

Synthesis of Chitosan from Crab's Shell Waste (*Portunus pelagicus*) in Mertasinga-Cirebon

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Abstract— The crab shell waste contains many chitins. When it processed in several chemical processes, it can produce chitosan, which has many benefits and economic value. Chitosan is a linear polysaccharide that consists of N-acetyl glucosamine and D-glucosamine monomers. This study aims to determine the optimum condition of temperature and time of the deacetylation process from the chitin crab shell (*Portunus pelagicus*) to be chitosan. There are three stages of making chitosan; *deproteination, demineralization, and deacetylation*. In the deproteination process, crab shell powder was added with a 3.5% NaOH at 65 oC while being stirred for 2 hours. In the demineralization process, crab shell powder was added by 1 N HCl solution for 30 minutes at room temperature. The deacetylation process was done by heating the chitin with NaOH 50% solution with a ratio of 1:10 (w/v) at 90o C for 2 hours. In every step of making chitosan when the stirring has been completed, then it's neutralized using aqua dest and dried in the oven for 24 hours. Chitosan that produced has the highest degree of deacetylation is 79.68%.

Keywords: *synthesis, portunus pelagicus, chitosan*

I. INTRODUCTION

A blue swimming crab is a group of crabs from the family Portunidae, which is part of the Crustacea of the Malacostraca class and the order Decapoda[1]. The blue swimming crab has a pair of hind legs with a shape like a paddle that is used to swim so that the crab is categorized into swimming crab groups. The crab has rough, wide, and flat texture. The male crab has blue dots, while the female crab has brown spots with the pattern and color intensity that are changed depending on the conditions of each crab. The eye of the crab protrudes in front of the carapace. The crab has sturdy and elongated claws; it also has spines that are slippery underneath[2].

Portunus pelagicus is one of the export commodities of the Indonesian fisheries sector, which is sold in the form of frozen or canned crabs. As a result of the activity of extracting meat by the small crab processing industry, a large amount of hard shell waste is produced, which can reach around 40-60% of the

total crab weight. The crab shell can be used as a mixture of animal feed, but this has not been able to maximize the use of the crab shell waste[3]. Located in a coastal area, the potential of natural wealth owned by Mertasinga Village, Gunung Djati, Cirebon is 75% of marine animals with crab as a major potential. As the small crab includes one of the major potentials of the village, there are residents who skin the crabs and sell the meat in other cities, islands and even abroad. The skinning process of the crabs produces a lot of shell waste, which pollutes the environment and creates unpleasant odors. Therefore it is necessary to utilize the waste from the crab's shell to decrease the waste; one of the efforts to reduce the waste is making the shell into chitosan.

Chitosan is a natural polysaccharide or biopolymer that has the chemical formula poly- (2-amino-2-deoxy-β- (1,4) -D-glucopyranose), which is biocompatible, biodegradable, non-toxic, so it can be used in various fields such as pharmaceutical industry, food, health, agriculture, textiles and so on. Chitosan can be used as a coagulant in wastewater treatment, moisturizer / facial creams, seed coatings, metal ion adsorbents, anti-cancer / anti-tumor, blood cholesterol control, additional components of animal feed, biopesticides, contact lenses, plaque inhibitors on teeth, accelerator of the healing of wounds and bones, fat solvents, food preservatives, food stabilizers and colors [4-6].

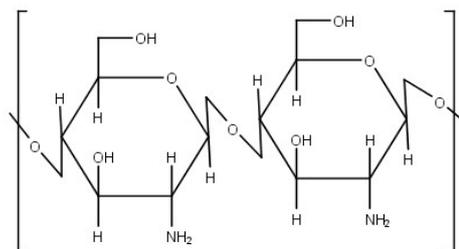


Fig. 1. Chitosan structure

Commonly isolated of chitosan from crustacean shell waste consists of three basic steps: demineralization (calcium carbonate and calcium phosphate separation), deproteinization

(protein separation) and deacetylation (remove acetyl groups). These three steps are the standard procedure for chitosan production[7]. Chitosan has two reactive groups, namely the OH group and the NH₂ group. Protonated chitosan interacts effectively with negatively charged biomolecules so that it can be used for adsorption of anionic dyes. Chitosan is a biopolymer of D-glucosamine that is produced from the chitin deacetylation process using strong alkali[5, 8]. In general, the degree of deacetylation for chitosan is around 60% and around 90-100% for chitosan that is fully deacetylation. This depends on the chitin raw material used, and the process carried out[9]. In the world market, the price of chitosan with a degree of deacetylation of 70% can reach US \$ 750 / kg[10]. Therefore, the crab shell waste in Mertasinga Village must be extracted into chitosan, so that it has high economic value.

