Isolation Of Chitosan From Cuttlefish Bones

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Abstract.
One of the potential natural resources in Indonesia is the abundance of natural resources in the marine sector. The survey results show that the sale of cuttlefish in the market indicates that the demand for cuttlefish is very high. Cuttlefish is a type of marine animal that is widely consumed by the public because of its very high protein content, but the part used from this cuttlefish is the meat, while the squid bones are discarded. Cuttlefish bone is one of the natural ingredients that contains chitin, and when the isolation process is carried out with the stages of deproteinization, demineralization, and deacetylation, it can produce chitosan compounds. The aim of the study was to isolate chitosan from cuttlefish bones. Isolation of chitosan includes 3 basic stages, namely deproteinization, demineralization, and deacetylation. Then the characteristics of chitosan from cuttlefish bones are analyzed, namely water content analysis, ash content, protein content, ninhydrin test, and FT-IR characteristics. The results of characteristic testing of 8.04% water content, 1.73% ash content, and 4.8% protein content, ninhydrin test results showed that cuttlefish bone chitosan had an amine group, and FT-IR results showed that the absorption bands of C-O and C-N groups. The conclusion of this study shows that chitosan can be isolated from cuttlefish bones.

Keywords: *chitosan, cuttlefish bone, isolation, characteristics*

I. INTRODUCTION

The abundant and diverse marine resources can be utilized optimally for human welfare. Marine organisms have high potential as efficacious ingredients that can be used for the development of the pharmaceutical field. Isolation is the process of separating compounds from natural materials using an appropriate solvent. Cuttlefish bone is one of the natural ingredients that contains chitin, and when the isolation process is carried out with the stages of deproteinization, demineralization, and deacetylation, it can produce chitosan compounds. Chitosan is biocompatible, biodegradable and nontoxic. [1]–[3].Chitosan can be used as an absorbent on heavy metals such as Zn, Cd, Cu, Pb, Mg and Fe, can be used as a wound healer. [4]. Chitosan is generally used in various chemical industries as a food preservative, fat solvent, pharmaceutical sector, metal ion adsorbent, seed coating, moisturizing agent, and as a coagulant in wastewater treatment, which is one type of biopolymer.

Due to the positively charged polycation content of chitosan, chitosan also has the ability to inhibit the growth of bacteria and molds.[5]. Chitosan can be produced from skin, crab shells, cuttlefish bones, and cartilage in squid by carrying out several processes, namely deacetylation. Deacetylation, or commonly called the removal of acetyl groups, is carried out at high temperatures and for a long time using strong alkalis. Due to its non-toxic, bioresorbable, biodegradable, and biocompatible properties, chitosan is usually combined with tooth and bone replacement materials[1]. Chitosan is generally insoluble in water but slightly soluble in hydrochloric acid and nitric acid and soluble in weak acids such as formic acid and stearic acid [6]. Chitosan is a biopolymer whose sources are abundant and include alternative resources that can be utilized as much as possible. The polycationic nature of chitosan is the basis for the use of chitosan in various fields, one of which is agriculture because of its biodegradable nature [7]. Isolation of chitosan from cuttlefish bone was made to utilize the waste of cuttlefish bone, which is usually not utilized and used.

II. METHOD

a. Sample

The sample used was cuttlefish bone obtained from the ulee lheu fish shelter in Aceh Besar. Samples were collected purposively.

b. Isolation of Chitosan from Cuttlefish Bone

Chitosan isolation includes 3 basic stages, namely deproteinization, demineralizationn and deacetylation. The mashed cuttlefish bone samples were soaked for 2 hours at 65ºC with 3.5% NaOH solvent with constant stirring at a ratio of 1:10 (w/v) (deproteinization). The results of the deproteinization were soaked for 30 minutes at room temperature with 1N HCl solvent with a ratio of 1:15 (w/v) (demineralization). Deacetylation was carried out using a temperature of 121ºC using a 50% sodium hydroxide solution for 15 minutes using a ratio of 1:10(w/v). The sample was then filtered, washed using distilled water until the pH became neutral and dried at 60^{o} C for 24 hours using an oven [8].

c. Characteristics of Chitosan from Cuttlefish Bone

The characteristics tested for chitosan from cuttlefish bones were water content analysis, ash content analysis, protein content analysis, ninhydrine test and chitosan characteristics using Fourier Transform Infra Red (FTIR) Spectrophotometry.

