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Abstract

Chitosan was extracted from grasshopper. The contents of this exoskeletons organism were extracted and analyzed to obtain chitosan and chitin. Chitosan extraction consists of four common steps such as demineralization, deproteinization, decolorization and N-deacetylation. Deacetylation of the different chitin produced was conducted by the conventional thermal method. From the analysis, the solubility shows insolubility in all different solvent used, the pH shows neutral at 7.28 for the chitin and the chitosan at 7.08. The ash content was obtained to be 20%, moisture content 19% and FTIR-Spectroscopy revealed 64% conversion which no effects against nematodes.

Keyword: chitosan, chitosan grasshopper, deacylation, FTIR.

Introduction

The biopolymer made by the combination of many molecules originated from living beings such as collagen, chitin, silk, alginate. Polysaccharides like cellulose, chitin and chitosan are commonly applied (Shanta et al., 2015). According to (Judson et al., 2013) chitin and chitosan is among the most common biopolymer sourced from exoskeleton of a crustaceans, fungi and insects. Being homopolymer of β(1-4) linked N-acetyl-D-glucosamine Austin which despite nitrogen presence it is still a cellulose with hydroxyl at C-2 replaced by an acetamito group ester (Thillai et al., 2017). Chitin deacetylase discovered and extracted from fungus mucor rouxii and its enzymes was associated with cell wall synthesis by converting chitin to chitosan.
Industrial processing of chitin is by extracting from crustaceans using acid to dissolve calcium carbonate followed by alkaline treatment to dissolve protein (Ricaudo, 2006). When the deacetylation is greater than 50%, the biopolymer becomes soluble in acidic aqueous solutions and behaves as a cationic polyelectrolyte due to $H^+$ protonated amine group (Jose et al., 2016). It is soluble in dilute acid at pKa value of 6.3 and acts as polycationic (Dario et al., 2017). Chitin and its associates have economic benefit due to its biological activities as well as agrochemical uses (Zouhour et al., 2011). Amino group is effective for complexing metals (Mince et al., 2012). Versatile modification of amino and hydroxyl group make it suitable as biomedical material (Gustavo et al., 2017). According to (Stephen et al., 2016) free amino group responsible for chemical and biochemical reactivity. It shows antiviral tendencies (Ashford, 1992). A variety of acylation reaction on it are possible by using different acylating agents (Manoj et al., 2013). The degree of deacetylation (%DD) can be determined by using UV, FTIR and NMR spectroscopic techniques (Sneha et al., 2014).

**Material and Methods**

**Reagent:** Sodium Hydroxide(NaOH), Hydrochloric acid(HCl), Potassium permanganate(KMnO$_4$), Oxalic Acid, Acetone, Acetic Acid, Distilled water.

**Sample Collection and Treatment:** Grasshopper was obtained from local market in Damaturu of Yobe state. Grasshopper samples were air dried in the laboratory; gravels and brushwood were removed before grinding with acid washed mortar and pestle. Then, the ground samples were sieved using 0.02mm sieve to obtain uniform particles size of the sample.

**Preparation of Chitin:** The sample of this species was pulverized into find powder and 150g was filtered through a 300um sieve, after the powder was obtained, the effort to extract chitosan from grasshopper was proceed by four methods which are as follows:

Firstly, prepared grasshopper was weight with PW-124 analytical balance to specific mass of 50g, then demineralization was done with 200ml of 1% HCl, in ratio of 1:14w/v at room temperature. The treatment was carried out for duration of 30 minutes; the solution was washed to neutrally with distilled water the residue was then collected with 300um.

Secondly, deprotonation was carried out with the residue whereby 200ml of 3.5% NaOH was added at room temperature, the treatment last for 30minutes. The solution was stirred with glass rod for 5 minutes, the residue was collected with 300um sieve and washed with distilled water until neutral pH was obtain using PHS-3D pH meter.

