The effect of deproteinization temperature and NaOH concentration on deacetylation step in optimizing extraction of chitosan from shrimp shells waste

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Abstract. Chitosan have been successfully produced from the dried shrimp home industry waste (Bangka, Indonesia). Extraction of chitosan was carried out in four steps: deproteinization, demineralization, decolorization and deacetylation of chitin. The effect of deproteinization temperature and NaOH concentration on deacetylation process was studied. The results shown that the increase of deproteinization from 30°C to 90°C causes the decrease of chitosan deacetylation degree (DD). The increase of deproteinization temperature triggers excess depolymerization which damages the chitin structure so that it has a negative effect on the chitosan DD. On the other hand, the increase of NaOH concentration from 20% to 60%, the chitosan DD increased. The diffusion rate of OH⁻ causes increment of OH⁻ attack to the amino group thus realizing the effective deacetylation of chitin. The highest chitosan DD was up to 88.89% is achieved under the optimized conditions of this process and the occurrence of deacetylation structurally demonstrated by the Fourier transform infrared (FTIR) characterization

1. Introduction
Shrimp is one of Indonesia's fisheries commodities, especially in the Bangka Belitung Islands Province [1]. Local people sell these commodities in raw or dried shrimp product which is known as Ebi. The Ebi shrimp are generally produced on a home industry scale from fresh shrimp (generally from Penaeus semisulcatus species) which has been boiled and peeled and then dried in the sun for 1-2 days. Based on the interviews and observations obtained that in every ten kilograms of fresh shrimp only produces an average of 0.8 - 1 kilograms of Ebi and shrimp shell waste are others.

Shrimp shell waste contains chemical compounds including: 30-40% of proteins, 30-50% of calcite, and 20-30% of chitin [2]. Chitin is a linear polymer composed of β-(1,4)-N-acetyl glucosamine and classified into α-, β-, and δ-chitin [3,4]. One of important derivative of chitin is chitosan are obtained by deacetylation of chitin under alkaline condition [5,6]. Chitosan have several unique physicochemical and biological properties including nontoxicity, antimicrobial activities, biodegradability, and biocompatibility [7-8]. Chitosan widely used in various fields such as medicine,
pharmaceutical, cosmetic, food preservation, agriculture and advanced materials include absorbent materials, biomaterials, tissue engineering, biosensor and etc [7-12].

Commonly, the extraction of chitosan includes deproteination, demineralization, and deacetylation step [13-16]. The deproteination step aims to reduce the protein content in the chitosan raw materials using an aqueous alkaline solution while heating. The demineralization step is intended to reduce calcite levels by using low concentration acids to obtain chitin. The subsequent deacetylation step aims to remove acetyl groups from chitin by heating in strong alkaline concentrations. In-addition before the deacetylation step, a decolorization process is carried out to bleach the chitin [17].

The one of quality indicator of chitosan is determined by value of degree of deacetylation (DD) [18]. This value describes the level of deacetylation of chitin to chitosan by breaking the chain of the acetyl group. The increment of DD value indicated that the effective deacetylation of chitin. It depending on the raw material and process parameters such as the concentration of alkaline solution, temperature, time reaction, solid-liquid ratio and etc. Optimizing extraction of chitosan including the deproteination, demineralization, and deacetylation step is one of the main factors that determine the DD value of chitosan. This work presents extraction of chitosan from cheap materials such as the dried shrimp home industry waste with focus on the effect of deproteinization temperature and NaOH concentration on deacetylation step.

2. Experimental Procedures
2.1 Materials
The NaOH (pellets, 99%), the HCl (37%), the NaClO (14% active chlorine) were purchased from MERCK. The high-pure water was used for preparation of all solution. In this study shrimp shells were obtained from the dried shrimp home industry waste (Bangka, Indonesia) and all shells were from a single species of shrimp (Penaeus semisulcatus).

2.2 Methods
2.2.1 Pre-treatment of the raw materials
The shrimp waste is rinsed several times using boiling water to eliminate all other related impurities then it was washed with distilled water and dried using sun drying (approximately 27 °C) for 4 hours. The raw materials treated were grounded to pass through a sieve 100 mesh.

2.2.2 Deproteinization step
The raw materials deproteinized using NaOH 2 N with a solid to solvent ratio of 1:6. In this step to study the effect of deproteinization temperature, the sample is heated at different temperatures 30°C, 60°C and 90°C while stirred for one hour. After each experiment, the mixture was filtered, washed with distilled water to remove excess of NaOH, and dried in an oven at 85°C overnight.

2.2.3 Demineralization step
The demineralization of deproteinized shrimp shells each temperature was performed using HCl 1.5 N with a solid to solvent ratio of 1:12 while stirred for one hour at room temperature. After each experiment, the mixture was filtered, washed with distilled water to remove excess of HCl, and dried in an oven at 85°C overnight. The residual solid product obtained was designated as chitin.

2.2.4 Decolorization step
The decolorization of chitin was performed using NaClO 5% with a solid to solvent ratio of 1:10 while stirred for 30 minutes at room temperature. The bleached chitin was filtered, washed with distilled water to remove excess of NaClO, and dried in an oven at 85°C.

2.2.5 Deacetylation step
In this step to study the effect NaOH concentration, the chitin isolated previously was deacetylated at different concentration 20%, 40%, and 60% while stirred for one hour at room temperature. After each
experiment, the mixture was filtered, washed with distilled water to remove excess of NaOH, and dried in an oven at 85°C overnight. The residual solid product obtained was designated as chitosan.

