**FUDMA Journal of Engineering and Technology**
**ISSN: 3092-9385**
**https://sites.google.com/fudutsinma.edu.ng/fjet**
**Volume 1, Issue 2, 2025, 885-897**

# Pretreatment and Characterization of Selected Precursors for Chitin/Chitosan Production

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***Abstract***

*This study investigated the pretreatment and characterization of three locally available biological precursors-grasshopper (Schistocerca gregaria), periwinkle shell (Tympanotonus fuscatus), and snail shell (Achatina fulica)-as potential raw materials for chitin/chitosan production. Comprehensive physicochemical analyses, including proximate composition, ultimate analysis, X-ray fluorescence (XRF), X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), Scanning electron microscopy with energy dispersive spectroscopy (SEM-EDS), Textual properties, and thermogravimetric analysis (TGA) were performed to assess the compositional, structural, morphological, and thermal properties of the precursors. Proximate analysis revealed moisture content ranging from 4.44 to 5.60 wt%, ash content from 9.15 to 95.90 wt%, lipid content from 0.24 to 8.58 wt%, crude protein from 2.50 to 25.00 wt%, and crude fiber from 0.57 to 20.62 wt%. XRF analysis indicated high CaO content in snail shell (92.08%) and periwinkle shell (90.89%), while grasshopper showed significantly lower CaO content (8.97%). XRD patterns confirmed aragonite crystalline form of calcium carbonate ($CaCO_3$) in both molluscan shells and calcite-like structure in the grasshopper. FTIR spectra revealed characteristic amide peaks in grasshopper samples and prominent carbonate stretching bands in shells. BET analysis revealed surface areas ranging from 141.05 to 331.53 $m^2/g$, characterized by mesoporous structures. SEM-EDS morphology confirmed the mineral-organic composite nature of all samples. TGA revealed two-stage thermal decomposition patterns with initial moisture loss (81-134°C) and organic matter degradation (368-387°C). The results demonstrated that grasshopper exhibits superior potential as a chitin precursor due to its higher organic content, lower mineral composition, and distinct chitin-related functional groups. However, all three precursors showed viability for chitin/chitosan production, requiring optimized demineralization, deproteinization, and deacetylation processes with varying acid and alkali concentrations tailored to their specific compositional characteristics. This research establishes a foundation for utilizing sustainable, locally-sourced biological waste materials as alternatives to conventional crustacean-based chitin production, promoting circular bioeconomy principles and addressing environmental challenges in Nigeria.*

***Keywords:*** *Chitin precursors, Grasshopper, Periwinkle shell, Snail shell, Characterization, Sustainable biomaterials.*

## 1.0 Introduction

Following cellulose, chitin is recognized as the richest natural polysaccharide, primarily present in the exoskeletons of arthropods, mollusks and fungal cell walls [1]. It's deacetylated form, chitosan, has become widely favoured because of its attributes, such, as biodegradability, biocompatibility, non-toxicity and antimicrobial effects [1]. These features render chitin and chitosan useful across multiple sectors, including pharmaceuticals, agriculture, food packaging, wastewater treatment and biomedicine. Traditionally, commercial chitin and chitosan are extracted from the shrimp and crab shells [2]. However, seasonal availability, high cost, and allergenic concerns have prompted the search for alternative and more sustainable raw materials.

The market value of snail and periwinkle in Nigeria, especially in the coastal region, is continually gaining advantage due to increasing awareness of nutritional compositions, easy access, and affordability amongst locals [3-4]. After consuming the meat, the shells were discarded as waste, thus contributing to environmental and human health challenges [5-6].

Amidst the environmental challenges, during the dry season in the southwest of the country, a large population of grasshoppers (*Zonocerus variegatus*) feeds on cassava plantations and defoliates, a development that can affect food security in an already precarious population [7-8]. Grasshoppers (*Schistocerca gregaria*) are easily harvested for food predominantly by the populace from the northwest, probably due to their taste and nutrients [9]. However, their use as food might endanger the health of consumers owing to the huge application of aerial insecticides to control locust swarms, which are considered agricultural pests [7]. Utilizing these bio-wastes and pests for chitin/chitosan production is expected to be a cost-effective and eco-friendly alternative, aligning with the principles of circular bioeconomy.

Received: 25/11/2025; Revised: 12/12/2025; Accepted: 18/12/2025; Published: 22/12/2025

To achieve environmental pollution control and safe agricultural pest control, some research efforts have successfully extracted chitin/chitosan from grasshopper, periwinkle shells, and snail shells. These include: grasshopper [10], grasshopper [11], periwinkle shells [5], periwinkle and snail shells [12], grasshopper [13]. The characterization of extracted chitin/chitosan is well documented; however, that of the raw precursors is underreported.

This study investigates the potential of three underexplored biomaterials-arthropod (grasshopper), molluscan (periwinkle shell and snail shell)-as novel sources of chitin and chitosan. The findings from this study are expected to provide insights into the suitability of these non-traditional sources and encourage the development of sustainable chitin/chitosan production routes using locally available biological resources.

## 2.0 Materials and Methods

### 2.1 Materials

The materials used in this research work include periwinkle shells (*Tympanotonus fuscatus),* snail shells (*Achatina fulica)*, and grasshoppers (*Schistocerca gregaria).* Reagents and consumables used include barium chloride ($BaCl_2$), copper sulphate ($CuSO_4$), distilled water, sulphuric acid ($H_2SO_4$), hydrochloric acid (HCl), sodium hydroxide (NaOH), and n-Hexane, all of which are analytical grade by Sigma-Adrich and Merck, Germany. Major apparatuses and instruments used include weighing machine (AR2130), oven (DHG 9101.SA), pH meter (PHS-3C), grinder (Kenwood- BLK-828), X-Ray Fluorescence (Genius-IF Xenemetrix), X-Ray Difractormeter (Miniflex 600), Scanning Electron Microscopy (Phenom ProX), FTIR Spectrometer (Shimadzu FITR 8500S), desiccator and crucibles, Distillation Apparatus (Z250325), Kjeldahl apparatus (HYP-308).

