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# Structural Characterization of α-Chitin Extracted from Selected Mollusk Shells

**Ejiro ProsperAwhin[1], Iruoghene Onosakponome[2]*, Alfred Ikechukwu Ajo[h1], Abigail Okezi Isoje[2], Gabriel Erhiga Odesa[3], Omoyele David Omolade[1], Prosper Chinyelum Okwuenu[4], Adefunke Olukemi Opajobi[1], Ugochukwu Enyinnaya Uzuegbu[1], Sabinus Oscar Onyebuchi Eze[5]**

[1]Department of Medical Biochemistry, Delta State University, Abraka, Nigeria
[2]Department of Biochemistry and Molecular Biology, Dennis Osadebay University, Asaba, Nigeria
[3]Department of Geology, Dennis Osadebay University, Asaba, Nigeria
[4]Department of Industrial Chemistry, Dennis Osadebay University, Asaba, Nigeria
5Department of Biochemistry, University of Nigeria, Nsukka, Nigeria

***Corresponding Author**: iruoghene.onosakponome@dou.edu.ng

**Abstract**

The present study was carried out to extract and characterize chitin from selected mollusk (periwinkles, oysters, cowries, and water snails) shell wastes. The extraction of chitin from the shells of the selected mollusks was carried out by the chemical method. The characterization of extracted chitin was carried out using X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FT-IR), and Scanning electron microscopy (SEM). The chitin yield from these shells ranged from 57.95 % to 70.9 % on a dry-weight basis. Analyses using XRD, FT-IR, and SEM confirmed that the chitin obtained was of the α-form. Using XRD, the extracted chitin's crystallite size and crystallinity index varied between 29.16 nm and 40.8 nm, and 16.73% to 44.36%, respectively. FTIR analysis revealed characteristic peaks at 1684.8 $cm^{-1}$ (C=O stretching, amide I) and 1785.4 $cm^{-1}$ (C=O stretching in the $NHCOCH_3$ group). SEM images showed a rough surface texture and a fibre-like structure of the extracted chitin. These findings indicate that the α-chitin produced can enhance mechanical interlocking, when used in composite materials, potentially enhancing bonding with substances. The findings of this study suggest that chitin is not only abundant in mollusk shell wastes, but the obtained chitin also possesses favourable structural properties for various applications.

**Keywords:** Chitin, Characterization, FTIR, XRD, SEM, Mollusk shell wastes

## Introduction

The consumption of mollusks has significantly increased over the years, making them a valuable source of polyunsaturated fatty acids (1). However, the outer shells of most mollusks are inedible, leading to the generation of substantial shell waste (2; 3). These shells are abundant in Nigeria's riverine areas, but a large portion is simply discarded into the sea or landfills, contributing to environmental pollution (4). Cadano *et al.* (5) estimated that 250,000 metric tons of waste are produced from aquatic animals, while Nirmal *et al.* (6) noted that only 30–40% of mollusks are processed for food, with the remaining 60–70% disposed of as waste or by-products. Globally, approximately 8 million tons of waste from mollusk shells are generated each year (7).

Recycling mollusk shell waste into useful products is an emerging concept aligned with the United Nations Sustainable Development Goals, converting waste resources into valuable biomass. Saravanan *et al.* (8) highlighted that periwinkle shell waste contains bioactive compounds, including proteins, polysaccharides (such as chitin), pigments, and lipids. Additionally, periwinkle shells are rich in calcium carbonate and minerals (9). The composition of

periwinkle shell waste typically includes 20 – 50% minerals (mainly calcium carbonate), 20–40% proteins, and 15 – 40% polysaccharides, with chitin being the predominant polysaccharide. Chitin is a cationic polymer composed of β-(1,4)-linked 2-acetamido-2-deoxy-D-glucopyranose units. Chitosan, a derivative of chitin, is produced through its deacetylation. Besides crustaceans, chitin is also found in insects (10) and mushrooms (11). The yield of chitin and chitosan varies by species (12). Notably, chitin is insoluble in water, while chitosan dissolves in acidic water systems. Mohan *et al.* (13) identified three allomorphs of chitin (α, β, γ-forms). Recent research by Saravanan *et al.* (14) has explored innovative applications of chitin and chitosan in the pharmaceutical and biotech industries.

