Indonesian Journal of Science and Pharmacy

Volume 1, Issue 3, Page 100-107, April 2024
e-ISSN 3025-5244

Isolation and Characterization of Chitosan from Kepah Shell Waste (*Polymesoda erosa*) Percut Sei Tuan, North Sumatera

Dara Indah Sari Sitorus
Ridwanto Ridwanto*

Faculty of Pharmacy, Universitas Muslim Nusantara Al-Washliyah, Garu II A, Harjosari 1, Sumatera Utara, 20147, Indonesia
*email: ridwanto@umnaw.ac.id

Keywords:
Chitin
Chitosan
Isolation
Shells

Received: January 2024
Accepted: Februari 2024
Published: April 2024

Abstract

Kepah clams (Polymesoda erosa) are one of the few aquatic animals that have a body and are protected by two cupped shells. Many people consume this shellfish meat, but many of the shells are thrown away by local people so that they only become waste. It is this shell waste that needs to be processed properly because it is known that shells are one of the fishery ingredients that contain chitin. One way to make kepah clams have a higher economic value is by isolating kepah clams into chitosan. The purpose of this study was to isolate and characterize chitosan from mussel shells and to determine functional groups using Fourier infrared (FTIR). The isolation methods used were deproteination, demineralization, depigmentation, deacetylation, and chitosan characterization. The results showed that the yield of the transformation of chitin into chitosan in cockles was 85.77% and had a brownish-white powder texture and no odor. The water content in the chitosan of the clam shells obtained was 1.53%, the ash content was 0.50%, and the degree of deacetylation obtained was 75%.

INTRODUCTION

The kepah clam with the Latin name Polymesoda erosa is widely consumed, but many of its shells are thrown away by local people so that they only become waste (1). This shellfish waste needs to be processed properly. Several previous studies have stated that shellfish is one of the fishery materials that contains chitin (2). The nature of chitin, which is non-toxic and easily degraded, encourages chitin modification with the aim of optimizing its use and expanding the field of chitin applications. One compound derived from chitin that has been widely developed because of its wide application is chitosan (3).

Chitosan is a cationic polymer that can bind strongly to anionic cargo, such as glycoproteins, in the mucosal layer. Chitosan is formed when the acetyl group in chitin is substituted by hydrogen to become an amine group (4). Chitosan can be used as a gelling agent in hydrogel or gel preparations (5). Apart from that, chitosan can be used as an adhesive agent, which can be used in mucoadhesive preparations. Apart from that, chitosan also has antimicrobial, preservative, anticholesterol functions and is still being developed in various kinds of research because of its many functions (6,7).

In general, chitosan isolation consists of separating deproteinized proteins, demineralized minerals, and depigmented pigments, while to obtain chitosan, it is continued with the deacetylation process (8). Chitosan has great potential to be used as a preservative because it has positively charged polycations that can inhibit microbial growth (9). Chitosan can be obtained from several animal shells, such as shrimp, lobster, crab, snail, feather shellfish, and so on. Fur shellfish waste has not been used so far. These clam shells can be processed into something of higher economic value, namely, chitosan.

Several studies on chitosan, namely the isolation of seashell (Anadara antiquata) shells, among others, produced chitosan with a degree of deacetylation of 90% (10). Isolation of chitosan from tofu clam shells, which obtained the percentage degree of deacetylation, which was 88.75% (11). The aim of this research is to isolate and characterize chitosan from kepah shells (Polymesoda erosa) and to determine the functional groups using Fourier transform infrared (FTIR).

METHODS

Sample

Kepah clam shell waste was obtained from the Percut Sei Tuan area, Deli Serdang District, in the amount of 4 kg, and then the waste was washed with clean, running water to remove the impurities contained in the shells. Then the shells are dried in the sun until completely dry. After drying, the samples were ground using a spice grinder, then sieved using a 40-mesh sieve until they had a powder-like texture.

Procedure for isolating chitin into chitosan

Deproteination

The deproteination process is carried out with the strong base NaOH. The protein will dissolve in the NaOH solution. The deproteination process aims to break the bonds between protein and chitin. Previously, the shell powder resulting from the sieve was reacted using 4% NaOH in a beaker with a ratio of 1:10 (w/v), heated to a temperature of 100°C for 1 hour using a magnetic stirrer, and then cooled. Next, the shell powder was filtered using filter paper and neutralized with distilled water to pH 7, then dried in an oven at 60°C for 4 hours. The residue obtained after drying is then cooled and weighed (12),

