Properties of Edible Surimi Film as Affected by Heat Treatment of Film-Forming Solution

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The effect of heat treatment of film-forming solutions on the properties of edible surimi films was investigated. The film-forming solutions prepared at pH 3 from frozen Alaska pollack surimi were heated to 45, 70 or 100°C to promote unfolding of surimi protein molecules. As a result, solubility, surface hydrophobicity, and reactive SH group of surimi proteins increased. After 45°C-treatment, the mechanical properties, film solubility, and protein solubility of surimi films were not affected and myosin heavy chain (MHC) of surimi proteins was degraded by endogenous acid proteinases. Conversely, at higher heating temperatures (70°C, 100°C), degradation of MHC was effectively inhibited and mechanical properties were improved, while the film solubility and protein solubility of surimi films decreased. It is revealed that the prevention of MHC degradation by heat treatment could improve mechanical properties of surimi films. The optimum condition was found to be heating the film-forming solutions (pH 3) at 70°C for 20 min.

Keywords: Edible film, Mechanical properties, Heat treatment, Alaska pollack surimi

Introduction

During the past decades, synthetic polymer films have been used in a wide range of applications, particularly protecting products such as foods or pharmaceuticals. However, these films are not biodegradable, and their accumulation leads to environmental pollution with serious ecological consequences. With the increasing global population and stress on limited resources and the environment, use of renewable resources to produce edible and biodegradable films that can improve product quality and reduce waste problems has been explored.

Edible films can be prepared from polysaccharides, proteins, and lipids. In general, the purposes of preparing edible films are to inhibit the migration of moisture, gases, aroma and lipids, to carry food ingredients, to improve mechanical integrity of foods, and to reduce the quantity of packaging material required for food products (Krochta and Mulder-Johnston, 1997). Thus, edible films with good mechanical properties can replace synthetic polymer films. Therefore, it is preferable that the strength of surimi films could be improved to the strength of low-density polyethylene (LDPE) films (Shiku et al., 2003), which is one of lower strength synthetic polymer films. In addition, the O₂ permeability of protein based films is lower than synthetic polymer films or polysaccharide based films (Cuq et al., 1998).

Among materials to prepare edible films, proteins have been extensively utilized because of their relative abundance, film-forming ability, and nutritional qualities. Edible films based on fish proteins can be produced from sarcoplasmic proteins (Iwata et al., 2000; Tanaka et al., 2001), myofibrillar proteins (Cuq et al., 1995; Shiku et al., 2003), stroma proteins (Jongjareonrak et al., 2006), surimi proteins (Shiku et al., 2004; Weng et al., 2006), and muscle proteins (Paschoalick et al., 2003; Hamaguchi et al., 2007).

Sarcoplasmic proteins are globular proteins containing most of the hydrophobic and SH groups hidden in the interior of molecules. Formation of sarcoplasmic protein films prepared from blue marlin (Makaira mazara) has mainly involved thermal treatment of film-forming solutions at temperature ranging between 55°C and 90°C (Iwata et al., 2000). Myofibrillar proteins are normally insoluble in water, but can become soluble by controlling pH of the solutions. At pH values outside of the isoelectric range, proteins are denatured, unfolded and solubilized, thus exposing interactive groups. Dispersed myofibrillar proteins are capable of forming a continuous matrix during drying (Cuq et al., 1995; Shiku et al., 2003). Cuq et al. (1995) reported that the thermal treatment was not influential on myofibrillar protein films prepared from film-forming solutions at 10~40°C.

