Isolation and Characterization of Chitosan from Coconut Crab Skin Origin of Halmahera Island with Ftir

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Abstract: This study aims to determine the isolation and characterization of chitosan from the skin of coconut crabs. Isolation of chitosan from coconut crabs was carried out through four stages, namely deproteination (4% NaOH, 80ºC), demineralization (HCl 1.25 N, 70ºC), depigmentation (4% NaOCl, 75ºC), deasitilation (50% NaOH, 80ºC). The degree of deasitilation of chitosan from walnut crabs is 82.52%. Chitosan FTIR spectrum analysis results of several main examples of wave numbers 3371.57 cm^{-1} which show symmetrical stretching vibrations due to overlapping OH and amines (NH), stretching vibrations of 1597.06 cm^{-1} caused by propagation of C=O and stretch vibrations 1651.07 cm^{-1} which shows secondary amide. Characterization of FTIR spectroscopy which showed the extraction of coconut crabs was chitosan.

1 INTRODUCTION

Halmahera Island has abundant natural resources in the fisheries sector, one of which is coconut crabs. The results of observations conducted in the markets showed that the sale of coconut crabs carried out was limited to the sale of the meat while the shell crabs shell was discarded and left alone until it roted without any utilization. If left unchecked it will cause environmental pollution and damage to environmental aesthetics. Crustacea (coconut crab) skin waste consists of three main components, namely protein (25% - 44%), calcium carbonate (45% -50%), and chitin (15% 20%) (Fohcher et al, 1992). The chitin content in shrimp skin waste is around 20-50% dry weight. Chitin polymers are composed of monomers; 2-acetamide-2-deoxy-D-Glucose (N-acetyl glucosamine) (Horton et al, 2002). The bond between chitin monomers is the glycoside bond in the β- (1-4) position. The structure of chitin molecules is a long straight chain. Chitin is the largest natural polymer in the world after cellulose (Yanming et al, 2001).

Chitosan [poly-2-amino-2-deoxy-β- (1-4) D-glucopiranose)] is a poly-aminosaccharide compound synthesized by partially removing 2-acetyl groups from chitin [poly (2 acetamido-2-deoxy-β-(1-4)-Dglukopiranosa)], linear biopolymers with 2000-5000 monomer units, bound together by β- (1-4) glycosidic bonds. Chitosan $(C_6H_{11}NO_4)n$ is a yellowish white amorphous solid, polyelectrolyte (Chen et all, 2007). Generally soluble in organic acids, the pH is around 4–6.5, insoluble at lower or higher pH. Solubility is influenced by molecular weight and degree of deacetylation (Mima et al., 1983).

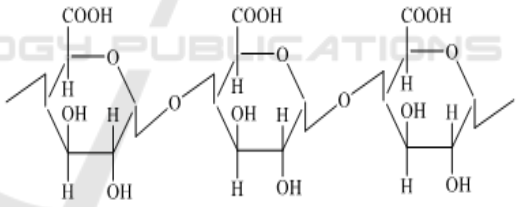

Figure 1: Molecular Structure of Chitosan.

Things related to the environment are by utilizing coconut crabs that form chitin and then transformed into chitosan which can be applied in various fields (Hargono et al., 2008). Since chitosan has high economic value, it is very important to conduct research to process skin into chitosan.

2 MATERIALS AND METHOD

2.1 Material

The materials used are coconut crab skin, acetic acid (CH_3COOH), hydrochloric acid (HCl), sodium

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hydroxide (NaOH), technical sodium hypochlorite (NaOCl), filter paper and aquades.

2.2 Tools

Petri dishes, pH meters, analytics, a set of reflux tools, glass plates, magnetic stirrers, shakers, ovens, mes machines, magnetic support devices, shakers, ovens, mes machines, supporting tools in the form of glassware, plastic and FTIR.

2.3 Experimental

2.3.1 Stages of Sample Preparation

Wash the skin of the coconut crabs with running water to remove the impurities that are attached, then dry them in an oven at 80˚C for 24 hours. After that the dried coconut crab skin is mashed to 100 mesh, then processed to get chitosan.

