Preparation and Characterization of Chitosan from *Schistocerca Gregaria* (Desert Locust)

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**ABSTRACT**

The current study prepared and characterized chitosan from *Schistocerca gregaria* (Desert Locust) by a chemical method. The *Schistocerca gregaria* (Desert Locust) chitin and chitosan have yield of 42% and 43% with acceptable physicochemical properties such as Moisture content: 6.0±0.014 and 5.0±0.007 %, Ash content: 2.20 and 2.0%, Protein content 12.0±0.028 and 9.01 ±0.021, Crude fibre8.95±0.041 and 8.95 ± 0.014%, Lipid content, 10.0 ±0.014 and10.0 ±0.014%, however, high carbohydrate, 60.5±0.01 and 65.0±0.01% are quantified in the obtained chitin and chitosan. properties of chitosan prepared were compared with that of the other sources chitosan extracted, the study revealed that (FTIR) analysis confirmed the structure of chitosan by the presence of their characteristic IR bands such as the N–H and O–H stretching bands around 3630 cm⁻¹, oxygen stretching (C=O-C) glycosidic linkage bands around 1125 cm⁻¹, (C-O) in primary OH group bands around 976 cm⁻¹, and 1634 cm⁻¹ corresponding to the free amino group (~NH2) at C2 of glucosamine with 69% degree of deacetylation. Also, the obtained XRD peaks were around 19.01, and 20.48° which indicated the amorphes structure. This indicates the successive formation of chitosan biopolymer. Therefore, the *Schistocerca gregaria* chitosan can be said to be of better quality and hence can replace commercial chitin and chitosan in terms of utilization and applications.

**Keywords:** Schistocerca gregaria (Desert Locust), Chitosan, characterization

1.0 INTRODUCTION

Biopolymers have gotten a lot of interest from both academic and industry, because it happens to be an appealing instrument for numerous biomedical applications, such as, tissue engineering, drug carrier vehicles, and sealants which is due to their biocompatibility and biodegradability (Bergmann et al., 2012). The most significant amount of scientific literature published in this area attests to the importance of chitin and chitosan as universal bio resources. Crab, shrimp and crayfish has been prepared for commercial production of chitin and chitosan and new sources like insect that include Schistocerca gregaria (Desert Locust) and their related species are great area of research (Bergmann et al., 2012).

1.1 Chitosan

Chitosan may be a polysaccharide, assured of the reduplication of component of 2-amine-2-deoxyβ-(1,4)-D-glucosamine as well as 2-acetamide-2-deoxy-β-(1,4)-D-glucosamine, achieved through limited deacetylation of chitin beneath alkaline environment (Elknidri, et al., 2018). The demineralization, deproteinization, and deacetylation are three phases in the chitosan extraction process (Srinivasan, et al., 2017). Chitosan is by far the most common cationic (-NH2) biopolymer on the planet (Ya and Yung 2020). Chitosan is soluble in inorganic and organic acids solutions (pH < 6), because of the protonation (H⁺) of its amino groups. Also due to its polycationic nature, it can combine alongside polyanions to form polyelectrolyte compound (Aranaz, et al., 2021). OH and NH2 functions in chitosan enable it for the creation of a variety of derivatives with enhanced characteristics for specific applications (Korma et al., 2018). Chitosan may be suitable biopolymer that could be utilized in a variety of industries. To minimize the need of chemical conservative also provide edible antimicrobial coatings, Chitosan is an antibacterial and wound-relieve chemical. Because of its unusual chemical, physical as well as biological features (Negm, et al., 2020). In 2023 a study was conducted to extract and
characterize chitosan from two types of shrimp shell (rough and smooth) by (Chengku et al., 2023). The study reported 30.09 ± 0.92 and 26.80 ± 1.05%, 20.75 ± 0.83 and 19.34 ± 1.30% yield of chitosan moisture content of 4.30 ± 0.07 and 4.71 ± 0.26 and ash content of 0.13 ± 0.01 b and 0.09 ± 0.01. for rough and smooth shrimp shells, It also revealed the presence of hydroxyl and amino groups which was certified from FTIR and a good crystallinity index was recorded using XRD of extracted chitosan. And the study concluded that the chitosan extracted from rough and smooth shrimp shells has the potential to be used as a biopolymer in different industrial applications. In 2022 a study was conducted to prepare and characterize the chitosan from white leg shrimp shell waste by (Liyanage, et al., 2022) the study reported (33.53%) of chitosan yield, 8.22 ± 0.66 (%) of moisture content, 1.08 ± 0.24 (%) of ash content, 80.43% of the degree of deacetylation and a good thermal stability also the FTIR analysis confirmed the structure of chitosan by the presence of their characteristic IR bands and X-ray diffraction analysis verified the crystalline structure of the obtained chitosan. Scanning electron microscopic (SEM) images of the extracted chitosan recognized the layers of flakes with porous and fibril structures. The study concluded that the chitosan with high quantity and quality can be prepared from white leg shrimp. But there is little information concerning the extraction of chitosan and chitin and from Schistocerca gregaria (Desert Locust), and its proximate composition. The global market for chitin and chitosan is expanding as demand for biopolymer products rises. Still the utilization of chitosan in some areas remains limited due to a considerable difficulty to access sufficient chitosan, Sub-sahara regions have more shortage due to lack of sea/aquatic animals, this has led to the scarcity of the polymer in these region (Luo et al., 2019). The present research aimed at extraction, preparation and characterization of chitosan from Schistocerca gregaria (Desert Locust). The structure of chitosan is presented schematically in Figure 1.