In a previous study, surimi films were successfully prepared from film-forming solutions with different pHs, and the effect of pH on mechanical properties of films was determined (Weng et al., 2006). As a result, it was revealed that fairly transparent films with good mechanical properties were successfully prepared from acidic film-forming solutions (pH 3). Furthermore, it was found that myosin heavy chain (MHC) of surimi proteins was degraded during the preparation of films due to the endogenous cathepsin D and cysteine proteinases. The concomitant decrease of surimi film strength with MHC degradation was also observed (Weng et al., 2007). MHC degradation
can be completely prevented by the addition of protease inhibitors such as pepstatin A and leupeptin. However, the use of these chemicals is not feasible for the production of edible surimi films from the standpoint of food safety. Heat treatment could thus be used as an alternative method to inhibit acid protease activities. At the same time, it is well-known that heat treatment modifies the three-dimensional structure of proteins, exposing SH and hydrophobic groups, which promote intra- or intermolecular S-S and hydrophobic interactions during the formation of protein films (Monahan et al., 1995; Perez-Gago and Krochta, 2001). Moreover, solubilization of proteins in the film-forming solution is an important factor to improve the mechanical properties of resulting edible films (Hamaguchi et al., 2007). However, there is little information concerning the thermal treatment of acidic film-forming solutions relating to the properties of edible protein films.

The objective of this study was to examine the effect of heat treatment of film-forming solutions on conformation and solubility of surimi proteins, mechanical properties of surimi films, and mechanism of film formation.

Materials and Methods

Materials  
Blocks (10 kg each) of ship-processed frozen Alaska pollack (*Theragra chalcogramma*) surimi (SA grade) were obtained from Maruha Co. (Tokyo, Japan) and stored at −50°C during the study. The surimi contained 16.5% protein, 4% sorbitol, 4% sucrose, and 0.3% sodium tripolyphosphate with 75% water content.

Determination of protein solubility and composition  
About 6 g of thawed surimi were stirred in 50 mL of distilled water at 1~5°C for 30 min. After the surimi solutions were dispersed thoroughly using a glass homogenizer (Sibata Scientific Technol. Co., Tokyo, Japan) and the pH of the solutions was adjusted to 3.0 with 1 M HCl, the solutions were heated in water baths held at 45, 70 or 100 °C for 5, 10, 15, 20, 25 or 30 min. Heated solutions were placed in an ice bath immediately after heating to avoid further denaturation. The surimi solutions thus prepared were centrifuged at 5000 × g for 30 min and the protein concentration in the supernatants was determined by the Lowry method (Lowry et al., 1951). In addition, the protein composition of the supernatants was determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE; Laemmli, 1970).

Surface hydrophobicity  
Surface hydrophobicity was determined using 1-anilino-8-naphthalene sulfonate (ANS; Wako Pure Chemical Ind. Co., Osaka, Japan) as a fluorescence probe. To 4 mL of the heated surimi solution (0.005% protein content), 40 μL of ANS solution (0.04% in 0.1 M phosphate buffer, pH 7.0) was added and incubated at 4°C for 10 min. After leaving at room temperature for 15 min, fluorescence intensities were measured at 365 nm (excitation) and 470 nm (emission) on a Shimadzu RF-1500 Fluorescence Spectrophotometer (Shimadzu Co., Kyoto, Japan). Surface hydrophobicity was expressed as the fluorescence intensity relative to that of the surimi solution without heating at pH 7.

Sulfhydryl (SH) content  
The SH group exposed on the surface of protein molecules was defined as reactive SH group (R-SH). The R-SH content of heated surimi solutions was determined by the method of Ellman (1959). One part of heated surimi solution was mixed with 19 parts of 10 mM EDTA in 0.1 M phosphate buffer (pH 6.8). To 4 mL of the mixture, 0.4 mL of 0.1% 5, 5′-dithiobis-2-nitrobenzoic acid (DTNB; Tokyo Kasei Kogyo Co., Tokyo, Japan) solution was added and incubated at 4°C for 1 h. The amount of R-SH was measured at 412 nm using a molar extinction coefficient of 13,600 M cm⁻¹. Total content of SH (T-SH) was determined by the Ellman’s reagent in 8 M urea-2% SDS (Runglerdkriangkrai et al., 1999).