2.3.2 Deproteination

Add 4% NaOH with a ratio of 1: 6 (b/v) to the skin of finely ground coconut crabs, then heated at 80˚C for 30 minutes. Then cool the resulting solution and filter it so that it gets a solid, after that the solid is dried at 80˚C for 24 hours (Roberts, 1992).

2.3.3 Demineralization

Mixing coconut crabs skin with HCl 1.25 N with a ratio of 1:20 (b/v), then heated at a temperature of 70 ˚C for 1 hour. The solution formed is then filtered so that it gets solids. The solid is washed with water to neutral pH, then dried at 80˚C for 24 hours. The product produced is chitin

2.3.4 Depigmentation

Depigmentation stages using 4% NaClO to remove impurities that may have been produced in the previous process

2.3.5 Deacetylation of Chitin to Chitosan

Mix chitin powder with 50% NaOH solution with a ratio of 1:10 (w / v) then heat it for 6 hours at a temperature of 80˚C. Washing solids obtained with distilled water to neutral pH after drying with an oven at 80˚C for 24 hours. The product formed from this process is chitosan. The chitosan obtained was then analyzed by FTIR to determine the Degree of Deacetylation (DD). Deacetylation degree calculations using equations:

$$DD = 100 - \left[\left(\frac{A_{1655}}{A_{3459}} \right) x \left(\frac{100}{1,33} \right) \right]$$

3 RESULTS AND DISCUSSION

3.1 Isolation of Chitosan Coconut Crabs

Coconut crabs skin is washed first to remove the dirt that sticks. After washing, the coconut crabs skin is dried in the sun to remove the water content. Then the coconut crabs skin is mashed with a blender (powder), aiming to simplify the deproteination, demineralization, depigmentation and deaetylation treatment processes.

3.2 Deproteination

The deproteination process aims to break the bonds between protein and chitin by adding NaOH. The skin of a 50 gram coconut crabs is dissolved with 500 ml of 4% NaOH then heated at 80ºC for 2 hours while continuing to stir. After being heated the solution is cooled for 30 minutes then filtered with a filter while washing with distilled water until the pH is neutral. Then the coconut crabs skin was dried in the oven for 24 hours and weighed. Deproteinization of coconut crabs skin was 31.86 grams so that the protein content contained in shrimp skin ranged from 36.28%. In this process, NaOH functions to break the intermolecular bond between chitin and protein, then the protein will bind to Na^+ proteinat which dissolves in water.

3.3 Demineralization

Demineralization to remove inorganic salts or minerals contained in the skin of coconut crabs. The main minerals contained in the skin of coconut crabs are $CaCO_3$ and Ca_3 $(PO_4)_2$. Marganov, 2003, minerals contained in the skin and shells of crustaceans (shrimp, crabs, etc.) are more easily separated than proteins because they are only physically bound. The mineral separation process is indicated by the formation of CO_2 gas in the form of air bubbles when the HCl solution is added in the sample, so that the addition of HCl into the sample is done in stages so that the sample does not overflow (Hendry, 2008). Reactions that occur:

$$Ca_3(PO_4)_{2(s)} + 6HCl_{(aq)} \longrightarrow 3CaCl_2(aq) + 2H_3PO_{(aq)}$$

$$Ca_3(PO_4)_{2(s)} + 6HCl_{(aq)} \longrightarrow 3CaCl_2(aq) + 2H_3PO_{(aq)}$$

$$H_2CO_{3(g)} \longrightarrow CO_{2(g)} + H_2O_{(I)}$$

3.4 Depigmentation

Depigmentation stages using sodium hipolorit (NaClO) to remove impurities that may be present in the previous process, and produce a yield of 91.04%. The product of this stage is called chitin and a further process is needed to obtain chitosan which is deacetylation.