### 2.2 Methods

### 2.2.1 Sample collection and labelling

The starting materials for this study was sourced locally in Nigeria during the dry season as follows: Periwinkle shell (*Tympanotonus fuscatus)* from Elele market, Ikwerre Local Government Area, River State with a location coordinate 5$^o$ 6’ 10” N, 6$^o$ 48’ 43” E; snail shell (*Achatina fulica)* from Warri, Warri South Local Government Area, Delta State with a location coordinate 5$^o$ 31’ 38” N, 5$^o$ 43’ 15” E; grasshopper (*Schistocerca gregaria)* from Rimi market, Kano Municipal Local Government Area, Kano State with a location coordinate 11$^o$ 59’ 47” N, 8$^o$ 31’ 29” E. The samples were collected, sorted, and placed in a polyethylene bag for preparation and further studies.

### 2.2.2 Sample preparation

Chitosan precursors (periwinkle shells, snail shells, and grasshopper) were cleaned under running water to remove dirt and organic matter. The washed samples were air-dried for 3 days and placed in a dryer to further dry at 60 $^O$C for 24 hours. The dried samples of grasshopper and periwinkle shell were pulverized with a milling machine (Kenwood BLK-828) into fine powder (150-300μm particle size) to increase the surface area. The snail shell was reduced to a small size with a mortar and pestle before being pulverized in a milling machine (Kenwood BLK-828). All samples were stored in an airtight container [14].

### 2.3 Proximate Composition Analysis

The prepared powdered samples of snail shell (SS), periwinkle shell (PS), and grasshopper (GH) were subjected to proximate analysis, and the components analyzed consist of moisture content, ash content, protein content, volatile matter, and crude fiber content. This was done according to standard and reported literature [11-16]. All reported proximate analysis data were measured in triplicate measurement

### 2.3.1 Percentage Moisture Content (% MC)

The percentage moisture content was determined by taking the weight of empty crucibles and recording it as $W_1$, then 5.00 g of each sample was weighed separately into crucibles, recorded as $W_2$, and dried in an oven (DHG 910SA made in England) at 105 °C for 4 h. The crucibles containing the samples were allowed to cool in a desiccator, and then the final weight of the crucibles and contents was recorded as $W_3$. The percentage moisture content (% MC) was calculated using equation (1) [11-12].

$$\% MC = \frac{(W_2 - W_3)}{(W_2 - W_1)} \times 100 \tag{1}$$

Where, $W_1$ is the Weight of empty crucible (g), $W_2$ is the Weight of crucible plus sample before heating (g), and $W_3$ is the Weight of crucible plus sample after heating (g)

### 2.3.2 Percentage Ash Content

To determine the percentage ash content, an empty cleaned gooch crucible ($W_1$) and 2 g of each sample were added separately to the crucibles and then weighed ($W_2$). Thereafter, the crucible containing samples was placed

Received: 25/11/2025; Revised: 12/12/2025; Accepted: 18/12/2025; Published: 22/12/2025

in an oven at 550 °C for 6 h. After the required period, the samples were allowed to cool in a desiccator and weighed again ($W_3$). Percentage ash content (% Ash) was calculated using equation (2) [16].

$$\% Ash = \frac{(W_3 - W_1)}{(W_2 - W_1)} \times 100 \qquad (2)$$

Where; $W_1$ is the Weight of empty crucible (g), $W_2$ is the Weight of crucible plus weight of sample (g), and $W_3$ is the Weight of crucible + Ash (g)

### 2.3.3 Percentage Protein Content (%CP)

The protein content of the sample was determined using Kjeldahl method with the aid of HYP-308 digestion block. Approximately 0.5 g of powdered sample was heated with 15 ml of concentrated tetraoxosulphate (VI) acid ($H_2SO_4$) and 0.5 g copper (II) tetraoxosulphate (IV) ($CuSO_4$) in a digestion flask, where organic nitrogen is converted to ammonium sulfate. About 10 ml of the digest was introduced into the distillation tube, and then 10 ml of 40 % NaOH was gradually added to make the solution alkaline, ammonia is released and distilled into boric acid solution. The 50 ml of trapped ammonia solution in borate was titrated against 0.1 M hydrochloric acid solution until there was a colour change to pink. Equations (3) and (4) were used to compute percentage nitrogen and percentage crude protein, respectively [13-14].

$$\% Nitrogen = \frac{volume\ of\ acid\ used \times Normality \times 1.4}{Weight\ of\ sample\ taken} \qquad (3)$$

$$\% Crude\ Protein = 6.25 \times \% N \qquad (4)$$

### 2.3.4 Percentage Crude Fiber Content (%CF)

The percentage crude fiber of each sample was determined by weighing 1 g of the powdered sample and adding 200 mL of 1.25% (w/v) tetraoxosulphate (VI) acid to the sample in a beaker, and then allowing it to boil for 30 min. After boiling, the mixture was filtered through a Buchner funnel, washed thoroughly with hot distilled water until acid-free. The residue was transferred back to the beaker, and then 200 mL of 1.25% (w/v) sodium hydroxide was added and allowed to boil for 30 min. The mixture was again filtered, washed with acetone to remove any fat residue. The residue was dried in an oven at 105 °C until constant. The dried residue was weighed as ($W_1$) before being transferred to a crucible for ashing in a muffle furnace operated at 550 $^oC$ for about 4 hours. After the ashing the residual was allowed to cool in a desiccator and weighed again ($W_2$). The % fiber was determined using Equation (5) [4, 14, 15].

$$\% CF = \frac{(W_1 - W_2)}{(Weight\ of\ Sample)} \times 100 \qquad (5)$$

Where; $W_1$ = Weight of residue after digestion and drying (g), $W_2$ = Weight of ash after incineration (g)

### 2.4 Ultimate Analysis/ Elemental Composition

The elemental composition, which comprises carbon content (C), hydrogen content (H), oxygen content (O), nitrogen content (N), and sulphur content (S) of the samples, is determined by the method of Onochie *et al.*, [18].

### 2.5 X-Ray Fluorescence (XRF)

The aliquot was analyzed using Genius – IF Xenemetrix XRF Equipment from the Laboratory of the National Steel Raw Materials Exploration Agency, Kaduna, Nigeria. The samples were loaded into the primary X–ray beam. Samples were transferred into a crystal chamber where diffracted secondary X–ray occurs with the aid of a collimator, and the percentage composition was obtained. Duplicate analyses were performed to obtain average values of the chemical composition of all already prepared samples [16].