Preparation of surimi films  
Film-forming solutions were prepared according to the method described in a previous study (Weng et al., 2006) with minor modifications. The supernatants of heated surimi solutions were mixed with distilled water to adjust the protein concentration to 2% (w/v). Glycerol was used as a plasticizer at the concentration of 30% (w/w) of protein. Air bubbles in solutions were removed by a Hybrid Mixer (HM-500; Keyence Co., Tokyo, Japan). The film-forming solution thus prepared (4 g) was then poured onto a rimmed silicone resin plate (50 × 50 mm) setting on a level surface and dried in a ventilated oven (Environmental Chamber, model H110 K-30DM; Seiwa Riko Co., Tokyo, Japan) at 25±0.5°C and 50±5% relative humidity (RH) for 24 h. After the water had been evaporated, the resulting films were manually peeled off.

Mechanical properties  
Film thickness was measured using a micrometer (Thickness Gage; Mitutoyo Co., Tokyo, Japan) to the nearest 0.005 mm at 6 random locations on the film. Precision of the thickness measurements was ±5%.

Prior to testing mechanical properties, films were conditioned for 48 h at 25±0.5°C and 50±5% RH. Tensile strength (TS) and percentage elongation at break (EAB) were determined using a Tensipresser (TTP-50BX II; Taketomo Electric Inc., Tokyo, Japan) operated according to ASTM standard method D 882-22 (ASTM, 1989). Two rectangular strips (width 20 mm; length 45 mm) were prepared from each film to determine their mechanical properties. Initial grip separation and mechanical crosshead speed were set at 30 mm and 1.0 mm/s, respectively.

SDS-PAGE  
SDS-PAGE was performed according to the method of Laemmli (1970). A 7.5% polyacrylamide gel (AE-6000, NPU-7.5L PAGE; Atto Co., Tokyo, Japan) was used. Gels were stained with 0.025% Coomassie Brilliant Blue R-250 (Merck, Darmstadt, Germany) in methanol/acetic acid/water (5:10:85%, v:v:v), then destained in methanol/acetic acid/water (30:10:60%, v:v:v). Page-Ruler™ Protein Ladder (Fermentas Life Sciences, Hanover, MD, USA) ranging from 10 to 200 kDa was used as a standard protein marker.

Film solubility and protein solubility in distilled water  
The solubility of surimi films in distilled water was determined according to the method of Shiku et al. (2003) with minor modifications. The initial dry matter of each film was determined by drying at 105°C for 24 h. The surimi
film samples were immersed in 10 mL of 0.1 M phosphate buffer (pH 7.0) with 0.02% (w/v) sodium azide. After immersion for 24 h at 30°C with continuous gentle stirring, undissolved surimi films were removed and dried at 105°C for 24 h. The weight of solubilized dry matter was calculated by subtracting the weight of insolubilized dry matter from the initial weight of dry matter. The protein concentration was determined by the Lowry method. Protein solubility was expressed as a percentage of total protein in the film solubilized at 30°C for 24 h.

**Protein solubility in various solvents** Surimi films were solubilized in four different solutions at pH 7 (Perezo-Mateos et al., 1997): 0.6 M NaCl (S1); 0.6 M NaCl + 1.5 M urea (S2); 0.6 M NaCl + 8 M urea (S3); and 0.6 M NaCl + 8 M urea + 0.5 M 2-mercaptoethanol (S4). Film powders (about 50 mg) were weighed and transferred into centrifuging tubes with 5 mL of four different solutions. The centrifuging tubes were gently shaken for 24 h at room temperature by using a reciprocal shaker. The solution was centrifuged at 9620 × g for 30 min and the protein concentration of the supernatant was determined in triplicate by the Lowry method.

**Statistical analysis** Statistical analysis on a completely randomized experimental design was performed using the General Linear Model procedure in the SPSS computer program (SPSS Statistical Software, Chicago, IL, USA). One-way analyses of variance (ANOVA) were carried out and mean comparisons were processed by Tukey-Kramer Honestly Significant Difference (HSD) test. Significance was defined as p<0.05.

