

Original article

Condition optimization of trypsin and chymotrypsin activities in Asian seabass (*Lates calcarifer*)

Winyoo Boonprasert, Prachaub Chaibu *, Kriangsak Meng-umphan, Jongkol Promya and Chanagun Chitmanat

Faculty of Fisheries Technology and Aquatic Resources, Maejo University, San Sai, Chiangmai, 50290, Thailand

* Correspondence: prachaub@mju.ac.th; Tel.: +66-1-5-9549-50

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Introduction

The Asian seabass is a carnivorous, is an economically important food fish in the tropical and subtropical regions in the Asia-Pacific. It is highly esteemed food taken mainly by artisanal fishermen. Because of its relatively high market value, it has become an attractive commodity of both large to small-scale aquaculture enterprises. It is important as a commercial and subsistence food fish. Therefore application of the digestive enzymes plays a role in the growth and survival of aquaculture. Trypsin and chymotrypsin are the 2 proteases that play the important role in protein digestion and involving in growth rate. In Asian seabass these two enzymes indicated as growth parameters by feed. Although enzyme assay at optimal condition is significant for its accuracy activity determination.

Trypsin is found in different isoforms in the pyloric caeca and intestine [1] and different distributions of these isoforms could determine genetic variation in protein and feed utilisation and growth performance of individuals in aquaculture [2]. The activity of trypsin showed a similar pattern as growth of juvenile Atlantic salmon during seasonal variation, and its secretion rate was related to the amount of feed passing through the gut [3]. A linear relationship between trypsin activity and protein digestibility was observed in rainbow trout (*Oncorhynchus mykiss*) [4]. Trypsin activity was reported to relate with growth rate in fish larvae fed high quality diets, such as in goldfish (*Carassius auratus*) [5], seabass [6] and red drum [7].

Different environmental or external factors, such as temperature [7], light dietary quality [8], growth hormone [9] and gene manipulation [8], induced differences in expression of trypsin (as well as chymotrypsin) and thus partly resulted in growth rate variations observed [6]. In this study, optimization of pH and temperature for trypsin and chymotrypsin in Asian seabass were investigated. There is less report of trypsin and chymotrypsin. Therefore, enzyme assay at

optimal condition is significant for its accuracy activity determination. Digestive enzyme distribution and activity in the digestive tracts of the Asian seabass.

Materials and methods

Sample preparation

Asian seabass (*Lates calcarifer*) were obtained from a local fish farm in Bang Pakong, Chachoengsao province, Thailand, stored in iced during transportation to laboratory. The intestine were separated into and stored at -80°C prior to extraction.

Preparation of crude enzyme extract

The enzymes extraction was performed according to Rungruangsak-Torrissen [2]. Frozen digestive tract was extracted in 50 mM Tris-HCl buffer pH 2-12 containing 200 mM NaCl (1:2 w/v) using a micro-homogenizer. The homogenate was then centrifuged at $15000 \times g$ for 30 min at 4°C . The supernatant of crude enzyme extract was collected after removing lipid and kept at -80°C in small portions.

Protein concentration

Protein concentration was determined by the method of Lowry et al. [10] using bovine serum albumin as a standard.

Optimum pH on activity of trypsin and chymotrypsin

Trypsin and chymotrypsin activity were determined using 1.25 mM benzoyl-L-arginine-*p*-nitroanilide (BAPNA) and 0.1 mM *N*-succinyl-Ala-Ala-Pro-Phe-*p*-nitroanilide (SAPNA), respectively, according to Rungruangsak-Torrissen [2]. Each substrate was dissolved in dimethylformamide (5% final concentration) before making up to final volume with 0.2 M Tris-HCl buffer at pH range of 2-12. The reaction mixture of 10 μl crude enzyme extract and 1000 μl substrate solution was incubated at 30°C for 30 min [11]. Increased absorbance of 1 min initial reaction

rate was measured at 410 nm. Both trypsin and chymotrypsin specific activities were expressed as $\mu\text{mol } p\text{-nitroaniline produced h}^{-1} \text{ mg protein}^{-1}$.

