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ISOLATION AND CHARACTERIZATION OF CHITOSAN FROM SEA AND FRESH WATER WASTE, NORTH SUMATERA PROVINCE, INDONESIA

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ABSTRACT

This study aims to manage shell and shell waste from fishery products into chitosan. The processing was carried out by isolating and characterizing chitosan from the shells of vaname shrimp (*Litopenaeus vanname*), the shells of the coral crab (*Charybdis ferryatus*) and the shells of the freshwater lobster (*Cherax quadricarinatus*). The isolation method was carried out in several stages starting with demineralization, deproteination, depigmentation, deacetylation and then characterization of the isolated chitosan. Characterization includes determining the degree of deacetylation, yield percentage, and absorption analysis of the isolated chitosan functional group using Fourier Transform Infrared (FT-IR) spectrophotometry. The isolation results obtained chitosan from vaname shrimp shell waste, coral crab shells and freshwater crayfish shells with a degree of deacetylation (%) of 82.84±0.13; 82.84±0.13; and 67.84±0.05. Chitosan from the isolation of vaname shrimp shell waste, coral crab shells is in accordance with the Standard National Indonesian (SNI) quality, while chitosan from freshwater crayfish shell waste is not suitable

Keywords: *Litopenaeus vanname* skin, *Charybdis feriatus* shell, *Cherax quadricarinatus* shell, SNI, and chitosan

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INTRODUCTION

North Sumatra Province has abundant marine potential, especially from catching fisheries and aquaculture. One of the potential marine catching fisheries are crabs, vaname shrimp, while the catch of freshwater aquaculture is lobster. These catching and aquaculture products contain waste that has not been processed so far. The catching waste can be processed into something productive that can be utilized. Crab is one of Indonesia's fishery commodities which is currently experiencing an increase in the production of waste in the form of hard shells (shells) of crabs which amount to around 40-60% of the total weight of the shells that have not been processed into something of economic value.[1]

The abundant shrimp and lobster shell waste can be used as a polyelectrolyte coagulant for wastewater treatment, fat binder, metal ion absorber,[2,3] microorganisms, microalgae, dyes, pesticide residues, PCB (biphenyl polychlorination), minerals and organic acids, affinity chromatography media, various coatings. natural fiber, antigenicity, polyate, natural preservative because it contains chitin which can be converted into chitosan.[4] The crustacean group contains a lot of chitin compounds which are contained in the shell and if demineralized it produces chitosan.[5,6] Chitosan can be used for food processing products, medicine, biotechnology and as food additives. materials of interest in biomedical and pharmaceutical applications such as antibacterial agents. The nature of chitosan is non-toxic, has biodegradability, absorption and the ability to form films and metal chelates and can be modified chemically and physically.[7,8]

EXPERIMENITAL

Materials

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The chemicals used were HCl, NaOH, NaOCl and instruments used magnetic stirrer, analytical balance, desiccator, Furnace, Kursh Cup, FT-IR_{KBr} spectrophotometry (Shimadzu IR Prestige-21 and Electrothermal 9100.

Sample Preparation

Marine and freshwater commodity wastes such as vaname shrimp (*Litopenaeus vanname*) shells, coral crab (*Charybdis ferryatus*) shells and freshwater lobster (*Cherax quadricarinatus*) shells were washed with running water. Then dried in the sun. After drying, the sample was mashed and sieved to have a texture like a powder with a size of 60 mesh.

Demineralization Process

Each sample was put into a glass beaker then added 1.5 M HCl solution with a ratio of 1:15 (w/v) between the solvent and the sample, then heated at a temperature of 60-70°C for 4 hours while stirring at a speed of 50 rpm using magnetic stirrer. The residue was separated and dried then cooled in a desiccator and then weighed.[9]

Deproteination Process

Each residue resulting from the demineralization process was put into a different glass beaker, then 3.5% NaOH solution was added with a ratio of 1:10 (w/v) between the solvent and the sample, then heated at a temperature of 60-70°C for 4 hours while stirring was carried out at a speed of 50 rpm using a magnetic stirrer. The residue obtained after drying is then cooled in a desiccator and then weighed.[10]

Depigmentation Process

The deproteination results were put into a glass beaker and then 0.315% NaOCl solution was added between the solvent and the sample (1:10 w/v), then heated at 40°C for 1 hour while stirring at 50 rpm using a magnetic stirrer. The dried residue was then cooled in a desiccator and then weighed.[11]

Chitin Deacetylation Process

Chitin was put into a glass beaker followed by the addition of 60% NaOH with a ratio of 1:20 (w/v), then heated at a temperature of 100-110°C for 4 hours while stirring at a speed of 50 rpm using a magnetic stirrer. The residue obtained after drying is then cooled in a desiccator and then weighed. The chitosan obtained from each sample was then identified qualitatively and quantitatively by FTIR analysis to determine the degree of deacetylation.[12]

Chitosan Yield

The yield of chitin into chitosan was determined based on the percentage by weight of chitosan obtained to the weight of chitin[13]:

chitosan yield = $\frac{m}{w}\ x\ 100\%$

Where m is the mass of isolated chitosan and w is the weight of chitin.

