

Improved Chitosan Production from Tiger Shrimp Shell Waste (*Penaeus monodon*) by Multistage Deacetylation Method and Effect of Bleaching

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Abstract: - Production of bleached chitosan from tiger shrimp shell waste (*Penaeus monodon*) by three stages deacetylation method has been carried out. The aims of this research were to know the bleaching effect to deacetylation degree and molecular weight of the chitosan and to know physical and chemical properties of bleached chitosan. Isolation of chitin from tiger shrimp shell waste comprised into three stages such as deproteination, demineralization and bleaching. The result showed deacetylation degree of chitosan for one stage, two stages, and three stages were 71%, 81% and 100%, respectively with molecular weights of 1.40×10^5 Da, 1.04×10^5 Da and 6.27×10^4 Da, respectively. Crystallinity index for the chitosans were 38%, 55%, and 67%, respectively. Bleached chitosan had a paler color. These indicated that repeated deacetylation increased the deacetylation degree and crystallinity index, but decreased the molecular weight. Bleaching process influenced molecular weight of chitosan.

Key-Words: - bleaching, chitosan, crystallinity index, deacetylation degree, molecular weight, repeated deacetylation, shrimp

1 Introduction

Shrimp is one of the marine life with high economic value. According to the Directorate General of Fisheries Cultivation [1], production of shrimp of Indonesia in year 2013 reached 619,400. The production of shrimp from year 2010 to 2013 increased on average by 19.46% per year.

Shrimp is generally exported in frozen form that has been discarded skin of the head and tail. These activities produce waste approximately 60-70% of the weight of the shrimp and this solid waste becomes an environmental issue. Approximately 35% of dried shrimp shells contain chitin. It can generate about 80% of chitosan [2].

Chitosan is a compound that is insoluble in water and the strong alkaline solution, slightly soluble in HCl and HNO₃ and H₃PO₄, and insoluble in H₂SO₄. Chitosan is non-toxic, readily biodegradable and are polyelectrolite [3]. Besides, chitosan can easily interact with other organic substances such as proteins [4]. Quality of chitosan is determined from several parameters. The degree of deacetylation is a

quality parameter that indicates an acetyl group which can be removed from yield of chitosan. High deacetylation degree of chitosan means that the acetyl group contained in the chitosan is weak. Deacetylation degree of chitosan varies between 56-99%, an average of 80% depending on the source and method of preparation [5].

Other quality parameters of chitosan are the color of a chitosan, wherein the application of chitosan also depends on the color of the chitosan. Chitosan with white color or looks clean has better quality so commonly used in the field of health and food industries. Process of whitening (bleaching) will reduce the content of astaxanthin in the chitosan compounds. However, the bleaching process will reduce the molecular weight of chitosan [6].

In this study, a deacetylation process was conducted using repeated deacetylation and bleaching process used reagents with the technical specifications to obtain chitosan with better quality and cheaper.

2 Materials and Methods

2.1 Materials

Tiger shrimp shell waste (*Peneaus monodon*) was obtained fresh from the Kendari Ocean Harbour. Sodium hydroxide was purchased from Brataco Chem. Hydrochloride acid, acetic acid, sodium hypochlorite, sodium acetate, acetone and ethanol were purchased from Merck. Deionized water was used in all experiments.

2.2 Isolation of chitin

Shrimp shells were washed and dried at room temperature, and crushed into pieces. The shell was demineralized with 1 N HCl for 0.5 h at ambient temperature with constant stirring and a solid to solvent ratio of 1:15 (w/v). It was washed until neutral in running tap water, rinsed with deionized water, and filtered to remove excess moisture.

Deproteinization involved stirring of the demineralized shell with dilute 3.5% NaOH with a solid to solvent ratio of 1:10 (w/v) for 2 h at 65 °C. The residue was then washed and filtered as above. The chitin residue was bleached with 0.13% hypochlorite solution with a solid to solvent ratio of 1:10 (w/v) for 1 h at ambient temperature. The colorized product was collected, washed as above and dried at 60 °C for 4 h.

2.3 Preparation of chitosan

Preparation of chitosan was achieved by deacetylation reaction of the chitin with 50% NaOH (w/w) solution at 120 °C for 3 h using a solid to solvent ratio of 1:10 (w/v) where chitin was stirred by electric drill. The residue was washed with deionized water to neutralize, then dried for 4 h at 60 °C. Subsequently, the product was obtained, then deacetylated again as above for 6 h and 9 h. The chitosans were denoted as CS-3, CS-6 and CS-9.

