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RESEARCH ARTICLE

THE EFFECT OF TEMPERATURE VARIATIONS ON THE DEACETYLATION PROCESS OF CHITOSAN CHARACTERISTICS FROM MUD CRAB (*SCYLLA SERRATA*) SHELL WASTE

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Abstract

Aim and objective: The aim of this study was to determine the yield value, moisturization, ash, and degree of deacetylation by the variations in deacetylation temperature effect on the quality of chitosan.
Methods: In the synthesis process of chitosan, the first step was demineralization, followed by deproteinization. Afterward, the deacetylation process synthesized the material with temperature variations of 80, 100, and 120ºC. Furthermore, it was characterized by moisture, ash content, and degree of deacetylation. The last step was the Fourier Transform Infra-Red Spectrophotometer (FT-IR) to determine the complete chitosan synthesis.
Results: The results of this study showed that the chitosan yield and water content met the requirements of the quality standards of chitosan. The values obtained were high, which was affected by the temperature. However, the ash content and degree of deacetylation did not meet the requirements of chitosan quality standards. FTIR data showed that the -OH functional group, a -NH group, and no -C=O group from the amide group were found to have characteristics of chitosan formation.
Conclusion: Mud crab shell waste is a potent raw material for making chitosan. The study also reported that the production of chitosan from mud crab shell waste is affected by temperature.
Keywords: Chitosan, chitin, deacetylation, *Scylla serrata*.

INTRODUCTION

Indonesia has a fairly extensive marine area with a fisheries potential of 6.4 million tons annually. Based on export data in Indonesia, about 12,608 mangrove crabs were exported worldwide in 2021 with a value of Rp. 2.542.163.271. Exports of Mangrove Crabs in 2022 (January-March), will be 5.405 pieces with a value of Rp. 969.989.920[1]. Increasing the volume of crab exports will increase the waste amount, including solid-shell waste[2,3].
Crab shell contains calcium carbonate (53.70-78.40%), protein (15.60-23.90%), and chitin (18.70-32.20%). Chitin can be converted into chitosan[4], and it will be economically more valuable if it is converted to chitosan, which is intended for various needs in many fields such as pharmaceuticals, food nutrition, cosmetics, the environment, biomedicine, and agriculture[18]. Chitosan is chemically a polysaccharide consisting of the monomer of N-acetylglucosamine and D-glucosamine[12].
In Indonesia, especially on the island of Sulawesi, many companies carry out mangrove crab export activities. One of the companies in Makassar is engaged in the crab export business, especially the *Scylla serrata* type mangrove crab, which produces crab shell waste during production. Most of the shell waste has not been utilized optimally and has even become waste, which also has the potential to contribute to polluting the environment. This is an opportunity for researchers to take the initiative to utilize crab shell waste by processing it into chitosan. Chitosan has economic value and is useful in several fields, such as the pharmaceutical sector[5,6].
Research on variations in deacetylation temperature is being carried out on samples of marine animals such as crab shells, cuttlefish, shrimp, etc. Therefore, research was carried out on the variation effect ofthe deacetylation temperature from mangrove crab shell waste to obtain the optimum deacetylation temperature. Quality of chitosan products are obtained for use in various fields, especially in the pharmaceutical sector.

MATERIALS AND METHODS

Mud crab (*S. serrata*) shell wastewas obtained from the crab export company CV. Zanama Indonesia based in Makassar. HCl (proanalyst) (Smart-lab), NaOH (pro

analyst) (Emsure), glacial acetic acid, distilled water (Brataco), Whatmann paper no. 42 (GE).

Methods:

The method of this study referred to Dali *et al.*[3], with small modifications[3]. Mud crab (*S. serrata*) shell waste was cleaned in tap water, dried, and then ground into a 60 mesh sieve with an average diameter of 0.356 mm. Afterward, the deproteination, demineralization, depigmentation, and deacetylation stages were carried out to complete this research.

