Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 104 (2007) 702–708
Food Chemistry
www.elsevier.com/locate/foodchem

Hydrolysis of surimi paste from walleye pollock (*Theragra chalcogramma*) by cysteine proteinase cathepsin L and effect of the proteinase inhibitor (E-64) on gelation

Yaqin Hu *, Katsuji Morioka, Yoshiaki Itoh

Laboratory of Aquatic Product Utilization, Graduate School of Agriculture, Kochi University, Monobeotsu 200, Nankoku, Kochi 783-8502, Japan

Received 7 August 2006; received in revised form 27 October 2006; accepted 1 December 2006

Abstract

The gel strength of kamaboko obtained from walleye pollock (*Theragra chalcogramma*) was much reduced when the surimi paste was incubated at 60 °C for 2 h. The proteinase inhibitor, E-64, could enhance the gel strength and, at 200 μg/g of surimi, appeared to be the most effective. Amino acid analysis of the corresponding kamaboko showed that E-64 suppressed the release of peptides. Electrophoretograms revealed the inhibition of MHC degradation by E-64. Actomyosin was extracted and subjected to Sepharose 6B gel filtration to obtain the actomyosin non-binding cathepsin L (L_{mix}). Studies of substrate specificity and the effect of activators and inhibitors confirmed that the thiol–cysteine enzyme obtained was crude cathepsin L. Its high heat-stability indicated its strong hydrolytic ability. L_{mix}, at 0.6 unit/g of surimi, greatly decreased walleye pollock kamaboko gel strength from 112 to 27.8 g/cm^2 when incubated at 60 °C for 2 h. The degradation was effectively inhibited by E-64 at 200 μg/g of surimi and the gel strength of the corresponding kamaboko was increased to 302 g/cm^2. The above results suggested that cathepsin L contributed to the modori phenomena in kamaboko processed from walleye pollock surimi.
© 2007 Elsevier Ltd. All rights reserved.

Keywords: Cathepsin L; Cysteine protease; Walleye pollock surimi; Hydrolysis; E-64; Gel strength

1. Introduction

The textural characteristics of kamaboko developed during the gelation of surimi are normally expressed as gel strength, which is the primary determinant for kamaboko quality and price. Surimi gel strengthening can generally be achieved by subjecting sols to setting at temperatures ranging from 0 to 40 °C prior to heating (An, Peters, & Seymour, 1996; Kamath, Lanier, Foegeding, & Hamann, 1992). The gelation and textural strengthening of salted surimi paste at temperatures of 0–40 °C is termed 'setting' (Lanier, 1992). Generally, setting can be performed at low (0–4 °C), medium (25 °C) or high (40 °C) temperatures (Benjakul, Visessanguan, & Leelapongwattana, 2003). High temperature setting is widely used to improve the gel property of surimi because a shorter processing time is required. However, protein degradation, induced by proteinases commonly active at 50–60 °C, can occur (Benjakul, Visessanguan, & Chantarasuwan, 2004). This leads to gel structure disintegration or softening, generally termed 'modori' (Jiang, 2000). Such proteolysis degradation of myofibrillar proteins has a detrimental effect on surimi quality and substantially lowers the gel strength (An et al., 1996).

Walleye pollock surimi (*Theragra chalcogramma*) is widely used in kamaboko processing. Kamath et al. (1992) reported that maximum production of cross-linked polymers occurred at the optimum setting temperature of 25 °C for walleye pollock surimi. While proteolysis of MHC occurred at all setting temperatures ranging from 4 to 80 °C, it was most marked between 50 and 60 °C. Thus,

* Corresponding author. Present address: College of Biological and Environmental Engineering, Zhejiang University of Technology, Hangzhou, Zhejiang 310014, PR China. Fax: +81 88 8645155.
E-mail address: huyaqin@yahoo.co.jp (Y. Hu).

0308-8146/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2006.12.032

it is crucial to elucidate the endogenous enzymes involved in the protein breakdown of walleye pollock surimi.

Lysosomal cathepsin L is well known to participate in the muscle softening of chum salmon during spawning migration (Yamashita & Konagaya, 1990). A cysteine protease was reported to contribute to muscle softening of arrowtooth flounder (Wasson, Babbit, An, & French, 1992). Cathepsin L remained in surimi and degraded myosin heavy chain in Pacific whiting (An, Weerasinghe, Seymour, & Morrissey, 1994). Previously, we found that cathepsin L still remained in actomyosin, despite its non-binding to actomyosin (Hu, Morioka, & Itoh, in press). The objective of this study was to examine the degradation, by cathepsin L, of walleye pollock surimi during processing into kamaboko.