2.4 FTIR analysis of chitosan

Fourier transform infrared spectra (4,000-500 cm⁻¹, resolution 4 cm⁻¹) of chitosans were recorded with a Shimadzu FTIR spectrometer.

2.5 Deacetylation degree of chitosan

The infrared spectroscopic method with a FTIR spectrometer used was applied to determine the deacetylation degree. The deacetylation degree was calculated from the following relationships [7].

$$\% \text{ DD} = \left[1 - \left\{ \left(\frac{A_{1660}}{A_{3450}} \right) \times \left(\frac{1}{1.33} \right) \right\} \right] \times 100\% \quad (1)$$

where DD is deacetylation degree; A_{1660 cm⁻¹} and A_{3450 cm⁻¹} are absolute heights of absorption bands of amide and hydroxyl groups.

2.6 Molecular weight of chitosan

Average molecular weight of chitosan is determined by determination of its intrinsic viscosity by Brook-field viscometer. 1 mg/ml (w/v) concentration of chitosan solution was prepared using 1% glacial acetic acid by a magnetic stirrer for a homogenous mixture of the polymer solution. The viscosity of the chitosan solution was analyzed using spindle RV-VI. The average molecular weight was obtained from Mark-Houwink equation:

$$[\eta] = K \times M^a \quad (2)$$

where $[\eta]$ is the intrinsic viscosity (mL.g⁻¹), M is the average molecular weight of the solution respectively and K and a are the Mark-Houwink constants specific for a given polymer K = 1.81 × 10⁻³ mL/g, a = 0.93 [8].

2.7 Crystallinity index of chitosan

Powders were further analyzed by X-ray diffraction to evaluate crystallinity [9]. X-ray diffraction spectra were collected on a Bruker D8 Advance X-ray diffractometer using CuK α radiation at 40 kV and 30 mA. The 2 θ scan range was 4–40° with a step size of 0.1° and a time/step of 1 s. The crystallinity index, CrI, of the samples was calculated as:

$$\text{CrI} = (I_{110} - I_{\text{am}})/I_{110} \quad (3)$$

where I₁₁₀ is the maximum intensity of the (110) diffraction peak at 2 θ = 20° and I_{am} is that of the amorphous diffraction signal at 2 θ = 16°. Two samples of each chitosan powder were evaluated and the CrI was reported as the average (n = 2) ± standard deviation.

3 Results and Discussion

3.1 Extraction of chitosan

Extraction of chitosan from shrimp shell requires harsh chemical treatments. The shrimp shell even though contains the majority of chitin, also has proteins and minerals. Proteins are removed by deproteinization and carbon and other salts are removed by demineralization. Chitin is a linear polymer of 2-acetamido-2-deoxy- β -D-glucopyranose (Glc NAc). This is deacetylated to form chitosan. It is a linear binary polymer of Glc NAc and 2-amino-2-deoxy- β -D-gluco-pyranose [10].

As shown in Table 1, the shrimp shell was found to contain 38.78% of chitin which close to 40% as reported by Patria [11] from shrimp shell.

Table 1 Shrimp shell composition

Component	Composition (%)
Protein	26.00
Mineral	12.73
Chitin	38.76
Chitosan	22.47

The mineral content (CaCO_3) of the shrimp shell was found to be 12.73% which lower than the range in the literature for shrimp as 20-30% [4]. Whereas, protein content slightly higher than reported in literature.

The moisture content of chitin was lower than that of the chitosan (Table 2). This was indicated that chitosan more hydrophilic than chitin. The ash content of chitin almost similar to chitosan. Ash measurement is an indicator of the effectiveness of the demineralization step for removal of calcium carbonate.

Table 2 Proximate analysis of chitin and chitosan

Compound	Moisture content (%)	Ash content (%)
Chitin	4.2	0.90
Chitosan	7.3	0.89

Increased solubility of chitosan is directly proportional to the increase in the degree of deacetylation. This is due to the acetyl group in chitin changed into the amine group by deacetylation. H ions on chitosan from the amine group made easily interact with solvent dilute acids through hydrogen bonding. [12]. As natural, chitin is formed crystalline which content high density polymer chains and form hydrogen bonding each

other [13]. Therefore, as shown in Table 3 that chitin has a lower solubility than chitosan. The presence of carboxyl groups in acetic acid will facilitate the dissolution of chitosan due to hydrogen interactions between the carboxyl groups with the amine group of chitosan [14].

Table 3 Solubility of chitin and chitosan in solvents

Solution	Chitin	Chitosan
Water	Insoluble	Insoluble
Ethanol	Insoluble	Insoluble
HCl 10 N	Insoluble	Insoluble
CH_3COOH	Insoluble	Soluble

Transformation chitin into chitosan prepared by the removal of acetyl groups of chitin into amine in chitosan which known as deacetylation reaction. This process was prepared by hydrolysis acetamide groups with strong base of 50% NaOH technical specification.

