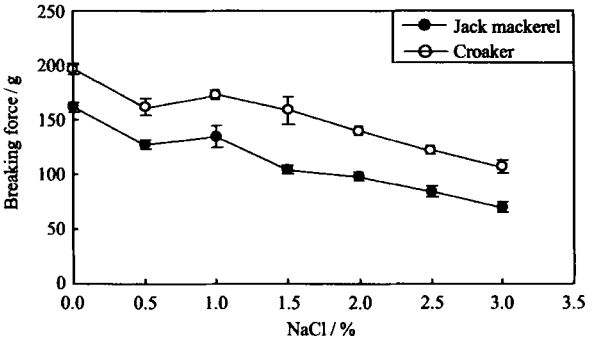

Fig.7 Effect of NaCl on breaking force of gel.

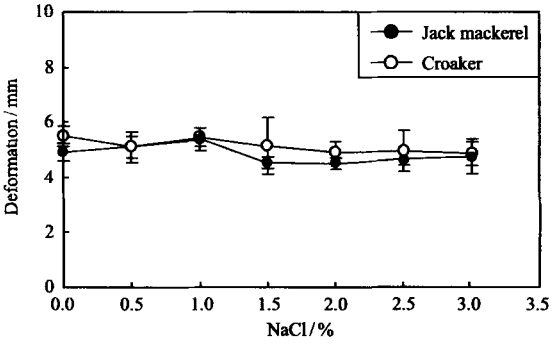

Fig.8 Effect of NaCl on deformation of gel.

3.6 Protein Patterns on SDS-PAGE

Different protein patterns were observed among fish proteins prepared using acid- or alkali-aided processing (Fig.9). A large molecular band was observed below the myosin heavy chain (MHC) from fish proteins

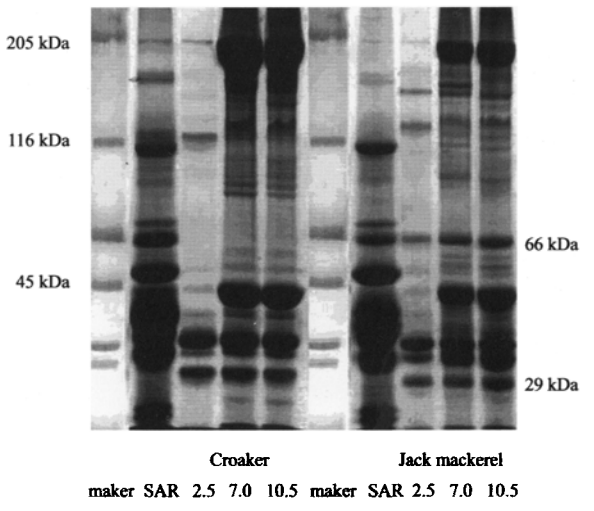

Fig.9 SDS-PAGE patterns of surimi from sarcoplasmic protein (SAR), conventional (7.0), acidic (2.5) and alkaline (10.5) processing of croaker and jack mackerel.

treated at pH 2.5. These protein patterns were similar to those in our previous report (Choi and Park, 2002). However, it was not clear whether the appearance of the small bands was the result of protease or acid hydrolysis.

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