**Results and Discussion**

**Effect of heating on solubility and SDS-PAGE pattern of surimi proteins** The film-forming solutions prepared at pH 3 from frozen Alaska pollack surimi were heated to 45, 70 or 100°C in order to inactivate endogenous acid proteinases in this study. Changes in the solubility of surimi proteins in distilled water at pH 3 during the heat treatment are shown in Fig. 1. According to a previous study (Weng et al., 2006), the solubility of surimi proteins in distilled water at pH 7 was about 10%, but it markedly increased to about 65% at pH 3. The highest solubility was attained by 100°C-heating for 20 min with a maximum value of approximately 100%, and the highest solubility at 45°C or 70°C was around 83% or 78%, respectively. These results clearly indicate that the solubility of surimi proteins can be noticeably improved by heat treatment at acidic pH. Surimi protein molecules become positively charged below their isoelectric points (pH 5.2~5.5; Weng et al., 2006), and the Brownian motion of protein molecules is intensified by heating. As a result of increased electrostatic repulsion between protein molecules, protein solubility by heating at pH 3 might be improved (Fig. 1).

The effect of heating temperature on the amount of MHC in the soluble surimi protein fractions obtained with different heat treatments was determined by SDS-PAGE (Fig. 2). At the optimum temperature of endogenous acid

![Fig. 1. Effect of heating temperature and time on the solubility of Alaska pollack surimi proteins in water at pH 3.](image)

![Fig. 2. SDS-PAGE patterns of film-forming solutions prepared from Alaska pollack surimi with different heating temperature and time.](image)

M: standard molecular weight mixture, MHC: myosin heavy chain.
Changes in total sulfhydryl groups (T-SH, mol/ g) and reactive sulfhydryl groups (R-SH, mol/ g) of surimi proteins by different heat treatment.

Values are mean ± standard deviation; n = 3. Any two means in the same row followed by the same letter are not significantly different (p > 0.05).

Table 1. Changes in total sulphydryl groups (T-SH, mol/10^3 g)* and reactive sulphydryl groups (R-SH, mol/10^3 g)* of surimi proteins by different heat treatment.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Control (pH 7)</th>
<th>Heating time (min)</th>
<th>0</th>
<th>5</th>
<th>10</th>
<th>15</th>
<th>20</th>
<th>25</th>
<th>30</th>
</tr>
</thead>
<tbody>
<tr>
<td>45</td>
<td>T-SH 7.5 ± 0.3*</td>
<td>7.5 ± 0.2*</td>
<td>7.5 ± 0.2*</td>
<td>7.4 ± 0.2*</td>
<td>7.4 ± 0.2*</td>
<td>7.5 ± 0.3*</td>
<td>7.4 ± 0.3*</td>
<td>7.5 ± 0.2*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>R-SH 3.9 ± 0.3*</td>
<td>4.7 ± 0.3*</td>
<td>5.9 ± 0.3*</td>
<td>6.9 ± 0.2*</td>
<td>7.4 ± 0.2*</td>
<td>7.5 ± 0.2*</td>
<td>7.5 ± 0.2*</td>
<td>7.5 ± 0.4*</td>
<td></td>
</tr>
<tr>
<td>70</td>
<td>T-SH 7.5 ± 0.3*</td>
<td>7.4 ± 0.2*</td>
<td>7.2 ± 0.3*</td>
<td>7.5 ± 0.2*</td>
<td>7.5 ± 0.3*</td>
<td>7.5 ± 0.2*</td>
<td>7.3 ± 0.2*</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>R-SH 3.9 ± 0.3*</td>
<td>4.6 ± 0.2*</td>
<td>6.4 ± 0.2*</td>
<td>7.2 ± 0.3*</td>
<td>6.9 ± 0.2*</td>
<td>6.9 ± 0.2*</td>
<td>7.1 ± 0.2*</td>
<td>7.2 ± 0.3*</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>T-SH 7.5 ± 0.3*</td>
<td>7.2 ± 0.3*</td>
<td>7.6 ± 0.3*</td>
<td>7.5 ± 0.2*</td>
<td>7.5 ± 0.3*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>R-SH 3.9 ± 0.3*</td>
<td>4.7 ± 0.1*</td>
<td>7.2 ± 0.3*</td>
<td>7.6 ± 0.3*</td>
<td>7.5 ± 0.2*</td>
<td>7.3 ± 0.3*</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Values are mean ± standard deviation; n = 3. Any two means in the same row followed by the same letter are not significantly different (p > 0.05).