Fig. 2 shows the infrared spectrum of chitin and multistep deacetylation of chitosan. When chitin deacetylation occurs, the band observed at 1656 cm^{-1} decreases, while a growth at 1597 cm^{-1} occurs, indicating the prevalence of NH_2 groups. FTIR spectrum of deacetylation of chitosan results showed changes during the process of deacetylation which indicated by variations infrared absorption of chitosan. The characteristic band at 1566 cm^{-1} is attributed to the amine group that increased during process of deacetylation improving from 1 x 3 h. Changes in absorption at 1665 cm^{-1} is getting weaker. FTIR spectra showed that the deacetylation of the chitosan gradually increased with a time of 2 x 3 h and 3 x 3 h produce chitosan with different infrared absorption spectrum [4].

3.2 Deacetylation degree of chitosan

The degree of deacetylation of the chitosan is a quality parameter that indicates the percentage of the acetyl group which can be removed from the yield of chitin and chitosan. The higher the degree of deacetylation of chitosan means low acetyl groups which increase interaction both the ion and the hydrogen bond [15].

Determination of deacetylation degree of chitosan was calculated by equation of Khan et al. [7] that comparing between absorption peak at 1650 cm^{-1} from amide group (characteristic of chitin) and peak at 3450 cm^{-1} from amine group (characteristic of chitosan).

Table 4 shows that the longer chitin deacetylation time, the higher deacetylation degree. The increasing deacetylation time gradually improved deacetylation degree of chitosan. This

indicated that physico-chemistry properties of chitosan is influenced by deacetylation time.

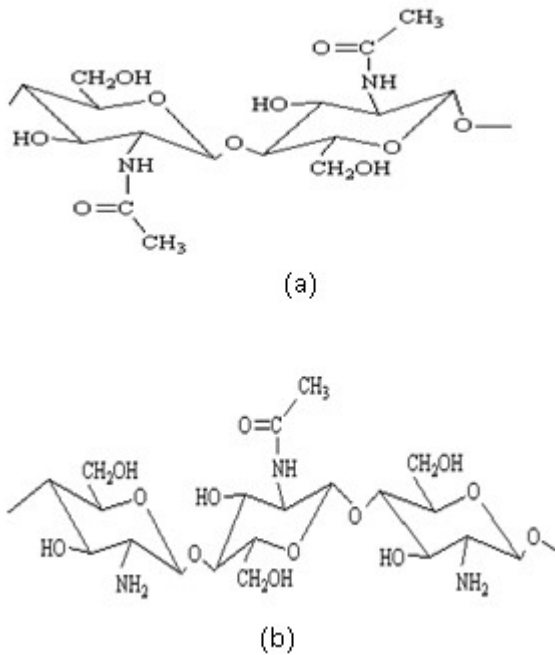


Fig. 1 Molecules structure of (a) chitin and (b) chitosan

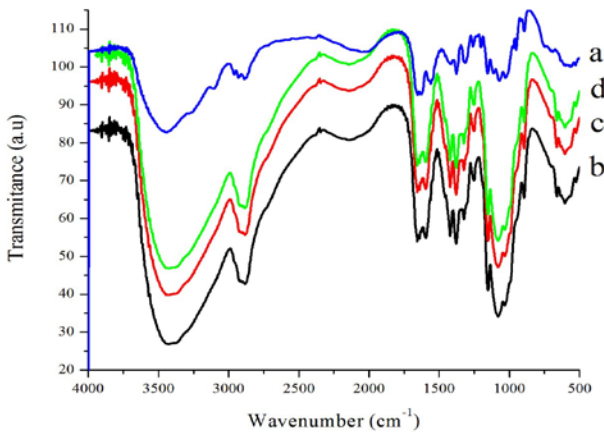


Fig. 2 FT-IR spectra of (a) Chitin, (b) CS-3, (c) CS-6 and (d) CS-9

Table 4. Deacetylation degree and molecular weight

Chitosan	Deacetylation Time (hour)	Deacetylation Degree (%)	Molecular weight (Da)
CS-3	1 x 3	71	1.40×10^5
CS-6	2 x 3	81	1.04×10^5
CS-9	3 x 3	100	6.27×10^4

3.3 Molecular weight of chitosan

The molecular weight of chitosan varied based on the source material and its preparation methods. Chitin have molecular weights typically greater than

one million Dalton while the molecular weight of chitosan in a range between 100 kDa - 1200 kDa, depending on the process and product quality [17].

