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Effect of endogenous acid proteinases on the properties of edible films prepared from Alaska pollack surimi

WuYin Weng, Patricia Yuca Hamaguchi, Kazufumi Osako, Munehiko Tanaka *

Department of Food Science and Technology, Tokyo University of Marine Science and Technology, Konan 4-5-7, Minato, Tokyo 108-8477, Japan

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Abstract

The existence of endogenous acid proteinases in Alaska pollack surimi and their effect on mechanical properties of surimi films were investigated. The optimum pH of acid proteinases involved in the degradation of myosin heavy chain (MHC) was 3.0, and the optimum temperature was 45 °C. The degradation of MHC was completely inhibited by pepstatin A together with any one of cysteine proteinase inhibitors, suggesting that acid proteinases present in surimi are mainly cathepsin D and cysteine proteinases. The concomitant decrease of surimi film strength with the extent of MHC degradation was observed, but surimi films were formed even when most of MHC was degraded. The main associative forces responsible for the surimi films prepared at pH 3.0 were ionic bonds and hydrophobic interactions.

Keywords: Acid proteinases; Surimi film; Myosin heavy chain; Hydrophobic interactions; Alaska pollack

1. Introduction

During the past decades, synthetic polymer films have been used in a wide range of application, such as protecting foods, pharmaceuticals, and other products. However, they are not biodegradable, and their accumulation leads to environmental pollution with serious ecological problems. With the increasing 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 purpose of preparing edible films is to inhibit the migration of moisture, gases, aromas, and lipids; and to carry food ingredients (Krochta & Mulder-Johnston, 1997). Thus, edible films with good mechanical properties can replace synthetic polymer films.

According to the Fisheries White Paper (Ministry of Agriculture, Forestry & Fisheries, Japan, 2006) published by the Japanese government, the production of frozen surimi and the consumption of surimi-based products have been gradually declining in Japan in recent years, partly due to the lack of the introduction of new products. In a previous study, preparation of edible/biodegradable films from frozen Alaska pollack surimi was successfully developed (Shiku, Hamaguchi, Benjakul, Visessanguan, & Tanaka, 2004). Surimi films were prepared from film-forming solutions with different pHs, and the effect of pH on mechanical properties of films was also determined (Weng, Hamaguchi, Shiku, & Tanaka, 2006). Under acidic conditions, it was revealed that the myosin heavy chain (MHC) in surimi proteins was degraded during the preparation of films, and hydrophobic interactions were the main associative forces involved for the formation of films (Weng et al., 2006). However, the effect of endogenous acid proteinases on the properties of edible surimi films has not been examined.

In this study, the type of acid proteinases in Alaska pollack surimi was first investigated. Secondly, in order to

* Corresponding author. Tel./fax: +81 3 5463 0611.
E-mail address: mune@kaiyodai.ac.jp (M. Tanaka).

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improve the mechanical properties of surimi films from acidic film-forming solutions, the effect of MHC degradation on physicochemical properties of edible/biodegradable surimi films was also investigated.

2. Materials and methods

2.1. Materials

2.1.1. Surimi

Blocks (10 kg each) of ship-processed frozen Alaska pollack (*Theragra chalcogramma*) surimi (SA grade) were obtained from Nichimo Co. (Tokyo, Japan) and stored at −30 °C during the study. This surimi contained 4% sorbitol, 4% sucrose, and 0.3% sodium tripolyphosphate with 75% water content.

2.1.2. Proteinase inhibitors used

Disodium dihydrogen ethylenediamine-*N*,*N*,*N*′,*N*′-tetraacetate (EDTA), ethylene glycol bis (β-aminoethylether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA), and *o*-phenanthroline were purchased from Wako Pure Chem. Ind. (Tokyo, Japan). Bean trypsin inhibitor (BTI), phenyl-methanesulphonyl fluoride (PMSF), benzamidine, *trans*-epoxysuccinyl-L-leucylamido (4-guanidino) butane (E64), antipain, leupeptin, and pepstatin A were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

2.2. Preparation of film-forming solutions

Film-forming solutions were prepared according to the method described in a previous study (Weng et al., 2006). The protein concentration of the film-forming solutions was fixed to 2% and glycerol as a plasticizer was added at 50% (w/w) of protein. After the surimi was stirred for 30 min in distilled water on ice, the film-forming solutions were dispersed thoroughly using a glass homogenizer (Sibata Scientific Technol. Co., Tokyo, Japan).