3.5 Deacetylation

Deacetylation is the process of removing acetyl groups (-$COCH_3$) from chitin using an alkaline solution to change to an amine group (-NH_2) (Sirait, 2002). Chitin has a long crystalline structure with strong hydrogen bonds between nitrogen atoms and carboxylic groups in adjacent chains (Muzzarelli, 1986). Termination of the bond between the acetyl group and the nitrogen group so that it turns into an amine group (-NH_2) needs to use NaOH with a concentration of 50% at 80°C for 4 hours. The use of alkali solutions with high concentrations and high temperatures during the deacetylation process can affect the degree of deacetylation produced (Kim et al., 2004).

Depigmentation stages using sodium hypochlorite (NaClO) to remove impurities that may be present in the previous process, and produce a yield of 91.04%. The product of this stage is called chitin and a further process is needed to obtain chitosan which is deacetylation.

3.6 Degrees of Deacitilation and Characterization of Chitosan with FTIR

3.6.1 Degree of Deacitylation of Chitosan

The FTIR spectrum analysis of chitosan from the skin of coconut crabs was carried out in the functional group and fingerprint region with a frequency of 4000 cm^{-1}-400 cm^{-1}. The degree of deacetylation of chitosan is determined by the base line method based on the FTIR spectrum, the formula used:

$$DD = 100 - \left[\left(\frac{A_{1655}}{A_{3459}} \right) \times \left(\frac{100}{1{,}33} \right) \right]$$

Where, A_{1655} shows absorption in the amide band, A_{3459} shows absorption in the hydroxyl band, and factor 1.33 shows the value of the ratio A_{1655}/A_{3459}.

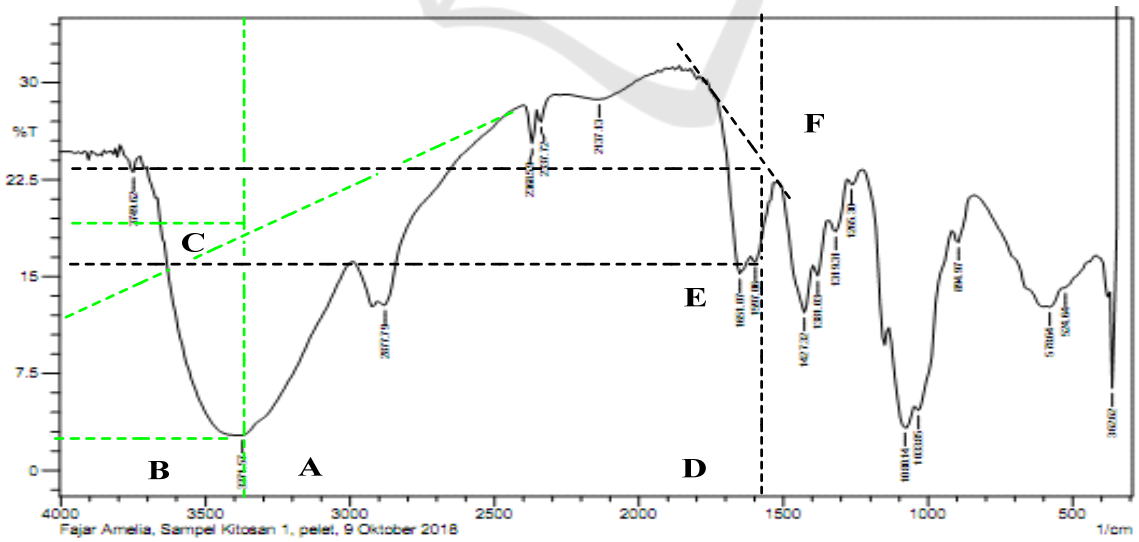

Figure 2: Calculation of the Degree of Deacetylation of Chitosan from the Skin of Coconut Crabs.