Understanding the structural components of chitin is essential for optimizing its extraction process and determining its suitable applications (12). Characterizing chitin from aquatic animal shells requires techniques such as X-ray diffractometry (XRD), energy-dispersive X-ray spectroscopy (EDX), FTIR, scanning electron microscopy (SEM), thermogravimetric analysis (TGA), and nuclear magnetic resonance spectroscopy (NMR) (12). Such characterization will provide insights into the chitin's substituents, surface morphology, mineral composition, and chemical properties, enhancing its applicability. This study aims to characterize chitin extracted from the shells of periwinkles, oysters, cowries, and water snails sourced from the shores of Warri, Delta State, Nigeria.

## Materials and Methods

### *Materials*

All chemicals and reagents used in this study were of analytical grade and sourced from Merck (Mumbai, India) and SD Fine Chemical Ltd. (Mumbai, India).

### *Collection and preparation of mollusk shells*

Mollusk shells—specifically those from periwinkles, oysters, cowries, and water snails—were collected from the Warri waterside in Delta State, Nigeria, with an average weight of 100 – 150 g. The shells were packaged in polyethylene bags and transported to the laboratory. In the laboratory, the shells were thoroughly washed with running tap water and then treated with boiling water at approximately 98 °C. After cleaning, the shells were dried in an oven at 60 °C until they reached a constant weight. Subsequently, the dried shells were crushed into powder using a high-speed mechanical blender (Philips Blender, Mumbai, India). The powdered samples were then sieved through a 60 µm mesh to ensure uniformity. The preparation of the powdered shells followed the method outlined by Alabaraoye *et al.* (15).

### *Extraction of chitin*

Chitin was extracted from mollusk shells through a three-step process: demineralization, deproteination, and decoloration (depigmentation).

### *Demineralization of chitin*

Demineralization was conducted following the method outlined by Abdou *et al.* (16) using 2 M hydrochloric acid (HCl). A sample of 100 grams of dried powdered shells was treated with 2 M HCl at room temperature, with constant agitation at 100 rpm. The mixture was allowed to stand for 24 hours to remove mineral content from the pulverized periwinkle shells. The chitin yield was determined by calculating the weight difference between the dried shells and the resulting chitin.

### *Deproteination of chitin*

The demineralized chitin underwent deproteination as described by Yugandhar and Ravi (17). Following the 24-hour demineralization period, the demineralized chitin was washed multiple times with distilled water to eliminate $CaCl_2$ and other water-soluble impurities until a neutral pH of 7 was achieved. The washed chitin was then treated with 1 M sodium hydroxide (NaOH) and refluxed at 60 °C for 24 hours to remove residual proteins and organic

materials. The resulting heterogeneous mixture contained chitin, which was subsequently separated by filtration using a vacuum pump. The precipitate was thoroughly washed with distilled water until the pH reached 7.0. The percentage yield of chitin was calculated according to the equation provided by Santos *et al.* (18).

$$\%\, Yield\ of\ chitin\ or\ chitosan\ = \frac{Weight\ of\ chitin\ obtained}{Initial\ weight\ of\ raw\ shell\ used\ for\ chitin\ extraction} \times 100$$

### *Decoloration (depigmentation) of chitin*

After 24 hours of deproteination, decoloration was performed following the method described by Sarbon *et al.* (19). The deproteinated shells were treated with 0.1 % hypochlorite solution at room temperature for 48 hours. The samples were then separated using vacuum filtration and washed with distilled water until achieving a neutral pH of 7.0. Finally, the samples were oven-dried at 60 °C until a stable weight was reached.

