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Short Paper

Comparison of protease activity in liver among several species of squid and cuttlefish

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Squid are widely distributed in the sea areas of the world, and their muscles are used for a variety of foodstuffs. On the other hand, squid livers are mostly discarded without use, although they are known to contain various and large amounts of enzymes.[1–4] Enzyme preparations originating from micro-organisms are frequently used in the pharmaceutical, chemical, and food industries. However, interest in utilization of natural materials has increased because of their high safety. Therefore, enzymes from squid liver, a natural product, will be preferable to those from micro-organisms at least for applications in the food industry. Recently, it was reported that angiotensin I-converting enzyme inhibitors (antihypertensive peptides) were produced during autolysis of squid liver and mantle muscle homogenates.[5] This report also indicates the effectiveness of squid proteases[2–4] as well as their muscle proteins in food processing. In order to utilize squid liver as a useful enzyme resource, the present study compared the potential of protease production in the livers among several species of squid and cuttlefish.

Table 1 shows samples used in this study. Sample nos. 1–5 were caught in sea areas far from Japan and were obtained in the frozen state at −20°C, while sample nos. 6–9, caught in Japan, were fresh enough to be obtained in iced storage without freezing. Although the freshness of each sample was different, this difference is not a critical problem for comparing the potential of proteolytic activity between these livers, as previously described.[6] Each liver was excised and subsequently homogenized. The homogenate was mixed with 15 parts (v/w) of cooled acetone (−20°C) and filtered with suction for removal of lipids. The precipitate collected on the filter was again washed with cooled acetone and then with cooled ether. The washed precipitate was dried completely under reduced pressure. The dried precipitate (100 mg) was mixed with 10 mL of 0.1 M sodium acetate buffer (pH 4.0) and the crude enzyme extract in the supernatant was recovered by centrifugation (10 000 *g*, 30 min, 4°C). After transporting the supernatant, the precipitate was again extracted with the same procedure. Both supernatants were combined and used as the crude enzyme extract from liver.[6]

Proteolytic activity of the crude enzyme extract was measured using each of 2% hemoglobin in 0.1 M sodium acetate buffer (pH 4.0) and 2% casein in 0.1 M phosphate buffer (pH 7.0) as the substrates.[6] An aliquot (0.2 mL) of the crude enzyme extract was added to 2 mL of 2% substrate solution and incubated at 37°C for 10–60 min, according to the potency of proteolytic activity. The enzyme reaction was stopped by adding 5 mL of 5% trichloroacetic acid. After standing for 30 min, the mixture was filtered. The digestion degree of the substrate in the filtrate was estimated by the method of Lowry *et al.*[7] using tyrosine as the standard. One unit of enzyme activity was defined as the amount of enzyme that liberated 1 μM of tyrosine per min.[8]

Table 2 shows the proteolytic activity in the crude enzyme extracts from the samples shown in Table 1. All the samples showed stronger activity at pH 4.0 than at pH 7.0, while significant differences among them were also observed in the potency of proteolytic activity, body- and liver weights, and liver percentages. It was reported that squid livers contain various types of cathepsin-like proteases.[1–4] These cathepsin analogs will participate as the major acid proteases in the crude enzyme extracts, although their enzymatic characterizations have not been carried out yet. The recovery of enzymes from the liver is dependent not only on specific enzyme activity but also on liver weight. Considering these factors, Antarctic flying squid (sample no. 1), jumbo flying squid (no. 3), purpleback squid (no. 4), and Japanese common squid (no. 8) will be more applicable to enzyme resources, at least for the preparation of proteases. Nevertheless, because all livers contained different but

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Table 1 Samples of squids and a cuttlefish

