연사 5

A New Process for Muscle Protein Isolate

Jae W. Park and Supawan Thawornchinsombut

Oregon State University

연사 5

A New Process for Muscle Protein Isolate

Submitted to Association of Korean Animal Food Products

Jae W. Park and Supawan Thawornchinsombut
Oregon State University
Dept of Food Science and Technology & OSU Seafood Lab Astoria, OR 97103 USA

1. Introduction: Processing Principle

The process for isolating the protein composition from a muscle source and recovering the protein composition using pH-shift was first patented by Hultin and Kelleher (1999). The effort was designed to better utilize low value raw materials including fatty, pelagic fish and deboned muscle tissue from fish and poultry processing. Considerable effort has also been made to produce a protein concentrate from underutilized fish species. The "conventional surimi" process has been successful in producing a stabilized protein food. In producing surimi, the muscle is ground and extensively washed and dewatered. During the washing process 20-30% of the fish muscle proteins, especially the sarcoplasmic proteins, are solubilized and generally not recovered from the wash water. A significant amount of myosin heavy chain was also soluble when mince was washed with fresh water during surimi manufacturing (Lin and Park 1996).

These novel processes have been developed in which proteins are isolated from animal muscle using acidic (pH 2-3) or alkaline (pH 10.5-11) solubilization of the proteins. The soluble proteins are subsequently recovered by isoelectric precipitation (pI ~ 5.5) (Hultin and Kelleher 1999; Hultin and others 2000; Choi and Park 2002; Kim and others 2003; Yongsawatdigul and Park 2004). Muscle tissue is ground and homogenized with enough water. The pH of the homogenate is adjusted to solubilize a major proportion, preferably all of the available protein and to reduce the viscosity to allow easy separation of insoluble materials from the solubilized composition.

Typically, the ratio of volume of aqueous liquid to weight of tissue is 5:1 to 9:1. This process differs from the conventional process in that major myofibrillar proteins are much less solubilized in the conventional process but water-soluble materials that lead to loss of quality of the product are removed. After the muscle proteins are solubilized, they are centrifuged at an adequate force (greater than 5000×g) to separate the membrane lipid and other insoluble fractions from neutral lipids and soluble proteins.

The neutral lipids can be skimmed off and the soluble supernatant, with the protein-rich fraction, is recovered by decanting.

The protein in the aqueous phase can be precipitated by adjusting its pH to about 5.0 or 5.5. The salt concentration can be optionally adjusted to aid precipitation. The total precipitated protein comprised of myofibrillar and sarcoplasmic proteins can be collected by centrifugation. At least 70% yield of the total animal muscle protein can be obtained as precipitated protein. The protein isolate can be adjusted to neutral pH with the addition of bicarbonate or other base. Cryoprotectants can be added to the precipitated protein to preserve the product during freezing and frozen storage (Hultin and Kelleher 1999; Hultin and others 2000).

This new protein recovery method offers several advantages over the traditional surimi process. The new process gives improved processing yield. Greater than 90% yields are generally obtained from fillets compared with 55-70% from the conventional process (Hultin and Kelleher 1999; Kim 2002; Undeland and others 2002). The improved yield results in less protein in the waste water, therefore environmental pollution is decreased. It is also clear that most of the sarcoplasmic proteins are recovered by this process as well. Furthermore, there was no indication that these sarcoplasmic proteins interfered with the gel formation of myofibrillar proteins and there is evidence that sarcoplasmic proteins may actually enhance myofibrillar protein gelation (Morioka and Shimizu 1990; Ko and Hwang 1995; Nowsad and others 1995).

A functional protein isolate can be produced with low or no lipid content since this process can remove the oxidation-prone membrane lipids by centrifugation, thus stabilizing the proteins against oxidation. One advantage to the elimination of lipid from the proteins is that lipid-soluble toxins i.e. polychlorinated biphenyls and polyaromatic hydrocarbons can be reduced in the final product.

The new process also increases productivity since the extensive washing and refining steps can be excluded. Undesired materials such as scale, skin, and bone are removed in the sediment of the first centrifugation. Furthermore, unlike the conventional method, this method does not require fresh or lean animal muscle as a starting material. Frozen or slightly damaged muscle tissue is still capable of producing a gel with excellent quality. On the other hand, in the manufacture of high quality surimi using the conventional method, high quality fish should be used (Hultin and Kelleher 1999; Hultin and others 2000; Hultin and Kelleher 2000).

2. Chemistry of pH-Treated Fish Proteins

Acidic or alkaline processing of muscle protein is expected to cause dramatic changes of protein conformation and function. According to conventional muscle chemistry, it is generally known that denatured muscle proteins contribute negative protein

functionalities. Conversely, it has been shown that the acid- or alkali-treated muscle protein isolate exhibited either comparative or improved gel qualities compared to proteins from conventional processes (Kim 2002, Underland and others 2002, Yongsawatdigul and Park 2004). Challenges for the unexplained evidence are to find out the mechanisms that help maintain or improve protein properties while they are exposed to severe pH conditions.

2.1 Muscle Proteins

Muscle tissue is composed of a number of different proteins that account for 15-22% of the total weight. Myofibrillar proteins, which make up around 60-70% of all the proteins in muscle, play a major structural and functional role in meat and other processed muscle foods as they have a tendency to interact with each other and other nonprotein ingredients (Xiong 1997). Altering the pH of a medium is one of the oldest known methods used to unfold proteins. As pH is increased or decreased away from the pI, the ionizable groups in proteins become increasingly charged up to a point where the charge repulsion causes the protein molecule to unfold. In addition to pH, ionic strength (IS) is also one of the most important parameters driving the changes of protein functionality. A typical U-shape of protein solubility was attained when Pacific whiting (PW) muscle protein suspensions were treated over a pH range of 2 to 12 at IS 10 mM. An increase in ionic strength to 600 mM enhanced protein solubility at pH between 6 and 10 and caused the pI shift of PW proteins toward the acidic side by about two pH units (~ pH 5.5 to 3.5) (Fig. 1) (Thawornchinsombut and Park 2004a). MHC was not stable to protein degradation by proteolytic enzymes and/or acid hydrolysis under acidic pH. In addition, MHC precipitated more as the salt content increased and

