Steam Flush Pressure: A New Modification Method to Extraction chitin from *Lingula sp*

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Abstract. Lingula sp is one of the bivalves, where it is very popular for people as food consumption. However, the high consumption of society is not accompanied by the utilization of residual waste from Lingula sp. In the composition of the structure of the compound, the shell of Lingula sp contains chitin compounds which are very potential to be applied in the field of biotechnology and have an economical value. The aims of this study to isolated chitin from Lingula sp shell used steam flush pressure method. Chitin isolation was carried out with three stages of the process, namely deproteination, decolorization and demineralization at 121 °C, a pressure of 15-20 psi with variations of time 5, 15, 30, 45 and 60 minutes. Chitin characterization showed that 60 minutes was the optimum treatment time with characteristics of 9.5% water content, 1.01% ash content, total N-content of 6.87% and deacetylation degree 33.19% with a yield of 5.06%. These results showed that the exploration of chitin potential contained in Lingula sp is very potential to be used as a new source. In addition, the steam flush pressure method is a breakthrough to isolation chitin.

1. Introduction

Chitin is a polysaccharide that is commonly found in nature after cellulose. Many chitin sources come from seafood wastes such as shrimp shells, crabs, squid, and lobsters [1]. In addition, chitin can also be found in the fungi of Mucor rouxii, Phycomyces, and Saccharomyces as compilers of cell walls [2]. Structurally chitin is found in three different forms, namely alpha, beta, and gamma. Alpha chitin is an arrangement of antiparallel GlcNAc with strong intermolecular bonds. Beta chitin is composed of chains that are parallel in parallel so that intermolecular tend to be weaker. The gamma structure is formed of three chains, namely two parallel chains and one anti-parallel chain from GlcNAc [3]. Structural characteristics can be found differently in each source. However, reports show that chitin isolation is more sourced from shells of shrimp and crabs. There are still many other sources that have not been fully exploited. Exploration and isolation of chitin from new sources can add insight to the development and application of chitin [4].

![Lingula sp](image-url)
The organisms in the Branchiopod phylum, Lingula sp, are one of the marine animals with shell structures containing chitin but have never been explored. Sponges of lingula shell consist of two parts with oval-shaped structure, long, smooth, and flexible. The presence of chitin content in the shell of Lingula sp is indicated by a flexible shell structure similar to nails [6]. According to Darmarini [7] chemically Brankopopods are classified into two different groups, namely calcareous one and the other is phosphate, where each of these chemical constituents binds to other organic compounds in composing shells.

Generally, chitin is not present in the form of pure compounds in nature. Chitin binds covalently with protein, minerals, and some pigments. Chitin isolation has been using thermochemistry. Thermochemistry utilizes chemical reagents by heating in stages of deproteination, decolorization, and demineralization [8]. However, the combination of physics-chemistry by using hot steam, pressure, and chemical solvents becomes a new combination in chitin isolation. This combination of methods is indicated to be a breakthrough in producing high-quality of chitin.

Therefore this study aimed to investigate the effectiveness of a combination of chitin isolation methods with hot steam, pressure, and alkaline solvents through the characterization of the chitin products produced.

2. Experimental
2.1 Material and Methods
Shell of Lingula sp, NaOH 5% (Merck), HCl 37% (Merck), NaOCl 0,5%, Chitin (Sigma), Aqua dest, Drying oven temperature 80 °C for 24 hours, Analytical balance, Fourier Transform Infra Red Shimadzu, Autoclave (121 ºC, 15-20 psi) Napco type 8000DES.

2.2 Sample Preparation
The Lingula sp shell is washed and dried. The dried shell is mashed with a grinder. Lingula sp shell powder was filtered with a 60 mesh sieve.

2.3 Deproteination
Lingula sp shell powder about 10 gram was weighed and then dissolved with 5% NaOH (1:10 w / v), then put it in the autoclave with a variation of treatment time 5, 15, 30, 45 and 60 minutes at 121ºC, 15-20 psi. The sample was filtered with a büchner filter, and the residue was washed with distilled water to a neutral pH filtrate. The sample was dried in an oven at 80 °C for 24 hours and then weighed [9].

2.4 Decolorization
The results of the deproteination stage were then weighed and dissolved into 0.5% NaOCl (1:10 w / v), put into the autoclave with a variation of treatment time 5, 15, 30, 45 and 60 minutes at 121 ºC, 15-20 psi. The sample was filtered with a büchner filter, and the residue was washed with distilled water to a neutral pH filtrate. The sample was dried in an oven at 80 °C for 24 hours, then weighed [9].

2.5 Demineralization
The results of the decolorization stage were weighed and dissolved into 1.5 M HCl by comparison (1:10 w / v), put into the autoclave with a variation of treatment time 5, 15, 30, 45 and 60 minutes at 121 ºC and pressure 15 -20 psi. The sample was filtered with a büchner filter, and the residue was washed with distilled water to a neutral pH filtrate. The sample was dried in an oven at 80 °C for 24 hours, then weighed [9].

