Effect of Ultrasonic Assisted on The Degree of Deacetylation of Chitosan Extracted from *Portunus Pelagicus*

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Article Info

Abstract

The technology for extracting chitin from shell and other materials needs to be continuously improved, including its conversion to chitosan. Chitosan is a biocompatible polymer, biodegradable, non-toxic, water-soluble at pH below 6.5, and it has protonated amino groups. The benefits of chitosan in industry, food and medicine make it necessary to fully study an efficient chitosan synthesis method and the results can be applied on an industrial scale. This study examined the effect of ultrasonic-assisted in increasing the degree of deacetylation of chitosan produced from Portunus pelagicus shell waste. The production process of chitosan goes through the stages of deproteination, demineralization and deacetylation. All these steps are ultrasound assisted processes with a frequency of 40 kHz through a digital ultrasonic cleaner. Ultrasonic-assisted chitin and chitosan were examined using FTIR spectrometry. The results showed that the ultrasonic method was able to increase the deacetylation degree of chitin with a value of 68.45±0.11% compared to 62.52±0.08% without ultrasonic. Application of ultrasonic assisted deacetylation gave a deacetylation degree of 85.35 ± 0.20%, higher than without ultrasonic 80.24 ± 0.19%. Physically, ultrasonic-assisted chitosan is smoother and brighter in color. The ultrasonic-assisted chitosan manufacturing method could increase the deacetylation degree and produce high grade chitosan.

INTRODUCTION

Chitin, the main structural component of the invertebrate exoskeleton, especially crustaceans. It is the second most abundant natural polysaccharide after cellulose. Its deacetylated derivative, known as chitosan ((1 → 4)-2-amino-2-deoxy-(d-glucose)), has important uses in the biomedical (Cheung et al., 2015; Karadeniz & Kim, 2014; Kong et al., 2010; Raafat & Sahl, 2009) and food agriculture (Raza et al., 2020; Shi & Tan, 2002; Tolve et al., 2019).

Chitosan is widely used in the food, medicine and cosmetic industries. Finding an efficient chitosan synthesis methods need to be thoroughly studied and the results can be applied on an industrial scale. The deacetylation degree of chitosan in industry is close to 100%. Currently, many methods are being developed to increase the degree of deacetylation. However, the results showed that the deacetylation degree is still below the average of 80% using conventional methods (Buanasari et al., 2019; Rahayu & Purnavita, 2017; Sartika, 2016). Furthermore, it is necessary to study the optimum conditions of the chitin extraction process and the technique of increasing the deacetylation degree of chitosan results. And technology that will be studied is ultrasonic. This technology is effective for the extraction, dissolving and formation of chitosan from fungi and other processes (Zhu et al., 2019). It is expected that ultrasonic can also increase the quantity and quality in the chemical synthesis process of chitosan from shells. Many previous studies have used high

intensity ultrasound to extract chitin (Kjartansson et al., 2006; Vallejo-Domínguez et al., 2021), to produce chitosan (Abiraman et al., 2017; Fiamingo et al., 2016; Wang et al., 2009), as an activator for polymerization reactions (Birolli et al., 2016; Guzmán et al., 2019; Kritchenkov et al., 2019), to reduce the molecular weight of more soluble polymers (Fiamingo et al., 2016; Trzciński & Staszewska, 2004), to produce nanoparticles and nanoparticles from chitin (Lu et al., 2013) and manufacturing microencapsulation (Anbinder et al., 2011; Behrouz et al., 2018; Raza et al., 2020; Sanna et al., 2015).

The effect of sonication on the degree of deacetylation of chitosan has rarely been studied. More studies are using sonication to form chitosan derivatives. Previous research stated that the degree of chitin acetylation from freshwater shrimp shells using sonification did not change, but the degree of chitosan acetylation decreased from 70.0 to 68.7 and 61.4% for sonication with a time of one and four hours. This still requires further study in determining the process conditions and raw materials used (Kjartansson et al., 2006). Another study stated that the use of sonification in the deproteinization process with a time variation of 10-40 minutes showed an increase in the degree of deacetylation of chitosan from 73.314 to 94.034% (Vallejo-Domínguez et al., 2021).

Previous sonication studies on chitin deacetylation using shrimp shells. Shrimp shell material has a high degree of deacetylation without sonication reaching more than 80% (Tolesa et al., 2019). Another source of chitin is *Portunus pelagicus* which is very abundant in the sea. The waste of its shell has not been widely used. This material provides a degree of deacetylation without sonication of less than 80% (Rahayu & Purnavita, 2017; Sartika, 2016).

