Food Chemistry 134 (2012) 829–835

Contents lists available at SciVerse ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Compositions and yield of lipids extracted from hepatopancreas of Pacific white shrimp (*Litopenaeus vannamei*) as affected by prior autolysis

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ARTICLE INFO

Article history:
Received 30 October 2011
Received in revised form 8 January 2012
Accepted 27 February 2012
Available online 12 March 2012

Keywords:
Autolysis
Hepatopancreas
White shrimp
Lipid
Oxidation
Hydrolysis
Carotenoid

ABSTRACT

Compositions and yield of lipids extracted from hepatopancreas of Pacific white shrimp (*Litopenaeus vannamei*) subjected to autolysis at 60 °C for different times (0, 30, 60, 90 120 and 150 min) were investigated. Extraction yield increased from 7.4% to 8.8% as autolysis time increased from 0 to 150 min. Coincidental increase in total carotenoid content was obtained with increasing autolysis time ($p < 0.05$). The increases in thiobarbituric acid-reactive substances (TBARS) and *p*-anisidine value (AV) of lipids were noticeable when autolysis time increased ($p < 0.05$). However, no changes in free fatty acid (FFA) content were observed within the first 60 min of autolysis ($p > 0.05$), but subsequently increased up to 150 min. ($p < 0.05$). No differences in fatty acid profiles of lipids extracted from hepatopancreas without and with 60 min prior autolysis were observed. Lipids extracted contained docosahexaenoic acid (DHA; C22:6($n-3$)) as the most abundant fatty acid, followed by eicosapentaenoic acid (EPA; C20:5($n-3$)). Therefore, prior autolysis at 60 °C for 60 min increased the extraction yield without negative effect on lipid quality.

1. Introduction

Thailand has been the world's leading exporter of cultivated shrimp since the mid-90s, (Lebel, Mungkung, Gheewala, & Lebel, 2010). White shrimp and white shrimp products are an economically important crustacean of Thailand. By the year of 2010, frozen white shrimp and white shrimp products were manufactured and exported, mostly to the USA and Japan, with a total amount of 407978 metric tons (Lebel et al., 2010). During shrimp processing, a large amount of by-products including cephalothorax, shell, etc. are generated. Shrimp cephalothorax has been used as raw material for shrimp hydrolysate, shrimp flavourant, carotenoid and chitin/chitosan (Armenta-López, Guerrero, & Huerta, 2002; De Holanda & Netto, 2006; Flores, Barrera-Rodríguez, Shirai, & Durán-de-Bazúa, 2007; Pan, 1990). Lipids of shrimp head and shell from Indian white shrimp (*Penaeus indicus*) were extracted with a yield of 9.8% (dry weight basis) (Ravichandran, Rameshkumar, & Prince, 2009). Krill oil was also extracted from krill, a shrimp-like crustacean (Sampalis et al., 2003). Similar to fish oil, krill oil is rich in long chain omega-3 polyunsaturated fatty acids, mainly docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), which have been associated with a wide-range of health benefits to humans (Duan, Jiang, Cherian, & Zhao, 2010; Matsumoto, Nakayama, Ishida, Kobayashi, & Kamata, 2009). Furthermore, krill oil contains vitamin E, vitamin A, vitamin D and canthaxanthin. The antioxidant potency of krill oil was found to be 48-fold more potent than fish oil (Sampalis et al., 2003). For shrimp shell, it has been intensively used for chitin and chitosan production (Percot, Viton, & Domard, 2003). Furthermore chitin/chitosan with bioactivity has been prepared (Dutta, Dutta, & Tripathi, 2004). Carotenoprotein was also recovered from black tiger shrimp cephalothorax (Sowmya, Rathinaraj, & Sachindra, 2011).

