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Title: Physicochemical Characteristics of Fish Myofibrillar and Sarcoplasmic Proteins Treated at Various pH Conditions.

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Protein solubility of Pacific whiting muscle with isoelectric point at pH 5.5was significantly affected by pH. The highest breaking force was measured from fish proteins treated at pH 11, while high deformation values were obtained at pH 2 and 11. Texture of gels made using the conventional method were quite inferior to gels made using fish proteins treated at pH 2 or 11, while color of conventional gels was significantly better than the other treatments. SDS-PAGE revealed that fish proteins were highly denatured during acid or alkali treatment. High cathepsin B-like activity was detected from acid-aided fish proteins. Strong cathepsin L-like activity was found in fish proteins treated at pH 10.5, which corresponded with the lower breaking force and deformation obtained from those samples. Disulfide bonds

contributed to high texture value in fish proteins treated at pH 11.

Physicochemical characteristics of sarcoplasmic proteins (SP) from rockfish and their interaction with Alaska pollock surimi (myofibrillar proteins) were investigated. Solubility of SP was significantly suppressed at acidic pH (2-4) plus high salt concentration (0.5 M NaCl). This was also supported by SDS-PAGE results (extensively degraded SP). DSC results revealed SP gave three endothermic transitions. The least amount of proteins was lost when treated at pH 2 or 3 followed by precipitation at pH 5.5. SP did not enhance the gelation properties of myofibrillar proteins, but positively contributed to gelation with myofibrillar proteins when compared to sucrose. Myofibrillar proteins were primary components contributing to heat-induced gelation.

Salt effect on acid- or alkali-treated surimi gel was investigated. Good gels were obtained without salt using acid- and alkali-treated fish proteins. Their texture properties decreased as NaCl content increased, unlike conventional surimi gels. Consequently, NaCl did not solubilize myofibrillar proteins once the fish proteins were treated by acid or alkali. Solubility was apparently not a key factor for the texture properties of acid- or alkali-treated surimi. Transglutaminase-mediated setting reaction was partially inactivated during acid or alkaline treatment. Acid-treated surimi gel gave the best color properties.

Physicochemical Characteristics of Fish Myofibrillar and Sarcoplasmic Proteins Treated at Various pH Conditions

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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Young S. Kim, Author

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CONTRIBUTION OF AUTHORS

Dr. Yeung J. Choi assisted with data collection and review. Biochemical analysis (SDS-PAGE and enzyme activity measurement), involved with the experimental design of Chapter 2 was guided by Dr. Choi's advice.

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INTRODUCTION

INTRODUCTION

In conventional surimi processing, repeated washing and dewatering are required to concentrate myofibrillar proteins. Consequently, relatively lower protein recovery and large quantities of wastewater are environmental and economic challenges. New surimi processing, such as the acid- or alkali-aided methods, demonstrates a high potential to provide good protein recovery and acceptable functional properties. Unlike conventional processing, these new methods utilize protein charges and isolate the protein by shifting the pH followed by centrifuging. As a result, water-soluble materials, such as sarcoplasmic proteins, are retained in the final products. Many factors are important with respect to acid- or alkali-aided surimi, for example, protein solubility, denaturation induced by pH or heat, gelation mechanism, chemical bonding, and roles of the sarcoplasmic proteins. These are reviewed and discussed in the following literature review.

SOLUBILITY

Solubility is the most important functional property of proteins in foods. Solubility is usually the first functional property investigated. Solubility can be used as an index of protein denaturation that may occur during extraction, processing or storage. However, many researchers use protein solubility as a second category of tests under the thermodynamic index of protein structure (Christen and Smith 2000).

Solubility is the result of the surface active properties of the proteins (Hall 1996). Proteins insoluble in water tend to have more hydrophobic groups and fewer charged or polar groups on the surface (Christen and Smith 2000). The solubility of a protein is, therefore, fundamentally related to the average hydrophobicity of the amino acid residues and the charge frequency (Fennema 1996). The proportion and distribution of surface hydrophilic and hydrophobic patches are, consequently, the main factors in determining the degree of solubility of the protein, rather than total hydrophobicity and charge density based on amino acid composition. The surface characteristics of a protein, however, are greatly affected by the environmental conditions around the protein, which influence the interrelations among physical forces (Hall 1996). Such environmental factors are pH, ionic strength, temperature, solvent component and other food components.

Protein solubility in aqueous solution is dependent on pH (Demodaran and Paraf 1997). The isoelectric point is the pH at which a protein has zero net charge in

solution (Christen and Smith 2000). For most of proteins, minimum solubility occurs at the isoelectric pH (Demodaran and Paraf 1997). At pH above or below the isoelectric point, the proteins acquire increasing net negative or positive charges. These net charges provide more binding sites for water and cause repulsion among the protein molecules to increase their surface for hydration, thus increasing protein solubility (Lin and Park 1998). However, some proteins (e.g., whey proteins) are highly soluble at their isoelectric pH. This is primarily because the exposed surfaces of these proteins contain a high ratio of hydrophilic to hydrophobic groups. Although these proteins are electrically neutral at their isoelectric pH, they contain a large number of charged and uncharged hydrophilic residues on the surface, and hydration of these polar residues creates hydration repulsion forces great enough to offset aggregation via hydrophobic interactions (Demodran and Paraf 1997).

Protein solubility is also a function of the concentration of dissolved salts in an aqueous solution (Christen and Smith 2000). Proteins generally first show an increase in solubility (salting-in) with increasing salt concentrations. This is followed by decreased solubility (salting-out) upon further addition of salt (Hultin and others 1995). Salting out might be explained as thus: When numerous salt ions are in solution, they must be neutralized or shielded from other charges. This is done by the water molecules. However, if enough water molecules are tied up with the salts, there are not enough water molecules left for the proteins. The protein molecules consequently interact with themselves and this leads to precipitation (Regenstein and Regenstein 1984). Salting-in is generally independent of ion type.

Salting-out, on the other hand, is strongly dependent on the salt that is used (Hultin and others 1995).

Other parameters that influence the degree of protein solubility are physical, chemical, and thermal treatments during processing, method of isolation, interaction with other food components, and mechanical treatments prior to solubility testing (Hall 1996). In addition, many different terms are used to designate protein solubility. Solubility tests are empirical and vary with the test conditions selected. In general, a protein is dispersed in water or buffer, the pH is adjusted, and the dispersion is centrifuged at a certain force for a specific time. Protein in the supernatant is quantified, usually by the Kjeldahl procedure or by a colorimetric assay (Christen and Smith 2000).

Myosin is usually extracted from comminuted meat with solutions of salt (about 0.5 M) and phosphate buffer (about pH 7). A higher concentration of salt is required to dissolve actomyosin than to dissolve myosin at pH values above the isoelectric point (e.g. at pH 6, 0.3 M salt for myosin and 0.6 M for actomyosin (Harris 1990). However, in surimi processing, myofibrillar proteins can be relatively soluble and are lost during extensive washing (Lin and Park 1996).

The loss of myofibrillar proteins during surimi processing could be due in part to the nature of their water solubility (Lin and Park 1996). Wu and others (1991) showed that the protein solubility of various minced washed fish samples was the highest when no salt was added. Stefansson and Hultin (1994) reported that essentially all of the proteins of cod muscle became soluble if the ionic strength was

sufficiently reduced, approaching near zero. A possible explanation for this phenomenon, which is consistently confirmed with data on the effects of ionic strength and pH, is the myofibrillar proteins have negative charges at neutral pH. In water, or solutions of very low ionic strength, the repulsive forces from these negatively charged side chains are sufficient to drive the individual protein molecules apart when sufficient water is available. The myofibrillar proteins of chicken breast muscle are also soluble at low salt concentrations and neutral pH if certain proteins (i.e., titin, C-protein, etc), which appear to inhibit the water solubility of the majority of the myofibrillar proteins, are first extracted (Dagher and others 2000). Unlike the situation with cod muscle proteins, simply reducing the ionic strength is not sufficient to allow the chicken breast muscle myofibrillar/cytoskeletal proteins to be solubilized in water. There are a set of inhibitory proteins that must be removed by selective solubilization before the other myofibrillar proteins become soluble in water (Chang and others 2001a).

The insolubility of proteins, due to the formation of insoluble aggregates decreased as their hydrophobicity increased. However, hydrophobicity is not the only structural factor that determines the functional behavior of a protein. There is a tendency for solubility to decrease with an increase in the number of free SH groups (Wagner and Anon 2000). Lin and Park (1998) also reported that the increased solubility correlated with the increased surface hydrophobicity and relative sulfhydryl content as well as the decreased α-helicity.

During frozen storage, in gadoids, protein solubility normally decreases.

Protein insolubilization is therefore thought to be caused by formaldehyde (HCHO)-mediated crosslinking of proteins. Protein crosslinking during frozen storage, consequently, could occur for sarcoplasmic and contractile proteins (Owusu-Ansah and Hultin 1992).

DENATURATION

Denaturation of proteins has been defined as a process in which the spatial arrangement of polypeptide chains within the molecule is changed from that typical of the native protein to a more disordered arrangement (Damodoran and Paraf 1997). A protein can be partially unfolded or denatured by environmental conditions such a very high or low pH, high salt concentrations, organic solvents, or temperature (Christen and Smith 2000). Subtle changes in structure, which do not drastically alter the molecular architecture of the protein, are usually regarded as conformational adaptability, whereas major changes in the secondary, tertiary, and quaternary structures without cleavage of backbone peptide bonds are regarded as denaturation (Fennema 1996). For most proteins, once a protein molecule begins to unfold with a further slight increase in denaturant concentration or temperature, or once a few interactions in the protein are broken, the whole molecule completely unfolds. This cooperative nature of unfolding suggests that globular proteins can exist only in the native or denatured state; that is, intermediate states are not possible (Fennema

1996).

Denatured protein molecules tend to aggregate and precipitate, and thus become insoluble (Christen and Smith 2000). Denaturation therefore is a pre-requisite for protein aggregation (i.e., gelations and coagulations). When the rate of protein aggregation is slow to cause denaturation, heat-denatured proteins are allowed to align in an ordered fashion to form a fine gel network, resulting in more elastic gels. However, in the case of Pacific whiting, because of high endogenous proteinases activities, slow heating rate can dismantle myofibrillar proteins resulting in lower gel texture (Yongsawatdigul and Park 1999).

Muscle pH affects not only the denaturation rate at high temperature but also the denaturation rate during frozen storage. It is apparent that at pH less than 6.5 the myofibrillar proteins are unstable and rapidly lose their ATPase activity, which is an indicator of gel forming ability (MacDonald and others 2000). Denaturation implicates damages to functionality and is usually measured as a loss of solubility (Wagner and Anon 1990). Viscosity has also been used to determine the degree of protein denaturation (Cofrades and others 1993).

Protein denaturation is accompanied by enthalpic changes that can be monitored by thermoanalytical techniques such as differential scanning calorimetry (Berli 1999). Denaturation temperature represents protein thermostability (Puppo and Anon 1999). Park and Lanier (1989) showed that addition of salt shifted the denaturation transitions to lower temperatures and decreased enthalpies of heat denaturation. These results suggested that addition of salt might cause a partial

unfolding of proteins that increases sensitivity to heat denaturation. Prolonged frozen storage can cause changes in the functional properties of muscle protein due to denaturation and/or aggregation of myofibrillar proteins. Cryoprotective compounds have therefore been used to prevent fish protein denaturation during frozen storage (Chang and Regenstein 1997).

PROTEIN GELATION

Protein gelation refers to the transformation of a protein from the sol state to a gel-like state. This transformation is facilitated by heat, enzymes, or divalent cations under appropriate conditions (Fennema 1996). Two of the most important factors that affect the gelation of myofibrillar proteins are salt concentration and pH (Feng and Hultin 2001).

Heat treatments of proteins weaken the bonds that maintain their secondary and tertiary molecular structures. As thermal denaturation occurs, hydrophobic areas of the protein molecules that are buried in the native conformation become exposed to the solvent. This molecular change generates an aggregation process of the partially unfolded protein molecules as a consequence of the imbalance between attractive and repulsive forces of particles (Berli and others 1999). Partial unfolding of the protein structure is accelerated by an increase in temperature, resulting in the aggregation of the unfolded regions between protein molecules to form a three

dimensional network (Visessanguan and others 2000). The interactions involved in network formation are primarily hydrogen bonds, hydrophobic and electrostatic interactions, and covalent bonds (Fennema 1996). The same protein may form a gel that appears either turbid or transparent, depending on the environmental conditions, and also exhibit different textural and water-holding capacity. Turbid protein gels are formed when charge repulsion between the molecules is low. Transparent gels are formed when the net charge is large and when a high degree of charge repulsion exist between molecules (Christen and Smith 2000).

At low ionic strength (<0.3 M), in the neutral pH range, myosin molecules assemble and form a filament, which possesses a structure similar to the thick filament observed *in vivo*. When the ionic strength is raised above 0.3 M, myosin molecules disperse individually and exist as monomers. It is widely agreed that solubilization of the myofibrillar proteins is an important feature of muscle protein gelation at salt concentrations equal to or greater than an ionic strength of 0.3 M. However, it has also been demonstrated that good fish gels can be made with relatively low salt concentrations (Chang and others 2001b). Consequently, good gels can be formed under conditions where myosin is not soluble, but the protein-containing structures must be disorganized or converted to a state in which the proteins can be solubilized by a proper solvent (Chang and others 2001a).

Since gel formation of muscle proteins results from interactions among the protein molecules, the changes in state of the myosin molecules must markedly affect the thermal gelation process (Sano and others 1990a). Thermal gelation of

myosin in the 30-45°C range is attributable to the tail portion of the molecule and gelation in the temperature range above 50°C is attributable to the head portion of the molecule (Sano and others 1990b).

CHEMICAL BONDING

It is believed that hydrogen bonds, hydrophobic interactions, disulfide bonds, and covalent bonds, other than disulfide, intervene differently in the formation of the gel network depending on the various parameters involved (Careche and others 1995). Hydrogen bonds are weaker dipole bonds that are not responsible for the gelation of myofiblillar proteins but are important in the stabilization of bound water within the hydrogel (Lanier 2000). Hydrogen and other polar bonds in fish gels have been reported to destabilize at high temperatures but stabilize at low temperatures (Park and others 1994). The role of hydrogen bonds in surimi gels is clearly supported by the fact that gel texture can increase as refrigeration storage is extended (Kim and Park 2000). The extent of their relative contribution of the various types of bonds to the overall aggregation and gelation process, though, is unclear and depends on experimental conditions, such as pH and salt concentration (Hoffmann and Mil 1997).

Hydrophobic interactions, in contrast, occur when nonpolar molecules are introduced into the polar environment of water (Park and others 1994).

Hydrophobic interactions and disulfide bond formation between protein molecules are primarily involved in the aggregation (Handa and others 2001). The intimate relationship of solubility and surface hydrophobicity reinforces the importance of hydrophobic interactions in aggregation-insolubilization (Wagner and others 2000). The increase in surface hydrophobicity of actomyosin during frozen storage might be due to the exposure of hydrophobic amino acids in the myosin molecules. Moreover, the presence of the cryoprotectants appeared to suppress the exposure of hydrophobic amino acids during frozen storage (Sompongse and others 1996a).

Covalent cross-linking of protein molecules can be brought by SH oxidation into S-S bonds and/or by SH-induced S-S interchange reactions (Shimada and Cheftel 1989). Sulfhydryl groups are considered to be the most reactive functional group in proteins, being easily oxidized to disulfide groups (Sultanbawa and Li-Chan 2001). Thiol oxidation reactions between exposed –SH or S– groups can occur under alkaline conditions. Thiol oxidation reactions occur in addition to disulfide interchange reactions at the higher pH values (Monohan and others 1995). The contribution of noncovalent interactions becomes increasingly important as pH values reach the isoelectric point and/or with higher salt concentrations (Hoffmann and Mil 1997). The disulfide bonds, as well as the formation of MHC dimmers, occur even in the presence of cryoprotectants during frozen storage, where no significant changes of actomyosin conformation such as Ca^{2+}-ATPase activity and surface hydrophobicity are observed (Sompongse and others 1996b). Potassium bromate induces disulfide crosslinking of proteins and EDC

[1-ethyl-3(3-dimethylaminopropyl)carbodiimide] induces crosslinking between amino and carboxyl groups (Lee and others 1997). The formation of disulfide bonds is key to the mechanism determining fracture strain (Errington and Foegeding 1998).

Low temperature setting (suwari) is very important in production of surimi-based products and affects the final textural strength and elasticity of fish muscle gels (Chan and others 1995). The optimum temperature for setting among species varied depending on the thermal stability of the myosin (Kamath and others 1992). The setting phenomenon of surimi has been attributed to the transglutaminase catalyzed cross-linking of the myosin heavy chains (Kim and others 1993). The setting period allows conformational changes in protein molecules, which may involve localized exposure and subsequent interaction of hydrophobic amino acid residues, resulting in the formation of a more elastic gel (Park and others 1996).