In this study, chitin will be extracted from the shell waste of *Portunus pelagicus* using a digital ultrasound cleaner. The chitin results obtained will be converted into high quality chitosan. The effect of ultrasonic assistance is expected to produce that.

MATERIALS AND METHODS

Materials

The main material of this research is the shells of *Portunus pelagicus* obtained from the crab processing waste industry in Rembang, Central Java, Indonesia. Other ingredients are sodium hydroxide (Merck), hydrochloric acid (Merck) and distilled water. The tools used in this study were digital ultrasonic cleaner, water analyzer (Radwag MAC50), UV-Vis spectrophotometer (Shimadzu2480), FTIR spectrometry (Perkin Elmer), electric scale (Sartorius), oven (Memmert), rotary evaporator (Scilogex).

Methods

Sample Preparation

The shell was separated from the rest of the meat and protein. The shells were rinsed with water until they were clean. They were then dried under the sun light. After that, they were then pollinated into 100 mesh sizes with an average moisture content of less than 10%. The materials were then stored at room temperature.

Chitin Isolation

Chitin isolation process, consists of deproteination and demineralization processes. The deproteinization stage used a modified Younes & Rinaudo, (2015) method. Crab shell powder plus 3.5% (1:10 grams/mL) NaOH solution was heated at 70ºC for two hours while stirring using a homogenizer (8,000 rpm). After being separated from the solution, the powder was then washed with distilled water until it was neutral. It was then dried using the oven until the weight was constant, the yield was then calculated. These steps were replicated by each process with and without ultrasonic assistance.

Demineralization stages. The deproteinated powder was then demineralized using 1N HCl solution (1:15 grams/mL) while stirring at room temperature for one hour. After being filtered, it was then washed with distilled water until neutral then dried using an oven until constant weight, and the water content was below 10%, the yield was calculated. The result obtained was chitin powder. These steps were then replicated by each process with and without ultrasonic assistance.

Chitosan Isolation

Isolation of chitosan was carried out by using deacetylation process. The deacetylation stage begins by heating the chitin powder in a 50% w/v NaOH solution with a ratio of 1:20 grams/mL at 90°C for two hours. After being filtered, it was then washed with distilled water. Then the drying

results were calculated in the form of chitosan powder. deacetylation degree analysis were then performed by using the Fourier Transform Infrared (FTIR) spectroscopy method. These steps are replicated by each process with and without ultrasonic assistance. The deacetylation process with non ultrasonic and ultrasonic-assisted is shown in Figure 1.
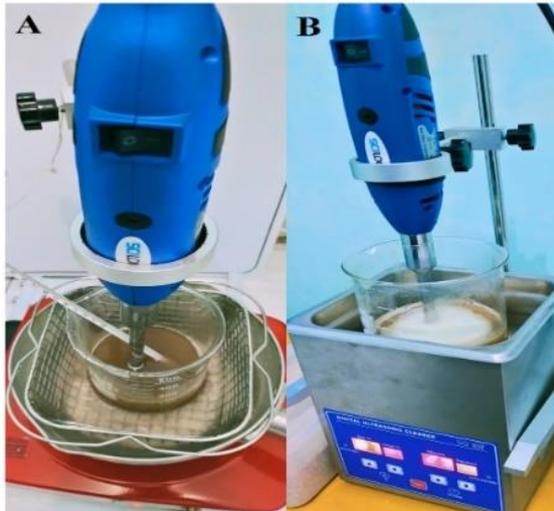
Figure 1. Deacetylation Process. (a) Non ultrasonic; (b). Ultrasonic assisted.

Chitosan Performance Test

Tests were carried out to determine the performance of chitosan produced as follows: The degree of deacetylation of chitosan can be measured by various methods. The method most commonly used is Fourier Transform Infrared (FTIR) spectroscopy. This technique provides several advantages, namely, it is relatively fast and has a high degree of accuracy compared to titrimetric techniques and other spectroscopic methods. The calculation of the degree of deacetylation (DD) was carried out by means of the base line according to (Roberts, 1992) based on the results of FTIR analysis using Eq. (1).

$$\%DD = 1 - \left[\left(\frac{A1655}{A3450} \right) x \left(\frac{1}{1.33} \right) \right] x \, 100\% \quad (1)$$

Where, A is the Absorbance, A_{1655}: Absorbance at a wavelength of 1655 cm^{-1} for absorption of amide or acetamide groups (-CH$_3$CONH); A_{3450}: Absorbance at a wavelength of 3450 cm^{-1} for hydroxy group (-OH) absorption and Factor 1.33: A_{1655}/A_{3450} ratio value for fully deacetylated chitosan.

