# 魚筋肉のプロテアーゼに関する研究IV

| | |
|---|---|
| 誌名 | 日本水産學會誌 |
| ISSN | 00215392 |
| 著者名 | 牧之段,保夫<br>池田,静徳 |
| 発行元 | 日本水産學會 |
| 巻/号 | 37巻6号 |
| 掲載ページ | p. 518-523 |
| 発行年月 | 1971年6月 |

# Studies on Fish Muscle Protease—IV. Relation between HIMODORI of KAMABOKO* and Muscle Proteinase

Yasuo MAKINODAN and Shizunori IKEDA**

(Received November 20, 1970)

A partially purified proteinase of barracuda muscle acted on myofibrillar proteins and the optimal activity was pH 7.5–8.0 (60°C), 55–60°C (pH 7.8). Optimum temperature of autolysis at pH 6.5 of crude muscle extracts of white croaker and lizard fish were near 60°C. Such proteinase activity remained about 70% in the bleached meat. And when the fish meat paste was pre-heated at 80°C where the muscle proteinase was inactivated, ASHI of KAMABOKO did not decrease the strength in subsequent heating at 60°C. From the above mentioned facts, we inferred that hydrolysis of meat protein by the muscle proteinase might be a factor causing HIMODORI.

When fish meat paste obtained by grinding fish meat with salt is cooked at various temperature, the meat paste heated at near 60°C shows lower ASHI (elasticity of KAMABOKO) in comparison with one heated in other temperature range[1]. Such a lowering of ASHI in special heating temperature range is named conventionally HIMODORI. For this mechanism, SHIMIDU *et al.*[1] and OKAMURA[2] considered heat coagulation of protein, and SHIMIZU[3] guessed the phenomenon might be due to the result of high-degree unfolding of peptide chain at near 55°C. However, the lowest gel strength occurs at 60–65°C. On the contrary, at near 80°C, where unfolding of protein molecule or heat coagulation of protein occur more remarkably, ASHI increases its strength. Accordingly, such a specific property of fish meat paste (HIMODORI) can not be explained adequately by only heating effect on muscle protein.

In fish muscle, as reported previously[4–6], a proteinase optimally active at 60–65°C on casein substrate exists. So there is a possibility that the proteinase in fish meat, in addition to heat effect on meat protein, may take part in HIMODORI of KAMABOKO.

In this report, we examined the mechanism of HIMODORI from a view point of participation of the enzyme.

## Materials and Methods

**Sample.** Each of used fish was in ex-rigor mortis and purchased from Maizuru fish market.

**Crude enzyme preparation.** The method of preparation of crude enzyme solution

* Japanese traditional food made by heating fish meat paste.

** Dept. Fish., Fac. Agr., Kyoto Univ., Maizuru, Japan (牧之段保夫・池田静徳: 京都大学農学部)

was the same as described previously[6].

**Measurement of proteolytic activity.** The method as described previously[6] was applied to the present proteinase assay, in which phosphate buffer (M/5 $NaH_2PO_4$—M/5 $Na_2HPO_4$) was used. Autolytic activity of crude enzyme preparation was determined as follow: 1 ml of crude preparation (20–30 mg protein/ml) was mixed with 3 ml of phosphate buffer and incubated at a fixed temperature for 2 hours. After incubation, $E_{660}$ of trichloroacetic acid soluble fraction was measured by the same method as described for the proteinase assay and the extinction of the 0 time was subtracted from that of the assay sample.

**Preparation of myofibrillar proteins.** The method given by SHIMIZU *et al.*[7] was used.

**Preparation of fish paste.** Minced meat with 3% of NaCl and 10% of deionized water to meat was ground in a mortar for 40 minutes. The obtained meat paste was packed with Kreharon tube of diameter 3 cm and offered to cooking.

**Measurement of strength of ASHI.** Strength of ASHI was determined by Food Rheometer[8] (Tabai Seisakusho Company Ltd.) or jelly strength tester (Chuo Riken Company Ltd.) and given as gel strength or jelly strength.

**Method of bleaching of meat in water.** Fish meat was minced through chopper plate of 3 mm mesh and washed with 15 times volume of deionized water 3 times each for 30 minutes. After washing, the mixture was centrifuged to dewater. Apart from this, minced meat was blended with a proper amount of water with Waring blendor. After standing for 30 minutes at 4°C, water was centrifuged (13,000×g, 30 minutes) off. This procedure was repeated to obtain blended bleached meat.