2.3. Degradation of surimi proteins

To investigate the degradation action of acid proteinases on surimi proteins at different pHs, the prepared film-forming solutions without any proteinase inhibitors were adjusted to pH 2.5–4.0 with 1 M HCl. Then the solutions were incubated in conical flasks at 35 °C for 90 min. After the incubation, 4 ml of 2% sodium dodecyl sulphate (SDS) – 8 M urea – 20 mM Tris–HCl (pH 8.8) mixture were added to 1 ml of the film-forming solution, and shaken overnight. The degradation of surimi proteins by acid proteinases at different pHs was observed by SDS-PAGE (Laemmli, 1970).

To investigate the effect of temperature on the degradation of surimi proteins, pH of the film-forming solutions was adjusted to 3.0 with 1 M HCl. Then the solutions were incubated at different temperatures (25, 30, 35, 40, 45, 50, 55 °C) for 150 min. With 20 min intervals, 1 ml of the film-forming solution was taken out and added into 4 ml of 2% SDS – 8 M urea – 20 mM Tris–HCl (pH 8.8) mixture. After SDS-PAGE was carried out, the SDS-PAGE gels were scanned by a scanner (Canoscan D 1230 U; Canon Co., Tokyo, Japan), and analyzed by a Scion Image Software (Scion Co., Frederick, MD, USA). The degradation rates of MHC by acid proteinases in surimi at different temperatures were determined by measuring the areas under the recorded peaks of MHC.

2.4. Inhibition of acid proteinases by different types of inhibitors

Different types of proteinase inhibitors were added into the prepared film-forming solutions before pH adjustment. After adjusting to pH 3 with 1 M HCl, the solutions were incubated at 35 °C for 90 min and the inhibition effect on the degradation of surimi proteins was detected by SDS-PAGE (Laemmli, 1970).

2.5. Surface hydrophobicity

Surface hydrophobicity was determined using 1-anilino-8-naphthalene sulphonate (ANS) (Wako Pure Chemical Ind. Co., Osaka, Japan) as a fluorescence probe (Kato & Nakai, 1980). To 4 ml of the film-forming solution (0.001% protein content), 40 μl of ANS solution (0.04% in 0.1 M phosphate buffer, pH 7.0) were 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 film-forming solution without inhibitors.

2.6. Film casting and drying

Air bubbles were removed from the film-forming solutions with and without proteinase inhibitors before casting by a HM-500 Hybrid Mixer (Keyence Co., Tokyo, Japan). Then the film-forming solutions thus prepared (4 g) was poured onto a rimmed silicone resin plate (50 × 50 mm) sitting on a level surface and dried in a ventilated oven at 25 ± 0.5 °C and 50 ± 5% relative humidity (RH) for 24 h (Environmental Chamber, model H110K-30DM; Seiwa Riko Co., Tokyo, Japan). After water had been evaporated, the resulting films were manually peeled off.

2.7. Mechanical properties

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

Prior to testing the 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-508X II, Taketomo Electric Inc., Tokyo, Japan) operated according to ASTM (1989) standard method D 882-22. Two rectangular strips (width 20 mm; length 45 mm) were prepared from each film to determine their mechanical properties. Initial grip separation and mechanical cross-head speed were set at 30 mm and 1.0 mm/s, respectively.

2.8. SDS-PAGE

SDS-PAGE was performed according to the method of Laemmli (1970). A 7.5% polyacrylamide gel (AE-6000, NPU-7.5L PAGEL, ATTO Co., Tokyo, Japan) was used. Film-forming solutions and films were dissolved in 2% SDS–8 M urea–2% mercaptoethanol–20 mM Tris–HCl (pH 8.8). 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). PageRuler™ Protein Ladder (Fermentas Life Sciences, Hanover, MD, USA) ranging from 10 to 200 kDa was used as a standard protein marker.

