

Proteolytic Properties of Crude Extracts from Internal Organs in the Japanese Anchovy (*Engraulis Japonicus*)

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The proteolytic activities of crude extracts from various internal organs in the Japanese anchovy were studied under different thermal and pH conditions. Activities in extracts from the intestine and pyloric caeca were high, and were also observed at relatively low levels even in the condition of being chilled in iced water to around 0°C. Hardly any activity was observed in extracts from the stomach, liver, ovaries and testes at the same temperature. In the pH condition, both of the activities of extracts from intestine and pyloric caeca increased in alkaline range, in contrast with the decrease seen in extracts from other organs.

Keywords: Japanese anchovy, crude extract, proteolysis, temperature, pH

1. Introduction

The Japanese anchovy, a small marine fish, is a predominant fisheries resource in Japan. Some are consumed fresh, but most are boiled or processed in a variety of ways. A major problem with anchovy storage is the rapid autolytic degradation of its abdominal tissue – a phenomenon often referred to as belly bursting¹⁾. This renders the fish unsuitable as a raw material due to the high autolytic activity of the internal organs. However, there may be other more positive ways to use this activity, such as in the aging of foods. The present study was undertaken to improve understanding of proteolytic activity in different organs of the anchovy in order to support identification of better uses for them in daily life.

2. Materials and Methods

Fish and crude extract

Japanese anchovies were bought from a common market in Shizuoka City. The fish weighed between 10.3 and 19.4 g each, and their lengths ranged from 10.4 to 13.3 cm. The internal organs of the fish in saline are shown in Figure 1. Crude extracts of each internal organs were prepared using the method of Yamashita²⁾ with slight modification. Briefly, pooled fractions of each organ from 20 to 30 fish were homogenized in five volumes of ice-cold distilled

water. The homogenates were centrifuged (20 min, 10,000 G, 4°C) and passed through filter paper. The resulting enzyme extracts were then stored at –30°C.

Chemicals

Analytical-grade casein, citric acid, copper (II) sulfate pentahydrate, disodium hydrogenphosphate 12-water, glycine, hydrochloric acid (HCl), maleic acid, polyoxyethylene (10) octylphenyl ether (Triton X-100), potassium chloride, sodium carbonate, sodium chloride, sodium hydroxide (NaOH), trichloroacetic acid (TCA), tris (hydroxymethyl) aminomethane hydrochloride (tris-HCl) and potassium (+)-tartrate-water (2/1) were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

Preparation of myofibril as substrate

Myofibril as substrate was prepared using the method of Seki *et al.*³⁾ with slight modification. Ordinary muscle from Japanese anchovy was collected, minced and stirred with five volumes of 20 mM Tris-maleate buffer, pH 7.0, containing 0.1 M KCl and then centrifuged at 3,000 rpm for five minutes. The precipitate was washed again with the same buffer and then centrifuged. This washing procedure was performed once more. The precipitate was homogenized four times at 10,000 rpm for 30 seconds with five volumes of the same buffer, with storage in ice. The suspension was then filtered through a piece of gauze. 1/20 by volume of 20% TritonX-100 was added to the suspension, which was then mixed in a test-tube mixer and left for 10 minutes

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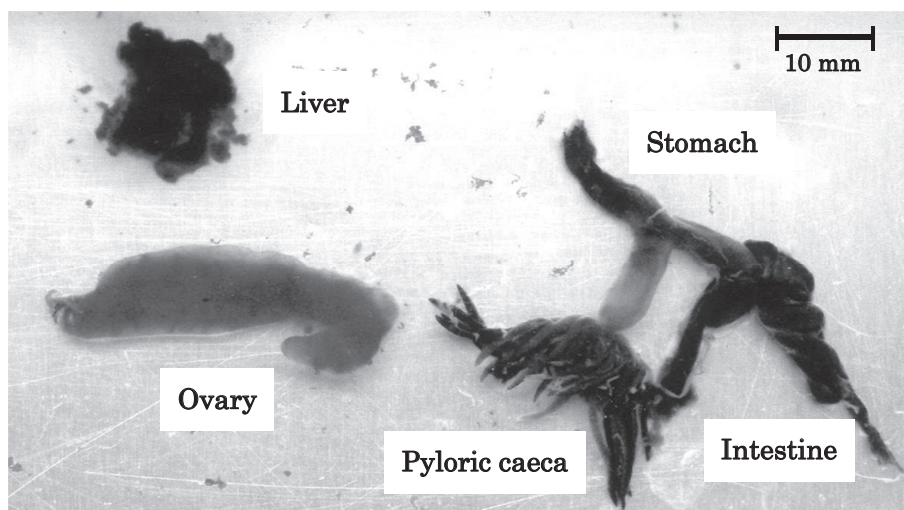


Fig. 1 Internal organs of Japanese anchovy in saline.

before being centrifuged at $600 \times g$ for 15 minutes. After centrifugation, the supernatant was removed and five volumes of the same buffer solution were added before being centrifuged at $600 \times g$ for 15 minutes. This washing procedure was repeated three times, and the precipitate was spread in a flat form with a thickness of about 5 mm in polyethylene bags and placed in a freezer. Once frozen, the flat myofibril was lyophilized and used as a substrate for the reaction of crude extract from various internal organs.