Optimum temperature on activity of trypsin and chymotrypsin

The optimum temperature on activity of trypsin and chymotrypsin preparation substrate as described above at optimum pH of Asian seabass. The reaction mixture of 10 μl crude enzyme extract and 1000 μl substrate solution was incubated at different temperature (30, 35, 40, 45, 50, 55, 60, 65, 70, 75 and 80°C) for 30 min [11], in a sonicator bath temperature control. Increased absorbance of 1 min initial reaction rate was measured at 410 nm. Both T and C specific activities were expressed as $\mu\text{mol } p\text{-nitroaniline produced h}^{-1} \text{ mg protein}^{-1}$.

Results and discussion

The optimum pH condition for hydrolysis of BAPNA by Asian seabass intestinal trypsin fraction was 8.0 and SAAPNA by chymotrypsin fraction was 5.0 (Fig. 1). Acidic pH values were more inhibitory to the enzyme than alkaline pH values. Similar results have been reported for trypsin from intestine bovine trypsin has an optimum pH of 8.2 [12], yellowfin tuna trypsin and chymotrypsin have an optimum pH of 8.0 [13], and anchovy trypsin and chymotrypsin was in the pH range of 8.0-9.0 and 7.5-8.5, respectively [7].

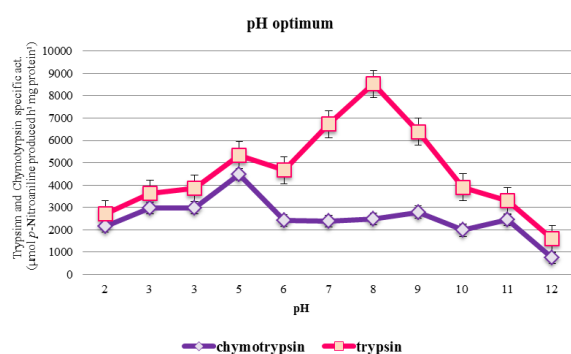


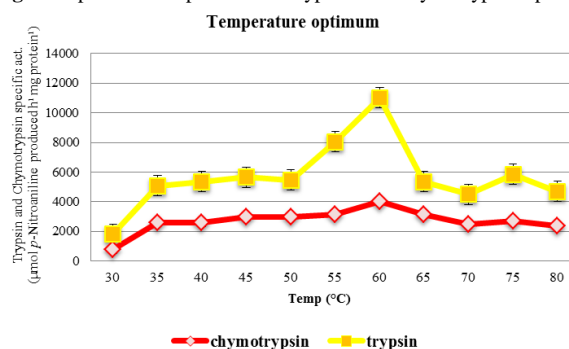
Fig. 1. Optimum pH of trypsin and chymotrypsin specific activity ($\mu\text{mol } p\text{-nitroaniline produced h}^{-1} \text{ mg protein}^{-1}$).

Temperature optimum

The optimum temperature for Asian seabass intestinal trypsin and chymotrypsin activities were 60°C at pH 8.0 and pH 5.0, respectively (Fig. 2). The pattern of Asian seabass trypsin activity was increased when the temperature was increased, it was correspond with anchovy trypsin and chymotrypsin had maximum activity for both specific substrates at 45°C and decreased when the temperature up to 50°C and inactive at 65°C [7]. It indicated that Asian seabass trypsin and chymotrypsin activities were maintained even at ambient temperature to 80°C. When Asian seabass

intestinal trypsin and chymotrypsin was held at 0 to 60°C for 30 min, activities were remained. Anchovy trypsin and chymotrypsin were stable at 0-50°C and loss activities at 60°C [7].

Fig. 2. Optimum temperature of trypsin and chymotrypsin specific



activity ($\mu\text{mol } p\text{-nitroaniline produced h}^{-1} \text{ mg protein}^{-1}$).

Acknowledgements

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