Chitin Deacetylation Shells of *Portunus pelagicus* L. Using Microwave Irradiation

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Abstract. Natural polymers such as chitosan are one of the interesting things to study or are widely used as industrial raw materials such as adsorbents, membranes, and edible films. *Portunus pelagicus* L. is one of the sources of chitosan from nature. The shell of *Portunus pelagicus* L. in Bangka Island are waste from the untapped food industry. Even though this shell can have high values because there are chitin and chitosan. Good chitosan has a high degree of deacetylation. So the research purpose of this study is to see the effect deacetylation process using a microwave. The microwave used was MARS 6 - Microwave Accelerated Reaction System (CEM) using an EasyPrep Plus vessel. Various in Microwave deacetylation process is the time at 1200 W and 100°C. The method for analyzing the structure and degree of deacetylation (DD) in this study uses Fourier Transform Infrared (FTIR) spectroscopy. The results showed that deacetylation using conventional methods for 120 minutes at a temperature 100°C had DD 76.67% while deacetylation using microwave for 40 minutes at a temperature of 100°C had a DD 77.89%. Microwave deacetylation is three times faster than conventional methods.

1. Introduction

Natural polymers such as chitosan are one of the interesting things to study or are widely used as industrial raw materials such as adsorbents, membranes and edible films [1]. Chitosan is a natural polymer in the form of a polysaccharide which is currently the most after cellulose. Chitosan can be produced through the waste of shrimp and crab shells which are very much found in Indonesian waters, especially in Bangka. The deacetylation process of chitin is the key to chitosan quality. At present, chitosan is mostly produced by conventional chemical methods [2]. Deacetylation process requires an alkaline solution with high concentration [3]. The temperature and time used are also high and long. This causes the deacetylation process to be less efficient. Therefore, technological innovation is needed to make the production process run more efficiently with more optimal results and shorter time. One of the technological innovations in chemical processes is using microwaves. Microwave radiation is already widely used in various food and chemical industries. Microwaves can be used as a source of energy for heating and drying material, and extracting natural compounds in the world of agriculture [4].

Based on previous research, microwave irradiation has many advantages, including relatively short startup and heating or reaction times, superior energy efficiency and process costs, easy and precise

processes, and better final product quality and can improve the quality of dry ingredients [5, 6]. Therefore this study the process of deacetylation of chitin into chitosan crab using microwaves.

2. Research methods

2.1 Preparation Chitin from Shell of Portunus pelagicus L.
The crab shell waste (*Portunus pelagicus L.*) from Jelutung Village is cleaned, dried and crushed with mortar to form a coarse powder. The powder is subsequently sieved, size of 80 mesh powders will be processed through deproteination, demineralization, and dechlorination [6].

2.2 Deacetylation Chitin
The chitin obtained was then deacetylated by two methods, namely by conventional and microwave methods. In this study, the conventional method of deacetylation was carried out by dissolving chitin in a 40% (w/v) NaOH solution. Comparison of chitin and NaOH solution is 1:20 (w/v). This process is carried out at a temperature of 100-120^{o}C for 2 hours. The precipitate formed is washed using aquadest until neutral pH and dried in the oven. Analysis of the degree of deacetylation (DD) of chitosan using FT-IR spectrophotometer. The chitosan obtained was weighed and analyzed physical properties [6]. Microwave method in this study was carried out with time variations: 10, 20, 30, and 40 minutes at 100^{o}C and 1200W. The microwave used was MARS 6 - Microwave Accelerated Reaction System (CEM) using an EasyPrep Plus vessel.

2.3 Chitosan Analysis
Chitosan obtained was analyzed by form, color, solubility, and degree of deacetylation (DD). The higher the DD, the better the conversion of chitin to chitosan. Based on the literature, the determination of DD using FT-IR spectroscopy, there are several differences in calculations based on the absorbance ratio. Commonly used equations are from Sabnis and Block [7] (equation 1). This equation sees the absorbance ratio of 1655 cm^{-1} and 3450 cm^{-1} with the following equation:

$$\% \text{ DD} = 97{,}67 - \left[\left(\frac{A_{1655}}{A_{3450}} x 26{,}486\right)\right] \quad (1)$$

DD analysis was also stated by Baxter [11]. The equation used also uses a ratio of absorbance of 1655 cm^{-1} and 3450 cm^{-1}. DD is calculated by equation 2.

