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Original Article

Comparison and selection of protease and lipase sources from visceral organs of three tuna species

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Abstract

Tuna viscera accounts for 7-8% of body weight and its value is still underutilized. This paper investigated on the influence of three tuna species and their individual visceral organ on the activities of three enzymes in tuna viscera. Results revealed that visceral enzymes of yellowfin tuna (*Thunnus albacares*) possessed the highest protease activity (3.09 $U.mg^{-1}$ protein) and lipase activity (0.05 $U.mg^{-1}$ protein) compared to those of skipjack tuna (*Katsuwonus pelamis*) and tonggol tuna (*Thunnus albacares*). None of them contained amylolytic activity. Among the individual visceral organ (stomach, liver, pancreas, spleen), spleen was the best source for protease (0.723 $U.mg^{-1}$ protein) while pancreas gave the highest value of lipase activity (0.03 $U.mg^{-1}$ protein).

Keywords: tuna viscera, protease, lipase, fish extract, enzyme application

1. Introduction

Thailand is the world largest producer and exporter of canned tuna product despite most of the raw materials are imported. There are five species of tuna used in the tuna canning industry : albacore (*Thunnus alaiunga*), yellowfin tuna (*Thunnus albacares*), skipjack tuna (*Katsuwonus pelamis*), tonggol tuna (*Thunnus* tonggol) and frigate tuna (*Auxis thazard*). During the process of tuna canning, vast amounts of liquid and solid wastes are generated. These include tuna precooking water, viscera, head, bone, blood and dark meat. For liquid waste, tuna precooking water is the major source and previously discharged directly to the wastewater treatment system causing the occurrence of red wastewater from the growth of photosynthetic bacteria (Prasertsan and Choorit, 1988). Besides solving this problem directly, the factories have turned to utilizing it for the production of fish extract employing an expensive imported production technology, whereby imported protease is added, thus increasing the production cost.

The solid wastes, on the other hand, are generally sold to a fish meal factory at a very low price. Tuna viscera accounts for 7-8% of the whole body weight (Prasertsan *et al*., 1988). Therefore, of the 400,000 tonnes of tuna processed, 3,200 tonnes of viscera were generated. Fish viscera were reported to be used as a source of enzymes particularly proteolytic digestive enzymes or serine protease (Heu *et al.*, 1995). Among industrial enzymes, proteases are most widely used and account for 60% of the industrial enzymes quantity (Haard, 1992). Commercial protease has been used in seafood processing plant for production of fish extract in Southern Thailand.

It is the aim of this work to find an alternative utilization of tuna viscera as enzyme source. Therefore, an investigation was carried out to determine protease and lipase activities present in the viscera of three tuna species employed in tuna canning factory. The application of crude enzymes from the selected source was used for production of fish extract.

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2. Materials and Methods

2.1 Source of enzymes

Whole viscera and individual viscera organ (stomach, liver, pancreas, spleen) of three tuna species : yellowfin tuna (*Thunnus albacares*), skipjack (*Katsuwonus pelamis*), and tonggol tuna (*Thunnus tonggol*) were used as source of enzymes in this study. They were kindly provided by two seafood processing factories in Songkhla region, Thailand. The viscera were kept frozen at -20°C in sealed plastic bags until needed for enzyme extraction. Each viscera organ of yellowfin tuna is illustrated in Figure 1.

2.2 Determination of enzyme activities

Protease activity was measured according to the procedure described by Hagihara *et al.* (1958) in which either casein or hemoglobin were used as substrate and the optical density was measured at the wavelength of 275 nm after incubation for 15 min at 37°C.

Lipase activity was measured by modified method of Winker and Stuckman (1979) using *p*-nitrophenyl palmitate as substrate and the optical density was measured at the wavelength of 410 nm after incubation at 37°C for 15 min.

Amylase activity was measured following the method of Pongsawasdi and Yagisawa (1988).

