

Study on Chitin Extraction from Crab Shells Waste

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Abstract: Chitin is the second most abundant natural polymer after cellulose. It occurs as a component of crustacean shells, insect exoskeletons, fungal cell walls and plankton. In this work, chitin was extracted from crab shells waste by chemical method. It includes two major steps such as demineralization and deproteinization step. These two steps were crucial for the elimination of calcium carbonate and other minerals as well as protein which are present in the shells. To extract the successive chitin, the sequence of these two treatment steps were varied in these experiments. In this study, the chemical compositions of crab shells waste were analyzed by X-ray fluorescence (XRF), the resultant chitins were characterized and analyzed by X-ray diffraction (XRD) and Fourier transform infrared spectroscopy (FTIR).

Keywords— Crab shells waste, Chitin, XRF, XRD, FTIR

1. INTRODUCTION

Chitin, poly(β -(1 \rightarrow 4)-N-acetyl-D-glucosamine) is a natural polysaccharide of major importance, first identified in 1884 (see. figure 1)[1]. Chitin is the major structural component of the exoskeleton of invertebrates and the cell walls of fungi[2]. The amount of chitin with respect to dry weight is the highest in crustaceans. Hence, crustacean shells are regarded the main source of chitin for the chemical industry[3]. The main components of crustacean shells are chitin (15-40%), protein (20-40%), calcium and magnesium carbonate (20-50%)[4]. Chitin is insoluble in water, in every common organic solvent and in acidic, basic and neutral aqueous solutions[3]. Chitin found in nature is normally packed into an orderly crystalline structure which made it strong and able to serve the exoskeleton purpose of the shells of arthropods. Chitin exists in three structural forms called alpha-, beta- and gamma- chitin. The packing and orientation of the chitin strands are different in each of the different types. Alpha-chitin, found in shrimp and crab shell, has anti parallel chitin strands that are packed tightly with both inter and intramolecular hydrogen bonding with makes it the strongest structure in all the three chitin structures found. Beta-chitin, found in squid pens, has parallel chitin strands that are pack looser and lack the intermolecular hydrogen bonding making it weaker. Gamma chitin found in the stomach lining of some mollusk, have a randomly orientated chitin stand. Of these three structural types alpha- and beta-chitin are the most abundant[5]. Traditional methods for the preparation of chitin include demineralization and deproteinization of the raw materials with strong acids and bases (e.g. HCl and NaOH)[3].

Chitin is non-toxic, odorless, biocompatible with living tissues, biodegradable, presenting antibacterial, moisture retaining and healing characteristics. Chitin and chitosan can be utilized in water purification, additives in cosmetics, antibacterial agents, pharmaceutical adjuvants, paper production, textile finishes, heavy metal chelating agents, membranes and biomedical applications such as wound dressings, separation membranes, antibacterial coatings, since they are harmless for the human body[6]. The objectives of this study are to determine the optimum condition of chitin

extraction from crab shells waste and to investigate the XRD and FTIR analysis of chitin.

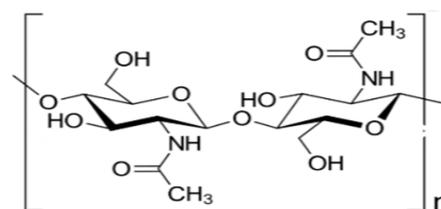


Figure 1. Chemical structure of Chitin

2. MATERIALS AND METHODS

2.1. Materials

In this work, the crab shells waste and chemicals were used as raw materials. Crab shells waste were collected from Crab World Company in Kyauktan Township. Hydrochloric acid (analytical grade) and sodium hydroxide (analytical grade) were used in chitin preparation process. Distilled water were also used to prepare desired concentration of chemical solution and to wash the sample.

2.2. Extraction of Chitin by Chemical Methods

In this investigation, the two experiments were carried out for extraction of chitin from crab shells by chemical method. This method is rather uncomplicated and simple chemicals such as NaOH and HCl can be used. Because NaOH is the preferential reagent and it is applied at concentration ranging from 0.125 to 5.0 M, at varying temperature (up to 160°C) and treatment duration (from few minutes up to few days) at chemical deproteinization step. Demineralization is easily achieved by using dilute hydrochloric acid because it involves the decomposition of calcium carbonate into the water-soluble calcium salts with the release of carbon dioxide. Demineralization treatments are often empirical and vary with the mineralization degree of each shell, extraction time, temperature, particle size, acid concentration and solute/solvent ratio. The latter depends on

the acid concentration, since it needs two molecules of HCl to convert one molecule of calcium carbonate into calcium chloride[7]. The experimental procedure of extracted chitin was described in Figure 2.