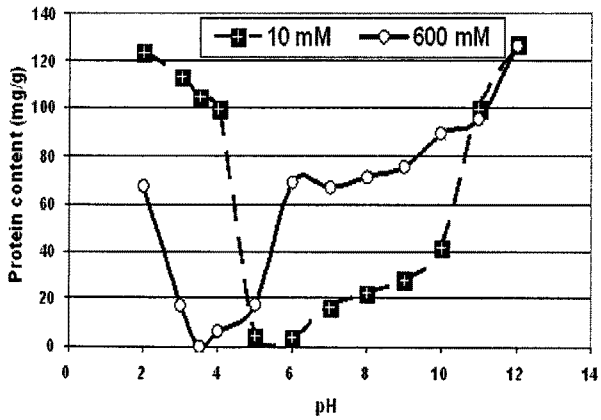

Fig. 1. Solubility of Pacific whiting muscle protein prepared at various pH and two IS levels. For pH 2, 3, and 12, IS was adjusted to 72, 12.5 and 32 mM NaCl instead of 10 mM due to technical difficulties, respectively (Thawornchinsombut and Park 2004d).

completely disappeared from the protein patterns at IS ≥ 150 mM (Fig. 2). On the other hand, under alkaline pH, MHC was more stable and the high pH might have partly contributed to some or partial polymerization at higher IS (> 150 mM NaCl) (Fig. 3) (Thawornchinsombut and Park 2004b).

The solubility of refolded cod myofibrillar proteins at pH 7.5 after acidic or alkaline treatment (pH 2.5 or 11) without precipitating at the pI exhibited significantly greater than the native proteins over the IS range between 0-600 mM KCl, whereas almost identical solubility profiles of native and refolded myosin were observed (Kristinsson and Hultin 2003a). However, the contradicting data were noted when alkali- or acid-treated proteins were precipitated at the pI before refolding at neutral pH. After acidic and alkaline treatments of rockfish protein isolates (pH 2-3/11-12 → pI → 7.0) the samples exhibited low solubility in both low and high ionic strength buffers (Fig. 4) (Yongsawatdigul and Park 2004). Low ionic strength buffer (50 mM KCl) was needed to extract sarcoplamic proteins, while high ionic strength buffer (0.6 M KCl) was required to solubilize myofibrillar proteins.

When mince sample (M) was washed conventionally, a majority of sarcoplasmic proteins was leached out, resulting in low sarcoplasmic protein and high myofibrillar protein content in the washed mince (WM). Therefore myofibrillar proteins were a main component in the WM. Although sarcoplasmic proteins remained in both alkali- (AKPI) and acid (ACPI)-treated protein isolates, the extraction of these proteins from AKPI and ACPI was much lower than that of M. Similarly, myofibrillar proteins of AKPI and ACPI were not as soluble as M and WM (Yongsawatdigul and Park 2004).

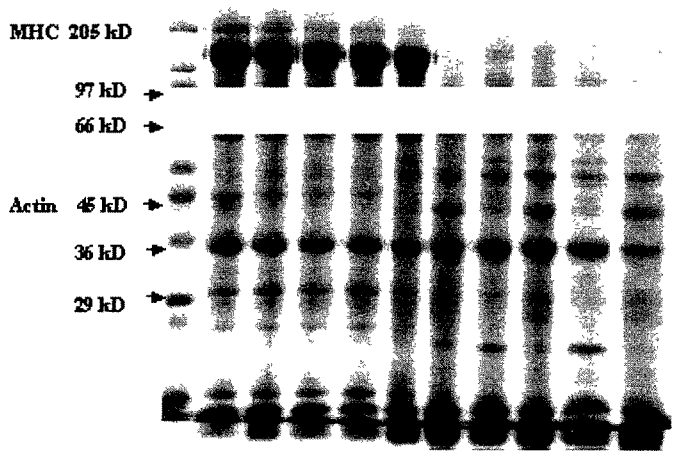

Fig. 2. SDS-PAGE patterns of Pacific whiting soluble proteins at pH 4 and various IS conditions. An equal quantity (25 μg of proteins) was applied per each well on the 10 % acrylamide separating gel (S: wide range mw standard; MHC: myosin heavy chain) (Thawornchinsombut and Park 2004c).

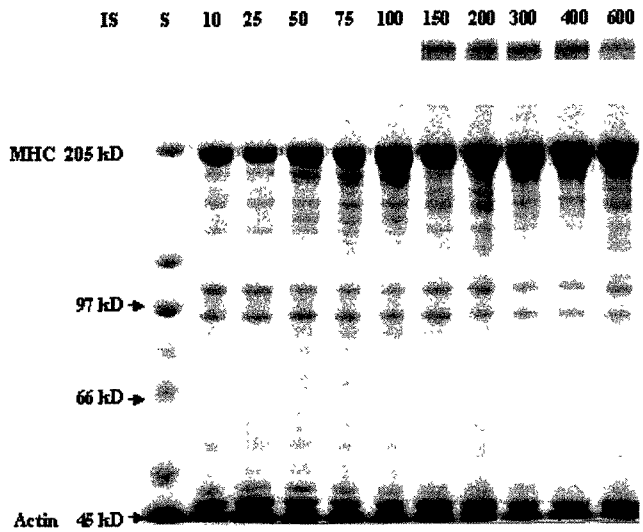

Fig. 3. SDS-PAGE patterns of Pacific whiting soluble proteins at pH 10 and various IS conditions. An equal quantity (25 μg of proteins) was applied per each well on the 6 % acrylamide separating gel (S: wide range MW standard; MHC: myosin heavy chain) (Thawornchinsombut and Park 2004c).

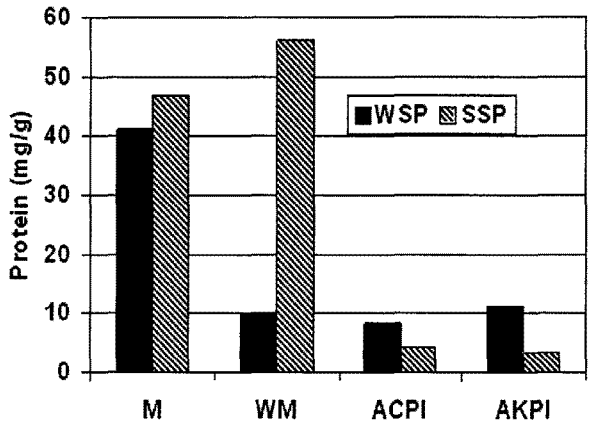

Fig. 4. Protein extractability of rockfish prepared by various treatments: M: mince; WM: washed mince; ACPI: acid-treated protein isolate; AK: alkali-treated protein isolate; WSP: water soluble proteins; SSP: salt soluble proteins (Yongsawatdigul and Park 2004).