The rate of transglutaminase-mediated cross-linking of MHC may be primarily dependent on the conformation of the substrate myosin at a given temperature rather than by the optimum temperature of the transglutaminase (Kamath and others 1992). Transglutaminase-mediated setting reaction in surimi was constrained more by the conformation of the substrate (i.e., myosin) than by that of the enzyme (Joseph and others 1994). The extent of covalent cross-linking in surimi sols affected by transglutaminase can be manipulated in several ways, such as addition of EDTA, variation of preincubation time and temperature, or addition of microbial

transglutaminase (Lee and others 1997). The endogenous transglutaminase requires Ca^{2+} to be effective and thus, can be inhibited with EDTA (Gulleland and others 1997). However, microbial transglutaminase made by Ajinomoto (Tokyo, Japan) is calcium independent (Lanier 2000). Cross-linking reactions mediated by transglutaminase lead to the formation of protein intra- and inter-molecular covalent bonds that are about twenty times stronger than hydrogen and hydrophobic interactions (Joseph 1994). Nowsad and others (1993) stated that cross-linked myosin heavy chain in suwari gel was partly formed by the aggregation of MHC through weak bonds such as hydrogen and hydrophobic bonds.

SARCOPLASMIC PROTEINS

The myofibrils of muscle cells are surrounded by and bathed in the sarcoplasm or intracellular fluid (Pearson and Young 1989). The sarcoplasmic protein pool consists of a large number of soluble proteins, most of which are enzymes and heme proteins, and individually do not warrant attention in a food context (Harris 1990). They consist of an extremely complex mixture containing over 1,000 different protein components that are involved in such diverse functions as protein synthesis and degradation, fatty acid oxidation, electron transport, phosphorylation, glycolysis, glycogenesis, and glycogenolysis among others (Pearson and Young 1989). However, major sarcoplasmic proteins of fish are glycolytic enzymes, which are the

same as for mammals (Nakagawa and others 1988).

Sarcoplasmic proteins include the heme proteins, which are responsible for the pigmentation of unleached mince. The heme proteins of blood and red muscle cells are hemoglobin and myoglobin, respectively. It is very difficult to leach all the myoglobin from dark, red muscle because it is deposited within the muscle cells. In contrast, hemoglobin, which is contained in the free-floating red blood cells of the bloodstream, is more easily removed when fish is processed in a relatively short period after harvesting (Lanier 2000). The content of myoglobin varies with muscle type within species, according to muscle function, and across species (Harris 1990).

All fish also contain trimethylamine oxide (TMAO), a water soluble nitrogenous compound used by fish for osmoregulation. TMAO demethylase is an enzyme that is especially prevalent in gadoid species, which degrades TMAO to formaldehyde and dimethylamine during frozen storage. Formaldehyde is a strong protein denaturant and thus, the gelling properties of surimi or minced fish can deteriorate rapidly if this enzyme system is actively present at a sufficient concentration. In addition, most fish also possess heat-stable proteolytic enzymes, such as cathepsin. This enzyme binds to the myofibrillar proteins, and in some cases the protease may be closely associated with the myofibrils (Lanier 2000).

Total sarcoplasmic content also depends on fish species. For example, sardine and mackerel, dark muscle fish, contain 35-40 %, and pollock, white muscle fish, contains 20-25 % sarcoplasmic proteins, respectively (Okada 1999). The sarcoplasmic proteins can be extracted with water or by 0.1μM NaCl solutions in a

pH range of 6.7-7.5 (Pearson and Young 1989). Ordinary fish muscle is generally rich in three sarcoplasmic proteins, with their molecular weight at 43 kDa, 40 kDa, and 35 kDa, respectively (Nakgawa and others 1988).

Many sarcoplasmic proteins are globular and would be expected to bind or trap more water upon denaturation. Sarcoplasmic proteins, consequently, do influence water holding capacity (Willson and Laack 1999). In addition, removal of sarcoplasmic proteins facilitated freeze-induced contraction of myofibrils, leading to textural hardening. Sarcoplasmic proteins retard sarcomere shrinkage resulting from freeze-induced contraction/protein cross-linking (Yoon and others 1991). Investigations using DSC indicate that the majority of sarcoplasmic proteins undergo thermal denaturation at temperatures between those of myosin and actin (Harris 1990).

In surimi processing, extensive washing is utilized to remove water soluble substances, mainly sarcoplasmic proteins (Lin and Park 1996). It was generally believed that the washing process is also good, not only for color and flavor, but for increasing the gel forming ability of fish meat (Okada 1964). However, the conventional surimi processing increases costs and causes water pollution (Okada 1999). Okada (1964) concluded that the improving effect of washing on the gel forming ability of fish meat is attributable to: 1) higher concentration of myosins, and 2) removal of water soluble proteins that are supposed to inhibit myosins from forming a gel network structure. Shimizu and Nishioka (1974) assumed that during heat denaturation, sarcoplasmic proteins bind to actomyosin and, as a result,

coagulation was observed.

Sarcoplasmic proteins from pelagic fish are not easily extracted at ionic strengths below 0.1, and are heat coagulable, whereas these proteins aggregated with actomyosin during heat denaturation at high ionic strength. At low ionic strengths sarcoplasmic proteins precipitated with myofibrillar proteins. In addition, enzymes remaining in washed muscle, especially of pelagic fish, may have an inhibitory effect on the gel values (Nakagawa and others 1989). Consequently, the lower gelling properties of surimi were related to the residual aldolase activity, a glycolytic enzyme (Nakagawa and others 1989).

Morioka and Shimizu (1990) reported that sarcoplasmic proteins do not interfere with gel formation of myofibrillar proteins, but positively contribute to it. Every sarcoplasmic component contributes to aggregation of actomyosin and supports gel formation (Morioka and Shimzu 1992). With an increased concentration of sarcoplasmic proteins the puncture strength of the gel progressively increased, while deformation of the gel changed little (Morioka and others 1992).

Morioka and others (1992) also reported that the strength of contribution of sarcoplasmic proteins to gelation depends on fish species. Morioka and Shimizu (1993) reported the high strength of sarcoplasmic gel is based mainly on a large amount of heat-coagulable proteins, especially those 94 kDa, 40 kDa, and 26 kDa components of sarcoplasmic proteins. Alaska pollock surimi was weakened by repeated washing, but was strengthened again by reincorporating the washwater. This dichotomy was resolved by finding the existence of transglutaminase (TGase)

in the sarcoplasmic protein (Nowsad and others 1995). TGase is an enzyme that catalyzes the polymerization and cross-linking of proteins through the formation of covalent bonds between protein molecules. This endogenous TGase in fish initiates setting and enhances gel strength (Lanior 2000).

Ko and Hwang (1995) also proceeded to state that the addition of sarcoplasmic proteins improves thermal gelation, while having a promotive effect on suwari and a restrictive effect on modori. They suggested that from the standpoint of thermal gelation and water pollution, sarcoplasmic proteins removal is not necessary. The washing process is wasteful in terms of recovery yield, because the water soluble proteins, i.e. sarcoplasmic proteins, free amino acids, etc. are lost (Morioka and others 1998). A high gel strength of myofibrillar proteins added with sarcoplasmic proteins is due to a large amount of the heat-coagulable proteins in the sarcoplasmic proteins (Morioka and others 1997). In addition, they showed the gel strength of myofibrillar proteins added with sarcoplasmic proteins is related to the composition of the sarcoplasmic proteins. The composition and content of the sarcoplasmic proteins, however, differ among fish species. Morioka and others (1998) stated that it is therefore advisable to reduce the number of times of washing and leave sarcoplasmic proteins to some extent for better gel strength and better protein recovery.

NEW APPROACHES FOR THE EFFECTIVE RECOVERY OF FISH PROTEINS (SURIMI) AND THEIR PHYSICOCHEMICAL CHARACTERISTICS

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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. Gel texture of surimi made using the conventional method were inferior to gels made using fish proteins treated at pH 2 or 11, while color of conventional gels was significantly better than the other test samples. SDS-PAGE revealed that fish proteins were highly denatured by acid or alkali treatment. High cathepsin B-like activity 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 word: Fish protein; pH; surimi; acid-aided processing; alkali-aided processing

INTRODUCTION

Conventional surimi processing from white flesh fish, such as Pacific whiting and Alaska pollock, typically utilizes 25-28% of total body weight. Conventional surimi is refined myofibrillar proteins processed by removing unnecessary foreign 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%) (Hultin 1999). 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, utilizing 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, with regards to yield, is that in the acid-aided process sarcoplasmic proteins (hemoglobulin, myoglobulin, and proteolytic enzymes) and other proteinous materials are not removed.

However, the acid-aided process was also applied to Pacific whiting

activation of cathepsin L enzymes, which are responsible for gel softening, was found (Choi and Park 2002). In contrast, 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 with our previous study, the application of alkaline pH for the solubilization of Pacific whiting muscle proteins was therefore needed. Since fish proteins treated at extremely low and high pH for acid- or alkali-aided applications, respectively, are likely to be denatured, our study focused on characterizing the biochemical and physical properties of fish proteins and comparing them to the conventional process.

MATERIALS AND METHODS

Materials

Pacific whiting (*Merluccius productus*) was obtained from Pacific Surimi JV (Warrenton, OR.). Fresh fillets were soaked in 70% sorbitol solution and stored in the freezer (-80°C). These samples were thawed, rinsed with refrigerated deionized water twice, and used for the various experiments.

Protein solubility

Protein solubility was measured by mixing 6 g of minced meat and 300 mL of refrigerated, deionized, distilled water in a homogenizer at setting 3 for 1 min (Power Gen 700, GLH 115, Fisher Scientific Inc., Pittsburgh, PA). Thirty mL of the homogenate, after measuring the protein content, were placed in centrifuge tubes and then the pH was adjusted to various points ranging from pH 1.5 – pH 12.0 using 0.2 N and 1 N HCl or NaOH solutions, respectively. The amount of HCl/NaOH solution used was recorded. The sample solutions were centrifuged at 27,000 x 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/mL) in the supernatant after pH adjustment. The Bradford dye binding method was used for protein determination (Bradford 1976).

Sample preparation

Based on the solubility study at various pH, rinsed fillets were subjected to conventional, acid-aided (pH 2 and 3), or alkali-aided (pH 10.5, 11, and 12) processing methods. For the conventional method, the fillets were mixed with distilled water at a 1 to 3 ratio. Three-cycle washing was applied but final washing was conducted using 0.3 % NaCl solution to facilitate easy dewatering. The

homogenate was centrifuged at 4,000 ×g for 20 min for each washing step. For acid- or alkali-aided, 2 N HCl or 2 N NaOH, was used to first solubilize the fish proteins after mixing the fillets with distilled water at a 1 to 9 ratio. Acidic or alkaline homogenates were centrifuged at 8,000 × 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 (4,000 ×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 2N NaOH. Temperature was maintained below 5°C. Fish protein samples were vacuum-packed and stored at –30°C until used.

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, containing 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 fluoresce intensity (RFI) of ANS-protein was measured with a spectrophotofluorometer (LS50B, Perkin Elmer, Norwalk, CT), 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 (SH) content

The SH group exposed on the surface of actomyosin protein molecule was measured as the reactive SH group (R-SH) (Riddles and others 1979). 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/mole/cm. The total SH content (T-SH) was determined by the method of Choi and Park (2002) with slight modifications. Actomyosin solution (0.25 mL) was mixed with 2.5 mL of 8M urea, 2% SDS, and 10 mM EDTA in 0.2 M tris-HCl buffer (pH 7.0). The sample solution was then mixed with 50 μL of Ellman's reagent and subsequently set in a water bath (40°C) for 15 min. The T-SH

was spectrophotometrically determined at 420 nm (Beckman DU640, Beckman Instruments Inc., Washington, DC).

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, respectively (Barret and Kirschke 1981). After centrifugation (10,000 × g, 30 min), the supernatant (400 μL) was diluted to 2 mL with 0.1% (w/v) Brij 35 and pre-incubated 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 μmole of aminomethylcoumarine within 1 min.

Gel preparation and analysis

Frozen surimi was partially thawed, comminuted with 1.5% beef plasma protein as an enzyme inhibitor, 2% salt, and enough water to maintain 78% moisture content. The communition was conducted at 5 °C for 6 min (with vacuum for the final 3 min). A small amount (~50g) 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 were measured by the punch test using a Sintech machine (Sintech 1/G, MTS, Cary, NC) using a 5 mm spherical probe with an initial probe speed of 6.0 cm/min after gels were equilibrated to room temperature. Breaking force (g) and deformation (mm) were recorded. A CIE Lab color scale was used to measure the degree of lightness (L*), redness or greenness (+/-a*), and yellowness or blueness (+/-b*) of the gels using a colorimeter (Model CR-300, Minolta, Japan). Whiteness was calculated as the whiteness index L*-3b* (Park 1994).

Oscillatory dynamic test

Non-destructive gelation properties of the three fish protein samples (conventional, acid-aided, and alkaline-aided) were evaluated using a cone and plate attached to a mechanical dynamic tester (CS-50 Rheometer, Bohlin Instruments, Inc.,

East Brunswick, NJ). Storage modulus (G') was measured at the test temperature ranges (10-90°C) and 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.

Electrophoresis (SDS-PAGE)

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 using SDS solution. The homogenates were incubated in an 85°C water bath for 30 min and centrifuged at 3,000 x g for 15 min. The protein concentration of the supernatant was determined by the Lowry method (Lowry and others 1951). SDS-PAGE was performed using 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 E. 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 glyceraldehydes-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 ($p \leq 0.05$) between treatment means. This was done using S-Plus 2000 Professional Release 3 (MathSoft Inc., Seattle, WA).

RESULTS AND DISCUSSION

Effect of pH on protein solubility

Solubility of Pacific whiting (PW) protein was the lowest at pH 5.5. A dramatic 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 our study, pH 5.5 was the isoelectric point where proteins have zero net charge in solution, resulting in minimum solubility and precipitation (Christen and Smith 2000). Around pH 5.5, a large number of precipitates after centrifugation were observed due to low electrostatic repulsion between protein molecules. Once the pH was shifted to either acidic or alkaline 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 certain points of ionic strength, 0.3 to 1.0 M sodium chloride (Hultin and others 1995) or 0.1 to 0.9 M salt (Dagher and others 2000) , a 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 due to increased electrostatic repulsion and the salting-in effect. However, below pH 3, protein solubility remained unchanged. Although the electrostatic repulsive force could still have increased, the salting-out effect, due to increased ionic strength, might have inhibited the solubility.

Adjusting the pH to 2 required about three times more quantity of 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 (Fennema 1996). 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 toward 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 protein was glutamic acid followed by aspartic acid, lysine, leucine, and arginine (Seo and

others 1998). The pK values of those amino acids correlated with the protein solubility curve (Fig 2.1).

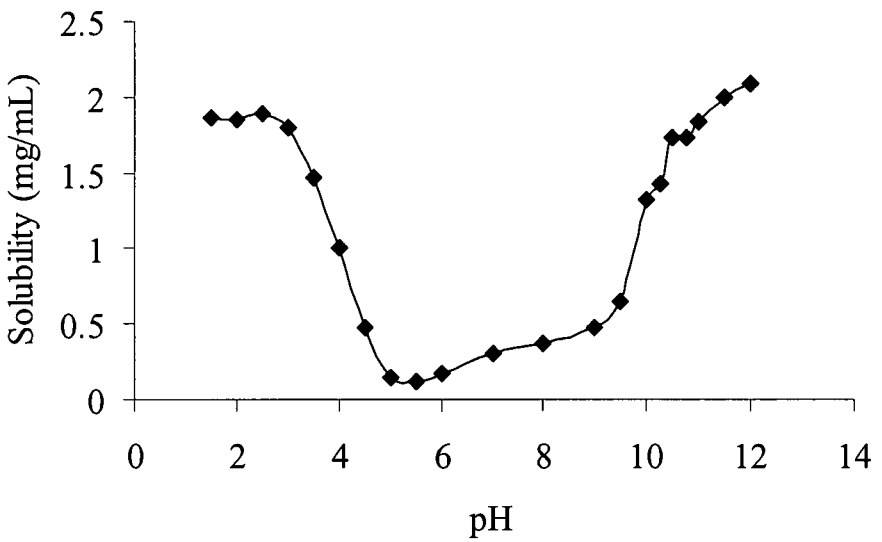

Fig 2.1 - Effect of pH on the protein solubility of Pacific whiting muscle.

Protein recovery

Protein recovery (yield) is shown in Fig 2.2. At pH 12, the highest protein recovery was obtained as a result of the highest protein solubility. According to our preliminary study, protein recovery at pH 10 was too low (no data provided). Even though the solubility at pH 10.5 was similar to that at pH 11 (Fig 2.1), the protein recovery was the lowest among all treatments, including the conventional method.