2.3 Effect of tuna species, viscera organ and buffer pH on the enzyme activities

Whole viscera and the individual viscera organ of the three tuna species (30 fishes for each species) were washed with sterile water and weighed for calculation of the percentage of each viscera organ. Cold 50 mM buffer solution of pH 2-11 were added in the 1:2 (w/v) ratio of viscera to buffer. They were prepared as following : pH 2.0-6.0 (citrate-phosphate buffer), pH 7.0-9.0 (Tris-HCl) and pH 10.0-11.0 (carbonate-bicarbonate buffer). The mixture was homogenized for 1-2 min before filtration through cheese cloth to remove solid residues. The fine particles left in the filtrate were removed by centrifugation at 2,800 *xg* for 30 min at 4°C. The supernatant (tuna viscera extract) was used as the crude enzyme and determined for activities of protease, lipase and amylolytic enzyme.

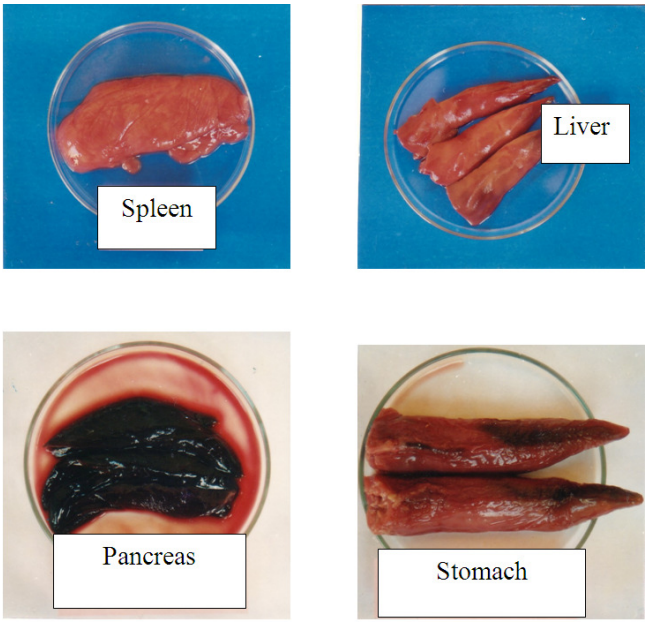

Figure 1. Individual viscera organ of yellowfin tuna (*Thunnus albacares*)

3. Results and Discussion

3.1 Effect of tuna species, viscera organ and buffer pH on the enzyme activities

Three tuna species were different in catching area, size and amount of viscera. Yellowfin tuna and skipjack tuna were catched from Indian Ocean and West Pacific Ocean while tonggol tuna was catched in the Gulf of Thailand. Their average (from 30 fish samples each) body weights were 2.20, 2.32 and 1.30 kg with the viscera yields of 7.05, 5.44 and 5.18%, respectively. The yield of yellowfin tuna viscera agreed with the previous report of 7-8% (Prasertsan *et al.*, 1988) with slightly lower than 8% in frigate mackerel (*Auxis rochei*) (Cano-Lopez *et al.*, 1987). The yields of the other two species were much lower.

Tuna species, viscera organ, and the pH of buffer used in enzyme extraction had a substantial influence on the protease activity (Figure 2) and lipase activity (Figure 3). Amylolytic activity was not detected in any source of enzymes used. It should be noted that lipase activity could be determined only in the pH range of 6.0-11.0 as the activity was too low in the pH range of 2.0-5.0. Both protease and lipase activities from all sources were highest at pH 10.0 for yellowfin tuna (*Thunnus albacares*) and skipjack tuna (*Katsuwonus pelamis*) while at pH 9.0 for tonggol tuna (*Thunnus tonggol*). Activities of both enzymes decreased sharply at pH over its optimum pH. The results clearly demonstrated that the enzymes from tuna viscera were serine protease which functioned best at alkaline pH (Shin and Zall, 1986).