### *Physicochemical properties of the extracted chitin*

### *Determination of moisture content*

The moisture content of the chitin was determined using the gravimetric method as described by Olafadehan *et al.* (20). A 1 g sample was placed in a pre-weighed container and dried in a hot air oven at 60 °C for 6 hours until it reached a constant weight. The mass of water was calculated by comparing the mass of the sample before and after drying. The moisture content (MC) was calculated using the following equation:

$$\%\, MC = (M - m) \times 100/M \quad \text{..... (Eq 2.)}$$

where *M* and *m* are the respective mass of wet and oven-dried samples (g).

### *Determination of ash content*

The ash content (% AC) of each sample was determined using the method outlined by Olafadehan *et al.* (20). A 1 g sample was incinerated in a pre-weighed crucible, which was then placed in an electric oven at 200 °C for approximately 18 hours. After cooling in a desiccator for 30 minutes (20, 21), the weight of the resulting white ash was recorded. The percentage ash content was calculated using the following equation:

$$\%\, AC = \left[\frac{M_1}{M_m \times \left(\frac{100-X}{100}\right)}\right] \times 100 \quad \text{..... (Eq 3.)}$$

where $M_1$ is the mass of residue (g)
, $M_m$ the weight of the test sample (g), *X* the % MC in the test sample.

### *Structural analysis of the extracted chitin*

### *Fourier transform infrared spectroscopy (FTIR)*

The Fourier Transform Infrared (FTIR) spectra of chitin samples were recorded using a Jasco FTIR 460Plus IR spectrometer (Japan) to analyze their chemical and structural properties. The samples were scanned at a resolution of 4 $cm^{-1}$ with 100 accumulations, measuring transmittance values (T %) over a wavelength range of 4000 to 650 $cm^{-1}$ at room temperature. The resulting spectra were processed using JASCO Spectra Manager software. For the analysis, the chitin samples were mixed with potassium bromide (KBr), and 16 scans were accumulated at the same resolution of 4 $cm^{-1}$. The amide group's carbonyl (C=O) stretching was assessed by calculating the ratio of the areas of the bands centered at 1660 $cm^{-1}$ and 3450 $cm^{-1}$, following the equation provided by Weipflog *et al.* (22).

$$AD\ (\%) = \frac{\frac{A1450}{A3450} \times 100}{1.33} \quad \text{..... (Eq 4.)}$$

### *X-Ray diffraction analysis of chitin*

X-ray diffraction (XRD) analysis was conducted using a Rigaku D/Max-III-C diffractometer (Japan). The chitin sample was exposed to an X-ray beam generated at 40 kV and 20 mA, utilizing non-monochromated Cu Kα radiation in a 2θ configuration at room temperature. The relative intensity was recorded over an angular range of 2 to 70° (2θ degrees). The crystallinity index (CrI) of the chitin was calculated using the Segal method where *Ic* is

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$$Crl\ (\%) = \frac{Ic-Ia}{Ic} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots \text{(Eq 5.)}$$

the intensity of the maximum intensity (crystalline portion) and *Ia* is the minimum intensity between major peaks (amorphous band). The size of the crystallites of each chitin sample was determined as well, using the Scherrer Equation

$$D(nm) = k\lambda/\beta cos\theta \ldots\ldots\ldots\ldots\ldots\ldots \text{(Eq 6.)}$$

where *D* is the size of the crystallites (nm), *k* = 0.94, λ is the wavelength, β is the width at half height of the peak analysed, while θ is the corresponding diffraction angle.

### *Scanning electron microscopy (SEM) analysis*

The surface morphology of chitin was examined using a scanning electron microscope (JEOL JSM – 630, Japan) operating at 20 kV. The surface of the dried chitin samples was coated with gold in a vacuum using a sputter coater prior to imaging.