2. Materials and methods

2.1. Chemicals and surimi material

Z-Phe-Arg-MCA, Z-Arg-Arg-MCA, Arg-MCA, Boc-Gln-Ala-Arg-MCA, and L-*trans*-epoxy-succinyl-leucylamido-(4-guanidino)-butane (E-64) were purchased from Peptide Institute Inc. (Osaka, Japan). All the other chemicals were of analytical grade. Frozen walleye pollock (*T. chalcogramma*) surimi (SS1 grade) was provided by a local surimi producer in Kochi, Japan and was transferred to the laboratory in ice. Frozen surimi was immediately used or kept at −80 °C for future use.

2.2. Actomyosin extraction

Actomyosin was prepared using the previous method (Hu et al., in press). Surimi was washed three times with low ionic strength buffer and was then treated with one cycle of dilution–precipitation. The sample of actomyosin obtained was resuspended in 0.6 mM NaCl–50 mM phosphate buffer (pH 7.0) and was ready for gel filtration.

2.3. Determination of protein concentration

Protein concentration was assayed by the Biuret method (Robinson & Hodgen, 1940) with bovine albumin as standard.

2.4. Assay of enzyme activity

Enzyme activity was measured by the previous method (Hu et al., in press), using a specific substrate of Z-Phe-Arg-MCA (Barrett & Kirschke, 1981). One unit was defined as 1 nmol AMC liberated within 30 min at 25 °C.

2.5. Sepharose 6B gel filtration of actomyosin (AM)

Sepharose 6B gel filtration was performed as previously reported (Hu et al., in press). The actomyosin solution was loaded onto an Excell SD450 column (2.6 × 40 cm) which was packed with Sepharose 6B (Pharmacia Fine Chemicals). The column was eluted at 0.5 ml/min. Fractions were collected (10 ml/tube) by a fraction-collector (Gilson 202, France). Cathepsin L activity was monitored in each fraction.

2.6. Surimi processing into kamaboko

Walleye pollock surimi was mixed with E-64 at 0, 200, 400, 600 and 800 μg/g surimi, respectively, and was then treated with NaCl to a final concentration of 2.5%. The final moisture content was adjusted to 80%. The resulting surimi was ground at 4 °C for 20 min using a mortar and pestle. Ground surimi was stuffed into stainless steel rings (diameter, 3.0 cm) and incubated at 60 °C for 2 h. Gel strength was estimated as the product of the tensile strength and the breaking extension (Itoh, Maekawa, Suwansakornkul, & Obatake, 1995).

2.7. Amino acid analysis of the trichloroacetic acid extract of kamaboko gel

Free amino acids and acidic soluble peptides were determined by the method of Itoh, Maekawa, Suwansakornkul, and Obatake (1997). Three grammes of heated gel were homogenized with 15 ml of 5% trichloroacetic acid solution at 10,000 rpm for 5 min using a Ace Homogenizer (Nihonseiki Kaisha). The filtrate of the resulting homogenate was used as an extract. The free amino acids were determined with a Hitachi 835 Autoaminoanalyzer. A portion of the extract was hydrolyzed in 6 M HCl at 110 °C for 24 h. The total amount of acid-soluble peptides was estimated by subtracting the total amount of free amino acids in the unhydrolyzed extract from that in the hydrolyzed one.

2.8. SDS–PAGE

The kamaboko gel was solubilized with 8 M urea, containing 2% SDS and 10% mercaptoethanol. The resulting surimi and actomyosin samples were treated with SDS-treatment solution (1:1, v/v). SDS-treatment solution was made of 0.05 M sodium phosphate buffer, containing 0.4% sodium dodecyl sulfate (SDS), 50% glycerol, 0.05% bromophenol blue (BPB) and 20% 2-mercaptoethanol. An aliquot of 10–40 μl from each sample was subjected to SDS–PAGE, using 5% polyacrylamide gel according to the method of Weber and Osborn (1969). Protein was stained with Coomassie Brilliant Blue R-250.

3. Results and discussion

3.1. Effect of E-64 on the gelation of walleye pollock surimi

Walleye pollock (fish within 24 h after death) was reported to contain a sacoplasmic-60 °C-modori inducing protease that induced the gel degradation and the breakdown of myosin heavy chain (Kinoshita, Toyohara, & Shi-

mizu, 1990). Liu, Kanoh, and Niwa (1996) found that cysteine inhibitors, such as *N*-ethylmaleimide and iodoacetamide, could suppress the formation of a protein component of 150 kDa which was presumed to be formed by the catalysis of a coexisting cysteine protease in Alaska pollock surimi.

To investigate whether cathepsin(s) are involved in the degradation of the protein, walleye pollock surimi was heated at 60 °C for 2 h in the presence or absence of E-64 and the gel strength was measured. E-64 was used because it and its analogues are well known to be the specific inhibitors of cysteine proteinases, including cathepsins B, H and L (Barrett et al., 1982). In this study, E-64 could increase the gel strength of walleye pollock kamaboko in a concentration-dependent manner (Fig. 1). Further increments of the specific inhibitor showed an overdose effect on kamaboko gel strength. The most suitable amount of E-64 appeared to be 200 μg/g surimi.