III. RESULT

The results of the isolation of chitosan at the processing stage:

Deproteination

The deproteination process aims to release protein bonds in cuttlefish bone powder. The deproteination process of cuttlefish bone powder is reacted with dilute NaOH and causes the protein to dissolve in the base so that the protein that is covalently bound to the functional group of the powder will be separated. A stirring and heating process is carried out to speed up the process of binding the end of the protein chain with NaOH, which causes the degradation process and protein deposition to take place perfectly [9].

Demineralization

In the demineralization stage, the minerals contained in the powder react with 1 N HCl. The process that occurs during the demineralization process is that the minerals contained in the powder react with HCl, resulting in the separation of minerals from the powder. The mineral separation process is indicated by the presence of an indicator, namely the formation of CO_2 gas in the form of air bubbles when the HCl solution is added to the sample, so that the addition of HCl in the demineralization process is carried out gradually so that the sample does not overflow [10].

Deacetylation

The deacetylation process is the transformation of chitin into chitosan through the hydrolysis of the acetyl group (-NH-COCH3) in chitin using a certain solution. The solution used is a strong NaOH solution. At this stage, the chitosan is still in the form of coarse chips and can be ground to a certain size. The chitin deacetylation process using concentrated NaOH solvent aims to change the acetyl group (-NH-COCH3) of chitin into an amine group (-NH2) in chitosan. This change can be detected by looking at changes in the infrared spectrum of chitin with its deacetylation results at certain wavelengths characterized [11].

The degree of deacetylation indicates the content of free amino groups in the polysaccharide. The deacetylation process will cause the removal of the acetyl group from the chitin molecule, producing chitosan with a high degree of chemical reactivity from the amino group. The dry powder resulting from the deacetylation process was characterized using an infrared spectrophotometer to identify the active groups present in the yield of 27.8%.This research uses raw materials in the form of cuttlefish bones. In order to determine (protein content, water content, and ash content), the chitosan from cuttlefish bone was characterized. Table 1 shows the results of the characterization of cuttlefish bone chitosan.

Table 1. Characteristics of cuttlefish bone chitosan

No.	Parameter	Isolated chitosan content (%)	Standard level of chitosan (EGRA,2010)
1.	Mousterizer content	8,04	≤ 10
2.	Ash content	1,73	≤ 3
3	Protein Level	4,8	≤ 6

The characteristic result of chitosan has a moisture content of 8.04%. water content is a parameter that used as a quality standard for chitosan. The amount of water content in chitosan will have an effect on the resistance of chitosan to the presence of microorganisms. The moisture content in chitosan is influenced by the process at the time of drying, drying time, the amount of chitosan being dried, and the surface area where the chitosan is dried[12]. The high water content of the results of this study is thought to be caused by the absorption of water vapor when the chitosan is open. This is because chitosan contains amino groups that have the ability to bind water molecules [6].The ash content of the chitosan research results reached 1.73%, which means the remaining mineral content is less. This shows that the demineralization process in the manufacture of chitosan has been going well, so that not many minerals are left. The ash content of chitosan is an important parameter.

A large ash content can affect the level of solubility and can reduce viscosity[13]. Ash content shows level of success demineralization, so that low ash content indicates purity of a chitosan. Determination of ash content aims to find out the content minerals found in shellfish blood. In addition, the ash content is also can be used to measure the solubility of chitosan in the solvent. If the ash content is high, then the minerals contained are still high and if the ash content is low, then the minerals contained on a small sample[14].Proof of the presence or absence of an amine group in chitosan was tested using a ninhydrine solution. The ninhydrin test, which is a qualitative test, was carried out to prove the presence of an amine group in chitosan. The sample that has an amine group will give a purple reaction result after the test using ninhydrin. Cuttlefish bone chitosan showed a change from cream color to purple color, which means that the cuttlefish bone chitosan sample had an amine group [15]. According to the results of the analysis using an FTIR spectrophotometer, chitin isolated from shrimp shells had been successfully synthesized into chitosan. Figure 1 shows the results of the analysis of the characterization of the chitosan functional group. Then Figure 2 shows the results of the analysis of the characteristics of the chitosan functional group from cuttlefish bone.

