Influence of Deacetylation Process in Chitosan Extract From Shrimp Shell Waste

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Abstract.
The increasing production of shrimp commodities in Bangka Belitung Island can cause shrimp shell waste and polluting the environment. Shrimp shell waste can further proceed into chitosan which has a wide range of functions in various fields. This current study aims to find out the influence of the deacetylation process in chitosan extracts from shrimp shell waste. The method of chitosan extraction by varying repetition of deacetylation process. The characterization of chitosan extracts by FTIR analysis to determine functional group and degree of deacetylation (DD). Based on FTIR spectra, repetition in the deacetylation process in chitosan extraction still produces chitosan extracts that do not fully transform into chitosan. However, it able to increase DD with the highest DD of chitosan extract constitutes 86.78% and can be used in the further application.

1. Introduction
In the last ten years, quantity of fishery production cultivation has experienced enhancement in Bangka Belitung Island. Aquaculture production in 2018 was recorded around 9,340.93 tons with the largest production contributed by vanamei shrimp commodity [1]. Shrimp in frozen conditions in the market have been separated from useless part such as shrimp shells. This shrimp shell produces a large amount of waste if it is not processed further. Although it degraded naturally, its large amounts in environment can cause unpleasant odors and take a longer time to decompose. Therefore, it is more valuable if it is recycled into other products as chitosan for reducing environmental issues [2], [3].

Chitosan is a polysaccharide which widely used in food, health, energy etc because it has excellent properties for instance non toxic, high mechanic and thermal stability and bioactivity [4], [5]. Various methods have been conduct to extract chitosan from shrimp shell waste [6], [7], [8]. Extraction of chitosan have three major process i.e deproteinization, demineralization and deacetylation. Modification method of chitosan extraction to obtain properties namely high degree of deacetylation, solubility, and yield percentage [9], [10].
Chitosan properties is determined by acetylated and non-acetylated glucosamine unit ratio. This ratio could be affected by deacetylation process including chitin isolation treatment, alkali concentration temperature and time of deacetylation. In addition, these treatment in deacetylation process produces different characteristic of chitosan which is have different application[11], [12], [13]. The reported that altering deacetylation times could increase degree of deacetylation gradually [14]. Therefore, this study aim to analyze influence repetition of deacetylation process in chitosan extract from shrimp shell waste.

2. Materials And Methods

2.1 Materials
Shrimp shell waste was collected from Pangkalpinang fish market, Bangka Belitung Island. Sample was fresh and carried to laboratory for further treatment. All chemicals were from analytical standard and purchased from Chemistry Laboratory, Universitas Bangka Belitung. All chemicals were used without further purification.

2.2 Methods

2.2.1 Shrimp shell waste preparation
Shrimp shell waste was washed with clean water properly and it was dried in the sun for 3-4 days. Dried sample was mashed by using a blender and sieved to 200 mesh size and formed shrimp shell powder. Next, shrimp shell powder was kept in tightly closed container for further treatment.

2.2.2 Extraction of Chitosan
Extraction of chitosan was modification method from Dompeipen [15] and Ramadhan [16]. Extraction of chitosan was in three step of process continuously namely deproteinization, demineralization and deacetylation. For deproteinization, 50 grams shell powder was dissolved with 6% NaOH at a volume ratio of 1:10 (w/v). The mixture was heated in vessel at 65°C for 2 hours, then it was cooled and filtered and obtained the solid. It was neutralized with distilled water and dried at 60°C in an oven. This process to segregate the protein bonds from the shrimp shell. The second process was demineralization to eliminate the minerals (CaCO₃) from shrimp shell powder. The deproteinization product was dissolved with 1 N HCl with volume ratio of 1:15 (w/v). The mixture was reacted in vessel at 60°C for 1 hour. It was cooled and filtered and obtained the solid. The solid was neutralized with distilled water and it was dried at a temperature of 60°C in an oven. This process product is chitin.

Lastly, deacetylation process to remove acetyl group from dry chitin flakes. Chitin was dissolved with 60% NaOH at a volume ratio of 1:10 (w/v). It was heated at 120°C for 3 hours. Then it was cooled and filtered and the residues were repeatedly washed with distilled water until pH 7. Next, neutral residues were dried at 60°C for 1 hour. Deacetylation process was repeated twice and three times.