### 2.6 Fourier transform Infrared Spectrophotometer (FT-IR)

For functional unit determination, Shimadzu Fourier Transform Infrared Spectrophotometer (FTIR) 8400S was used from the Spectral Laboratory Services, Kaduna State, Nigeria. Ten milligrams of the sample was weighed and homogenized with 0.01 g KBr anhydrous in a mortar and pestle. The mixtures were pressed in a vacuum hydraulic (Graseby Specac) at 1.2 psi to obtain transparent pellets. The spectra were measured in the wave number range of 4000-650 $cm^{-1}$ at 4 $cm^{-1}$ resolution and 32 scans [19].

### 2.7 X-Ray Diffractometer (XRD)

The fractions of various samples were analyzed using Rigaku Miniflex 600 Benchtop XRD Japan, from the Laboratory of the National Steel Raw Materials Exploration Agency, Kaduna, Nigeria. The crystal structure present was analyzed by employing Cu-Kα radiation at 2θ angles $2^o$ - $70^o$. The Scanning was ended after obtaining optimum values by ensuring that the scan axis and angle between the incident x-rays and the sample surface no longer changed significantly [6].

### 2.8 Textual Properties of Selected Chitin Precursors

The textural qualities of the samples were measured using Quantachrome NOVA 4200e2003, USA. Brunauer-Emmett-Teller (BET) technique for precursor materials outgassing was performed on the sample. Moisture and volatiles that might impair the isotherms were removed at 300 $^{o}$C for 3 hrs in a vacuum. Following that, the sample was subjected to nitrogen at 77 K at various incremental pressures[17-19].

### 2.9 Scanning Electron Microscopy (SEM-EDS)

To observe the morphology of the samples, a Phenom ProX G5 2017 Netherlands was used, from the Laboratory of the National Steel Raw Materials Exploration Agency, Kaduna, Nigeria. The sample was speckled on a sample stub. It was then taken to a sputter coater (quorum-Q150R Plus E) with 5 nm of gold. It was then placed on a charge reduction sample holder and introduced into the column of the SEM machine. The sample holder was viewed from a NavCam before it was sent to SEM mode. After adjustment of image brightness and contrast, images with different magnifications were stored in a USB stick [6].

### 2.10 Thermogravimetric Analysis (TGA)/Differential Thermal Analysis (DTA)

The thermal properties of the samples were measured using TGA4000 PerkinElmer. The TGA machine was calibrated; about 5 mg of each sample was weighed into an alumina pan crucible and placed in the analysis chamber (furnace). The samples were heated in the presence of nitrogen gas at approximately 20 mL/min flow rate from room temperature to 1000 $^{o}$C [21].

## 3.0 Results and Discussion

### 3.1 Proximate Composition

Proximate composition analysis is necessary in evaluating processing time, economic viability, and also ascertaining their potential to serve as raw material for various technological applications [16]. Table 1 presents the results of the proximate analysis of GH, PS, and SS, and their compositional variances.

Table 1. Proximate composition of selected chitin/chitosan precursor

| Parameter | Moisture (%) | Ash (%) | Crude Protein (%) | Crude Fibre (%) | Lipid (%) | Reference |
|---|---|---|---|---|---|---|
| Grasshopper (GH) | 5.59±0.23 | 9.15±2.78 | 25.00±1.25 | 20.63±1.02 | 8.58±1.55 | This work |
| | 5.10 | 1.00 | 64.51 | 17.00 | 12.00 | [15] |
| | 5.33±1.16 | 11.50±3.04 | 2.19±0.87 | 2.03±1.01 | 49.33±2.08 | [9] |
| | 5.67±0.58 | 9.83±0.76 | 57.33±0.15 | 10.33±0.29 | 10.67±0.76 | [22] |
| Periwinkle shell (PS) | 4.44±0.23 | 91.83±0.29 | 2.5±1.25 | 2.58±0.75 | 0.32±0.23 | This work |
| | 2.50 | 9.10 | 0.34 | 0.43 | NA | [6] |
| | 35.51±1.31 | 5.44±1.14 | 25.0±1.09 | 4.46±0.41 | NA | [23] |
| Snail shell (SS) | 5.60±0.76 | 95.90±1.79 | 2.92±1.23 | 0.57±0.13 | 0.24±0.16 | This work |
| | 3.00 | 10.00 | 0.30 | 3.96 | NA | [24] |
| | 0.43±0.13 | 94.85±0.11 | 3.18±0.58 | 0.63±0.02 | NA | [25] |

Source: Salisu *et al.*, 2025, Note: NA (Not available).

The moisture content result varies slightly among the three chitosan sources. According to Table 1, GH showed a moisture content of 5.59% which is consistent with 5.667, 5.33, and 5.1% reported by Suleiman *et al.* [22], Sani *et al.* [9], and Olayele *et al.* [15], respectively. PS was recorded to have 4.44%, which is higher than 2.5% reported by Oyawoye *et al.* [6] and far less than 35.51% reported by Idowu and Olatokunbo [23], and the snail shell was observed to have 5.60%, which is higher than both 0.43 and 3.0% reported by Nkansah and Opoku [25] and Jatto *et al.* [24], respectively. The differences in values could be attributed to biological origin, environmental conditions, and processing conditions. This low moisture observed in the study of materials is reported to be helpful for storage stability and processing, as it reduces the risk of microbial degradation during storage before chitin extraction [9, 26].

The ash content is a major sign of inorganic mineral components (Ca, K, and Mg) [9]. The study revealed the ash content of GH, PS, and SS to be 9.15, 91.83, and 95.90%, respectively. The obtained value for the PS is significantly higher than 9.1 and 5.44% reported by Oyawoye *et al.* [6] and Idowu and Olatokunbo [23], respectively. The results obtained for GH in this study are higher than 1.0% observed by Olaleye and Asuquo [15] but align with 9.833% reported by Suleiman *et al.* [22] and slightly lower than 11.50% reported by Sani *et al.* [9]. In the case of SS, the value obtained aligns closely with 94.85% reported by Nkansah *et al.* [25] and is significantly higher than 10% reported by Jatto *et al.* [24]. The observed variations in ash values are attributed to differences in mineral content [9]. This parameter is reported to be significant for the demineralization step in chitosan production, as higher ash content will require thorough acid treatment to remove calcium carbonate and other minerals [26].