Similar results have been demonstrated in PW protein isolate using either acidic or alkaline treatment (Kim 2002; Thawornchinsombut and Park 2004c,d). Low extractability indicated the denaturation and aggregation of proteins. These results probably indicated that the isoelectric protein precipitation caused substantial structural changes of muscle proteins resulting in denaturation and aggregation of both sarcoplasmic and myofibrillar proteins. Interestingly, these low water and salt extractable proteins how-

ever, exhibited better or comparative gel qualities than conventional surimi (Kim 2002; Thawornchinsombut and Park 2004 c,d; Yongsawatdigul and Park 2004).

Soluble PW muscle proteins extracted at alkaline pH (11-12) contained lower sulfhydryl (SH) group content than those extracted at acidic pH (2-4) or neutral pH. The decrease in SH content in alkaline conditions was also in accordance with the formation of high molecular weight polymers. This result perhaps indicated that SH groups became more susceptible to oxidation at alkaline pH resulting in the formation of disulfide bonds (Thawornchinsombut and Park 2004a). Kristinsson and Hultin (2002a) reported an increase in reactive SH groups for cod myosin after alkaline treatment, which could aid in the formation of stronger gel than that of surimi. Several studies reported significantly harder gels for protein isolate made with the alkaline process when compared to the acid process (Undeland and others 2002; Kim and others 2003; Thawornchinsombut and Park 2004c; Yongsawatdigul and Park 2004).

2.2 Sarcoplasmic Proteins

As previously described, the new method can provide extremely high yields because it solubilizes nearly all myofibrillar and sarcoplasmic proteins through homogenization and pH adjustment before recovery by isoelectric precipitation. In contrast, the conventional process removes sarcoplasmic proteins through washing and dewatering, which results in a significant protein loss, excessive water usage, and contributes to waste water problems.

No absolute agreements, however, have been made regarding the role of sarcoplasmic proteins in the gelation of myofibrillar proteins. It has been reported that the heat coagulative sarcoplasmic proteins adhere to myofibrillar proteins when the fish muscle is heated and thus, impede the formation of fish protein gels (Shimizu and Nishioka 1974). Okada (1964) also reported that sarcoplasmic proteins inhibit gel formation of myofibrillar proteins. On the other hand, recent studies indicated that water-soluble sarcoplasmic proteins from mackerel actually increase gel strength (Morioka and Shimizu 1990). The addition of sarcoplasmic proteins also improved thermal gelation of milkfish, and has a positive effect on suwari and a restrictive effect on modori (Ko and Hwang 1995).

Nomura and others (1995) speculated that modori (gel softening at 40℃) inhibition for Hilgendorf saucord (*Hilgendorf saucord*) was possibly due to the sarcoplasmic protein fraction from Princes small porgy (*Chelidoperca hirundinacea*) and bigeyed greeneye (*Chlorophthalmus albatrossis*). In addition, Alaska pollock surimi was weakened by repeated washing but was strengthened again when the removed fraction was reincorporated into the surimi (Nowsad and others 1995). This was likely due to the function of transglutaminase in the sarcoplasmic proteins.

While there is still controversy regarding the role of sarcoplasmic proteins from conventional surimi processing, the better understanding of sarcoplasmic proteins from the pH-driven fish protein isolation process and its role has been discussed limitedly. Kim and others (2004) studied the influence of pH and salt on the physicochemical characteristics of sarcoplasmic proteins from rockfish. They found the solubility and molecular weight distribution of sarcoplasmic proteins were independent of pH. High NaCl concentration (0.5 M) significantly affected solubility and protein patterns only under acidic conditions. Sarcoplasmic proteins appeared to be more stable against pH (especially neutral and alkaline) and salt than myofibrillar proteins. When sarcoplasmic proteins were treated to pH 2 or 3 and then shifted to pH 5.5, the least amount of proteins was lost in the supernatant fraction (Fig. 5).

This significantly higher recovery of sarcoplasmic proteins at pH 2 and 3 could mainly result from the large amount of aggregates through exposed hydrophobic sites. As the band around 17 kDa disappered from the supernatant under acidic condition, it reflected that these heme proteins were more precipitated (Kim and others 2004). This led to a higher concentration of heme proteins (denatured form) in the acid protein isolate (ACPI) than alkaline protein isolate (AKPI) and thus the proteins were highly susceptible to oxidation since denatured heme proteins are very active catalysts of lipid oxidation. The alkaline condition on the other hand suppressed pro-oxidative activity of heme proteins (Kristinsson 2002a,b). Inclusion of sarcoplasmic proteins into myofibrillar

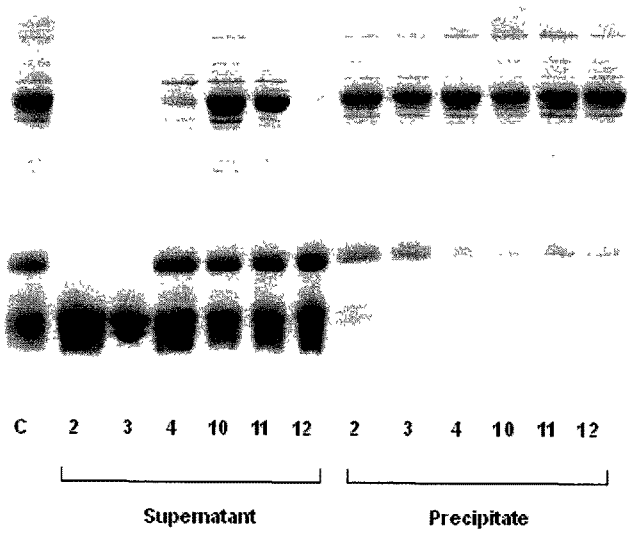

Fig. 5. Protein patterns of supernatants and precipitates of pH-treated sarcoplasmic protein (SP) after pH readjustment to 5.5. C (control) indicates SP solution (0% NaCl, pH 5.5). The number indicates the pH at which samples were adjustedtreated before recovered (Kim and others 2004).

proteins (surimi) improved fracture texture properties (Fig. 6), but it negatively affected color properties due to increased heme protein concentrations (Kim and others 2004).