2.6 Characterization of Chitin
The characteristics of chitin and chitosan were determined to determine the properties of chitin and chitosan compounds. The parameters of analysis were water content and ash content by the gravimetric method [10], total N-content with the Kjeldahl method [1], and the degree of deacetylation (% DD). For the degree of deacetylation (% DD) from chitin and chitosan analyzed using FTIR (Fourier Transform Infra Red) by Moore and Robert [Moore and Robert, 1980]. The degree of
The deacetylation of chitin is calculated according to the following equation: % DD = (1 - [(A1655/A3450) x (1/1.33)]) x 100% derived from this absorbance. [11].

3. Result and Discussion

The chitin isolation process starts at the sample preparation stage by conducting a drying process to reduce the water content contained in the shells of Lingula sp. The shell of Lingula sp is smoothed using a grinder to reduce the sample particle size. The sample powder is filtered with 60 mesh sieves to produce similar particle sizes. Smaller particle size has an impact on the contact surface to be wider so that the effectiveness of the isolation results obtained can increase [12].

Deproteinization is a process to separate the bonds between protein and chitin by using 5% NaOH with variations in treatment time. The resulting residue is dissolved Na-proteinate. The results of the deproteinization process are characterized by changes in the color of the sample powder from white bone to light brown. Deproteinization powder is dried at 80 °C for 24 hours. Dry samples show different weight differences from each treatment process. Its shows that the degradation process of proteins occurs in the sample. The yield of each treatment for the sample can be seen in table 1.

| Sample | Deproteination Weight (gram) | Rendemen (%) |
|--------|-----------------------------|--------------|
| 5 second | 7.43 | 74.30 |
| 15 second | 5.49 | 54.91 |
| 30 second | 7.25 | 72.50 |
| 45 second | 7.36 | 73.60 |
| 60 second | 6.95 | 69.47 |

Decolorization stage was aimed at removing pigments or dyes in chitin. In Lingula sp, the pigment binds to the protein. At deproteinization at high temperatures and pressures, the pigment-free bond of protein dominates to pink mixed with yellow due to heating. The length of treatment provides a broader contact effect between chitin and solvent so that the longer the pores open, the material becomes more significant than before. Increase of pore size will make it easier for the solvent to bind the pigment [13]. The use of inorganic solvents gives a striking change to the color produced in chitin before and after decolorization. It is happening because inorganic solvents NaOCl as a strong oxidizer will oxidize carotenoids in chitin in a faster time and with a whiter color result than organic solvents. The oxidizer will break the double bond in astaxanthin, causing a color change. Sample weights obtained from the decolorization process shown in table 2.

| Sample | Decolorization Weight (gram) | Rendemen (%) |
|--------|-----------------------------|--------------|
| 5 second | 5.55 | 74.70 |
| 15 second | 3.61 | 65.88 |
| 30 second | 5.57 | 76.84 |
| 45 second | 5.49 | 74.63 |
| 60 second | 5.44 | 78.33 |

The demineralization process aims to remove minerals in the shell of Lingula sp. The demineralization process results in calcium carbonate and calcium phosphate reacting with hydrochloric acid to form calcium chloride, carbonic acid and phosphoric acid which is a 4 compound soluble in water, while the insoluble residues are chitin compounds. The mineral dissolution reaction that occurs is written in the reaction equation (1) and (2) [14].

\[
\text{CaCO}_3(s) + 2\text{HCl}(l) \rightarrow \text{CaCl}_2(l) + \text{H}_2\text{O}(l) + \text{CO}_2(g) \quad (1) \\
\text{Ca}_3(\text{PO}_4)_2(s) + 4\text{HCl}(aq) \rightarrow 2\text{CaCl}_2(aq) + \text{Ca(H}_2\text{PO}_4)_2(aq) \quad (2)
\]
The carbon dioxide produced is seen from the foam formed in the demineralization process. So HCl must be poured in stages to avoid CO2 overflow. The resulting yellowish brown precipitate with different yields from each treatment process as in table 3.

**Table 3. Demineralization results with variations in treatment times**

| Sample     | Demineralization Weight (gram) | Rendemen (%) |
|------------|--------------------------------|--------------|
| 5 second   | 0.5015                         | 9.03         |
| 15 second  | 0.3473                         | 9.60         |
| 30 second  | 0.4155                         | 7.46         |
| 45 second  | 0.30003                        | 5.46         |
| 60 second  | 0.2755                         | 5.06         |

Chitin obtained from the isolation process was gradually characterized by several variables, including water content, ash content, N-total, and deacetylation degree (DD).