The Protein content differs significantly among the chitosan precursors, and follows the order GH (25%) > SS (2.92%) > PS (2.5%). Comparing with other literature, the obtained value for GH is higher than 2.19% reported by Sani *et al.*, [9] but lower than 57.33 and 64.51% reported by Suleiman *et al.*, [22] and Olaleye and Asuquo [15] respectively. The result of the PS is higher than 0.34% reported by Oyawoye *et al.*, [6] and lower than 25.00% reported by Idowu and Olatokunbo [23]; while that of the SS was found to be higher than both 3.18 and 0.30% reported by Nkansah *et al.*, [25] and Jatto *et al.*, [24], respectively. The differences in values can be attributed to biological nature, habitat, and feed among the species. This observation also suggests the requirements of deproteinization during chitin extraction [26].

The results of crude fibre vary among the precursors and follow the order for GH (20.63%) > PS (2.58%) > SS (0.57%). The obtained value of crude fibre in GH was substantially higher than 2.03, 10.333, and 17.0% reported by Sani *et al.* [9], Suleiman *et al.*, [22], and Olaleye and Asuquo [15], respectively. The obtained percentage for the PS was lower than 4.46% reported by Idowu and Olatokunbo [23] and lower than the 0.43% obtained value by Oyawoye *et al.*, [6], while, the value measured for the SS was lower than 0.63 and 3.96% reported by Nkansah *et al.*, [25] and Jatto *et al.*, [24], respectively. This was due to species differences, sample preparations, and analytical methods. In insects, this high fibre content is largely attributable to the presence of chitin in the exoskeleton, suggesting that GH may be a more promising source of chitin [27].

Lipid content was observed across chitosan sources, with GH containing 8.58%, which is lower than 49.33, 12.0, and 10.67% reported by Sani *et al.*, [9], Olaleye *et al.*, [15] and Suleiman *et al.*, [22], respectively. PS sample was observed to be 0.32%, and the SS with the least, 0.24%. The value of lipid content for shell samples was very low, and there is limited literature reporting. The difference in lipid content can be attributed to the fact that grasshoppers are active insects with significant fat storage for metabolic functions, while snail and periwinkle shells are mineral structures with low organic and lipid content [9, 25]. It was suggested that to obtain pure chitin, the lipid must be removed during the extraction process [19, 26].

### 3.2 Ultimate Analysis/Elemental Composition

The ultimate analysis determines the elemental composition of a material, which includes the percentage carbon (C), hydrogen (H), oxygen (O), nitrogen (N), and sulphur (S).

Table 2. Elemental composition of selected chitin/chitosan precursors.

| Material | C (%) | H (%) | O (%) | N (%) | S (%) | References |
|---|---|---|---|---|---|---|
| Grasshopper (GH) | 54.57±0.94 | 1.13±0.03 | 39.55±0.96 | 4.00±0.20 | 0.75±0.01 | This work |
| Snail shell (SS) | 37.73±0.01 | 1.16±0.02 | 60.64±0.03 | 0.40±0.20 | 0.07±0.01 | This work |
| Periwinkle shell (PS) | 29.56±0.32 | 1.02±0.01 | 68.77±0.38 | 0.47±0.20 | 0.18±0.01 | This work |
| Blue Crab | 30.00-40.00 | 4.00-6.00 | 30.00-40.00 | 6.00-7.00 | 0.00-1.00 | [28] |

Source: Salisu *et al.*, 2025

The elemental composition of chitosan precursors is significant as it underlies both the biochemical synthesis and the resulting properties of chitin [10]. Table 2 shows the elemental composition of GH, SS, and PS, respectively.

The carbon content for GH, SS, and PS was observed to be 54.57, 37.73, and 29.56%, respectively. The values for PS and SS were observed to fall within the range 30.00-40.00% reported for crustacean species (blue crab) by Demir *et al.*, [28]. The value of GH was higher, and this was attributed to the differences in biological composition, suggesting a combination of organic matter and mineral components [5, 29]. Research studies indicate that materials with higher carbon content have more potential for chitin production [30].

The hydrogen percentage for chitosan precursors was found to be approximately the same. The low values obtained are compared to the range 4.00-6.00% reported by Demir *et al.*, [28] for blue crab. The observed differences may arise from biological compositions and structural differences [5].

The oxygen content of the GH, SS, and PS was observed to be 39.55, 60.64, and 68.77%, respectively. The values of PS and SS were found to be substantially higher than GH. Comparing with other literature, the value for GH aligns with the range value 30.00-40.00% reported by Demir *et al.*, [28], however, differs from the values for SS and PS. The obtained values and differences observed can be attributed to the presence of high mineral content and other oxidized materials in the shells, as reported by Elegbede *et al.*, [4] and Nkansah *et al.*, [25], respectively.

In PS and SS, oxygen is often found bonded with the $CaCO_3$ organic chitin molecule, while in the GH, the oxygen reflects the polysaccharide (chitin) and protein matrix composition [5, 26, 31].

The obtained nitrogen content for GH revealed a value of 4.00% which is higher than both 0.40 and 0.47% for SS and PS, respectively. These values differ from 6.00-7.00% reported for the blue crab precursor [28]. The large significant difference observed in nitrogen content can be attributed to protein content or treatment, and affects the degree of deacetylation and molecular weight of the produced chitosan [26].

The sulphur content was determined to be consistent across the chitosan precursors with GH (0.75%) > SS (0.18%) > PS (0.07%). These values fall within the range 0-1.00% reported by [28]. According to Owoyemi *et al.*, [32]. The presence of sulphur content in chitosan sources originated from amino acids and vitamins present in the samples.

### 3.3 X-Ray Fluorescence (XRF)

X-ray fluorescence is a non-destructive analytical technique used to determine the elemental composition of a wide range of material samples. Table 3 shows the elemental oxide composition of samples of periwinkle shell (PS), snail shell (SS), and grasshopper (GH) using X-ray fluorescence (XRF).