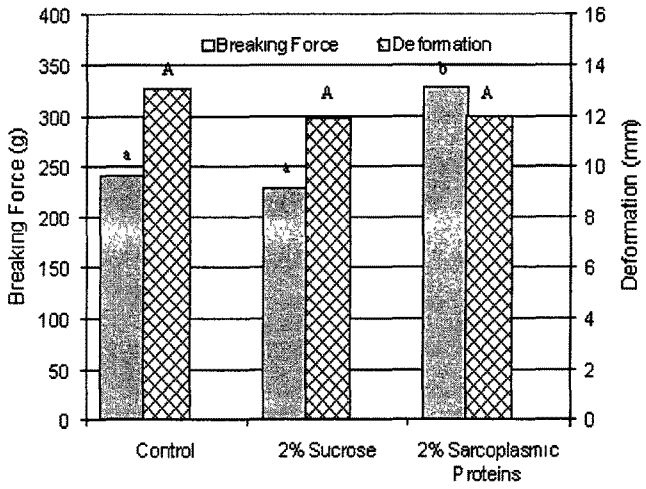

Fig. 6. Fracture properties of pollock surimi as affected by the addition of SP or sucrose. Different alphabetical letters indicate a significant difference ($p \leq 0.05$) (Kim and others 2004).

3. Characteristics of Fish Protein Isolate (FPI)

3.1 Biochemical Properties

3.1.1 Protein Unfolding/Denaturation

Similar hydrophobicity was found among fish protein isolate samples except pH 2 and 12 treatment (Fig. 7) (Kim and others 2003). Normally, hydrophobicity values are higher at acidic pH compared to neutral or alkaline pH (Alizadeh-pasdar and Li-Chan 2000). Das and Kinsella (1989) reported at different pH values (2.8, 4.3, 5.0, 7.6, and 9.7) the highest hydrophobicity was measured at pH 2.8 and showed that with an increase in pH, hydrophobicity decreased drastically.

Thawornchinsombut and Park (2004b) compared protein hydrophobicity of acid-treated fish proteins analyzed by two probes [ANS (1-anilinonaphthalene-8-sulfonic acid) and PRODAN (6-propionyl-2-(dimethylamino)-naphthalene)]. They reported that fish proteins treated at pH 4 exhibited extremely high hydrophobicity at 10 mM NaCl when the ANS probe (ANS-S_o) was used: 10 times larger than using a PRODAN-S_o probe. Since at acidic pH (pH 4) the ANS probe yields negative sulfonate anions (pK_a ~ 3.6) while proteins have a net positive charge, the ANS anion can bind prominently to the cationic group of the proteins. If the hydrophobic regions of the protein are adjacent to charged side chain residues, electrostatic interactions of the anionic ANS and the protein charges possibly strengthen the hydrophobic interactions between ANS and

protein (Greene 1984; Haskard and Li-Chan 1998). They concluded, therefore, that the use of an anionic ANS fluorescent probe needs to be more carefully considered for measuring protein surface hydrophobicity under acidic conditions.

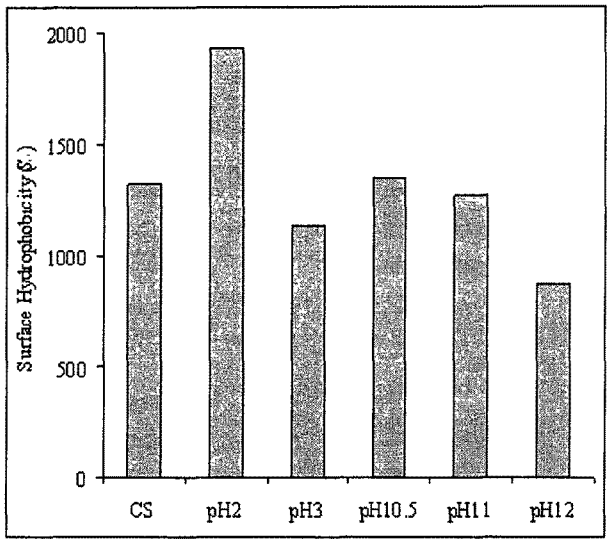

Fig. 7. Surface hydrophobicity of PW protein isolates prepared at various pH. S_0 is expressed by initial slopes of relative fluorescence intensity vs. protein concentration plot in the presence of ANS (1-anilino-8-naphthalenesulfonate). CS is conventional surimi (Kim 2002).

Conventional surimi from PW demonstrated the lowest S_o value although its IS was comparable to refolded acid- or alkali-treated FPI at IS 400 mM (adjusted to pH 7.0). Similar findings have been reported for cod myosin (Kristinsson and Hultin 2003b) and rockfish muscle proteins treated at low and high pH (Yongsawatdigul and Park 2004).

Changed protein conformation induced by chemical denaturation might not be fully refolded back to its native form. A study using Raman spectroscopy revealed that the Raman spectra of pH 11-treated rockfish protein isolates were significantly different from the conventional surimi even though the pH was adjusted to 7.0 before analysis (Thawornchinsombut and others 2004). More exposed tyrosine residues and peptide backbone stretching were proposed for alkali-treated FPI. Under the pH-shift treatment the conformation of the myosin head did not revert to its native state upon refolding (Kristinsson and Hultin 2003a). This might have contributed to increased availability of hydrophobic patches on the protein molecules for probe bindings.

The measurement of ATPase activity estimates the degree of protein denaturation. Decreased activity of these enzymes is probably due to the denaturation of the ATP-binding site in myosin (Hamai and Konno 1990). There was no measurable ATPase activity of PW proteins isolated at acidic pH (Table 1) (Choi and Park 2002). Kristinsson and Hultin (2003a) discovered the dissociation of myosin light chain in acid-treated cod

Table 1: ATPase activities of PW proteins recovered using conventional and acid-aided processing

ATPase activities (μ mole Pi/min/mg protein)	Conventional processing		Acid-aided processing
	1-washing	3-washing	
Ca-ATPase	0.128	0.095	ND
Mg-ATPase	0.218	0.126	ND

ND: Not detectable.