## Results and Discussion

**HIMODORI of KAMABOKO.** According to the reports[2,9], the temperature range of HIMODORI differs among fish species. Thus, the strength of ASHI was the lowest at 60°C for white croaker[9], *Argyrosomus argentatus*, and at 50°C for horse mackerel[2], *Trachurus japonicus*, but as to pollack, *Theragra chalcogramma*, HIMODORI is not observed[9]. If revealing temperature range of HIMODORI differs among fish species, this may be important in considering the mechanism. Therefore, fish meat pastes prepared from barracuda, *Sphyraena pinguis*, white croaker, lizard fish, *Saurida undosquamis*, horse mackerel and cod, *Gadus macrocephalus*, were heated in water baths of various temperatures for 1 hour and after cooling the strengths of ASHI were measured. Results are shown in Fig. 1. Irrespective of fish species ASHI was the lowest at 60–65°C and the difference among species in the temperature range in which HIMODORI appeared was not observed. But there was found a case where the phenomenon was not so conspicuous even when the same species was used. There is a report that the effect of heating tem-

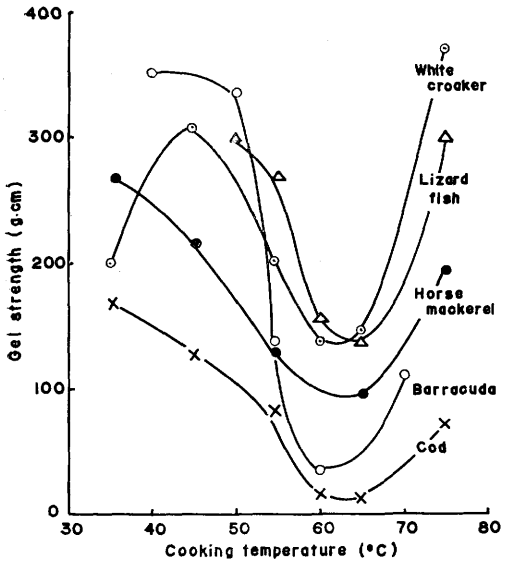


Fig. 1. Influence of cooking temperature on the gel strength of various fish meat pastes.

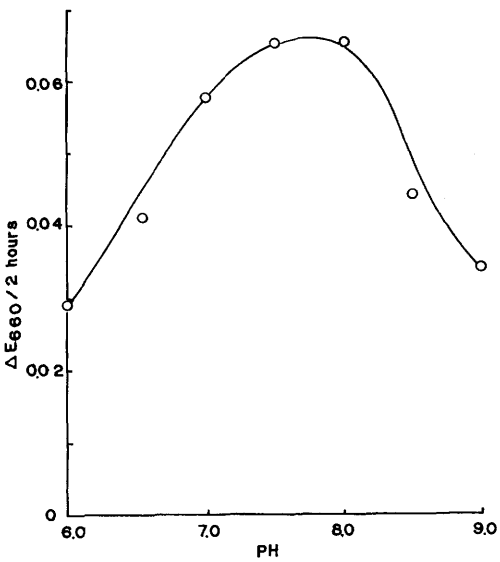


Fig. 2. pH-activity curve of barracuda muscle proteinase on myofibrillar proteins at 60°C. The buffers were M/5 $NaH_2PO_4$–M/5 $Na_2HPO_4$ (below pH 8.0) and M/10 $KH_2PO_4$–M/20 $Na_2B_4O_7$ (pH 8.5 and 9.0). Specific activity ($\Delta E_{660}$/2 hours/mg protein at 60°C at pH 7.8) of the partially purified enzyme was 2.06. The reaction mixture contained about 10 mg myofibrillar protein.

perature for the formation of ASHI differs with freshness of fish[9]. The difference of appearance of HIMODORI observed in same fish species may be attributed to the qualitative change of muscle proteins after death, but the mechanism is not clear.

**Action of fish muscle proteinase on the muscle protein.** The proteinase active optimally in slightly alkaline pH range found in fish muscle[4] seems to take part in HIMODORI of KAMABOKO. So, at first, the action of the barracuda muscle proteinase on myofibrillar proteins prepared from the same fish muscle was examined. The enzyme preparation used in this experiment was a partially purified one obtained by heat treatment (63–65°C) of crude preparation, ammonium sulfate fractionation (40–65%), acetone fractionation (0–30%) and gel filtration (Sephadex G–200). As shown in Fig. 2, optimum pH on myofibrillar proteins was 7.5–8.0, and it is worthy to note that the enzyme has 40–60% of the maximum activity at pH 6.5, near to pH of KAMABOKO. The optimum temperature at pH 7.8 for 2 hour reaction was 55–60°C (Fig. 3). As shown in Fig. 3, temperature—activity curve (○——○) was in striking contrast to gel strength curve (×---×) given from Fig. 1 for reference, and gel was destroyed remarkably in optimum temperature range of the enzyme. Similar relations were also observed between temperature—autolytic activity curves of crude extract of white croaker meat and lizard fish

