The Use of Different Deacetylation Temperature Toward Quality of Chitosan Mud Crab Shell (Scylla serrata)

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Abstract. This research aims to determine the chemical composition (proximate) of chitosan and chitin. The best deacetylation temperature for obtaining chitosan, and the yield of chitosan and chitin from mud crab. This research was conducted in two stages, that is: 1) Preparation of mud crab flour and 2) Extraction and analysis of chitin and chitosan. The experimental design used for chitosan isolation was Completely Randomized Design (CRD). The process of deacetylation chitin becomes chitosan by using 50% NaOH with varying heating temperatures (120, 130, and 140°C). Parameters observed were yield, moisture content, ash content, and degrees of deacetylation. The result showed that the best chitosan was obtained by chitin deacetylation process into chitosan using a temperature of 130°C (KO2). Characteristic quality of the chitosan mud crab shell produced are KO1: yield 61.00%, moisture content 6.47%, ash content 17.18% (db), and degree of deacetylation 49.63%. KO2: yield 59.94%, moisture content 6.48%, ash content 14.85% (db) with degree of deacetylation 51.13%. KO3: yield 53.97, moisture content 6.54%, ash content 14.66% (db) and degree of deacetylation 52.63%. Characteristic quality of chitin included yield was 27.81%, moisture content 7.29%, ash 44.05%, and degree of deacetylation 33.09%.

1. Introduction

Waste increased is produced by crab products, especially solid waste in the form of crab shells. Data shows that 1,000 tons of crab shell waste are generated per year. Crab shell is a waste that has the highest chitin content (70%) compared to crustaceans, insects, worms, and fungi [1].

Chitosan isolation took place through the stages of demineralization, deproteination, and deacetylation. The deacetylation step aims to remove the acetyl group from chitin so that more amine groups remain. The number of acetyl groups released indicates the effectiveness of the deacetylation process. The magnitude of the deacetylation process is expressed as the degree of deacetylation or Degree of Deacetylation [2].

The purer the chitosan or chitin produced, the higher the quality of the chitosan produced. The quality of chitosan can be seen from the value of water content and low ash content with a high degree of deacetylation. The degree of destilation is one of the important quality parameters of chitin or chitosan [3]. The higher the value of the degree of deacetylation, the higher the selling value and usefulness. The parameters that affect the value of the degree of deacetylation are base concentration, base strength, temperature, repetition of the deacetylation process (re-deacetylation), and time. This study aims to obtain the best deacetylation temperature in the manufacture of mud crab shell chitosan
and to determine the quality of chitosan and chitin products which include yield, moisture content, ash content and degree of deacetylation.

This study aims: 1) to determine the chemical composition (proximate) of chitosan and chitin, 2) The best deacetylation temperature for obtaining chitosan, and 3) Yield of chitosan and chitin from mud crab.

2. Methods
The main material used in this research is crab shell waste from one of the Seafood restaurants in Pekanbaru. Other ingredients are aquades, HCl, NaOH, H$_2$O$_2$. The tools used distillator, desiccator, burette, furnace, glass plate, spectrophotometer, etc.

The method of this study was experimental in two steps, they were 1) Preparation of mud crab flour and 2) Extraction and analysis of chitin and chitosan using 50% NaOH with varying heating temperatures 120°C (KO1), 130°C (KO2), and 140°C (KO3). The research design used a completely randomized design (CRD).

2.1. Extraction and analysis of chitin
Chitin extraction refers to the procedure Suptijah [3]. The mashed crab shell flour was demineralized with a 1.5 N HCl solution. It was precipitated and washed repeatedly with distilled water until the pH was neutral. deproteinized with 3.5 N solution, then precipitated and washed repeatedly with distilled water until the pH is neutral. followed by the decoration process. The solids obtained were dried in an oven at 60°C for 6 hours. Then the chemical composition (proximate) measurements are carried out including ash and water. Determination of the degree of deacetylation using FTIR spectroscopy [4].

2.2. Extraction and analysis of chitosan
Extraction of chitosan also refers to the procedure of [3]. Chitin was added with 50% NaOH with a ratio of 1:20 (w/v) between chitin and solvent, then precipitated. The obtained solids were washed repeatedly using distilled water until the pH was neutral. The solids were dried in an oven at 60°C for 6 hours. Then the chemical composition (proximate) measurements are carried out including protein, fat, ash, water, and carbohydrates. Protein analysis used the Kjeldahl method, fat using the Soxhlet method, ash [5], moisture [5], and Crude Fiber (by difference).

2.3. Data Analysis
The collected data from each treatment were analyzed using analysis of variance (ANOVA).

3. Results And Discussion

3.1. Mud Crab Chitin (Scylla serrata)
The results of the chemical composition analysis (proximate) of mud crab chitin flour are presented in Table 1.

| Parameters                  | Study results | Chitin quality standards* |
|-----------------------------|---------------|---------------------------|
| Water content (% bb)        | 7.29          | < 10                      |
| Ash content (% bk)          | 44.05         | < 2                       |
| Deacetylation degree (%)    | 33.09         | 15-70                     |
The water content of chitin in the mud crab shells produced is 7.29% and has met the chitin standard set by [6] which is a maximum of 10%. According to [7], chitin on the market is expected to have a moisture content of no more than 10% to prevent mold damage.