Degree of deacetylation

The degree of deacetylation is the process of eliminating the acetyl group from the chitin compound using a strong base solution with a high temperature. The greater the loss of acetyl groups, the higher the quality of the chitosan compounds produced. The calculation of the degree of deacetylation is based on the FT-IR absorption values as follows[14,15]

$$\%\ DD = 100 - \left[\left(\frac{A_{1655}}{A_{3450}}\right) x\ 115\right]$$

Information :

A_{1655} = Absorbance at wavelength 1655 of amide/acetamide (CH_3CONH)

A_{3450} = Absorbance at wavelength 3450 of the hydroxyl (-OH) group

RESULTS AND DISCUSSION

Chitosan Isolation

Demineralization was the first process carried out to remove $CaCO_3$ and other mineral compounds contained in the sample. Mineral elimination process by adding dilute HCl solution at room temperature. The demineralization achieved was characterized by the release of CO_2 and the dissolution of calcium and in phosphate contained in the sample and fixed and insoluble chitin compounds. This demineralization process plays an important role in the isolation of chitin. The results of this stage greatly affect the quality of the chitin produced. The deproteination process aims to break the bond between protein and chitin by adding NaOH solution.[16] The deproteination process undergoes a reaction characterized by bubbles on the surface of the solution, thickens and the solution forms a reddish color. The depigmentation process is the stage of removing pigment (dyes) from the sample. The dark colored pigment in shrimp waste is called crustacyan which is a lipoprotein compound, where the lipid group is a carotenoid compound known as astaxanthin.[11]

Deacetylation aims to break the acetyl group (-$NHCOCH_3$) bound to nitrogen in the structure of chitin compounds to increase the percentage of amine groups, thus this process shows the conversion process of chitin polymer compounds into chitosan[11] Figures-1 and 2. The degree of acetylation of each sample is shown in Table-1. Yield percentage of each isolated chitosan is shown in Table-2.

(a) (b) (c)

Fig.-1: Isolated chitin from waste; (a) Vaname Shrimp (*Litopenaeus vanname*) Shells, (b) Coral Crab (*Charybdis ferryatus*) Shells And Freshwater Lobster (*Cherax quadricarinatus*) Shells

(a) (b) (c)

Fig.- 2: Isolated Chitosan; (a) Vaname Shrimp (*Litopenaeus vanname*) Shells, (b) Coral Crab (*Charybdis ferryatus*) Shells And Freshwater Lobster (*Cherax quadricarinatus*) Shells

Table-1: Result of calculation of degree of acetylation

No.	Chitin Source	Degree of Deacetylation (%)
1.	Vaname shrimp (*Litopenaeus vanname*) shells	82.78 ± 0.10
2.	Coral crab (*Charybdis ferryatus*) shells	82.84 ± 0.13
3.	Freshwater lobster (*Cherax quadricarinatus*) shells	67.84 ± 0.05

Calculation with mean ± SD; n = 3

Table-2: Yield (%) of isolated chitosan

No.	Chitosan Source	Yield (%)
1.	Vaname shrimp (*Litopenaeus vanname*) shells	75.94 ± 0.04
2.	Coral crab (*Charybdis ferryatus*) shells	55.74 ± 0.05
3.	Freshwater lobster (*Cherax quadricarinatus*) shells	68.75 ± 0.04

Calculation with mean ± SD; n = 3

Testing the Purity of Isolated Chitosan Using FTIR

The results of testing the purity of chitosan isolated from vaname shrimp (*Litopenaeus vanname*) shells (Figure-1). The results of the isolation using Infrared spectrophotometry showed absorption at wave numbers 3750-3000 cm^{-1} (stretch O-H and N-H amine), namely 3475.15 cm^{-1}, 3282.84 cm^{-1}, and 3074.53 cm^{-1}. The absorption at wave numbers 2400-2100 cm^{-1} (stretch -C≡C, C≡N) is 2333.87 cm^{-1} and 2137 cm^{-1}. Furthermore, the absorption at wave number 1675-1500 cm^{-1} (stretch C=C aliphatic, C=O amide and C=N) is 1651.07 cm^{-1}. Then the absorption appears at the wave number of 1475-1300 cm^{-1} (C-H bending) which is 1431.18 cm^{-1}. Then there was absorption at wave number 1250-1000 cm^{-1} (CN stretching vibration) which is 1080.14 cm^{-1}. The absorption at wave number 1470-1350 cm^{-1} (CH bending vibration) was 1431.18 cm^{-1}.