![Figure 1: Structure of chitosan](https://fugus-ijsgs.com.ng)

### 2. MATERIAL AND METHOD

#### 2.1 Materials

Dried, matured *Schistocerca gregaria* (Desert Locust) which were used for chitosan preparation was purchased from the Old market of Sokoto State. It was prepared by removing the internal organs, the materials were washed, dried inside oven for 2 hours at 50°C and crushed using motor as well as pestle. A powdered sample was obtained, and it was sieved (Chang et al., 2011).

#### 2.2 preparation of chitosan

The prepared exoskeletons were demineralized using 5% HCl (1:15 w/v) at 30°C for 60 mins. The mixture was cooled, filtered, the residue was washed, rinsed 3 times with distilled water to neutrality and then dried (Kaya et al., 2013). After that the demineralized exoskeleton were deproteinized using 3% NaOH solution (1:50w/v). at 30°C for 60 mins, The mixture was filtered via filter paper then the residue was washed with distilled water. The chitin was dried (Andressa, et al., 2021). The chitin obtained from the above process was deacetylated using 50% NaOH (1:50 w/v) solution at 120°C for 120 mins. The resultant chitosan was washed completely to neutrality using distilled water and oven dried for 24 hours at 50°C (Amelia, et al., 2021). The Structural diagram of chitosan preparation are presented in figure 2 below.
2.4 Yield of chitin and chitosan
The yield of chitin and chitosan for the entire process (exoskeleton-to-chitosan) was calculated using the equation 1 and 2 below as described by (Luo, et al., 2019).

Chitin Yield(%) = Dried chitin (g) / Raw exoskeleton used (g) x100……………(1)
Chitosan Yield(%) = Dried chitosan (g) / Dried chitin used(g) x100…………..(2)

2.5 Determination of Proximate composition
(Ash content, Moisture content, Crude fiber, Total Carbohydrate, Protein content, and Crude lipid content) was determine using standard analytical procedure as described by methods of (AOAC, 2005).

2.6 Characterization of the Prepared Chitosan

The prepared chitosan was characterized using FTIR and XRD, and the Degree of deacetylation (DDA) Was assessed through Fourier transform infrared spectroscopy and it was calculated from the Fourier infrared spectra the using equation below as described by (Domszy et al.,1985).

\[ \text{DEGREE OF DEACETYLATION} = 100 \times \left(1 - \frac{1634}{A_{3630}} \right) \times \frac{1.33}{100} \]

3.0 RESULT AND DISCUSSION

3.1 Result
The result of chitosan extraction process from Schistocerca gregaria (Desert Locust) exoskeleton are presented in Table 1. below, and the result of Proximate composition of Raw, Chitin and chitosan of Schistocerca gregaria (Desert Locust) are presented in Table 2 below.