Thirdly, the product obtained from deprotonation was decolorized with 100ml of 1% KMnO$_4$ for 1hour, the product was collected with sieve washed to neutralize and 100ml of 1% acetone was also added to the solution and allow to stand for 1 hour and the product was collected with 300um sieve and washed with distilled water, the product was dried under oven at 40°C for 30 minutes and chitin was obtained.
Finally, N-deacetylation (from Chitin to Chitosan). The chitin (10 g) was put into 100ml of 50% NaOH at 60°C for 8 hours to prepare crude Chitosan. After filtration, the residue was washed with hot distilled water at 60°C for three times. The crude Chitosan was obtained by drying in an air oven at 50°C for 2 hours. Pure chitin sample of 40g were refluxed in 100ml 50% NaOH solution at 130°C for 2 hours. The product were filtered and washed repeatedly with distilled water and dried at 50°C for 2 hours (Murat et al., 2015).

Figure: Schematic reaction of Chitin to Chitosan

**pH:** the pH measurement of the chitosan solutions was carried out using a PHS-3 pH meter. 20ml solution was added to 10g of grasshopper weighed into a 50ml beaker. The suspension was allowed to stand for 30 minutes with occasional stirring. The suspension was again allowed to stand for another 30 minutes undisturbed to allow the fine particles to settle. The supernatant liquid was decanted into a clean 50ml beaker and the pH was determined using an electrical PHS-3D pH meter.

**Solubility:** chitosan is usually tested in acetic acid by dissolving it in 1% or 0.1M acetic acid. It demonstrated that the amount of acid needed depends on the quantity of chitosan to be dissolved. The solubility of chitosan was demonstrated in various solutions like distilled water, acetone, ethanol, acetic acid and petroleum ether.

**The degree of acetylation and deacetylation of chitosan:** determined using an infrared ray spectroscopy, applying the formula as stated by (Murat et al., 2015)

\[
DDA = \frac{A_{164.084}}{A_{97.862}} \times 100
\]

**Moisture Content** is based on drying a sample in an oven and determining moisture content by the weight difference between dry and wet material. 2 g of previously ground sample was weighed out and placed in drying oven at 105°C for at least 3 hours after which the sample was allowed to cool in a dryer. The sample was weighed again, taking care not to expose the sample to the atmosphere. The following calculation was made afterwards (Shanta et al., 2015)

\[
\text{Moisture content (\%)} = \frac{(B-A)-(C-A)}{B-A} \times 100
\]

Where:
- \(A\) = Weight of clean, dry scale pan (g)
- \(B\) = Weight of scale pan + wet sample (g)
- \(C\) = Weight of scale pan + dry sample (g)

**Ash Content** 2 g of the dried powdered sample was weighed (\(W_1\)) into pre-weighed empty crucible (\(W_0\)) and placed into a muffle furnace at 550°C for 5 hours. The ash was cooled in a desiccator and weighed (\(W_2\)). The weight of the ash will then be
determined by the difference between the powdered dried sample, pre-weighed and the ash in the crucible. Percentage ash was obtained by the following equation:

\[
\text{Ash content (\%) } = \frac{W_2 - W_0}{W_1 - W_0} \times 100
\]

Where:
- \( W_2 \) = Weight of empty crucible (g)
- \( W_0 \) = Weight of crucible + powdered sample (g)
- \( W_1 \) = Weight of crucible + ash sample (g)

_Agricultural Application of Chitosan_ Beans seed has been planted at three different portions, in which two have been combined with chitosan and the other one without chitosan to observe the effect of nematodes against the plant.

**Result**

_Table: 1 pH values for Chitin and Chitosan_

<table>
<thead>
<tr>
<th>S/N</th>
<th>Chitin</th>
<th>Chitosan</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7.25</td>
<td>7.00</td>
</tr>
<tr>
<td>2</td>
<td>7.25</td>
<td>7.25</td>
</tr>
<tr>
<td>3</td>
<td>7.28</td>
<td>7.00</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td><strong>7.26</strong></td>
<td><strong>7.08</strong></td>
</tr>
</tbody>
</table>
### Table 2: Chitosan Moisture and Ash Content Value

<table>
<thead>
<tr>
<th>S/n</th>
<th>Chitosan</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Moisture Content</td>
<td>19%</td>
</tr>
<tr>
<td>2</td>
<td>Ash Content</td>
<td>20%</td>
</tr>
<tr>
<td>3</td>
<td>% chitosan yield</td>
<td>64%</td>
</tr>
</tbody>
</table>