II. MATERIALS AND METHODS

A. Tools, raw materials and chemicals

The tools used were glassware from the laboratory, filter paper, universal indicators, ovens, hot plate stirrers, magnetic stirrers, and Fourier Transform Infrared (FTIR). The raw material used was a small crab shell taken from the village of Mertasinga, Gunungjati, Cirebon. The chemicals used for the deproteination process were NaOH p. a (Merck) with a concentration of 1.0 N; 2.0 N and 3.5%, then for the demineralization process HCl p.a (37%, Merck) was used with a concentration of 1.0 N; and 1.5 N for deacetylation NaOH p. a (Merck) was used with a higher concentration of 50%. In addition, aqua dest or distilled water was also used for washing samples at each stage.

B. Sample preparation

The crabs were boiled and then skinned. After that, they were separated from the flesh. The small crab shells that had been separated from the meat was then cleaned from the remnants of the meat that was still attached, then dried in the sun, then ground to produce fine crab shell powder.

C. Synthesis of chitosan

Chitin and chitosan were made in three stages; deproteination, demineralization, and deacetylation with different procedures depending on the concentration of chemicals used and their comparison with samples. Even the heating temperature used was also different so that the best chitosan was gained.

1) Deproteination.

Fine swimming crab shell powder was added with a solution of NaOH 2.0 N with a ratio of 1: 6 between the sample and a solvent, then it was heated at a temperature of 80oC while being stirred using a hot plate stirrer for 1 hour. After that, it was washed to reach neutral pH using aqua dest and filtered to take the sediment. Next, it was dried in an oven at 80oC for 24 hours and weighed.

Demineralization. The crab shell powder obtained from the deproteination process was added with 1.5 N HCl solution little by little with a ratio of 1:12 the sample and solvent, then it was heated at room temperature while being stirred for 1 hour.

After that, it was washed to reach neutral pH using aqua dest and filtered. Lastly, it was dried in the oven at 80 oC for 24 hours and weighed.

Deacetylation. Chitin obtained from demineralization was added with 50% NaOH with a ratio of 1:10 between the sample and a solvent and heated at a temperature of 90 oC while being stirred for 2 hours. After that, it was washed until neutral pH had been reached using aqua dest and filtered. Lastly, it was dried in an oven at 80 oC for 24 hours and weighed. Finally, it was stored in a desiccator until chitosan was ready to use[8].

2) Deproteination.

The shell powder was added with a 3.5% NaOH at a ratio of 1:10 (w / v). Then it was heated at 65 oC while being stirred for 2 hours. After that, it was washed using aqua dest and separated by decantation. Washing was done continuously until the pH was neutral, then it was filtered using filter paper. Lastly, it was dried in an oven at 90 oC for 24 hours and weighed.

Demineralization. The crab shell powder obtained from the deproteination process was added by 1 N HCl solution little by little with a ratio of 1:15 (w / v) and stirred for 30 minutes at room temperature. After that, it was washed using aqua dest and separated by decantation. Washing was carried out continuously until the pH was neutral, then it was filtered. Next, it was dried in an oven at 90 oC for 24 hours. Lastly, the chitin was weighed.

Deacetylation. Chitin obtained from mineral removal results was added by 50% NaOH solution with a ratio of 1:10 (w / v), then heated at a temperature of 90 oC while being stirred for 2 hours, then washed using aqua dest. After that, it was separated by decantation. Washing was done continuously until the pH was neutral, then filtered. Then it was dried in an oven at 90 oC for 24 hours and weighed. Lastly, the chitosan was stored in a desiccator[5].

3) Deproteination.

The protein removal process was done by adding the shell powder into 1 N NaOH solution with a ratio of 1:15 (w / v). Then it was heated at 80 ° C while being stirred using a hot plate stirrer for 3 hours. After that, it was washed using aqua dest and decanted. Washing using aqua dest was done until a neutral pH was obtained. Next, it was filtered using filter paper. Lastly, it was dried in an oven at 80 oC for 24 hours and weighed.

