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Optimization of extraction of chitosan and carotenoids from shrimp waste

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Abstract
This study describes pigments and chitosan extraction from shrimp wastes under different methods. Chitosan were extracted and measured from shrimp waste according to the conventional method. But in step of deproteinization, three process acid, alkaline, and enzyme extraction was used. Carotenoids are also evaluated as by product. The degree of deacetylation and the moisture percent was also determined. The total chitosan level for acid, enzyme, and alkaline methods were 1.95±0.014, 1.33±0.014, and 0.183±0.007 mg/ml and total carotenoids 44.77±0.2, 184.58±0.447, and 85.17±0.9 respectively. These results demonstrated that good yields can be obtained enzymatic treatment. Subject to economic advantages, enzymatic can replace the other methods.

Keywords: Shrimp Waste, Extraction Methods, Chitosan, Carotenoids

1. Introduction
About 50% of shrimp total body weight is waste. Shrimp waste is the main byproduct in fishery industries [3]. These wastes are environmental contaminants. Therefore, utilization of these wastes can prevent environmental contamination. Carotenoids have been documented for health promoting function. Astaxanthin, the main carotenoid found in shrimp, its antioxidant activity was reported to be higher than that of carotene, carotene and lutein and is higher than tocopherol against certain reactive oxygen species [4]. The carotenoid of shrimp wastes can be used as a source of coloring and flavoring agent in marine products [5]. This pigment could also be used the food industries. Because of the concern about safety of synthetic antioxidants in foods, searching natural antioxidant for replacing has been attracted by food industries.
Chitosan is copolymer of glucosamine and N-acetylglucosamine prepared from chitin is a major component of the shells of crustaceans, by deacetylation [6]. It is the universally accepted non-toxic chitosan. Chitosan applications is in many areas include wastewater treatment, food, agriculture, cosmetic and pharmaceutical industries [10]. Chitosan exhibit pharmacology as antimicrobial, hypocholesterolemic, drug delivery and would healing [8].
This study mainly focuses on chitosan extraction. Three techniques for extraction chitosan were used and compared the bioactivity of chitosan of shrimp wastes. Carotenoids as byproduct in these techniques were also surveyed.

1.1 The test materials
The shrimp wastes, *Penaeus semisulcatus*, were collected from the processing plants. Then, the wastes were air dried in the shade and powdered.

1.2 The experimental design
2 g of sample was placed in a test tube and dissolved in 0.1 N HCL for 24h at room temperature for demineralisation treatment. In deproteinization step, three methods were used. The residue was placed in 1 N NaOH, 10% of Trypsin and Trichloroacetic acid (TCA). In these step complex protein-carotenoids was separated. In enzymatic method; 10% of trypsin was added to waste. pH was adjusted to 8 for enzyme activity and heated at 37 °C for 4 h. In this step, the carotenoid-protein compounds are removed from wastes. Then, the hydrolysate was centrifuged and the supernatant was used for determination of total carotenoids. The remaining of this process is chitin. The chitin obtained from this process should change to chitosan. Chitin changes to chitosan in deacetylation process. In this process, the acetyl groups were removed from the chitin. For this purpose, the residue is placed in 50% NaOH and boiled at 100 °C for 2 h.

1.3 Determination of total carotenoids
The supernatant in deproteinization step in three methods was calculated for carotenoid yield. Total carotenoids were determined by β carotene standard curve and by spectrophotometric method at 470 nm [11].

1.4 Determination of chitosan yield
The chitosan obtained was assessed according to the previous method [2]. Briefly, a stock solution was prepared by dissolving 100mg of chitosan in 100 ml of %1 aqueous acetic acid. The solutions are made from this stock solution. The solutions of chitosan was mixed with 0.5M NaNO2 reagent and the mixture was heated at 80 °C for 30 min. PH was adjusted to 8. Then, thiobarbituric acid was added and heated at 80 °C for 10 min in water bath. The absorbance of the supernatant was measured at 555 nm.