2.9. Protein solubility in various solvents

Surimi film were solubilized in four different solutions at pH 7 (Perez-Mateos, Lourenco, Montero, & Borderia, 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 (Lowry, Rosebrough, Farr, & Randall, 1951).

2.10. 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, USA). One-way analyses of variance (ANOVA) were carried out and mean comparisons (10 determinations) were run by Duncan's multiple range test (Stell & Torrie, 1980).

3. Results and discussion

3.1. Characterization of endogenous acid proteinases in surimi

In order to elucidate the existence of acid proteinases in Alaska pollack surimi and their effect on the degradation of surimi proteins, the film-forming solutions without any proteinase inhibitors were incubated at different pHs (2.5, 3.0, 3.5, 4.0 and 4.5) for 90 min at 35 °C. The results are shown in Fig. 1. Acidification of the film-forming solutions resulted in changes of SDS-PAGE patterns which were strongly pH dependent. As pH decreased below 4.5, a dramatic decrease in intensity of the MHC band was observed at pH 3.0–3.5 with the appearance of new bands at 130–150 kDa and 70 kDa. The degradation of MHC was most prominent at pH 3.0. On the other hand, actin was almost completely degraded below pH 3.0. As the reduction of protein bands in SDS-PAGE is an indicator of proteolytic activity (Yongsawatdigul, Park, Virulhakul, & Viratchakul, 2000), these results point to the action of acid proteinases on myofibrillar proteins of Alaska pollack surimi at the optimum pH 3.0. It is similar to the catheptic activity in muscle extracts of rex and petrale sole (Geist & Crawford, 1974) and to carp muscle cathepsin D (Makinodan, Akasaka, Toyohara, & Ikeda, 1982).

Since MHC represents 60% of muscle myofibrillar proteins (William & John, 1977), the extent of MHC degradation was determined to find the optimum temperature (Fig. 2). The optimum temperature for the degradation of MHC by acid proteinases in surimi was found to be around 45 °C at pH 3.0. According to Kang and Lanier (2000), eight lysosomal cathepsins, which are responsible for the intracellular protein degradation, are known to exist in fish muscle. Of eight lysosomal cathepsins, cathepsins D, E and L are acid proteinases. The optimum temperature of cathepsin D was reported to be 45 °C for three species of Pacific sole (Geist & Crawford, 1974), and 50 °C for carp (Makinodan et al., 1982). On the other hand, cathepsin E does not have proteolytic activity against intact fish muscle proteins (Yamashita & Konagaya, 1992), and cathepsin L has an optimum temperature of 55 °C (An, Weerasinghe, Seymour, & Morrissey, 1994). From the results of optimum pH and temperature, it can be also

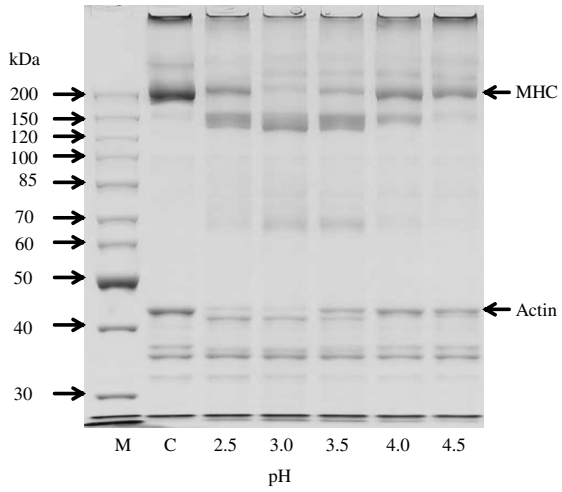

Fig. 1. Effect of pH on SDS-PAGE patterns of the film-forming solutions prepared from Alaska pollack surimi. M: marker, C: the film-forming solutions prepared from Alaska pollack surimi at pH 7.