Assay of proteolytic activity

Proteolytic activity was assessed using the method of Yamashita²⁾ and Tsukamasa *et al.*⁴⁾ with slight modification. In the experiments, four 0.2-M buffer solutions produced with reference to paper⁵⁾ (glycine-HCl buffer (pH 1.0–2.5), phosphate-citrate buffer (pH 2.5–8.0), tris-HCl buffer (pH 8.0–10.5) and glycine-NaOH buffer (pH 10.5–13.0)) and two substrate solutions (5% casein and 20% myofibril) were used. The reactive mixture was prepared with 500 μL of 0.2-M buffer solution, 250 μL of substrate solution and 250 μL of crude extract, and was then incubated for 30 min at 0, 5, 20 and 30°C. The reaction was stopped via the addition of 5 mL of 6% TCA. After filtration, TCA-soluble peptides in the filtrate were evaluated using the method of Lowry *et al.*⁶⁾. Proteolytic activity was expressed as the absorbance of the reaction liquid at 750 nm or relative values with the highest absorbance as a base of 100%.

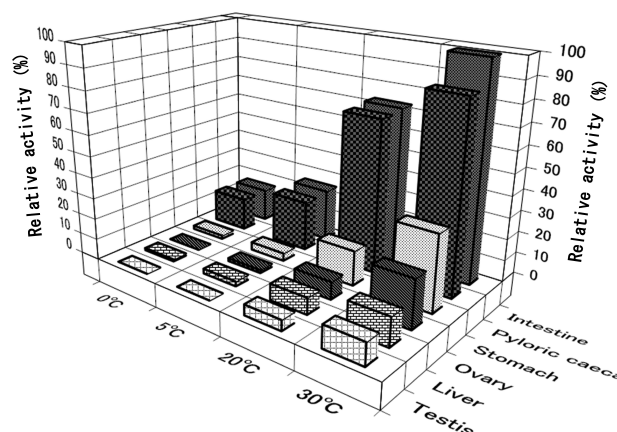


Fig. 2 Proteolytic dependency on temperature of crude extracts from internal organs of Japanese anchovy. Scale values represent averages for two to four samples. Casein was used for the substrate of the enzymatic reaction.

3. Results and Discussion

The relationships between reactive temperature and the proteolytic activity of the crude extracts from the internal organs of the fish for the casein substrate are shown in Figure 2. Activity increased for all organs as the reactive temperature rose. Activity in the intestine and pyloric caeca was higher than in other organs, and relatively low activity (as little as 15% of the highest value) was observed even with chilling to around 0°C in iced water. The results observed here indicate that the intestine and pyloric caeca are particularly important for autolysis. Relationships between pH and activity in the crude extracts for the casein substrate are shown in Figure 3. Activity in the intestine and pyloric caeca increased as the pH value rose,

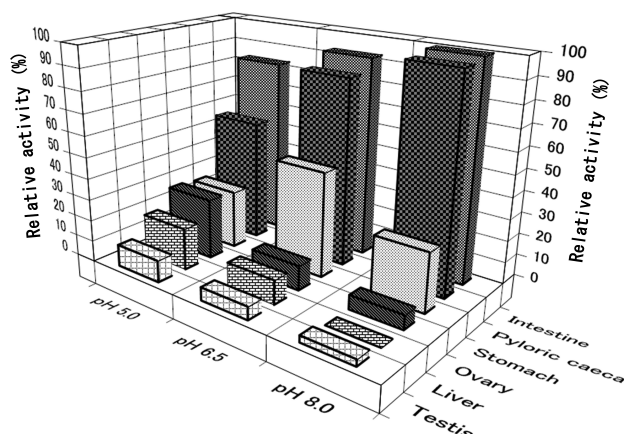


Fig. 3 Proteolytic dependency on pH of the crude extracts from internal organs of Japanese anchovy. Scale values represent averages for five to six samples. Casein was used for the substrate of the enzymatic reaction.