Demineralization

This process aims to remove the compounds and mineral components contained in the shells. The deproteinized shell powder was reacted with 2N HCl in a beaker with a ratio of 1:5 (w/v) using a magnetic stirrer for 1 hour at a temperature of 100°C. Next, the shell powder was filtered using filter paper and neutralized with distilled water to pH 7. The resulting solid was dried in an oven at 60°C for 4 hours. The residue obtained after drying is then weighed (13)

Depigmentation

The demineralized shell powder was reacted with 4% NaOCl in a beaker with a ratio of 1:10 (w/v) for 1 hour at room temperature. The shell powder was then filtered using filter paper and neutralized with distilled water to pH 7. The resulting solid was dried in an oven at 60°C for 4 hours. The residue obtained after drying is then cooled and weighed (13).

Deacetylation of Chitin to Chitosan

The depigmented shell powder was reacted with 50% NaOH in a beaker with a ratio of 1:20 (w/v) for 1 hour at a temperature of 100°C. After the temperature cools, it is filtered and neutralized with distilled water to pH 7. The resulting solid is dried in an oven at 100°C for 80 minutes until it becomes powder. The residue obtained after drying is then cooled and weighed (14).

FTIR Test

Identification using FT-IR (Fourier Transform Infra Red) can prove the presence of chitin and chitosan; the isolation results can be analyzed by making pellets with KBr and then observing the IR spectrum with FT-IR..

Characterization of Kepah Shell Chitosan

Chitosan characterization can be done in the following ways: organoleptic (texture, color), transformation of chitin into chitosan, water content, ash content, solubility level of chitosan, and degree of deacetylation.

Organoleptic

Kepah clam shell powder that has gone through the demineralization, deproteination, and

deacetylation processes is seen for its texture and color to see whether it meets the values of the Indonesian National Standard.

Rendement

The yield of chitin transformation into chitosan can be determined based on the percentage of the weight of chitosan produced to the weight of chitin obtained (15).

Water content

As much as 0.5 grams of chitosan can be put into a porcelain cup whose empty weight is known. Then the chitosan was placed in an oven at 105°C for 2 hours, then placed in a desiccator for 30 minutes, and then weighed (16). This treatment can be carried out until the weight remains constant. Water content can be calculated using the following equation:

$$\%\ Water\ content = \frac{A - B}{C} x\ 100\%$$

Information :

A = weight of container + wet sample (g)

B = weight of container + dry sample (g)

C = wet sample weight (g)

Ash Content

A total of 0.5 grams of chitosan was put into a crucible cup whose empty weight was known. Then the chitosan is heated in a furnace at 500°C for up to 4 hours until it completely turns to ash (Pratiwi & Ridwanto, 2022). The ashed chitosan is then put into a desiccator until room temperature and then weighed. Ash content can be calculated using the following equation:

$$\%\ Ash\ Content = \frac{A-B}{C} x\ 100\%$$

Information :

A = weight of container + wet sample (g)

B = weight of container + dry sample (g)

C = wet sample weight (g)

Chitosan Solubility

Chitosan was dissolved in acetic acid with a concentration of 2% in a ratio of 1:100 (g/ml) and stirred until homogeneous (15).

Degree of Deacetylation

The chitosan that was obtained was tested using FTIR spectroscopy, and the resulting peak was compared with the peak of commercial chitosan. Based on the peak obtained, the degree of deacetylation is then calculated by comparing the absorbance wavelength of the amide group with the absorbance wavelength of the amine group (17).

RESULT AND DISCUSSION

Isolation of Kepah Shellfish Chitosan

Kepah clam shell waste taken in the Percut Sei Tuan area, Deli Serdang district, first started with sample preparation. 4 kg of kepah clam shells yields 3.5 kg of powder. The results of deproteination showed a decrease in sample weight, and the final result was 90.26 g with a yield of 90.26%. The results of the demineralization process showed a decrease in sample weight of 39.11% after deproteination. From the results of the depigmentation process, the sample weight was 32.68 g compared to the sample weight after the demineralization process, namely 33.72 g. The depigmentation results showed that the weight of the sample decreased by 96.92% after the depigmentation process.

Testing the Purity of Chitosan Isolated from Kepah Shellfish Using FTIR

The results of the isolation of feather clam chitosan using FT-IR analysis can be seen in Figure 1.