RESULTS AND DISCUSSION

In this research, the first step was material preparation, in this case the material was a crab shell powder that has passed 100 mesh. The sample was powdery, brownish white, with a pH of 7.6 and a moisture content of 7.129%.

Chitin Isolation

The deproteination process aims to break the bond between the powder and the protein contained in the sample using a 3.5% NaOH solution with a ratio of 1:10 grams/mL for one hour. The results of chitin isolation are presented in Table 1 and Figure 2.

Table 1. The yield of chitin in the process of deproteination and demineralization.

No	Process	Yield (%)	
		Non-ultrasonic	Ultrasonic-assisted
1	Deproteination	32	34
2	Demineralization	12.69	12.99

Figure 2. Chitin powder. (a) Non ultrasonic; (a). Ultrasonic assisted.

The results showed that the chitin from the two processes showed different physical appearance. The physical appearance of chitin resulting from these two processes was carried out through observation. Chitin produced from the ultrasonic process is whiter than chitin without ultrasonic. An ultrasonic are able to remove colored matter and some of the remaining protein material bound to the matrix more completely than without the sonication. The size of the chitin powder with the ultrasonic assisted process is also smaller than that without ultrasonic. This is in accordance with Fiamingo et al., (2016); Vallejo-Domínguez et al., (2021), which found that the longer the sonication process was able to reduce the color and size of the powder.
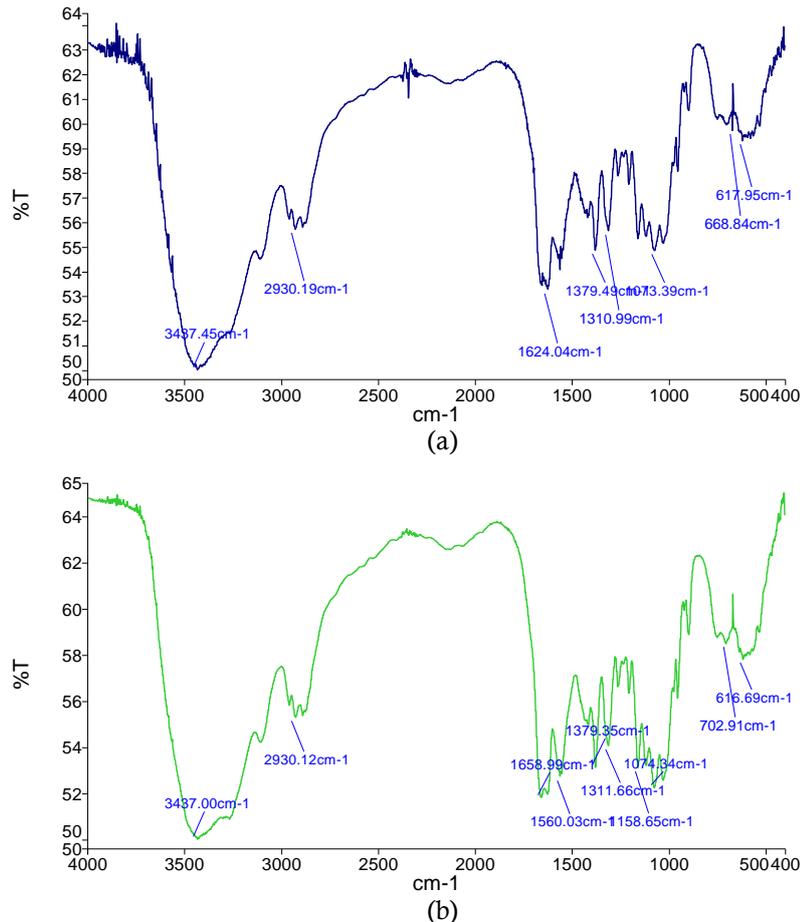
Figure 3. Chitin FTIR Spectra. (a). Non ultrasonic; (b). Ultrasonic assisted.

The yield percent obtained in the deproteination and demineralization processes were 32; 12.69% with ultrasonic and 34; 12.99% without ultrasonic. In this study, the ultrasonic assisted process was able to maintain yield. This contradicts with Kjartansson et al., (2006), who stated that sonication of shrimp chitin for four hours caused small yields. In this study, the sonication time was carried out for one hour, so that it was still able to maintain the yield.

