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Biotechnology-Driven Extraction and Characterisation of Chitosan from the West African River Prawn (*Macrobrachium vollenhovenii*) and American Cockroach (*Periplaneta americana*) using a Modified Approach

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Abstract

Chitosan is a natural biopolymer derived from the deacetylation of chitin, a structural polysaccharide abundantly found in the shells and exoskeletons of crustaceans, insects, and other arthropods. Its unique physicochemical properties have led to widespread applications in biomedical, pharmaceutical, agricultural, and industrial sectors. In Nigeria, abundant freshwater crustaceans and insects remain underutilized as sources of chitosan. This study evaluated the potential of West African river prawns (*Macrobrachium vollenhovenii*) and American cockroaches (*Periplaneta americana*) as local chitosan sources using a modified chemical extraction process. Exoskeletal materials were pretreated, demineralized, deproteinized, and subjected to autoclave-assisted alkaline deacetylation (50% NaOH, 121 °C, 15 psi, 30 min). Chitin yields were 29.53% for prawns and 17.78% for cockroaches, while chitosan yields were 28.13% and 11.56%, respectively. Fourier Transform Infrared Spectroscopy confirmed characteristic functional groups of chitosan in both sources. The degree of deacetylation (DD) was 68.79% for prawn-derived chitosan and 81.21% for cockroach-derived chitosan, indicating effective conversion of N-acetyl-D-glucosamine to D-glucosamine units. These findings demonstrate that both species are viable alternative sources for chitosan production, with pressurized deacetylation enhancing yield and polymer quality. This approach provides a scalable, reproducible strategy for sustainable chitosan extraction in Nigeria, supporting potential applications in biotechnology, medicine, and industry

Keywords; Chitin, Chitosan, Biopolymer, Deacetylation, Extraction, Nigeria

Introduction

Chitosan is a natural biopolymer, composed of β-(1-4)-linked D-glucosamine and N-acetyl-D-glucosamine units (Aibani et al., 2021). It is derived from the deacetylation of chitin which is abundantly found in the exoskeletons of crustaceans, insects, fungi and other organisms (Jiménez-Gómez & Cecilia, 2020a; Kaur & Dhillon, 2014). It has garnered significant attention globally. The demand for chitosan is increasing with its market value projected to reach $15.1 billion by 2030 (Vieira *et al*., 2023). Chitosan is extensively being utilized across diverse sectors, including biomedical and pharmaceutical sciences, food technology, agriculture, water purification, textile engineering, and environmental protection (Harugade et al., 2023; Shariatinia, 2019; Thambiliyagodage et al., 2023). Globally,.

The industrial production of chitosan often relies on utilising crustacean exoskeleton, with the chemical method of extraction, involving sequential chemical treatments: demineralization, deproteinization, and deacetylation (Mersmann et al., 2025; Yi et al., 2024). These steps, particularly deacetylation, are time-consuming, often requiring several hours to over 24 hours to achieve high degrees of deacetylation and purity (Pellis et al., 2022). Furthermore, the use of marine crustaceans in chitosan production is constrained by the seasonality of harvesting cycles (Ma et al., 2021), presenting freshwater species and insects as a valuable alternative owing to their local availability and favourable shell composition (Liyanage et al., 2023; Sáenz-Mendoza et al., 2023).

In Nigeria, insects and crustaceans are abundant yet underutilised sources for chitosan production. The African giant river prawn (*Macrobrachium vollenhovenii*), widely distributed across West Africa and harvested year-round by local fishermen, remains largely unstudied for its suitability in chitosan production. Similarly, cockroaches, although regarded as a pest, possess bioactive properties and offer untapped potential as an alternative source of chitosan. These species therefore represent promising local raw materials for sustainable chitosan production in Nigeria. Therefore, this study extracts chitosan from locally sourced West African river prawns (*M. vollenhovenii*) and American cockroaches (*Periplaneta americana*) using a modified chemical process.

Methodology

Sample Collection and Preparation

A total of fifty-two (52) adult cockroaches (*Periplaneta americana*) were collected from residential areas in Ibadan, Nigeria, and maintained in an insectary under controlled conditions (27 ± 2 °C, continuous darkness) for 30–90 days with a diet of dry bread, fruits, and water. In addition, twenty (20) African river prawns (*Macrobrachium vollenhovenii*), comprising both adults and juveniles, were obtained from Eleyele Lake, Ibadan.