Among all shrimp products, there is currently an increasing demand for whole shrimp without hepatopancreas. Hepatopancreas is removed and recovered by a vacuum sucking machine. Hepatopancreas can be the major source of lipids, carotenoid, etc. (Andrew, 1979; Pacheco et al., 2009). Additionally, shrimp hepatopancreas has been known to be an excellent source of proteases, especially trypsin (Oh, Kim, Kim, & Kim, 2000; Sriket, Benjakul, & Visessanguan, 2011a). Since hepatopancreas contains high amount of proteases, lipoproteins and carotenoprotein, autolysis mediated by endogenous proteases might increase the extraction yield of lipids as well as carotenoid. However, little information about lipids and carotenoids from Pacific white shrimp hepatopancreas has been reported. Therefore, the aim of the present study was to investigate the impact of autolysis on extraction efficiency and properties of lipids from the hepatopancreas of white shrimp, a by-product from a shrimp processing plant.

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http://dx.doi.org/10.1016/j.foodchem.2012.02.188

2. Materials and methods

2.1. Chemicals

Palmitic acid, cupric acetate, *p*-anisidine, ammonium thiocyanate, cupric acetate and pyridine were purchased from Sigma (St. Louis. MO, USA). Trichloroacetic acid, anhydrous sodium sulphate, isooctane and ferrous chloride were obtained from Merck (Darmstadt, Germany). 2-Thiobarbituric acid and 1,1,3,3-tetramethoxypropane were procured from Fluka (Buchs, Switzerland). Methanol, ethanol, chloroform, petroleum ether, hydrochloric acid, sulphuric acid and ammonium thiocyanate were purchased from Lab-Scan (Bangkok, Thailand). Astaxanthin was obtained from Dr. Ehrenstorfer GmbH (Augsburg, Germany).

2.2. Collection and preparation of hepatopancreas from Pacific white shrimp

Hepatopancreas of Pacific white shrimp (*Litopenaeus vannamei*) was obtained from the Sea Wealth Frozen Food Co., Ltd. in Songkhla. Pooled hepatopancreas was placed in a polyethylene bag and transported on ice to the Department of Food Technology, Prince of Songkla University, Hat Yai, Songkhla within approximately 2 h. The samples were stored at −18 °C until use, but the storage time was not longer than 1 month. Hepatopancreas was analysed for moisture, ash, fat and protein contents according to the method of AOAC (2000). The values were expressed as % (wet weight basis). Prior to analyses or extraction, hepatopancreas was ground using a blender (Phillips, Guangzhou, China) for 30 s.

2.3. Autolysis study of hepatopancreas

Ground hepatopancreas (45 g) was placed in a 500 ml-beaker and incubated at different temperatures (30, 40, 50, 55, 60, 65, 70 and 80 °C) for 30 min. After the designated incubation time, autolysis was terminated by addition of 5 ml of 50% trichloroacetic acid (TCA). The mixtures were homogenised at a speed of 11,000 rpm for 2 min using an IKA Labortechnik homogeniser (Selangor, Malaysia). Thereafter, the homogenate was centrifuged at 3000 × *g* for 15 min using a refrigerated centrifuge (Beckman Coulter, Avanti J-E Centrifuge, Fullerton, CA, USA). TCA-soluble peptides in the supernatant were determined using the Lowry's method (Lowry, Rosebrough, Farr, & Randall, 1951).

2.4. Effect of autolysis time on extraction and properties of lipids

Hepatopancreas was incubated at the optimal temperature for different times (0, 30, 60, 90, 120 and 150 min). The samples were cooled suddenly in iced water, followed by lipid extraction.

Lipid was extracted by the Bligh and Dyer method (Bligh & Dyer, 1959). Sample (25 g) was homogenised with 200 ml of a chloroform:methanol:distilled water mixture (50:100:50, v/v/v) at a speed of 9500 rpm for 2 min at 4 °C. The homogenate was added with 50 ml of chloroform and homogenised at 9500 rpm for 1 min. Thereafter, 25 ml of distilled water were added and homogenised at the same speed for 30 s. The homogenate was centrifuged at 3000 × *g* at 4 °C for 15 min and transferred into a separating flask. The chloroform phase was drained off into the 125 ml Erlenmeyer flask containing 2–5 g of anhydrous sodium sulphate, shaken very well, and decanted into a round-bottom flask through a Whatman No.4 filter paper (Whatman International Ltd., Maidstone, England). The solvent was evaporated at 25 °C using an EYELA rotary evaporator N-1000 (Tokyo Rikakikai, Co. Ltd, Tokyo, Japan) and the residual solvent was removed by nitrogen flushing. The yield was calculated and expressed as the percentage of lipids extracted. Lipid samples were collected in a vial, flushed with nitrogen gas, sealed tightly and kept at −40 °C until analysis.

