Extraction of degradable bio polymer materials from shrimp shell wastes by two different methods

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Abstract. Chitosan is one of the most widespread biopolymer materials available in nature which is extracted from chitin. The main source of chitosan is the chitin that is extracted from exoskeletons of crustacean, such as shrimp and crabs, which are found in a huge amount of shells waste that produced from seafood companies around the world. The chitosan has several applications such as pharmaceutical, fertilizer and edible coating in food industries. The quality of the chitosan’s depends on its extraction method, so in this research work we have studied the effect of grounding shrimp shell waste before the extraction step. The chemical and physical properties of the extracted chitosan were studied by using different techniques such as Fourier transform infrared spectroscopy (FTIR), and Thermogravimetric analysis (TGA). The study showed that the films and powder produced from non-ground samples showed a higher purity of chitosan and better thermal stability than the chitosan produced from ground samples.

1. Introduction

Chitosan is a natural biopolymer material produced from chitin biopolymer, which is the second natural abundant biopolymer found in nature after cellulose. Chitin has been found in many sources such as marine crustaceans, insects, some algae, and fungi [1]. Chitosan is non-toxic, biodegradable and antimicrobial material which has many beneficial applications in many fields such as food industry, water treatment, manufacturing of membranes, cosmetics, antifungal and antibacterial products [2]. Chitosan is a bi-product extracted from chitin by using alkali treatment which involves three main steps. The first step is deproteinization (DP) step, which removes proteins and undesired materials such as pigments from shrimp shells. This is followed by demineralization (DM), which removes all minerals (e.g. CaCO3) by using acidic solution, then the deacetylation step (DD) which removes acetyl groups from the polymeric chain. The produced chitosan’s quality depends on the conditions of extraction such as alkali concentrations and reaction time of acid with samples [3].
In this study, we explored the effect of the mechanical treatment of the shrimp shell waste on the yield of chitosan extracted. Degradable biopolymer films were made from chitosan using two different methods of extraction. The first method was based on grounding shrimp shell waste as shown in figure 1a, whilst the second method relied on treatment of shrimp shells without groundings shown in Figure 1b. The research tested the hypothesis that grounding the shells increases the surface area and would thus affect the properties of the chitosan produced. Various procedures have been adopted to obtain chitosan from crustaceans. Although chemical methods are widely used to extract chitosan and chitin from the raw materials shrimp wastes [4], there is little information about how to make the production process more efficient. Chitosan extraction usually takes place by enzymatic or chemical methods. Demineralization usually takes place by HCl and deproteinization by NaOH. The sequence of these extraction steps differs, although in most chitin producing industries the deproteinization step is carried out before demineralization [5].

![Figure 1. a) ground shrimp, b) whole shrimp without grounding.](image)

Divya. K, et al reported a higher w/w % yield of high purity chitosan and the difference in yield was possibly due to the increased time of reaction [6]. Deproteinization and demineralization steps were repeated twice and, the final deacetylation of chitin at room temperature lasted 3 days. The characteristics of produced chitosan were in accordance with the commercial standard. The obtained chitosan had low viscosity, high degree of deacetylation (DD) and a denser crystalline structure. Chitosan with such properties have many commercial applications and greater scope of industrial applications.

M. S. Hossain and A. Iqbal reported chitosan produced from shrimp waste by a chemical method that started with the demineralization step before deproteinization and deacetylation steps [7]. Carrying out demineralization with different concentrations of HCl and NaOH in deproteinization steps, the results showed that 3% HCl used in demineralization and 4% NaOH in the deproteinization were the suitable concentration for these methods, respectively, at ambient temperature (28±2°C)without a heating process. The degree of deacetylation (DD) process depends on NaOH concentration. Due to the fact that the highly bonded acetyl groups in chitin is difficult to remove, higher concentration of NaOH and higher temperature are required. In this case, the increase of NaOH concentration improved the deacetylation process where the highest deacetylation degree (81.24%) was reached at a NaOH concentration of 60%. The improvement in deacetylation degree was not observed with NaOH concentration more than 60% since the process become inefficient especially in the final washing to obtain chitosan. Among the treatments used in the study 4% NaOH and 3% HCl were found to most successfully extract chitin. The highest deacetylate yield of chitosan with maximum solubility was done by 60% NaOH treatment, whilst high quality chitosan DD 79.57% and 97.02% solubility with minimum chemical usage was yielded by 50% NaOH treatment.