### Table 3: solubility values of chitosan

<table>
<thead>
<tr>
<th>S/N</th>
<th>Reagent</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Acetic acid</td>
<td>Insoluble</td>
</tr>
<tr>
<td>2</td>
<td>Ethanol</td>
<td>Insoluble</td>
</tr>
<tr>
<td>3</td>
<td>Petroleum ether</td>
<td>Insoluble</td>
</tr>
<tr>
<td>4</td>
<td>Water</td>
<td>Insoluble</td>
</tr>
<tr>
<td>5</td>
<td>Acetone</td>
<td>Insoluble</td>
</tr>
</tbody>
</table>
Discussion of Result

Analysis of pH: from the result obtain above, the pH values for the chitin range between 7.26-7.08 which show that the chitin is neutral while for the chitosan it ranges from 7.00-7.06 is also neutral. Which indicate complete demineralization of chitin and deacetylation of chitosan.
**Moisture Content:** the moisture content of chitosan is 19% which is obtained from the analysis, which show that the moisture in the chitin is very low.

**Ash Content:** ash content is one of important parameters that determine the quality of chitosan. The lower the ash content then the higher chitosan quality. The ash content of grasshopper chitosan was determined to be 20% which indicate that the chitosan is of higher quality.

**Solubility:** the chitosan was dissolved in different solvents such as acetone, ethanol, petroleum ether, water and acetic acid. It shows insoluble in all the solvent, which indicate that chitosan deacetylation is 64% because the removal of acetyl group is not completed by demineralization process. When the degree of deacetylation is greater than 90%, the biopolymer becomes soluble in acidic aqueous solutions and behaves as a cationic polyelectrolyte due to the protonation of amine groups in the presence of H\(^+\) ion.

**FTIR Analysis:** from interpretation of FTIR, it can be said that all the functional groups which are added during synthesis have been identified in the of peaks. This indicates the successive formation of chitin biopolymer. During acid hydrolysis of chitin desired temperature is maintained to remove minerals such as calcium carbonate and proteins. It can also be interfered that as shell powder to acid ratio increases, yield and product appearance also increase. The grasshopper chitin shows an intensive peak at 1000-1350cm\(^{-1}\) which correspond to the N-H deformation of amide 1, the band at 1313. cm\(^{-1}\) are attributed to vibration of amide in band 1, at 1690 -1640 cm\(^{-1}\) attributed to C=O group by H bond. The sharp peak at 1551,52 cm\(^{-1}\) corresponds to asymmetric deformation of CH\(_3\) group but at 1040 -1250 cm\(^{-1}\) correspond to a peak of -O-. The FTIR result of chitosan, the spectra correspond to the deacetylated sample with NaOH for 2hrs, which show a spectrum of broad band of N-H from 3500-3300 cm\(^{-1}\) which indicated the presence of primary amine. The band at 1577 cm\(^{-1}\) has a large intensity than 1640 cm\(^{-1}\) when deacetylation occur, which indicate that the band observed at 1640 cm\(^{-1}\) decrease while the growth at 1577 cm\(^{-1}\) occur indicate the presence of NH\(_2\).

**Agricultural Application of Chitosan:** the beans seeds mixed and planted with chitin, chitosan and without both were attacked and destroyed by the nematodes this shows at 64% chitosan conversion has no effect against nematode infection.

**Summary and Conclusion**
The chitosan obtained and prepared using demineralization, deprotonation, decolorization followed by deacetylation. Its pH, solubility, moisture, ash and FTIR spectra was carried out and obtained. The percentage conversion yield shoes no effect against nematode. The result for the chemical reaction as it offers a clean, cheap, and convenient method for extracting chitosan from chitin extracted from grasshopper. Within the results in this work, the conclusion was reached that grasshopper are an excellent source for chitin. The yields and acetylation degree of chitosan increase with increasing the concentration of NaOH solution, the temperature, and the length of treatment. The chitosan obtained showed the highest degree of deacetylation.
REFERENCES