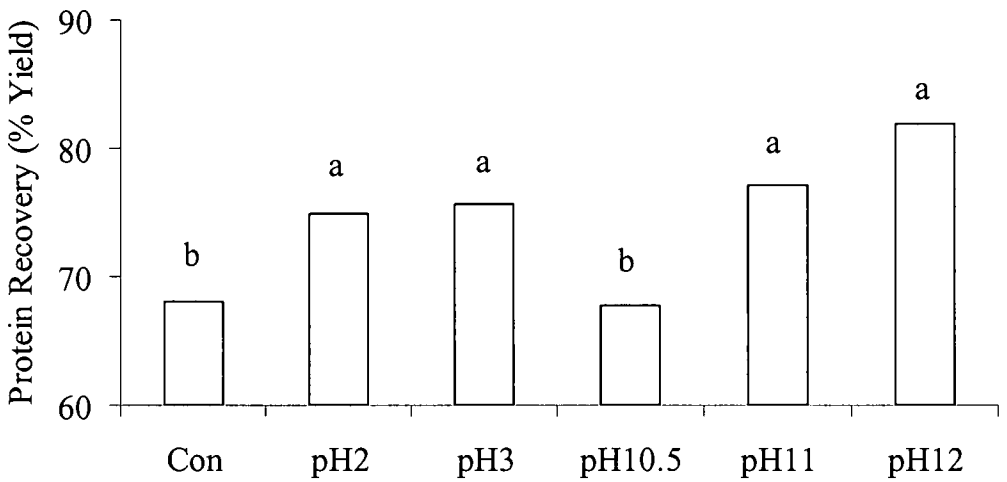

Fig 2.2 - Protein recovery (yield) at various treatments. The yield is expressed as percentage weight of recovered fish protein based on fish fillet. Con represents fish proteins prepared using conventional method. The pH 2 and pH 3 represent acid-treated fish proteins at pH 2 and pH 3, respectively. The pH 10.5, 11, and 12 represent alkali-treated fish proteins at pH 10.5, 11, and 12, respectively. All samples were prepared at 78% moisture, 2% NaCl, and 1.5 % BPP. Different alphabetical letters indicate a significant difference ($p < 0.05$).

The discrepancy at pH 10.5 could partially be due to protein extractability being affected by the mixing ratio. Fish proteins were highly soluble with a 50-fold dilution rate at pH 10.5 (Fig 2.1). However, the extracting solvent used in the alkali-aided sample (pH10.5) might have not been sufficient enough because the sample was prepared using only a 10-fold dilution factor. Dagher and others (2000) reported that at pH 9.2, maximal protein solubility was seen at dilutions by 36-fold or greater. Solubility at these dilutions was ten times the amount of solubilization that

was observed at a dilution of 1:12. In contrast, they observed that at pH 8.5 dilution factors did not significantly affect solubility. The solubility, depending on pH, is therefore dramatically affected by dilution factors.

Protein recovery from the conventional method was lower than either the acid- or alkali-aided processes, except at pH 10.5. A significant difference in yield might be due to the nature of the washing system. The conventional method washed out almost all sarcoplasmic proteins and retained most myofibrillar proteins with extensive washing, while the acid- or alkali-aided method kept both sarcoplasmic and myofibrillar proteins during the process. It is also important to understand a significant amount of myofibrillar proteins, which are generally considered as water-insoluble and salt-soluble proteins, can be washed away when washing is repeated (Lin and Park 1996; Stefansson and Hultin 1994). Therefore, the lower recovery from the conventional method is partly due to the water solubility of the myofibrillar proteins.

Hydrophobicity

Similar hydrophobicity was found among fish protein samples, except for the pH 2 and pH 12 treatments (Fig 2.3). 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 others

(2000) also confirmed that hydrophobicity values are higher at acidic pH compared to neutral or alkaline pH. Das and Kinsella (1989) reported that the highest hydrophobicity of β-lactoglobulin was measured at pH 2.8 and hydrophobicity drastically decreased as pH increased. The high surface hydrophobicity at very low acidic pH was attributable to noncovalent monomer-dimer transitions rather than to substantial changes in the protein secondary structures (Lin and Park 1998).

When fish proteins were treated at pH 12, hydrophobicity was the lowest

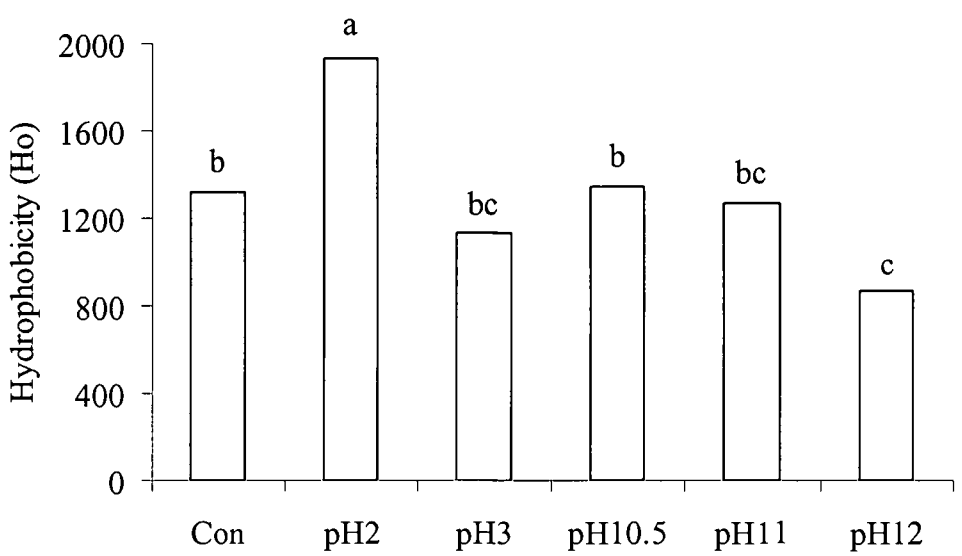

Figure 2.3- Hydrophobicity of fish proteins at various treatments. Samples were treated at various pH conditions during protein recovery, and then adjusted to pH 7.0. Ho is expressed by initial slopes of relative fluorescence intensity vs. protein concentration plot in the presence of ANS (1-anilino-8-naphthalenesulfonate). Different alphabetical letters indicate a significant difference ($p \leq 0.05$).

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 (Petruccelli and Anon 1995). According to literature information (Ishizaki and others 1993; Wagner and others 2000), the formation of aggregates decreased the surface hydrophobicity of proteins.

SH content

The total SH content of acid- or alkali-aided fish proteins was lower than that of fish proteins prepared using the conventional method (Fig 2.4). All myofibrillar proteins contain sulfhydryl groups and particularly the myosin molecule contains 42 thiol (SH) groups (Hofmann and Hamm 1978; Buttkus 1970; Smyth and others 1998). Because acid- or alkali-treated fish proteins retaine all sarcoplasmic proteins, resulting in a higher ratio of sarcoplasmic protein to myofibrillar protein compared to fish proteins of conventional method, the total SH content of fish proteins prepared using the conventional method was the highest among all samples.

Total and reactive SH concentration gradually decreased as pH increased from 10.5 to 12. This trend was probably due to the conversion of cystine and/or cysteine residues to degraded products, such as H_2S and dehydroalanine (Monahan and others

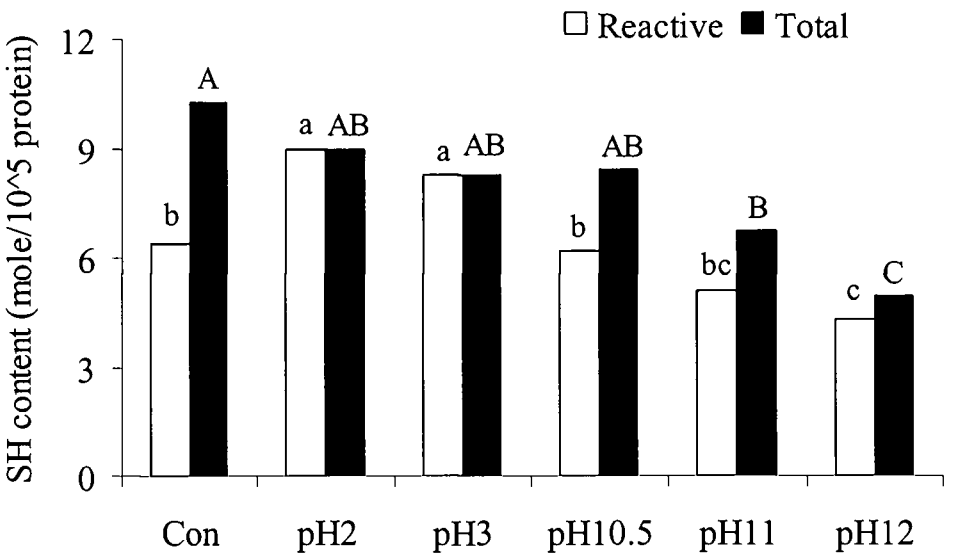

Figure 2.4 – Total and reactive SH content of fish proteins at various treatments. Samples were treated at various pH conditions during protein recovery, and then adjusted to pH 7.0. Different alphabetical letters indicate a significant difference ($p \leq 0.05$). Small letters are for reactive SH activity, while capital letters for total SH activity.

1995). Thiol oxidation reactions might have already occurred in addition to disulfide interchange reactions during alkaline treatment, resulting in reduced SH concentration.

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 (Monahan and others 1995). Disulfide interchange reactions are favored at high pH. On the other hand, SH/S-S interchange reactions are inhibited at low pH (Monahan and others 1995; Errington and Foegeding 1998). Exposure to moderately high pH followed by readjustment 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 (Damodoran and Paraf 1994). Presumably, alkali-treated fish proteins contained more intermolecular disulfide bonds than acid-treated fish proteins or fish proteins prepared using conventional method.

Cathepsin activities

Cathepsin L-like activities were found in all samples (Fig 2.5). The lowest activity was observed from fish proteins prepared using the conventional method. An and others (1994) reported that during surimi processing, washing could remove cathepsin B and H, but not cathepsin L. However, washing steps did reduce the amount of cathepsin L-like enzymes in our study. Choi and Park (2002) reported that cathepsin L activities of water soluble proteins after three washing cycles were higher than that of water soluble proteins after one washing cycle. Cathepsin L probably leached out gradually during the washing cycle. Washing, therefore, might

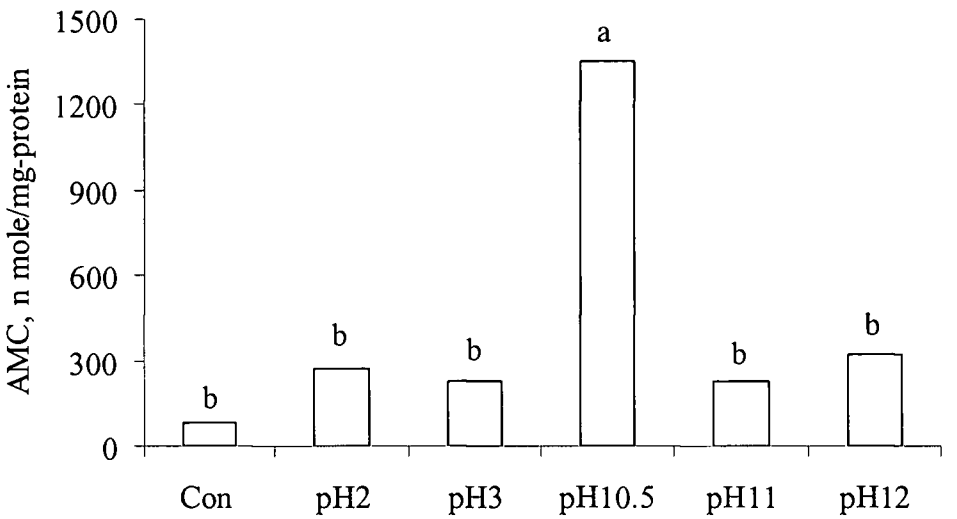

Figure 2.5 – Cathepsin L-like activities of fish proteins at various treatments. Samples were treated at various pH conditions during protein recovery, and then adjusted to pH 7.0. The measurement was conducted at pH 5.5. Different alphabetical letters indicate a significant difference ($p \leq 0.05$).

have removed a significant amount of cathepsin L-like enzymes.

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 (Lanier 2000). 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 reactivated when the pH was re-adjusted to 7.0 and further activated during testing at pH 5.5.

Cathepsin B-like enzymes appeared to be highly activated during acid

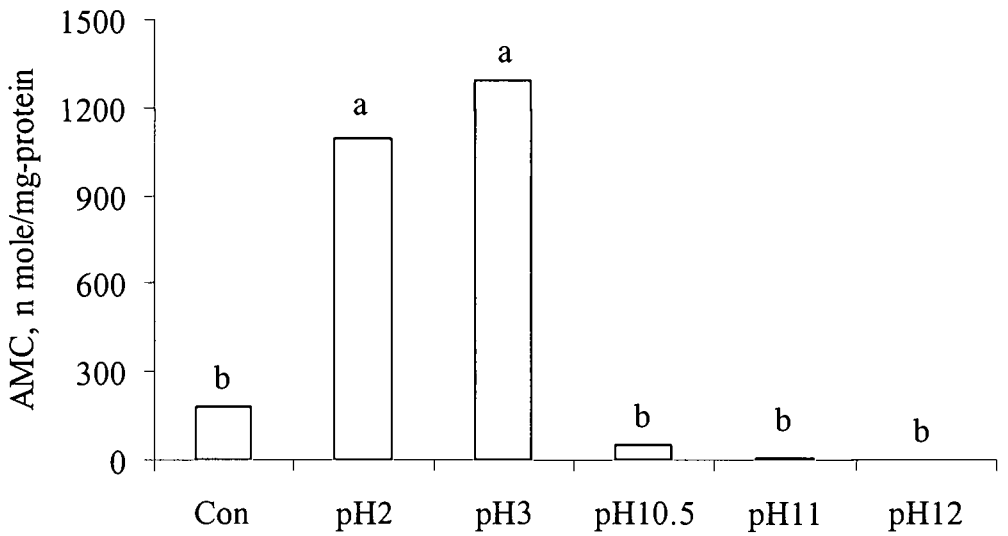

Figure 2.6 – Cathepsin B-like activities of fish proteins at various treatments. Samples were treated at various pH conditions during protein recovery, and then adjusted to pH 7.0. The measurement was conducted at pH 6.0. Different alphabetical letters indicate a significant difference ($p \leq 0.05$).

treatment (Fig 2.6). Conventional washing could not remove all cathepsin B-like enzymes. However, the alkaline process dramatically removed them. Especially at pH 12, no activities were detected. 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 (Kang and Lanier 2000). Cathepsin B-like could therefore not tolerant alkaline conditions.

Cathepsin H-like activities were not detected in any samples (no data reported). It was presumably easily washed away during conventional washing (An and others

1994). This enzyme was also 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 2.7). Fish proteins treated at pH 12 demonstrated the worst texture followed by pH 10.5. Gels at pH 12 did not show gel-like appearance. They appeared to be coagulants as indicated by low deformation value. Extensive formation of degraded products from cysteine and cystine occurred due to extremely high pH treatment, resulting in thermal coagulation (Monahan and others 1995). Presumably, pH 12 was too strong to make gel formation.