$$\% \text{ DD} = 100 - \left(\frac{A_{1655}}{A_{3450}} x 115\right) \quad (2)$$

Other DD analysis is determined by the baseline method from Domszy and Roberts equation [8] (equation 3). This equation looks at the relationship of wave number absorbance of 1588 cm-1 to the absorption of the amide/acetamide group with the absorbance of 3410 cm^{-1} for the absorption of hydroxide (OH) groups.

$$\% \text{ DD}=100-\left[\left(\frac{A_{1588}}{A_{3410}} x 100\right)/1{,}33\right] \quad (3)$$

3. Result and Discussion

3.1 Chitin Isolation from Shells of Portunus pelagicus L.
Isolation of chitin from crab shell waste (*Portunus pelagicus L.*) through stages of deproteination, demineralization and dechlorination. In the deproteination stage, the mashed, and sifted crab shell powder is added with NaOH solution. The NaOH solution aims to break the bonds between proteins and chitin. At this stage, a thick brown solution occurs. This is because the released protein binds to Na^{+} ions which are soluble in water while chitin will precipitate as insoluble in water. The precipitate formed was then demineralized by dissolving it in 500 mL 1M HCl. The addition of HCl is done gradually because CO^{2} gas bubbles will form. Demineralization aims to remove inorganic salts present in crab shells such as $CaCO_3^{\neg}$ and $Ca_3(PO_4)_2$. HCl will react with $CaCO_3^{\neg}$ so that it produces CO_2 and $CaCl_2$

salts which are soluble in water. Dechlorination to remove color pigments in chitin. In this process, brown chitin is added with sodium hypochlorite (NaOCl) solution so that the chitin color turns white. The chitin is then dried to obtain chitin powder. This chitin powder was analyzed using an FT-IR spectrophotometer.

The chitin FT-IR spectrum in this study showed that calcium carbonate ($CaCO_3$) crab shells had decomposed into carbon dioxide; this was due to the reaction with HCl and heating for 1 hour. If there are $CaCO_3$, the FTIR spectrum will produce a peak at the wave number 1390 and 1796 cm^{-1} [9]. The chitin amide group extracted from *Portunus pelagicus L.* is shown at wave number 1637 and 1554 cm^{-1}. Beside, the amide group is visible at wave number 1312 cm^{-1}, which is a vibration of the C-N amide stretching group. The characteristic of chitin extracted is similar to chitin from shrimp. The bond absorbance in chitin as a result of extracting crab shells (*Portunus pelagicus L.*) is presented in Table 1.

Table 1. Analysis of functional groups of chitin crab shells (*Portunus pelagicus L.*)

Wave number (cm^{-1}) Chitin extracted	Wave number (cm^{-1}) Chitin shrimp shell [10]	Vibration Mode
3433	3475	OH *stretching*
3262	3265	NH *stretching*
3104	3105	NH *stretching*
2916	2883	C-H *stretching*
2225	2165	
1637	1660	C=O *band*
1554	1554	NH *band*
1378	1431	CH_3 *wagging*
1014	1072	C-O-C *stretching*
707	707	N-H

3.2 Chitin Deacetylation Using Conventional Methods

Chitin deacetylation is the removal of the acetyl group ($COCH_3$) which is bound to the amine group ($-NH_2$). Deacetylation using conventional methods is by dissolving chitin in NaOH solution 40% (w / v) with a ratio of chitin and NaOH solution (1:20 (w / v)) for 2 hours at 100 ° C. Chitosan from conventional results has a creamy white color with a powdery texture and can dissolve in 1% acetic acid. The structure analysis of chitosan can be seen in Figure 1. Chitosan characteristics were shown in the FT-IR spectrum at the absorbance of 3356 (broad) and 2915 cm^{-1}. The wave number corresponds to the -OH, NH, and CH (stretching) groups. The absorbance at wave number 1639 and 1578 cm^{-1} indicates the presence of an amide group from chitosan. This shows that the acetamide group has not all been hydrolyzed. Wave number 1373 cm^{-1} shows the vibration of the CH_2OH group [11]. While the characteristics of carbohydrate (group C-O-C) marked the absorbance at 1031 cm-1. Vibration-OH group that is characteristic of carbohydrates is observed at wave number 643 cm-1. The results of chitosan are also by previous studies, such as chitosan from shrimp [10, 11]. The effectiveness of changing chitin to chitosan is seen from the degree of deacetylation (DD). The DD using the conventional method is 76.67%.

