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Bioactive Compounds from By-Products of Shrimp Processing Industry in Vietnam

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ABSTRACT

Shrimp heads from the shrimp processing industry in Vietnam can be a good source for production of bioactive compounds. It is estimated that there is an amount of 200,000 metric tons of shrimp by-products annually from two main species: black tiger shrimp (*Penaeus monodon*) and white shrimp (*Penaeus vannamei*). We found that the shrimp by-products can be used as raw materials for extraction of chitosan and lipid-mineral-rich carotenoprotein by combining chemical and biological treatment. Chitosan has been recognized as a multifunctional bioactive compound and has been used widely in food, biotechnology, cosmetics and medicine. Lipid-mineral-rich carotenoprotein can be used as a supplement in food and aqua-feed with good nutrional and functional properties. Besides, the characteristics of these products have been evaluated and reported. These results proved that high quality chitosan and lipid-mineral-rich carotenoprotein can be obtained from shrimp by-products.

Key words: shrimp by-products, chitosan, lipid-mineral-rich carotenoprotein

INTRODUCTION

Vietnam is a major exporter of shrimp to the world market thanks to a rise in shrimp aquaculture farming in recent years. The average annual shrimp export from 2005 to 2008 is over 160 thousand metric tons, worth approximately US$ 1.5 billion/year. Shrimp is usually processed to obtain shrimp meat for export. The 35 - 45% leftovers are shells and heads considered as by-products. As a result, shrimp processing leads to massive amounts of shrimp biowaste, estimated to be more than 200,000 metric tons (wet weight) per year. The major components of the shrimp by-products are protein, chitin (deacetylated chitosan), lipid, minerals. It contains also a small amount of valuable carotenoids. Chitosan, a valuable bioactive compound, has widely used in food, agriculture, biotechnology, cosmetics, medicine and waste treatment[1,2]. Other components from shrimp processing waste are minerals, lipid and carotenoprotein that have been widely used as functional ingredients in food and aquafeed[3,4].

Currently shrimp heads in Vietnam is mainly used for in chitin recovery and very limited amount used for chitosan production. The other bioactive conponents such as carotenoprotein, minerals (mainly calcium), lipid have not been recovered and just run out to the wastewater system. This impropreate treatment has not only reached to the efficient utilization of the shrimp by-products but also led to the big environmental issues caused by this treatment. This research presents novel process for extraction of chitosan and lipid-mineral-rich carotenoprotein from shrimp heads in order to achieve better utilization of shrimp by-products to produce bioactive compounds.

MATERIALS AND METHODS

Shrimp heads as a by-product of white shrimp species (*Penaeus vannamei*) was collected from seafood processing factories in Khanh Hoa province, central Vietnam. After collection, the shrimp waste is transported to the laboratory in iced condition. The by-product then was washed under running water and ground in a knife mill to obtain pieces of 0.3 to 0.5 cm. Portions (10 kg) were packed into plastic bags and frozen at -20^{o}C until use. Enzyme Alcalase® Food Grade was generously provided by Novozyme (Denmark). The enzyme is produced from submerged fermentation of *Bacillus licheniformis*.

I. *Alcalase Deproteinization (the 1st stage)*

The ground shrimp heads (10kg) was thawed and mixed well with 5l warm distilled water (50℃). The deproteininization by Alcalase was carried out with conditions as follows: Enzyme/waste ratio of 0.2% v/w, pH 8, 55℃, treatment duration for 8 h. The deproteinization was carried out in plastic tanks. The adjustment of pH was done by using 1N NaOH. The reaction was stopped at 90^{o}C in 5 min. Then the partially deproteinized chitin in solid form was separated by

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filtration. Lipid-mineral-rich carotenoprotein was recovered from the supernatant by isoelectric point precipitation at pH 4 - 4.5 with adding chitosan at concentration of 100 ppm as coagulant and flocculant. Lipid-mineral-rich carotenoprotein was further freeze-dried.

II. *Further Deproteinization with NaOH and Demineralization by HCl to Obtain Chitin*

The partially deproteinized shrimp heads was further treated with diluted NaOH at a concentration of 2% (w/v) for 12h with ratio of solid/liquid ratio of 1/5 (w/v) at room temperature in order to remove the remaining protein. The deproteinized shrimp heads was then demineralized by soaking in 4% HCl solution for 12 h with solid/liquid ratio of 1/5 (w/v) at room temperature to get chitin.

III. *Chitosan Preparation*

Chitosan was prepared by chemical method in 50% (v/v) NaOH at 65°C for 20 h[5]. Then chitosan was purified by dissolving in 1% lactic acid and precipitated by 4% NaOH at pH 10. The purified chitosan was freeze-dried.