Chitin Preparation

Deproteination

The deproteination process was performed at 80°C, followed by adding a 1 N NaOH solution (1:10 g/mL) while stirring for 60 minutes. Then, the solution was filtered to remove the precipitate, and the precipitate was washed by using distilled water until the pH was neutral. The next process was to filter, and the sediment was dried.

Demineralization

The material from deproteination was prepared to perform mineral removal using the demineralization process. The demineralization process was performed at 80°C using an HCl solution (1:10) while stirring for 120 minutes. Then, it was filtered to remove the precipitation. The precipitate was washed with distilled water until the pH was neutral, then it was filtered and dried.

Deacetylation Process

The deacetylation process from chitin into chitosan was performed at three different temperature variations, namely at 80, 100, and 120°C. The chitin was added into a NaOH solution with a concentration of 50% (by weight) at temperatures of 80, 100, and 120°C, respectively, while stirring for 60 minutes. The result was a filtered slurry and the precipitation was washed with distilled water, then a dilute HCl solution was added so that the pH was neutral and then dried.

The chitosan that was obtained was tested, including water and ash content. Finally, an FT-IR spectro-photometer was used to determine the deacetylation degree.

Chitosan Characterization

Chitosan Yield

The chitosan yield was obtained by calculating the ratio of the weight of the final product to the initial material from the mud crab shell waste.

Water content

The water content method was based on The AOAC (Association of Analytical Communities) method; it was carried out by heating as follows: The sample was weighed in a glass flask with a constant weight of 0.5 g. Then, the material weighed was heated in the oven at 100-105ºC for 1-2 hours. Afterward, it was cooled in a desiccator for approximately 30 minutes, and then it was weighed.

Ash content

The ash content test was performed in constant weight of glass flask. After that, the material was put in the oven and heated at 105°C for 1 hour. The step was repeated several times until a constant weight was found. Then, 0.5 grams of chitosan sample was placed in a flask of known weight at 600°C for 1 hour. Then, the chitosan was ashed until room temperature.

Degree of deacetylation

The degree of deacetylation showed the percentage of acetyl groups lost from chitin into chitosan. The chitosan product was characterized using an FT-IR spectrophotometer in the functional group and fingerprint area 4000-400 cm^{-1}. The degree of deacetylation (DD) from the FT-IR data was calculated by comparison of the absorbance at 1655 cm^{-1}wavelengthfor the amide-NH group with the primary amine group at 3450 cm^{-1} wavelength.

RESULTS AND DISCUSSION

Optimation of chitosan production in the deacetylation process with three different temperature variations was performed at 80, 100, and 120ºC with yields of 17.11, 19.21, and 18.98%, respectively. This research informed that the temperature factor more influenced the chitosan yield; a temperature of 80ºC produced a higher yield than temperatures of 100ºC and 120ºC. A heating temperature that is too high in the deacetylation process will degrade the polymer into a low molecular weight polymer.

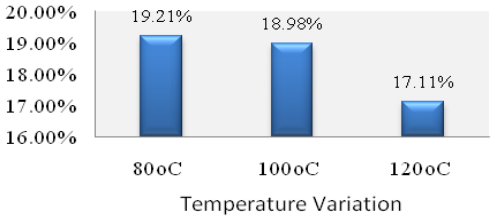

Figure 1: Chitosan yield.

That temperature can speed up the deacetylation reaction, but if the temperature is too high, it can cause excessive release of acetylation chains in chitin, resulting in the formation of fine chitosan particles, which are then dissolved in the NaOH solution during the deacetylation process. Progresses and causes a decrease in chitosan mass. The NaOH solution at a higher temperature, namely 120ºC, evaporates more quickly than the NaOH solution at 100ºC and 80ºC, so the NaOH solution at a higher temperature runs out more quickly than a lower temperature[9,18,19]. The result showed that the water content of chitosan known at 80, 100, and 120ºC was 1.4, 1.6, and 1.9%, respectively. The water content was relatively low. However, this meets the chitosan quality requirements set by Protan Biopolymer, namely ≤10%. Muhammad reported that water content is the most important parameter in determining the quality of chitosan[9].