Table 3: Elemental oxide composition of selected chitin/chitosan precursors.

| Material/Parameter | CaO | $P_2O_5$ | $K_2O$ | MgO | $Al_2O_3$ | $SiO_2$ | $Fe_2O_3$ | $SO_3$ | References |
|---|---|---|---|---|---|---|---|---|---|
| PS (%) | 90.89 | NA | NA | NA | 3.96 | 2.50 | 0.59 | 0.20 | This work |
| | 87.60 | 0.01 | 0.1 | 3.9 | 2.6 | 1.8 | 0.8 | 0.3 | [29] |
| SS (%) | 92.08 | NA | 0.06 | 0.95 | 3.41 | 1.36 | 0.14 | 0.36 | This work |
| | 97.14 | 0.004 | 0.212 | 0.152 | 0.180 | 0.628 | 0.409 | 0.073 | [33] |
| | 84.38 | 0.08 | 0.15 | 0.06 | 1.22 | 0.69 | 0.07 | NA | [32] |
| GH | 10.05 | 10.38 | 20.75 | 8.97 | 0.35 | 15.29 | 3.50 | 18.89 | This work |

Source: Salisu *et al.,* 2025, NA: Not available

The results of both PS and SS confirm the presence of calcium in the form of CaO as the predominant component, with values of 90.89% and 92.08%, respectively. This observation is in agreement with the values 87.6%, 97.14% and 84.38% reported by Adah *et al.*, [29], Laskar *et al.*, [33], and Owoyemi *et al.*, [32], respectively. The high amount of CaO is attributed to high mineral content in the materials and thus, the need for acid treatment to isolate the organic polymer (chitin) beneath it [5]. For the GH the value of CaO is 10.05% indicating the presence of calcium from the exoskeleton [34]. Other oxides recorded include $P_2O_5$ (10.38%), MgO (8.9%), $K_2O$ (20.75%), and $Fe_2O_3$ (16.6%). There is limited literature on the oxide value of grasshopper as it is predominantly organic material; however, the presence of elements such as (Ca, P, K, Mg, Fe) was confirmed by [35]. Other oxide values of the shells that do not reasonably affect chitin quality were found in trace amounts, including: $SiO_2$ (2.50%) in PS and 1.36% in SS. The results are slightly higher than 1.8% and 0.69% reported by Adah *et al.*, [29], and Owoyemi *et al.*, [32], respectively. Additionally, around 3.96% and 3.41% of $Al_2O_3$ were found in PS and SS, respectively, which is slightly higher compared to 2.6% and 1.22% reported by Adah *et al.*, [29], and Owoyemi *et al.*, [32], respectively. The difference in the minor oxide value can be influenced by the species environment, such as seawater [36].

### 3.4 Fourier-transform infrared (FT-IR)

The FT-IR is conducted to gain information regarding the molecular structure and chemical composition of the material [16]. The FTIR spectra of PS, SS, and GH are shown in Figure 1.

Received: 25/11/2025; Revised: 12/12/2025; Accepted: 18/12/2025; Published: 22/12/2025

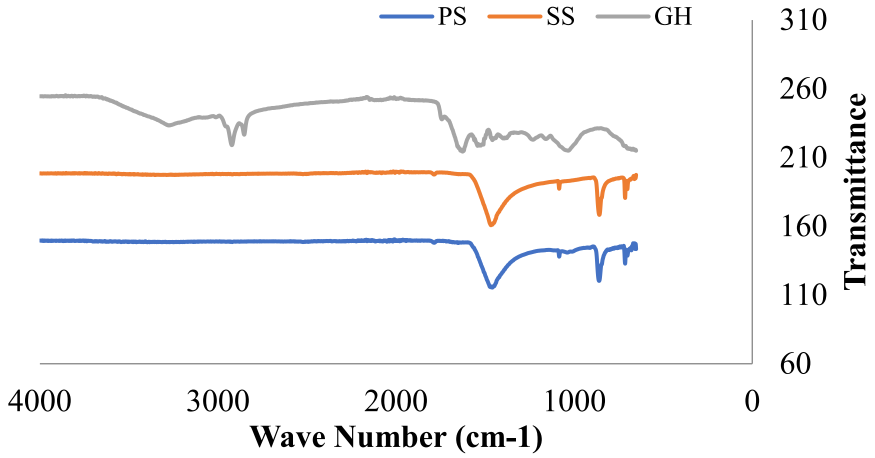

Figure 1: FTIR spectrum for PS, SS, and GH

The FT-IR spectra of mollusk shells (SS and PS) revealed four similar strong bands, although with slight differences around 1457-1466, 1082, 859-857, 711-719 cm$^{-1}$, assigned to carbonate stretching ($CO_3^{-2}$), indicating the presence of aragonite or calcite form of $CaCO_3$ [5, 21,38]. These peaks are expected to disappear with acid treatment during demineralization in chitosan production [5]. Other vibrational peaks that appears around 3397-3800 cm$^{-1}$ can be attributed to O-H or N-H stretching of the organic matrix, the peak at 2521 cm$^{-1}$ can be associated with carbonate group ($HCO_2^{3-}$), while peaks at 1787 cm$^{-1}$ can be attributed to C=O stretching indicating presence of carbonate ions and that at 1645 cm$^{-1}$ is considered to be an Amide I arises from C=O stretching vibrations of protein [37]. The FT-IR of arthropod (GH) revealed vibration peaks at 3274 and 3011 cm$^{-1}$ that corresponds to O-H stretching and carbon related compounds, peaks at 2922 and 2853 cm$^{-1}$ relate to C-H stretching, indicating the presence of fat, peak at 1742 cm$^{-1}$ represents ester group (C=O) vibration of an acetyl group, while those at 1625 and 1541 cm$^{-1}$ can be attributed to Amide I and Amide II stretching arising from peptide of protein, the one at 1230 cm$^{-1}$ corresponds to Amide III and phosphate vibrations and the peak at 1032 cm$^{-1}$ related to C-O-C stretching of polysaccharide [38].

## 3.5 X-Ray Diffraction (XRD)

The X-ray diffraction is conducted to gain information regarding the structural and physical characteristics of crystalline materials [21]. The XRD patterns of GH, PS, and SS are shown in Figure 2.