The kamaboko obtained was subjected to amino acid analysis to examine the internal changes of protein. Fig. 2 shows the effect of E-64 on the amino acids in the corresponding kamaboko. In the heated control, peptides and free amino acids increased more obviously than they did in unheated control, and the main amino acids in the released peptides were glycine, alanine, lysine, glutamic acid, serine and isoleucine (data not shown). The increase was efficiently suppressed by the addition of E-64. And the inhibition was enhanced by increasing the amount of E-64.

For further investigation of the protein degradation occurring in the kamaboko, SDS–PAGE was performed. Compared to that of control, after heating the sample at 60 °C for 2 h, the intensity of the MHC (myosin heavy chain) band on SDS–PAGE gel was substantially reduced. Many minor bands appeared between the bands of MHC and AC, indicating severe degradation of MHC because of hydrolysis by endogenous enzymes (Fig. 3, lane 0). E-64, at 200 μg/g of surimi, could effectively inhibit the degradation according to the electrophoretogram (Fig. 3, lane 200). Further increase of E-64 did not lead to any further inhibition effect (Fig. 3, lane 400, 600 and 800).

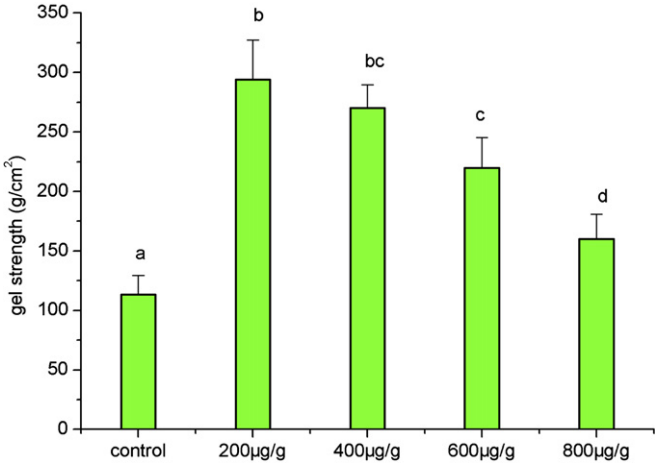

Fig. 1. Effect of E-64 on the gel strength of kamaboko. Lowercase a, b, c, d are means of five determinations. Different letters are significantly different ($p < 0.05$). To walleye pollock surimi, appropriate amount of E-64 (μg/g surimi) was added, and the moisture was adjusted to 80% and NaCl to 2.5%, before incubation at 60 °C for 2 h.

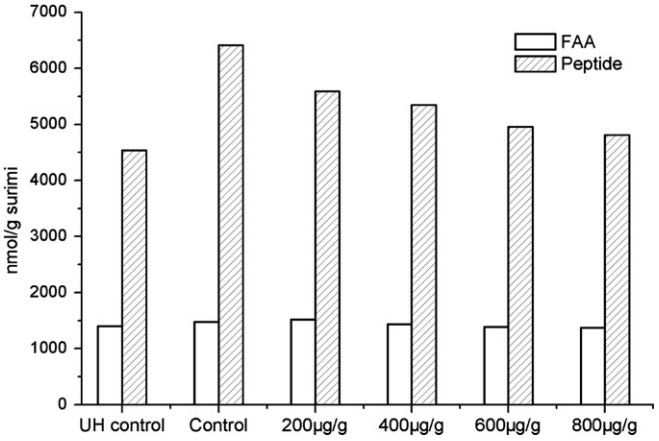

Fig. 2. Effect of E-64 on the total FAA and total acidic soluble peptides in kamaboko. Walleye pollock surimi was incubated with 200, 400, 600, 800 μg E-64/g surimi at 60 °C for 2 h. UH control: unheated control; FAA: free amino acid.