Comparison among the individual viscera organ, spleen of the yellowfin tuna gave the highest protease activity (53.38 U ml^{-1} with the specific activity of 2.56 U mg^{-1} protein) while pancreas gave the highest lipase activity (0.72 U ml^{-1} with the specific activity of 0.03 U mg^{-1} protein). However, these enzyme activities were lower than those extracted from the whole viscera of all three tuna species using its optimum pH buffer (Table 1). For lipase activities, whole viscera of the yellowfin tuna exhibited the highest lipase activity (1.26 U ml^{-1} with a specific activity of 0.05 U mg^{-1} protein), followed by the those of tonggol tuna (0.86 U ml^{-1} and 0.03 U mg^{-1}, respectively) and skipjack tuna (0.53

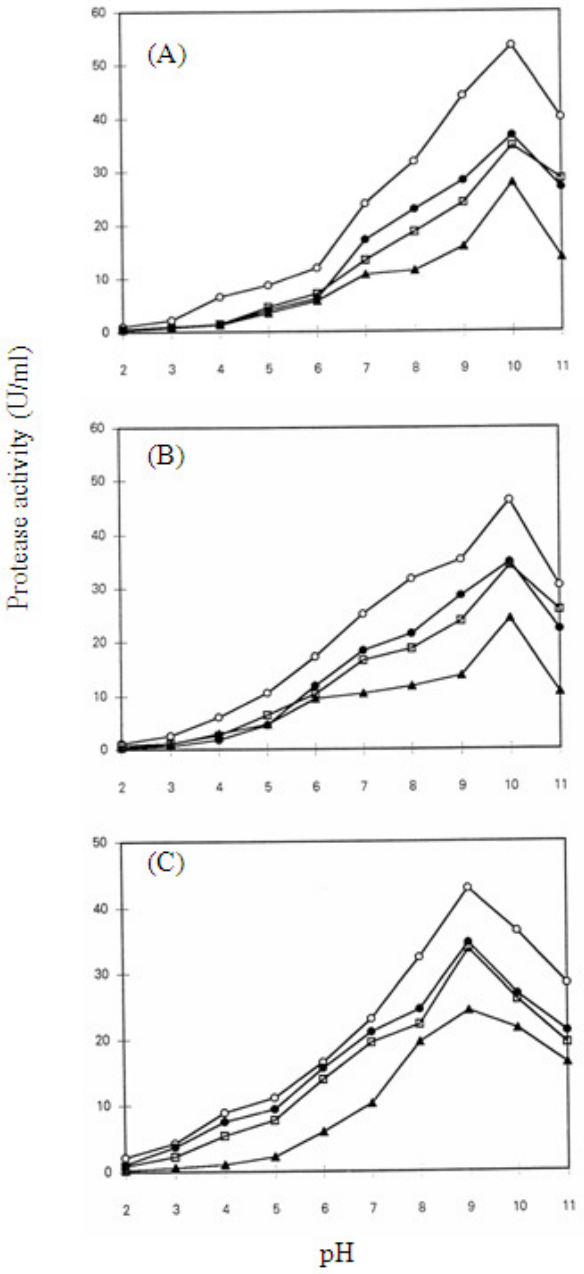

Figure 2. Effect of enzyme extracted buffer pH on protease activity from stomach (▲), spleen (O), liver (●) and pancrease (□) from yellowfin tuna (*Thunnus albacares*) (A), skipjack tuna (*Katsuwonus pelamis*) (B), and tonggol tuna (*Thunnus tonggol*) (C)