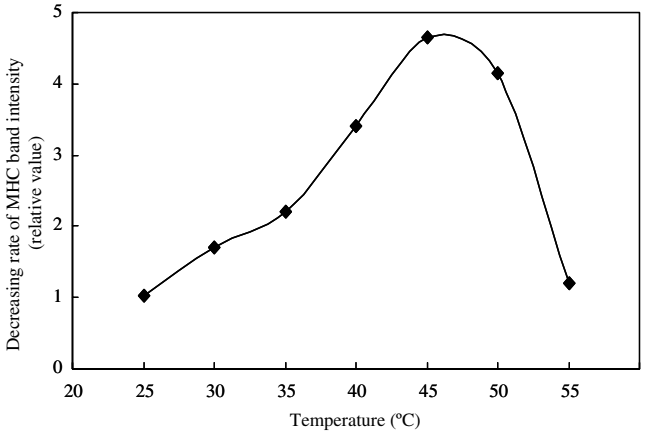

Fig. 2. Effect of temperature on the decreasing rate of MHC band intensities.

concluded that one of endogenous acid proteinases in Alaska pollack surimi is cathepsin D.

In order to further characterize acid proteinases in surimi responsible for the degradation of surimi proteins, various proteinase inhibitors (the final concentration is shown in Table 1) were added into the film-forming solutions. As depicted in Fig. 3, the addition of disodium dihydrogen ethylenediamine-*N*,*N*,*N*′,*N*′-tetraacetate (EDTA), ethylene glycol bis(β-aminoethylether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA) or phenanthroline did not alter the SDS-PAGE patterns, indicating that endogenous acid proteinases in surimi do not belong to a family of metalloproteinases. Additionally, serine proteinase inhibitors, such as BTI, PMSF and benzamidine, did not cause any inhibition on the degradation of MHC and actin. On the other hand, antipain, E64 and leupeptin which are cysteine proteinase inhibitors suppressed the degradation of MHC to some extent. Pepstatin A, an inhibitor for aspartic proteinases, gave considerable inhibition on MHC and actin degradation, indicating the presence of cathepsin D which is a carboxyl endopeptidase. The same phenomenon was reported in the previous study of carp muscle cathepsin D using pepstatin A (Makinodan et al., 1982). It is of relevance to note that there was no degradation of MHC bands in SDS-PAGE when leupeptin and pepstatin A were both present in the film-forming solutions. The similar SDS-PAGE patterns were also observed by the combined addition of E64 and pepstatin A, or antipain and pepstatin A (data not shown). These results strongly suggest that endogenous acid proteinases in surimi are a mixture of cathepsin D and cysteine proteinases, and these data agree with earlier findings by Saunders (1994) on the degradation of proteins in mutton, beef and chicken muscles.

3.2. Effects of proteinase inhibitors on properties of surimi films

Table 1 presents tensile strength (TS) and elongation at break (EAB) of surimi films prepared with and without dif-

Table 1
Effect of proteinase inhibitors on tensile strength (TS) and elongation at break (EAB) of surimi films prepared at pH 3.0[A]

	Control	EDTA	EGTA	Phenanthroline	BTI	PMSF	Benzamidine	E64	Antipain	Leupeptin	Pepstatin A	Leupeptin + Pepstatin A
Concentration	–	5 mM	5 mM	5 mM	100 μg/ml	5 mM	5 mM	0.005 mM	0.005 mM	0.01 mM	0.01 mM	0.01 + 0.01 mM
TS (MPa)	4.7 ± 0.3^{a}	4.8 ± 0.3^{a}	4.6 ± 0.2^{a}	4.5 ± 0.1^{a}	4.9 ± 0.2^{a}	4.9 ± 0.3^{a}	4.8 ± 0.2^{a}	5.0 ± 0.4^{ab}	5.2 ± 0.4^{ab}	5.5 ± 0.5^{ab}	5.6 ± 0.7^{b}	7.1 ± 0.4^{c}
EAB (%)	158.3 ± 7.2^{a}	158.3 ± 12.0^{a}	137.9 ± 17.3^{b}	139.9 ± 20.3^{b}	150.8 ± 15.6^{a}	155.2 ± 14.5^{a}	154.6 ± 14.0^{a}	138.7 ± 18.3^{b}	145.3 ± 15.9^{ab}	161.6 ± 8.7^{a}	154.2 ± 15.6^{a}	146.7 ± 13.2^{ab}

[A] Means ± standard deviation. Any two means in the same row followed by the same letter are not significantly different ($p > 0.05$).