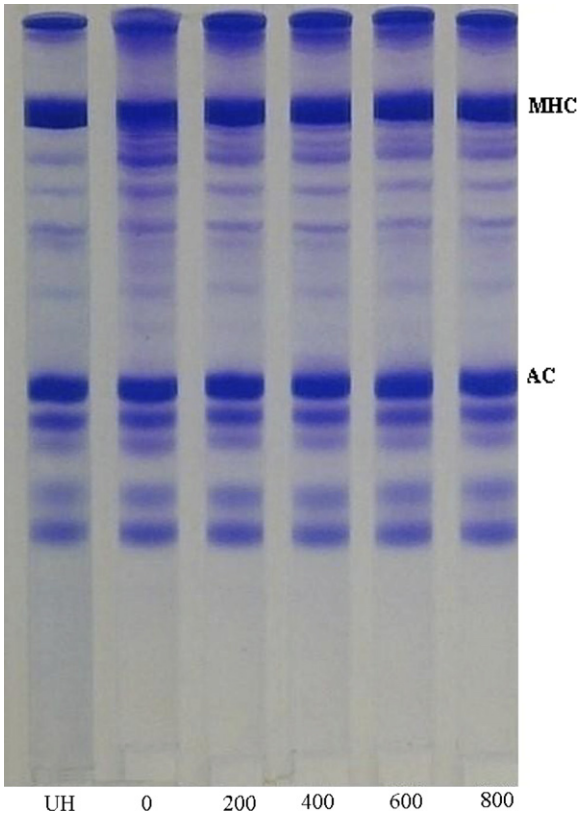

Fig. 3. SDS–PAGE patterns on E-64 inhibition of the autolysis in kamaboko manufacture. MHC: myosin heavy chain; AC: actin; UH: unheated control; 0: heated control; 200, 400, 600 and 800 mean that, to these samples, 200, 400, 600 and 800 μg E-64/g surimi were added. The surimi was heated at 60 °C for 2 h.

From the above results, we could conclude that cysteine type proteases, including cathepsins B, H and L, are involved in the degradation of protein in walleye pollock surimi. On the other hand, E-64 could not stop the degradation of MHC completely, suggesting that there could be other types of hydrolytic protease(s) involved in walleye pollock surimi as well as the cysteine type.

3.2. L_{mix} and some characterizations

Actomyosin was extracted and subjected to Sepharose 6B gel filtration. We previously reported that the main peak of cathepsin L was obviously separated from that of actomyosin (Fig. 4). Fractions showing the activity of cathepsin L were pooled and referred to as partially purified cathepsin L (L_{mix}). Its optimal temperature and pH were monitored to be 45 °C and 5.0, respectively (data not shown), which was consistent with that in mackerel (Lee, Chen, & Jiang, 1993). Substrate specificity and the effect of activators and inhibitors were investigated to characterize the crude enzyme obtained.

Substrate specificity of the partially purified cathepsin L was examined using synthetic substrates specific for cathepin L, B, H and trypsin or trypsin-like, including Z-Phe-Arg-MCA, Z-Arg-Arg-MCA, Arg-MCA and Boc-Gln-Ala-Arg-MCA.

The hydrolytic activity on the synthetic substrates was determined according to the method of assay of enzyme activity. Substrate solutions were added to the final concentrations of 0.1, 0.5, 1.0, 1.5 and 2.0 mM, respectively (Fig. 5).

The partially purified proteinase could strongly hydrolyze Z-Phe-Arg-MCA, a specific substrate commonly used to assay cathepsin L activity. Arginine is a very efficient P1 residue for the hydrolysis of methylcoumarin substrates for cathepsins (Mason, Green, & Barrett, 1985). Cathepsin L has only endopeptidase activity and preferentially cleaves peptide bonds with hydrophobic amino acid residues at P2 and P3 (Kargel et al., 1980, 1981). Purified cathepsin L hydrolyzed only Z-Phe-Arg-MCA, suggesting that a

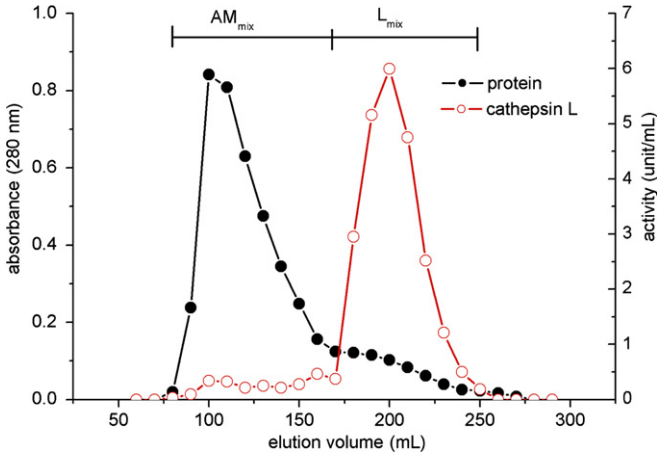

Fig. 4. Sepharose 6B gel filtration of actomyosin (Hu et al., in press).