N.S. Mahmoud et al. [8] showed the effect of using organic acids in the demineralization on the deproteinized chitin instead of using inorganic acid HCl. The study showed that the effectiveness of
using lactic and/or acetic acids for demineralization of shrimp shells was comparable to that of using hydrochloric acid. Since organic acids are environmental friendly and the chitin properties are maintained, the advantages of using organic acids in the demineralization step would result in: (a) production of important products as acetate and lactate salts besides chitin production. (b) Successful removing of minerals, (c) maintaining natural chitin properties and (d) reduction in the purification cost [9], however the conventional HCl was used in this study.

2. Experimental section

2.1. Materials
The raw shrimp shell waste was bought from local fish market, Al-Obour city, Egypt. The shrimp shell waste was obtained from mix of species of shrimp. All of the reagents used were of a high analytical grade which purchased from Sigma-Aldrich chemical company; Sodium Hydroxide pellets (NaOH, pure), Hydrochloric acid (HCl, 35-38%), Acetic Acid (CH₃COOH, 100%).

2.2. Methods of extraction
Approximately 2000 g of shrimp shells waste was washed several times with tap water then deionized water until the shell waste were clean and then the shells were dried at room temperature. The dried shrimp shell waste was divided into two samples (A) and (B) each weighing ~1000 g, (A) was ground with an electric grinder and (B) was left as the whole exoskeleton with head and tail without grinding. The following procedures were applied for both (A) and (B). Chitin was extracted from shrimp shells by deproteinized followed by demineralization steps [10]. Shells were deproteinized by boiling several times in 1 M NaOH solution and demineralized using 1 M HCl. Then chitin produced was deacetylation using 50% NaOH to obtain chitosan.

2.3. Preparation of chitosan film by casting
For each sample, 2g of chitosan was added to 1 % CH₃COOH then boiled for 30 mins, and then poured into flat flexible plastic cover [11]. The samples were left to dry at room temperature, until solid films were produced as shown in figure 2.

2.4 Characterization Tests
2.4.1 pH and Solubility. The pH measurement of the chitosan solutions was carried out using a pH meter (EC500). Solubility of the chitosan was tested by dispersing 0.5 g of chitosan in 1% acetic acid and heating the mixture to 100°C with continuous stirring. If the formed chitosan was not soluble then the degree of deacetylation would not be considered acceptable and the deacetylation process would need to be repeated.

2.4.2 Fourier transformation Infrared (FT-IR) Spectra. FT-IR spectral analysis of chitosan is used to detect the functional groups of chitin and chitosan and to determine the degree of deacetylation DD, but it is valid only when the value of DD is not more than 55% [12]. The spectral region was scanned between 4000 and 400 cm⁻¹. Potassium bromide KBr pellets were used in preparing specimens. Dried powdery chitosan was mixed with KBr powder and pressed in vacuo to form a homogeneous disc with a thickness of 0.5 mm. The chitin concentration in the samples was 2%, calculated with respect to KBr.
2.4.3 $^1$HNMR spectroscopic analysis. $^1$HNMR spectroscopy is used for determining DD of chitin/chitosan using the following equation [13]:

$$DD\% = \frac{1}{3} X \text{ICH}_3 + \frac{1}{6} X (\text{H}_2-\text{H}_6) \times 100$$

The chitosan was dissolved in DMSO/trifloroacetic acid and the spectroscopic analysis carried out on a Bruker Avance III instrument (500 MHz). Nomenclature is given as recommended by Harris et al [13]. Chemical shifts ($\delta$) are given in ppm and coupling constants $J$ in Hz. Relax delay 1.00 sec. pulse 45.0 degree. The chitosan samples were with concentration between 0 and 10 ppm.

2.4.4 Thermal Gravimetric Analysis (TGA). Thermal stability of chitosan powders and films was measured by Thermal gravimetric analysis (TGA) Using a Hi-Res TGA 2950 Thermogravimetric Analyzer from TA Instruments Company (Tokyo, Japan. The analyses were made by increasing the temperature from room temperature to $800^\circ C$ in an inert nitrogen atmosphere with a flow rate of 60 mL/min and warming rate of 10 $^\circ C$/min

2.4.5 Zeta potential. Zeta potential of the particles was recorded on a Particle Sizing Systems\ZPW388-V2.14\zpW388.tbl. The wavelength used was 632.8 nm, and sample temperature of 230°C

2.4.6 Measurement of Viscosity. Viscosity was measured using Brookfield viscometer 1% chitosan solution was prepared using 1% acetic acid. Measurement was made using a No. 5 spindle at 50 rpm at 25°C with values reported in centipoises (cPs) units.