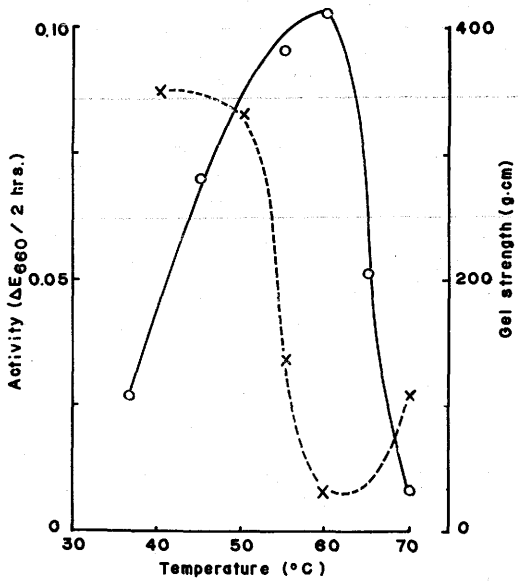


Fig. 3. Temperature-activity curve of barracuda muscle proteinase on myofibrillar proteins at pH 7.8 (○——○). ×---×: Gel strength curve of barracude meat paste (from Fig. 1).

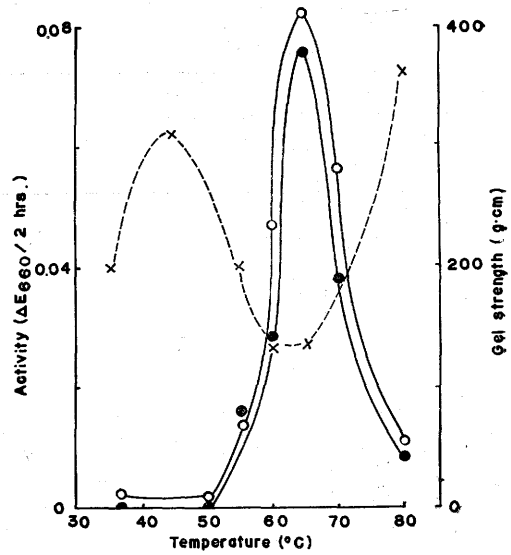


Fig. 4. Influence of temperature on the autolysis of crude muscule protein extract of white croaker at pH 6.5 (●——●) and at pH 7.8 (○——○). ×---×: Gel strength curve of white croaker meat paste (from Fig. 1).

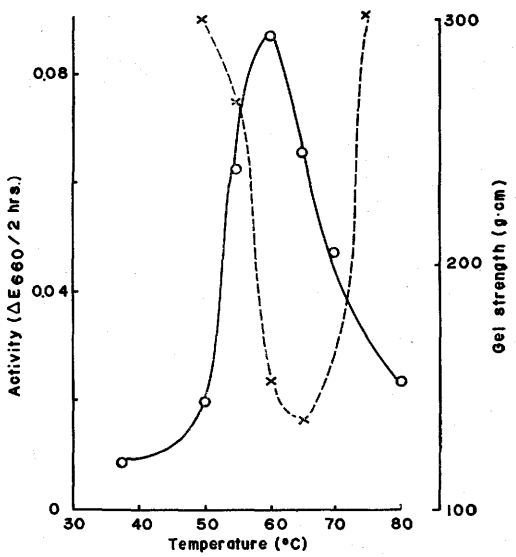


Fig. 5. Influence of temperature on the autolysis of crude muscle protein extract of lizard fish at pH 6.5 (○——○). ×---×: Gel strength curve of lizard fish meat past (from Fig. 1).

meat and gel strength curves of KAMABOKO. Figs. 4 and 5 show the results with white croaker and lizard fish respectively. During incubation, myofibrillar proteins or endogenous proteins aggregated softly. Like this, enzyme assay in this experiment was performed in temperature range accompanied by heat coagulation of fish meat protein. Therefore the results may not show properties of the enzyme for meat protein precisely. However, the enzyme itself is considered to be fairly stable with much protein at 60° or 65°C[6] and so action of the enzyme on such coagulated protein, in one sense, seems to reveal the profile of the proteinase ac-

**Table 1.** Removal of proteinase from minced meat by bleaching in water (lizard fish).

| Meat offered bleaching | Frequency of experiment | Residual activity* (%) |
|---|---|---|
| Minced through chopper plate of diameter 3 mm | 8 | 72(53–88) |
| Minced with Waring blendor | 6 | 28(24–33) |

* No bleaching (control) or bleaching meat were homogenized with 6 times of deionized water. After the activity was measured as to one ml of homogenate, total activity for one gram of meat was calculated. Values are mean residual value showed as per cent to control. Values in parentheses denote range of values.

tion in meat paste during heating process. Such enzymic hydrolysis of meat protein is probably a factor of the decrease of ASHI.