Enzyme activity, surface hydrophobicity, and surface SH showed a significant relationship with the textural properties. Between two similar acidic pHs, 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 as compared to pH 3. Kim and Park (2000) 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

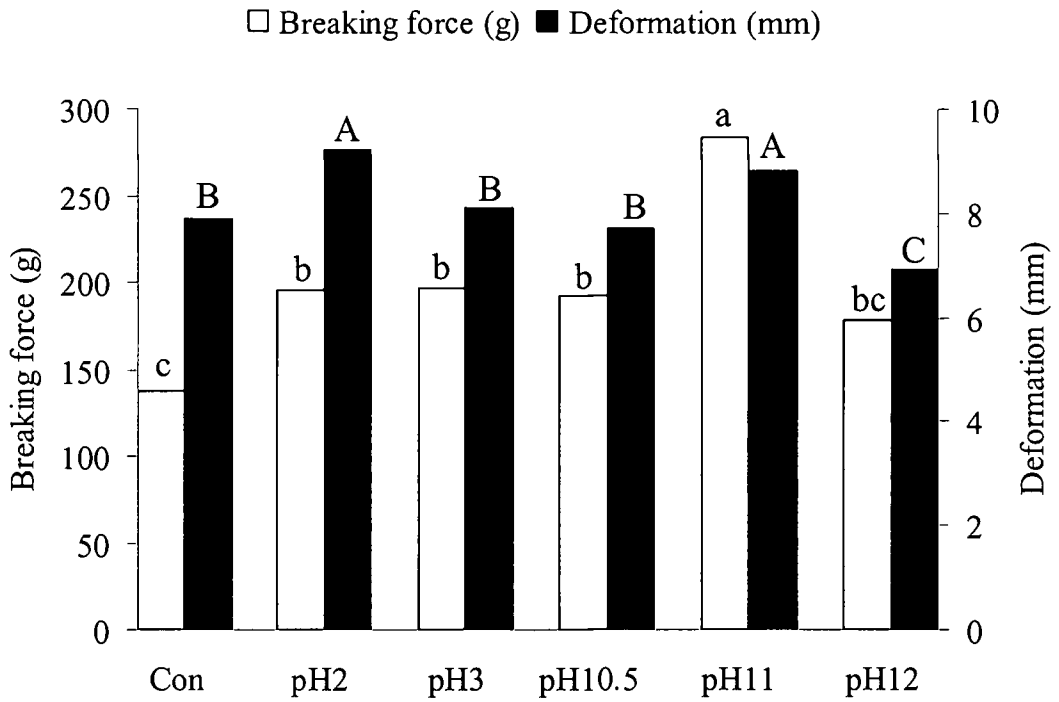

Figure 2.7 – Textural properties of fish protein gels prepared after various treatments. Samples were treated at various pH conditions during protein recovery, and then adjusted to pH 7.0. Different alphabetical letters indicate a significant difference ($p \leq 0.05$). Gels were prepared with 1.5% beef plasma protein.

disulfide bonding formation, as indicated by the reduced surface SH concentration. However, pH 10.5 treatment did not give high texture value. This was probably due to high cathepsin L-like activities (Fig 2.5), 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

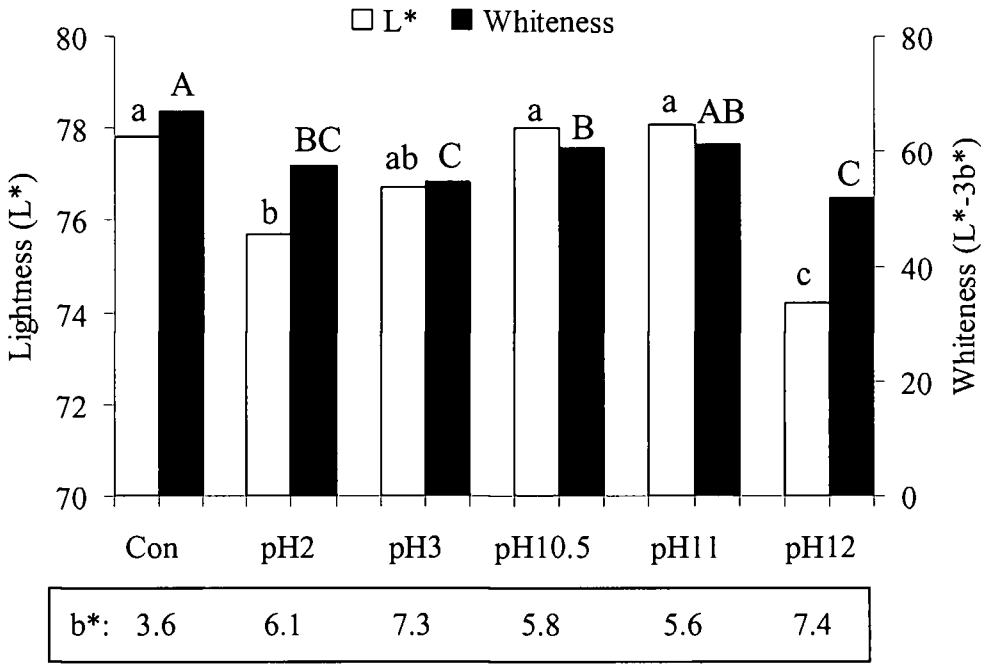

Figure 2.8 – Comparison of lightness (L*), yellowness (+b*), and whiteness (L*-3b*) at various treatments. Samples were treated at various pH conditions during protein recovery, and then adjusted to pH 7.0. Different alphabetical letters indicate a significant difference ($p \leq 0.05$). Gels were prepared with 1.5% beef plasma protein.

and gels prepared using the conventional washing method (Fig 2.8). The lowest yellow hue (b*) was obtained from gels prepared using the conventional method. Consequently, conventional gels had the highest whiteness value, while gels with pH 12 had the lowest whiteness value.

Fish protein gels were visually quite different from each other. Conventional gels were the best, gels treated at pH 10.5 and 11 were quite comparable, and gels

treated at pH 2, 3, and 12 were unacceptable. Based on the fact that acid- or alkali-treated gels contained sarcoplasmic proteins (for example, 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

Unlike fish proteins prepared using the conventional method, 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 2.9). Storage modulus, G', is a measurement of energy recovered per cycle of sinusoidal shear deformation (Yongsawatdigul and Park 1999). The patterns of slopes on acid- or alkali-aided fish proteins were very similar. However, fish proteins from the conventional method exhibited uniquely different results compared to the other methods. The curve from conventional surimi showed two valleys (around 30°C and 45°C) while the others had only one (30°C). A small drop of G' values starting at 25°C indicated the unfolding of α-helices of the myosin molecules in the tail portion (Ogawa and others 1995). When G' reached the bottom of the second valley at around 45°C, it

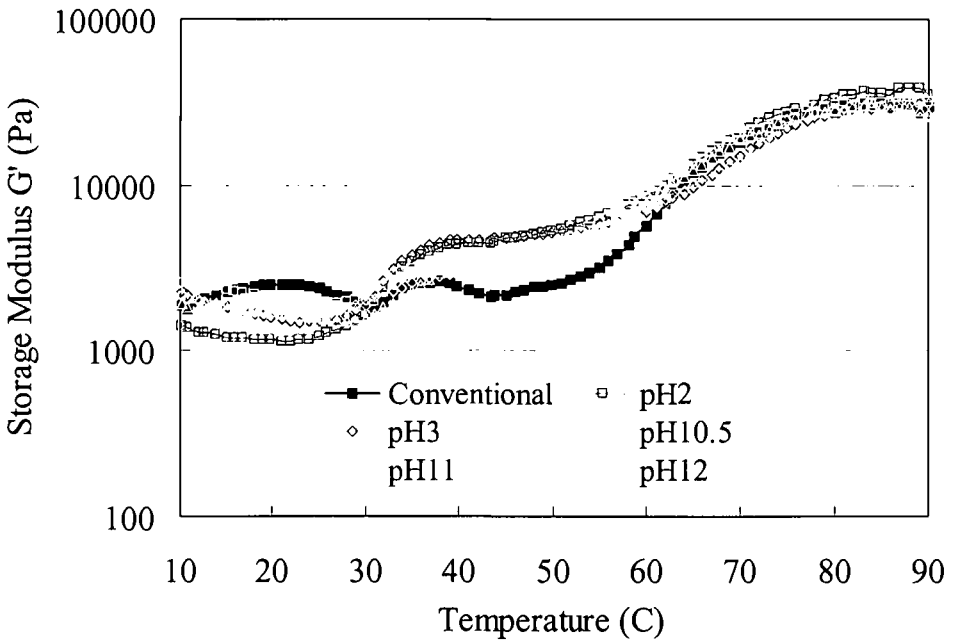

Figure 2.9 – Changes in storage modulus during linear heating of fish proteins pastes. Samples were treated at various pH conditions during protein recovery, and then adjusted to pH 7.0. Pastes were prepared with 1.5% beef plasma protein.

indicated 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 the conventional method, fish proteins-treated with acid or alkali showed no changes in G' around 45°C, but rather demonstrated 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 the gelation mechanisms of conventional and the new pH-driven methods, respectively, were quite different.

Protein patterns on SDS-PAGE

Different protein patterns were observed among fish proteins prepared using conventional, acid-, and alkali-aided methods (Fig 2.10). 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 (Choi and Park 2002). 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 Pacific whiting hydrolyzed the myosin heavy chain, resulting in fragments and/or smaller peptides (An and others 1994). 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.

CONCLUSION

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 re-natured through adjusting pH to neutral. The gelation mechanism of acid- or alkali-treated fish proteins was quite different from that of fish proteins

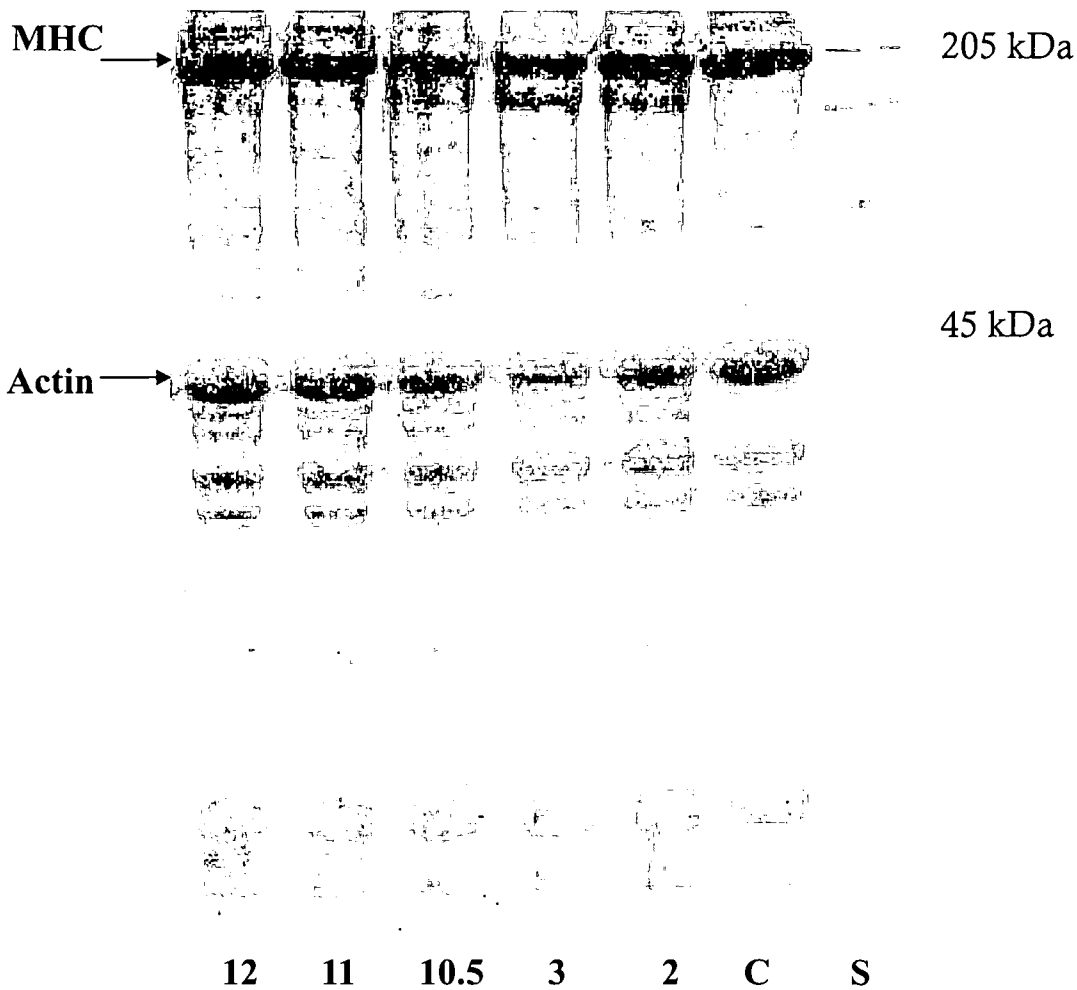

Figure 2.10 – SDS-PAGE patterns of fish proteins after various treatments. Samples were treated at various pH conditions during protein recovery, and then adjusted to pH 7.0. The number indicates pH and S denotes high molecular standard. Gels were prepared with 1.5% beef plasma protein.

prepared using the conventional method. The highest protein recovery was obtained for the alkali-aided method at pH 12. Color quality of acid- or alkali-treated fish protein gels was inferior to that of fish protein gels prepared using the conventional method. 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 bonds formation.

CHARACTERISTICS OF SARCOPLASMIC PROTIENS AT VARIOUS pH AND THEIR INTERACTION WITH MYOFIBRILLAR PROTEINS

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ABSTRACT

Physicochemical characteristics of sarcoplasmic proteins (SP) from rockfish and their interaction with Alaska pollock surimi (myofibrillar proteins) were investigated. Solubility of SP was suppressed significantly at acidic pH (2-4) plus high salt concentration (0.5 M NaCl). It was also supported by SDS-PAGE results (extensively degraded SP). DSC results revealed SP gave three endothermic transitions. The least amount of proteins was lost when treated at pH 2 or 3 followed by precipitation at pH 5.5. SP did not enhance the gelation properties of myofiblillar proteins, but positively contributed to gelation with myofiblillar proteins when compared to sucrose. Myofibrillar proteins were the primary components contributing to heat-induced gelation.

Key word: Sarcoplasmic proteins; myofibrillar proteins; gelation; pH; NaCl

INTRODUCTION

New surimi processing methods, acid- or alkali-aided, have significant advantages over conventional processing. This revolutionary approach can contribute high yields, reduce water usage, and reduce wastewater problems. The new method can provide extremely high yields (35-40%) because it solubilizes almost all myofibrillar and sarcoplasmic proteins before centrifugal recovery. While the conventional process removes sarcoplasmic proteins through washing and dewatering, the new pH-driven process retains sarcoplasmic proteins in the system.

Fish muscle protein is composed of 20-40% sarcoplasmic proteins depending on species (Okada 1999). Considering this high proportion of sarcoplasmic proteins on fish muscle, there is a significant opportunity to utilize these proteins. Water-soluble sarcoplasmic proteins are removed in the conventional process and therefore are not never utilized, rather they further pollute wash water, resulting in problematic wastewater treatment.

No absolute agreements have been made regarding the role of sarcoplasmic proteins in the gelation of myofibrillar proteins. The heat coagulative sarcoplasmic proteins adhere to myofiblillar proteins when the fish muscle is heated and impede the formation of fish protein gels (Shimizu and Nishioka 1974). Okada (1964) also reported sarcoplasmic proteins inhibit the gel formation of myofibrillar proteins. On the other hand, recent studies indicated that water-soluble sarcoplasmic proteins from mackerel rather than interfering with gel formation ability of myofibrillar

proteins, 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°C) inhibition for Higendorf suacord (*hilgendorf saucord*), may be contained in the sarcoplasmic protein fraction of Princes small porgy (*Chelidoperca hirundinacea*) and bigeyed greeneye (*Chlorophthalmus albatrossis*). Alaska pollock surimi was weakened by repeated washing but was strengthened again when the removed fraction was added back (Nowsad and others 1995). This was likely due to the function of transglutaminase in the sarcoplasmic proteins.

While there is still controversy over the role of sarcoplasmic proteins from conventional surimi processing, the nature of sarcoplasmic proteins from pH-driven surimi processing and its role have not been discussed yet. Therefore, a better understanding of sarcoplasmic proteins during the pH-driven protein recovery would be important. The objectives of this study were 1) to investigate the influence of pH and salt on sarcoplasmic proteins from rockfish, and 2) to determine the effects of sarcoplasmic proteins in the gel matrix of myofibrillar proteins.

MATERIALS AND METHODS

Materials

Fresh rockfish (*Sebastes flavidus*) was obtained from Pacific Surimi JV (Warrenton, OR). Medium grade frozen surimi made from Alaska pollock (*Theraga chalcogramma*), about 1 yr old, was obtained from Trident Seafoods (Seattle, WA) as a myofibrillar protein material.

Sample preparation

For the preparation of sarcoplasmic protein, rockfish was homogenized in deionized water with a 1:1 mixing ratio. Homogenate was centrifuged at 10,000 x g at 4°C for 20 min. The supernatant was subjected to gradual freezing at -18°C in order to concentrate sarcoplasmic protein. Then freeze-drying was applied (FreeZone12, Labconco, Kansas City, MO) . Freeze-dried sarcoplasmic powder was kept at –18°C until the following experiments were conducted.

Solubility measurement

One gram of freeze-dried sarcoplasmic proteins was mixed with 15 mL cold deionized water. The pH of the mixture was adjusted to 2, 3, 4, 5, 5.5, 6, 7, 8, 9, 10, 11, and 12, respectively using either 1N HCl or NaOH. The final volume was

brought to 20 mL using deionized water and the pH was adjusted precisely to each pH. The mixture was continuously stirred for 30 min and centrifuged at 10.000 x g at 4°C for 20 min. The protein concentration of the supernatant was determined using the Bradford method (1976) with bovine serum albumin as a standard. Solubility of sarcoplasmic proteins at each pH was calculated based the protein concentration (mg) per unit volume (mL) of the supernatant. Solubility measurement at various NaCl concentrations (0, 0.1, and 0.5M NaCl) was also conducted.

Protein loss while shifting pH

This study was conducted to determine the nature of sarcoplasmic proteins as pH was adjusted to 5.5 after solubilization. One gram of freeze-dried sarcoplasmic proteins was mixed with 15 mL cold deionized water. The mixture was adjusted to 2, 3, 4, 10, 11, and 12, respectively using either 1N HCl or NaOH and then was stirred for 30 min at refrigerated temperature. The pH of the mixture was re-adjusted to 5.5. The final volume of the mixture was adjusted to 25 mL and was continuously stirred for 20 min before centrifuging at 10.000 x g at 4 °C for 20 min. The protein concentration of the supernatant was obtained using the Bradford method and the difference was reported as protein loss.

Electrophoresis (SDS-PAGE)

Various sarcoplasmic protein (SP) treatments were subjected to SDS-PAGE: 1) soluble SP fractions at various pH; 2) SP after re-adjusting pH to 5.5. For surimi gels (with or without SP) and SP precipitate after pH readjustment to 5.5, samples (3 g) were homogenized in 5% (w/v) SDS solution (85°C) at a speed setting of 3 using a homogenizer (Ultra-Turrax T25; IKA Working Inc., Willington, NC) for 1 min. The homogenates were adjusted to a final volume of 30 mL before incubating in an 85°C water bath for 30 min and then centrifuged at 3,000 x g for 15 min. The protein concentration of the supernatant was determined using the Lowry method (Lowry and others 1951).