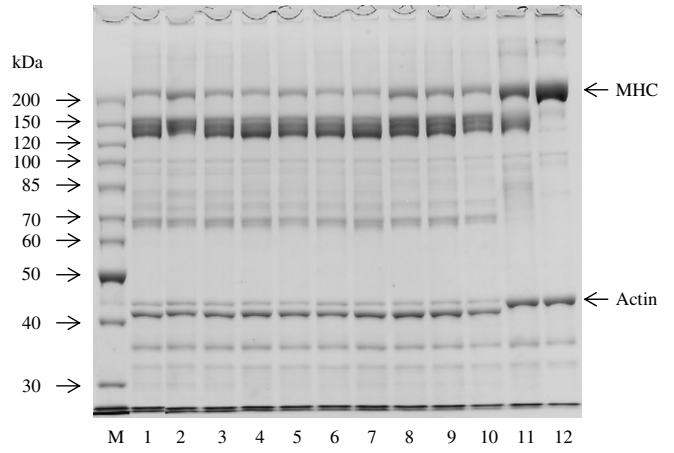

Fig. 3. SDS-PAGE of the film-forming solutions prepared from Alaska pollack surimi treated with different proteinase inhibitors. M: marker, 1: control; 2: EDTA; 3: EGTA; 4: phenanthroline; 5: BTI; 6: PMSF; 7: benzamidine ; 8: antipain; 9: E64; 10: leupeptin; 11: pepstatin A; 12: pepstatin A and leupeptin.

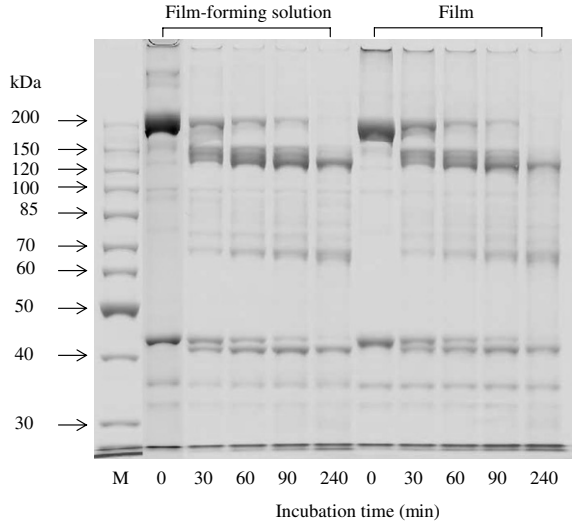

Fig. 4. Patterns of protein components during the incubation at 35 °C of the film-forming solutions prepared from Alaska pollack surimi and the surimi films.

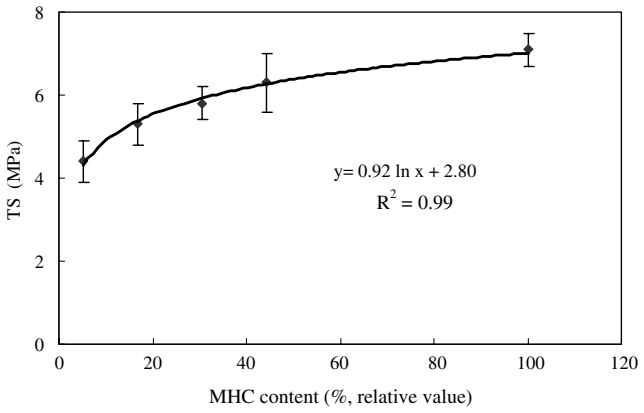

Fig. 5. Relation between the tensile strength (TS) of surimi films and the content of myosin heavy chain (MHC).

ferent types of proteinase inhibitors at pH 3.0. Compared to the control film without inhibitors, TS were not altered by the addition of metal proteinase inhibitors or serine proteinase inhibitors to the film-forming solutions. On the other hand, TS increased slightly with the addition of cysteine proteinase inhibitors or pepstatin A in the film-forming solutions. When cysteine proteinase inhibitors were present together with pepstatin A in the surimi film-forming solution, TS became the highest. These results suggest that TS of surimi films is related to the degradation extent of MHC. On the other hand, any significant effect of inhibitors was not detected on EAB of surimi films. Generally, the increase of TS is accompanied with reduction of EAB (Gennadios, Weller, & Testin, 1993). However, these tendencies were not observed in this study.