Table 1: chitin and chitosan yield extracted Schistocerca gregaria (Desert Locust) exoskeleton

<table>
<thead>
<tr>
<th>S/N</th>
<th>Extraction process</th>
<th>Schistocerca gregaria (Desert Locust)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Chitin yield (%)</td>
<td>42%</td>
</tr>
<tr>
<td>2</td>
<td>Chitosan yield (%)</td>
<td>43%</td>
</tr>
</tbody>
</table>

Table 2. Proximate composition of raw material, chitin and chitosan extracted from Schistocerca gregaria (Desert Locust)

<table>
<thead>
<tr>
<th>S/N</th>
<th>Composition Analysis (%)</th>
<th>Raw Schistocerca gregaria (Desert Locust)</th>
<th>Schistocerca gregaria (Desert Locust) Chitin</th>
<th>Schistocerca gregaria (Desert Locust) Chitosan</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ash content (%)</td>
<td>6.51 ± 0.141c</td>
<td>2.205 ± 0.007b</td>
<td>2.01 ± 0.007a</td>
<td>0.001</td>
</tr>
<tr>
<td>2</td>
<td>Moisture content (%)</td>
<td>3.01 ± 0.007a</td>
<td>6.01 ± 0.014c</td>
<td>5.00 ± 0.007a</td>
<td>0.001</td>
</tr>
<tr>
<td>3</td>
<td>Protein content (%)</td>
<td>39.015 ± 0.021c</td>
<td>12.02 ± 0.028b</td>
<td>9.015 ± 0.021a</td>
<td>0.001</td>
</tr>
<tr>
<td>4</td>
<td>Crude fibre (%)</td>
<td>9.015 ± 0.021b</td>
<td>8.940 ± 0.041a</td>
<td>8.940 ±0.141a</td>
<td>0.032</td>
</tr>
<tr>
<td>5</td>
<td>Lipid content (%)</td>
<td>12.01 ± 0.041b</td>
<td>10.01 ±0.014a</td>
<td>10.01 ±0.014a</td>
<td>0.001</td>
</tr>
<tr>
<td>6</td>
<td>Carbohydrate (%)</td>
<td>26.798±0.003a</td>
<td>60.510 ±0.0141b</td>
<td>65.015 ± 0.0213c</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Note: values are expressed as mean ± standard deviation, p value < 0.05 show significant difference. Values in the same row bearing similar super script are significantly different at p<0.05).

3.2 DISCUSSION

3.2.1 Chitosan Extraction Process From Schistocerca Gregaria (Desert Locust)
Schistocerca gregaria (Desert Locust) exoskeleton chitin was successfully extracted, purified and converted to chitosan, the sample were analyzed after each step of the chitin purification process to determine changes in their composition, in terms of minerals, proteins, and chitin content. Overall, the extraction and purification processing steps produced Schistocerca gregaria (Desert Locust) chitin and chitosan with low protein and ash compositions, in comparison to their original compositions (Table 2).

3.2.1.1 Chitin Yield (%)
After the demineralization and deproteinization, it was found that the chitin content of the...
Schistocerca gregaria (Desert Locust) exoskeleton was 42% which is relatively higher than 19.34 ± 1.30% reported for rough and smooth shrimp shells of the L. vannamei species (Che et al., 2023). But close to that of commercially utilized such as crabs, and shrimp that varies between 13 and 42% as reported by (S. parthiban and Al-Khateeb, 2003). This findings shows that Schistocerca gregaria (Desert Locust) exoskeleton can be evaluated as alternative source of chitin and its derivatives.

3.2.1.2 Chitosan Yield (%) 
Chitosan was prepared from chitin through deacetylation process and it was found that the chitosan content of the Schistocerca gregaria (Desert Locust) exoskeleton was 43%. Which is higher than 33.53%. Reported for raw mealworm’s cuticles of edible insects Chitosan by (Lucas et al., 2021).