Adapted from Choi and Park (2002).

myosin after refolding at neutrality while only half of the light chains were dissociated in alkali-treated samples. Choi and Park (2002) also proposed that myosin light chain, containing ATP active sites, is partially damaged and dissociated during acid-aided processing. The ATPase activity begins to decrease at approximately pH 9 as a result of the denaturation of the myosin molecule as well as loss of the alkali light chain (Pearson and Young 1989).

3.1.2 Proteolytic Enzyme Activities

In the acid- and alkali-aided processes, myofibrillar protein degradation was significantly more pronounced after acidification than alkalinization (Undeland and others 2002; Kim and others 2003). Cortes-Ruiz and others (2001) also reported proteolytic activity in acid produced proteins from Bristly sardine. Cathepsins L and B were found to be active in Pacific whiting. However, only cathepsin L, which still remains after the washing steps of the conventional surimi process, causes the gel-weakening phenomenon upon slow heating (An and others 1994).

Choi and Park (2002) discovered that after acid solubilization followed by isoelectrical precipitation cathepsins were retained along with myofibrillar proteins. Cathepsin L-like was highly activated by the alkaline process, especially at pH 10.5 (Fig. 8). The lowest cathepsin L-like activity was observed from PW proteins prepared using the conventional method. A possible explanation of this discrepancy is that cathepsin L-like might be reactivated once the pH was readjusted to 7.0. (Kim and others 2003).

Cathepsin B-like enzymes appeared to be highly activated at acid treatment (Fig. 9). Conventional washing could not remove all cathepsin B-like enzymes. However, the alkaline process removed them dramatically. Especially at pH 12, no activity was detected (Fig. 9). Most lysosomal proteinases are active at acidic pH (Jiang and others 1997). Cathepsin B has maximal activity at pH 6.0 and is unstable above pH 7.0 (Park 2000). Cathepsin B-like enzymes could therefore not tolerate alkaline conditions (Kim and others 2003). Cathepsin H-like activities were not detected in either ACPI or AKPI.

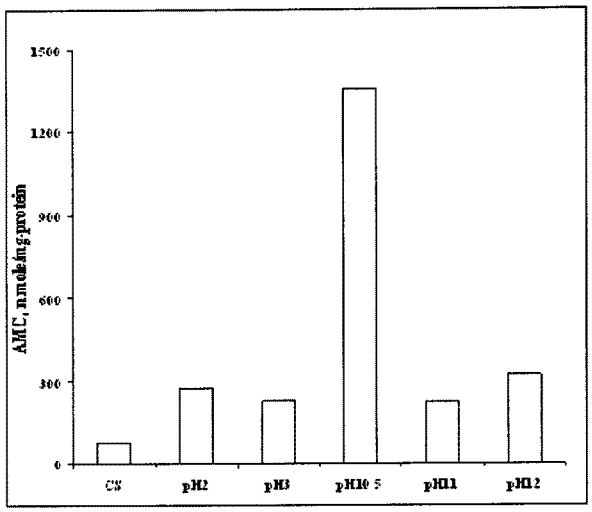

Fig. 8. Cathepsin L-like activities of PW protein isolates prepared at various pH. The measurement was conducted at pH 5.5 (Kim 2002).

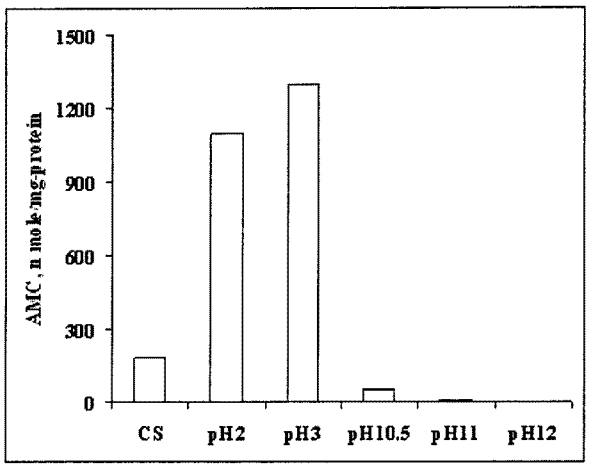

Fig. 9. Cathepsin B-like activities of PW protein isolates prepared at various pH. The measurement was conducted at pH 6.0 (Kim 2002).

These are presumably easily washed away during conventional washing. This enzyme was also likely damaged by acid or alkali treatment (Kim and others 2003).

A large molecular band was observed right below the myosin heavy chain (MHC) from gels of ACPI prepared at pH 2 and 3 (Fig.10). ACPI gels also showed one additional small band under the actin band. These protein patterns were the same as the results of Choi and Park (2002). Concurrent results were also observed by Undeland and others (2002) in ACPI from herring light muscle while hydrolysis was negligible in AKPI.

However, it was not clear that those band generations were due to high cathepsin B-like activities or acid hydrolysis.

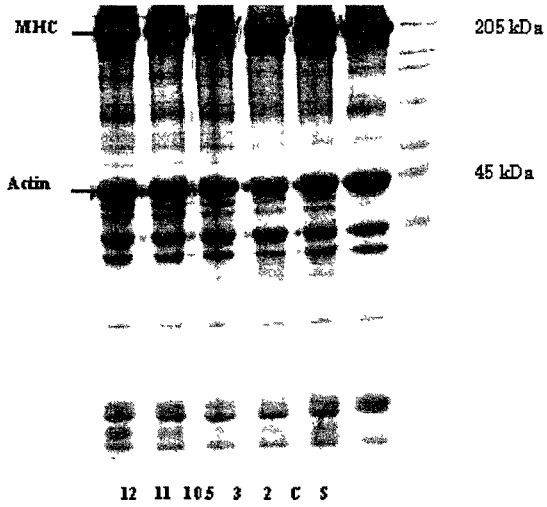

Fig. 10. SDS-PAGE patterns of PW protein isolates prepared at various pH. The number indicates pH and S denotes high molecular weight standard (Kim 2002).