2.3 Characterization of chitosan

The FTIR spectra of chitosan were recorded using FTIR spectrophotometer (Thermo Fisher Scientific, Nicolet 8700) in the range 600 - 4000 cm\(^{-1}\). The degree of deacetylation of sample are calculated using base line method which is based on sample FTIR spectra according to the equation [19]:

\[
DD = (100 - \left( \frac{A_{1655}}{A_{3450}} x \frac{100}{1.33} \right)) \%
\]

Where, \(A_{1655}\) is the absorbance of amide I band (acetyl band), \(A_{3450}\) is the absorbance of hydroxyl band, and a factor of 1.33 shows the value of the \(A_{1655}/A_{3450}\) ratio for chitin deacetylation completely.

3. Results and Discussion

The FTIR spectra of chitosan samples obtained under different treatment are illustrated in Figure 1.

![Figure 1. FTIR spectra of chitosan samples which are described in Table 1.](image)

The broad band at about 3450 cm\(^{-1}\) corresponded to the vibrational OH stretching. The bands nearly disappeared at 3260 and 3107 cm\(^{-1}\) originating from stretching of N-H after deacetylation. It causes the regular hydrogen bond of N-H in the unreacted chitin disturbed the deacetylation reaction. The successful deacetylation demonstrated by reduction of band at 1655 cm\(^{-1}\) and 1310 cm\(^{-1}\) assigned to the stretching of C=O in amide bond and CO-NH bending vibration respectively [20-23]. A significant difference was seen in part 3 where the reduction of the band at 1655 and 1310 cm\(^{-1}\) is higher than others. It indicated that more chitin was deacetylated. This also corresponded to the increment of deacetylation degree of chitosan. Furthermore, the stretching vibrations of the glycosidic bond of chitosan polysaccharide structure at 1021 and 856 cm\(^{-1}\) in part 3 higher than others also indicated the effective deacetylation of chitin [24]. The intense peak at 1556 cm\(^{-1}\) arising from the N-H deformation of amide II and the band at 1430 cm\(^{-1}\) originating from the stretching vibrations of the C-H deformation of alkanes [25].
Table 1. Chitosan deacetylation degree of sample

| Sample | Deproteinization temperature (°C) | NaOH concentration (%) | Degree of deacetylation (%) |
|--------|----------------------------------|------------------------|----------------------------|
| 1      | 30                               | 20                     | 65.72                      |
| 2      | 30                               | 40                     | 67.44                      |
| 3      | 30                               | 60                     | 88.98                      |
| 4      | 60                               | 20                     | 64.31                      |
| 5      | 60                               | 40                     | 66.27                      |
| 6      | 60                               | 60                     | 69.49                      |
| 7      | 90                               | 20                     | 63.75                      |
| 8      | 90                               | 40                     | 65.56                      |
| 9      | 90                               | 60                     | 68.32                      |

Deproteinization temperature is one of the most factors that affect the DD chitosan. In this work, the shrimp waste (raw materials) deproteinized at different temperatures 30°C, 60°C and 90°C respectively. The DD chitosan decrease with the increase of deproteinization temperature as shown in Table 1. The increasing of deproteinization temperature causes the reactivity of system was increased at the same NaOH concentration. This condition supposes leads to an exceeded depolymerization reaction which damages the chitin structure. In shrimp shells, chitino-proteins represented of association of chitin and proteins. The stable of this association make the proteins are not easily removed [17, 26-27]. The basic treatment using to remove these proteins most often NaOH solutions, at varying concentrations during a long treatment at high temperatures. However, high-temperature treatment has a negative impact on the chitosan DD. The highest of chitosan DD is reached at the deproteinization temperature was 30°C and gradually decreases when the temperature rises to the final temperature was 90°C at the same NaOH concentration shown in Figure 2 (a).

![Figure 2](image)

(a) Effect of deproteinization temperature (a) and NaOH concentration (b) on deacetylation degree.

As shown in Table 1, with the increase of NaOH concentration from 20% to 60% at the same deproteinization temperature, the chitosan DD increased gradually 63.75% to 88.98%. The highest of
chitosan DD is reached at the final NaOH concentration was 60% shown in Figure 2 (b). The deacetylation reaction of chitin mostly influenced by the steric hindrance from the natural chitin structure [28]. The steric hindrance formed by compact structure of natural chitin which obstructs the attack of OH⁻ to the amino group. Furthermore, the diffusion rate of OH⁻ to the surface and the inside of chitin particle would be closely depends on alkali concentration. Therefore, the increase of NaOH concentration facilitated OH⁻ to resolves the steric hindrance and achieve the chitin deacetylation [29-30].

4. Conclusion
Chitosan have been successfully produced from the dried shrimp home industry waste (Bangka, Indonesia). The effect of deproteinization temperature and NaOH concentration on deacetylation step was studied. In this work, significantly indicated that increasing of deproteinization temperature causes the decrease of chitosan DD. On the other hand, the chitosan DD increase while increasing NaOH concentration on the deacetylation step. The highest chitosan DD was 88.89% is achieved at deproteinization temperature of 30°C and NaOH concentration of 60%. However, further studies are needed to maximize degree of deacetylation chitosan such reaction times and solid-liquid ratio due to its contribution to the effectiveness of reaction rate.

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