## Results and Discussion

Chitin extraction was conducted chemically, based on the method described by Alabaraoye *et al.* (23) with slight modifications. In this study, demineralization was achieved using a dilute HCl solution to eliminate $CaCO_3$ and $Ca_3(PO_4)_2$. The shell samples were treated with 2 M HCl at ambient temperature (approximately 30 °C) for 24 hours. This process effectively decomposed calcium carbonate into water-soluble calcium, releasing carbon dioxide (24). Rajathy *et al.* (24) reported that HCl is commonly used for extracting β-chitin from mollusk shells.

Following demineralization, the shells were deproteinated using 1 M sodium hydroxide (NaOH) and refluxed at 60 °C for 24 hours to remove residual proteins and organic materials. The resulting solid was washed with distilled water to neutrality. It was then dried to a constant weight at room temperature over 48 hours. Consistent with our findings, Alabaraoye *et al.* (23) extracted chitin from crustaceans using 3 % NaOH and HCl for deproteination and demineralization, respectively. Similarly, Adekanmi *et al.* (25) subjected mollusk shell powder to NaOH for deproteination, while Mohan *et al.* (13) varied NaOH concentrations between 0.4 and 3 M for 24 hours during chitin extraction.

After deproteination, the shells were treated with 0.1 % hypochlorite at room temperature for 48 hours to eliminate colored pigments, enhancing the purity of the chitin. The final chitin yield was calculated based on the processed shells.

Table 1: Chitin yield (%)

| Mollusk shells | Chitin yield (%) |
|---|---|
| Periwinkle | 68.8 |
| Oyster | 70.9 |
| Cowry | 63.4 |
| Water snail | 58.0 |

The weight of chitin extracted from 100 g of dried mollusk shell powder varied based on the dry weight of the shells. Chitin yields ranged from 57.95 % to 70.9 %, with the highest yield obtained from oyster shells (70.9 %), followed by periwinkle shells (68.8 %). Water snail shells exhibited the lowest yield at 57.95 %, as shown in Table 1. The relatively high yields may be attributed to the lower concentration of HCl used, which was insufficient to remove most minerals from the shell waste. In contrast, previous studies reported lower chitin yields from other sources, such as squid pens (*Sepioteuthis lessoniana*) at 39.7 % (26) and cuttlefish bones (*Sepia aculeata*) at 21 % (27). Notably, Sudatta *et al.* (28) achieved a maximum yield of 80.15 % from Pinna bicolor powder.

Moisture content (MC) reflects the percentage of water retained within a sample. The MC of the shells was calculated and shown in Table 2. This indicates that oyster shell waste retained more water than the other mollusk shell wastes. All the mollusk shells used in this study complied with KFDA (29), which states that the moisture content of a typical commer-

cial chitosan biopolymer should not exceed 10 %. The variability in moisture content among the biopolymers may be related to the differing amounts of moisture absorbed during the extraction process. Abubakar *et al.* (30) noted that higher moisture content can reduce a material's effectiveness as an absorbent. Consequently, periwinkle and water snail shell wastes may serve as better adsorbents compared to the chitin derived from oyster and cowry shell wastes.

Ash content refers to the inorganic residue left after organic matter has been burned off. Khan *et al.* (31) indicated that ash content can interfere with adsorption processes. The ash content for the shells was measured and shown in Table 2. Olafadehan *et al.* (20) reported an ash content of 3.5 % from shrimp shell wastes, which aligns with our findings. This study suggests that water snail and oyster shell wastes may function as better adsorbents compared to cowry shell waste.