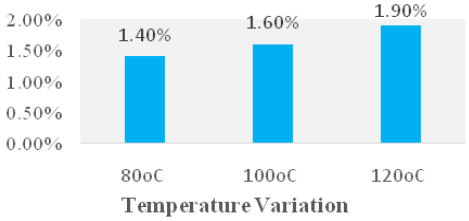

Figure 2: The water content of chitosan.

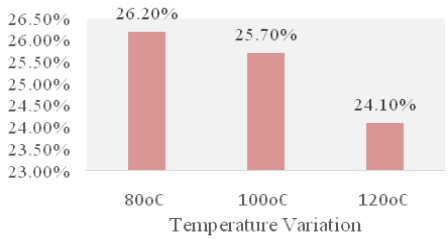

Figure 3: Ash content of chitosan.

The water in chitosan allows a swelling process to occur in the chitosan, where the hygroscopic nature of chitosan is due to the ability of the chitosan amine groups to bind water molecules[9,18]. Less water content can suppress or reduce damage to chitosan, for example, by avoiding the activity of microorganisms[9,18-21].

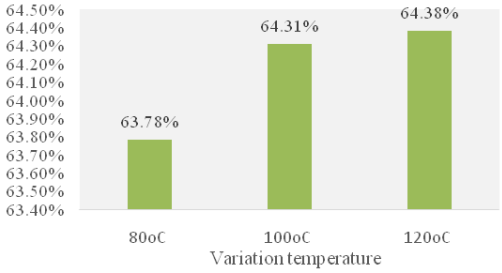

Figure 4: Deacetylation degree.

If the water content is higher, it will be more vulnerable and have a relatively short shelf life. The drying process influences the water content contained in chitosan, the drying time taken, the amount of chitosan dried, and the surface area where the chitosan is dried. The ash content of chitosan is a parameter to determine the minerals contained in chitosan. This can affect solubility, viscosity, and the characteristics of the product. The ash content obtained at 80, 100, and 120ºC temperatures was 26.2, 25.7, and 24.1%, respectively. This result did not meet the established quality requirements, namely ≤ 2%. The solvent concentration and the stirring time could influence it. The ash content may also be because mud crab shells contain many minerals[3,9], such as calcium carbonate (53.70-78.40%), protein (15.60-23.90%), and chitin (18.70-32.20%)[2,3,9].

The degree of deacetylation at 80, 100, and 120ºC was 63.78, 64.31, 64.38%, respectively. It was also investigated using FT-IR data. The FT-IR showed that temperatures of 80, 100, and 120ºC did not meet the quality standard requirements for the degree of deacetylation from the Protan Laboratory was ≥70%. The highest degree of deacetylation at 120ºC was 64.38%. This is relevant to research reported by Dali *et al.*[3], that the degree of deacetylation is influenced by temperature. The higher the temperature, the more acetyl groups are released from chitin, thereby increasing the degree of deacetylation of chitosan. However, the low deacetylation degree showed a low degree of deacetylation. Determination of the degree of deacetylation was performed using FT-IR to determine the functional groups, namely -NH, -OH, and C-C functional groups. The -CH and -C=O for chitin.

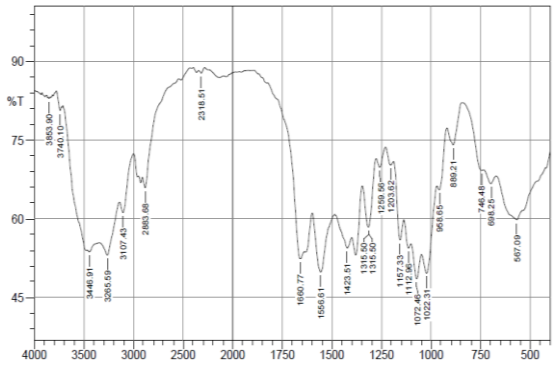

Figure 5: FT-IR data of Chitosan at 80ºC.