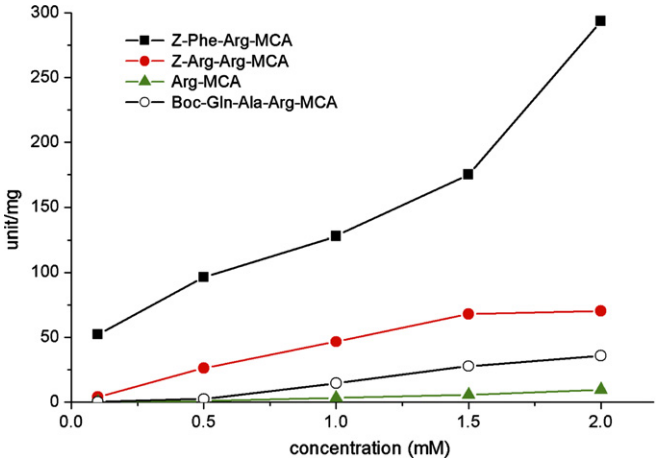

Fig. 5. Activity of L_{mix} on various synthetic substrates.

hydrophobic amino acid was necessary for substrate specificity of arrowtooth flounder proteinases (Visessanguan, Benjakul, & An, 2003). The partially purified proteinase also showed some hydrolytic activities on Z-Arg-Arg-MCA, Z-Arg-MCA and Boc-Gln-Ala-Arg-MCA specific substrates for cathepsins B, H and trypsin or trypsin-like. The hydrolytic activity was obvious when the substrates were at high concentrations. This was considered to be the contamination of cathepsin B, H and trypsin or trypsin-like in L_{mix}.

The partially purified cathepsin L from walleye pollock surimi was incubated for 15 min at 25 °C with an equal volume of 0.2 mM E-64, 2 mM iodoacetic acid, 0.2 g/l soybean trypsin inhibitor, 0.2 mM leupeptin, 4 mM DTT and 4 mM EDTA, to give the final concentrations listed in Table 1. A sample incubated with an equal volume of deionized water was used as control. The remaining activity was analyzed.

Partial or complete inhibition was observed in the presence of thiol-blocking agents, E-64 and iodoacetic acid (Table 1). Enzymatic activity was enhanced by thiol-activating agents, such as DTT and EDTA, used for specific activation of cysteine proteinase activity. Proteinase inhibitor for serine protease, STI, did not show distinctive effects on the activity of cathepsin L in AM and L_{mix}. The above results confirmed that the partially purified proteinase was a thiol cysteine-type proteinase.

Table 1
Effects of inhibitors and activators on activity of cathepsin L in AM and L_{mix}

Inhibitor/activator	Concentration	AM (%)	L_{mix} (%)
Control	–	100	100
E-64	0.1 mM	0	0
Leupeptin	0.1 mM	0	0
Iodoacetic acid	1 mM	0	0
STI	0.1 g/l	113	103
EDTA	2 mM	100	111
DTT	2 mM	325	311
EDTA + DTT	2 mM each	250	234

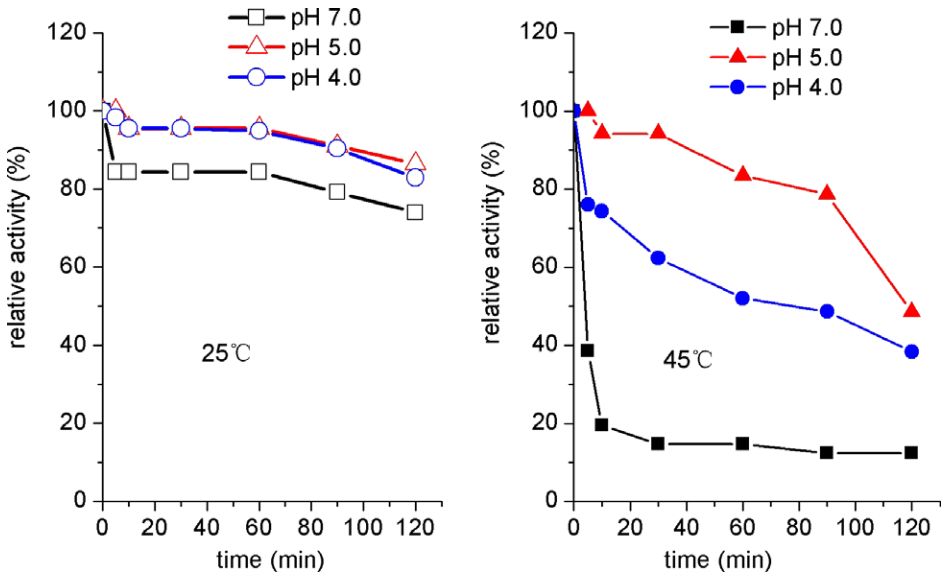

Fig. 6. Heat stability of L_{mix}. L_{mix} was adjusted to objective pH and incubated at 25 or 45 °C with different time intervals. The remaining activity was monitored at 25 °C, 30 min.

L_{mix} was adjusted to pH 4.0, pH 5.0 and pH 7.0 with 7% acetic acid, and was thus incubated at 25 or 45 °C for 0, 5, 10, 30, 60, 90, 120 min. The remaining activity was monitored at 25 °C, 30 min as the method of assay of enzyme activity.

