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Short communication

Synthesizing and Characterization of Natural Biopolymer *Chitosan* Derived from Shrimp Type, *Penaeus monodon*

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ABSTRACT. *In this study natural biopolymer "chitosan" was synthesized using locally available shrimp type of Penaeus monodon. Synthesis of chitosan involved four main stages; preconditioning, demineralization, deprotenisation and deacetylation. The first stage, "preconditioning" process, is a new step introduced in this research. Effect of deacetylation conditions such as alkali concentration, number of times deacetylation was performed and reaction temperature was investigated. Yields of chitin and chitosan from P. monodon were calculated. Chitosan was characterized using Fourier Transform Infrared (FTIR) Spectroscopy and X-ray diffraction. Degree of deacetylation of synthesized powder was calculated using FTIR spectra. Both characterization techniques confirm the existence of chitosan.*

Key words: *Chitin, chitosan, degree of deacetylation, Penaus monodon, shrimp shell*

INTRODUCTION

Use of natural biopolymers for diversified applications in life sciences has several advantages such as availability from replenishable agricultural or marine food resources, biocompatibility and biodegradability. Nevertheless, they bring ecological safety and the possibility of preparing a variety of chemically or enzymatically modified derivatives for specific end uses. Polysaccharides, as a class of natural macromolecules, have the tendency to be extremely bioactive, and are generally derived from agricultural feedstock or crustacean shell wastes.

Chitosan is a linear polysaccharide, composed of glucosamine units linked by β(1–4) glycosidic bonds. The application potential of chitosan is multidimensional. It has already shown potential use in food and nutrition, biotechnology, material science, drugs and pharmaceuticals, agriculture and environmental protection and recently in gene therapy too. Chitosan in generally valued 20-40 USD/kg .

Thirty different shrimp species including Indian white shrimp *(Penaeus indicus),* Banana prawn (*P. merguiensis*), Giant tiger prawn (*P. monodon*), and Green tiger prawn (*P. semisulcatus*) are available in Sri Lanka. The shrimp processing industry turns out tones of head and shell waste per annum. Decaying of shells makes bad odor. Therefore, shrimp processing industries have the problem with disposing shell waste. At present only a small quantity of shell waste is utilized for animal feed in Sri Lanka. Thus, there is very high potential to extract chitosan from shrimp waste.

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The contents of chitin, protein, minerals, and carotenoids in the shell waste varies widely depend on peeling conditions during processing as well as the species, the part of organism, state of their nutrition and stage of reproductive cycle (Synowiecki & Nadia, 2003). In this study we used a modified procedure with additional step of preconditioning for extracting chitosan from species *P. monodon*.

MATERIALS AND METHODS

The shrimp wastes were purchased from local seafood processing industry. A preconditioning process was introduced as the first step to the common procedure of chitosan extraction. This pretreatment aims to remove loosely bound protein prior to deproteination process. In the preconditioning, the skeletal matrix structure is first weakened which makes easier to remove solubilized protein by washing with water. Therefore, during deproteination process less alkali concentration can be used. At the preconditioning stage, shrimp shells were allowed to soak in 0.05 M acetic acid solution for 24 h. Then the shells were washed thoroughly with water and dried to remove excess water. Dried shells were demineralized using 0.68 M HCL at ambient temperature for 6 hours. The residue was washed with distilled water until pH 6.5-7.5 range was obtained and then the residue was dried. After that the demineralized shrimp shell was deproteinized using 0.62 M NaOH solution at ambient temperature for 16 hours. Then residue was washed thoroughly with water followed with distilled water until pH 6.5-7.5 range was obtained. The chitin was dried, ground and screened with 150 μm sieve. Then chitin yield from *P. monodon* was calcualated. The chitin obtain from the above process was deacetylated in 12.5 M and 15 M NaOH for 20 h at different reaction temperatures as shown in Table 1. After deacetylation, the chitosan was washed thoroughly with water followed with distilled water until pH 6.5-7.5 range was obtained. Then the yield of chitosan was calculated.

Table. 1. Deacetylation process parameters

Sample name	Sample size (g)	NaOH (mol/l)	Reaction temperature	No. of times of deacetylation
CHO-01	10	12.5	55	1
CHO-02	10	12.5	65	1
CHO-03	10	12.5	90	1
CHO-04	10	12.5	65	2
CHO-05	10	15	65	1

The extracted chitosan was characterized by Fourier transformed infrared (FTIR) spectroscopy (Bruker Alpha-T) in the range of 400 to 4000 per cm. The degree of deacetylation (DD) of the sample was calculated using FTIR spectra (Mohammad, 2007).

The crystallinity of chitosan in powder form was studied by X-ray diffraction method (Bruker D8) using Cu Kα radiation generated at 40 kV and 40 mA at scanning speed of 0.3 2θ/min within a range of 5^0 to 35^0.

RESULTS AND DISCUSSION

The preconditioning pretreatment aims to remove loosely bound protein prior to deproteination process. Therefore, during deproteination process less alkali concentration can be used. This process leads to less exposure of chitin and chitosan to strong alkali solution and therefore, results less chemical contamination in the final product.

It was found that chitin yield was 41.6 % and the yield of chitosan from that chitin was 55.8 %. Hence, Sri Lankan *P. monodon* gives a considerable amount of yield of chitin and chitosan. Fig. 1 shows FTIR spectra of chitosan derived from the above mentioned process. The chitin showed an intense peak at 1564 per cm which correspond to the N-H deformation of amide II. The band at 1639 per cm corresponds to the amide I stretching of C=O group (Zouhour *et al*., 2010).