Demineralization. The process of removing minerals was done by adding the deproteinated powder with 1 N HCl solution little by little in a ratio of 1:15 (w / v). Then it was stirred at room temperature for 1 hour and washed with aqua dest, then decanted. Washing using aqua dest was done until a neutral pH was obtained. After that, it was filtered and dried in an oven at 80 oC for 24 hours and weighed to obtain the yield. Lastly, the chitin obtained from the process was weighed as much as 0.5 grams for FTIR testing.

Deacetylation. The acetyl group from the chitin was removed by adding 50% NaOH with a ratio of 1:15 (w / v). Then it was heated at 90 oC while being stirred for 2 hours. Next, it was washed with distilled water and decanted.

Washing using aqua dest was done until a neutral pH was obtained. Next, it was filtered and dried in an oven at 80 oC for 24 hours. Lastly, it was weighed and stored in a desiccator[11].

III. CHARACTERIZATION OF CHITOSAN WITH FTIR ANALYSIS

Chitosan characterization using FTIR analysis aims to discover the functional groups that are found as a result of the process of chitosan making. It was obtained by comparing the FTIR spectrum produced during the process with the standard chitosan spectrum or by looking at the correlation map for the establishment of functional groups in the infrared spectrum.

IV. PROPERTIES OF CHITOSAN

A. Degree of deacetylation

The degree of deacetylation shows how much the acetyl group has been removed from the chitin polymer chain to produce an amine group and form chitosan. The degree of deacetylation that was obtained from the FTIR spectrum uses the baseline method formulated by Baxter. The degree of deacetylation was calculated based on the ratio between absorbance at 1655 cm-1 and absorbance at 3450 cm-1. Measurement of the degree of deacetylation was based on a curve drawn by a spectrophotometer. The highest peak (Po) and the lowest peak (P) were recorded and measured by the selected baseline. Absorbance ratio was calculated by the formula:

$$A = \log \frac{P_0}{P} \tag{1}$$

Po / P is the ratio of absorbance at 1655 cm-1 with an absorbance of 3450 cm-1. The value of the degree of deacetylation can be calculated by the formula:

$$DD = \left\{ 1 - \left(\frac{A_{1650}}{A_{3450}} \times \frac{1}{1.33} \right) \right\} \times 100 \tag{2}$$

B. Water content

The test of water content in chitosan used a gravimetric method, which was done by drying in the oven at 105 oC for 3 hours. Water content was obtained from the deviation between the weights of chitosan before drying with the weight of chitosan which had reached a constant after it had been dried using the formula:

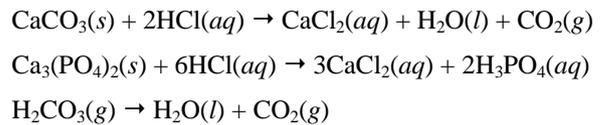
$$\text{Water content} = \frac{\text{wet weight} - \text{dry weight}}{\text{dry weight}} \times 100$$

V. RESULTS AND DISCUSSION

A. Synthesis of chitosan

At the first step of making chitosan, deproteination has been done to remove proteins using a strong base solution,

NaOH, to break the hydrogen bonds that occurred between chitin and protein. This hydrogen bond was formed between the carboxyl groups found in proteins and the amino groups found in chitin and vice versa. OH- ion from NaOH solution bind the H+ ions that were contained in the -NH3+ group of amino acids, then Na+ ions bind to the ends of the chain of negatively charged amino acids, namely the group -COO- to -COONa to produce Na-proteinate. The second step was demineralization, which is the process of removing minerals found in depleted crab shell samples using a strong acid, HCl. Adding HCl must be done slowly because it produces quite a lot of foam and air bubbles. This indicates that the reaction really happens because when HCl reacts with minerals contained in the crab shell, especially CaCO3 and Ca3(PO4)2, it will produce carbon dioxide gas[5]. The reaction is as follows:



The deacetylation process was the last step in the making of chitosan, which aims to eliminate the acetyl group in chitin that has been obtained from demineralization. In this deacetylation process, a strong base solution was used again with a higher concentration of NaOH. This was done because the bond between the group -COO- with the nitrogen atom could be broken through the hydrolysis reaction mechanism shown in Figure 2 so that it produced chitosan, which had an amino group. The initial reaction is an addition, in which the OH group enters into the group -NHCOCH3, and then elimination changes to the group -CH3COO- so that it produces chitosan[12].