The proteases from Pacific whiting hydrolyzed not only the myosin heavy chain but also the resulting fragments into smaller peptides (An and others 1994). Many other small molecular weight bands, which were not detected on fish protein gels from the conventional surimi method, appeared on both acid- and alkali-aided fish proteins gels. Those proteins were derived from sarcoplasmic protein and degradation products through acid or alkaline treatment (Kim and others 2003).

3.2 Texture Properties

The best textural properties were obtained from fish proteins treated at pH 11 followed by pH 2 (Fig. 11) (Kim and others 2003). Yongsawatdigul and Park (2004) and Thawornchinsombut and Park (2004c) also reported that the highest gel breaking force was obtained from AKPI (pH 11). Myofibrillar proteins, especially actomyosin, contribute to the elasticity of muscle protein gels. Removal of sarcoplasmic proteins resulted in higher myofibrillar protein concentration, which consequently increased the breaking force of washed mince in the conventional method. The greater extent of disulfide formation of myofibrillar and sarcoplasmic proteins induced by alkaline solubilization could contribute to higher breaking force and deformation of AKPI gels (Kim and others 2003, Thawornchinsombut and Park 2004c; Yongsawatdigul and Park 2004). Smyth and others (1998) reported that intermolecular disulfide bonds of the 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 the gel.

Lower breaking force and deformation were observed in the ACPI samples (Fig. 11). Although rheological changes of ACPI resembled those of AKPI (Fig 12), the extent of disulfide formation was much lower. This might have resulted in lower breaking force and deformation for ACPI. Choi and Park (2002) and Yongsawatdigul and Park (2004) also reported low breaking force and deformation of gels obtained from the acid solubilization process of Pacific whiting and rockfish, respectively. In contrast, Hultin and Kelleher (1999) showed high shear stress and shear strain of gels prepared from acid solubilization of Atlantic mackerel and cod.

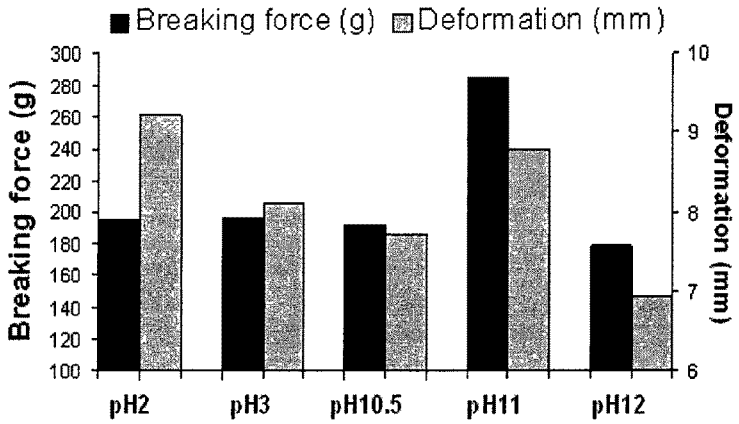

Fig. 11. Textural properties of PW protein isolates prepared at various pH (Kim 2002).

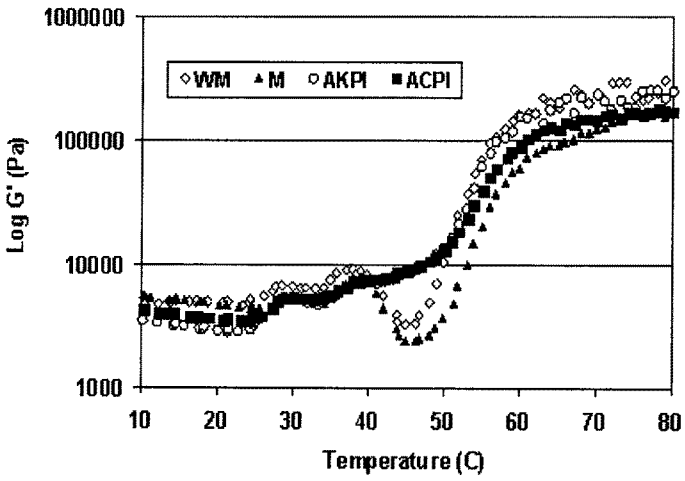

Fig. 12. Changes in storage moduli (G') of rockfish muscle proteins prepared by various treatments. WM: washed mince; M: mince; AC: acid-treated protein isolate; AK: alkali-treated protein isolate (Yongsawatdigul and Park 2004).

3.3 Rheological properties

Storage modulus (G') of ACPI and AKPI showed a different pattern from the native protein. However, the patterns of slopes on acid- and alkali-aided fish proteins were similar. Unlike conventional samples, WM (washed mince) and M (unwashed mince), G' of ACPI and AKPI increased continuously from 25°C without a decline starting at 39°C and a sharp increase starting at 46°C (Fig. 12) (Yongsawatdigul and Park 2004). Similar observations were found by Kim and others (2003).

Since a decline of G' was attributed to denaturation of light meromyosin (Xiong and Blanchard 1994), it could be assumed that light meromyosin might have undergone denaturation during acid and alkali treatments as suggested by the DSC results (Table 2). Therefore, viscoelastic properties of ACPI and AKPI were mainly contributed by aggregation of denatured muscle protein, which was previously induced by acidic and alkaline conditions. 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 chicken breast myosin had a long filamentous structure at pH 5.4 and formed a fine strand gel structure through myosin head interactions, exhibiting higher rigidity. Therefore, muscle proteins could readily aggregate to form elastic gel networks after isoelectric precipitation.

3. 4. Thermodynamic Properties Measured Using DSC

The thermogram of PW conventional surimi contained four endothermic transitions. The temperatures at which maximum heat input occurred (T_m) were 35.4, 41.2, 51.1, and 68.8℃, respectively. The first three and the last transition were assumedly assigned to myosin domains and actin denaturation, respectively (Esturk 2003). Depending on species (Howell and others 1991; Ogawa and others 1993), the environment (pH, ionic strength) (Wright and Wilding 1984; Howell and others 1991), as well as scanning rate (Thawornchinsombut and Park 2004c), isolated myosin may show various peaks at different T_m.