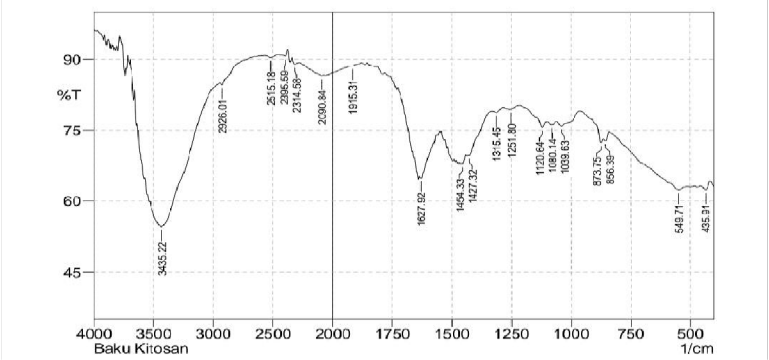

Fig 1. Spectrum of the analysis of the standard functional group of chitosan

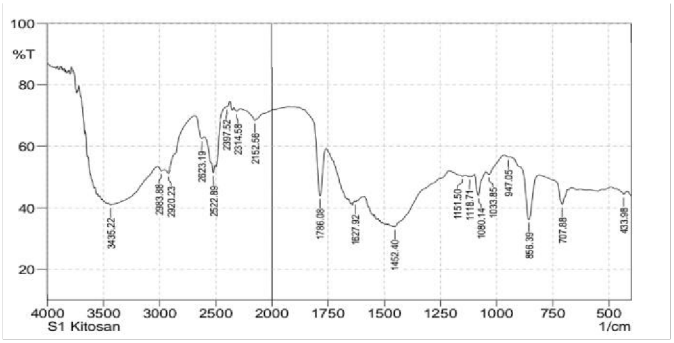

Fig 2. Spectrum of the analysis of the functional group of cuttlefish bone chitosan

The results of the functional group analysis on cuttlefish bone chitosan isolates provided absorption by utilizing infrared spectrophotometry whose wave number of 3435.22 cm-1 was expressed as the N-H and O-H stretching groups of chitosan where these groups appeared in the 3000-3500 range. With the peak of N-H absorption, where the structure of chitosan is the main characteristic. Apart from that, the wavenumber having a peak can also be referred to as the NH marking of the primary aliphatic amine stretching. At the absorption peak at wave number 2920.23 cm-1 1 indicates the presence of –C-H sp3 bonds. At the absorption peak of 1452.4 cm-1 which is referred to as the N-H bending of the primary aliphatic amine there are also two peaks that vary slightly compared to the -N-H stretching of the primary aliphatic amine and at the absorption peak of 1151.5 cm-1 which shows the presence of C-O and C-N groups. The absorption bands of the C-O and C-N groups have a lot of speakers that can be said to overlap each other so that in the fingerprint region it is difficult to identify [16].

IV. CONCLUSION

Chitosan can be isolated from cuttlefish bones as evidenced by the results of the characterization tests carried out showing results that are in accordance with the literature or previous research.

REFERENCE

[1] I. D. Sartika, 'Isolasi dan karakterisasi kitosan dari cangkang rajungan (Portunus pelagicus)', ***Jurnal Biosains Pascasarjana*****,** vol. 18, no. 2, pp. 98–111, 2016.