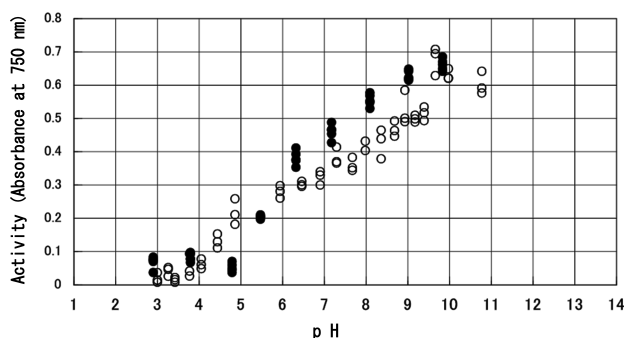


Fig. 4 Proteolytic dependency on pH of the crude extracts from intestine (●) and pyloric caeca (○) of Japanese anchovy. Dots represent measured values. Myofibril was used for the substrate of the enzymatic reaction.

whereas liver, ovary and testis samples showed opposite tendencies. In the stomach, activity was the highest at pH 6.5, but a report on the anchovy *Engraulis encrasicolus*¹⁾ suggests the existence of an acidic pH range in which activity is higher than in the neutral range (pH 6.5). Figure 4 shows the relationships between pH (from 2.91 to 10.78) and activity in the intestine and pyloric caeca for the myofibril substrate of this fish. Both increased in the alkaline range to about pH 10. A high correlation between activity and pH values was observed in each organ ($r =$

0.96 for the intestine, 0.98 for the pyloric caeca), and similar tendencies were seen.

One aspect of rapid autolytic degradation of abdominal tissue (often called belly bursting) renders the fish unsuitable as a raw material¹⁾. This phenomenon appears to be caused by leakage of proteolytic enzymes from the intestine and pyloric caeca to the ventral muscle. In this study, it was supposed that proteolytic activity in the intestine and pyloric caeca of the Japanese anchovy would be at relatively low levels even during periods of storage in iced water (0°C) and increased in alkaline range. To avoid degradation due to autolysis in a fresh state, it may be advisable to keep the fish slightly above its freezing point in a slightly acidic range so that the risk of muscle denaturation is reduced.

References

- 1) Aurora Martinez and A. Girdberg: Autolytic degradation of belly tissue in anchovy (*Engraulis encrasicolus*). *International Journal of Food Science and Technology*, 23, 185–194 (1988)
- 2) Michiaki Yamashita: Assay of proteolytic activity in fish muscle. *Bulletin of National Research Institute of Fisheries Science*, 8, 111–122 (1996)
- 3) Nobuo Seki, Yoshio Oogane, and Takahiro Watanabe: Changes in ATPase Activities and Other Properties of Sardine Myofibrillar protein during ice-storage. *Bulletin of the Japanese Society of Scientific Fisheries*, 46(5), 607–615 (1980)
- 4) Yasuyuki Tsukamasa, Takayuki Nakagawa, Kazuhiro Masuda, Masashi Ando, and Yasuo Makinodan: Presence of cysteine endopeptidase in the pyloric caeca of pacific mackerel *Scomber japonicus* and Its Purification and Characterization. *Fisheries Science*, 64(4), 627–632 (1998)
- 5) R. Munilla-Moran and F. Saborido-Rey: Digestive enzymes in marine species. I. Protrinsase activities in gut from redfish (*Sebastes mentella*), seabream (*Sparus aurata*) and turbot (*Scophthalmus maximus*). *Comparative Biochemistry and Physiology*, 113B(2), 395–402 (1996)
- 6) Oliver. H. Rowry, Nira. J. Rosebrough, A. Lewis Farr, and Rose J. Randall : Protein measurement with the folin phenol reagent. *Journal of Biological Chemistry*, 193, 256–275 (1951)

〔研究ノート〕

カタクチイワシ内臓粗抽出液のタンパク質分解特性

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要 旨

カタクチイワシは肉質が軟弱であり脆弱化が速く、内臓プロテアーゼのタンパク質分解活性が高いことが報告されており、自己消化による品質劣化は、外観的に腹部における皮膚の穿孔としても観察される。カタクチイワシの食利用上、この内臓プロテアーゼの影響の除去が重要課題であるが、本研究では、安価に入手できるこの魚の食利用法をより豊かにすることを見据えた基礎検討として、各臓器から抽出した粗抽出液のタンパク質分解特性を調べた。

まず、多数のカタクチイワシから胃、幽門垂、腸、肝臓、卵巣および精巣を分取して、個々の粗抽出液を調製した。その後、種々の温度(0, 5, 20, 30℃)および水素イオン指数(pH 2.91~10.78)条件下で、それらのタンパク質分解活性を測定した。結果として、腸および幽門垂粗抽出液において特に活性が高く、反応温度の低下に伴って活性は低下したものの、他の臓器群においては活性が殆ど失われていた0℃付近においても、最高値に対して15%程度の活性の残存が認められた。また、腸および幽門垂粗抽出液のみに、塩基性pH領域での活性の上昇があり、反応溶液として最高pHであった10周辺まで連続して認められた。

キーワード：カタクチイワシ、粗抽出液、タンパク質分解、温度、水素イオン指数