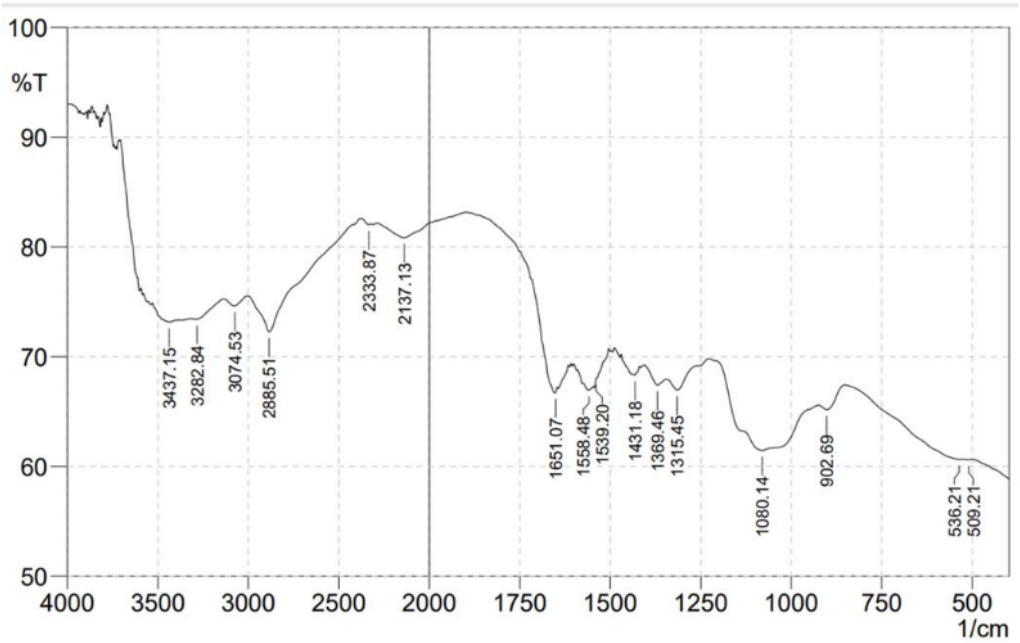

Fig.-1. Results of FT-IR_{KBr} Spectra of Chitosan Vaname Shrimp (*Litopenaeus vanname*) Shells

The results of testing the purity of chitosan isolated from Coral crab (*Charybdis ferryatus*) shells (Figure-2). The results of the isolation using Infrared spectrophotometry showed absorption at wave numbers 3750-3000 cm^{-1} (stretch O-H and N-H amine), namely 3448.72 cm^{-1}, 3286.70 cm^{-1}, and 3059.10 cm^{-1}. The absorption at wave numbers 2400-2100 cm^{-1} (stretch -C≡C, C≡N) is 2137.13 cm^{-1} and 2067.70 cm^{-1}. Furthermore, there was absorption at wave number 1675-1500 cm^{-1} (stretch C=C aliphatic, C=O amide and C=N) which is 1651.07 cm^{-1} and 1558.48 cm^{-1}. Then the absorption appears at the wave number of 1475-1300 cm^{-1} (CH bending) which is 145.04 cm^{-1}. Then there was absorption at wave number 1250-1000 cm^{-1} (CN stretching vibration) which is 1145.72 cm^{-1}.

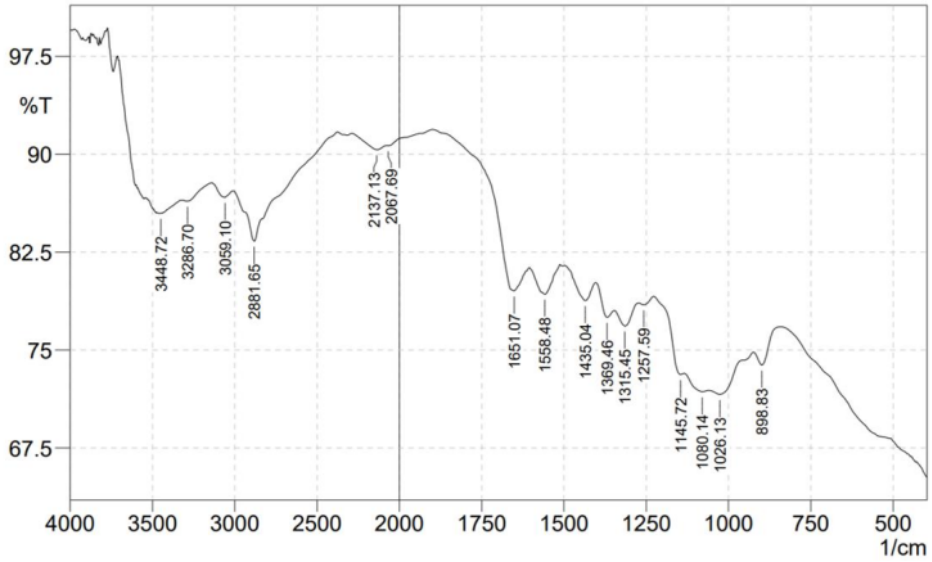

Fig.-2. Results of FT-IR$_{KBr}$ Spectra of Chitosan Coral Crab (*Charybdis ferryatus*) Shells