The deproteination process shows that the amount of protein bound by Na^+ ions forms sodium proteinate. In this process, it is about 68-64% dissolved in water. There were some bubbles on the surface and thickened slightly. That indicates that the protein was separated during this process.

The demineralization process was aimed to separate the chitin from minerals in the crab shell, such as calcium carbonate and tricalcium phosphate. Soaking at room temperature is mostly done to minimize hydrolysis, or the breakdown of water molecules into H^+ and OH^- in the polymer chains (Wang et al., 2009). The yields were 12.69

and 12.99%, this shows that the amount of minerals that react with HCl is 60.34% which is indicated by the appearance of foam.

The results of analysis using FTIR for deproteination and demineralization results also show that ultrasonic work can improve the quality of chitin which can be seen from the degree of deacetylation. The results of the examination using FTIR are presented in Figure 3. The characteristic absorption band of chitin could be observed in both samples. In this study, the -OH vibration is reflected in the region of 3434–3438 cm^{-1}. The NH_2 and NH amide groups were in the regions of 1624–1658 cm^{-1} and 1533–1560 cm^{-1}, respectively. Chitin fingerprints could be identified, the C-O-C band stretching in the region of 1158–1159 cm^{-1} and C-O at 1073–1074 cm^{-1}. Other studies have also reported the same absorption bands of chitin obtained from a variety of methods and materials (Buanasari et al., 2019; Vallejo-Domínguez et al., 2021).

The results of chitin from the above FTIR data were used to calculate the degree of deacetylation. The calculation of the deacetylation

degree from the results of this process is presented in Table 2.

Table 2. The degree of deacetylation of chitin.

No	Process	DD(%)
1	Non-Ultrasonic	62.52±0.08
2	Ultrasonic Assisted	68.45±0.11

The deproteination and demineralization processes that were carried out with three replications showed that the ultrasonic assisted process was able to increase the degree of chitin deacetylation obtained in this process. This is because the sound waves added to the process are able to penetrate cells and help remove the proteins and minerals present in the material (Ren et al., 2019; Vallejo-Domínguez et al., 2021).

Chitosan Isolation

Chitosan which is formed in the form of powder, brownish white, has a pH of 7.5 and an average moisture content of 9.341%. At this stage a deacetylated chitin is produced which has a slightly alkaline pH as explained by (Muzzarelli, 1973) that chitosan is an alkaline polysaccharide due to the use of a high concentration of NaOH solution even though it has been washed slowly and long. The chitosan powder is presented in Figure 4.

Figure 4. Chitosan powder. (a). Non ultrasonic; (b). Ultrasonic assisted.

Chitosan powder produced by ultrasonic assisted process has a finer shape with a lighter color. Ultrasonic is able to break down particles and make them smoother (Cravotto et al., 2005; Nouri et al., 2016; Vallejo-Domínguez et al., 2021).

The functional groups that compose chitosan can be known according to the wave number (cm^{-1}) shown in Figure 5.

Figure 5. shows that each peak contains a distinctive absorption band and matches other chitosan studies (Vallejo-Domínguez et al., 2021; Younes & Rinaudo, 2015). Peak area data of

chitosan FTIR results non-ultrasonic and ultrasonic-assisted are presented in the Table 3. The stretching of –OH could be found in the regions of 3434–3435 cm^{-1}. The asymmetric stretching of CH_2 and the symmetric stretching of CH are in the range of 2916–2928 cm^{-1} and 2867–2882 cm^{-1}, respectively. The peak of the NH_2 absorption band for amides could be observed in the region of 1626–1653 cm^{-1}. An absorption band of about 1540 cm^{-1} was not found indicating the absence of protein, as reported in the literature (Mohammed et al., 2013; Vallejo-Domínguez et al., 2021). The fingerprints of the C-O-C asymmetric stretching band and the C-O symmetrical stretching along with CH_3 were in the area of 1378–1379 cm^{-1} and 1075–1085 cm^{-1}, respectively, which showed the presence of chitosan.

Table 3. Chitosan FTIR peak area data.

Non-ultrasonic			Ultrasonic-assisted		
Peak A	X (cm^{-1})	Y (%T)	Peak B	X (cm^{-1})	Y (%T)
1	3435.48	50.05	1	3435.08	50.00
2	2928, 31	56,11	2	2916,50	54.12
3	2867,50	56,17	3	2882,50	54,18
4	1626.19	53.76	4	1653.49	55.01
5	1379.41	55.04	5	1379.24	56.41
6	1314.76	56.17	6	1085.75	54.92
7	1075.24	54.17	7	671.03	60.41
8	597.41	58.44	8	662.66	60.22

The calculation of the deacetylation degree from chitosan is presented in Table 4.