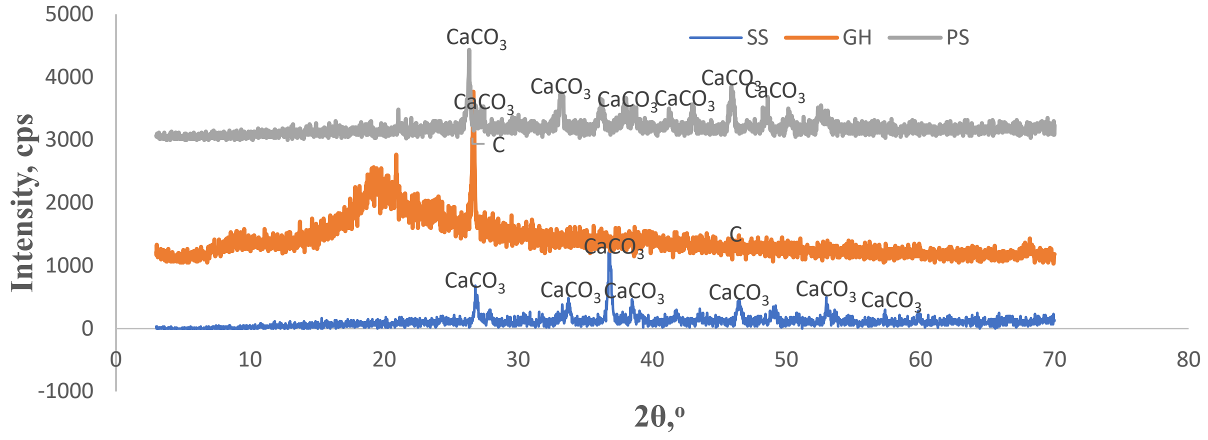

Figure 2: XRD analysis of PS, SS, and GH

For SS, prominent diffraction peaks (2θ) are observed at 26.34, 27.46, 33.36, 36.28, 45.9, and 48.6 °, indicating the presence of aragonite, a metastable form of $CaCO_3$ [21]. In the case of PS, prominent peaks were observed at 26.82, 33.74, 36.86, 38.48, 46.48, 52.98, and 57.36 ° indicating the presence of $CaCO_3$ with aragonite crystalline form. This result is in agreement with Oyawoye *et al.*, [6], who reported a major peak at 26.2 ° and Gbenbor *et al.*, [5], who also reported a prominent peak at 27.07, 35.84, 42.72°. In the grasshopper spectrum, the highest diffraction peak was observed around 26.58 °. According to Mohan *et al.*, [31], this indicates the presence of carbon in graphite form that could be attributed to the abundance of chitins, lipids, and proteins, all of which are carbon-rich organic compounds.

Received: 25/11/2025; Revised: 12/12/2025; Accepted: 18/12/2025; Published: 22/12/2025

### 3.6 Textual Properties of Selected Chitin/Chitosan Precursors.

Textual properties analysis was carried out to determine the surface area, pore size, and porosity characteristics. These properties have a direct impact on the efficiency and quality of extracted chitosan [20]. The results of the analysis are presented in Table 4.

Table 4. Surface textual properties of precursors: grasshopper (GH), periwinkle shell (PS) and snail shell (SS)

| Sample | Surface area ($S_{BET}$), $m^2/g$ | Average pore width(nm) | Micropore volume (cc/g) | Pore diameter (nm) | Pore volume (cc/g) | Reference |
|---|---|---|---|---|---|---|
| Grasshopper (GH) | 141.046 | 5.246 | 0.068 | 2.442 | 0.086 | This work |
| Periwinkle shell (PS) | 213.214<br>434.90<br>628.3 | 5.272<br>5.74<br>2.45 | 0.094<br>0.10<br>0.37 | 2.411<br>NA<br>2.11 | 0.136<br>0.27<br>NA | This work<br>[39]<br>[40] |
| Snail shell (SS) | 331.533<br>356.34 | 5.767<br>6.366 | 0.135<br>NA | 2.118<br>NA | 0.201<br>0.1826 | This work<br>[41] |

Source: Salisu *et al.*, NA: Not available

According to Table 4, the surface area $S_{BET}$ for GH, PS, and SS was found to be 141.05, 213.21, and 331.53 $m^2/g$, respectively. In comparison with other literature, the value for PS was significantly lower than 434.90 and 628.3 $m^2/g$ reported by Nworie *et al.*, [39] and Nkwoada and Onyedika [40], respectively. The value of SS was consistent with 356.34 $m^2/g$ reported by [41]. This observation demonstrated that the porosity differs between precursors.

The surface area was in the order of PS > SS > GH. This indicates that the PS has a greater surface area than the other precursors. Average pore widths were revealed to be consistent between the chitosan precursors, with GH, PS, and SS having 5.246, 5.272, and 5.767 nm. Comparatively, the value of PS aligns with 5.74 nm reported by [39]; however, lower than 2.45 nm reported by [40]. The value of SS was slightly lower than 6.366 nm reported by [41]. All values fall within the range 2-50 nm, suggesting a mesoporous structure [42]. The pore diameter was observed to be consistent among the GH, PS, and SS with corresponding values 2.442, 2.41, and 2.118 nm, respectively. The value of PS align with 2.11 nm reported by [40]. According to Datta *et al.*, [42] a high surface area of precursors indicates availability of reactive surface for chemical reactions such as deproteinization, demineralization and deacetylation in chitin/chitosan extraction, affecting extraction yield and quality.

The micropore volume and total pore volume were observed to be highest for SS with a value of 0.135 and 0.201 cc/g followed by PS with a value of 0.094 and 0.136 cc/g, least for GH with a value of 0.068 and 0.086 cc/g. The micropore volume for PS was consistent with 0.10 cc/g reported by Nworie *et al.*, [39], but lower than 0.37 cc/g as reported by Nkoada and Onyedika [40]. While the total pore volume for PS was lower than 0.27 cc/g reported by Nworie *et al.*, [39] and that of SS align with 0.1826 cc/g reported by [41]. This shows SS has more developed micro and mesoporosity, enhancing surface accessibility.

### 3.7 Scanning Electron Microscopy (SEM-EDS)

The SEM-EDS is conducted to gain information regarding the morphological characterization of the surfaces of organic and inorganic samples [5]. Figure 3 shows the SEM-EDS of PS, SS, and GH.