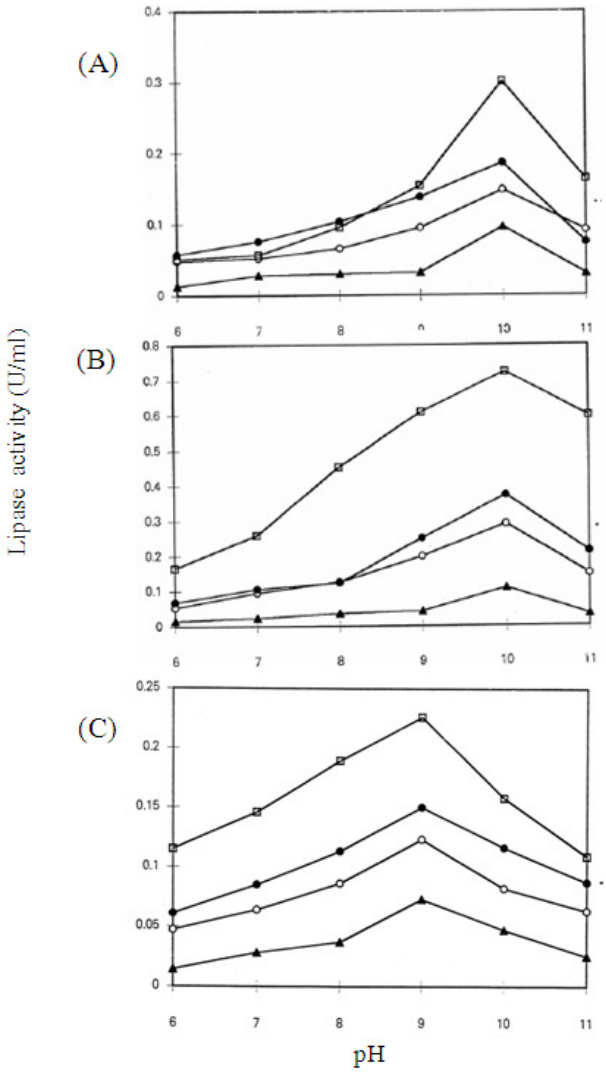

Figure 3. Effect of enzyme extracted buffer pH on lipase activity of enzymes from stomach (▲), spleen (O), liver (●) and pancrease (□) of skipjack tuna (*Katsuwonus pelamis*) (A), yellowfin tuna (*Thunnus albacares*) (B) and tonggol tuna (*Thunnus tonggol*) (C)

U ml^{-1} and 0.02 U mg^{-1}, respectively) at pH 10.0 and 9.0, respectively.

For protease activities, whole viscera of the yellowfin tuna exhibited the highest protease activity (72.17 U ml^{-1} with a specific activity of 3.09 U mg^{-1} protein), followed by the those of skipjack tuna (60.53 U ml^{-1} and 2.39 U mg^{-1}, respectively) and tonggol tuna (48.53 U ml^{-1} and 2.30 U mg^{-1}, respectively) at pH 10.0 and 9.0, respectively. These specific protease activities (2.30-3.09 U mg^{-1}) were higher than the trypsin activity of viscera from anchovy (*Engraulis encrasicholus*) (0.2 U mg^{-1}) (Martinez *et al.*, 1988) and chymotrypsin activity of caeca from rainbow trout (*Oncorhynchus mykiss*) (0.64 U mg^{-1}) (Kristjansson and Nielson, 1991) but lower than that of chymotrypsin from Atlantic cod (*Gadus morhua*) viscera (10.7 U mg^{-1}) (Asgeirsson and Bjarnason, 1991).

The enzymes from all sources of the three tuna species showed their highest protease activities at alkaline pH (pH 9.0-10.0). These were in agreement with the previous report that extraction of protein in alkaline condition especially at pH 10.0 would enhance the activities of the enzyme from fish viscera and the digestive tract (Kim *et al.*, 1994). Nevertheless, the highest specific activity of enzyme from gustric mucosa of Polar cod (*Boreogadus saida*) was achieved at a pH of 7.3 (Meinke *et al.*, 1972).

Table 1. Comparison on the highest specific activities of protease and lipase extracted from the whole viscera of three tuna species at their optimum buffer pH

Tuna species	Optimum pH	Protease		Lipase	
		Activity (Unit ml^{-1})	Specific activity (Unit mg^{-1})	Activity (Unit ml^{-1})	Specific activity (Unit mg^{-1})
Yellowfin (*Thunnus albacares*)	10	72.17±0.05	3.089±0.003	1.258±0.011	0.0538±0.0007
Skipjack (*Katsuwonus pelamis*)	9	48.53±0.08	2.304±0.005	0.527±0.008	0.0221±0.0004
Tonggol (*Thunnus tonggol*)	10	60.53±0.06	2.399±0.002	0.855±0.008	0.0338±0.0003

4. Conclusion

Whole viscera of yellowing tuna (*Thunnus albacares*) possessed the highest protease and lipase activities and none of the three tuna species contained amylolytic activity. Among the individual visceral organ (stomach, liver, pancreas, spleen), spleen was the best source for protease while pancreas gave the highest value of lipase activity.

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