Table 2: Properties of chitin extracted from mollusk shells

| Mollusk shells | Moisture Content (%) | Ash Content (%) | Degree of acetylation (AD) (%) | Crystalline index (Crl) (%) | Crystalline size (nm) |
|---|---|---|---|---|---|
| Periwinkle | 0.89 | 3.65 | 80 | 59.55 | 40.8 |
| Oyster | 1.35 | 3.21 | 71 | 60.13 | 38.8 |
| Cowry | 1.16 | 4.57 | 69 | 55.70 | 29.2 |
| Water snail | 0.93 | 3.09 | 78 | 51.43 | 31.0 |

### *Chitin characterization*

#### *Fourier Transform Infrared Spectroscopy (FTIR)*

FTIR is a spectroscopic technique used to differentiate between α-chitin and β-chitin by analyzing the amide I band. The FTIR spectra of chitin extracted from oyster, cowry, periwinkle, and water snail shells are presented in Figure 1 (A, B, C, D). The analysis reveals the characteristic wavelengths of the extracted chitin, detailed in Tables 3 and illustrated in Figures 1(A, B, C, D).

Previous research has identified the presence of α, β, and γ polymorphic forms of chitin (32). In this study, the chitin extracted from mollusk shells exhibited peaks at specific signature wavelengths: 1684.8 $cm^{-1}$ (C=O stretching, amide I), 1785.4 $cm^{-1}$ (C=O stretching in the $NHCOCH_3$ group), 2504.8 $cm^{-1}$ (C-H stretching), 3645.3 $cm^{-1}$ (O-H stretching), and 3671.3 $cm^{-1}$ (NH-asymmetric stretching of primary amines). All chitin samples extracted from oyster, cowry, periwinkle, and water snail shells were identified as the α-form, as indicated by a weak single amide I band around 1684.8 $cm^{-1}$ for water snail shell waste and 1785.4 $cm^{-1}$ for the other samples. The spectra from oyster, periwinkle, and cowry shells showed structural similarities at 1785.4 $cm^{-1}$ (C=O stretching in the $NHCOCH_3$ group). While there were no significant differences among the chitin samples extracted from the various mollusks, some variations in peak wavelengths were observed, attributed to differences in natural sources and the extraction processes used. Additionally, a band at 2523.4 $cm^{-1}$ (C-H in the pyranose ring) was noted. In agreement with our findings, Olafadehan *et al.* (20) reported that chitin extracted from shrimp and crab shells exhibited bands around 2079.9 and 2113.4 $cm^{-1}$ (aliphatic compounds), 1430 and 1425 $cm^{-1}$ ($CH_2$ and $CH_3$ deformation), 857.3 $cm^{-1}$ (along-chain vibrations), and 711.9 $cm^{-1}$ (CH ring stretching, saccharide rings). Degree of chitin acetylation was assessed based on the FTIR spectra, as shown in Table 2.

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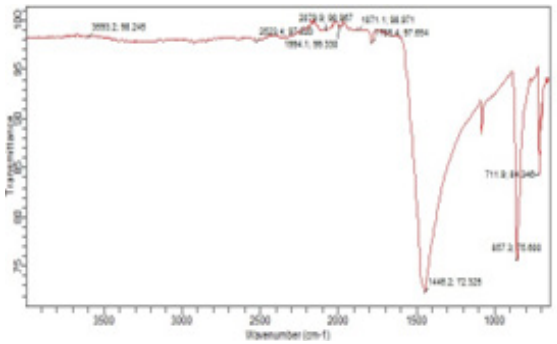
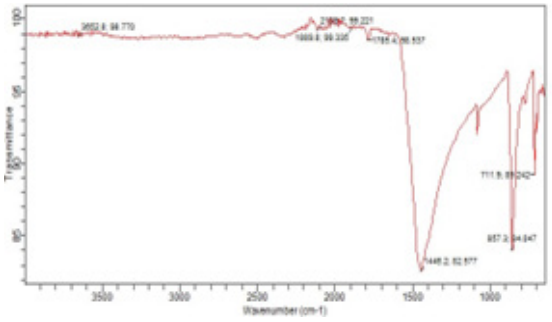
Table 3: FTIR bands of chitin extracted from mollusk shell waste