The results of testing the purity of chitosan isolated from freshwater lobster (*Cherax quadricarinatus*) shells (Figure-3). The results of the isolation using Infrared spectrophotometry showed absorption at wave numbers 3750-3000 cm^{-1} (stretch O-H and N-H amine), namely 3410.15 cm^{-1}, 3290.56 cm^{-1}, and 3105.39 cm^{-1}. The absorption at wave numbers 2400-2100 cm^{-1} (stretch -C≡C, C≡N) is 2314.59 cm^{-1} and 2129.41 cm^{-1}. Furthermore, the absorption at wave number 1675-1500 cm^{-1} (stretch C=C aliphatic, C=O amide and C=N) is 1647.21 cm^{-1}. Then the absorption appears at the wave number of 1475-1300 cm^{-1} (C-H bending) which is 1315.45 cm^{-1}. Then there was absorption at wave number 1250-1000 cm^{-1} (CN stretching vibration) which is 1068.56 cm^{-1}. The absorption at wave number 1470-1350 cm^{-1} (CH bending vibration) is 1377.17 cm^{-1}.

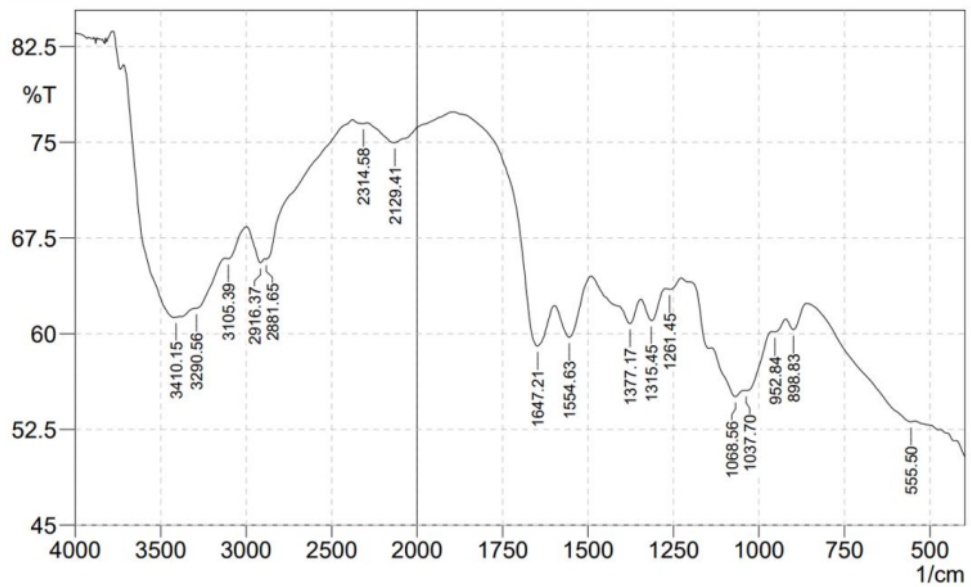

Fig.-3. Results of FT-IR$_{KBr}$ Spectra of Chitosan Freshwater lobster (*Cherax quadricarinatus*) shells

The isolated chitosan obtained was in accordance with the chitosan quality standard based on the results of characterization with FT-IR_{Kr} for the amine functional group, indicated by the presence of absorption at a wave number of 3448.72 cm^{-1} with a degree of deacetylation of 82.78±0.10 for vaname shrimp (*Litopenaeus vanname*) shells, 3448.72 cm^{-1} with a degree of deacetylation of 82.84±0.13 for coral crab (*Charybdis ferryatus*) shells and 3410.15 cm^{-1} with a degree of deacetylation of 67.84±0.05 for freshwater lobster (*Cherax quadricarinatus*) shells. The results of the isolation and characterization of the chitosan obtained were in conformity with the Standard National Indonesian (SNI) for vaname shrimp shells and small crab shells based on the degree of deacetylation of more than 75%.[17]

CONCLUSION

Ated chitosan has 82.84±0.13 degree of deacetylation for vaname shrimp (*Litopenaeus vanname*) shells and 82.84±0.13 for coral crab (*Charybdis ferryatus*) shells and was in accordance with the quality of the Standard National Indonesian (SNI), however for freshwater lobster (*Cherax quadricarinatus*) shells chitosan has 67.84±0.05 degree of deacetylation and it was not appropriate.

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