SDS-PAGE was performed using 5% polyacrylamide stacking gel and 15% polyacrylamide running gel. Gels were stained in Coomassie brilliant blue, and de-stained in a solution (50% methanol: 7% acetic acid: 43% distilled water (v / v / v)). A wide range standard mixture containing myosin of rabbit muscle (205 kDa), β-galactosidase of *E. 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), glyceraldehydes-3-phosphate dehydrogenase of rabbit muscle (36 kDa), carbonic anhydrase of bovine erythrocytes (29 kDa), trypsinogen of bovine pancreas (24 kDa), tripsin inhibitor of soybean, α-lactalbumin of bovine milk (14.2 kDa), and aprotinin of bovine lung (6.5 kDa) was used.

Oscillatory dynamic rheology

Non-destructive gelation properties of fish protein paste samples, containing 0, 1, 3, and 5% of SP, were evaluated using a cone and plate attached to a oscillatory dynamic rheometer (CS-50, Bohlin Instruments, Inc., East Brunswick, NJ). Storage modulus (G') was measured with temperature sweep (10-90°C) at a heating rate of 1°C/min. A solvent trap with wet sponge inside was used to prevent moisture evaporation during measurement. Based on our preliminary calibration to determine the linear viscoelastic region, 1 Pa torque value for stress and 0.1 Hz for frequency were selected.

Differential scanning calorimetry (DSC)

Differential scanning calorimetry was performed on micro DSC (Setaram, Lyon, France). The instrument was calibrated for temperature accuracy using deionized water and naphthalene. Samples weighing around 0.5g with an accuracy of ±0.1mg were sealed in a stainless steel sample cylinder. Another calibration with samples was performed along with an empty reference cylinder to determine the amount of deionized water required as reference. Samples were scanned with a reference cylinder containing water at a heating rate of 1 °C/min over a temperature range between 20-90 °C.

Gel preparation and gel analysis

Frozen pollock surimi was partially thawed, comminuted with various content of SP (0, 1, 2, 3, and 5%), 2% salt, and enough water to maintain 78% moisture content. Additional batches were made to account for the effect of sarcoplasmic protein content. Four levels (1-5%) of SP were replaced by sucrose, which we assumed did not affect gel formation of myofibrillar proteins, but stabilized the myofibrillar proteins. Paste was then stuffed into plastic tubes and cooked at 90°C for 15 min. Gels were refrigerated overnight. Texture properties were measured using the punch test with a Sintech machine (Sintech 1/G, MTS, Cary, NC) using a 5 mm spherical probe with an initial probe speed of 6.0 cm/min after gels were equilibrated to room temperature. Breaking force (g) and deformation (mm) were recorded. A CIE Lab 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 the whiteness index L*-3b* (Park 1994).

RESULTS AND DISCUSSION

Effect of pH and salt on solubility

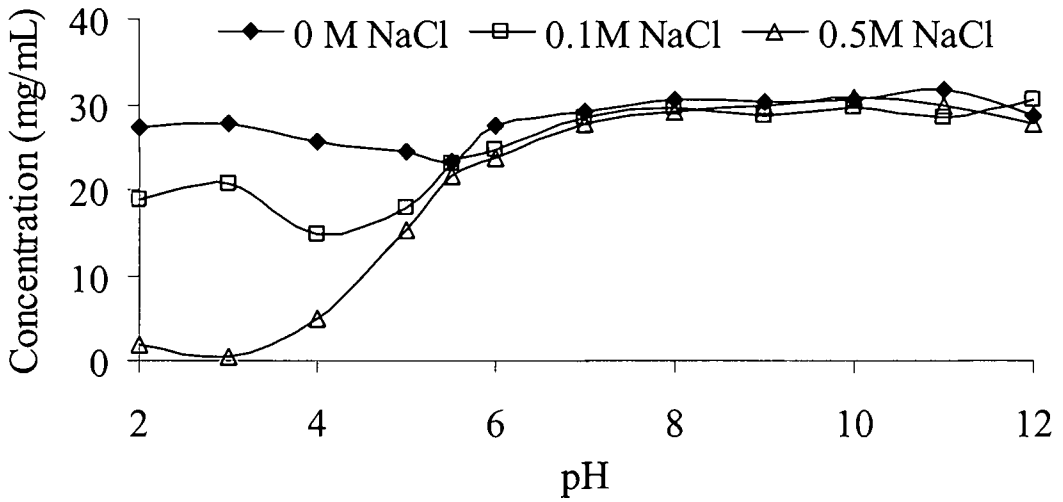

Figure 3.1 – SP solubility at various pHs in different NaCl concentration.

The solubility of SP in 0 M NaCl solution was constant over the pH range of 2 to 12 (Fig 3.1). A slight decrease of solubility was found at around pH 5.5, which indicates isoelectric point (pI). Solubility varies greatly with pH, due to changes in molecular charges. At pH values above the pI, a protein becomes more negatively charged, whereas at pH values below the pI, a protein becomes more positively charged. As the pH is shifted away from the pI, toward either higher or lower pH values, electrostatic repulsion between molecules increases and solubility is enhanced (Christen and Smith 2000). However, the solubility curve of SP in 0 M NaCl solution was not a typical U-shape.

Some proteins (for example, whey proteins) are highly soluble at their isoelectric pH because the exposed surfaces of these proteins contain a high ratio of

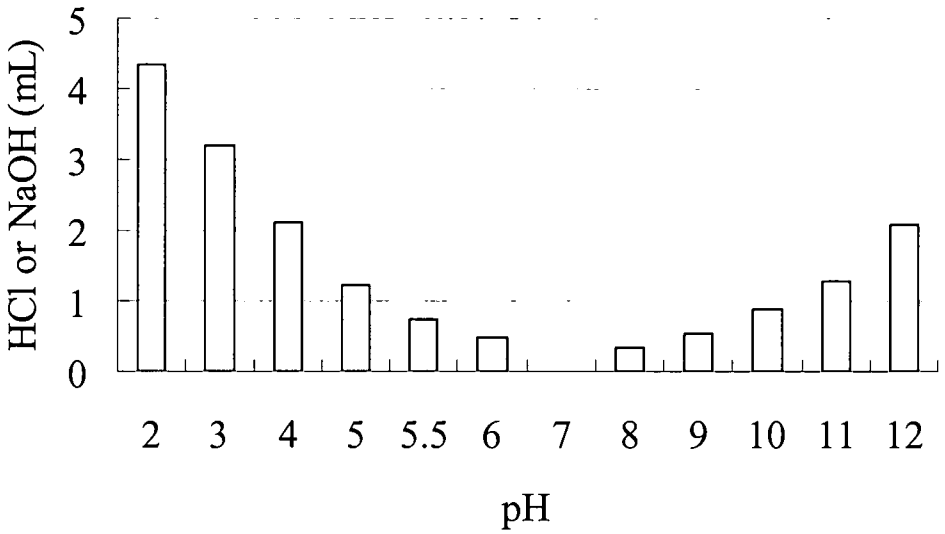

Figure 3.2 – The required amount of 1.0N HCl or NaOH to adjust to each pHs. Initial pH of SP-P solution was about 7.0.

hydrophilic to hydrophobic groups (Damodaran and Paraf 1997). Presumably, SP contains a large number of hydrophilic groups on the protein surface and hydration of these polar residues produces hydration repulsion forces great enough to offset aggregation via hydrophobic interactions (Damodaran and Paraf 1997).

Considering buffering capacity, SP is composed of acidic proteins, like the majority of food proteins, where the sum of aspartic acid and glutamic acid is greater than the sum of lysine, arginine, and histidine residues (Fennema 1996). Therefore, the pI of SP was slightly acidic and adjusting the pH to acidic conditions required more quantity of 1.0 N HCl than that of 1.0 N NaOH required to adjust the pH to the basic conditions (Fig 3.2).

When salt was introduced to the system, the solubility curves were changed. In 0.1M NaCl solution, the isoelectric point was shifted to around pH 4 to 5. The solubility decreased as the pH shifted below pH 5.5. In 0.5M NaCl solution, the isoelectric point was further shifted to the acidic direction, at about pH 3. The solubility also dramatically decreased at acidic conditions. Especially at pH 2 and 3, large precipitates were observed resulting in no sign of soluble fractions. Probably chloride ion from NaCl enhances hydrophobic interactions (protein-protein) on the protein surface, and hydration repulsion forces arising from the charged residues are no longer greater than the hydrophobic interaction force, resulting in extremely low solubility near the pI.

Protein loss while shifting pH

When SP was treated to pH 2 or 3 followed by shifting to pH 5.5, the least amount of proteins was lost further indicating that acid-aided processing could recover SP more efficiently than alkali-aided processing (Fig 3.3). The pH 10 treatment, before recovering at pH 5.5, gave the largest protein loss followed by pH 11, 4, and 12. This dramatic change was likely due to molecular changes occurring at acidic treatment and extremely high pH treatment, pH 12 as discussed below.

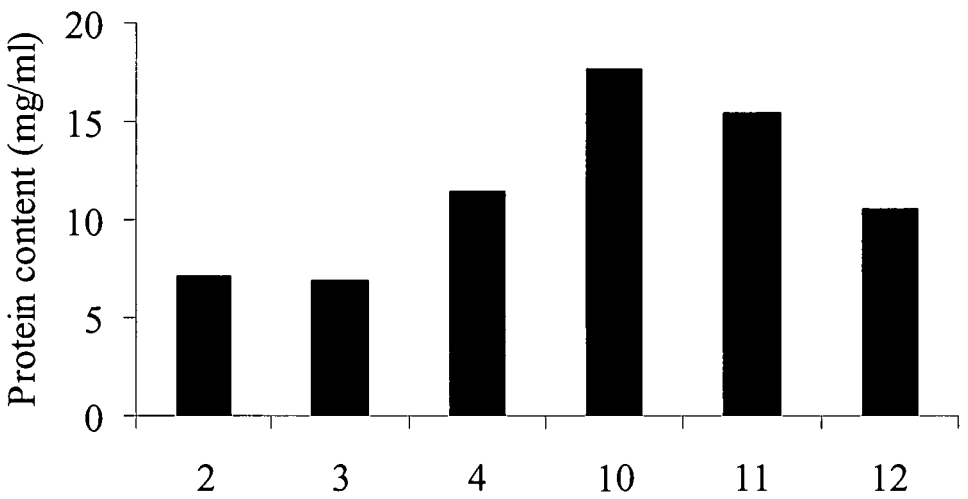

Figure 3.3 – Protein loss at various pH treatment. Protein loss is expressed as protein concentration (mg/mL) in supernatant after pH readjustment to 5.5.

Oscillatory Dynamic properties

The changes of storage modulus, G', during linear heating rate were different depending on SP concentration in the surimi paste (Fig 3.4). At initial heating stage, 10-30 °C, the higher concentration of SP exhibited the lower G' value. Unfolding, as indicated by reduction of G', was started at around 38°C for all samples. Unfolding was ended and gelation was started at around 45°C. Gelation was completed after 75°C. These unfolding and gelation temperature profiles were very similar among samples regardless of SP concentration. However, the depth of the valleys at around 45°C, indicating the start of gelation, was significantly different

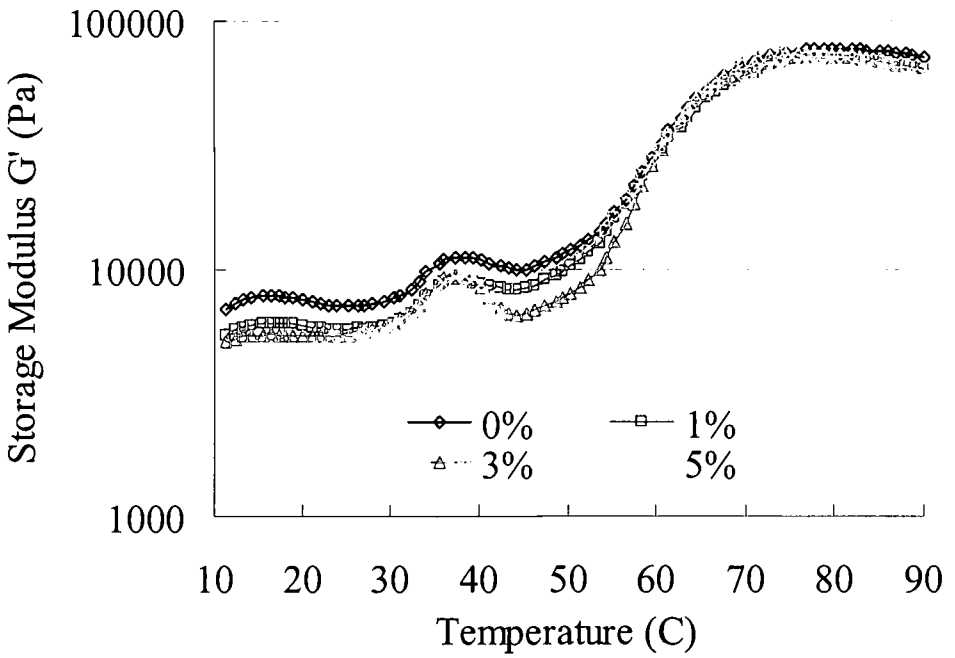

Figure 3.4 – Changes in storage modulus during linear heating rate of surimi at various SP content.

among samples. The higher the concentration of SP content, the deeper the valley. This trend seems to be correlated with the myofibrillar protein concentration. Lower myofibrillar protein concentration gave the deeper valley.

Figure 3.6 showed loss modulus values (G") at starting temperatures, especially 15°C had a strong correlation (R^2=0.99) with the addition of SP. Interestingly, G" values at 15°C were strongly correlated (R^2=0.96) with texture properties, particularly breaking force values (Fig 3.7). Viscosity properties of fish proteins may have good correlation with gelation properties. However, further

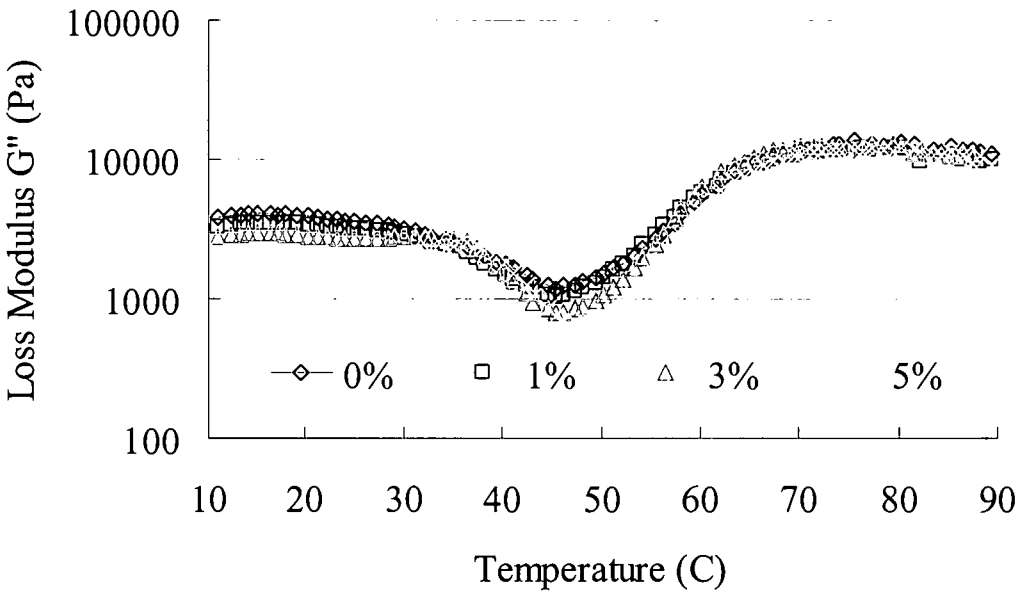

Figure 3.5 – Changes in loss modulus during linear heating rate of surimi at various SP content.

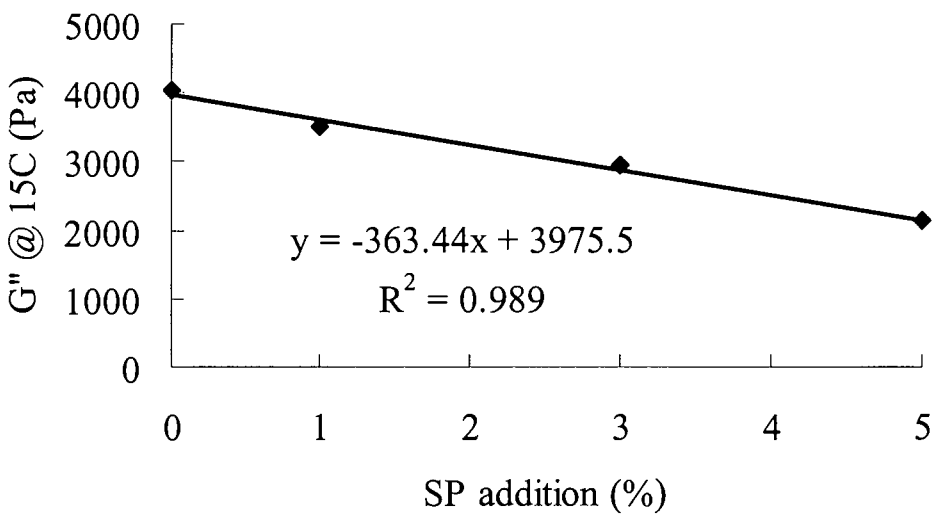

Figure 3.6 – Relationship between G" values at 15°C and SP content.

