

Original article

## Effective extraction of carotenoprotein from Pacific white shrimp (*Litopenaeus vannamei*) shells using albacore tuna (*Thunnus alalunga*) spleen trypsin

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### Introduction

Carotenoprotein has been reported to have bioactivity. Studies have been carried out on the recovery of carotenoprotein from shrimp waste by different techniques [1]. However, there are many disadvantages to those methods such as low protein content, high ash and chitin levels and low stability of carotenoprotein. To increase the extraction efficiency, hydrolysis processes mediated by proteinases have been implemented. Based on our previous study, proteinases from albacore tuna spleen were partitioned using aqueous two-phase system (ATPS), which were identified as trypsin-like serine proteinases [2]. However, no information regarding the use of partitioned trypsin from albacore tuna spleen as a food-processing aid, particularly to recover the bioactive components from fish/shellfish-processing wastes, has been reported. Therefore, this study aimed to investigate its efficacy in carotenoprotein extraction from Pacific white shrimp shells.

### Materials and methods

Carotenoprotein from the shells of Pacific white shrimp was extracted by the method of Klomklao et al. [3].

The reaction was initiated by adding partitioned trypsin at a level of 0.2 and 0.8 units/g sample. The mixtures were shaken continuously at 200 rpm in a shaking water bath at 25°C for various times intervals (0, 15, 30, 45, 60 and 120 min). The carotenoprotein was extracted in the same manner as previously described.

Different amounts of partitioned trypsin (0, 0.05, 0.1, 0.15, 0.3, 0.5, 0.7, 0.8, 0.9, 1.0, 1.2 units/g sample) were used. The hydrolytic reaction was carried out at 25°C for 45 min, and the extraction of carotenoprotein was performed as previously described.

Ground Pacific white shrimp shell was blended with buffer at a ratio of 1:0.5, 1:1, 1:2, 1:3, 1:4 and 1:5 (w/v). Partitioned albacore tuna trypsin at a level of 0.8 units/g sample was added and the reaction was maintained for 45 min at 25°C. The carotenoprotein was extracted in the same manner as previously described. The protein contents of the samples were measured by Biuret method [4]. Total carotenoid content was determined according to the method of [3].

Protein, ash and fat contents of shrimp shells and the carotenoprotein were determined according to the method of AOAC (2012).

Chitin was measured according to the method of Senphan et al. [1].

Amino acid composition of carotenoprotein was analysed according to the method of Senphan et al. [1].

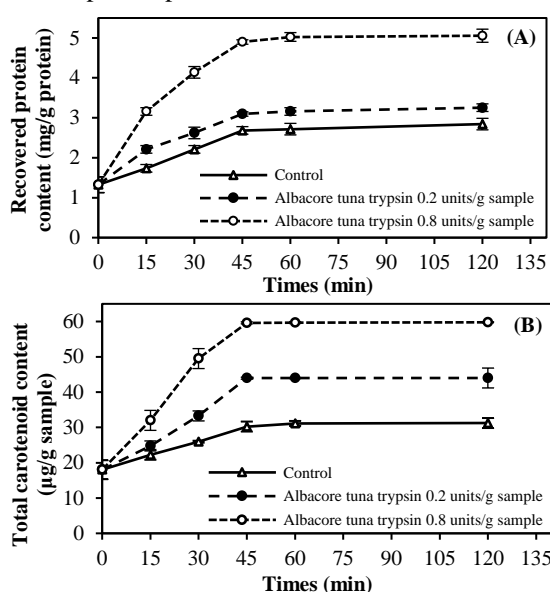
### Results and discussion

At the same hydrolysis time, the recovered protein and carotenoids contents of shrimp shells hydrolysed with partitioned trypsin was higher than that of the control ( $P < 0.05$ ) (Fig. 1). This indicated that the addition of partitioned albacore tuna trypsin to the shrimp shells homogenate was effective in improving the carotenoprotein recovery. From the results, the hydrolysis