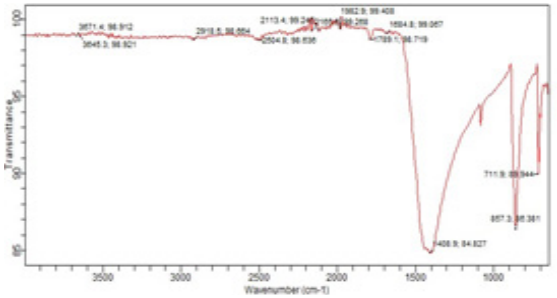
| Mollusk Shell | FTIR wavelength ($cm^{-1}$) |
|---|---|
| Periwinkle Chitin | 711.9, 859.3, 1446.2, 1785.4, 1889.8, 2109.7, 3652.8 |
| Oyster chitin | 711.9, 857.3, 1446.2, 1785.4, 1871.1, 1994.1, 2079.9, 2523.4, 3593.2 |
| Cowry shell | 711.9, 857.3, 1446.2, 1795.2, 1889.8., 1982.9, 2113.4, 2918.5 |
| Water snail | 711.9, 857.3, 1408.9, 1684.8, 1789.1, 1982.9, 2165, 2113.4, 2504.8, 2918.5, 3645.3, 3671.4 |



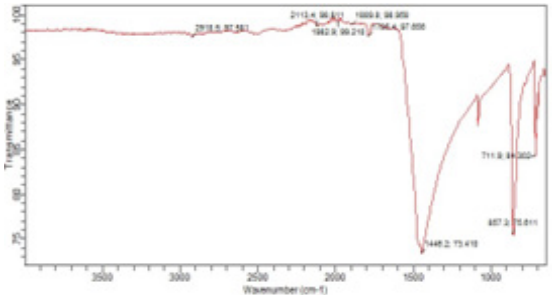
[A] Chitin from Oyster Shells



[B] Chitin from Periwinkle Shells



[C] Chitin from Water Snail Shells



[D] Chitin from Cowry Shells

Figure 1: FTIR analysis of chitin extracted from mollusk shell waste

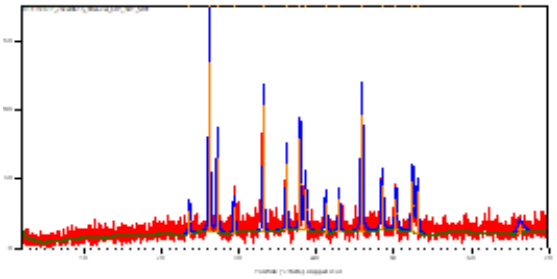
The degree of acetylation (AD) of chitin was assessed from the spectral data presented in Table 2. The findings revealed that chitin extracted from periwinkle shell waste exhibited the highest AD, followed by that from water snail chitin (Table 2). This difference may be attributed to the sodium hydroxide used during deproteination, which likely enhances the AD. The AD is a critical parameter, as it influences the physicochemical properties of chitin, including solubility, crystallinity, and reactivity. A higher AD generally signifies a greater presence of acetyl groups, which can improve specific characteristics beneficial for applications in biopolymers, pharmaceuticals, and agriculture (33). Previous studies have reported that the AD values of chitin derived from marine seashell waste range from 51.61% to 91% (15). The AD values for all chitin samples in this study fall within the ranges established in earlier research (15; 20; 33).

