Production of Chitosan from *Amusium sp* Scallop Shell Waste

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Abstract. Chitosan is one of the natural polysaccharides, which is produced from chitin by deacetylation process. In this study, chitosan was produced from *Amusium sp* scallop shell waste. First, chitin was isolated by extraction via deproteinization using alkaline solution followed by demineralization using acid solution. Thereafter, chitosan was resulted from deacetylation of chitin using a high concentration of alkaline solution. The chemical structure of chitin and chitosan products was characterized using fourier transform infrared spectroscopy (FTIR).

Keywords: chitin, chitosan, *Amusium sp* scallop shell waste

1. Introduction

Indonesia is an archipelago with abundant marine products. Along with the increasing use of marine resources, solid wastes as by products cause new problems. Therefore, it should be properly handled to avoid environmental damage. One of the fishery product found in Indonesia is *Amusium sp* scallop. It was reported by Susilowati et al. [1] that *Amusium sp* scallop consists of meat (19-28% wt.), shell (53-65% wt.) and liquids (9-25% wt.).

Chitosan has wide range of industrial applications, e.g. in cosmetics, agriculture, food, pharmacy, biomedical, paper industries and as absorbent materials for wastewater treatment [2-4]. Chitosan is generally prevalent in marine invertebrates, terrestrial, and fungi of the genus *Mucor, Phycomyces, and Saccharomyces*. The existence of chitosan in nature is generally bound with protein, minerals and various pigments. It is soluble in aqueous acidic medium due to the presence of amino groups [5].

Many publications have been reported about chitosan production, but most of them used shrimp shell as raw material. The objective of the present work is to isolate the useful polymers chitin from the waste shell of *amusium sp* scallop and further used for chitosan production. Considering the content of scallop shell, it is possible to be used for chitin production and further processed into chitosan.

2. Materials and Methods

2.1. Materials

The scallop shell waste was obtained from Tambak Lorok Fish Market, Semarang, Indonesia. NaOH, CH₂COOH, and HCl were purchased from Merck (Hohenbrunn, Germany). Commercial chitosan as reference was purchased from Biotech Surendo, Cirebon, Indonesia. Distilled water for all experiments was produced from a home-made pure water unit.
2.2. Methods

The scallop shell waste was initially washed and dried until a constant weight has been reached. It was then ground followed by sieving using a 250 μm sieve resulting in scallops shell powder (SSP). Thereafter, deproteinization was performed by dissolving SSP in NaOH solution (ratio: 1 g to 4 ml) with different NaOH concentrations (1-5%), time of deproteination (20-140 minutes) and temperature (30-90°C). The solution was then filtered to obtain the remaining solid, which was further processed by washing using distilled water until a neutral pH was reached. The solid was dried in oven at 40°C for 24 hours.

Demineralization was performed after deproteination has completed. Deproteinated-SSP was dissolved in acid solution (ratio: 1 g to 5 ml) with different HCl concentrations (0.5-4M), time demineralization (15-120 minutes) and temperature (30-90°C). The solution was then filtered to obtain the remaining solid, which was chitin. Chitin was further processed by washing using distilled water until a neutral pH was reached. The chitin was dried in oven at 40°C for 24 hours. In order to obtain chitosan, deacetylation followed the method proposed by Kurita [6] was performed. Chitin was dissolved in 50% NaOH solution (ratio: 1 g to 20 ml) at 90°C. Chitosan was then washed with distilled water until a neutral pH was obtained. Finally, it was dried until reached a constant weight.

2.3 Analysis

Protein content was determined by proximate analysis using Kjedahl method [7]. Ash content of dried chitin was performed by burning in a muffle furnace at 700 °C for 6 hours and weighing after it has been cooled to room temperature and placed in desiccator. The degree of deacetylation (DD %) of chitosan was determined using a FTIR. It was calculated from the intensity of the absorption band at 1655 cm⁻¹ (amide I band) with the band at 3450 cm⁻¹ (OH band) as an internal standard. The DD % was calculated using Baxter’s equation [8], DD % = 100 – [(A1655/A3450) x 115].

3. Results and discussions

3.1 Chitin Isolation from Scallop Shell Waste

The results from deproteination experiments are presented in Figures 1-3. Figure 1 shows that the increase in NaOH concentration decreased protein content of SSP indicating deproteination increased. It can be explained that the higher NaOH concentration the higher ability to break protein-SSP powder bond. Nevertheless, beyond 4% the increase in NaOH concentration did not increase deproteination anymore. Figure 2 shows the effect of temperature on protein content of SSP. It is seen that protein content of SSP decreased significantly when the temperature was increased from 30-40°C. A plateau condition of protein content was observed for the temperature increase higher than 40°C. The increase in time of deproteination decreased the protein content indicating more protein has been released (Figure 3). However increasing deproteination time higher than 60 minutes did not decrease protein significantly. These 60 minutes seems to be the optimum deproteination time.

![Figure 1. The effect of NaOH concentration on protein content of SSP (t=120 min, T=70°C)](image-url)
3.2 Demineralization

Deproteinated-SSP was further demineralized using HCl solution. The results are presented Figures 4-6. As HCl concentration was increased the ash content of SSP decreased indicating more minerals react with HCl forming chloride salt, which was dissolved in water (Figure 4). The increase in temperature decreased ash content indicating more minerals have been released (Figure 5). The ash content decreased with increasing demineralization time. However, beyond 60°C the decrease in ash content could not be seen anymore (Figure 6).
3.3 Chitin Deacetylation

Deacetylation of chitin resulted in chitosan with yield was 9.7% and degree of deacetylation was 71.8%. Further characterization using FTIR is shown by Figure 7. In general, chitin and chitosan showed similar IR spectra. Specific peak, which characterize both of them is around 3400-3450 cm\(^{-1}\) attributed to \(-\text{NH}_2\) and \(-\text{OH}\) groups. This peak also indicates intermolecular hydrogen bonding [9]. The two bands at around 1680 and 1625 cm\(^{-1}\) indicate amide I and C=O groups were observed in chitin spectra. In addition peak at 1380 indicates amide III (C-N stretch). After deacetylation of chitin, peak at 1680 disappeared and new peak at 1565 was observed. Further, peak shifting from 1380 to 1430 was also observed. This suggests that deacetylation process was confirmed and chitosan was really produced. Further characteristics of chitosan are peak at 1030-1050 cm\(^{-1}\) indicating CO stretching, peak at \(-1430\) cm\(^{-1}\) indicating CH\(_2\) bonding, peak at \(-1560\) cm\(^{-1}\) indicating amide II band, peak at 1620 cm-1 indicating amide I band, peak at 2860 indicating CH stretching were observed.
4. Conclusion
Synthesis of chitosan from Amusium sp scallop shell waste was performed. Deproteination and demineralization of SSP were influenced by the concentration of alkaline or acid solution, temperature, and deproteination/demineralization time. The yield obtained for the synthesis of chitosan was 9.7% with DD was 71.8%. The chemical characteristic was similar with the commercial chitosan.

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