To reveal the relation of the degradation extent of MHC to the mechanical properties of surimi films, the films with different contents of MHC were prepared. The surimi films with no MHC degradation was obtained by the addition of both pepstatin A and leupeptin into the film-forming solution before pH adjustment (pH 3.0) and incubation at 35 °C for 240 min. The surimi films with various extents of MHC degradation were prepared by adding pepstatin A and leupeptin into the prepared pH 3.0 film-forming solutions after incubating at 35 °C for 30, 60, 90 and 240 min. Protein subunits of surimi films were analyzed by SDS-PAGE (Fig. 4). It is obvious from Fig. 4 that the degradation of MHC took place during the incubation at 35 °C of the film-forming solutions, but it was not significant during the drying process of film formation.

Fig. 5 shows the relationship between TS of surimi films and the relative content of MHC in surimi films. A high correlation between TS and MHC content ($r^2 = 0.99$) was observed and expressed by the following equation: TS (MPa) = 0.92 ln (relative MHC content) +2.80. However, surimi films maintained relatively high TS even when most of MHC was degraded (Fig. 5). Thus, certain factors other than MHC matrix formation seemed to contribute the strength development of the surimi film.

3.3. Mechanism of film formation

The distribution and extents of inter- and intramolecular interactions, which give rise to a three-dimensional network structure of the films, should affect their mechanical properties. Therefore, the solubility of the films in the following four different denaturing solutions was determined to reveal the main associative force involved in the film formation at pH 3.0 (Fig. 6). The solutions employed in this study were 0.6 M NaCl solution (S1) that disrupts ionic bonds, 0.6 M NaCl and 1.5 M urea solution (S2) that disrupts hydrogen bonds, 0.6 M NaCl and 8 M urea solution (S3) that disrupts hydrophobic interactions, and 0.6 M NaCl, 8 M urea and 0.5 M mercaptoethanol solution (S4) that disrupts disulphide bonds. As given in Fig. 6, S1 and particularly S3 increased with increasing incubation time

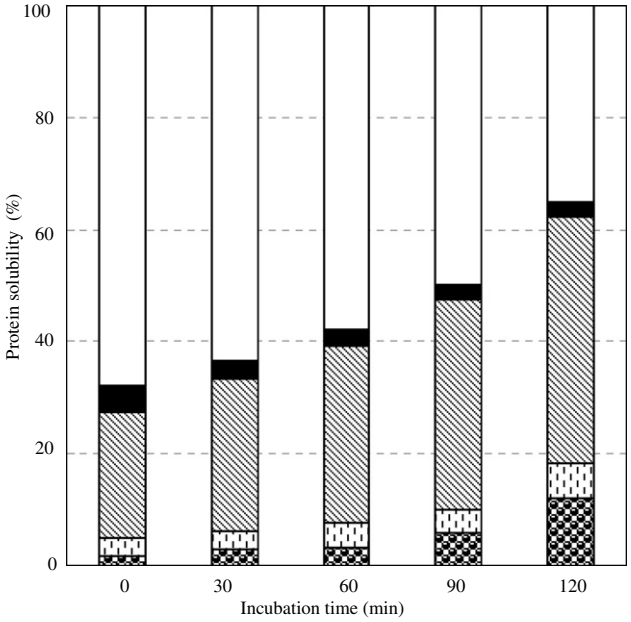

Fig. 6. Protein solubility of surimi films with different MHC contents in various protein denaturant solutions. ▩ S1: 0.6 M NaCl; ▨ S2: 0.6 M NaCl + 1.5 M urea; ▧ S3: 0.6 M NaCl + 8.0 M urea ■ S4: 0.6 M NaCl + 8.0 M + 0.5 M 2-mercaptoethanol; □: Insoluble fraction.