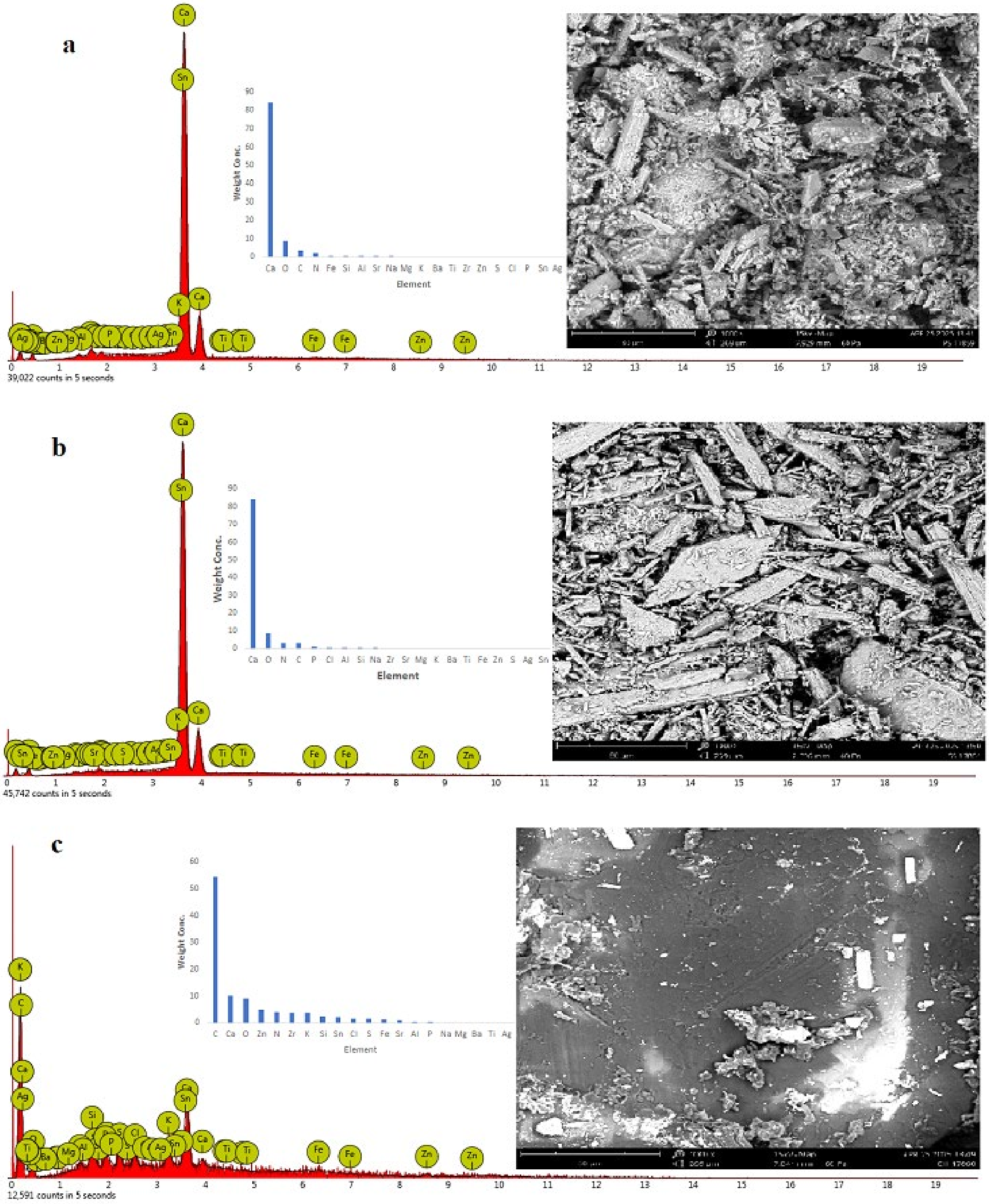

Figure 3: SEM-EDS analysis of (a) PS, (b) SS, and (c) GH

According to Figure 3a, the PS exhibits a rod-like and granular particulate shape with an irregular, rough surface. The rough surface can be attributed to mechanical milling and the presence of $CaCO_3$ [5]. Some portion appears as fibrillar clusters. This is most likely due to chitin microfibrillar embedded in $CaCO_3$ matrix [12]. According to Figure 3b, the SS shows a noticeable Irregular rod-like particles and also rod-like structure with straight edges. According to Laskar *et al.*, [33] this feature indicates a crystalline mineral phase, consistent with aragonite. The EDS results revealed that calcium-related compounds dominated the test samples, followed by oxygen, carbon and nitrogen for both periwinkle shell and snail shell, corroborating the findings of SEM, FTIR, XRD and XRF as presented in Figure 3a and 3b.

The SEM Image of GH in Figure 3c, revealed a surface appearing rough and irregular with a mixture of fine particles and irregular fragments. While the EDS shows a high percentage of carbon (74.52%) follow by oxygen (9.23%), nitrogen (4.6%), and calcium (4.11%). The observation is consistent with organic polymeric material (chitin, protein) and mineral content (calcium content) detected by SEM morphology [37].

Received: 25/11/2025; Revised: 12/12/2025; Accepted: 18/12/2025; Published: 22/12/2025

### 3.8 Thermogravimetric Analysis (TGA)/Differential Thermal Analysis (DTA)

Thermogravimetric analysis and Differential thermal analysis provide an insight into thermal stability and composition of the raw materials by determining weight changes as a function of temperature increase [16]. Figure 4a-c shows the TGA/DTA thermogram for PS, SS, and GH.