### *X-ray Diffraction (XRD)*

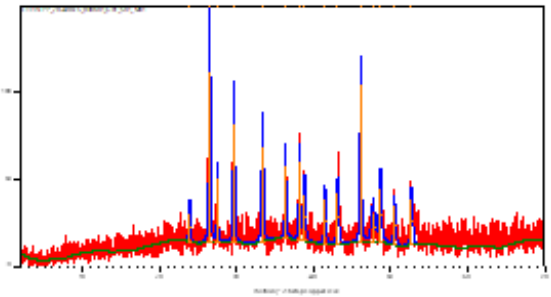
X-ray diffraction (XRD) reveals crucial details about crystalline materials' structural arrangement, crystal orientation, size, and crystallinity (34). Figure 2 (A, B, C, D) displays the XRD peaks for chitin extracted from mollusk shell waste. In this study, we observed a total

of fifteen peaks for periwinkle shells, including three prominent peaks at 26.5°, 29.6°, and 46.1°, along with twelve weaker peaks at 23.8°, 27.5°, 33.3°, 36.3°, 38.1°, 38.8°, 41.4°, 43.1°, 47.7°, 48.6°, 50.5°, and 52.7°. For chitin extracted from oyster shells, the XRD spectra displayed three sharp peaks at 26.2°, 33.2°, and 45.9°, as well as four weaker peaks at 23.6°, 29.4°, 41.2°, and 50.3°. Chitin derived from water snail shells showed two strong peaks at 26.8° and 46.4°, with weaker peaks between 27.9° and 43.5°. In the case of cowry shells, a significant peak was found at 30.2°, accompanied by eight weaker peaks at 27.0°, 33.9°, 36.9°, 40.2°, 43.8°, 46.6°, 48.3°, and 49.4°. All chitin samples exhibited sharp peaks between 26.2° and 46.4°, confirming the presence of the α-form of chitin and indicating a denser crystalline structure. Previous studies by Sajomsang and Gonil (35) reported XRD peaks for α-chitin structures at around 26°, aligning with our findings. Sudatta *et al.* (28) noted strong peaks between 23° and 50°, including peaks at 29.3°, 19.63°, and 20° for pen shells, and a peak at 20.04° for horse mussels (36). The peaks observed in our chitin samples closely resemble those reported in other studies (37; 38).

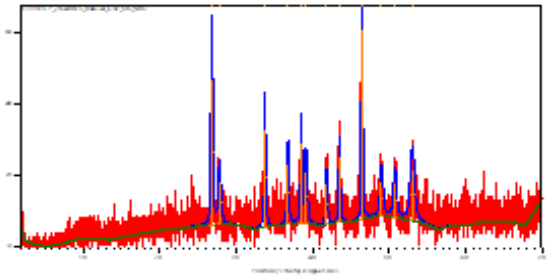
The crystallite size of chitin from the studied mollusk shells ranged from 29.16 nm to 40.8 nm (Table 2), with periwinkle-derived chitin exhibiting the largest size (40.8 nm) and cowry-derived chitin the smallest. The crystallinity index (CrI) is crucial for assessing the effectiveness of chitin and its derivatives in various applications. The CrI values for the extracted chitins ranged from 51.43 % to 60.13 % (Table 2). Notably, chitin extracted from water snail shells had the lowest CrI at 51.43 %, while oyster shell-derived chitin had the highest CrI at 60.13 %, indicating that oyster shell chitin is more crystalline and has a better chain alignment compared to the other sources studied. Previous research has shown CrI values for chitin ranging from 47 % to 91 %, depending on species and extraction methods (33; 39). High crystallinity in chitin is beneficial for formulating chitin nanofibrils, particularly for applications in the cosmetic and biomedical fields (22).