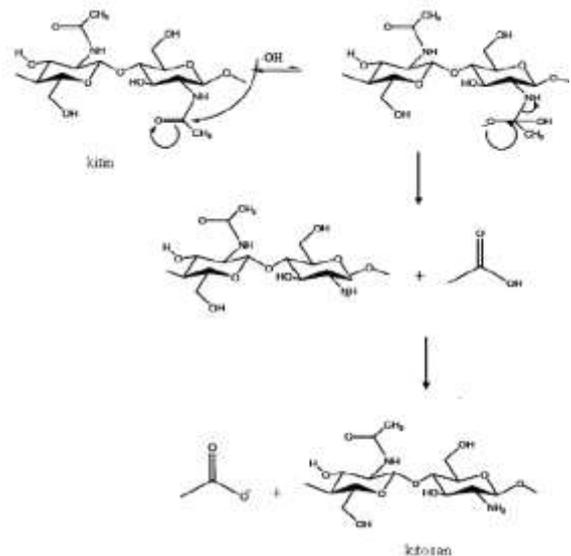


Fig. 2. The mechanism of the hydrolysis reaction of chitin to chitosan

At the end of each stage of making chitosan, it must be washed using aqua dest until the pH is neutral so that the sample resulting from the addition of a material which is always different in terms of acid or base at each stage is not damaged. For example, at the end of deproteination, it had

been reacted with alkaline NaOH so it must be neutralized in the demineralization stage using HCl so that the sample was not damaged due to a very drastic decrease from pH 14 to pH 1.

TABLE I. THE YIELD AT EVERY STAGE OF CHITOSAN MAKING

	The yield of deproteination (%)	The yield of demineralization (%)	The yield of deacetylation (%)
Group 1	71.11	18.9	71.54
Group 2	79.4	23.1	42.86
Group 3	69.94	18	69.97

^a Yield: final weight/ initial weight x 100%

Based on the yield shown in Table 1 it can be seen that the smallest yield exists during the demineralization process because the minerals contained in the crab shell are very high. Consequently, the minerals that have been bound by HCl dissolved during the washing process. Small crab shell waste still contains quite a lot of chemical compounds, including protein 30-40%, minerals (CaCO₃) 30-50%, and Chitin 20-30% [3].

B. Characterization and properties of chitosan

Chitin and chitosan characterization using FTIR spectrophotometer was used to determine the functional groups that are specific in both chitin and chitosan. From the results of the FTIR spectrum of group 2 chitin in Figure 3 it can be seen that there are the absorption bands of wave 3448.72 cm⁻¹ to 468.70 cm⁻¹. Typical functional groups in chitin are hydroxy groups found in wave 3448.72 cm⁻¹ and amide groups at wave 1658.78 cm⁻¹ and 1635.64 cm⁻¹. The absorption band for the amide group is quite sharp and slightly widened, and there is also an absorption band at the wave 1566.20 cm⁻¹. This shows C = O carbonyl of the sharp acetamide, which means that chitin is well-formed.

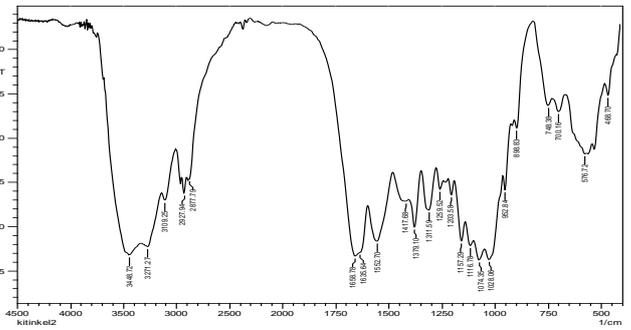


Fig. 3. Result of FITR chitin spectrum

Figure 4 shows the results of the FTIR spectrum of group 2 chitosan, which is quite different from the spectrum of chitin. In the chitosan, the absorption spectrum for amide and C = O becomes weaker at wave 1658.78 cm⁻¹ and 1566.20 cm⁻¹ and loss of absorption at wave 1635.64 cm⁻¹. This indicates that amide groups are getting weaker. In addition, the wave 3294.42 cm⁻¹ and 3448.72 cm⁻¹ become wider due to overlapping OH and NH groups from primary amines. This indicates that the formation of chitosan in group 2 has occurred well. Table 2 shows the conclusions from the results of the chitosan functional group analysis in each group, which has not much difference in the value of the wavenumbers. However, there is a significant difference in group 3. There are two amide groups uptakes that are still quite sharp. It can be said that the process of deacetylation is incomplete.