[2] K. Gurning, W. Haryadi, and H. Sastrohamidjojo, 'ISOLATION AND CHARACTERIZATION OF ANTIOXIDANT COMPOUNDS OF BANGUN-BANGUN (Coleus amboinicus, L.) LEAVES FROM NORTH SUMATERA, INDONESIA', *RJC*, vol. 14, no. 01, pp. 248–253, 2021, doi: 10.31788/RJC.2021.1416077.

[3] A. Shanmugam, K. Kathiresan, and L. Nayak, 'Preparation, characterization and antibacterial activity of chitosan and phosphorylated chitosan from cuttlebone of Sepia kobiensis (Hoyle, 1885)', *Biotechnology Reports*, vol. 9, pp. 25–30, 2016.

[4] E. J. Dompeipen, 'Isolasi dan identifikasi kitin dan kitosan dari kulit udang Windu (Penaeus monodon) dengan spektroskopi inframerah', *Majalah Biam*, vol. 13, no. 1, pp. 31–41, 2017.

[5] B. Andhika and I. Syauqiah, 'Pemanfaatan Kitosan Dari Limbah Cangkang Bekicot (Achatina Fulica) Sebagai Adsorben Logam Berat Seng (Zn)', *Konversi*, vol. 5, no. 1, pp. 24–29, 2016.

[6] E. Cahyono, 'Karakteristik kitosan dari limbah cangkang udang windu (Panaeus monodon)', *Akuatika Indonesia*, vol. 3, no. 2, pp. 96–102, 2018.

[7] A. Wiyarsi and E. Priyambodo, 'Pengaruh konsentrasi kitosan dari cangkang udang terhadap efisiensi penjerapan logam berat', *Laporan Penelitian.* ***Jurusan Pendidikan Kimia FMIPA. Yogyakarta****: Universitas Negeri Yogyakarta*, 2009.

[8] B. V. Gaikwad, J. M. Koli, and A. S. Desai, 'Isolation and characterization of chitosan from crab (Scylla serrata) shell waste', ***Int. J. Sci. Appl. Res*****,** vol. 2, no. 6, 2015.

[9] A. M. Silalahi, A. Fadholah, and L. O. Artanti, 'Isolasi dan Identifikasi Kitin Dan Kitosan Dari Cangkang Susuh Kura (Sulcospira testudinaria)', ***Pharmaceutical Journal of Islamic Pharmacy*****,** vol. 4, no. 1, pp. 1–9, 2020.

[10] K. Divya and M. S. Jisha, 'Chitosan nanoparticles preparation and applications', *Environmental chemistry letters*, vol. 16, no. 1, pp. 101–112, 2018.

[11] D. I. Sánchez-Machado, J. López-Cervantes, M. A. Correa-Murrieta, R. G. Sánchez-Duarte, P. Cruz-Flores, and G. S. de la Mora-López, 'Chitosan', in *Nonvitamin and nonmineral nutritional supplements*, Elsevier, 2019, pp. 485–493.

[12] S. Agustina, I. M. D. Swantara, and I. N. Suartha, 'Isolasi kitin, karakterisasi, dan sintesis kitosan dari kulit udang', ***Jurnal Kimia*****,** vol. 9, no. 2, pp. 271–278, 2015.

[13] A. Heras, 'CHITOSAN: A NATURAL POLYMER WITH POTENTIAL INDUSTRIAL APPLICATIONS'.

[14] U. Mardiana, 'ISOLASI DAN KARAKTERISASI KITOSAN PADA KERANG DARAH (Anadara granosa)', ***Journal of BTH Medical Laboratory Technology***, vol. 1, no. 1, 2021.

[15] K. Hayati, C. C. Setyaningrum, and S. Fatimah, 'Pengaruh Penambahan Kitosan terhadap Karakteristik Plastik Biodegradable dari Limbah Nata de Coco dengan Metode Inversi Fasa', ***Jurnal Rekayasa Bahan Alam dan Energi Berkelanjutan*****,** vol. 4, no. 1, pp. 9–14, 2020.

[16] R. J. Fessenden and J. S. Fessenden, 'Kimia Organik Jilid 1', 1982.