Sample no.	Japanese name	English name	Scientific name	Fishing ground
1	*Minamisurumeika*	Antarctic flying squid	*Todarodes filippovae*	Argentina
2	*Takoika*	Boreopacific gonate squid	*Gonatopsis borealis*	North Pacific Ocean
3	*Amerikaooakaika*	Jumbo flying squid	*Dosidicus gigas*	Peru
4	*Tobiika*	Purpleback flying squid	*Symlectoteuthis ouralaniensis*	Peru
5	*Arugentin-irekkusu*	Argentine shortfin squid	*Illex argentinus*	Argentina
6	*Akaika*	Neon flying squid	*Ommastrephes bartrami*	Yamaguchi, Japan
7	*Yariika*	Spear squid	*Loligo bleekeri*	Yamaguchi, Japan
8	*Surumeika*	Japanese common squid	*Todarodes pacificus*	Yamaguchi, Japan
9	*Kouika*	Golden cuttlefish	*Sepia esculenta*	Yamaguchi, Japan

Table 2 Proteolytic activity in crude enzyme extracts from livers of squids and a cuttlefish shown in Table 1

Sample no.	Bodyweight (g)	Liver weight (g)	Liver (%)*	Proteolytic activity (units/g of liver weight)	
				pH 4.0	pH 7.0
1	404.0±99.4	82.3±34.4	20.7±6.7	17.3±3.1	4.8±0.5
2	364.0±77.0	32.1±13.9	7.6±2.5	8.5±1.0	2.0±0.1
3	630.0±212.1	69.4±28.3	12.5±8.7	14.8±0.1	3.5±1.0
4	450.0±70.7	44.4±20.6	11.8±7.5	17.2±3.8	5.9±0.9
5	302.5±31.0	26.0±4.9	8.3±2.1	16.3±3.1	4.3±0.3
6	334.6±16.8	8.9±0.4	2.7±0.1	9.8±0.8	1.5±0.1
7	127.6±9.0	3.5±0.8	2.7±0.1	14.8±2.5	3.6±1.3
8	543.6±91.4	67.6±18.7	12.3±1.7	14.9±0.8	2.5±0.8
9	510.5±20.5	29.3±5.4	5.8±1.3	12.4±4.1	2.7±1.0

Mean±SD (n=3–5).
*Liver (%)=(liver weight/ bodyweight)×100.

significant amounts of proteases, enzyme recovery from livers, of which weight is known to vary seasonally,[1] may be improved dramatically.

At the present stage of the experiment, we have not compared protease production potentials of squid liver with those of other applicable organisms such as mammals and plants. Moreover, the present study deals only with protease and not with other enzymes, but squid livers are supposed to be useful as resources for various enzymes.

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REFERENCES

1. Suyama M, Konosu S, Hamabe M, Okuda Y. *Utilization of Squid (Ikanoriyou)*. Kouseisyakouseikaku, Tokyo, 1973; 80–104 (in Japanese).
2. Takahashi T. Biochemical studies on the viscera of cuttle-fish, *Ommastrephes sloani pacificus*-VI. On some properties of proteolytic enzymes of the liver. *Nippon Suisan Gakkaishi* 1961; **27**: 85–90.
3. Inaba T, Shindo N, Fujii M. Purification of cathepsin B from squid liver. *Agric. Biol. Chem.* 1976; **40**: 1159–1165.
4. Makinodan Y, Nakagawa T, Hujita M. Effect of cathepsins on textural change during ripening of ika-shiokara (salted squid preserves). *Nippon Suisan Gakkaishi* 1993; **59**: 1625–1629.
5. Wako Y, Ishikawa S, Muramoto K. Angiotensin I-converting enzyme inhibitors in autolysates of squid liver and mantle muscle. *Biosci. Biotechnol. Biochem.* 1996; **60**: 1353–1355.
6. Hatate H, Amagoi H, Takahashi J. Proteolytic activity in crude enzyme extracts from livers of squids and cuttlefish. *J. Natl Fish. Univ.* 1999; **47**: 121–127 (in Japanese).
7. Lowry OH, Rosebrough NJ, Farr AL, Randal RJ. Protein measurement with the folin phenol reagent. *J. Biol. Chem.* 1951; **193**: 265–275.
8. Hara K, Takeda T, Sakai K, Ishihara T. Existence of cysteine protease activity in an acidic pH range in fish. *Nippon Suisan Gakkaishi* 1987; **53**: 1295–1300.