Partially purified cathepsin L (L_{mix}) was sensitive to pH (Fig. 6). Under acidic conditions, L_{mix} was heat-stable. At pH 4.0 and 5.0, its activity decreased slowly and remained at 38.86% and 48.54%, respectively, after incubation at 45 °C for 120 min. It is well known that cathepsin L is an acidic enzyme and cannot be exposed for a long time to neutral and alkaline conditions. In this study, at pH 7.0, activity of cathepsin L decreased sharply about 90% activity was inactivated after incubation at 45 °C for 10 min.

On the other hand, at low temperatures, under either acidic or neutral conditions, cathepsin L appeared to be very stable. After incubation at 25 °C for 120 min, it still kept 73–86% of its original activity. Visessanguan et al. (2003) reported the purified cathepsin L in arrowtooth flounder lost 80% of its activity after incubation at an optimum temperature of 55 °C within 10 min. Compared to that in arrowtooth flounder, cathepsin L appeared to be a highly heat-stable enzyme in walleye pollock surimi. Purified enzymes are known to be much more sensitive than are crude ones (Seymour, Morrissey, Peters, & An, 1994) and the variation of fish species might be another way to explain the difference.

The profile of heat-dependence showed that actomyosin non-binding cathepsin L was much more heat-stable and might contribute to the degradation of protein in walleye pollock surimi and surimi-based products.

3.3. Hydrolysis of L_{mix} in walleye pollock kamaboko

After Sepharose 6B gel filtration, L_{mix} fractions were pooled and the total activity was measured. The L_{mix} obtained was carefully mixed with walleye pollock surimi; thus the final concentration of L_{mix} was 0.6 unit/g of surimi. Surimi, with L_{mix} added in the presence or absence of E-64, was adjusted to a moisture content of 80% and NaCl 2.5% for making kamaboko (60 °C, 2 h).

The gel strength of the kamaboko obtained was evaluated (Fig. 7). Due to hydrolysis by the endogenous enzymes, the gel strength of heated control was very low, 112 g/cm^2. Superaddition of L_{mix} sharply lowered the gel strength of kamaboko to 27.8 g/cm^2, while addition of E-64 strongly enhanced the gel strength and the value was increased to 302 g/cm^2.

SDS–PAGE electrophoretograms clearly showed the degradation of MHC by L_{mix} (Fig. 8, lane H + L). The MHC in kamaboko treated with L_{mix} (0.6 unit/g surimi) was severely degraded and many minor bands appeared between MHC and AC, indicating the strong proteolysis

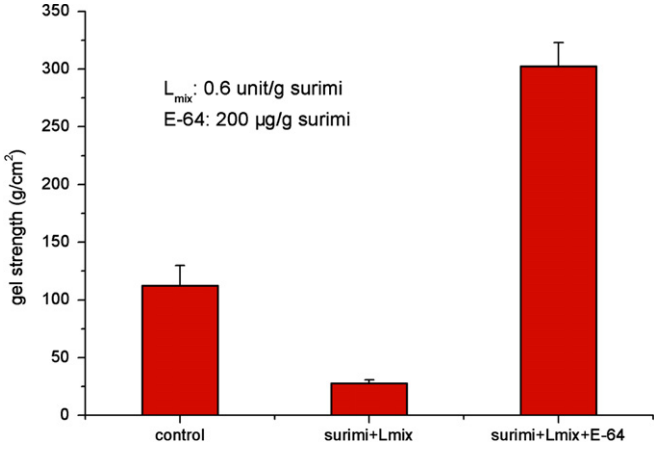

Fig. 7. Effect of L_{mix} hydrolysis on gel strength of kamaboko. Surimi was incubated with L_{mix} in the presence or absence of E-64 at 60 °C for 2 h. Bars express standard deviations of five determinations.