**Table 4. Comparison of characteristics of chitin from the variation of treatment time with standard chitin (according to protan laboratories).**

| Sample     | Water content (%) | Ash content (%) | N-Total (%) | DD (%) |
|------------|-------------------|-----------------|-------------|--------|
| 5 second   | 10.0              | 2.13            | 4.7         | 19.3   |
| 15 second  | 9.77              | 1.98            | 6.02        | 20.95  |
| 30 second  | 10.18             | 1.34            | 5.29        | 31.38  |
| 45 second  | 9.26              | 1.56            | 7.76        | 31.22  |
| 60 second  | 9.5               | 1.01            | 6.87        | 33.19  |
| Standard of Protan Lab* | <10       | <2              | 6-7         | 15-70  |

Based on the data in table 4 shows that the variation in the time of treatment of samples at the time of the 60-minute treatment became the best treatment time with a percentage of water content 9.5%; ash content 1.01%, N-Total 6.87%. Some other data for the time of treatment indicate a standard characterization variable that is not yet in line with the standard so that it shows the time the treatment needs to be optimized.

Chitin obtained from deproteination results was analyzed by FTIR spectrophotometer to determine the main functional groups contained in chitin; besides that the results of further FTIR measurements were used to determine the degree of deacetylation of chitin. According to Protan Laboratories [15], chitin, which has good quality standards, is expected to have a degree of deacetylation in the range of 15-70%. It is following the results of the research obtained, where all the chitin isolated from Lingula sp shells had deacetylation levels according to established standards, but the highest degree of deacetylation was obtained at the 60 minute treatment, as shown in table 4.
Figure 2. Spectra of FTIR absorption of chitin samples at various treatment times

Based on the analysis of the absorption results obtained an interpretation of functional groups that absorb the isolated chitin compounds compared to standard chitin (Sigma) can be seen in Table 5.

Table 5. Comparison of functional groups that absorb the infrared spectrum of chitin sigma (standard) and chitin at variations in treatment times.

| Functional Groups   | Wavenumber (cm⁻¹) |
|---------------------|-------------------|
|                     | Sigma Chitin (standard) | Sampel 5 sec | Sampel 15 sec | Sampel 30 sec | Sampel 45 sec | Sampel 60 sec |
| O-H strech          | 3481,3446         | 3568,31      | 3446,79      | 3446,79      | 3446,79       | 3446,79       |
| N-H strech          | 3269              | 3448,72      | 3282,35      | 3282,35      | 3282,6        | 3282,84       |
| C-H strech aliphatic| 2929              | 2927,94      | 2931,80      | 2931,80      | 2931,80       | 2931,80       |
| C=O (amida I)       | 1666, 1633        | 1654,92      | 1654,92      | 1656,85      | 1653          | 1654,92       |
| N-H bend (amide II) | 1558              | 1560,41      | 1558,48      | 1558,48      | 1560,41       | 1560,41       |
| CH₃ sym             | 1379              | 1377,17      | 1379,10      | 1379,10      | 1379,10       | 1379,10       |
| C-N strech          | 1311              | 1317,38      | 1317,38      | 1317,38      | 1317,38       | 1315,45       |
| C-O-C in cyclic     | 1203, 1261        | 1261,45      | 1261,45      | 1261,45      | 1259,52       | 1259,52       |
| C-O-C strech on dialkyl | 1157, 1114     | 1116,78      | 1114,86      | 1114,86      | 1118,71       | 1116,78       |

Based on the analysis of functional group absorption for the optimum treatment process, which at 60 minutes showed that the absorption intensity at wave number was around 3446.79 cm⁻¹ which showed OH stretch groups. The N-H stretch group shows in 3282.84 cm⁻¹; 2931.80 cm⁻¹ which shows the C-H stretching group; 1654.92 cm⁻¹ which shows the C = O stretching group (NHCH₃, Amida I); 1560.41 cm⁻¹ which shows the N-H bend group (NHCH₃, Amida II) and uptake at 1379.10 cm⁻¹ which shows that the CH₃ symmetry group is getting lower with increasing chitosan DD.
addition, the higher DD chitin. The absorption peak around 3265 & 3473, 3446 cm⁻¹ which shows the –OH and –NH₂ groups wider. It is due to the higher DD chitin, the greater the number of –NH₂ groups. Degradation of the acetyl group caused a shift in smaller wavenumbers in the absorption area of about 3446.79 cm⁻¹ and absorption of about 1654.92 cm⁻¹, the effect of which was an increase in DD of chitin.

4. Conclusions
The results of chitin isolation from the shell of Lingula sp showed optimum effectiveness at the time of 60 minutes of treatment. The characteristic values of chitin produced following standard of chitin, namely: 9.5% moisture content, 1.01% ash content, 6.87% N-total and 33.19% deacetylation degree with the appearance of white, brown color samples with powder form.

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