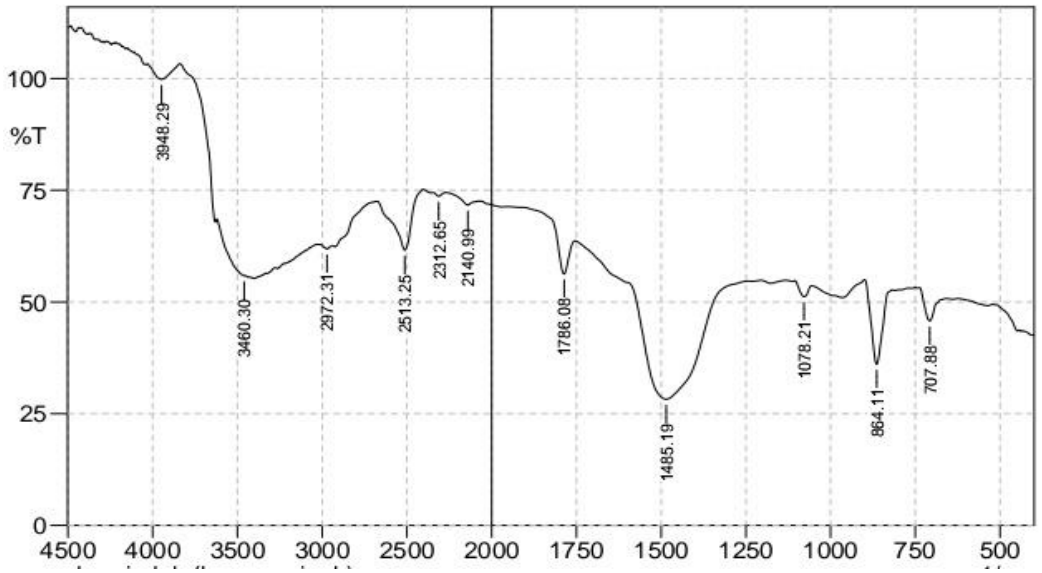

Figure 1. FTIR Analysis Results of Kepah Shellfish Chitosan

Chitosan Characterization

The chitosan results obtained from the gradual isolation process were then characterized in several specific ways. Chitosan Isolation and Characterization Results can be seen in Table 1.

Table 1 Chitosan Isolation Characterization Results and Comparison with SNI

Specification	Shellfish (*Polymesoda erosa*)	Indonesian national standards (SNI)
Organoleptic (Color, Texture, odor)	Light brown, powder, no odor	Light brown to white, Fine flakes and powder, no odor
Water content	1,53%	≤ 12%
Ash content	0,50%	≤5%
Solubility of chitosan in acid	Soluble	Soluble
Degree of deacetylation	75%	≥ 75%

The deproteination process is carried out to remove the protein contained in kepah clam shell powder. In this process, the proteins contained in the kepah clam shell are kept in an alkaline environment so that the proteins that are covalently bound to the chitin functional groups will be separated. The use of a NaOH solution with a high concentration and temperature will be more effective in removing proteins and causing the deacetylation process to occur. The kepah shellfish produced from the sieving process is 100 grams. The results of deproteination showed a decrease in sample weight and the final result was 86.21 g and a yield of 86.21% was obtained.

Based on the results of research that has been carried out, the mineral separation process is characterized by the formation of CO_2 in the form of air bubbles when the HCl solution is added to the sample, so that the addition of HCl is carried out gradually so that the sample does not overflow.From the de-mineralization process, the kepah shellfish obtained a sample weight of 33.72 g compared to the sample weight after the deproteinization process, namely 86.21 g. The results of the demineralization process showed a decrease in sample weight of 39.11% after deproteinization.

This shows that degradation of the mineral content contained in the sample has occurred. The demineralization process plays an important role in chitin isolation. The results of this process greatly influence the quality, especially in determining the ash content. The lower the chitin ash content obtained, the better the quality of the chitin produced.

The depigmentation process of kepah clams is based on the results of research that has been carried out. The release of color pigments contained in the sample is marked by a change in the color of kepah clam shell powder, which was originally gray to white. The addition of NaOCl will cause the carotenoid compounds contained in chitin to dissolve, resulting in a change in the color of the residue from gray to white. Depigmentation aims to provide an attractive appearance to the resulting chitosan product. From the results of the depigmentation process, the sample weight was 32.68 g compared to the sample weight after the demineralization process, namely 33.72 g. The results of the depigmentation process showed a decrease in sample weight of 96.91% after demineralization.

Based on the results of the research that has been carried out, the purity test results of kepah shell chitosan isolated using infrared spectrophotometry show that there is absorption at a wave number of 3460.30 cm^{-1} (O-H and N-H amines). There is absorption at wave number 2972.31 cm^{-1} (C-H). Furthermore, there is absorption at wave number 1768.08 cm^{-1} (C=O). Absorption at wave number 1078.21 cm^{-1} (C-O-C). The quality of chitosan can be determined by the percentage degree of deacetylation. In this study, a deacetylation degree of 75% was obtained in kepah clam chitosan.