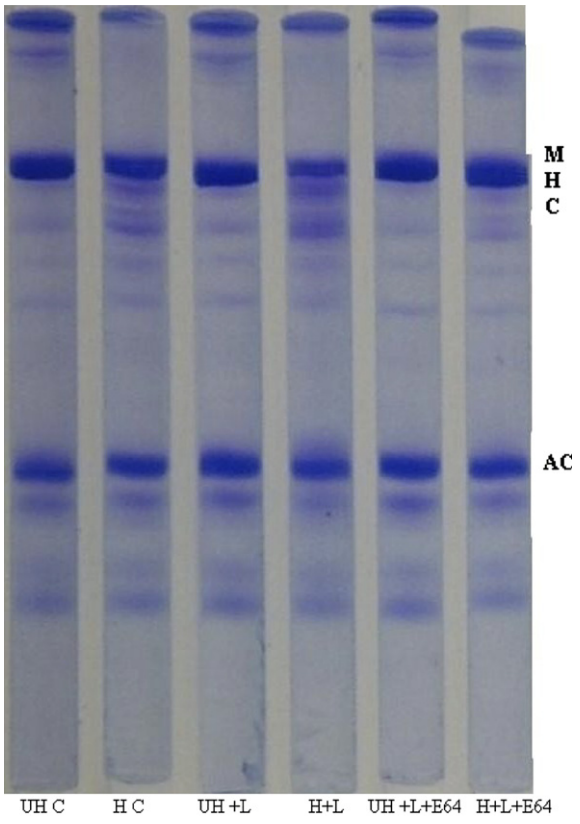

Fig. 8. SDS–PAGE pattern on L_{mix} hydrolysis of surimi paste. MHC: myosin heavy chain; AC: actin; UHC: unheated control; HC: heated control; UH + L: unheated control of surimi treated with L_{mix}; H + L: heated surimi treated with L_{mix}; UH + L + E64: unheated control of surimi treated with L_{mix} and E-64; H + L + E64: heated surimi treated with L_{mix} and E-64. The amount of L_{mix} was 0.6 unit/g of surimi and the amount of E-64 was 200 μg/g of surimi. Samples were heated at 60 °C for 2 h.

of L_{mix}. On the other hand, E-64 could effectively inhibit the degradation of MHC, but could not stop it completely, suggesting the existence of other types of endogenous enzymes that could not be inhibited by E-64. Calpain- and serine-type proteases were reported to closely relate to the protein degradation in Alaska pollock paste setting at 30 °C for 5 h (Liu, Kanoh et al., 1996; Liu, Nowsad, Kanoh, & Niwa, 1996).

Cathepsin L was reported to be predominant in Pacific whiting surimi and to degrade the proteins. Purified Pacific whiting cathepsin L hydrolyzed myofibrils, myosin and native and heated-denatured collagen (An et al., 1994). Yamashita and Konagaya (1991) reported that cathepsin L was capable of hydrolyzing the major muscle structural proteins, such as connectin, nebulin, myosin, collagen, α-actinin and tropin T/I. The MHC of rabbit liver AM could be degraded into several fragments by rat liver cathepsin L (Matsukura, Nishimuro, & Kato, 1981) or by rabbit liver cathepsin L (Mikami, Whiting, Taylor, Macievicz, & Etherington, 1987). According to Jiang, Lee, and Chen (1996), cathepsin L from mackerel muscle could degrade both MHC and actin. In this study, it was clear that cathepsin L could degrade MHC. Actin seemed to be resistant to hydrolysis by cathepsin L (Figs. 3 and 8). The SDS–PAGE pattern, using 10% gel, also gave the same result (data not shown).

4. Conclusion

Modori occurs in walleye pollock surimi incubated at 60 °C for 2 h. E-64, at 200 μg/g of surimi, could effectively suppress the hydrolysis by the endogenous enzymes. Partially purified cathepsin L was obtained from Sepharose 6B gel filtration. Studies of substrate specificity and the effects of activators and inhibitors confirmed that the enzyme obtained was crude cathepsin L, a thiol–cysteine protease. Its high heat-stability indicates its strong hydrolytic ability. Gel strength of walleye pollock surimi was greatly decreased by the crude cathepsin L, suggesting that cathepsin L contributed to the modori phenomenon in walleye pollock surimi. E-64 was effective in inhibiting the degradation and increased the gel strength of the corresponding kamaboko. On the other hand, E-64 could not stop the MHC degradation completely, suggesting the existence of other types of endogenous proteolytic enzymes in walleye pollock surimi.

References

An, H., Peters, M. Y., & Seymour, T. (1996). Roles of endogenous enzymes in surimi gelation. *Trends in Food Science and Technology, 7*, 321–327.

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.

Barrett, A. J., & Kirschke, H. (1981). Cathepsin B, cathepsin H and cathepsin L. *Method Enzymology, 80*, 535–561.

Barrett, A. J., Kembhavi, A. A., Brown, M. A., Kirschke, H., Knight, C. G., Tamai, M., et al. (1982). L-*trans*-Epoxy-succinyl-leucylamido (4-guanidino) butane (e-64) and its analogues as inhibitors of cysteine proteinases including cathepsin B, H and L. *Biochemical Journal, 201*, 189–198.

Benjakul, S., Visessanguan, W., & Chantarasuwan, C. (2004). Effect of high temperature setting on gelling characteristic of surimi from some tropical fish. *International Journal of Food and Science Technology, 39*, 671–680.

Benjakul, S., Visessanguan, W., & Leelapongwattana, K. (2003). Purification and characterization of heat-stable alkaline proteinase from bigeye snapper (*Priacanthus macracanthus*) muscle. *Comparative Biochemistry and Physiology B – Biochemistry and Molecular Biology, 134*(4), 579–591.

Hu, Y., Morioka, K., & Itoh, Y. (in press). Actomyosin non-binding cathepsin L in walleye pollock surimi. *Journal of Food Biochemistry*.