2.5. Analyses

2.5.1. Measurement of total carotenoid content

Total carotenoid content in the lipid samples was determined according to the method of Saito and Regier (1971) with a slight modification. Lipid (30 mg) was mixed with 10 ml of petroleum ether and the mixture was allowed to stand for 30 min. The absorbance of the extract, appropriately diluted, was measured at 468 nm using a UV-1601 spectrophotometer (Shimadzu, Kyoto, Japan). The concentration (*C*) of carotenoid in the sample was calculated using the equation given by Saito and Regier (1971).

$$C(\mu g/g \text{ lipid}) = \frac{A_{468} \times \text{vol of extract} \times \text{dilution factor}}{0.2 \times \text{weight of sample used in gram}}$$

where 0.2 is the A_{468} of 1 μg/ml standard astaxanthin.

2.5.2. Measurement of peroxide value (PV)

Peroxide value was determined using the ferric thiocyanate method (Chaijan, Benjakul, Visessanguan, & Faustman, 2006). To 50 μl of lipid sample (10-fold dilution), 2.35 ml of 75% ethanol (v/v), 50 μl of 30% ammonium thiocyanate (w/v) and 50 μl of 20 mM ferrous chloride solution in 3.5% HCl (w/v) were added and mixed thoroughly. After 3 min, the absorbance of the coloured solution was read at 500 nm using a spectrophotometer. Blank was prepared in the same manner, except the distilled water was used instead of ferrous chloride. PV was expressed as A_{500} after blank substraction.

2.5.3. Measurement of thiobarbituric acid-reactive substances (TBARS)

Thiobarbituric acid-reactive substances (TBARS) were determined as described by Buege and Aust (1978). Lipid sample (0.5 g) was mixed with 2.5 ml of a solution containing 0.375% thiobarbituric acid (w/v), 15% trichloroacetic acid (w/v) and 0.25 M HCl. The mixture was heated in boiling water (95–100 °C) for 10 min to develop a pink colour, cooled with running tap water and centrifuged at 3600 × *g* at 25 °C for 20 min. The absorbance of the supernatant was measured at 532 nm using a spectrophotometer. A standard curve was prepared using 1,1,3,3-tetramethoxypropane at the concentrations ranging from 0 to 6 ppm. TBARS was calculated and expressed as mg malonaldehyde/kg sample.

2.5.4. Measurement of p-anisidine value (AnV)

p-anisidine value of lipid sample was analysed according to the method of AOCS (AOCS, 1990). Lipid sample (100 mg) was dissolved in 25 ml of isooctane. The solution (2.5 ml) was mixed with 0.5 ml of 0.5% (w/v) *p*-anisidine in acetic acid for 10 min. The absorbance was read at 350 nm using a spectrophotometer. The *p*-anisidine value was calculated using the following formula:

$$p\text{-anisidine value} = 25 \times \left(\frac{(1.2 \times A_2) - A_1}{W}\right)$$

where A_1 and A_2 are the absorbance at 350 nm before and after adding *p*-anisidine, respectively; *W* = weight of sample (g)

2.5.5. Measurement of free fatty acid

Free fatty acid (FFA) content was determined according to the method of Lowry and Tinsley (1976). Lipid sample (0.1 g) was added with 5 ml of isooctane and swirled vigorously to dissolve the sample. The mixture was then treated with 1 ml of 5% (w/v) cupric acetate–pyridine reagent, prepared by dissolving 5 g of the reagent grade cupric acetate in 100 ml of water, filtering and