Organoleptic testing, or sensory testing, is a method of testing using the human senses. Organoleptics have a very important role in implementing quality. With the organoleptic characteristics of chitosan, it can provide an indication of quality deterioration and other damage. At this organoleptic stage, what is seen is the color, texture, and smell of the chitosan that has been isolated after the sample powder has gone through the processes of deproteination, demineralization, and deacetylation of chitin to become chitosan.

The color of the isolated chitosan is light brown. This meets SNI requirements, where good colors for chitosan range from light brown to white. Meanwhile, the chitosan texture is like flakes. The isolated ki-tosan does not produce an odor that disturbs the sense of smell; it can even be said to be odorless. This can be interpreted as having met SNI requirements where good chitosan does not cause odors.

The transformation rate of chitin into chitosan is determined based on the weight percentage of chitosan produced compared to the weight of chitin obtained. The transformation rate of chitin into chitosan in kepah clams is 85.77%, has a brownish-white powder texture, and is odorless. The water content of chitosan is influenced by the humidity of the air around the storage area because chitosan easily absorbs water vapor and the surrounding air. The chitosan polymer groups (amine, N-acetyl, and hydroxyl groups) will hydrogen bond with H_2O from the air (18). SNI for Non-Food Fishery Products stipulates that the quality standard for water

content in chitosan is ≤ 12%. From the table, it is known that the water content of the kepah shell chitosan that was obtained was 1.53%. These results indicate that the water content of the isolated chitosan that was obtained met the predetermined requirements and did not exceed the maximum limit of chitosan quality standards.

The water content is not influenced by the concentration of NaOH or temperature during the ongoing deacetylation process. The water content contained in chitosan is influenced by the drying process, the length of time the drying is carried out, the number of samples dried, and the surface area where the chitosan is dried. The SNI for non-food fishery products (2013) sets the quality standard for chitosan ash content at ≤ 5%. From the table, it is known that the ash content of the kepah shellfish was 0.50%. These results indicate that the ash content of the chitosan resulting from the isolation obtained meets the specified requirements and does not exceed the maximum limit of the ki-tosan quality standard.

Chitosan solubility is one of the main parameters in assessing chitosan quality. The solubility of chitosan was observed by comparing the clarity of the chitosan solution with the clarity of the solvent. The results of this research show that chitosan can dissolve in 2% acetic acid. Chitosan can dissolve in weak acids, possibly due to the bond between the carboxyl group and the amine group of chitosan. Other factors, such as temperature and soaking time, also influence the solubility of chitosan (18). The higher the solubility of chitosan in 2% acetic acid, the better the quality of the chitosan produced.

The degree of deacetylation is the most important parameter to determine the level of purity of chitosan. The degree of deacetylation indicates the presence of acetyl groups that can be removed from chitin to produce chitosan. A high degree of deacetylation indicates that the acetyl groups contained in chitosan are low, which means that the process of deacetylation of chitin into chitosan has been carried out perfectly. The SNI for Non-Food Fishery Products (2018) stipulates that the quality standard for the degree of deacetylation of chitosan is a minimum of 75%, and the degree of deacetylation of ki-tosan from kepah clam shells obtained is 75%, where chitosan meets the requirements set out in accordance with the SNI.

CONCLUSION

The results of kepah clam shell chitosan obtained a degree of deacetylation of 75%. Which states that the results meet the requirements of the Indonesian National Standard, namely % DD of chitosan > 75%. Based on the results of the characterization of chitosan from kepah clam shells, it shows a light brown, fine powder and has no odor. The solubility test shows that chitosan dissolves in acetic acid. The water content and ash content obtained were 1.53% and 0.50%, respectively.

REFERENCES

1. Ondu AF, DM EAJ, Sunarsih S. Efektifitas Citrus aurantifolia swingle dan Averrhoa bilimbi dalam Menurunkan Konsentrasi Timbal pada Kerang Kalandue (Polymesoda sp) dari Teluk Kendari. HIGIENE: Jurnal Kesehatan Lingkungan. 2019;5(1):1–13.
2. Rachmadianty A, Aryati F, Sastyarina Y. Isolasi dan Karakterisasi Kitin dari Limbah Cangkang Kerang Asia (Corbicula fluminea): Isolation and Characterization of Chitin from Asian Shell Waste (Corbicula fluminea). In:

Proceeding of Mulawarman Pharmaceuticals Conferences. 2021. p. 31–6.