In experiment I, crab shells waste were washed out with water to remove dirt and body tissue, then sun-drying. Twenty grams of dried shells were treated with 1N of NaOH solution at 80°C for 3 hrs in deproteinization. Then, deproteinized sample was treated with 0.5N of HCl and 1N of HCl at room temperature for 3 hrs. The purified chitin was dried in an oven at 60°C until it was constant weight.

of crab shells powder were treated with 7% HCl with continuous stirring and heated at 60°C for 3 hrs to remove mineral content and then treated with 5% NaOH to reduce nitrogen content of protein. After that the sample was filtered, washed repeatedly with distilled water to remove any traces of chemicals and soluble impurities. The filtered sample was dried in an oven at 70°C for 3 hrs. Finally, the dried demineralized, deproteinized and deodorized sample of chitin was obtained.

3. RESULTS AND DISCUSSION

3.1. Analysis of Crab Shells Waste

The chemical compositions of crab shells waste was analyzed by X-ray Fluorescence at Land Use Division (Yangon). The results was as shown in Table 1.

As the result, the component of calcium was the highest content of this composition in raw crab shells.

Table 1. Chemical Compositions of Crab Shells Waste

Composition	Percent (%)
Moisture	1.097
Nitrogen	0.78
P ₂ O ₅	2.777
K ₂ O	0.045
Ca	28
Mg	2.4
Fe	0.087
Cu	0.007
S	0.41
Na	0.778

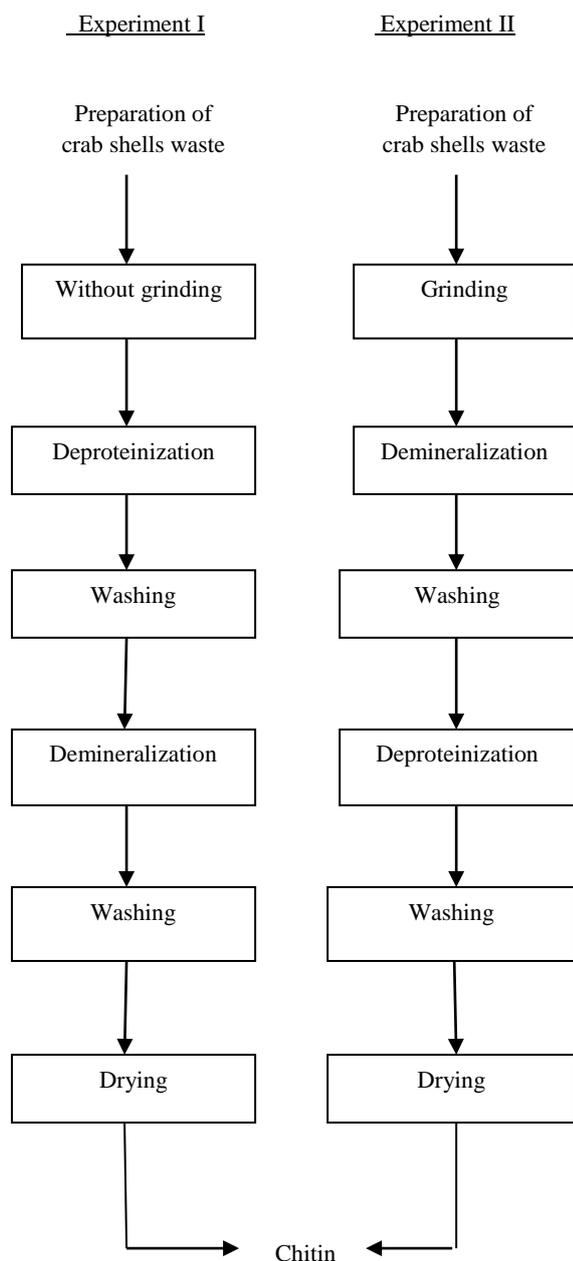


Figure 2. Extraction of Chitin from Crab Shells Waste

3.2. Conditional Results of Extracted Chitin in Experiment I

The conditional results of extracted chitin in experiment I were shown in Table 2. In this experiment, the two samples were treated with same concentration of NaOH solution in deproteinization at 80° C and different concentration of HCl solution in demineralization at room temperature. The demineralization step, the solute and solvent were used as the same ratio for these samples. The resultant samples from this experiment were analyzed and characterized by XRD and FTIR as shown in Figure 3 and Figure 4. In XRD results, the sharp peak intensity of S₁ at two-theta(degree) is 28.9 and the sharp peak intensity of S₂ at two-theta(degree) is 19.22. So, it indicates that although S₁ was formed as calcium carbonate but also S₂ was formed as chitin. In standard FTIR

of chitin, the wavelength pattern of all the functional groups express as amide I groups were $1660-1680\text{ cm}^{-1}$, amide II groups and amide III groups were $1560-1530\text{ cm}^{-1}$ and 952 cm^{-1} , the hydroxyl group was 3448 cm^{-1} . From the results of FTIR for S_1 , the wavelength pattern of amide I group was 1795 cm^{-1} , amide II group was 1626 cm^{-1} and amide III groups was 1035 cm^{-1} respectively. So, the over range of wavelength pattern indicates that the resultant product cannot be determined as chitin. FTIR results for S_2 , amide I group was 1672 cm^{-1} , amide II group 1550 cm^{-1} and amide III was 952 cm^{-1} . The range of wavelength pattern indicates that the resultant product can be determined as chitin.