2.2.3 Characterization of Chitosan
Chitosan extract was confirmed with characterization by Fourier transform infrared spectroscopy (FT-IR) which its spectra would be compare with spectra of commercial chitosan. FTIR analysis of sample was conducted using Bruker Alpha with Standard KBr beamsplitter. Next, degree of deacetylation (DD) of chitosan was calculated by baseline A method from Domszy dan Robert (1984) which equation given below [17]:

\[
DD\ (\%) = \left[ 1 - \frac{A_{1655}}{A_{3450}} \times \frac{1}{1.33} \right]
\]  

(1)

Where \(A_{1655}\) is the absorbance of amide I vibration; and \(A_{3450}\), the absorbance of OH; 1.33 is a constant of ratio of \(A_{1655}/A_{3450}\) for fullyN-acetylated chitosan
3. Result And Discussion

3.1 FTIR Analysis

Chitosan extract was carried out through the deacetylation process. Deacetylation is a process to remove the acetyl group present in chitin to transform it become chitosan [11]. Figure 1 represent FTIR spectra commercial chitosan and chiosan extract with different alliteration of deacetylation process and Table 1 depicts their bands description. FTIR spectrum comparison between chitosan extracts and commercial chitosan (the standard of chitosan) aims to confirm whether the chitosan has been extracted from the shrimp waste.

![Figure 1. FTIR spectra of commercial and chitosan extract (chitosan DA 1: chitosan extract with one deacetylation process; chitosan DA 2 : chitosan extract with twice deacetylation process; chitosan DA 3 : chitosan extract with three times deacetylation process)](image)

**Table 1.** Comparison assignment between commercial and chitosan extracts

| Functional Groups (Type of Vibration) | Chitosan DA 1 | Chitosan DA 2 | Chitosan DA 3 | Commercial chitosan |
|--------------------------------------|---------------|---------------|---------------|---------------------|
| -OH Overlap (vs) NH<sub>2</sub>      | 3440          | 3442          | 3442          | 3443                |
| (vs) C-H aliphatic                   | 2920          | 2921          | 2921          | 2923                |
| (v) C=O [Secondary Amide]            | 1658          | 1658          | 1657          | 1660                |
| (v) C=O [Secondary Amide]            | 1626          | 1620          | 1628          | -                   |
| (vs) C-O                             | 1157          | 1156          | 1158          | 1158                |
| (v) C-O(C-O-C)                       | 1070          | 1074          | 1070          | 1077                |
| ω β-1,4-glycosidic                   | 897           | 897           | 897           | 897                 |

The FTIR spectrum of chitosan extract from different treatment illustrated six major peaks while chitosan commercial has five major peaks corresponding to their functional group. Chitosan extract establish extra peak than commercial chitosan in band at 1620 cm<sup>-1</sup> to 1628 cm<sup>-1</sup> is due to C=O groups vibration from secondary amide [14], [8]. This extra band indicated chitosan extract not fully transform into chitosan but it still has another compound such as chitin [18]. Chitosan extracts with different treatment have similar absorption pattern. Stretching vibration of overlap OH and amines (NH<sub>2</sub>) bands ranging from 3440 cm<sup>-1</sup> to 3443 cm<sup>-1</sup> and peak at 2920 cm<sup>-1</sup> to 2923 cm<sup>-1</sup> emblematic C-H groups stretching. Absorption bands at 1657 cm<sup>-1</sup> to 1660 cm<sup>-1</sup> associated C=O groups from secondary amide.

In addition, the bands located at 1156 cm<sup>-1</sup> to 1158 cm<sup>-1</sup> is mainly associated with stretching of C-O groups. Else, peaks band at 1070 cm<sup>-1</sup> to 1077 cm<sup>-1</sup> are related to C-O groups from C-O-C bridge.
vibration and it was characteristic from chitosan. Lastly, all chitosan samples have same bands in fingerprint area at 897 cm\(^{-1}\) corresponding to \(\beta\)-1, 4-glycosidic. Peak absorption about 897 cm\(^{-1}\) and 1156 cm\(^{-1}\) confirmed ring of pyranose and saccharide structure in chitosan [18].

3.2 Degree of Deacetylation (DD)
Chitosan characterization required perseverance of its degree of deacetylation (DD) and it was calculated from FTIR spectrum. This technique offers faster measurements, nondestructive and convenient for both soluble and nonsoluble substance [19], [20]. The equation to determine DD from equation 1. DD values from different deacetylation process and commercial chitosan were represented in Table 2.

| Type of Chitosan | Degree Of Deacetylation (DD) |
|-----------------|------------------------------|
| Commercial Chitosan | 91% |
| Chitosan DA 1     | 80.73 % |
| Chitosan DA 2     | 86.78% |
| Chitosan DA 3     | 87.28% |

According to Table 2 showed that chitosan DA 3 (chitosan extracts with three times deacetylation process) had the highest degree deacetylation reached 87.28% and although they still had lower degree of deacetylation from commercial chitosan. The DD of chitosan DA 3 is considered high because it between 85% and 95% [21]. Repetition in deacetylation process caused increasing DD chitosan extract. The degree of deacetylation rise due to the number of the acetyl groups reduce because NH\(_2\) functional groups protonation on the C\(_2\) position of the repeating unit D-glucosamine [22].

4. Conclusion
The study confirm that repetition in deacetylation process affect chitosan extract which it rise percentage of degree of deacetylation until 87.28% whereas it still lower than commercial chitosan and not fully transform into chitosan. Further investigation needed to study an increase of chitosan degree of deacetylation by different parameters in method.

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