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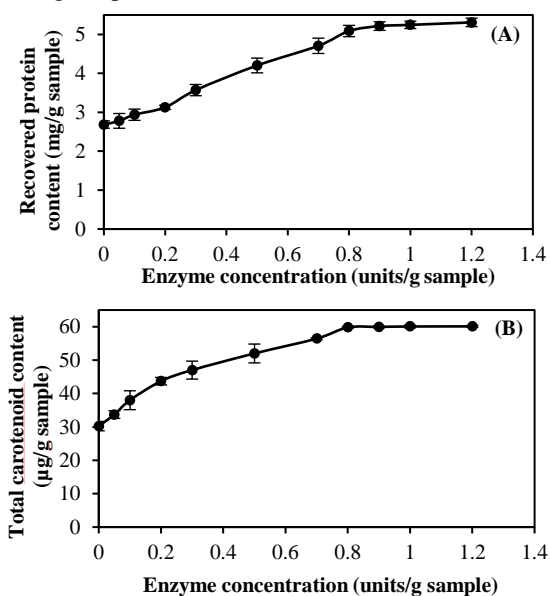
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time of 45 min was found to be optimal for carotenoprotein production.



**Fig. 1.** Recovered protein (A) and total carotenoid (B) contents of Pacific white shrimp shells without and with the aid of trypsin from albacore tuna spleen at a level of 0.2 and 0.8 units/g sample for various times at 25°C. Bars represent standard deviation from triplicate determinations.

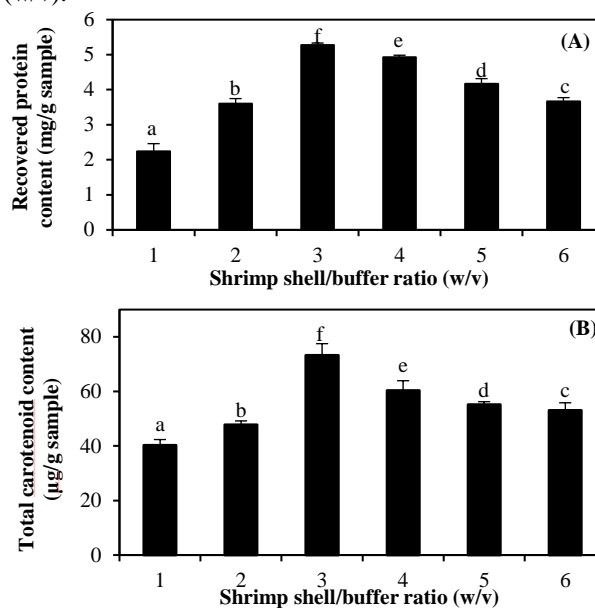
The recovered protein and total carotenoid contents increased with increasing partitioned trypsin concentrations ( $P < 0.05$ ) (Fig. 2). The optimum level of trypsin from albacore tuna spleen for carotenoprotein extraction was 0.8 units/g sample.



**Fig. 2.** Recovered protein (A) and total carotenoid (B) contents of Pacific white shrimp shells without and with the aid of trypsin from albacore tuna spleen at different levels. The hydrolytic reaction was conducted at 25°C for 45 min. Bars represent standard deviation from triplicate determinations.

The increase in ratio up to 1:2 significantly increased both protein and carotenoid contents ( $P < 0.05$ ) (Fig. 3). From the results, the optimum conditions for carotenoprotein extraction using partitioned albacore

tuna trypsin were 0.8 units/g sample for 45 min at 25°C and Pacific white shrimp shells/buffer ratio of 1:2 (w/v).



**Fig. 3.** Effect of shrimp shells/buffer ratio on recovered protein (A) and total carotenoid (B) contents for Pacific white shrimp shells treated with albacore tuna trypsin at a level of 0.8 units  $g^{-1}$  sample. The hydrolytic reaction was conducted at 25°C for 45 min. Different letters indicate significant differences ( $P < 0.05$ ). Bars represent the standard deviation from triplicate determinations.

Albacore tuna trypsin showed similar recovery efficacy of protein or carotenoids, compared with bovine trypsin ( $P > 0.05$ ) (data not shown). Therefore, albacore tuna trypsin has a potential application for the extraction of carotenoproteins from shrimp shells.

Carotenoprotein consisted of 72.37% protein, 18.79% lipid, 7.14% ash, 1.61% chitin, and 73.25 µg total astaxanthin/g sample. It was rich in essential amino acid and Asp/Asn and Glu/Gln were found as dominant amino acids.

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