Table 2. Results of Experiment I

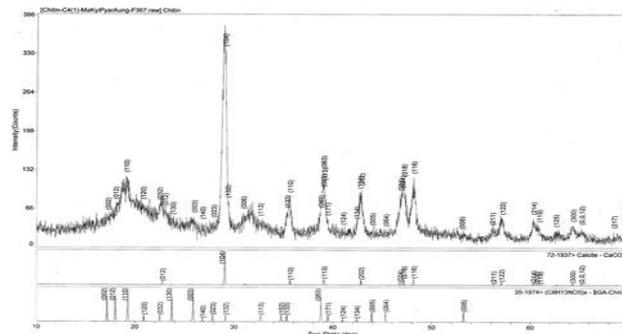
No	Concentration of deproteinization at 80°C	Concentration of demineralization at R.T	Sample to solvent ratio(w/v)	Color
S_1	1N NaOH	0.5N HCl	1:15	Pink
S_2	1N NaOH	1N HCl	1:15	Pink

3.3. Conditional Results of Extracted Chitin in Experiment II

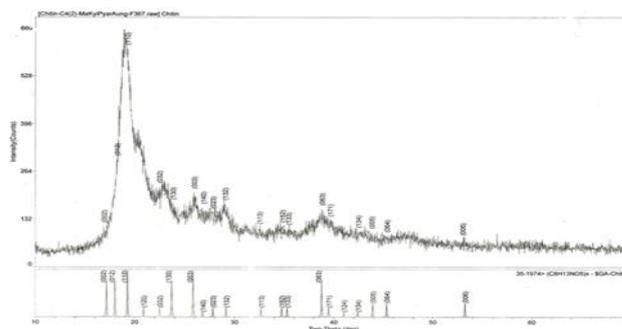
The conditional results of extracted chitin in experiment II were shown in Table 3. In this experiment, the two samples which had 30 mesh size were treated with the same concentration of HCl solution in demineralization and NaOH solution in deproteinization at the same temperature. In demineralization step, the solute and solvent were used different ratio. The resultant samples from this experiment were analyzed and characterized by XRD and FTIR as shown in Figure 5 and Figure 6. As all of the results of XRD, the sharp peak intensity of chitin was 19.22 at two-theta (degree). Therefore, all the samples such as S_3, S_4 were determined as chitin. According to the standard FTIR pattern, the ranges of wavelength in FTIR results for all the samples indicate that the resultant product can be determined as successive formation of biopolymer.

TABLE 3. RESULTS OF EXPERIMENT II

No	Concentration of demineralization at 60°C	Sample to solvent ratio(w/v)	Concentration of deproteinization at 60°C	Color
S_3	7% HCl	1:10	5% NaOH	White
S_4	7% HCl	1:15	5% NaOH	White

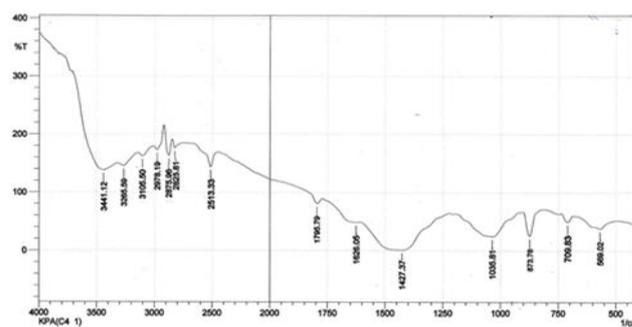


(a)

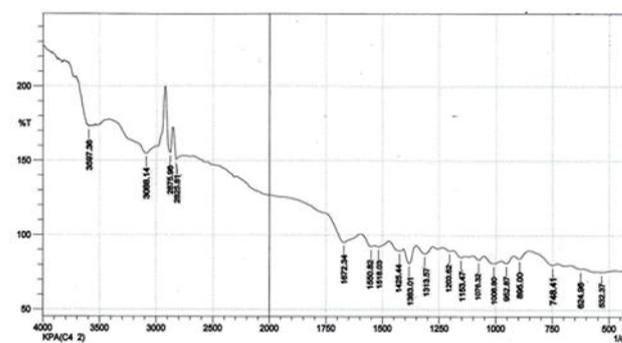


(b)