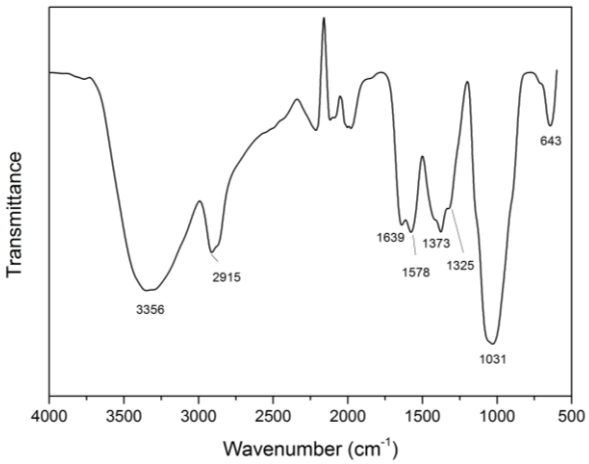

Figure 1. The FT-IR spectrum of crab chitosan (*Portunus pelagicus L.*) using the conventional method

3.3 Deacetylation Using Microwave Irradiation

Based on the results of FT-IR spectrum (Figure 2), chitosan microwave results have absorbance at the wave number 3350-3340 (broad) and 2882 cm^{-1} indicating the presence of OH groups, NH, and CH (stretching). The OH and NH groups overlap at wave number 3350-3340 (broad). The absorbance at wave number 1642 and 1557 cm^{-1} indicates the presence of an amide group from chitosan. Whereas the vibration of the CH_2OH group is shown at wave number 1376 cm^{-1}[11]. Absorbance at 1031 cm^{-1} is vibration from the group C-O-C or C-OH carbohydrate of chitosan results microwave.

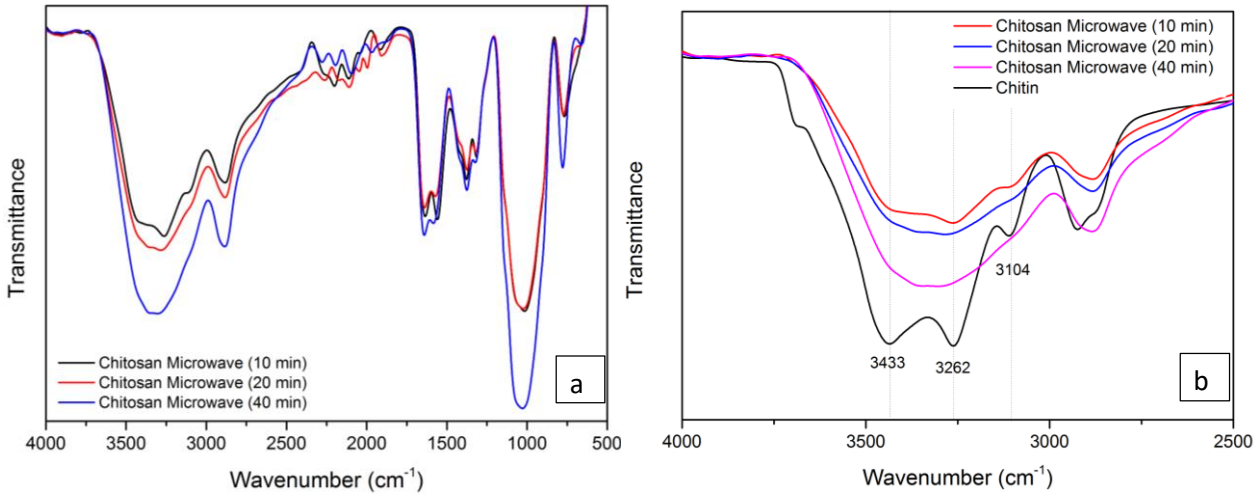

Figure 2. The FT-IR spectrum of: (a) Chitosan (*Portunus pelagicus L.*) microwave irradiation, (b) Chitosan microwave with chitin

FT-IR spectrum from the deacetylation reaction using the microwave for 10 minutes showed that the chitosan produced still resembled chitin. This is indicated by the presence of chitin-like absorbance at wave number 3433, 3262, and 3104 cm^{-1} (Figure 2(b)). The absorbance is the vibration of the OH and NH groups from stretching amides. Even though the absorbance was not as strong as chitin, it showed that the deacetylation reaction using a microwave had not been completed within 10 minutes. While the results of microwave chitosan are 20 minutes, the absorbance that resembles chitin does not appear. This shows that a lot of chitosan starts from the microwave deacetylation process for 20 minutes. The absorbance that characterizes chitosan is stronger than the results of the microwave 40 minutes. This is indicated by the loss of absorbance at 3433 and 3262 cm-1, as well as the appearance of absorbance from the vibrations of OH and NH groups that overlap at wave number 3350-3340 cm-1 (broad).