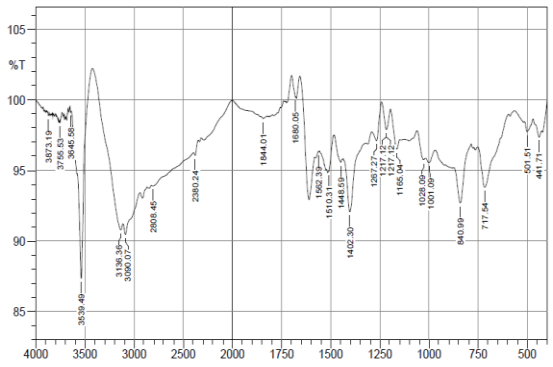

Figure 6: FT-IR data of Chitosan at 100ºC.

The results of FT-IR detection are depicted in the form of the peaks of the functional groups at their respective wave numbers. The specific absorbance at 1655 cm^{-1} wavelength for the amide-NH group with the primary amine group at 3450 cm^{-1} wavelength. The determination value was 1.33, which shows the complete deacetylation process. The FT-IR data at 80ºC in Figure 5 displayed that the peak sharp and strong at 1300-1000 cm^{-1} will give rise to a-CO as an ester absorption, 1556.61 -C=C Aromatic, 698.25 to 958.65 -CH alkene, 1315.5 NO_2, 3740.1 and 3853.9 -OH phenol. From the functional group analysis, it can be concluded that chitosan has been successfully synthesized. The FT-IR data at 100ºC in Figure 6 showed that the peak sharp and strong at 1300-1000 cm^{-1} will give rise to a -CO (Ester) absorption, 1510.31 -C=C Aromatic, 3136.36 and 3539.49 with -NH amide groups and amine, 2380.24 nitrile.

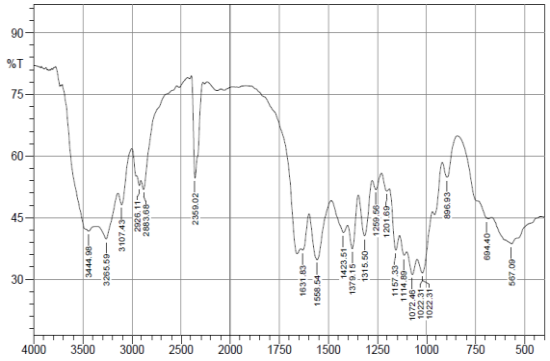

Figure 7: FT-IR data of Chitosan at 120ºC.

Through this functional group analysis, it can be concluded qualitatively that chitosan has been successfully synthesized. The FT-IR data at 120ºC in Figure 7 showed that the peak sharp and strong at 1300-1000 cm^{-1} will give rise to -CO as an ester absorption, 1558.54 -C=C Aromatic, 3107.43 -C=H, 3265.59 and 3444.98 indicates the presence -OH phenol. Based on this functional group analysis, it can be concluded qualitatively that chitosan has been successfully synthesized.

Limitations of the study

The limitation of this research is the demineralization process needs to try a higher concentration of solvent to decrease ash content to be able to increase the deacetylation degree.

CONCLUSIONS

The chitosan yield value obtained decreases with increasing temperature, the water content value of chitosan obtained increases with increasing deacetylation temperature, while the ash content value decreases as the deacetylation temperature increases and the value of the degree of chitosan deacetylation obtained is that the higher the temperature, the higher the degree of deacetylation.

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AUTHOR'S CONTRIBUTION

HS: Write the original draft, methodology, and investigation. **ATO:** formal analysis, data curation, conceptualization. **AN:** write, review, and edit methodology and discussion. Final article was reviewed and approved by all authors.

DATA AVAILABILITY

Anyone can seek access to the data by contacting the respective author.

CONFLICT OF INTEREST

There is no conflict of interest in this work.

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