1.5 Qualification determination of degree of deacetylation of chitosan yield
Degree of deacetylation is one of the main parameters of chitosan. This parameter was assayed according to published method. Chitosan (0.1 grams) was dissolved in 10 mL of %1 aqueous acetic acid. The solutions of chitosan were mixed with 200 ml of bromocresol. The free amino group have reactive binding site for Bromocresol [1]. These samples were measured at the 589 nm.

1.6 Determination of moisture of chitosan yield
The moisture was measured by drying the samples at 110 °C in 10 h. The weight loss of samples is considered as moisture.

2. Result and Discussion
The main purpose of the present work was the production of chitosan with high quality, but pigment was also determined as by-product. For pigment, Table 1, the results of experiments are shown. The concentration of carotenoid pigment in the extracts was calculated using the standard curve obtained by commercial β carotene.

$Y = 100.41x - 0.4073$, $R^2 = 1$

The more content of pigments exited in extracts of enzyme treatment. The level of carotenoid pigment in this group was 184.58±0.447. The amount of pigments was significantly more than other groups ($P<0.05$). Therefore, enzyme extraction of shrimp waste could be a potential source for pigments. The amount of pigment was followed by alkaline treatment (Table 1).

Table 1: Level of total carotenoid

Extraction Method	Pigments Content (ppm)
Pigments extracted by trypsin	184.58±0.447
Pigments extracted by TCA	44.77±0.2
Pigments extracted by alkaline treatment	85.17±0.9

Table 2: Level of total chitosan and moisture at different procedure. The amount in enzymatic and TCA methods, successive procedure shown

Extraction Method	Chitosan Content (mg/ml)	Moisture%
Chitosan extracted by trypsin	1.33±0.014	0.11±0.007
Chitosan extracted by TCA	1.95±0.014	0.05 ±0.002
Chitosan extracted by alkaline treatment	0.183±0.007	0.15±0.007

For chitosan, Table 2 showed that TCA extraction was more effective as compared with other methods. The extracted chitosan would be more than other techniques. Furthermore, in TCA extraction, chitosan obtained was more soluble.

Table 3: The comparison of the three procedure in deacetylation level

Extraction Method	Absorbance unit (nm)
Chitosan extracted by trypsin	0.788±0.01
Chitosan extracted by TCA	0.347±0.003
Chitosan extracted by alkaline treatment	0.213±0.01

Degree of deacetylation affects the chemical, physical and biological properties such as adsorption and encapsulation [8]. The increasing in DD and soluble product is achieving by applying enzyme (Table 2). The solubility depends to the percentage of degree of deacetylation value. The disadvantage of chitosan is its poor solubility in water or organic solvents. Use of chitosan in the food industry and biomedical application will be faced with problem because of its weak solubility. In enzyme extraction, chitosan obtained also was soluble. The hydrolyzed chitosan is readily soluble, because of its shorter chain lengths and free amino groups in D-glucosamine units [7]. It is possible, enzyme hydrolyzed chitosan.

Moisture is an important factor in chitosan. It affects the durability and texture of chitosan. The result showed that the lower moisture percent in TCA extraction. Several methods are available for extraction of chitosan from shrimp waste. The variations of the quality of chitosan depend to these methods. Due to extensive use chitosan in various industries consistently produce it with high quality is considered.

The recovery of carotenoids and chitosan extraction from the waste would improve the economics of the shrimp processing plant. The use of acids and bases are not guaranteed, environmentally safe for extraction of pigments and chitosan. Therefore, there is a need to develop a suitable extraction method [9]. In this survey, weak acid (0.1N) unlike other methods were used in demineralization step. Acid 1 N is commonly used. Acids and bases are entering into an environment and cause severe injuries. Optimum conditions for enzymatic activity is necessary are not readily available in the environment. The other advantage of this method is the solubility of chitosan.

Cost of TCA is disadvantage of this process. Regarding to solubility of this product that it is not necessary to apply other methods for increasing the solubility, this method is more economical process. For prepare water soluble chitosan, the solution of acetic acid and NaOH is required.

In conclusion, subject to the amount of chitosan and carotenoids yield and degree of deacetylation, enzymatic can replace the other methods.

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