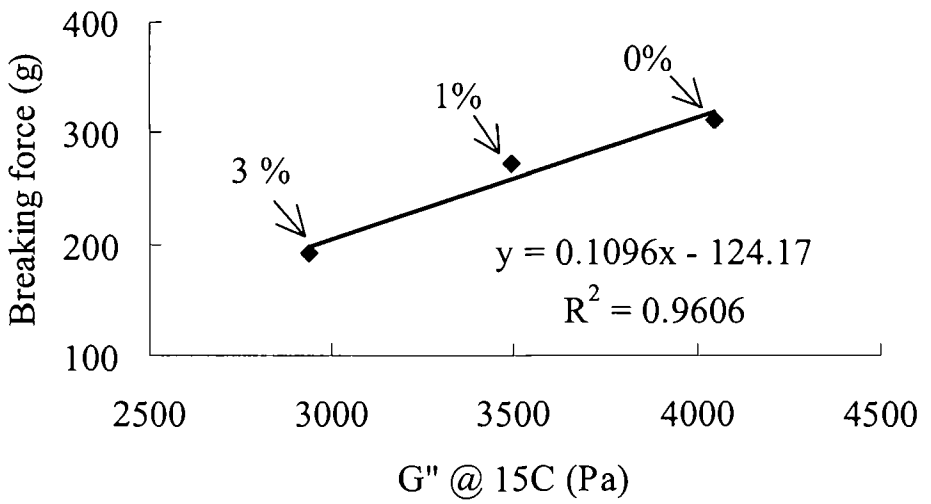

Figure 3.7 – Relationship between G" values at 15°C and breaking force values.

investigation may be necessary in order to determine these correlations.

DSC

Regardless of salt concentration, SP solution samples exhibited an exothermic peak at 38.5°C and 37.2°C, respectively (Fig 3.8). This may happen as a result of aggregation. However, further studies are necessarily to be confirmed. The large second peak (endothermic) was observed at 74.2°C for samples without salt and at 72.1°C for samples with 2% NaCl. The addition of NaCl reduced not only denaturation temperatures, but also aggregation temperatures. The addition of salt

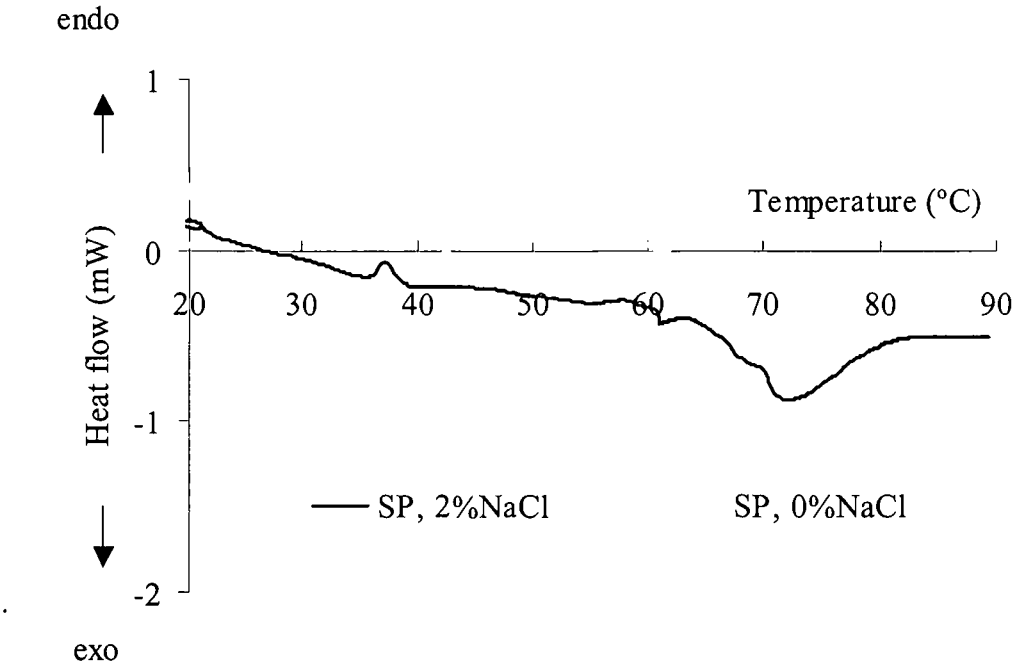

Figure 3.8– DSC thermograms of SP solution (3% SP, pH7.0).

generally lowers the denaturation temperature (Park and others 1988; Chen 1995). This indicates that sarcoplasmic proteins can also be destabilized in the presence of NaCl.

Table 3.1 and Figure 3.9 show the effect of SP on the differential scanning calorimetric properties of the myofibrillar proteins. Angsupanich and others (1999) reported four endothermic transitions at 34.2, 43.8, 50.6, and 64.9°C, respectively, were observed in cod myofibrillar proteins (7.3±0.9% protein concentration, pH≈7, I= 0.3). They concluded the first three peaks attributed to the myosin transitions and the last peak attributed to the actin transitions. In our study, presumably peak 2, 4,

Table 3.1 – Transition temperature (°C) of peaks at various SP concentration in surimi.

SP conc. (%)	Peak 1	Peak 2	Peak 3	Peak 4	Peak 5	Peak 6	Peak 7
	Td	Td	Td	Td	Td	Td	Td
0	36.8	45.8	51.9	62.6	67.0		75.3
1	37.6	46.2	50.8	64.4	67.3	70.3	
3	37.6	46.1	53.0	65.4	68.4	70.0	
5	38.3	45.3	53.3	65.5		69.7	

and 5 attribute to myosin transition, and peak 7 attributes to actin transition (Table 3.1). Peak 4 was the major peak, which is assigned to the denaturation of myosin at 62.6-65.5 °C. Presumably, SP gave three peaks, 1, 3, and 6 (Table 3.1). When the SP concentration increased from 0 to 5 %, peak 1, 3, and 6 appeared more clearly. However, at the same time, myosin and actin peaks were weakened or disappeared with increased SP concentration due to lowered myofibrillar protein concentration. SP properties in surimi on DSC were quite similar to other protein additives, such as beef plasma, egg white, and whey protein in terms of exothermic contribution. The reduction in enthalpy was due to increased protein aggregation (Park 1994).

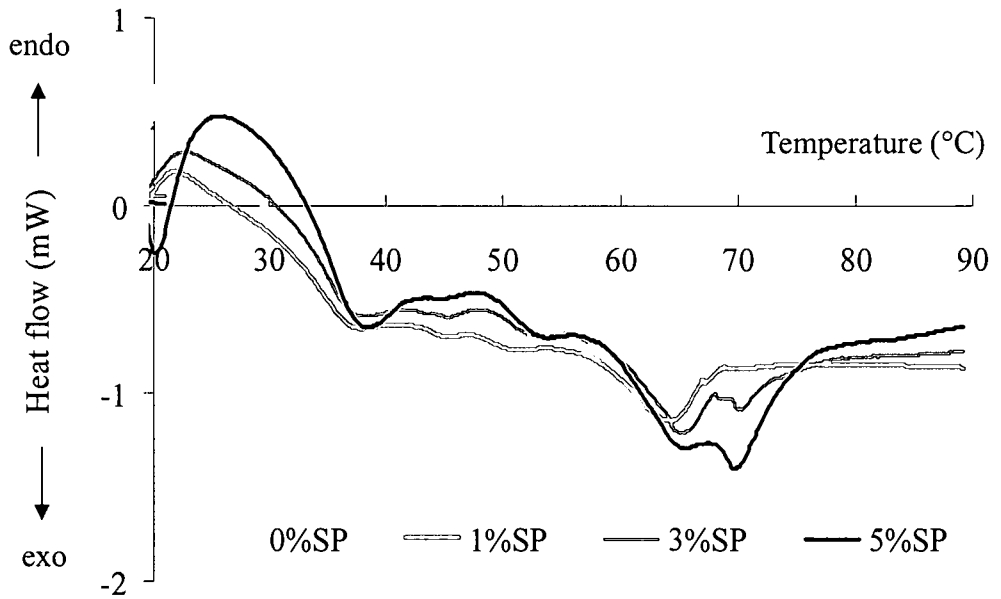

Figure 3.9 – DSC thermograms of pollock surimi with various % of SP.

Gel properties

When the SP content increased, both breaking force and deformation values decreased (Fig 3.10). However, texture properties of SP-added gels were superior to sucrose-added gels up to 2 % concentration. SP did not enhance the gelation of myofibrillar proteins, but SP apparently contributed to gel formation of myofibrillar proteins up to 2% addition. At 3% addition, there was no significant difference between SP-added gels and sucrose-added gels. At 5% addition, no measurable gel was obtained from either SP or sucrose-added samples.

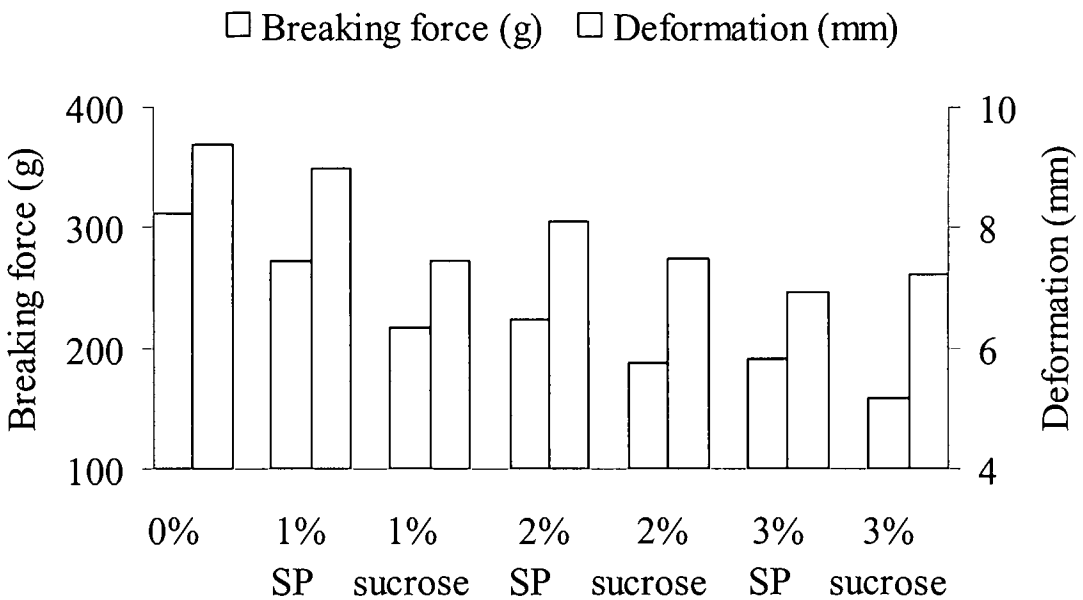

Figure 3.10 – Effects of additives to pollock surimi on textural properties.

There is one unclear issue with regards to whether SP adversely affects surimi gelation (Lanier 2000). Okada (1964) concluded that the removal of SP by washing in surimi processing would be necessary because washing brings forth a high concentration of myosin by removing SP, which otherwise inhibits myosin from gel formation. Shimizu and Nishioka (1974) mentioned SP binds and coagulates with actomyosin during cooking, resulting in decreased gel forming ability of surimi. In our study, SP did not interfere the gelation of myofibrillar proteins, but positively contributed to the gelation. However, the gel forming ability of SP was much weaker than the myofibrillar proteins.

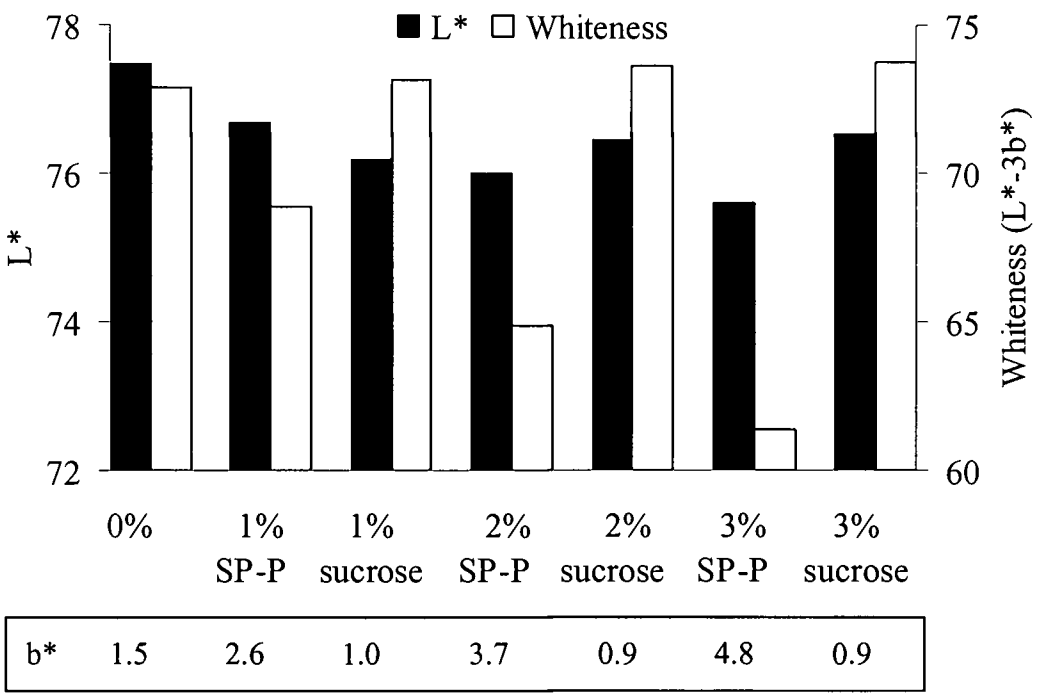

Figure 3.11 –Effects of additives to pollock surimi on color quality.

Our results were supported by several studies (Morioka and Shimizu 1990, Morioka and others 1997, 1998). It must be remembered that the gelation properties of SP highly depend on fish species and on the composition of SP (Morioka and others 1992, 1997). It can be assumed that there is a certain minimum requirement of myofibrillar proteins content to obtain decent gels regardless of SP addition.

Color quality of gels decreased sharply when the SP concentration increased (Fig 3.11). Whiteness value significantly decreased due to the addition of SP. The

decline of color value from whitish to yellowish hue is mainly due to the increased b* value. This is probably due to the inclusion of heme proteins, which are responsible for the pigmentation of fish muscle.

SDS-PAGE

The protein patterns of SP in 0 M NaCl solution at various pHs were very similar to each other (Fig 3.12). The major sarcoplasmic bands were 43kDa, 40kDa

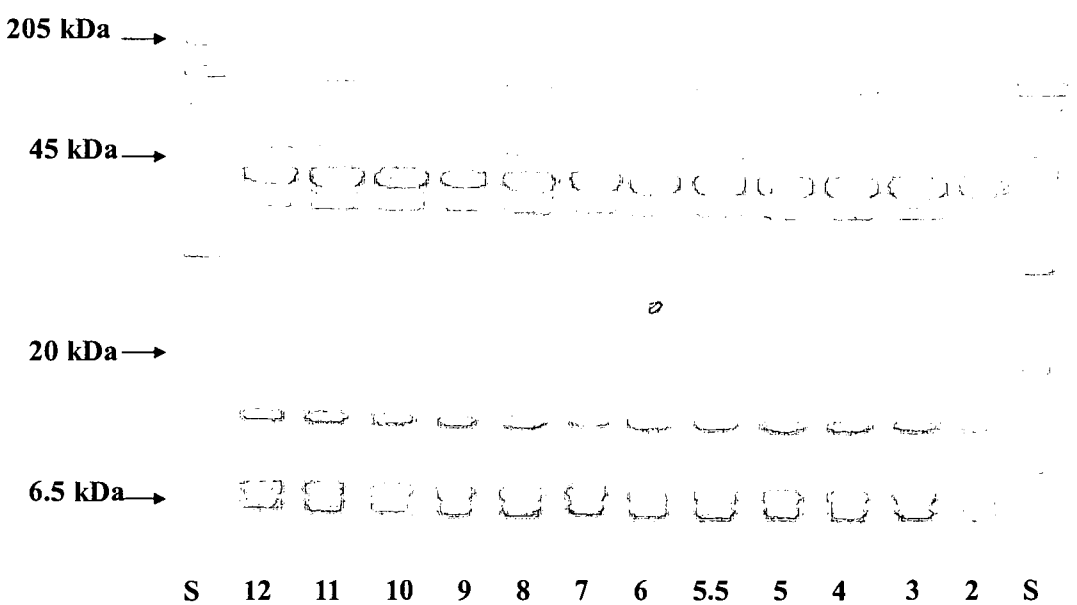

Figure 3.12 – Protein patterns of SP in 0M NaCl solution. The number indicates pH and S denotes wide range molecular standard.

