Optimisation of Deacetylation Process for Chitosan Production from Red Snapper (Lutjanus sp.) Scale Wastes

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Abstract. Red snapper (Lutjanus sp.) is common tropical fish that known as important source of marine product in particular Indonesia. This research aimed to optimise the chitosan synthesis from the red snapper scale waste through deacetylation process. Method in this research was divided into three stages which were deproteination, demineralization, and deacetylation. Deproteination stage was done with solution containing 4.2% w/v NaOH and heated at 60° C for 5 hours and followed by the demineralization stage with solution containing 52% v/v 2 N HCl at room temperature for 6 hours. The comparison between fish scales and solutions was 1:6. After that, process continued with the deacetylation. Several treatment during the deacetylation process were taken into consideration to determine the effective concentration for yielding optimum chitosan output. Chitosan produced were having moisture content of 2.88%, ash content of 1.10%, and nitrogen content of 0.0136%. Optimal Degree of Deacetylation (DDA) was up to 90.83% that obtained by heating treatment at a temperature of 110° C with solution containing 80% NaOH for 4 hours, and comparison between chitin : solution was 1 : 3. This result indicated that chitosan extracted from red snapper scale is very potential and can be applied to industry.

1. Introduction
Crustacea shell waste is known as chitosan source that important for commercial use due to its high content and availability. Chitosan usually is used in medical and pharmaceutical industries. Chitin has close relation to protein, mineral, lipid and pigment [1]. Chitin is natural polysaccharide with chemical structure (poli [β- (1 → 4) -2- acetamido-2-deoxy-glucose]), mainly found in shell of crustacea namely crab, shrimp, insect cuticle and fungi cell wall. Chitin is also biopolymer with chemical structure resemble to cellulose and it is the primary component of exoskeleton. Beside from crabs and shrimp shell, chitin has been successfully isolated from carp (Cyprinus carpio L.) scale [2]. Furthermore, chitin and chitosan has been extracted from tilapia (Tilapia nilotica) scale [3,4]. It was also confirmed that chitin and chitosan can be extracted from scale of Labeo rohita, likewise extraction of chitosan and carboxymetil chitosan from scale of same species [5,6].

From above studies, chitosan and chiton are most likely extracted from freshwater fishes. Due to limited information about extraction similar compound on saltwater fishes in particular red snapper, investigation on method for extracting and characterizing chitosan is urgent. Therefore, this paper
aims to investigate the optimum deacetylation process for producing chitosan from red snapper (*Lutjanus* sp.).

### 2. Experimental Method

#### 2.1. Preparation of Fish Scale

Scales were washed using running water to remove wastes and contaminants from scales. Contaminants will affect the chitin and chitosan quality. After being cleaned and washed, scales were dried out under natural sunlight. Using of furnace in drying out will cause discoloration on fish scale sand cause scales turn yellow and will affect chitin and chitosan contents. Dry scales then kept in the watertight container to keep the moisture.

#### 2.2. Deproteinase and Demineralisation

During the deproteination stage, scales were dissolved in the NaOH 4.2% (w/v) with ratio of scale to NaOH was 1:6. The solution was stirred for 5 hours at 60 °C and sieved for obtaining residu. This residu then washed with aquadest to obtain neutral pH. During the demineralisation, the washed solution then dissolved in solution containing 52% v/v 2 N HCl at room temperature for 6 hours with ratio of 1:6. The solution then washed with aquadest to obtain neutral pH. The result from deproteination and demineralization of fish scales was chitin.

#### 2.3. Deacetylation

This stage aimed to produce chitosan. The deacetylation process were conducted by using treatments combine the concentration of NaOH (60, 70, and 80%), temperature (110, 120, an 130 °C) and duration (4 and 6 hours) difference (Table 1). The purpose of combining those treatment was to find which treatment can produce chitosan with high DDA value (> 75%). In these treatments, the chitin was added by NaOH (ranged from 60 to 80 %) and then boiled at temperature 110-130 °C from for 4 to 6 hours.

#### 2.4. Measurement of ash content, nitrogen content, and DDA

Quality of chitosan can be seen from several parameters. Some parameters like ash content, nitrogen content and DDA are important characters in determining chitosan wheter it is good or not [7]. Ash and nitrogen content were analysed using proximate analysis while DDA was using FTIR. For ash content, chitosan were placed on the petri dish and heated inside the furnace at 100-105 °C for 30 minutes. Ash content from chitosan can be calculated using formula below

\[
\% \text{ ash} = \frac{W_3 - W_1}{W_2 - W_1} \times 100 \%
\]

(1)

where, \(W_3\) is weight of chitosan ash and petri dish, \(W_2\) is weight of chitosan and petri dish, and \(W_1\) is weight of petri dish only.