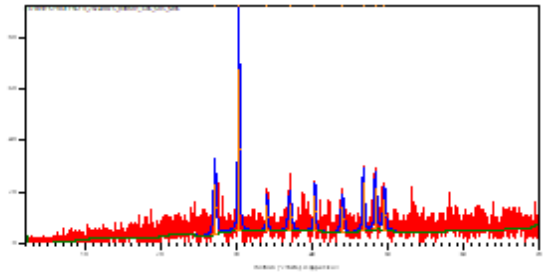
[A] Chitin from Oyster Shells



[B] Chitin from Periwinkle Shells



[C] Chitin from Water Snail Shells

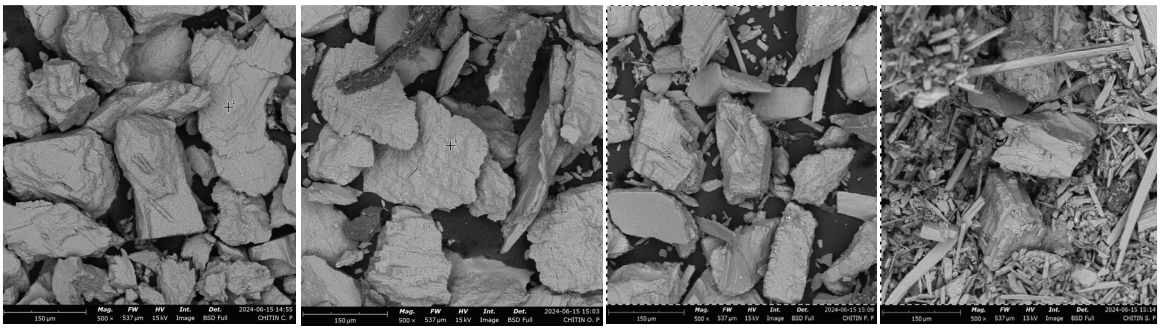


[D] Chitin from Cowry Shells

Figure 2: XRD analysis of chitin extracted from mollusk shell waste

### *Scanning electron microscopy (SEM)*

Surface morphology plays a crucial role in the effective utilization of chitin. Scanning electron microscopy. This study used scanning electron microscopy (SEM) to analyze the microstructures of chitin obtained from different sources, including periwinkle, cowry, oyster, and water snail shells Fig. 3A–D. The SEM images reveal that the chitin extracted from periwinkle and cowry shells features irregular, uneven sharp edges and a brick-like structure. In contrast, the oyster shell exhibits a corrugated, cohesive overlapping surface, suggesting a more complex architecture than the rest mollusks. The chitin from water snail shell waste displays a rough, irregular surface with densely packed features and fiber-like structures, which may result from the crushing process used to prepare these aggregates. This rough surface texture is likely to enhance the material's mechanical interlocking, while the fiber-like structures can increase the overall strength and durability of the chitin when used in composite applications, potentially improving bonding with other materials, such as cement. The uneven shape observed in SEM images may reflect the presence of different morphological forms, suggesting that chitin does not possess a uniform structure across different species or even within the same organism. Majekodunmi *et al.* (41) noted that chitin isolated from the mollusks, *Mytilus edulis* and *Lecanicillium attenuatum* showed variations in size and shape at low magnification, as well as brick-like structures at higher magnifications. Other studies have identified distinct surface morphologies of chitin, including rough (20) and irregular brick-like structures (42). The rough surface of chitin particles established in this study can enhance adhesion to cementitious matrices, thereby increasing mechanical strength. Furthermore, Lakshmi *et al.* (43) reported that an increase in pore density on the chitin surface enhances its capacity as a sorbent for metal ions.



[A] Chitin from Oyster Shells [B] Chitin from Periwinkle Shells [C] Chitin from Water Snail Shells D] Chitin from Cowry Shells

Figure 3: SEM analysis of chitin extracted from mollusk shell waste

## Conclusion

This study successfully extracted and characterized chitin from mollusk shell waste, providing a comprehensive assessment of its yield, chemical properties, and structural characteristics. The extraction process employed—combining demineralization with HCl and deproteination using NaOH—proved effective in maximizing chitin recovery. The resulting chitin exhibited favorable properties, including low moisture and ash content, high degrees of acetylation, and significant crystallinity. The findings underscore the potential of mollusk shell waste as a sustainable source of chitin, which could be leveraged in various applications, particularly in biopolymers, pharmaceuticals, and composite materials.

**Conflict of interest**

No conflict of interest was declared

**Authors' Contributions**

This research work was executed in collaboration among all the authors. All the authors read and approved the final manuscript.

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