AKPI (pH 11), which were adjusted to pH 7.0 after recovery at pH 5.5 and contained cryoprotectants (7C), showed three endothermic peaks with T_m around 33.5-34.7 (P1), 46.2-47.8 (P2 & P3), and 66.5-68 °C (P4), respectively (Table 2) (Thawornchinsombut and Park 2004d). The first peak perhaps consisted of the myosin domain and sarcoplasmic proteins. Collagens and connective tissues would also be exhibited in the first peak (Hastings and others 1985) if they were not removed through centrifugation. Badii and Howell (2003) observed the transition temperature of cod connective tissue at 34.9 °C. Peak 2 actually resulted from two endothermic transitions (Table 2). Actin was highly sensitive to the pH-shift method since it appeared at T_m ~ 66-68 °C with a small endothermic transition (< 0.04 J/g) (Thawornchinsombut and Park 2004d).

Howell and others (1991) reported that T_m of actin was similar in all fish species, regardless of habitat temperature, and was destabilized by increasing both pH and ionic strength.

Yongsawatdigul and Park (2004) reported that no endothermic transitions were observed in either AKPI or ACPI and suggested that myosin and actin could have undergone alkali- and acid-induced denaturation. The different results could be due to the different sensitivities of the instruments. For the study by Yongsawatdigul and Park (2004), the conventional DSC was much less sensitive compared to the micro DSC used by Esturk (2003) and Thawornchinsombut and Park (2004d). In addition, a faster scanning rate (10 °C/min) and less amount of sample were applied (20 mg) for the conventional DSC as compared to the micro DSC (1 °C/min and 500 mg)

Table 2. Peak maximum temperatures and enthalpies for denaturation of protein components in experimental samples.

Treatments*	Parameters	P1		P2 & P3	
		Means	SD	Means	SD
7C	T_m[a]	34.4	0.4	47.4	0.4
	ΔH[b]	0.180	0.008	0.200	0.013
5C	T_m	33.5	0.1	46.2	0.1
	ΔH	0.189	0.005	0.180	0.005
7C-F	T_m	34.7	0.1	47.8	0.2
	ΔH	0.185	0.002	0.212	0.019
5C-F	T_m	33.9	0.2	46.3	0.7
	ΔH	0.185	0.018	0.194	0.039
7NC-F	T_m	35.2	0.3	48.2	0.3
				53.2	0.4
	ΔH	0.204	0.006	0.303	0.005
5NC-F	T_m	33.9	0.1	46.7	0.3
				54.0	0.4
	ΔH	0.188	0.008	0.231	0.002

Means and standard deviations (SD) were calculated based on at least 2 replicates.

[a] T_m = Peak maximum temperature.

[b] ΔH = Enthalpy in Joules/g total protein.

*All treatments were adjusted to equal cryoprotectant content and pH 7.0 before analysis.

5 = frozen storage at pH 5.5.

7 = frozen storage at pH 7.0.

C = with cryoprotectants.

NC = without cryoprotectants.

F = with 3 freeze/thaw cycles (freezing at -18 ± 2 °C, 18 h; thawing at 4 ± 2 °C, 6 h).

4. Conclusion

The new process of fish protein isolation using pH-shift exhibited several advantages over the traditional method for fish protein concentration (surimi). Unlike conventional surimi, this fish protein isolate (FPI) contains both myofibrillar and sarcoplasmic proteins. Myofibrillar proteins demonstrated changes of protein properties when treated at acidic or alkaline conditions whereas sarcoplasmic proteins were independent of pH. Solubility and protein patterns of sarcoplasmic proteins were altered considerably by high salt concentration (0.5 M NaCl).

FPI is chemically unfolded while pH is shifted to either acidic or alkaline conditions. Unfolded proteins induced by chemical treatment are perhaps refolded when the pH is readjusted to 7, but are not identical to its native form. Myofibrillar protein degradation is more predominant in ACPI than AKPI. Cathepsin L-like enzymes were more active in the alkaline process (pH 10.5), while cathepsin B-like enzymes appeared to be more activated in the acidic process (pH 2 and 3).

AKPI demonstrated superior gel texture to ACPI. Intermolecular disulfide bonds probably played a meaningful role in gel formation of fish protein isolate especially AKPI. Rheological properties of ACPI and AKPI were different from conventional surimi (CS). However, rheological changes of ACPI and AKPI resembled CS. Viscoelastic properties of fish protein isolate were mainly contributed by aggregation of denatured protein, which was previously induced by acidic and alkaline conditions.

References

Alizadeh-Pasdar N, Li-Chan ECY. 2000. Comparison of protein surface hydrophobicity measured at various pH values using three different fluorescence probes. J Agric Food Chem 48:328-334.

An H, Weerasinghe V, Seymour TA, Morrissey MT. 1994. Cathepsin degradation of Pacific whiting surimi proteins. J Food Sci 59:1013-1017, 1033.

Badii F, Howell NK. 2003. Elucidation of the effect of formaldehyde and lipids on frozen stored cod collagen by FT-Raman spectroscopy and differential scanning calorimetry. J Agric Food Chem 51:1440-1446.

Choi YJ, Park JW. 2002. Acid-aided protein recovery from enzyme-rich pacific whiting. J Food Sci 67:2962-2967.

Cortes-Ruiz JA, Pacheco-Aguilar R, Garcia-Sanchez G, Lugo-Sanchez ME. 2001. Functional characterization of a protein concentrate from bristley sardine made under acidic conditions. J Aquatic Food Prod Technol 10(4):5-23.

Das KP, Kinsella JE. 1989. pH dependent emulsifying properties of β-lactoglobulin. J Dispers Sci Technol 10(1):77-102.

Esturk O. 2003. Characterization of rheological properties and thermal stability of fish myofibrillar proteins. [PhD Dissertation]. Corvallis, OR: Oregon State University.

Greene FC. 1984. Interactions of anionic and cationic fluorescent probes with proteins: The effect of charge. J Protein Chem 3(2):167-179.

Hamai M, Konno K. 1990. Structural stability of fish myosin subfragment-1. Comp Biochem Phisiol 95B:225-259.

Haskard CA, Li-Chan ECY. 1998. Hydrophobicity of bovine serum albumin and ovalbumin determined using uncharged (PRODAN) and anionic (ANS^-) fluorescent probes. J Agric Food Chem 46:2671-2677.

Hastings RJ, Rodger GW, Park R, Matthews AD, Anderson EM. 1985. Differential scanning calorimetry of fish muscle: The effect of processing and species variation. J Food Sci 50:503-506, 510.