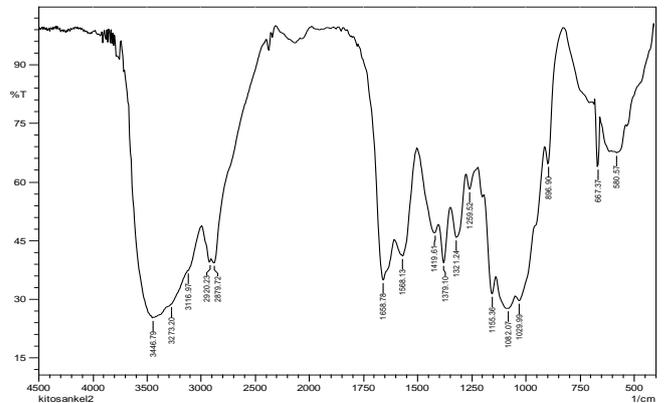


Fig. 4. Result of FITR chitosan spectrum

TABLE II. THE RESULTS OF THE ANALYSIS OF CHITOSAN FUNCTIONAL GROUPS FROM CRAB SHELLS

Functional group	Wavenumber (cm ⁻¹) group 1	Wave number (cm ⁻¹) group 2	Wave number (cm ⁻¹) group. 3	Wave number (cm ⁻¹) literature
Formation of NH ₂ primary amide	580.57, 667.37 and 896,90	594.08, 667.37 and 896,90	580.57, 667.37 and 896,90	750-600
Symmetrical C-O-C	1029.99	1031.92	1029.99	1050-950
C-O from primary alcohol	1082.07	1093.64	1078.21	1085-1030
C-O from a secondary alcohol	1155.36	1153.43	1155.36	1125-1085
C-O-C asymmetrical	1259.52	1261.45	1259.52	1150-1060
C-H amine	1321.24	1321.24	1319.31	1359-1000
-CH ₃	1379.10	1379.10	1379.10	1390-1370
-CH	1419.61	1419.61	1421.54	1350-1480
C=O carbonyl	1568.13	1566.20	1566.20	1530-1580
N-H primary amide	1658.78	1658.78	1625.99 and 1660.71	1650-1550
C-H stretching from alkane	2879.72, 2920.23 and 3116.97	2879.72 and 2920.23	2885.51, 2922.16 and 3115.04	3000-2850
O-H/N-H stretching from primary amine	3273.20 and 3446.79	3294.42 and 3448.72	3271.27 and 3446.79	3450-3250

The degree of deacetylation and water content obtained in the three groups has met the standard of chitosan requirements. The water content in the standard chitosan is less than 10%, while the degree of deacetylation is more than 70%. The water content obtained has the same value in the three groups, 5.2%, while for the degree of deacetylation is shown in Figure 5 The best degree of deacetylation is in group 2 which is 79.68% so that the procedure can be used as a reference for the next stage which is the production of chitosan from crab shells.

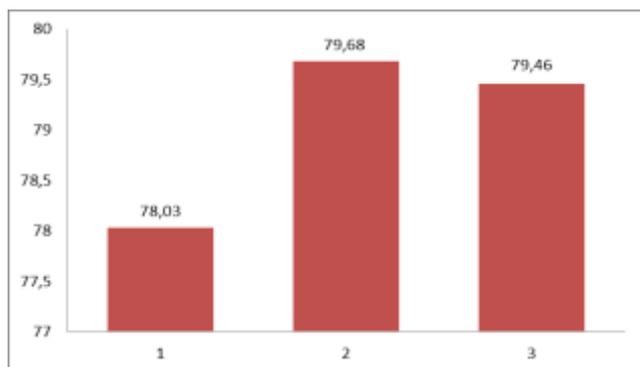


Fig. 5. The degree of chitosan deacetylation in each group

VI. CONCLUSIONS

Synthesis of chitosan from crab shells obtained from the village of Mertasinga Kecamatan Gunung Djati Kab. Cirebon with the last step, deacetylation using 50% NaOH with a ratio of 1:15 (w / v) at 90° C for 2 hours has fulfilled the standard of chitosan requirements to produce the best degree of deacetylation of 79.68%.

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