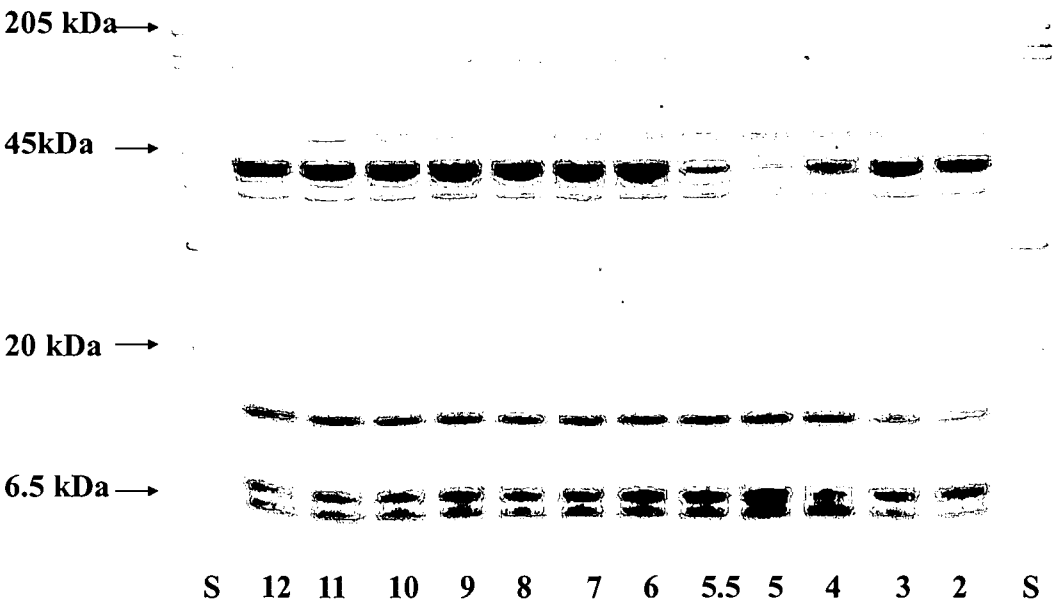

Figure 3.13 – Protein patterns of SP in 0.1M NaCl solutions. The number indicates pH and S denotes wide range molecular standard.

, 17kDa, 11kDa, and 8kDa, respectively. No degradation occurred in SP components even at extremely high or low pH. Based on SDS-PAGE, the patterns of the sarcoplasmic fractions were fairly consistent as the pH was shifted to either the acid or alkaline side.

When NaCl was added (0.1M), however, some SP components were not solubilized well under mild acidic condition, especially at pH 4 and 5 (Fig 3.13). This data correlates well with the solubility curve (Fig 3.1). Because of low solubility of certain protein components in SP under mild acidic and low salt concentration, the total protein solubility was suppressed. When more NaCl was

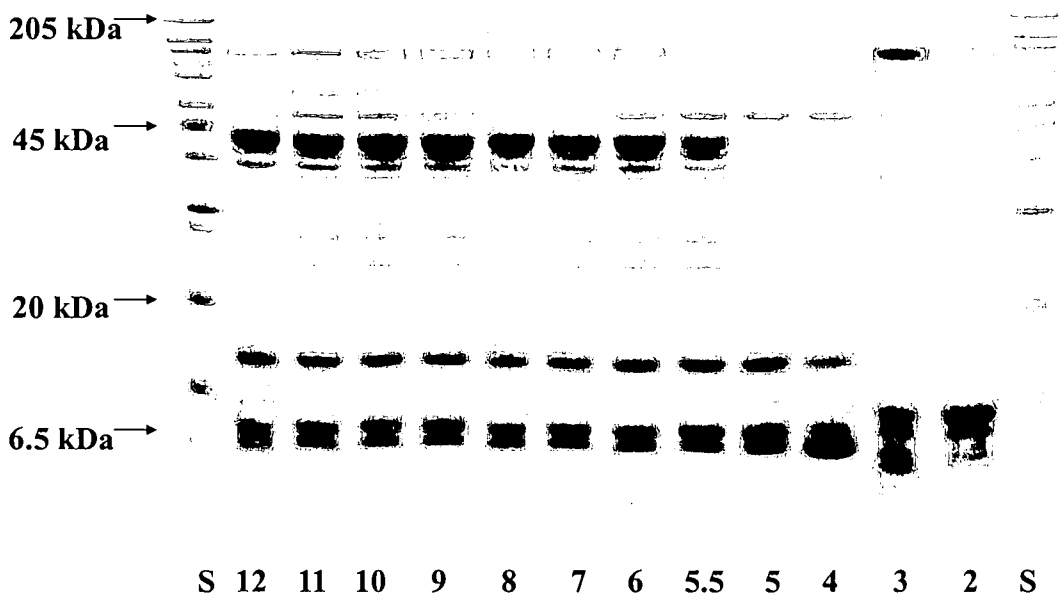

Figure 3.14 – Protein patterns of SP in 0.5M NaCl solutions. The number indicates pH and S denotes wide range molecular standard.

introduced to the system (0.5M NaCl), protein patterns were significantly changed under acidic condition (Fig 3.14).

At pH 2, 3, and 4, the 43kDa protein band disappeared. Moreover, at pH 2 and 3, many SP components were degraded. In contrast, at alkaline condition, each SP component was very stable regardless of NaCl concentration. Figure 3.15 revealed that less protein loss at pH 2 and 3 was mainly due to significant recovery of proteins, 43, 40, and 17 kDa, respectively, upon pH readjustment to 5.5.

Visual color appearance was quite different among pH treatments (data not shown) especially due to the contribution of myoglobin, which was seen at 17 kDa.

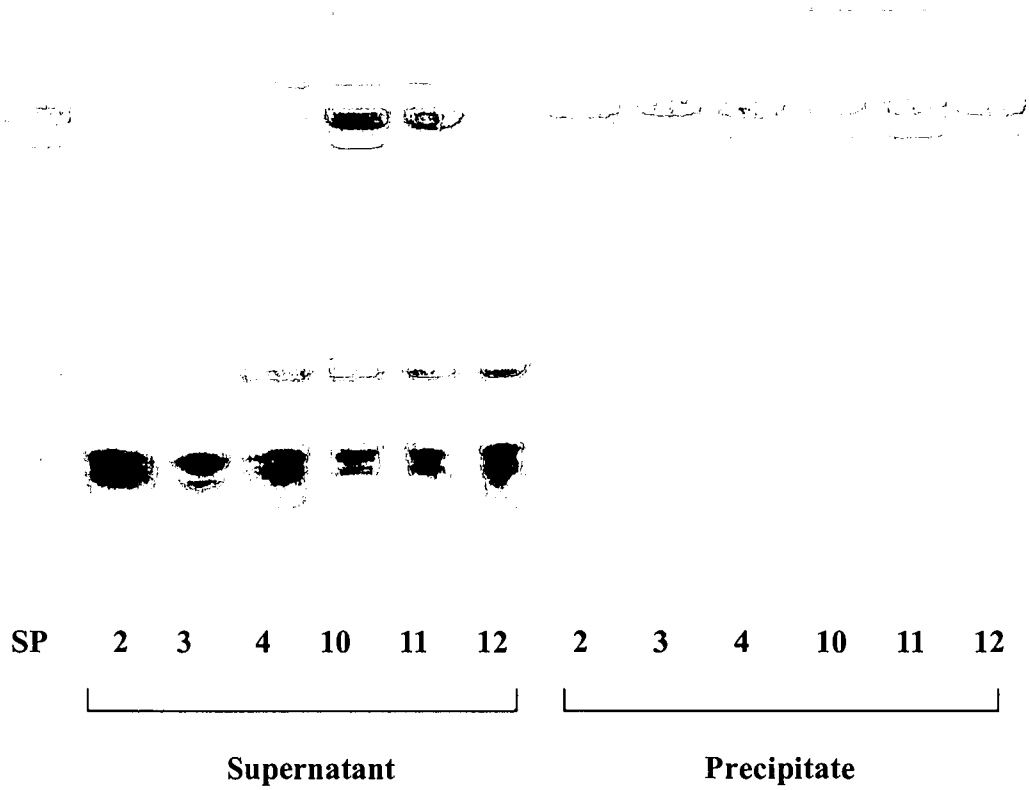

Figure 3.15 – Protein patterns of SP after pH readjustment to 5.5. SP indicates sarcoplasmic solution (0% NaCl pH 5.5). The number indicates the pH at which samples were adjusted.

Maillard browning reaction might also have been involved, particularly at pH 12.

When SP was added to pollock surimi more SP bands were visualized. No degradation of myofibrillar proteins by addition of SP was observed. Probably, each SP components contributed to the gelation mechanism. However, judging from this result, it is difficult to conclude whether SP adhered to the myofibrillar

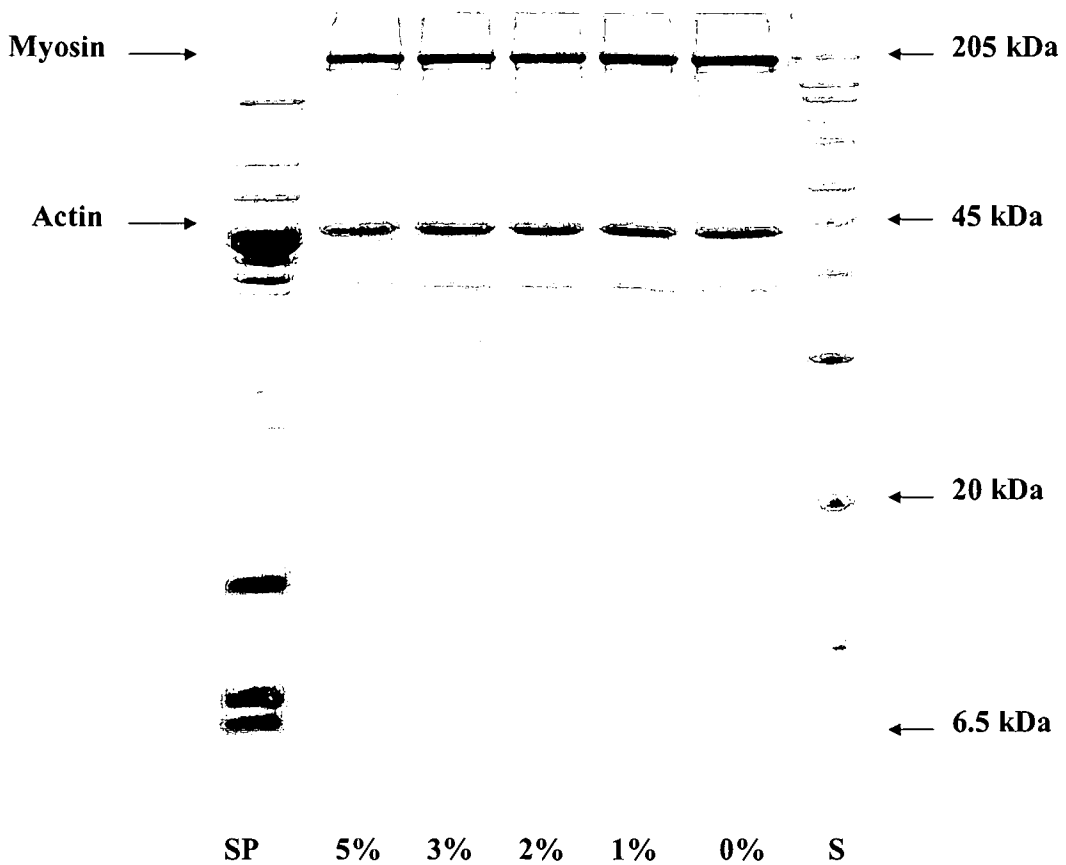

Figure 3.16 – Protein patterns of pollock surimi with various SP concentrations. SP indicates sarcoplasmic solution (2% NaCl pH7.0). Each number of percentages represents the concentration of SP in pollock surimi gel. S donates wide range molecular standard.

proteins and consequently interfered with the gelation of myofibrillar proteins or not.

CONCLUSIONS

Solubility and protein patterns of SP were independent of pH when no salt was added. High NaCl concentration significantly affected the solubility and protein patterns only under acidic condition. SP appeared to be more stable against pH (especially neutral and alkaline) and salt than myofibrillar proteins. According to DSC results, SP attributed to three peaks in surimi. Furthermore, SP addition to pollock surimi did not improve fracture texture properties because the inclusion of SP reduced the quantity of myofibrillar proteins proportionally.

GELATION PROPERTIES OF SPECIAL FISH MUSCLE PROTEINS WITHOUT SALT

Young S. Kim and Jae W. Park

To be submitted to Journal of Food Science

ABSTRACT

Salt effect on acid- or alkali-treated surimi gel was investigated. Good gels were obtained without salt using acid- and alkali-treated fish proteins. Texture properties decreased as NaCl content increased, unlike conventional surimi gels. Consequently, NaCl apparently did not solubilize myofibrillar proteins once the fish proteins were treated by acid or alkali. Solubility, however, was not a key factor for the texture properties of acid- or alkali-treated surimi. Endogenous transglutaminase was partially inactivated during acid or alkaline treatment. Acid-treated surimi gel gave the best color properties.

Key word: Salt, pH shift, fish protein, setting

INTRODUCTION

One of the major undesirable factors of surimi seafood is relatively high sodium content. Many consumers are interested in reducing their sodium intake. Thus, the salt content of surimi seafood has received considerable attention (Chang and others 2001).

New processing approaches of acid- or alkali-aided fish proteins have been previously studied. These methods utilize protein denaturation and subsequent renaturation by pH readjustment using strong acid or alkali. For the conventional method of gel preparation, salt is used to extract myofibrillar proteins and initiate the unfolding before cooking. However, if myofibrillar proteins are already denatured as shown in our acid- and alkali-aided studies (Choi and Park 2002), salt addition may not be required. The removal of salt could completely or partially result in low sodium chloride content. Our hypotheses in dealing with denatured fish proteins were: 1) salt may not be required to obtain gels from acid- and alkali-treated fish proteins, 2) the setting effect may still be able to contribute to high gel quality in unsalted acid- or alkali-aided fish proteins.

The objectives of this study were to investigate the role of salt for gelation of acid- and alkali-treated fish proteins. In addition, the setting effect of acid- and alkali-aided fish proteins was studied.

MATERIALS AND METHODS

Materials and sample preparation

Due to the limited availability of fresh fish samples, high grade frozen surimi (12 mo old) made from Alaska pollock (*Theraga chalcogramma*) was used (American Seafood, Seattle, WA). After partially thawing, the surimi was homogenized with distilled water at a 1 to 9 ratio for the acid or alkali treatment. The acid or alkali treatment was done using 2N HCl or 2N NaOH to adjust to pH 2 or 11, respectively. Then the pH of the acidic or alkaline homogenates was readjusted to 5.5. Dewatering was conducted using centrifugation (4,000×g, 20 min). The solid fraction of the fish protein was collected. 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 2N NaOH. The temperature was maintained below 5°C. Fish protein samples were vacuum-packed and stored at –30°C until used.

Gel preparation and gel properties

Fish protein samples were adjusted to 78 % moisture content and 0, 1, 2, or 3%

salt, respectively for gel preparation. The pH of all samples was adjusted to 7.0 using 1N NaOH. Paste was stuffed into stainless steel tubes. In order to observe the setting (suwari) effect, the samples were divided into two parts. One was cooked at 90°C for 15 min immediately after stuffing. The other was kept at 5°C for 16 to 18 h followed by cooking at 90°C for 15 min. Cooked gels were refrigerated overnight.

Texture properties were measured by the punch test using a Sintech machine (Sintech 1/G, MTS, Cary, NC) using a 5 mm spherical probe with an initial probe speed of 6.0 cm/min. The breaking force (g) and the deformation (mm) at fracture were recorded. A CIE Lab 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, Japan). The instrument was standardized using calibration plates. Whiteness was calculated using the whiteness index, L*-3b* (Park 1994).

Solubility measurements

Solubility of surimi was determined in two different solutions; 20 mM tris-HCl (pH 7.0) and 20 mM tris-HCl (pH 7.0) containing 0.6 M NaCl. Surimi sol (3.0 g) (moisture content was adjusted to 78 %) was mixed and homogenized with the above solution (27.0 mL). The sample solutions were centrifuged at 27,000 x g at 4-6 °C

for 20 min. The Bradford dye binding method was used for protein determination (Bradford 1976).

Salinity

Salinity was evaluated to determine the difference between conventional surimi gels and acid- or alkali-aided surimi gels. Salinity was measured using a conductivity meter (YSI Model 3100, Yellow Springs, OH) after diluting samples by 20-fold and filtering through Whatman #54 filter paper (Kent, England). A standard curve was made using 0.005-0.05% NaCl solutions.