Howell BK, Matthews AD, Donnelly AP. 1991. Thermal stability of fish myofibrils: A differential scanning calorimetric study. Int J Food Sci and Technol 26:283-295.

Hultin HO, Kelleher SD, inventors; Advanced Protein Technologies, Inc., assignee. 1999 Dec 21. Process for isolating a protein composition from a muscle source and protein composition. U.S. patent 6,005,073.

Hultin HO, Kelleher SD, inventors; University of Massachusetts, assignee. 2000 Oct 24. High efficiency alkaline protein extraction. U.S. patent 6,136,959.

Hultin HO, Kelleher, SD. 2000. Surimi processing from dark muscle fish. In: Park JW, editor. Surimi and surimi seafood. New York: Marcel Dekker Inc. p 59-77.

Hultin HO, Kelleher, SD, Feng Y, Kristinsson HG, Richards MP, Undeland IE, Ke S. 2000. High efficiency alkaline protein extraction. U.S. patent application No. 60/230,397.

Jiang ST, Lee B, Tsao C, Lee J. 1997. Mackerel cathepsins B and L effects on thermal degradation of surimi. J Food Sci 62:310-315

Kim YS. 2002. Physicochemical characteristics of fish myofibrillar and sarcoplasmic proteins treated at various pH conditions. [MS Thesis]. Corvallis, OR: Oregon State University.

Kim YS, Park JW, Choi YJ. 2003. New approaches for the effective recovery of fish proteins and their physicochemical characteristics. Fisheries Sci 69:1231-1239.

Kim YS, Yongsawatdigul J, Park JW, Thawornchinsombut S. 2004. Sarcoplasmic proteins at various pH conditions and their interaction with myofibrillar proteins. J. Food Biochem. Forth coming.

Ko W-C, Hwang M-S. 1995. Contribution of milkfish sarcoplasmic protein to the thermal gelation of myofibrillar protein. Fisheries Sci 61:75-78.

Kristinsson HG. 2002a. Conformational and functional changes of hemoglobin and myosin induced by pH: Functional role in fish quality. [PhD Dissertation] Amherst, MA: University of Massachusetts at Amherst.

Kristinsson HG. 2002b. Acid-induced unfolding of flounder hemoglobin: An evidence for a molten globular state with enhanced pro-oxidative activity. J Agric Food Chem 50:7669-7676.

Kristinsson HG, Hultin HO. 2003a. Changes in conformation and subunit assembly of cod myosin at low and high pH and after subsequent refolding. J Agric Food Chem 51:7187-7196.

Kristinsson HG, Hultin HO. 2003b. Effect of low and high pH treatment on the functional properties of cod muscle proteins. J Food Sci 68:917-922.

Lin TM, Park JW. 1996. Extraction of proteins from pacific whiting mince at various washing conditions. J Food Sci 61:432-438.

Morioka K, Shimizu Y. 1990. Contribution of sarcoplasmic proteins to gel formation of fish meat. Nippon Suisan Gakkaishi 56: 929-933.

Morita JI, Choe IS, Yamamoto K, Samejima K, Yasui T. 1987. Heat-induced gelation of myosin from leg and breast muscle of chicken. Agric Biol Chem 51(11): 2895-2900.

Nomura AA, Itoh Y, Toyoda H, Obatake A. 1995. Inhibitory effect of sarcoplasmic protein fraction of fish meat upon modori-phenomenon induced at about 40 °C by washing meat. Nippon Suisan Gakkaishi 61: 744-749.

Nowsad AA, Kanoh S, Niwa E. 1995. Setting of surimi paste in which transglutaminase is inactivated by *N*-ethylmaleimide. Fisheries Sci 62:189-191.

Nowsad AA, Katoh S, Kanoh S, Niwa E. 1995. Effect of sarcoplasmic proteins on the setting of transglutaminase-free paste. Fish. Sci. 61, 1039-1040.

Okada M. 1964. Effect of washing on the gelly forming ability of fish meat. Bulletin of the Japanese Society of Scientific Fisheries 30(3): 255-261.

Park JW. 2000. Surimi and surimi seafood. New York: Marcel Dekker Inc. P 448-453.

Pearson AM, Young RB. 1989. Muscle and meat biochemistry. Sandiego, Ca: Academic Press Inc. 444 p.

Shimizu Y, Nishioka F. 1974. Interactions between horse mackerel actomyosin and sarcoplasmic proteins during heat coagulation. Nippon Suisan Gakkaishi 40: 231-234.

Smyth AB, Smith DM, O'Neill E. 1998. Disulfide bonds influence the heat-induced gel properties of chicken breast muscle myosin. J Food Sci 63: 584-588.

Thawornchinsombut S, Park JW. 2004a. Role of pH in solubility and conformational changes of Pacific whiting muscle proteins. J Food Biochem 28:135-154.

Thawornchinsombut S, Park JW. 2004b. Role of ionic strength in biochemical properties of soluble fish proteins isolated from cryoprotected Pacific whiting mince. J. Food Biochem. Forth coming.

Thawornchinsombut S, Park JW. 2004c. Effect of ionic strength on gelation characteristics of acid- and alkali-treated pacific whiting fish protein isolates. Unpublished data.

Thawornchinsombut S, Park JW. 2004d. Frozen stability of alkali-treated fish protein isolate. Unpublished data.

Thawornchinsombut S, Park JW, Meng G, Li-Chan ECY. 2004. Raman spectroscopy determines structural changes of fish proteins recovered at alkaline pH. Unpublished data.

Undeland I, Kelleher SD, and Hultin HO. 2002. Recovery of functional proteins from herring (*Clupea harengus*) light muscle by acid or alkaline solubilization process. J Agric Food Chem 50:7371-7379.

Xiong YL. 1997. Structure-function relationships of muscle proteins. In: Damodaran S, Paraf A, editors. Food proteins and their applications. New York: Marcel Dekker, Inc. p 341-392.

Xiong YL, Blanchard, SP. 1994. Myofibrillar proteins gelation: viscoelastic changes related to heating procedures. J Food Sci 59: 734-738.

Yongsawatdigul J, Park JW. 2004. Effects of alkaline and acid solubilization on gelation characteristics of Rockfish muscle proteins. J Food Sci. To be submitted.