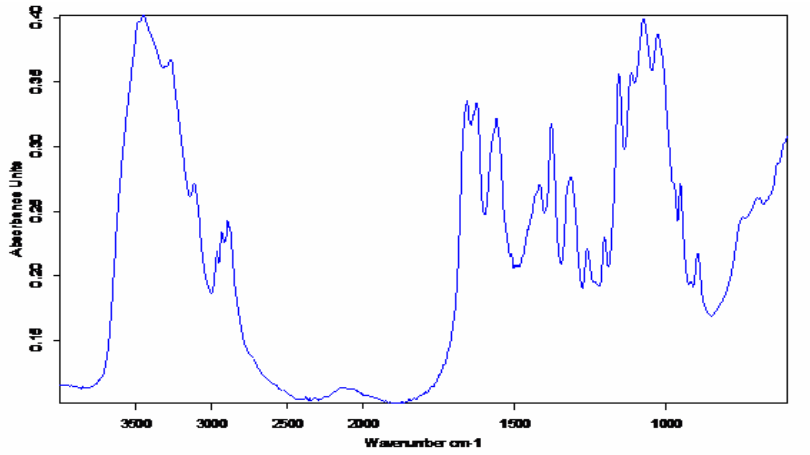

Fig. 1. FTIR spectra of Chitosan

Degree of deacetylation of synthesized chitosan powder were determined by the peak at 1655 per cm as measuring band and the peak at 3450 per cm as reference band. The O-H stretching bond was appeared at 3450 per cm. Chitin and chitosan both have this peak, it is independent of deacetylation. Therefore, it was used as reference band. The peak at 1655 per cm correspond to C=O stretching in secondary amide and its intensity dependent of deacetylation (Kathleen & Paul, 2004). Therefore, it was used as measuring band. The DD values of synthesized powder are shown in Table 2. CHO-02 and CHO-05 samples were synthesized at same reaction temperature (65 °C) and deacetylation was carried out only once. But NaOH concentration was changed from 12.5 M to 15 M. As a result of change in NaOH concentration DD increase from 48.5 % to 51 %. CHO- 01, CHO-02 and CHO-03 were synthesized using same 12.5 M NaOH concentration and deacetylation was performed only once. But the reaction temperatures were 55 °C, 65 °C and 90 °C, respectively. As a result of these changes in temperature the resultant DD values ware increased to 47 %, 48.5 % and 51.9 % at respective temperatures. CHO-02 and CHO-04 were synthesized at the same reaction temperature and alkali concentration. But the deacetylation process had been carried out only once for CHO-02 and twice for CHO-04. As a result of doing two consecutive deacetylation process DD is increased from 48.5 % to 54.4 %. Hence, slight increase in DD values can be observed with increasing NaOH concentration, reaction time and number of deacetylations performed.

Table 2. Variation of Degree of deacetylation with reaction conditions

Sample name	NaOH (mol/L)	Reaction temperature (°C)	No. of times of deacetylation	Degree of deacetylation (%)
CHO-01	12.5	55	1	47.0
CHO-02	12.5	65	1	48.5
CHO-03	12.5	90	1	51.9
CHO-04	12.5	65	2	54.4
CHO-05	15.0	65	1	51.0

During the deacetylation reaction, acetyl group of the chitin react with NaOH and produce an amine group. This is a reversible reaction and, when NaOH concentration is increased the reaction is biased towards the forward direction by producing more chitosan. As a result, DD will be increased. Similarly, when temperature is increased reactivity energy of the reaction increased in the forward direction. When number of times of deacetylation is increased it gives better opportunity to produce more amine groups. Hence, DD will increase with increasing above parameters.

Fig.2 shows the X-ray diffractrogams of chitosan. The chitosan sample shows two sharp peaks approximately at 10^0 and 20^0 (2θ). The sharp peaks indicate that synthesized chitosan has high crystalinity. One of the major physical characteristics that determine the functional properties of chitosan is the crystalinity (Trang *et al.*, 2006).

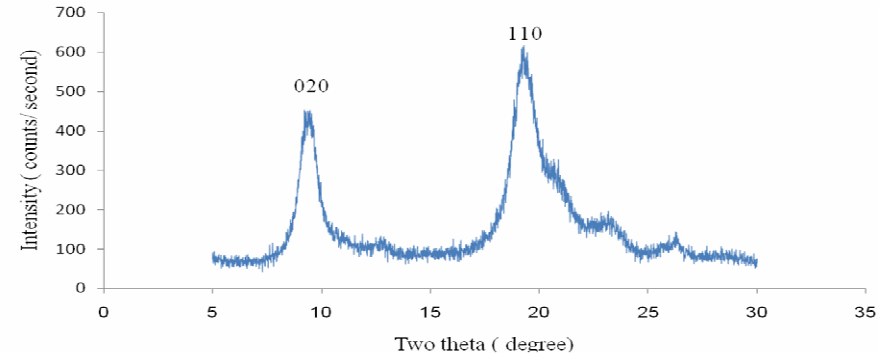

Fig. 2. X-ray diffraction spectra of *c*hitosan

The strong reflections at 2θ around 9-10^0 and 19-20^0 corresponds to (020) and (110) planes of chitosan (Jolanta *et al.*, 2010).

CONCLUSIONS

Chitosan was successfully extracted from Sri Lankan shrimp species *P. monodon* by introducing a new preconditioning step to the conventional route. Degree of deacetylation values calculated using FTIR spectrum depends on critical parameters such as reaction temperature, reaction time, number of times of deacetylation and alkali concentration. X-ray diffraction patterns indicated characteristics reflection peaks of chitosan with high crystalinity.

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