Exoskeletal materials (cockroach cuticles and prawn shells) were manually separated, washed thoroughly with distilled water to remove adhering impurities, and oven-dried at 35–37 °C until constant weight. The dried materials were subsequently ground into fine powder for use in chitin extraction.

Chitosan Extraction technique

Chitosan extraction technique was performed following the method described by Sagheer et al. (2009), with modifications (Table 2).

The exoskeleton (cockroach cuticles and prawn shells) was treated with acid (1 M $HCl_{(aq)}$) at a liquid-to-solid ratio of 20:1. The acid treatment facilitated the removal of mineral components. The mixture (exoskeleton and acid) was passed through a 20 µm cloth sieve. The obtained exoskeletal residue was washed repeatedly with distilled water until the pH of the residue became neutral. The exoskeleton was dried to a constant weight at oven temperature (35-37°C). This procedure is the demineralization step.

The dried exoskeleton was deproteinized by treating the demineralized exoskeleton with alkaline solution (1 M $NaOH_{(aq)}$) at a liquid-to-solid ratio of 20:1. The mixture was passed through a 20 µm cloth sieve. The residue was washed with distilled water until the pH became neutral, and the resulting chitin was dried to a constant weight at oven temperature (35-37 °C). The chitin yield was calculated as (Weight of Chitin / Weight of Starting Material) × 100.

The obtained chitin was treated with 50% $NaOH_{(aq)}$ (w/v) under pressurized alkaline conditions, as indicated in Table 3. This treatment facilitated the removal of acetyl groups, resulting in the formation of chitosan. Following treatment, the chitosan were washed with distilled water until neutral pH was achieved and then dried to a constant weight at oven temperature (35-37°C). The chitosan yield was calculated as (Weight of Chitosan / Weight of Starting Material) × 100

Fourier Transform Infrared Spectroscopy (FTIR) was then used to characterize the extracted chitosan using a PerkinElmer FT-IR Spectrophotometer (UATR Two) over the wavelength range of 4000–400 cm^{-1}. The spectra were examined to identify the characteristic functional groups of chitosan and to confirm the reduction of amide bands, which indicated successful deacetylation of chitin. The degree of deacetylation (DD) of the obtained chitosan was further quantified from the absorbance band ratios, using the equation described by Sabnis and Block (1997): **DD (%)** = 97.67 - [26.486 x (A1658/A3450)] (Sabnis & Block, 1997).

Table 1: Demineralization and Deproteinization Treatment Conditions for Cockroach and Prawn Exoskeletons

Exoskeleton Sample	Sample Initial Dry Weight (DW)g	Pre-treatment	Treatment Condition for Chitin
American Cockroach	9	Degutting, washing, grinding, drying; 1M HCL (Rm. Temp, 12hrs)	1M HCL (Rm. Temp, 3hrs), 1M NaOH (100℃, 3hrs)
African River Prawn	15	Deshelling, washing, grinding, drying	1M HCL (Rm. Temp, 24hrs), 1M NaOH (100℃, 3hrs)

Table 2: Deacetylation Treatment Conditions for Obtained Cockroach and Prawn Chitin

Chitin Sample	Sample Initial Dry Weight (DW)g	Treatment Condition for Chitosan
American Cockroach	9	50% NaOH (121℃, 15psi, 30mins)
African River Prawn	15	50% NaOH (121℃, 15psi, 30mins)

Results

The extraction of chitin yielded 17.78% from *Periplaneta americana* (cockroach) and 29.53% from *Macrobrachium vollenhovenii* (prawn), indicating a higher recovery from prawn exoskeletons (Table 3). Subsequent deacetylation produced chitosan yields of 11.56% and 28.13% from cockroach and prawn samples, respectively (Table 4), confirming prawn as the superior source in terms of extraction efficiency. Fourier-Transform Infrared (FTIR) spectroscopy revealed that chitosan obtained from both sources exhibited absorption bands consistent with characteristic functional groups of chitosan, including O–H, N–H, C–H, and C=O stretches. The observed spectra closely matched those of reference chitosan (Sigma Aldrich), with only minor shifts in band positions (Figures 1–2; Table 5), thereby validating the structural identity of the extracted polymers. Despite the higher yield obtained from prawn exoskeletons, cockroach-derived chitosan exhibited a greater degree of deacetylation (81.21%) compared to prawn-derived chitosan (68.79%) (Table 6).