proteinases (45°C; Weng et al., 2007), the intensity of the MHC band was apparently reduced by heating with the appearance of lower molecular weight bands at 130 ~ 150 kDa and 65 kDa, suggesting that degradation of MHC took place in the heated surimi solutions. However, degradation of MHC was suppressed by 70°C-heating. In the case of 100°C-heating, the degradation of MHC was prevented at the beginning, but MHC was degraded after 10 min of heating due to acid hydrolysis at pH 3. Furthermore, it is obvious from Figure 2 that high molecular weight fractions (HMWF) that were too large to enter the polyacrylamide gel were also broken down by endogenous acid proteinases. However, the increased formation of HMWF by 70°C-heating (Fig. 2) indicates the possibility of MHC polymerization by the heat treatment of film-forming solutions.

Effect of heating on surface hydrophobicity and reactive SH groups In order to ensure the unfolding of surimi protein molecules upon heating, surface hydrophobicity was measured by using ANS. The results (Fig. 3) indicate that continuous heating caused a concomitant increase in surface hydrophobicity at each heating temperature, and that surface hydrophobicity markedly increased at 45°C and 100°C after heating for 10 min and 30 min, respectively. The results obtained in this study are in agreement with our previous findings (Weng et al., 2007) that larger surface hydrophobicity was partly due to the degradation of MHC (Fig. 2). Furthermore, Lin and Park (1998) revealed that the solubility of myosin was improved when the surface hydrophobicity of protein molecules increased. Therefore, it can be concluded that the heat treatment of surimi solutions causes the unfolding of surimi proteins, thus increasing protein solubility at pH 3 (Fig. 1).

T-SH shows the oxidation of SH groups and changes in R-SH content represent the behavior of the surface SH groups of the molecules upon heating (Runglerdkriangkrai et al., 1999). T-SH was constant (about 7.5 mol/10^3 g protein) irrespective of pH or heating temperature (Table 1). In contrast, R-SH content at pH 7 was about 3.9 mol/10^3 g protein, but increased to about 4.7 mol/10^3 g protein at pH 3 (Table 1). Furthermore, R-SH content increased at each temperature during the initial heating period (Table 1), suggesting that the buried SH groups were easily exposed to the surface of protein molecules by pH adjustment or heating. Moreover, it was observed that the initial increasing rate of R-SH content by 100°C-heating was the
Heat Treatment of Edible Surimi Film

Table 2. Effect of heat treatment on tensile strength (TS) and elongation at break (EAB) of surimi films prepared at pH 3.0.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Heating time (min)</th>
<th>TS (MPa)</th>
<th>Heating time (min)</th>
<th>EAB (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>45</td>
<td>0</td>
<td>4.4 ± 0.5*</td>
<td>10</td>
<td>4.1 ± 0.3*</td>
</tr>
<tr>
<td>70</td>
<td>5</td>
<td>4.4 ± 0.5*</td>
<td>15</td>
<td>4.1 ± 0.3*</td>
</tr>
<tr>
<td>100</td>
<td>10</td>
<td>4.4 ± 0.5*</td>
<td>20</td>
<td>3.4 ± 0.2*</td>
</tr>
</tbody>
</table>

* Values are mean ± standard deviation; n = 10. Any two means in the same row followed by the same letter are not significantly different (p > 0.05).

