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Characteristics and antioxidative activity of carotenoprotein from shells of Pacific white shrimp extracted using hepatopancreas proteases

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ABSTRACT

Carotenoprotein from shells of Pacific white shrimp (*Litopenaeus vannamei*) was extracted with the aid of proteases from hepatopancreas of the same species at various levels (5–30 units/g shrimp shell) for different times (0–180 min). Recovery of carotenoprotein increased with increasing protease levels and hydrolysis times, but mainly reached the plateau at 120 min of hydrolysis. Carotenoprotein contained astaxanthin and astaxanthin-diester as major carotenoids. Carotenoids extracted from carotenoprotein showed increasing ABTS, DPPH radical scavenging activities, FRAP and metal chelating activity when the concentrations increased up to 5 mg/ml ($p<0.05$). Carotenoprotein consisted of 73.58% protein, 21.87% lipids and 2.63% ash contents. It was rich in essential amino acids and Asp/Asn and Glu/Gln were found as dominant amino acids. Therefore, carotenoprotein from shell of Pacific white shrimp could be successfully extracted using shrimp hepatopancreas proteases and could serve as the value-added nutritive food ingredients or as the animal feed.

1. Introduction

Pacific white shrimp (*Litopenaeus vannamei*) accounts for 90% of the global aquaculture shrimp production. Thailand has been the world's leading exporter of cultivated shrimp since the mid-90s, (Lebel, Mungkung, Gheewala, & Lebel 2010). Pacific white shrimp and its products have become economically important for Thailand. By the year 2010, frozen Pacific white shrimp and products were manufactured and exported totally for 407,978 metric tons, particularly to USA and Japan (Lebel, Mungkung, Gheewala, & Lebel 2010; Senphan & Benjakul, 2012). During shrimp processing, a large amount of by-products including cephalothorax, shell, etc. is generated. To exploit those left-over, cephalothoraxes have been used as raw material for production of shrimp hydrolysate, shrimp flavorant, carotenoid and chitin/chitosan (Armenta-López, Guerrero, & Huerta 2002; Bueno-Solano et al., 2009; Gildberg & Stenberg, 2001; Ravi Kumar, 2000; Sowmya, Rathinaraj, & Sachindra, 2011). Shrimp shell has been intensively used for carotenoprotein extraction (Armenta & Guerrero-Legarreta, 2009; Babu, Chakrabarti, & Surya Sambasivarao, 2008; Cano-Lopez, Simpson, & Haard, 1987; Chakrabarti, 2002; Klomklao et al., 2009; Sila, Nasri & Bougatef, 2012; Sowmya et al., 2011).

Carotenoid and carotenoproteins have been recognised as the natural colourant. Furthermore, they have been reported

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to have bioactivity, e.g. antioxidative, antimicrobial, etc. (Sowmya & Sachindra, 2012). Carotenoids were extracted using traditional solvent extraction, supercritical fluid extraction (Babu et al., 2008) and vegetable oils (Sowmya & Sachindra, 2012). To increase the extraction efficiency, hydrolysis process mediated by protease has been implemented. Cano-Lopez et al. (1987) reported the use of Atlantic cod trypsin or bovine trypsin for the extraction of carotenoproteins from shrimp processing wastes. Chakrabarti (2002) also used trypsin, pepsin and papain to extract carotenoprotein from brown shrimp waste.

Among all shrimp products, whole shrimp without hepatopancreas currently is of increasing demand. Along the process, hepatopancreas is removed by vacuum sucking machine. Shrimp hepatopancreas can be the major source of proteases, especially trypsin and chymotrypsin (Hernandez-Cortes, Whitaker & Garcia-Carreno, 1997; Sriket et al., 2012). Protease in hepatopancreas from freshwater prawn actively hydrolysed various proteinaceous substrates (Sriket et al., 2012). Appropriate hydrolysis condition could lead to the increasing extraction yield of carotenoprotein from shrimp shells. Therefore, the present study aimed to investigate the impact of hydrolysis using protease from hepatopancreas on the extraction of carotenoproteins from Pacific white shrimp and to characterise the resulting carotenoproteins.