Table 3: Yield at Each Stage of Chitin Extraction and Percentage Chitin Yield from the Different Extraction Procedures

Sample	Initial Weight (g)	Weight after Demineralization (g)	Weight after Deproteination (g)	Chitin Yield (%)
American Cockroach	9	-	1.60	17.78
African River Prawn	15	6.81	4.43	29.53

Table 4: Overview of Chitosan Extraction Yields from Deacetylation of Chitin using the Different Procedures

Sample	Initial Dry Weight of Sample (g)	Dry Weight of Chitin (g)	Dry Weight after Deacetylation (g)	Chitosan Yield (%)
American Cockroach	9	1.60	1.04	11.56

African River Prawn	15	4.43	4.22	28.13

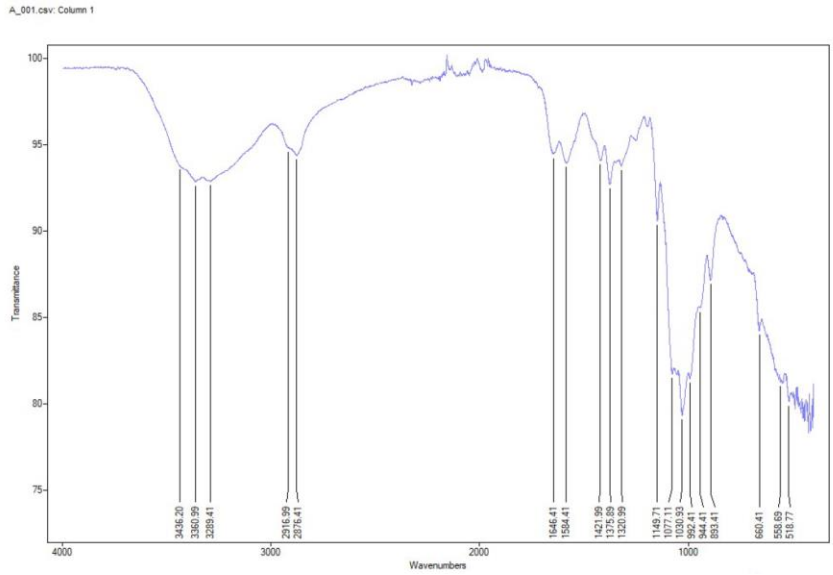

Figure 1: FTIR Spectra of Cockroach Chitosan

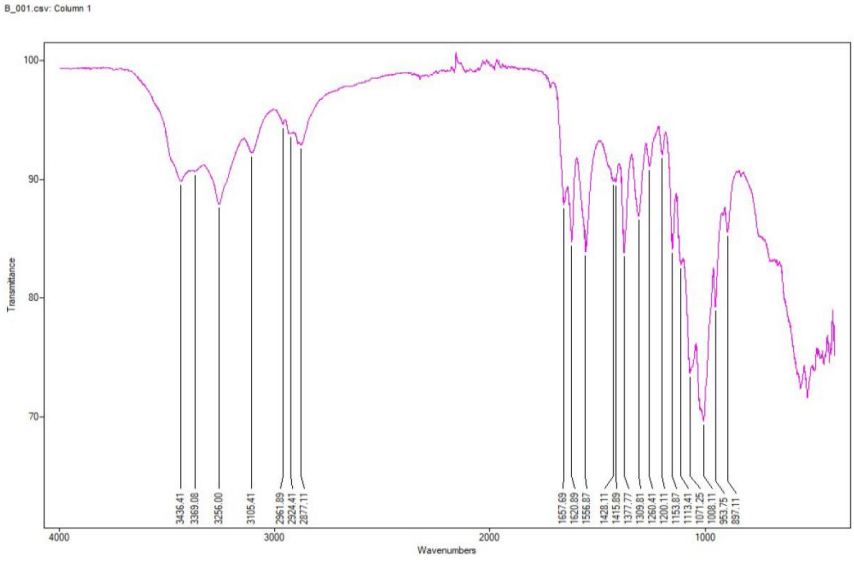

Figure 2: FTIR Spectra of Prawn Chitosan

Table 5: A Comparison Between the FTIR Absorption Wavelength of The Extracted Chitosan in The Present Study and A Reference Chitosan from Sigma Aldrich