In general, the use of high temperatures (> 150 °C) and high concentration acid such as hydrochloric acid during the preparation process can cause depolymerization (polymer chain termination) on chitosan molecular chain resulting in a decrease molecular weight [6].

Molecular weight of chitosan from multistep deacetylation was calculated by viscoscopic method using Ostwald viscometer. This measurement was prepared by comparing between the flow time of pure solvent (acetic acid) with flow of chitosan solution. The results of deacetylation degree are shown in Table 4.

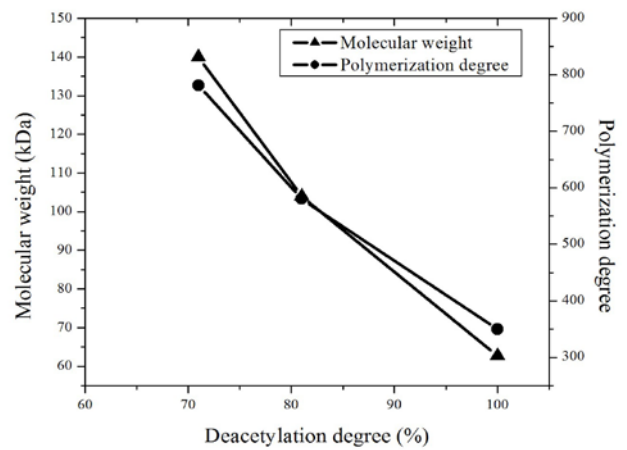


Fig. 3. Molecular weight and polymerization degree of chitosan

The utilization of hypochlorite in the bleaching process significantly caused decreases in molecular weight of chitosan. The hypochlorite causes depolymerization which resulted in a decrease in the molecular weight of chitosan. According to No [2] that the excessive utilization of bleaching agent can cause a decrease in molecular weight of chitosan. Fig. 3 shows that polymerization degree and thus molecular weight decrease in each chitosan.

3.4 Crystallinity index of chitosan

Jaworska et al. [9] reported that the chitin was much more crystalline than deacetylated materials. They have similar lattice those are 2θ at 10 ° and 20° but chitosan has lower intensity than chitin. The enlargement of peak is influenced by the heterogeneous structure of polymer chains, degree of ordering of macromolecules and crystallinity degree [18]. The determination of crystallinity index

of chitosan was analyzed by X-ray diffraction and calculated by equation 3. Fig. 5 shows XRD patterns of chitosan with different chitosans.

The degree of crystallinity depends on the deacetylation degree of the chitosan: the higher the deacetylation degree, the higher the degree of crystallinity [12]. This may be attributed to the fact that chains of chitosan with higher deacetylation degree are more flexible and have fewer large acetyl side groups. These are consistent with the results of the present study. In contrast, the contact angles of chitosans decreased with the increasing deacetylation degree indicating that the hydrophilicity of chitosan increased with increasing deacetylation degree.

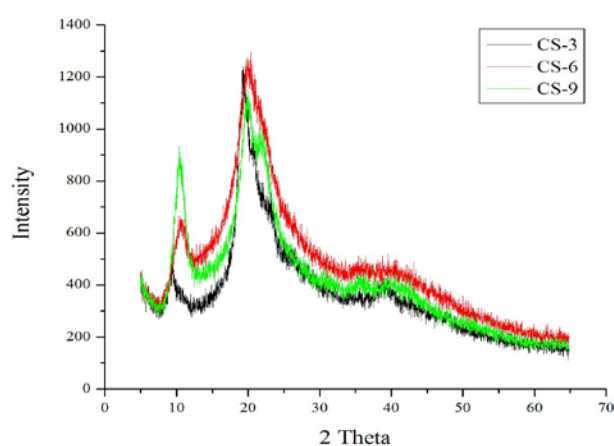


Fig. 5. XRD patterns of CS-3, CS-6 and CS-9

Table 5 Crystallinity index of chitosan in various of deacetylation degree

Chitosan	Deacetylation degree (%)	Crystallinity index (%)
CS-3	71	38
CS-6	81	55
CS-9	100	67

Table 5 shows that the increasing deacetylation time produced chitosan with high deacetylation degree and crystallinity index.

4 Conclusion

In conclusion, chitosan with different deacetylation degree and molecular weight has been conducted from gradually deacetylated chitin. The deacetylation of 1 x 3, 2 x 3 and 3 x 3 h resulted in deacetylation degree of 71, 81 and 100 %, respectively.

It shows that increase deacetylation time significantly increased deacetylation degree and crystallinity index, but decrease the molecular weight. In addition, the utilization of bleaching agent decreases molecular weight of chitosan.

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