3.2.2 Proximate composition of Raw materials, Chitin and Chitosan Extracted from Schistocerca Gregaria (Desert Locust) 
3.2.2.1 Ash content (%) 
From Table 2, the result revealed that the Schistocerca gregaria (Desert Locust) exoskeleton contained 6.5% ±0.141 ash, while chitin and chitosan has ash content of 2.20±0.07 and 2.0 ±0.07 respectively extracted from this species, which was relatively Lower than 6.77% reported for house cricket (B. portentosus) with chitin and chitosan ash content of 1.0% reported by (Ibitoye, et al., 2018). the ash content obtained for Schistocerca gregaria (Desert Locust) exoskeleton in the present study was also relatively lower than 18.11% for white leg shrimp shells reported by (Mittal et al., 2021).The ash content of chitin and chitosan is indicative of the effectiveness of the method used for removal of inorganic materials.

3.2.2.2 Moisture Content (%) 
From Table 2, the Raw sample of Schistocerca gregaria (Desert Locust) Exoskeleton contained 3.0% ±0.007 moisture on a dried basis, which is Lower than, 21.51%, 20.57% and 22.59% respectively for shrimp, crab and squilla reported by (Parthiban, et al., 2017), while chitin and chitosan has moisture content of 6.0±0.014 and 5.0±0.007 % which is relatively lower than 8.3, 9.32 and 9.10% reported by (Parthiban et al., 2017) for chitin obtained from shrimp, crab and squilla.

3.2.2.4 Protein content 
From Table 2 The Protein content% of Raw, Chitin and chitosan of Schistocerca gregaria (Desert Locust) exoskeleton was found to be 39.0 ±0.021, 12.0±0.028 and 9.01 ±0.021 which was higher than 10.63 ± 0.01%, 0.9 ± 0.02% and 0.2 ± 0.03%, for dry shells, chitin and chitosan reported for Blue Crab by (Zaghbiba et al., 2021) and 8.1, 0.39 ± 0.2 and 0.30 ± 0. Reported for Pelagicus Shell, chitin and chitosan By (Amelia, et al., 2021). but raw exoskeleton protein was relatively low compared to the 51.29% for white leg shrimp shells reported by (Mittal et al., 2021). Part of the remaining protein may be strongly linked to chitin, making it difficult to remove.

3.2.2.5 Crude Fibre 
From Table 2 the raw, chitin and chitosan of schistocerca gregaria (Desert Locust) exoskeleton was found to be 9.0 ± 0.02, 8.95±0.041 and 8.95 ± 0.014 crude fibre. It was also found that the fibre content of the chitosan and chitin was higher than that of the raw sample, probably the removal of more matter from the sample to get chitin and chitosan could have led to the presence of more fibre in the chitosan.

3.2.2.6 Lipid Content 
From the Table 2 the lipid content of of Raw, Chitin and chitosan of Schistocerca gregaria (Desert Locust) exoskeleton contained 12.0± 0.04, 10.0 ±0.014 and10.0 ±0.014 of lipid content this shows that the lipid content was reduced to 10±0.01 and10±0.01 in chitin and chitosan and this is attributed to alkaline treatment of the raw sample at the deproteinization step in the chitin purification. The lipid content for raw exoskeleton was found to be higher than 5.4± 0.58, 1.02±0.01 and 0.3±0.01 for dry shells, chitin and chitosan reported for Blue Crab (Portunus segnis) Shell by (Imen Zaghbiba, 2021).

3.2.2.7 Carbohydrate 
From the Table 2 the carbohydrate content of Raw, Chitin and chitosan of Schistocerca gregaria
(Desert Locust) exoskeleton was found to be 26.8±0.1, 60.5±0.01 and 65.0±0.01, but relatively close to 15.73, 75.12 ± 20.3  54.67 ± 1.7 Reported for Pelagicus Shell chitosan By (Amelia, et al., 2021). Chitin exist in association with proteins and inorganic materials Component in the exoskeleton of insect. Increase in percentage of carbohydrate Component indicating the success of the purification process, since protein and minerals are eliminated during the process.

**Result of Characterization of the Produced Chitosan**

Results of FTIR and XRD analysis of chitosan prepared from *Schistocerca gregaria* (Desert Locust) exoskeleton are presented in fig. 3 and 4 below.