Statistical Analysis

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 ($p \leq 0.05$) between treatment means. This was done using S-Plus 2000 Professional Release 3 (MathSoft Inc., Seattle, WA).

RESULTS AND DISCUSSION

The maximum gel strength of conventional surimi without setting was obtained at 2% NaCl addition. The main function of salt is to help the myofibrillar proteins become solubilized and subsequently improve gel formation. The maximum gelling effect of pollock surimi at neutral pH can usually be obtained at 2-3% NaCl (Chung and others 1993). Salt also increases dissociation of actomyosin through physical cuts or tears of the muscle fibers during comminuting. The dissociated myosin is available to participate in gel matrix formation and to bind water (Park and others 1996).

Conventional surimi gel with 3% NaCl was slightly weaker than that with 2% NaCl. The decreased texture properties of conventional surimi gel at higher salt concentration were probably due to the use of aged frozen surimi. The proteins might have already been partially unfolded. Alterations in myofibrillar proteins and their functionality have been observed in frozen muscle and isolated protein systems in terms of protein solubility and decreased gel-forming ability (Park and others 1988). In addition, higher concentration (3%) could have given a salting-out effect resulting in some protein aggregation.

Texture properties were dramatically improved after setting except for the sample without salt (0%). Covalent dipeptide linkages are not the only protein-protein interactions that stabilize the low temperature-induced set gel. Intermolecular hydrophobic interactions contribute to the low temperature setting

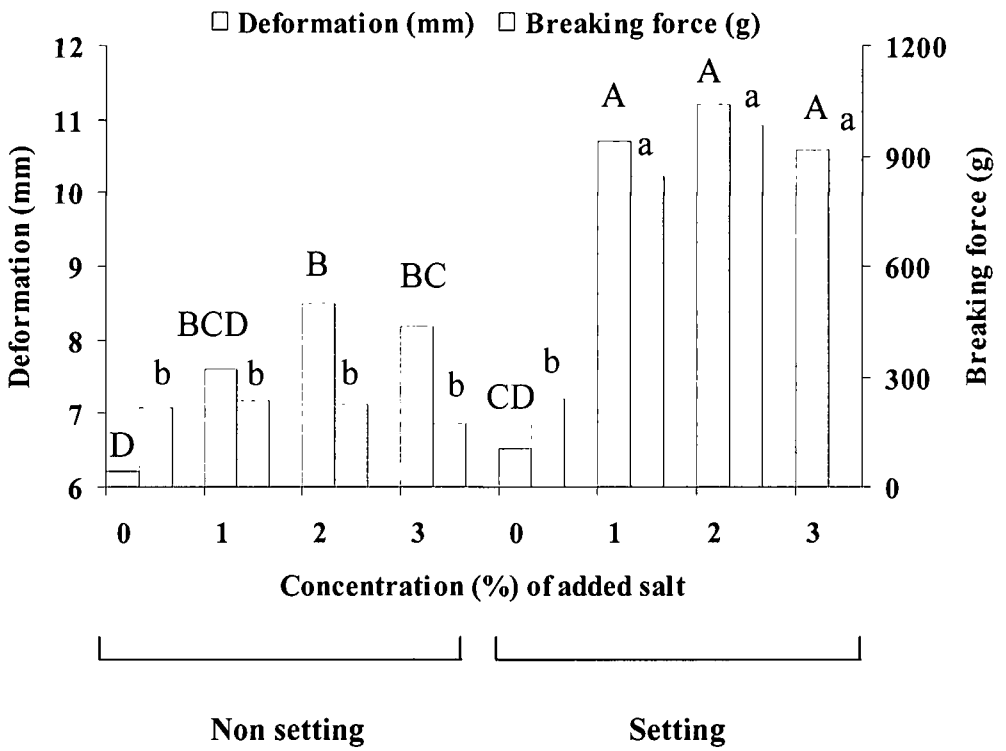

Fig 4.1- Texture properties of conventional surimi gel at various salt concentrations. Non setting = 90°C for 15 min, Setting = set gel at 4°C for 16-18 hrs followed by cooking at 90°C for 15 min. Means having the same letter are not significantly different ($p>0.05$).

reaction as well (Lanier 2000). The setting period allows conformation changes in the protein molecules that may involve localized exposure and subsequent interaction of hydrophobic amino acid residues resulting in formation of a more elastic gel (Park and others 1996). The requirement of salt addition to surimi paste to induce setting also supports the role of hydrophobic interactions in setting because

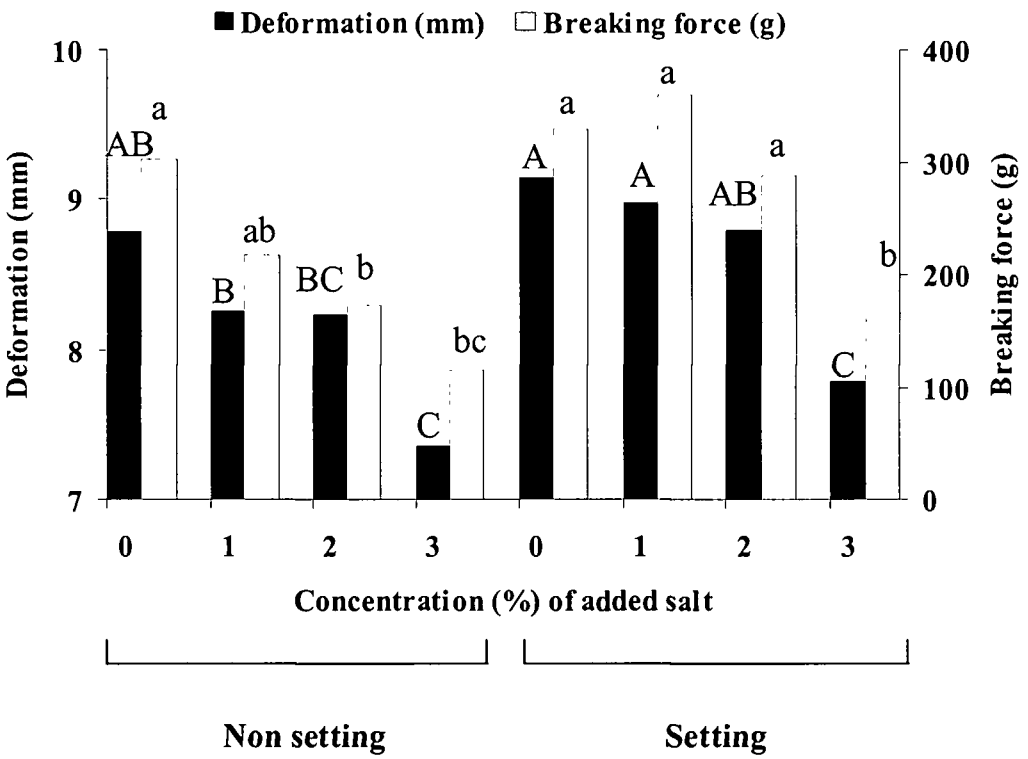

Fig 4.2- Texture properties of acid treated surimi gel at various salt concentrations. Non setting = 90°C for 15 min, Setting = set gel at 4°C for 16-18 hrs followed by cooking at 90°C for 15 min. Means having the same letter are not significantly different ($p>0.05$).

certain salts, such as sodium chloride, act with water molecules to strengthen the hydrophobic interactions between proteins (Lanier 2000). Unlike conventional surimi, both breaking force and deformation values were decreased in acid treated samples as NaCl content increased (Fig 4.2). Table 4.1 indicates NaCl did not significantly solubilize myofibrillar proteins in acid-aided surimi. Salt also strongly

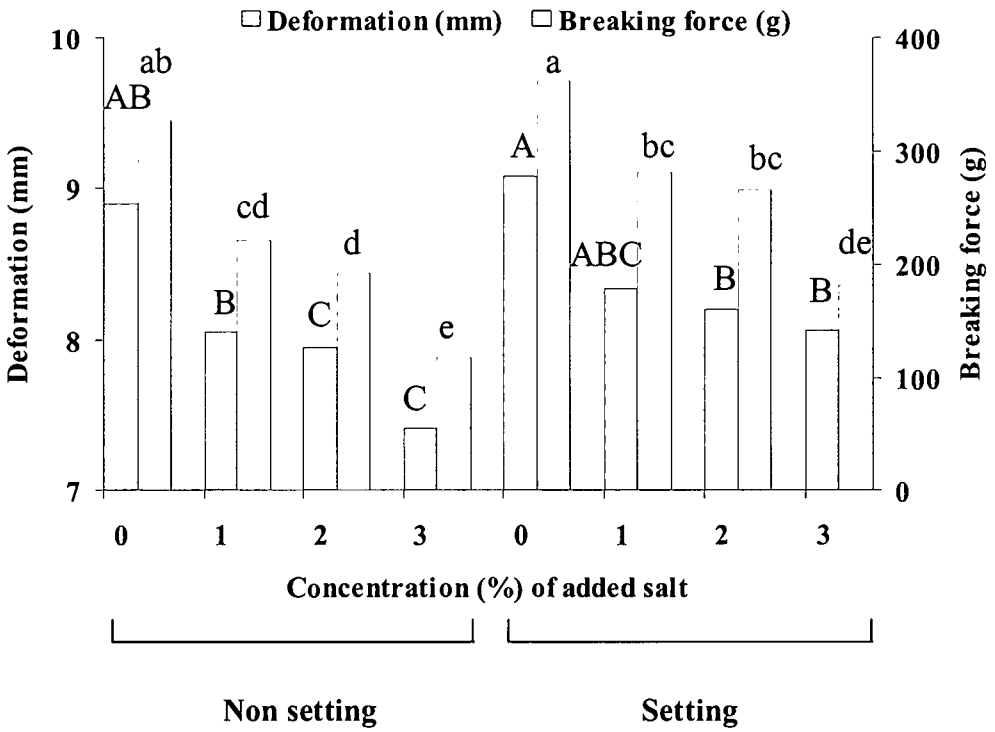

Fig 4.3- Texture properties of alkaline treated surimi gel at various salt concentrations. Non setting = 90°C for 15 min, Setting = set gel at 4°C for 16-18 hrs followed by cooking at 90°C for 15 min. Means having the same letter are not significantly different ($p>0.05$).

increases undesirable electrostatic and hydrophobic forces (Lin and Park 1998) and consequently, it negatively affects gelation since acid-aided surimi is completely unfolded before comminuting with salt. In addition, due to reduced content of total protein by adding salt, texture properties could also have been reduced. Reduced effect of setting in acid treated surimi was observed. However, comparing with

Table 4.1- Salinity and solubility of surimi.

		Solubility (mg/mL)		
	Salinity (mM)	Tris A	Tris B	B/A
Conventional	52.1±2.2	0.77±0.01	4.62±0.03	6.00±0.05
Acid	36.0±0.6	1.39±0.04	1.76±0.00	1.27±0.03
Alkali	29.6±1.4	1.85±0.00	2.39±0.02	1.29±0.01

Salinity measurement was performed using surimi gels (0% NaCl added, 78% moisture). Solubility measurement was conducted using surimi paste (78% moisture). Tris A: tris-HCl buffer (pH 7.0) containing no salt. Tris B: tris-HCl containing 0.6 M NaCl.

conventional surimi, the setting effect was quite low. Presumably, during strong acid treatment, transglutaminase-mediated setting reaction could have been partially inactivated. Alkali treated surimi also showed results similar to acid-treated surimi (Fig 4.3 and Table 4.1). During acidic or alkaline treatment, NaCl content did not increase according to the salinity data (Table 4.1).

Color properties of surimi gels were shown in Table 4.2. The a* values were relatively constant regardless of salt concentration and setting in all samples. Park (1994) reported that a* value of pollock and whiting gel was very consistent

Table 4.2- Color properties of surimi gels at various salt concentrations.

		Non-set				Set			
	Salt (%)	0	1	2	3	0	1	2	3
Conventional	L*	73.3^{c}	78.2^{ab}	78.3^{a}	79.1^{a}	74.8^{bc}	79.0^{a}	75.9^{abc}	76.8^{abc}
	a*	-4.9^{bc}	-4.6^{ab}	-4.5^{ab}	-4.2^{a}	-5.0^{bc}	-4.8^{ab}	-5.2^{c}	-4.9^{bc}
	b*	1.0^{ab}	2.2^{a}	2.2^{a}	2.4^{a}	1.1^{ab}	2.3^{a}	0.0^{b}	0.7^{ab}
	Whiteness	70.4^{c}	71.7^{c}	71.6^{c}	71.9^{bc}	71.6^{c}	72.0^{bc}	75.9^{a}	74.7^{ab}
Acid-aided	L*	75.7^{a}	78.0^{a}	77.9^{a}	77.1^{a}	77.8^{a}	78.1^{a}	77.7^{a}	77.0^{a}
	a*	-4.3^{ab}	-4.2^{ab}	-4.3^{ab}	-4.2^{ab}	-4.1^{a}	-4.4^{ab}	-4.4^{ab}	-4.5^{b}
	b*	1.2^{a}	0.7^{ab}	-0.2^{bc}	-0.4^{bc}	1.4^{a}	0.4^{abc}	-0.7^{c}	-0.9^{c}
	Whiteness	72.2^{c}	76.0^{ab}	78.3^{a}	78.2^{a}	73.6^{bc}	77.1^{ab}	79.6^{a}	79.6^{a}
Alkali-aided	L*	75.2^{a}	78.2^{a}	77.3^{a}	76.4^{a}	75.2^{a}	78.5^{a}	77.6^{a}	77.0^{a}
	a*	-4.3^{ab}	-4.2^{a}	-4.3^{ab}	-4.4^{ab}	-4.3^{ab}	-4.3^{ab}	-4.5^{ab}	-4.7^{b}
	b*	1.7^{a}	1.8^{a}	1.0^{ab}	0.3^{ab}	1.5^{a}	1.8^{a}	0.6^{ab}	-0.5^{b}
	Whiteness	70.2^{c}	72.7^{bc}	74.3^{abc}	75.6^{ab}	70.8^{c}	72.9^{bc}	75.9^{ab}	78.6^{a}

Non-set indicates non set surimi gel, Set indicates set surimi gel, Whiteness = L* - 3b*.Means having the same letter are not significantly different ($p>0.05$).

regardless of cooling/setting conditions, moisture content, sample size, or frozen storage.

No significant difference of the L* values for acid- or alkali-aided surimi was found at various salt concentrations or setting conditions. The b* values gradually increased as salt concentration increased in acid- or alkali-aided surimi gels. As the

b* value decreased, whiteness values increased. However, acid-treated surimi gel gave the highest whiteness values under the same salt and setting conditions. Protein may form a gel that appears either turbid or transparent depending on the environmental conditions. Manipulation of environmental conditions can be used to change the gel properties (Christen and Smith 2000).

Since acid- or alkali-treated surimi is denatured as our previous study indicated, certainly, those surimi give different protein network, resulting in different color properties. Salt also attributes significantly to color value due to the electrostatic forces. At low salt concentration, because the attractive forces between molecules are low, normally transparent gels are formed. At high salt concentration, because of the high attractive forces between protein molecules, turbid gels are formed (Christen and Smith 2000). In our results, a significant increase of whiteness values was observed upon salt addition for every treatment. Setting also slightly contributed to increased whiteness values for all treatments. Gel color often depended on the protein-protein interactions in the medium (Choi and others 2000).

CONCLUSIONS

Salt was necessary to increase the gelation properties of conventional surimi gel. In contrast, salt negatively affected the gelation properties in acid- and alkali-treated surimi gel. Transglutaminase-mediated setting reaction was

apparently inactivated by acidic or alkaline treatment. Further studies may be needed to investigate the role of cryoprotectants in this pH-drive protein recovery.

SUMMARY

New surimi processing, acid- or alkali-aided method, is a very effective approach in terms of increasing of fish protein recovery compared to the conventional method. While the conventional surimi method avoids denaturation during processing, the new approaches induce fish proteins denaturation through strong acid or alkaline treatments. This different processing condition between the conventional and the new methods generates distinguishable protein conformation, characteristics, and composition in the resulting surimi. The gelation mechanism of acid- or alkali-treated fish proteins was significantly different from that of fish proteins prepared using the conventional method. The new methods, however, cannot inactivate proteolytic enzymes, specifically cathepsin L activities, as we hypothesized. However, alkaline treatment, especially at pH11 could demonstrate good gels, probably due to enhanced disulfide bonds formation.

The role of sarcoplasmic proteins is very important in acid- or alkali-aided surimi since the new methods can significantly retain this protein in surimi. Sarcoplasmic proteins appeared to be more stable against pH (especially neutral and alkaline) and salt than myofibrillar proteins. The least amount of sarcoplasmic proteins was lost when treated at pH 2 or 3 followed by precipitation at pH 5.5. Sarcoplasmic protein addition to pollock surimi, however, did not improve fracture texture properties, although neither did not interfere with the gelation of myofibrillar

proteins.

Salt negatively affected the gelation properties of acid- and alkali-treated surimi gels. Transglutaminase-mediated setting reaction was apparently inactivated by acidic or alkaline treatment. Further studies may be needed to investigate the role of cryoprotectants in this pH-drive protein recovery.

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