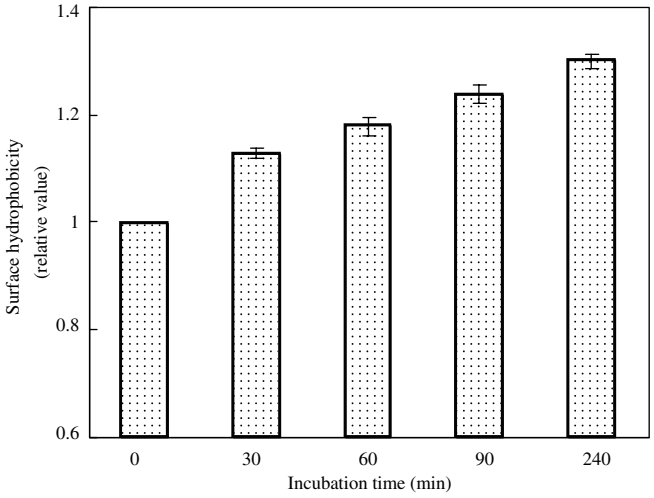

Fig. 7. Changes of relative surface hydrophobicity during the incubation at 35 °C of the film-forming solutions prepared from Alaska pollack surimi.

at 35 °C, suggesting that ionic bonds and hydrophobic interactions played an important role for the formation of surimi films when MHC was degraded. On the contrary, hydrogen bonds and disulfide bonds were not significantly involved in the formation of surimi films at pH 3.0, irrespective of the MHC degradation. As the results of MHC degradation, the proportion of insoluble fraction of protein in surimi films decreased (Fig. 6), and the main associative force for the formation of surimi films became ionic bonds and hydrophobic interactions.

In order to further clarify the involvement of hydrophobic interactions, the surface hydrophobicity of the film-forming solutions was measured (Fig. 7). It is apparent from Fig. 7 that the surface hydrophobicity increased during the incubation at 35 °C due to the breakdown of MHC molecules. Nielsen and Nielsen (2001) reported that the digestion of β-chain of oxidized insulin by cathepsin D resulted in preferential cleavage at bulky hydrophobic amino acids. Moreover, Schwenke (1997) also revealed the exposure of buried hydrophobic groups as a result of limited proteolysis of proteins, which may accelerate the formation of the hydrophobic–hydrophobic interaction. It can be concluded that the hydrophobic interactions are the main associative force for the formation of surimi films, especially when MHC were degraded by acid proteinases to a larger extent. The results obtained in this study lead to the following hypothesis for the formation of surimi films. Surimi proteins are unfolded at pH 3.0 and expose more hydrophobic groups to water. Furthermore, the degradation of surimi proteins especially MHC by endogenous acid proteinases promotes more exposure of hydrophobic groups, which extend out of water into air at the water/air interface. As water evaporates from the surface of film-forming solutions, the protein concentration increases and protein–protein interactions particularly hydrophobic interactions can be accelerated. This conclusion indicates that the interface of the surimi film facing to the air might have different moisture affinity from that facing to the silicone sheet, suggesting that the film has two surfaces of hydrophobic and hydrophilic properties.

4. Conclusions

The existence of endogenous acid proteinases in Alaska pollack surimi and their effect on the mechanical properties of surimi films were investigated. The optimum pH of acid proteinases involved in the degradation of MHC was 3.0, and the optimum temperature was 45 °C. The degradation of MHC was completely inhibited by pepstatin A together with any one of cysteine proteinase inhibitors, suggesting that acid proteinases present in surimi are mainly cathepsin D and cysteine proteinases. The results also suggested that theses enzymes are strongly associated with the myofibrillar proteins and are not removed by extensive washing during the production of frozen surimi (Cuq, Aymard, Cuq, & Guilbert, 1995). The strength of surimi films was related to the content of MHC, but films were formed even when most of MHC was degraded. The main forces involved in the formation of surimi films at pH 3.0 were ionic bonds and hydrophobic interactions.