Reference Chitosan (Olafadehan *et al*., 2021)	**Extracted AC Chitosan**	**Extracted ARP Chitosan**	**Functional Groups**
Wave number (cm^{-1})	**Wave number (cm^{-1})**	**Wave number (cm^{-1})**	
3428	3360 - 3289	3436	(O-H) group ($-NH_2$) group
2923	2916	2924	(CH_2) in CH_2OH group
2880	2876	2877	(C─H) in pyranose ring
1667-1623	1646	1657	(C=O) in the $NHCOCH_3$ group (Amide I band)
-	1584	1556	(NH_2) in $NHCOCH_3$ group (amide II band)
1422	1421	1428	(CH_2) in CH_2OH group
1380	1375	1377	(CH_3) in $NHCOCH_3$ group
1322	1320	1309	(C–H) in pyranose ring
1155 – 1077	1149 – 1077	1159 - 1071	(C–O–C) (glycosidic linkage)
1031	1030	1023	(C–O) in primary OH group
897	893	897	Pyranose ring skeletal vibrations

Table 6: The Degree of Deacetylation (DD) of the Chitosan Obtained

Sample	DD of Chitosan (%)
AC	81.21
ARP	68.79

Discussion

The extraction and characterisation of chitosan from alternative biological sources remains a subject of growing interest due to the increasing demand for biodegradable and non-toxic biomaterials. Insects and crustaceans represent promising reservoirs of chitin and its derivative chitosan, with yields often influenced by species type, structural composition, and extraction methodology. In the present study, *Periplaneta americana* (cockroach) and *Macrobrachium vollenhovenii* (African river prawn) were evaluated as sources for chitosan production.

The findings from our study showed the yield of chitin from cockroach as 17.18%. This is higher in comparison to reports from previous studies which showed an average yield range of 12.17 – 15% from cockroaches (Kim *et al*., 2017; Basseri *et al*., 2019). Similarly, the African river prawn chitin yield obtained in this present study (29.53%) was observed to be higher than the yields from previous studies ranging from 8.28-26.08% (Kjartansson et al. 2006; Balkhande and Ratnakar, 2019; Olafadehan et al., 2021). Additionally, other chitin from other species particularly, tiger prawns in the Arabian Gulf presented a yield of 19.13% (Sagheer et al., 2009). Currently, our yield of 29.53% presents the highest chitin yield in literature. The observed difference in chitin yield both from prawn and cockroach can be attributed to environmental factors such as diet, humidity, temperature, and other ecological pressures known to influence exoskeleton thickness and rigidity. Since these environmental factors vary by geography, the local conditions under which the cockroaches in our study developed are particularly relevant**.** Nigeria, characterized by its relatively high humidity and temperature (Mobolade & Pourvahidi, 2020), could have provided favourable conditions that promoted the development of thicker, more rigid exoskeletons in cockroaches, thereby contributing to the increased chitin yield observed in our study. These findings imply that chitin yield is not a fixed biological constant but rather a variable product influenced by environmental conditions. Consequently, geographical location and prevailing ecological factors should be considered when assessing the potential of arthropods populations as sources of chitin. This also suggests that tropical regions such as Nigeria may serve as

advantageous sites for sourcing chitin, given its favourable environmental conditions that enhance exoskeleton robustness and, by extension, chitin yield.