Table 3. Film solubility and protein solubility of surimi films prepared from Alaska pollack surimi with different heat treatments.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Heating time (min)</th>
<th>Film solubility (%)</th>
<th>Protein solubility (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>45</td>
<td>0</td>
<td>21.3 ± 3.0*</td>
<td>18.6 ± 3.1*</td>
</tr>
<tr>
<td>70</td>
<td>5</td>
<td>19.7 ± 3.1*</td>
<td>16.14 ± 0.5*</td>
</tr>
<tr>
<td>100</td>
<td>10</td>
<td>21.0 ± 1.2*</td>
<td>12.1 ± 1.8*</td>
</tr>
</tbody>
</table>

* Values are mean ± standard deviation; n = 4. Any two means in the same row followed by the same letter are not significantly different (p > 0.05).

The content of R-SH became equal to the T-SH content of native surimi proteins by continuous heating (Table 1), indicating that the heat treatment of surimi solutions could induce surimi proteins to unfold completely at pH 3.

Effect of heating on mechanical properties of surimi films Table 2 presents TS and EAB of surimi films prepared from film-forming solutions heated at different temperatures at pH 3. In the case of 45°C-heating, no marked changes of TS and EAB were observed, irrespective of heating time. This result is in agreement with the report by Cuq et al. (1995), who investigated the influence of heating myofibrillar protein solutions of Atlantic sardine meat on the properties of edible films. In this study, the highest TS and EAB were obtained from film-forming solutions heated at 70°C for 20 min. However, TS and EAB increased by heating at 100°C for 5 min but decreased after 10 min of heating. It is known that protein denaturation can affect protein-protein interactions, physical properties, and polymer morphology of protein films. Improved physical properties through heat treatment were observed in whey protein films (Stuchell and Krochta, 1994), wheat gluten films (Ali et al., 1997), and pea protein films (Choi and Han, 2001). However, extensive heating reduced the strength of films prepared from tilapia muscle proteins (Garcia and Sobral, 2005). A similar tendency was observed in this study. It can be concluded that the heat treatment of surimi film-forming solutions at 70°C for 20 min is the optimum condition to improve the mechanical properties of edible surimi films.

Effect of heating on film solubility and protein solubility Film solubility can be viewed as measures of the water resistance and integrity of a film (Rhim et al., 2000). Table 3 presents film solubility and protein solubility as related to different heating treatments. Films were not dissolved or broken apart after 24 h of incubation at 30°C regardless of the heating condition of film-forming solutions, suggesting that the protein network of surimi films prepared in this study is quite stable and that films can be applied as barriers to control the transfer of moisture. The film solubility of surimi films was not significantly affected when prepared by heating at 45°C (Table 3). However, film solubility decreased by 70°C-heating but increased by 100°C-heating (Table 3). Moreover, the highest solubility of surimi films was brought about by 100°C-heating and the lowest solubility was attained by 70°C-heating. These results can lead us to conclude that the higher solubility of films is ascribable to a weaker structure (Fig. 2, Table 3).

The protein solubility of surimi films in distilled water was also investigated in order to predict the type of protein-protein interactions responsible for the formation of films, as proteins dissolved in distilled water are not strongly involved in the protein network (Mauri and
The protein solubility of surimi films prepared at pH 3 by 45°C-heating were unchanged during the treatment (Table 3), but decreased during the initial 10-min heating at 70°C. In the case of 100°C-heating, the protein solubility of films decreased at the beginning of heating, followed by the gradual increase (Table 3). From these results, together with SDS-PAGE patterns (Fig. 2) of the film-forming solutions and TS (Table 2) of surimi films, it is concluded that the mechanism of film formation may be changed with heating treatments. In addition, the difference between film solubility and protein solubility may also correspond with the presence of glycerol added to surimi films as a plasticizer.

Mechanism of surimi film formation To examine the protein composition of the films prepared from Alaska pollack surimi by heating at pH 3, SDS-PAGE patterns of protein subunits were determined (Fig. 4). In the case of 45°C-heating, MHC and actin were degraded to a large extent by endogenous acid proteinases in surimi films irrespective of heating time and their mechanical properties were not significantly changed as mentioned above (Table 2). Compared with Fig. 2, it is obvious that the degradation of MHC proceeded even during the film drying period at 25°C, with the appearance of the 65 kDa band. This band could be formed from the 130~150 kDa bands as the result of acid proteinase activity during the drying of film-forming solutions.