2.4.7 Calculation of Yield Percentage. The amount of chitosan extracted from 1000g of dry shrimp shell wastes was calculated as yield percentage as following:

$$Yield \% = \frac{\text{Mass of dry chitosan extracted}}{\text{mass of dry shell waste}} \times 100.$$  

3. Results and Discussion

3.1. pH and Solubility test

The pH of dissolved chitosan from method A was around 11-12, while the pH from (B) was about 9-10. When the solubility test was conducted Both extracted methods produced chitosan which dissolved, as shown in figure 3 but chitosan from method A took a longer time to dissolve than from method B.
3.2. Fourier transformation Infrared (FT-IR) Spectra

FTIR analysis was conducted to observe structural changes that occurred as a result of chitosan produced by two different methods. It was based on the identification of the functional groups present in chitosan molecules, which are given in Table 1 with the corresponding absorption bands (in cm$^{-1}$). In order to fully characterize the starting materials, chitosan A and B were compared with the spectra of pure high molecular weight chitosan (HMWC) and low molecular weight chitosan (LMWC). The first step in the procedure is the conversion of chitin into chitosan, which was confirmed through the disappearance or decrease of peaks that are related to secondary amides (thus to chitin) in both experimental procedures. The FT-IR spectra of the two chitosan samples produced from methods A and B are shown in figure 4 and revealed that there is a reduction in the band at 1629 cm$^{-1}$ in CsA (Chitosan Method A), as it transformed from chitin to chitosan. In the pure LMWC and HMWC samples no band was observed between 1650 and 1900 cm$^{-1}$, which showed that oxidative groups such as carboxylic aldehyde, or carbonyl groups did not exist in these samples.

But in CsA a new absorption ban showed at 1797.5 cm$^{-1}$ which is attributed to the absorption of the carboxylic group (–COOH). These results suggest the rupture of β-(1, 4) glycoside linkage in macromolecule of CsA. Furthermore, significant decreases in the intensity of 1629 cm$^{-1}$ showed the effective of the deacetylation process. The FT-IR spectrum of chitosan from method B (CsB) showed the characteristic absorption bands at 3442.31, 2921.63 cm$^{-1}$ corresponding to NH and CH stretching. The bands around 1643.05, 1589.06 and 1421.28 cm$^{-1}$ were assigned to amide I, II and III, respectively, while the characteristic bands attributed to the saccharide structure appeared at 1079.94 cm$^{-1}$ (antisymmetric stretching of the C-O-C bridge). Absorption band at 1643.05 cm$^{-1}$ assigned to the increase of deacetylation degree following with the same increase of intensity at 1589.06 cm$^{-1}$ which shows free amine group NH$_2$, as shown in table 1.

**Table 1.** Comparison between FTIR spectral Patterns between commercial and extracted chitosan
3.3. $^1$HNMR

The $^1$HNMR spectra are accurate and precise for measuring high degree of deacetylation (DDA), which is usually difficult to measure using other techniques like IR or titration of dilute solutions of Chitosan samples dissolved in DMSO solvent. The $^1$HNMR spectrum of method A, revealed peaks at $\delta$=4.79 (H1), $\delta$ =2. (H2), $\delta$ =3.43~3.686 (H3, H4, H5, H6), $\delta$ =2.1 (NHCOCH3) ppm, while in method B; the 1HNMR spectrum revealed peaks at the same positions but with lower intensities, as shown in Figure 5. The Peaks appeared at 2.5, 3.5 ppm related to the solvent. The DD of method A and Method B results are shown in Table 2. The higher DD for method B ties in with the greater solubility reported found in the solubility test.

| Commercial Chitosan | Experimental Chitosan | Vibration Modes |
|---------------------|-----------------------|----------------|
| High MWT            | Low MWT               |                |
| 3446.17             | 3432.67               | NH$_2$OH in pyranose ring |
| 2923.56             | 2921.63               | CH2 in CH2OH group |
| 2360.44             | 2362.37               | (C=O) |
| 2333.45             | 2339.23               | (C=O) in NHCOCH3 group (Amide I band) |
| 1646.91             | 1652.7                | *NH$_2$ in amino group |
| 1396.21             | 1378.85               | CH$_3$ |
| 1149.37             | 1153.22               | –C-H complex vibrations of NHCO group (Amide III band) |
| 1076.08             | 1076.08               | (C-O-C) (glycosidic linkage) |
| 896.737             | 896.737               | pyranose ring skeletal vibrations |