Itoh, Y., Maekawa, T., Suwansakornkul, P., & Obatake, A. (1995). Seasonal variation of gel forming characteristics of three lizard fish species. *Fish. Sci., 61*, 942–947.

Itoh, Y., Maekawa, T., Suwansakornkul, P., & Obatake, A. (1997). The effect of meat-washing on the gel formation and on the polymerizing and degrading behavior of lizardfish meat proteins at 40 and 60 °C. *Fish. Sci., 63*, 286–290.

Jiang, S. T. (2000). Enzymes and their effects on seafood texture. In *Seafood enzymes: Utilization and influence on postharvest seafood quality*. New York: Marcel Dekker, Inc.

Jiang, S. T., Lee, J. J., & Chen, H. C. (1996). Protolysis of actomyosin by cathepsin B, L, L-like, and X from mackerel. *Journal of Agriculture and Food Chemistry, 44*(3), 769–773.

Kamath, G. G., Lanier, T. C., Foegeding, E. A., & Hamann, D. D. (1992). Nondisulfide covalent cross-linking of myosin heavy chain in setting of Alaska pollock and Atlantic croaker surimi. *Journal of Food Biochemistry, 16*, 151–172.

Kargel, H. J., Dettmer, R., Etzold, G., Kirschke, H., Bohley, P., & Langner, J. (1980). Action of cathepsin L on the oxidized B-chain of bovine insulin. *FEBS Letter, 114*, 257–260.

Kargel, H. J., Dettmer, R., Etzold, G., Kirschke, H., Bohley, P., & Langner, J. (1981). Action of rat liver cathepsin L on glucagons. *Acta Biologica et Medica Germanica, 40*, 1139–1143.

Kinoshita, M., Toyohara, H., & Shimizu, Y. (1990). Diverse distribution of four distinct types of modori (gel-degradation)-inducing proteinases among fish species. *Nippon Suisan Gakkaishi, 56*(9), 1485–1492.

Lanier, T. C. (1992). Measurement of surimi composition and functional properties. In *Surimi Technology*. New York: Marcel Dekker, Inc.

Lee, J. J., Chen, H. C., & Jiang, S. T. (1993). Purification and characterization of proteinases identified as cathepsin L and L-like (58KDa) proteinase from mackerel. *Bioscience, Biotechnology and Biochemistry, 57*, 1470–1476.

Liu, D., Kanoh, S., & Niwa, E. (1996). Effect of cysteine protease inhibitor on the setting of Alaska pollock surimi paste. *Nippon Suisan Gakkaishi, 62*, 275–279.

Liu, D., Nowsad, A. A., Kanoh, S., & Niwa, E. (1996). Effect of serine protease inhibitor on the setting of alaska pollock surimi paste. *Nippon Suisan Gakkaishi, 62*, 791–795.

Mason, R. W., Green, G. D. J., & Barrett, A. J. (1985). Human liver cathepsin L. *Biochemical Journal, 226*, 233–241.

Matsukura, U., Nishimuro, A., & Kato, H. (1981). Mode of degradation of proteins by an endogenous protease, cathepsin L. *Biochimica et Biophysica Acta, 662*, 41–47.

Mikami, M., Whiting, A. H., Taylor, M. A. J., Macievicz, R. A., & Etherington, D. J. (1987). Degradation of myofibrils from rabbit, chicken and beef by cathepsin L and lysosomal lysates. *Meat Science, 21*, 81–97.

Robinson, H. W., & Hodgen, C. G. (1940). The Biuret reaction in the determination of serum protein. I. A study of the condition necessary for the production of the stable color which bears a quantitative relationship to the protein concentration. *Journal of Biology Chemistry, 135*, 707–725.

Seymour, T., Morrissey, M. T., Peters, M. T., & An, H. (1994). Purification and characterization of pacific whiting proteases. *Journal of Agriculture and Food Chemistry, 42*, 1427–2421.

Visessanguan, W., Benjakul, S., & An, H. (2003). Purification and characterization of cathepsin L in arrowtooth flounder muscle. *Comparative Biochemistry and Physiology, 134B*, 477–487.

Wasson, D. H., Babbit, J. K., An, S., & French, J. S. (1992). Characterization of a heat stable protease from arrow-tooth flounder. *Journal of Aquatic Food and Production Technology, 1*, 167–182.

Weber, K., & Osborn, M. (1969). The reliability of molecular weight determination by dodecyl sulfate–polyacrylamide gel electrophoresis. *Journal of Biological Chemistry, 244*, 4406–4412.

Yamashita, M., & Konagaya, S. (1990). Participation of cathepsin L into extensive softening of the muscle of chum salmon caught during spawning migration. *Nippon Suisan Gakkaishi, 56*, 1271–1277.

Yamashita, M., & Konagaya, S. (1991). Hydrolytic action of salmon cathepsin B and L to muscle softening. *Nippon Suisan Gakkaishi, 57*, 1917–1922.