3. Ridwanto R, Rosa VD, Rani Z, Fauzi ZPA. Utilization of Chitosan from Fresh Water Lobster (Cherax quadricarinatus) Shells in Anti-Acne Gel Preparations. Trends in Sciences. 2024;21(2):7243–7243.
4. Ridwanto R, Saragih DS, Rani Z, Pulungan AF, Syahputra RA, Kaban VE, et al. TOXICITY TEST OF VANAME SHRIMP (Litopenaeus vannamei) SKIN CHITOSAN USING BRINE SHRIMP LETHALITY TEST (BSLT) METHOD. Rasayan Journal of Chemistry. 2023;16(4).
5. Rani Z, Nasution HM, Kaban VE, Nasri N, Karo NB. Antibacterial activity of freshwater lobster (Cherax quadricarinatus) shell chitosan gel preparation against Escherichia coli and Staphylococcus aureus. Journal of Applied Pharmaceutical Science. 2023;13(2):146–53.
6. Imtihani HN, Wahyuono RA, Permatasari SN. Biopolimer kitosan dan penggunaannya dalam formulasi obat. Penerbit Graniti; 2020.
7. Nurhayati N, Ridwanto R, Daulay AS, Syahputra RA, Rani Z. Utilization Of Chitosan As A Natural Preservative Against Catfish. International Journal of Science, Technology & Management. 2022;3(5):1396–401.
8. Ridwanto R, Pratiwi A, Rani Z. Isolation and Toxicity Test of Chitosan from Green Mussels (Perna viridis L.) With Brine Shrimp Lethality Test (BSLT) Method. Jurnal Sains dan Kesehatan. 2023;5(5):759–65.
9. Masindi T, Herdyastuti N. Karakterisasi Kitosan dari Cangkang Kerang Darah (Anadara Granosa)[Characterization Chitosan from the Shells of Blood Clams (Anadara Granosa)]. J Chem. 2017;6(3):137–42.
10. Sepvina NI, Ridwanto R, Rani Z. Uji Toksisitas Kitosan Cangkang Kerang Bulu (Anadara antiquata) Dengan Metode Brine Shrimp Lethality Test (BSLT). Jurnal Ilmiah Ibnu Sina. 2022;7(2):380–9.
11. Pratiwi A, Ridwanto R. UJI TOKSISITAS KITOSAN CANGKANG KERANG TAHU (Meretrix meretrix L.) DENGAN METODE BRINE SHRIMP LETHALITY TEST (BSLT). Jurnal Farmasi Klinik dan Sains. 2022;2(2):7–13.
12. Windari NNR, Fauziah SI, Juniar AE, Purnomo T. Biobakterisida Kitosan Cangkang Kerang Darah sebagai Anti Bakteri Ralstonia solanacearum. In: Proceeding Biology Education Conference: Biology, Science, Enviromental, and Learning. 2019. p. 280–4.
13. Walke S, Srivastava G, Nikalje M, Doshi J, Kumar R, Ravetkar S, et al. Physicochemical and functional characterization of chitosan prepared from shrimp shells and investigation of its antibacterial, antioxidant and tetanus toxoid entrapment efficiency. Int J Pharm Sci Rev Res. 2014;26(2):215–25.
14. Celikci N, Ziba CA, Dolaz M. Synthesis and characterization of carboxymethyl shrimp chitosan (CMSCh) from waste shrimp shell. MANAS Journal of Engineering. 2020;8(2).
15. Agustina S, Kurniasih Y. Pembuatan kitosan dari cangkang udang dan aplikasinya sebagai adsorben untuk menurunkan kadar logam Cu. In: Prosiding Seminar Nasional MIPA. 2013.

16. Baharuddin S, Isnaeni D. Isolasi dan uji aktivitas kitosan cangkang kerang bulu (Anadara inflata) sebagai antibakteri terhadap Staphylococcus epidermidis dan Escherichia coli. MPI (Media Pharmaceutica Indonesiana). 2020;3(2):60–9.
17. Andini A, Prayekti E, Triasmoro F, Kamaliyah IN. Pengaruh Penggunaan Jenis Pelarut dalam Uji Sitotoksistas Metode Brine Shrimp Lethality Test (BSLT) pada Wound Dressing Kolagen-Kitosan. Al-Kimiya: Jurnal ilmu Kimia dan Terapan. 2021;8(1):15–20.
18. Dompeipen EJ, Kaimudin M, Dewa RP. Isolasi kitin dan kitosan dari limbah kulit udang. Majalah Biam. 2016;12(1):32–9.