Table 4. The degree of deacetylation of chitosan.

No	Process	Yield (%)	DD (%)
1	Non-ultrasonic	83.28	80.24±0.19
2	Ultrasonic-assisted	72.55	85.35±0.20

The results of ultrasonic assisted chitosan showed a smaller yield compared to non-ultrasonic processes, but it had a higher degree of acetylation. This is similar to previous studies which state that the degree of chitosan acetylation increases with the longer the sonication duration (Vallejo-Domínguez et al., 2021). The absorption bands of the NH_2 (1626–1653 cm^{-1} groups showed a higher intensity, indicating a higher degree of deacetylation by the ultrasonic assisted process. It is also increased by ultrasonic because the sound waves used are able to help the chemical reaction process that occurs
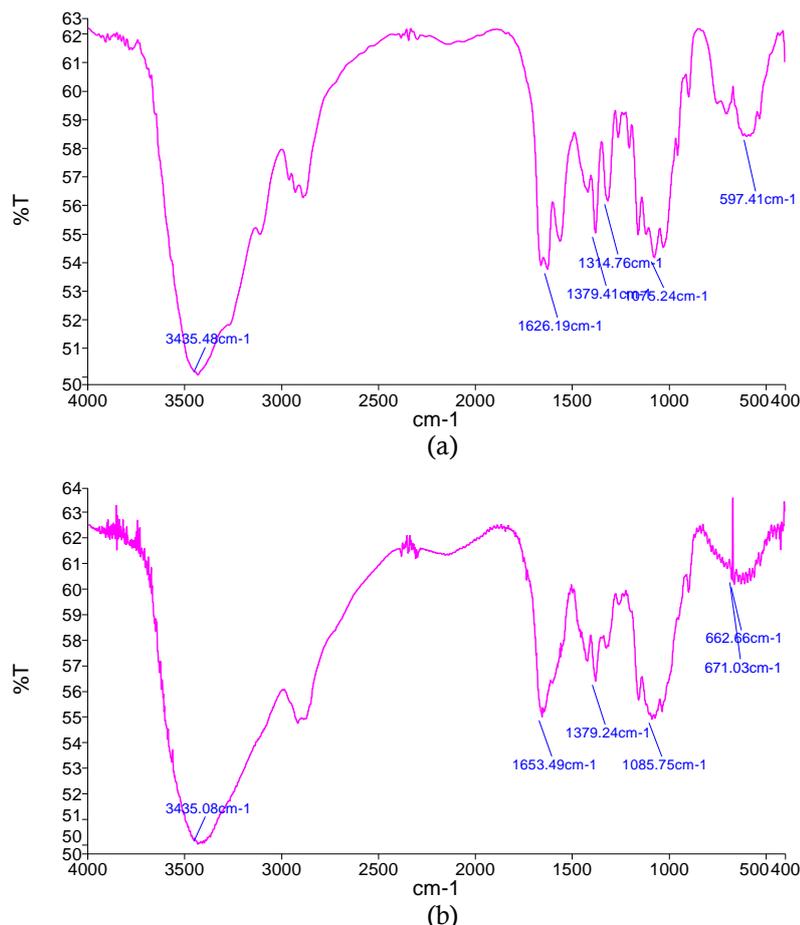
Figure **5**. Chitin FTIR Spectra. (a). Non ultrasonic; (b). Ultrasonic assisted.

increasingly intense. This is consistent with many previous studies that utilize ultrasonics to enhance polymerization reactions (Fiamingo et al., 2016; Muxika et al., 2017), the formation of nanoparticles (Philibert et al., 2017; Wei et al., 2018) and encapsulation process (Yang et al., 2020).

CONCLUSION

The ultrasonic-assisted method could increase the degree of deacetylation in the isolation process of chitin and chitosan. Isolation of chitin with the ultrasonic assisted method was able to increase the degree of deacetylation of chitin by $68.45 \pm 0.11\%$ compared to without sonication $62.52 \pm 0.08\%$. Application of ultrasonic assisted deacetylation gave a deacetylation degree of $85.35 \pm 0.20\%$, higher than without ultrasonic $80.24 \pm 0.19\%$. Physically, ultrasonic assisted chitosan is smoother and brighter in color. This method is recommended for the production of chitosan in laboratories and industry.

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