**Removal of proteinase from minced meat by bleaching in water.** For that muscle proteinase may be related to HIMODORI, it must be elucidated that the enzyme remains in meat paste without much removal during bleaching treatment which is a process in KAMABOKO making. Table 1 shows the residual rate of muscle proteinase after minced meat was submitted to water bleaching. When muscle was ground finely by Waring blendor, the enzyme was removed remarkably, but by the ordinary water bleaching about 70% of the enzyme remained in the bleached meat. And the remaining enzyme is considered to affect on meat protein during subsequent processing.

**Effect of pre-heating of fish meat paste on HIMODORI.** Supposing that the enzyme participates to HIMODORI, the phenomenon may not occur if meat paste is precooked at such a temperature as the enzyme loses all of the activity. Accordingly, we compared the strength of ASHI of KAMABOKO obtained by pre-heating lizard fish meat paste at 40, 60 or 80°C for 20 minutes (Fig. 6). As expected, the sample pre-heated at 80°C did not decreased ASHI during subsequent heating at 60°C. On the other hand, the sample pre-heated at low temperature where the enzyme was not inactivated lost ASHI gradually by the following heating at 60°C. Shimizu *et*

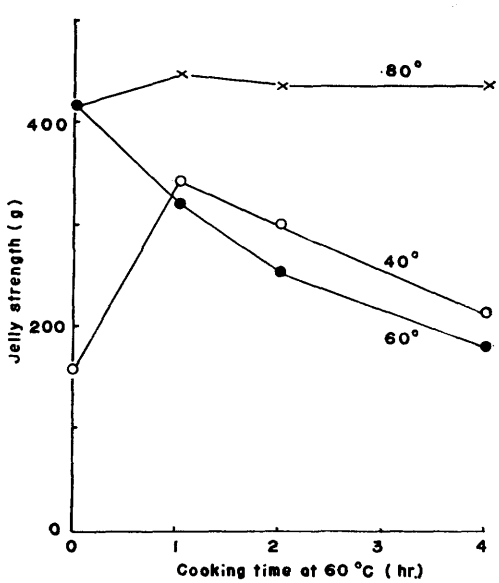


**Fig. 6.** Influence of pre-cooking on the change of jelly strength during cooking at 60°C of lizard fish meat paste. Samples were pre-cooked at 40, 60, or 80°C for 20 minutes.

*al.*[10] reported the same result on the effect of pre-heating of meat paste on HIMODORI.

Many facts mentioned above support the possibility that muscle proteinase may participate in HIMODORI. Fish meat paste forms certain elastic structure when it is heated. However, if it is heated at near 60°C which is optimum temperature of the proteinase, myofibrillar proteins which make gel structure maybe sustain the enzymic hydrolysis, consequently ASHI is probably destroyed.

## References


1) W. SHIMIDU, Y. SHIMIZU and T. NISHIDE: *Bull. Res. Inst. Food Sci.,* Kyoto Univ., No. 23, 1–8 (1960).
2) K. OKAMURA: This Bull., **27**, 755–762 (1961).
3) Y. SHIMIZU: in "Gyoniku Tanpaku ni kansuru Genryogakuteki Kenkyu" p. 150 (1961).
4) Y. MAKINODAN, M. YAMAMOTO and W. SHIMIDU: This Bull., **29**, 776–780 (1963).
5) Y. MAKINODAN and S. IKEDA: *ibid.,* **35**, 672–676 (1969).
6) Y. MAKINODAN and S. IKEDA: *ibid.,* **35**, 749–757 (1969).
7) Y. SHIMIZU and W. SHIMIDU: *ibid.,* **26**, 806–809 (1960).
8) Y. SHIMIZU and W. SHIMIDU: *ibid.,* **26**, 911–916 (1960).
9) M. OKADA: *Bull. Tokai Reg. Fish. Res. Lab.,* No. 24, 73–78 (1959).
10) Y. SHIMIZU, H. YOSHIMOTO and W. SHIMIDU: This Bull., **28**, 610–615 (1962).