Figure 3. XRD pattern of Chitin (a) S_1 (b) S_2

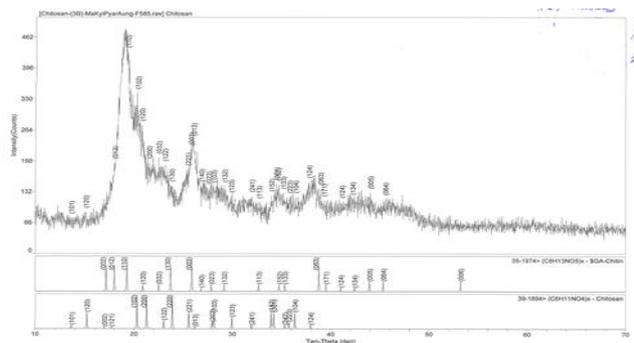


(a)

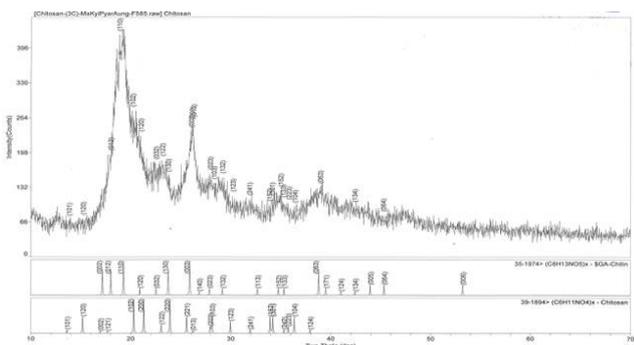


(b)

Figure 4. FTIR pattern of Chitin (a) S_1 (b) S_2

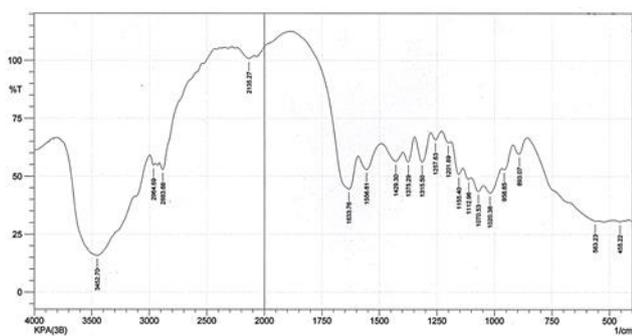


(a)

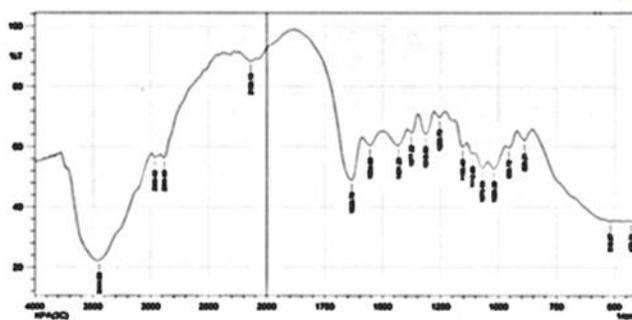


(b)

Figure 5. XRD pattern of Chitin (a) S₃ (b) S₄



(a)



(b)

Figure 6. FTIR pattern of Chitin (a) S₃ (b) S₄

4. COMPARISON OF CHITIN YIELD

The comparison of chitin yield in experiment I and II were shown in Table 4.

Table 4. Chitin Yield

Sample	S ₂	S ₃	S ₄
Yield	0.97 g	1.8 g	1.5 g
Yield%	4.9%	6%	5%

The yield of extracted chitin from crab shells was between 4.9% and 6%. Although sample (S₃) was the highest yield percent of chitin product but also S₂ and S₄ were not acceptable in percentage of yield. Therefore, S₃ was better than other samples in yield percent of product chitin. Among the results, it can be said that S₃ was favorable result for prepared chitosan because it was obtained the highest yield percent.

5. CONCLUSION

In this study, the two experiments were carried out to extract chitin. When increasing the acid concentration, the results of experiment II were better than experiment I in the chitin formation. The sharp peak intensity of XRD patterns for all samples in experiment II were formed as chitin at two-theta. From the results of FTIR, all the functional groups which have been identified in the form of peak that include amide, carbonyl and hydroxyl groups successfully. According to the experimental results, it can be investigated that the successive formation of chitin depend on the process parameters such as degree of each shell, extraction time, temperature, particle size, acid concentration and solute/solvent ratio. Among the results, sample (S₃) was the highest percentage of yield was obtained at 6%. In conclusion, the extracted chitin which is the highest percentage of yield can be used to produce chitin-derived product, such as chitosan.

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