IV. *Characterization of Chitosan and Lipid-mineral-rich Carotenoprotein*

Chitin content in the waste was estimated by method of Chakrabarti[6]. Protein, lipid and minerals were determined using FAO analytical method[7]. Amino acid analysis is measured by LC/MS/MS. Viscosity is measured using Brookfield Viscometer. Residual protein content of chitosan was determined by Microbiuret method[8]. Degree of deacetylation of chitosan was measured by UV method[9]. Bulk density and dye binding capacity were assayed following the procedure of Cho *et al*.[10] and was computed as g/mL. Water and fat binding capacity were measured using the method of No *et al*.[11]. Extraction and determination of total carotenoid content from shrimp waste and the obtained protein sediment was using method taken from Sachindra *et al*.[12]

Each treatment is carried out in triplicate, and all determinations were carried out in triplicate. All data are expressed as means ± SD. Data were analyzed by an analysis of variance ($p < 0.05$). The results were processed using Minitab software.

RESULTS AND DISCUSSION

I. *Chemical Composition of Shrimp Heads*

Chemical composition of shrimp heads is shown in Table 1. The average dry matter of shrimp heads was 22.5 ± 1.2%. Shrimp heads contained 4 main components: chitin, protein, minerals, lipid and small amount of valuable carotenoids. In which, protein accounts for the highest amount of 54.4%, minerals of 21.2%, lipid of 11.9% and chitin of 9.3%.

Table 1. Valuable components of shrimp heads

Components	Contents (%)*
Chitin (%)	9.3 ± 0.8
Protein (%)	54.4 ± 1.8
Minerals (%)	21.2 ± 1.6
Lipid (%)	11.9 ± 1.4
Carotenoids (mg/kg)	206 ± 14

*based on dry basis.

The protein content of this shrimp heads is higher than that of processing waste from shrimp *Xiphopenaeus kroyeri*[13]. These figures have shown that shrimp heads is a good source for recovery of chitin to produce chitosan and lipid-mineral-rich protein.

II. *Characterization of Chitosan and Lipid-mineral-rich Carotenoprotein Extracted from Shrimp Heads*

The chitosan and purified chitosan were characterized and the results are presented in Table 2. The chitosan shows good quality, the protein and ash content is less than 1% as required[14]. Degree of deacetylation of shrimp reached 82% and chitosan has high viscosity. The high viscosity of chitosan due to the light treatment conditions of enzyme combined with dilute NaOH.

Table 2. Characteristics of chitosan and purified chitosan

Characteristics*	Chitosan	Purified chitosan
Ash (%)	0.91 ± 0.2	0.17 ± 0.03
Protein (%)	0.93 ± 0.1	0.23 ± 0.02
Degree of deacetylation (%)	82.3 ± 0.5	81.8 ± 0.4
Viscosity (cps)	1214 ± 52	980 ± 50
Solubility (%)	99.1 ± 0.2	99.8 ± 0.2
Turbidity (NTU)	18.3 ± 0.8	15.3 ± 0.7
Bulk density (g/mL)	0.51 ± 0.01	0.31 ± 0.02
Water binding capacity (%)	484 ± 30	570 ± 30
Fat binding capacity	216 ± 13	230 ± 13
Dye binding capacity (%)		
- Crystal violet (cationic)	33.5 ± 2.8	24.6 ± 3.8
- Orange II (anionic)	48.1 ± 2.7	63.5 ± 4.2

*based on dry basis; ND: not determined.

This chitosan has high solubility and turbidity is low (Table 2). With these characteristics, this chitosan has shown good quality and can be used in food and agriculture. However, chitosan for application for functional foods and medicine need more purity and therefore purification process has been used and the characteristics of purified chitosan is presented in Table 2. The purified chitosan shows higher quality than chitosan,

especially lower ash and protein content, higher dye binding capacity. Depending on the requirement for any application, the right chitosan with suitable purity is selected.

Another product of this process is lipid-mineral-rich carotenorotein. This product was characterized for sensory values, protein, lipid, minerals contents and amino acid patterns. The results of various observations are presented in Table 3 and 4.

Table 3. Characteristics of the lipid-rich carotenoprotein

Characteristics	
Color	Reddish
Smell	Shrimp powder
Minerals content (%)	12.73 ± 1.6
Protein content (%)	33.83 ± 2.3
Lipid content (%)	31.07 ± 1.4
Chitin content (%)	6.4 ± 1.2
Carotenoid content (mg/kg)	886 ± 14

Table 4. Amino acid composition in the lipid-rich carotenoprotein

Amino acids	(mg/kg)
Alanine	16207
Glycine	16596
Serine	5378
Proline	9464
Valine	7964
Threonine	0.00
Trans-4 hydroxy-L-proline	0.00
Leucine-Isoleucine	11473
Methionine	1164
Phenylalanine	3323
Arginine	0.00
Aspartic acid	19306
Glutamic acid	30453
Tryptophan	253.5
Cysteine	271.3
Lysine	10104
Histidine	1950
Tyrosine	7388
Cystine	0.00

The protein in the lipid-mineral-rich carotenoprotein has good amino acid composition with high amount of glutamic acid (Table 4). Previous study also showed that shrimp waste had high protein content with good amino acid balance and glutamic acid was the abundant amino acid in shrimp protein[15]. Besides, this carotenoid-protein complex can make carotenoid (mainly astaxanthin) more stable than the carotenoid alone[6].

CONCLUSIONS

Chitosan and lipid-rich carotenoprotein with their well-known functional properties can be extracted from shrimp heads which is available in large amount from shrimp processing industry.

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