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References

American Society for Testing and Materials (1989). *Annual book of ASTM standards*. Philadelphia, USA: ASTM.

An, H., Weerasinghe, V., Seymour, T. A., & Morrissey, M. T. (1994). Cathepsin degradation of Pacific whiting surimi proteins. *Journal of Food Science, 59*, 1013–1017.

Cuq, B., Aymard, C., Cuq, J. L., & Guilbert, S. (1995). Edible packaging films based on fish myofibrillar proteins: Formulation and functional properties. *Journal of Food Science, 60*, 1369–1374.

Geist, G. M., & Crawford, D. L. (1974). Muscle cathepsins in three species of Pacific sole. *Journal of Food Science, 39*, 548–551.

Gennadios, A., Weller, C. L., & Testin, R. F. (1993). Property modification of edible wheat gluten-based films. *American Society of Agricultural Engineers, 32*, 465–470.

Kang, I. C., & Lanier, I. S. (2000). Heat-induced softening of surimi gels by proteinases. In *Surimi and Surimi Seafood* (pp. 445–474). New York: Marcel Dekker.

Kato, A., & Nakai, S. (1980). Hydrophobicity determined by a fluorescence probe method and its correlation with surface properties of proteins. *Biochimica and Biophysica Acta, 624*, 13–20.

Krochta, J. M., & Mulder-Johnston, C. D. (1997). Edible and biodegradable polymer films: Challenges and opportunities. *Food Technology, 51*, 61–74.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature, 227*, 680–685.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry, 193*, 265–275.

Makinodan, Y., Akasaka, T., Toyohara, H., & Ikeda, S. (1982). Purification and properties of carp muscle cathepsin D. *Journal of Food Science, 47*, 647–652.

Ministry of Agriculture, Forestry & Fisheries, Japan (2006). Annual report on the developments in the fisheries industry in FY2005. Fisheries Policy Department, Fisheries Agency.

Nielsen, L. B., & Nielsen, H. H. (2001). Purification and characterization of cathepsin D from herring muscle (*Clupea harengus*). *Comparative Biochemistry and Physiology, 128B*, 351–363.

Perez-Mateos, M., Lourenco, H., Montero, P., & Borderia, J. (1997). Rheological and biochemical characteristics of high-pressure and heat-induced gel from blue whiting muscle proteins. *Journal of Agricultural and Food Chemistry, 45*, 44–49.

Saunders, A. B. (1994). The effect of acidification on myofibrillar proteins. *Meat Science, 37*, 271–280.

Schwenke, K. D. (1997). Enzyme and chemical modification of proteins. In S. Damodaran & A. Paraf (Eds.), *Food Proteins and Their Application* (pp. 393–423). New York: Marcel Dekker.

Shiku, Y., Hamaguchi, P. Y., Benjakul, S., Visessanguan, W., & Tanaka, M. (2004). Effect of surimi quality on properties of edible films based on Alaska pollack. *Food Chemistry, 86*, 493–499.

Stell, R. D. D., & Torrie, J. H. (1980). *Principle and procedure of statistics: A biometrical approach*. New York: Mc Graw-Hill, pp. 862.

Weng, W. Y., Hamaguchi, P. Y., Shiku, Y., & Tanaka, M. (2006). Preparation and characterization of edible films based on Alaska pollack surimi. *Nippon Shokuhin Kagaku Kogaku Kaishi, 53*, 103–108.

William, N. S., & John, W. C. B. (1977). Degradation of myofibrillar proteins by cathepsins B and D. *Biochemical Journal, 167*, 811–820.

Yamashita, M., & Konagaya, S. (1992). Differentiation and localization of catheptic proteinases responsible for extensive autolysis of mature chum salmon muscle (*Oncorhynchus keta*). *Comparative Biochemistry and Physiology, 103B*, 999–1003.

Yongsawatdigul, J., Park, J. W., Virulhakul, P., & Viratchakul, S. (2000). Proteolytic degradation of tropical tilapia surimi. *Journal of Food Science, 65*, 129–133.