2. Materials and methods

2.1. Chemicals

2,2-Azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) and 2,2-diphenyl-1-picryl hydrazyl (DPPH), N-α-Benzoyl-DL-arginine 4-nitroanilide hydrochloride (BAPNA) and 3-(2-pyridyl)-5–6-diphenyl-1,2,4-triazine-4′,4″-disulphonic acid sodium salt (ferrozine) were purchased from Sigma (St. Louis, MO, USA). Anhydrous sodium sulphate, ferric chloride and ferrous chloride were obtained from Merck (Darmstadt, Germany). Methanol, hexane, acetone, petroleum ether, hydrochloric acid and sulphuric acid were procured from Lab-Scan (Bangkok, Thailand). Astaxanthin was obtained from Dr. Ehrenstorfer GmbH (Augsburg, Germany).

2.2. Preparation of shells from Pacific white shrimp

Shells of Pacific white shrimp (*Litopenaeus vannamei*), obtained from the Sea wealth frozen food Co., Ltd., Songkhla province, Thailand, were transported to the Department of Food Technology, Prince of Songkla University, Hat Yai, Songkhla within 2 h. Upon arrival, the samples were washed with tap water, followed by drying in hot air oven (Binder FED115, Tuttlingen, Germany) for 6 h at 65 °C. The dried samples were then ground to obtain the particle size of 1.0–2.0 mm. Ground shells were placed in polyethylene bag and kept in dark.

2.3. Preparation of crude extract from hepatopancreas of Pacific white shrimp

Pooled hepatopancreases of Pacific white shrimp were obtained from the Sea wealth frozen food Co., Ltd., Songkhla province. Samples were placed in polyethylene bag and imbedded in a polystyrene box containing ice with an ice/sample ratio of 2:1 (w/w) during transportation for approximately 2 h. Upon arrival, the samples were stored at −18 °C until use, but not longer than 1 month.

Prior to extraction of proteases, hepatopancreases of Pacific white shrimp were powdered in liquid nitrogen. Samples were then defatted by stirring in three volumes of acetone at −20 °C for 30 min according to the method of Kishimura and Hayashi (2002). The homogenate was filtered in vacuo on Whatman No. 1 filter paper (Whatman International Ltd., Maidstone, England). The residue obtained was then stirred in two volumes of acetone at −20 °C for another 30 min, and then the residue was left at room temperature until dried and free of acetone odour.

To prepare the crude extract, acetone powder was suspended in 10 mM Tris–HCl, pH 8.0 containing 1 mM $CaCl_2$ at a ratio of 1:50 (w/v) and stirred continuously at 4 °C for 3 h. The suspension was centrifuged for 10 min at 4 °C at 10,000 × *g* to remove the tissue debris using a refrigerated centrifuge (Beckman Coulter, Avanti J-E Centrifuge, Fullerton, CA, USA). The supernatant was lyophilised using a SCANVAC CoolSafe™ freeze-dryer (CoolSafe 55, ScanLaf A/S, Lynge, Denmark). Before use, the lyophilised sample (10 g) was dissolved with 50 ml of cold distilled water (4 °C) and the solution is referred to as "crude extract".

2.4. Assay for trypsin activity

Trypsin activity of crude extract was measured using BAPNA as a substrate according to the method of Khantaphant and Benjakul (2008). A 200 μl of sample was mixed with 200 μl of distilled water and 1000 μl of reaction buffer (50 mM Tris–HCl buffer, pH 8.0, containing 10 mM $CaCl_2$). The reaction was initiated by adding 200 μl of 2 mg/ml BAPNA to the reaction mixture. After incubation for 20 min at 60 °C, 200 μl of 30% acetic acid (v/v) were added to terminate the reaction. Production of *p*-nitroaniline was measured by monitoring the absorbance of reaction mixture at 410 nm (A_{410}) using a UV-1601 spectrophotometer (Shimadzu, Kyoto, Japan). A blank was conducted in the same manner except that the sample was added after addition of 30% acetic acid. One unit was defined as the amount of trypsin causing an increase of 1.0 in A_{410}/min.

2.5. Extraction of carotenoprotein using crude extract from hepatopancreas

Carotenoprotein from shrimp shell was extracted with the aid of crude extract from hepatopancreas. Ground shrimp shell (25 g) was mixed with five volumes of distilled water adjusted to pH 8 using 1.0 M NaOH and pre-incubated at 60 °C. The mixture was added with crude extract from hepatopancreas to obtain different levels of protease (5, 10, 20 and 30 units/g shrimp shell). The mixtures were shaken