Chitosan known for its unique properties has garnered significant application in industries and medicines. Our study used a pressurized deacetylation method to extract chitosan from the exoskeleton of African River Prawn and Cockroach. We obtained a chitosan yield of 11.56% from cockroach chitin. Studies using conventional deacetylation technique from different populations reported a chitosan yield range of 7.28 - 7.4% (Kim *et al*. 2017; Basseri *et al*. 2019). Also, we obtained a chitosan yield of 28.13% from the exoskeleton of African river prawn. This also exceeded those reported for *Macrobrachium rosenbergii* by Vupputuri et al. (2016) (25.99%) and from shrimp shell wastes by Mohanasrinivasan et al. (2020) (17%) and Olafadehan et al. (2021) (16.93%). Taken together, our study, recorded considerably higher yields of cockroach chitosan. This enhanced yield can be attributed to the pressurized deacetylation process, which likely increases the penetration of alkaline solution into the chitin matrix, accelerates the removal of acetyl groups, and reduces the formation of residual impurities. The combination of heat, pressure, and optimized reaction time improves the efficiency of the chitin-to-chitosan conversion, resulting in both higher recovery and greater purity of the final product. These findings emphasize that processing parameters including pressure, temperature, and reaction duration play a critical role in maximizing yield and quality, highlighting the potential of pressurized deacetylation as a superior method for industrial-scale chitosan production.

The functional groups present in chitosan, such as hydroxyl (-OH), amino ($-NH_2$), and acetyl ($-COCH_3$) groups, contribute to its wide biological applicability, including interactions with cell membranes, proteins, and DNA, as well as roles in drug delivery, surface modification, and bioconjugation (Dash et al., 2011). FTIR spectra analysis of the chitosan obtained in this study revealed absorption bands corresponding to characteristic functional groups of chitosan. The spectra of chitosan extracted from cockroach samples showed close similarities to those reported by Basseri et al. (2019) and Badawy and Mohamed (2015). Likewise, the FTIR spectra of chitosan extracted from prawn samples exhibited absorption bands comparable to those reported by Kjartansson et al. (2006) and Balkhande and Ratnakar (2019).

Importantly, chitosan quality is further graded by the degree of deacetylation (DD)**,** which quantifies the conversion of N-acetyl-D-glucosamine units in chitin to D-glucosamine units (Novikov et al., 2023). DD influences physicochemical properties, solubility, and bioactivity, making it a key determinant of functional applicability (Aranaz et al., 2021). In the present study, cockroach-derived chitosan exhibited a degree of deacetylation (DD) of 81.21%, exceeding the 70% reported by Badawy and Mohamed (2015) but slightly lower than the 90.43% documented by Kim et al. (2017), likely reflecting differences in deacetylation duration and processing conditions.

For prawn-derived chitosan, the DD value was 68.79%. This was lower than the 74.82% reported by Mohanasrinivasan et al. (2013) and the 70–85% range reported by Ahing and Wid (2016) for shrimp shells in Sabah. In contrast, Isa et al. (2012) reported a DD value of 50.64%, while Olafadehan et al. (2021) achieved a markedly higher value of 89.73% from local shrimp sources in Nigeria. The variations in DD values observed across studies are likely attributable to differences in the source of chitin (species, habitat, and environmental conditions) as well as the extent and method of deacetylation employed (Sánchez-Machado et al., 2024). The relatively high DD observed in both chitosan sources confirms the efficiency of pressurized deacetylation in producing chitosan with a substantial conversion of N-acetyl-D-glucosamine to D-glucosamine units. This indicates that, beyond enhancing yield, pressurized deacetylation reliably generates chitosan with desirable physicochemical properties, underscoring its potential for scalable production from diverse arthropod exoskeletons under environmentally favorable conditions.

2.2 Conclusion

We present the first evidence of the application of pressurized deacetylation in chitosan extraction from locally sourced West African river prawns (*Macrobrachium vollenhovenii*) and American cockroaches (*Periplaneta americana*). Our findings demonstrate that this method not only enhances chitosan yield but also produces polymers with high degrees of deacetylation, confirming efficient conversion of N-acetyl-D-glucosamine to D-glucosamine units. Cockroach-derived chitosan achieved a DD of 81.21%, while prawn-derived chitosan reached 68.79%, both exceeding and matching values reported using conventional deacetylation techniques. Taken together, these results establish pressurized deacetylation as a highly efficient, scalable, and reproducible method

for chitosan extraction from alternative arthropod sources, with potential applications in biomedical, pharmaceutical, and industrial sectors. This approach offers a viable strategy for sustainable chitosan production, particularly in regions with abundant local arthropod resources

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