* no band found

Table 2. The DD of method A and Method B

| Method   | DD  |
|----------|-----|
| Method A | 57% |
| Method B | 70% |
3.4. Zeta potential

The magnitude of zeta potentials as seen in Figure 6, shows method B averaged 48.98, while the average for method A was 37.88. The higher magnitude of zeta potential in method B. The magnitude of zeta potential reflects the degree of electrostatic repulsion between charged particles. For dispersion system, high degree of zeta potential, negative or positive indicates resistance of the particles to aggregation, and this denotes an apparently stable system [14].

3.5. Thermogravimetric (TGA)

The thermal stability of chitosan particles was investigated by thermogravimetric analysis. The changes in sample weight with the increase of the temperature are presented in Figure 7. Chitosan weight loss curves associated with the decomposition of chitosan are in the range 200–450 °C15. In our study, there is a weight loss up to 100 °C in all samples due to elimination of adsorbed water that shows hydrophobic character. Amongst all the samples, method B the non-ground chitosan has more residual weight after TGA and is thus considered to be the most stable chitosan in this study. Samples showed a significant weight loss from 270 °C up to 500 °C. This can be attributed to the pyrolytic decomposition, dehydration of the saccharide rings and depolymerisation of the polysaccharide structure16. Analysed the gases evolved during TGA using FTIR, the complex gaseous mixture released during the degradation of chitosan was mainly composed of CO₂, NH₃, CO₂, CH₃COOH and CH₄. The release of H₂O, NH₃, CO₂, CO and CH₃COOH was observed in the temperature range 2500 °C - 450 °C and this assigned to the pyrolytic degradation of chitosan. NH₃ was released at lower temperature than the other gases. While the release of CH₄ took place in the range from 450 °C -750 °C. As suggested in literature17-19, this step suggests a modification of the material when the structure is decreased due the release of methane and forms graphite-like structures through a hydrogenation mechanism.
According to the TGA rate of wt% loss curve of chitosan-CsA (blue line shown in Figure 7a (a), there are multi-degradation stages. In the first stage, weight loss starts at ~<100°C and continues, reaching at the second stage its maximum weight loss (77. %/°C) at 307 °C and at 355 °C another peak where it lost 63%/°C. The last degradation stage curve at 639 °C with a weight loss percentage of 30%/°C which indicates the elimination of CO₂ gas. The chitosan from method (A) film also showed similar multi degradation steps starting at less than 100°C up to 700°C reaching its maximum weight loss 79 %/°C at 290°C as shown in Figure 7a (b). In the rate of weight loss curve of chitosan from method (B) as shown in Figure 7b (c), there two degradation stages reaching its maximum weight loss 66%/°C at 307°C. The films produced from Method B are illustrated in Figure 7b(d) and revealed three degradation stages. First degradation stage below 100°C, maximum rate weight loss at 283°C with highest percentage 67%/°C, and then last degradation stage at 468°C with weight loss 42.17%/°C. The residues of the four samples were recorded as shown in table 3.

| Samples residue      | weight(mg) |
|----------------------|------------|
| Chitosan A           | 0.3127     |
| Chitosan film A      | 0.5191     |
| Chitosan B           | 0.6209     |
| Chitosan B film      | 1.187      |

3.6 Measurement of viscosity

The viscosity of chitosan A (ƞ) was found to be 24 cP, whilst Chitosan B showed a viscosity (ƞ) of 180 cP. Hence method B, produced chitosan with higher viscosity.
3.7. Calculation of Yield Percentage

Amount of Chitosan extracted from the ground waste method was 187 g from 1000 g of shell wastes, whilst chitosan from the whole exoskeleton method produced 226.2 g from 1000 g of shell wastes. Hence the yield of CsA was lower (18.7 wt. %) than that of CsB (22.62 wt. %).

![TGA graphs](image_url)

Figure 7. TGA of chitosan particles produced a) CsA, b) CsB
4. Conclusion
Chitosan produced from whole, non-ground dried exoskeleton (method B) showed a higher degree of deacetylation, a higher purity of chitosan, and enhanced thermal stability compared to chitosan from produced from ground dried shrimp waste (method A). Using shrimp shell waste as a whole shell showed a higher degree of control of the extraction procedure. With the chemical treatment described in this paper, a larger yield of chitosan was produced from non-ground than ground shrimp shell method.

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