### 3.3.1 Fourier Transform Infrared Spectroscopy (FT-IR)

FT-IR analysis was performed to confirm the structure of the chitosan prepared from *Schistocerca gregaria* (Desert Locust) exoskeleton through the identification of bands characteristic of this polymer. The results are presented in figure 3. The obtained IR spectra in the current study is almost similar to the IR spectra for house cricket and shrimp shells chitosan reported by Ibitoye et al., (2018) and Sewvandi and Adikary (2011). Also the characteristic peaks confirming the identity of chitosan were detected, such as the N–H and O–H stretching bands around 3630 cm⁻¹, oxygen stretching (C–O–C) glycosidic linkage bands around 1125 cm⁻¹, (C–O) in primary OH group bands around 976 cm⁻¹, and 1634 cm⁻¹ corresponding to the free amino group (−NH₂) at C₂ of glucosamine The yielded results were closed to the 3358, 1653, 1151 and 940 cm⁻¹ band peaks obtained in the studies conducted earlier for commercial shrimp chitosan by Ibitoye et al., (2018). (Mohammed, et al., 2013) reported the amide I band as 1658/cm and the amine band as 1632/cm for prawn shells chitosan. It was observed that the1540 cm⁻¹ absorption band which is attribute to protein was absent, hence confirming the effectiveness of deproteinization, also the presence of peak around 1634cm⁻¹ which is the characteristic of amino group proving the success of deacetylation and this. This indicates the successive formation of chitosan biopolymer. explanation was backed up by high DDA of chitosan.

### 3.3.1 X-ray Diffractometry (XRD)

The XRD analysis was performed to reveal the crystalline structure of the chitosan from *Schistocerca gregaria* (Desert Locust). From the result a total of two peaks, including one sharp and one weak peak, around 19.01, and 20.48° and many noisy peaks were observed as indicated in (Figure 4) the appearance of only two peaks and many noisy peaks were ascribed to the amorphores structure, this indicated the destruction of
crystalline structure of the chitosan, Which were close to those from other studies. Two sharp peaks were observed in the chitosan produced from C. barbarus at approximately 10.92° and 20.08° (Kaya et al., 2014). It was observed that peak around 26, 27 and 28 which is attribute to the remaining minerals which as reported by (Kaya, et al., 2014) was absent, and this indicated the effectiveness of demineralization process.

3.3.3 Determination Degree of Deacetylation (DDA)
Degree of deacetylation can be determine by employing FTIR. Two spectral peaks, the OH peak (~3630 cm⁻¹) and the –CO–NH (1634 cm⁻¹) of the Amide I band, were used to calculate the DDA of the Schistocerca gregaria (Desert Locust) exoskeleton chitosans which was found to be 69%.

4.0 CONCLUSION
Extraction of chitin from Schistocerca gregaria (Desert Locust) using chemical method has produced a good quantity chitin and chitosan, (yield of 42 and 43%) with acceptable proximate composition (Moisture content: 6.0 and 5.0%, Ash content: 2.20 and 2.0%, Protein content 12.0 and 9.0%, Crude fibre 8.95.0 and 8.95%, lipid content, 10.0 and 10.0%, however, high carbohydrate 60 and 65% are quantified in the obtained chitin and chitosan. The FTIR study confirmed the structure of chitosan by the presence of their characteristic IR bands such as the N–H and O–H stretching bands, oxygen stretching (C=O–C) glycosidic linkage bands, (C–O) in primary OH group bands and amino group (–NH2) at C2 of glucosamine with 69% degree of deacetylation. Also the obtained XRD patterns indicated the amorphoses structureof the chitosan, This indicates the successive formation of chitosan biopolymer. Therefore, Schistocerca gregaria (Desert Locust) chitosan can be said to be of better quality and can replace commercial chitin and chitosan in terms of utilization and applications. This result will contribute to the knowledge of the development of Schistocerca gregaria (Desert Locust) by revealing that it contains almost fully developed chitin and chitosan structures that have similar properties to those found in other sources.

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