Determination of the degree of deacetylation of coconut crabs:

$$DD = 100 - \left[\left(\frac{A_{1655}}{A_{3459}} \right) \right]$$

Where,

$$A_{1655} = \log \left(\frac{DF}{DE} \right)$$

$$A_{1655} = \log \left(\frac{21,3}{16,9} \right) = 0,10$$

$$A_{3450} = \log \left(\frac{AC}{AB} \right)$$

$$A_{3450} = \log \left(\frac{19}{7} \right) = 0,43$$

So that, the value of the degree of deacetylation obtained:

$$DD = 100 - \left[\left(\frac{0,10}{0,43} \right) x \left(\frac{100}{1,33} \right) \right]$$

DD = 100 - 17,48
DD = 82,52%

Degree of deasitilation of coconut crabs skin is 82.52%, indicating the provision of an amine group (NH_2) as an active group. The degree of deacetylation is carried out to determine the formation of chitosan from chitin. The degree of deacetylation of chitosan in this study was 82.52%, which was determined based on the FTIR spectrophotometer method with the Base Line method as proposed by Baxter et al (Khan et al., 2002). The degree of deacetylation is still in accordance with the value of deacetylation degree according to Protan Laboratory which states that the degree of deacetylation of chitin to chitosan usually ranges from 70-100%.

3.6.2 Characterization of Coconut Crabs Skin with FTIR Spectrophotometer

In figure 3 shows the FTIR spectrum of the skin of coconut crabs in the area 400 - 4000 cm^{-1}. Chitosan produced from coconut crabs was characterized by FTIR spectroscopy. The chitosan IR spectrum is presented in Figure 3, to identify the functional groups. Characteristic absorption of chitosan is at wave number 3371.57 cm^{-1} which indicates the presence of hydrogen bonds from the -OH group which overlap with the -NH range (Guibal, 2004). Uptake at 2877.79 cm^{-1} indicates the existence of a range of vibrations from the -CH, while the tensile vibration -CH appears at a stirring number of 1381.03 cm^{-1}. -NH gulp vibration appears at wave number 1597.08 cm^{-1}. C-O obstacle vibration is one of the characteristic absorption of polysaccharides. Appears at wave number 1080.14 cm^{-1}. Based on the picture, it is also seen that the absorption in the area of 1651.07 cm^{-1} is getting weaker and this indicates that diacetylation is perfectly close (Marguerite, 2006).

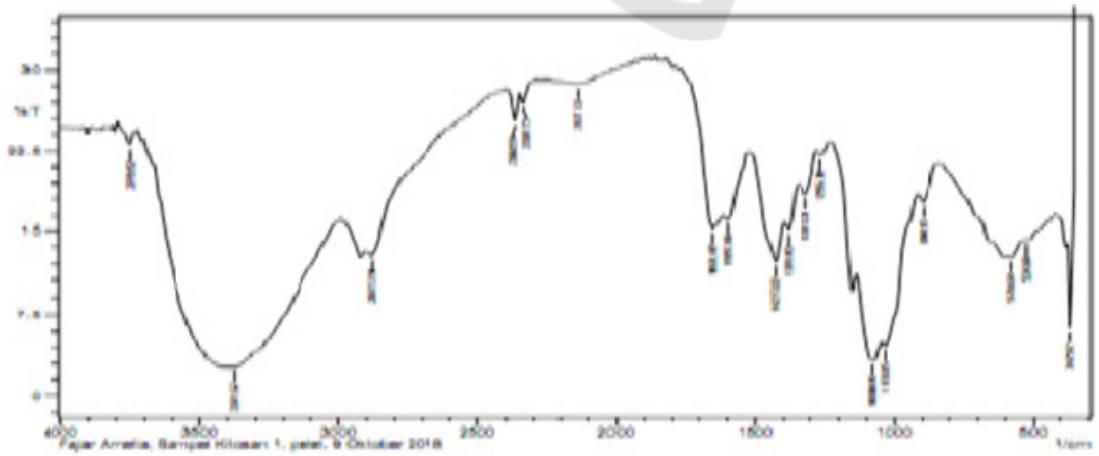

Figure 3\: Chitosan FTIR Spectrum from Coconut Crabs Skin.

4 CONCLUSION

This study has succeeded in isolating chitosan compounds from the skin of coconut crabs through the process of deproteinization reaction with NaOH, demineralization with HCl, depigmentation with NaOCl and deacetylation with NaOH. The degree of deacetylation from the isolation of chitosan from coconut crabs skin was 82.52%. The results of infrared spectroscopy characterization showed that the extracted compound was chitosan.

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