In this study, DD measurements were calculated using the Sabnis and Block, Baxter, and Domzy & Roberts equations. Beside, the quality of chitosan can be seen from its solubility to acetic acid. DD results and physical-analysis are presented in Table 2.

Table 2. The results of the analysis of physical properties and the DD of chitosan.

Methods	Reaction Times (Min)	Solution color	Solubility in acetic acid 1%	DD (%)		
				Equation 1	Equation 2	Equation 3
Microwave	10	turbid	slightly soluble	73,02	-7,04	34,25
	20	transparent	soluble	74,93	1,24	40,13
	30	transparent	soluble	74,77	0,55	38,22
	40	transparent	soluble	77,89	14,13	49,09
Conventional	120	transparent	soluble	76,67	8,80	38,22
Commercial	-	transparent	soluble	82,59	34,5	59,45

The DD equation 1 has the highest value, while equation 2 has the smallest value. DD value difference is probably due to humidity chitosan produced [15]. The results show that the length of time microwave irradiation tends to increase the DD of chitosan produced. The most significant increase in DD occurs at 40 minutes. Increasing longer time is expected to increase DD even though significant increase does not accompany it. This is because chitosan can undergo a degradation reaction so that the resulting molecular weight of chitosan is low [10]. When viewed from the physical properties of chitosan, the results of this study have transparent and excellent solubility in 1% acetic acid. Although the effect of microwave 10 minutes of chitosan have a slightly soluble in acetic acid. This is because the deacetylation process of chitin to chitosan has not run entirely.

3.4 Comparison of Chitosan results from Conventional methods with Microwave irradiation

The structure of chitosan from deacetylation using microwave irradiation has the same characteristics as conventional and commercial chitosan (Figure 3).

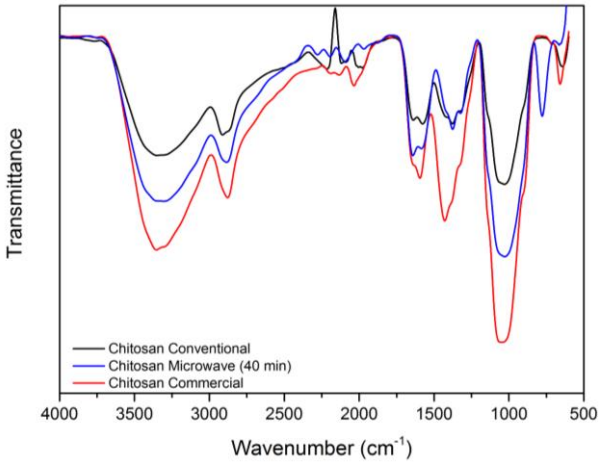

Figure 3. The FT-IR spectrum of chitosan of microwave and conventional

All three have similarities that characterize the absorbance of chitosan, which is at wave number 3350-3340 (broad) and 2882 cm^{-1} indicating the presence groups of OH, NH, and CH (stretching). The OH and NH groups overlap at wave number 3350-3340 (broad). The difference in the FT-IR spectrum from all three is only on the absorbance produced, while the peak produced has a similar wave number value.

Chitosan from microwave irradiation with 40 minutes has a higher absorbance than conventional chitosan with a reaction time of 120 minutes (2 hours). This causes DD chitosan microwave irradiation is higher than conventional chitosan. Deacetylation using microwave has a reaction time three times

faster than conventional methods. The use of microwaves results in inter-atomic vibrations in molecules. This results in increased kinetic energy in the molecule so that intermolecular collisions increase. Therefore, the use of microwave irradiation is better than the conventional method in terms of time and chitosan produced.

4. Conclusion

Deacetylation using conventional methods for 120 minutes at 100^{o}C have a DD of 76.67% while microwave chitosan at 100^{o}C for 40 minutes have a DD of 77.89%. The use of microwaves results in inter-atomic vibrations in molecules. This results in increased kinetic energy in the molecule so that intermolecular collisions increase. Therefore, the use of microwave irradiation is better than the conventional method in terms of time and chitosan produced.

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