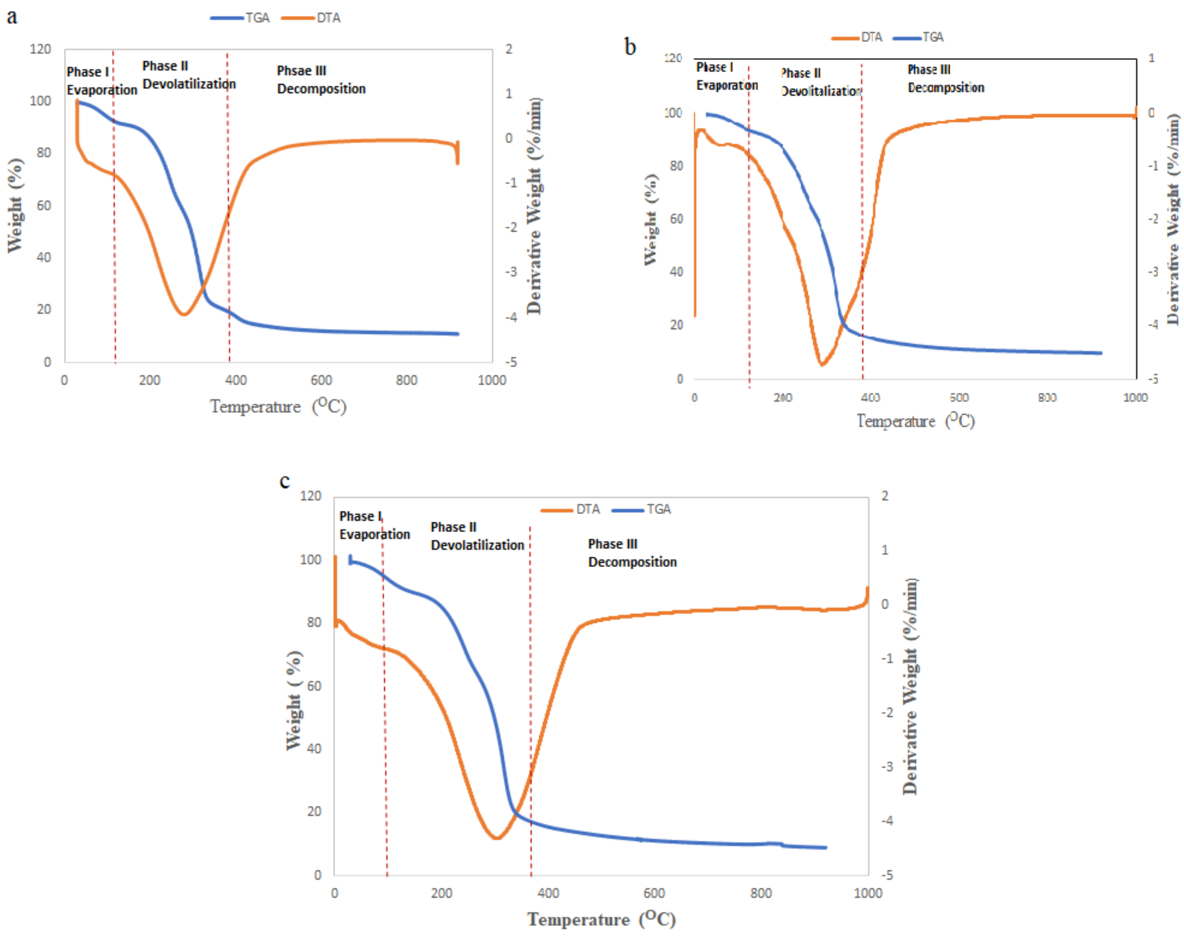

Figure 4: TGA/DTA of (a) PS, (b) SS, (c) GH.

Two major weight losses were observed, with the first between 84-134, 83-130, and 81-124 °C for PS, SS, and GH, respectively. These observed temperatures are within the ranges of 30-100, 27.7- 320, and 0-150 °C for PS, SS, and GH as reported by Gbenebor *et al.*, [5], Kolawole *et al.*, [43] and Kaya *et al.*, [10], respectively. The observed weight loss could be attributed to the loss of moisture present in the samples, as revealed in the proximate analysis presented in the previous section and supported by the findings reported by [5] and [21]. The second weight loss was observed at temperature values 386, 387, and 368 °C for PS, SS, and GH, respectively. This second stage of weight loss is attributed to decomposition of organic matter [40]. Other research on GH also reported two-stage mass losses [10]. While the DTA curves show a sharp, deep endothermic trough indicating heat absorption due to decomposition of organic and initial inorganic transformation [5]. The maximum DTA was observed at 282, 278, and 288 °C for PS, SS, and GH, respectively, which could be due to the release of structurally bound water and slight decomposition [5].

## 4.0 Conclusion

This research effectively identified the characteristics of three accessible biological sources-GH (*Schistocerca gregaria*), PS (*Tympanotonus fuscatus*), and SS (*Achatina fulica*)-to evaluate their potential as feedstocks for chitin/chitosan synthesis. Detailed investigations, comprising proximate composition, analysis, XRF, XRD, FTIR, SEM-EDS, BET, and TGA/DTA uncovered unique physicochemical traits that guide the best extraction methods for each source.

The proximate analysis revealed differences in composition across the samples, with GH containing elevated protein (25.00%) and crude fiber levels (20.63%), whereas both molluscan shells displayed markedly greater ash content (91.83% and 95.90% for PS and SS, respectively), reflecting their high mineral content. The prevalence of CaO (90.89-92.08%) in the samples, as verified by XRF and XRD assessments, underscores the need for thorough demineralization procedures. In contrast, the comparatively lower CaO concentration (10.05%) in GH indicates a decreased demand for acid in processing.

FTIR examination verified the existence of functional groups linked to chitin and protein in GH, showing distinct amide peaks (Amide I at 1625 $cm^{-1}$ and Amide II at 1541 $cm^{-1}$), whereas molluscan shells displayed notable carbonate stretching bands characteristic of $CaCO_3$. XRD profiles indicated an aragonite crystalline structure in both shell samples and a calcite-like formation in the GH. The textual property analysis revealed surface areas between 141.05 and 331.53 $m^2/g$ with all samples demonstrating frameworks conducive to chemical treatment penetration. SEM-EDS examination corroborated the findings showing the mineral-composite nature of the precursors.

Thermal analysis shows two-stage decomposition forms for all samples, with initial weight loss attributed to moisture removal (81-134°C) and subsequent degradation of organic matter (368-387°C), providing insights into optimal processing temperatures and thermal stability.

Considering these characteristics, GH stands out as a notably favorable precursor because of its elevated organic material, lesser mineral content, and distinct chitin-associated functional groups, indicating a possibly greater chitin output with milder demineralization needs relative to molluscan shells. Nonetheless, all three precursors prove suitable for chitin/chitosan synthesis with processing conditions-especially acid and alkali concentration, for demineralization, deproteinization, and deacetylation, necessitating optimization according to their unique compositional characteristics

This research contributes to the development of sustainable, locally-sourced alternatives to conventional crustacean-based chitin production, addressing environmental challenges related to bio-waste disposal while enhancing circular bioeconomy principles. The use of these underexploited biomaterials not only provides cost-effective raw material choices but also provides solutions to agricultural pest management and waste valorization in Nigeria. Future work should focus on optimizing extraction conditions specific to each precursor and evaluating the quality and functional properties of the extracted chitin/chitosan for various industrial applications.

## 5.0 Acknowledgements

This research did not receive any specific grants from funding agencies in the public, commercial, or non-profit sectors. The authors sincerely thank the Department of Chemical Engineering, Faculty of Engineering, University of Abuja, for supporting and providing the enabling environment needed for this study. The authors also wish to thank Mr. Bulus Baba Musa and Anas Ibrahim for technical support.

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