Chitin ash content obtained from this study was 44.05%. The chitin ash content does not meet the chitin quality standard set by [6] which is a maximum of 2%. High ash content can be caused by washing not using running water and less constant stirring during the demineralization process [8]. High ash content will affect the solubility and also the viscosity of chitin. The results of the FTIR spectra of chitin in mud crab can be seen in Figure 1.

![FTIR spectra of mud crab chitin](image)

**Figure 1.** FTIR spectra of mud crab chitin

The degree of deacetylation of chitin produced is 33.09% and has met the chitin standard of Protan Laboratories which is about 15-70%. According with [9] that chitin is a slightly deacetylated N-acetyl glucosamine polymer that is greater than 25% and less than 70%.

### 3.2. Mud Crab Chitosan (Scylla serrata)

The yield of mud crab chitosan using different deacetylation temperatures (120, 130, and 140°C) is presented in Table 2.

| Repetition | Treatment | KO1 | KO2 | KO3 |
|------------|-----------|-----|-----|-----|
| 1          | 65.54     | 59.36 | 52.82 |
| 2          | 66.73     | 61.00 | 53.36 |
| 3          | 65.73     | 59.82 | 55.73 |
| **Average** | **66.00**<sup>A</sup> | **59.94**<sup>h</sup> | **53.97**<sup>C</sup> |

The highest yield was in KO1 (66.00%) and the difference was seen in each treatment. The higher the deacetylation temperature used, the lower the yield value. The difference in the yield of chitosan produced is thought to be influenced by the process of breaking the acetyl bond in the chitosan.
hydrolysis process which causes a decrease in molecular size so that the molecular weight of chitosan becomes lighter [10].

The water content of chitosan in mud crab shells using different deacetylation temperatures (120, 130, and 140°C) is presented in Table 3.

**Table 3.** The average value of the water content of chitosan.

| Repetition | KO1  | KO2  | KO3  |
|------------|------|------|------|
| 1          | 6.42 | 6.46 | 6.48 |
| 2          | 6.52 | 6.56 | 6.55 |
| 3          | 6.47 | 6.42 | 6.59 |
| Average    | 6.47\(^a\) | 6.48\(^a\) | 6.54\(^a\) |

Description: KO1 (120°C), KO2 (130°C) dan KO3 (140°C).

The water content of the chitosan produced ranges from 6.47-6.54% and this has met the quality standards of chitosan set by [6] and [11] which stated that the water content of chitosan is a maximum of 10%. The chitosan drying process was carried out uniformly for all treatments, namely by heating using an oven at a temperature of ± 60°C for ± 6 hours so that the moisture content obtained was not much different. The chitosan ash content of mud crab shells using different deacetylation temperatures (120, 130, and 140) is presented in Table 4.

**Table 4.** The average value ash content of chitosan.

| Repetition | KO1  | KO2  | KO3  |
|------------|------|------|------|
| 1          | 16.21| 13.96| 13.69|
| 2          | 15.94| 14.20| 13.93|
| 3          | 16.05| 13.52| 13.49|
| Average    | 17.18\(^a\) | 14.85\(^a\) | 14.66\(^a\) |

Description: KO1 (120°C), KO2 (130°C) dan KO3 (140°C).

The chitosan ash content obtained from the results of this study is 14.66-17.18%. The ash content value does not meet the chitosan standard set by [6] which is a maximum of 2% and [11] is <1%. The amount of ash content produced shows that the demineralization process is less than perfect and the minerals contained in the sample have not been completely lost. The factors that affect the effectiveness of the ash content are the concentration of the solvent and the length of time for stirring [12]. The value of the degree of deacetylation of mud crab chitosan produced is presented in Figure 2.
The degree of deacetylation of chitosan produced from this study ranged from 49.63% - 52.63% where this result was far below the quality standard of chitosan required by [6] and [11] who stated that the degree of deacetylation of chitosan was >70%. The degree of deacetylation is a parameter of chitosan quality, thus the chitosan obtained in this study is of very low quality or still close to chitin.

4. Conclusions
Based on the results of the research that has been done, the following conclusions can be drawn.

1. The best chitosan was obtained by deacetylation of chitin into chitosan using a temperature of 130°C (KO2). The quality characteristics of mangrove crab shell chitosan produced were KO1: yield 61.00%, water content 6.47%, ash content 17.18% (db) and degree of deacetylation 49.63%. KO2: yield 59.94%, water content 6.48%, ash content 14.85% (db) with a degree of deacetylation of 51.13%. Meanwhile, for KO3: the yield is 53.97, the water content is 6.54%, the ash content is 14.66% (db) and the degree of deacetylation is 52.63%.

2. The resulting chitin is a creamy white powder. The quality characteristics of mangrove crab chitin from the research included yield of 27.81%, moisture content of 7.29% wt, ash 44.05% wk and degree of deacetylation of 33.09%. The chitin produced has met the quality standards set by [6] except for the ash content.

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