Nitrogen content was can be calculated based on formula

\[
\% \text{ nitrogen} = \frac{(V_a - V_b) \times N \times 14.007}{W \times 1000} \times 100 \%
\]

(2)
where, $V_a$ is sample titration volume of HCl, $V_b$ is void sample volume of HCl, $N$ is normality of HCl, and $W$ is chitosan weight.

Degree of deacetylation (DDA) influences the physical, chemical and biological properties of chitosan, such as acid base and electrostatic characteristics, biodegradability, self aggregation, sorption properties, and the ability to chelate metal ions [8]. DDA was measured by using the equation below [9].

$$%\text{DDA}=100 - \left( \frac{A_{1655}}{A_{3450}} \times \frac{100}{1.33} \right)$$  

(3)

where, $A_{1655}$ is chitosan absorbance at 1655/cm wavelength and $A_{3450}$ is chitosan absorbance at 3450/cm wavelength.

3. Result and Discussion

3.1. Ash content on chitosan extracted from red snapper scale waste

Ash content is one of important parameters that determine the quality of chitosan. The lower ash content then the higher chitosan quality. The ash content determined by the deacetylation process and the chitosan raw material. Based on the measurement, the ash content of chitosan synthesized from red snapper scale has percentage of ash ranged from 1.1% to 1.88% (Figure 1).

![Figure 1. Ash content on chitosan extracted from red snapper scale waste](image)

The lowest ash content which is 1.1% found in the deacetylation process that added by NaOH 80% boiled at temperature 110 °C for 4 hours (treatment 5). Likewise, the highest ash content which is 1.88% found in the deacetylation process that added by NaOH 60% boiled at temperature 110 °C for 4 hours (treatment 1). From the graph it can be drawn from treatment 10, 13 and 16 that higher temperature combined with the extensive boiling time will increase the ash content and lower the chitosan quality. Ash content of chitosan from red snapper has fulfilled the ash content maximum quality standard which is equal to 5% because red snapper scales has ash content below 5% [7]. This result also contrast to some researches which showed higher content. Content of ash on chitosan extracted from Nigerian shrimp was 6.41% while in mussel shell was 36.87% [11,12]

3.2. Nitrogent content on chitosan extracted from red snapper scale waste

In general, nitrogen content of chitosan from red snapper were very low (Figure 2). The lowest nitrogen content which is 0.0136% found in the deacetylation process that added by NaOH 80% boiled at temperature 110 °C for 4 hours. Likewise, the highest ash content which is 0.0278% found in the deacetylation process that added by NaOH 80% boiled at temperature 110 °C for 6 hours.
Nitrogen content of chitosan from red snapper below the minimum quality standard which is equal to 5 % [7]. Nitrogen content on this research were also contrast to others. In chitosan extracted from Nigerian shrimp content of nitrogen was 2.71% while in mussel shell was 2.29% [11,12]

**Figure 2.** Nitrogen content on chitosan extracted from red snapper scale waste

3.3. *DDA of chitosan extracted from red snapper scale waste*

Based on the measurement, the DDA of chitosan synthesized from red snapper has DDA ranged from 79.32 % to 90.83 % (Figure 3). The lowest DDA which is 79.32 % found in the deacetylation process that added by NaOH 60 % boiled at temperature 110 °C for 4 hours (treatment 1). Likewise, the highest DDA which is 90.83 % found in the deacetylation process that added by NaOH 80 % boiled at temperature 110 °C for 4 hours (treatment 5). However, the boiling temperature and duration were not affect the DDA. From the graphic, the temperature increment from 110 to 120 and 130 °C combined with the boiling time extension did not increase the DDA. It can be seen from the graphic, there is 6 peaks represented in the treatment 5, 6, 10, 11, 15 and 17. Those treatments have similarity which influenced by adding the NaOH from 60 to 80%. Therefore, it can be drawn that NaOH concentration is more important than temperature and boiling duration during the deacetylation process. DDA of chitosan from red snapper has fulfilled the DDA minimum quality standard which is equal to 75 % [7].

**Figure 3.** DDA on chitosan extracted from red snapper scale waste

Content of ash and protein among all treatments performed decreased with increasing of DDA (see treatment 5). Reaction with NaOH can decrease protein level, resulting purer chitosan [10].
4. Conclusion
Chitosan synthesized from red snapper scale has high DDA and low ash contents. Deacetylation process by adding chitosan with NaOH 80 % and boiled at temperature 110 °C for 4 hours will increase the DDA and reduce the ash content.

5. References
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