When the film-forming solutions were heated at 70°C for 5 min, it was found that MHC and actin were degraded (Fig. 4), suggesting that endogenous acid proteinases in surimi were not inactivated. The temperature of the film-forming solutions prepared by heating for 5 min at 70°C was approximately 50°C, which is not high enough to inhibit proteinase activity. However, it is of relevance to note that, after heating at 70°C for more than 10 min, there were no degradations of HMWF, MHC, and actin (Figs. 2,
The solubility of surimi films in the following four different denaturing solutions was determined to reveal the associative forces involved in the formation of surimi films prepared by different heat treatments. The denaturing solutions employed in this study were a 0.6 M NaCl solution (S1) that disrupts ionic bonds, a 0.6 M NaCl and 1.5 M urea solution (S2) that disrupts hydrogen bonds, a 0.6 M NaCl and 8 M urea solution (S3) that disrupts hydrophobic interactions, and a 0.6 M NaCl, 8 M urea and 0.5 M mercaptoethanol solution (S4) that disrupts disulfide bonds (Perez-Mateos et al., 1997). Figure 5 illustrates the protein solubility of surimi films in these denaturing solutions. In the case of 45°C-heating, surimi films were completely solubilized in S3, suggesting that the surimi films are formed through ionic bonds, hydrogen bonds, and hydrophobic interactions. Furthermore, the solubilities of surimi films in S1, S2 and S3 were almost constant regardless of heating time at 45°C, demonstrating that the main associative force for the formation of surimi films was hydrophobic interactions. In the case of 70°C-heating, the protein fraction soluble in S1 decreased slightly by the heat treatment and the insoluble fraction was detected by heating after 10 min. The same phenomenon was observed in surimi films prepared from the film-forming solution heated for 5 min at 100°C. The occurrence of the insoluble fraction was in coincidence with the presence of HMWF on SDS-PAGE (Fig. 4). However, the solubility in S3 was not improved by the addition of mercaptoethanol (S4), suggesting that SS/SH exchange reaction did not take place during the formation of surimi films at pH 3, irrespective of heating conditions. This could be due to lower reactivity of the sulfhydryl residues at an acid pH (Wada et al., 2006).

The above results lead to the following hypothesis for the film formation. When pH of the film-forming solution for edible surimi films is adjusted to 3 which is the optimum pH of endogenous acid proteinases in Alaska pollock surimi, the degradation of MHC initiates some exposure of sulfhydryl and hydrophobic groups. During heating at above 70°C, protein conformation is further disrupted, and sulfhydryl and hydrophobic groups are exposed completely. During the drying phase of film preparation, totally denatured surimi proteins then interact to form films, especially through hydrophobic interactions.

Conclusions

The unfolding of surimi protein molecules was promoted by heating the film-forming solutions at pH 3, thus increasing the solubility, surface hydrophobicity, and number of reactive SH group of surimi proteins. The mechanical properties, film solubility, and protein solubility of surimi films were not affected by the heat treatment of film-forming solutions at 45°C, although MHC of surimi proteins was degraded by endogenous acid proteinases. In contrast, the degradation of MHC was effectively inhibited by the heat treatment of film-forming solutions at higher temperatures (70°C and 100°C). Interactions between the hydrophobic amino acid residues gave rise to the improved mechanical properties of surimi films, leading to decreased film solubility and protein solubility of surimi films. However, excessive heating caused the reduction of film strength partly due to acid hydrolysis of MHC at pH 3. From the results obtained in this study, it is revealed that the prevention of MHC during the formation of edible surimi films is essential to improve mechanical properties, and the optimum condition is found to be heating acidic film-forming solutions (pH 3) at 70°C for 20 min.


