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COMPARATIVE STUDIES OF THE PHYSICOCHEMICAL PROPERTIES OF CHITOSAN FROM COMMERCIAL AND BIOWASTE SOURCES.

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Abstract
Chitosan is a bio-polymer that consists of β-(1-4-2- acetamido-2-deoxy-D-glucose) glucosamine units, which has found its usefulness in several applications such as agricultural, textiles, food, biomedical and pharmaceutical industries. Proximate composition and Fourier Transform Infrared Spectroscopic (FTIR) characterization of chitosan extracted from periwinkle shell using Moringa oleifera as agent of deproteination and deacetylation (CM) and commercial chitosan (CC) purchased from MarkNature USA were studied. The aim was to utilize local waste, periwinkle shell and naturally sourced alkali as deproteination and deacetylation agents to synthesize chitosan and compare the physicochemical properties with the commercially procured chitosan. Moisture, ash and protein contents were determined using standard methods. The percentage yield, solubility, degree of deacetylation, water and fat binding capacities were also determined using their standard methods. Characterization was done using FTIR. CM and CC respectively had moisture content (1.51 and 6.94) %, ash content (3.92 and 1.87) % and protein content (2.29 and 1.70) %, water binding capacity (1078 and 970) %, fat binding capacity (294.11 and 177.61) %. The percentage yield and degree of deacetylation of CM were 87 % and 78.30 respectively. Both CM and CC were soluble in 1% acetic acid. The FTIR spectra of CM and CC gave characteristic free hydroxyl (OH) band at (3774 and 4037.00) cm^{-1} respectively, aliphatic –OH- absorption bands of (3470 and 3430.17) cm^{-1}, aliphatic CH_3 absorption bands of (2927 and 2966) cm^{-1} respectively. The study has shown that chitosan prepared from periwinkle shell using naturally sourced alkali showed good physicochemical properties when compared with commercial chitosan.
***Keywords**: Periwinkle shell; Natural alkali; Chitosan; Proximate composition; Transform Infrared Spectroscopy*

Introduction

Food processing generates a large quantity of wastes which can cause environmental and human problems. Most of which are still underutilized and are substances of high useful value (Hamed *et al.*, 2010). The seafood industry annually generates about 106 tons of waste, most of which is destined for composting or to be converted into low value-added products such as animal feed and fertilizers (Schmitz *et al.*, 2019). The crustacean shell is one of the prominent sources of chitin and chitosan because of the abundance of the biomaterial and the large-scale industrial extraction process. According to (FAO, 2019) report, the waste generated in 2017 from crustacean shells was 8.4 million tonnes (Adekanmi *et al.*, 2023). As a result of large amounts of shells littering the environment, there is the urgent need to recycle them which results in the production of chitosan through chemical deacetylation of chitin (Amoo *et al.*, 2019).

Approximately 2000 tons of chitosan is produced annually, whose main source of extraction is from shrimp and crab shell residues (Muñoz et al, 2018). The shell of selected crustaceans was reported by Abdulwadud *et al.* to consist of 30%–40% protein, 30%–50% carbonate and phosphate of calcium and 20%–30% chitin (Kumari *et al.*, 2015). Periwinkle shell wastes like other crustaceans and molluscs contain some amounts of chitin which can be further deacetylated into chitosan (Gbenebor *et al.*, 2017). One common shell food consumed in southern part of Nigeria is periwinkle (*Tympanotonus fuscatus*) as a source of protein (Ekpo *et al.*, 2021). They are gastropods of the phylum Mollusca and are found in lagoons, estuaries, mangroves, and swamps in Nigeria (Paul *et al.*, 2022). The empty shells are poorly utilized

and it adds to environmental menace (Ugoeze and Chukwu, 2015). Periwinkle shells are abundant in Nigeria and can be utilized as source for chitosan.

Chitosan is defined as a linear polysaccharide formed by the random distribution of two monosaccharides, D-glucosamine and D-N-acetyl-glucosamine, which are joined together by a ß-(1–4) bond (Jayakumar *et al.*, 2010). Chitosan is a natural polycationic polysaccharides obtained from deacetylation of chitin from crustacean, molluscs and some fungi. Chitosan is chemically and thermally difficult to degrade but are highly stable (Amoo *et al.*, 2019). In its crystalline form, chitosan is normally insoluble in aqueous solutions above pH 7; however, in dilute acids (pH 6.0), the protonated free amino groups on glucosamine facilitate solubility of the molecule (Di Martino *et al.*, 2005). Chitosan's charge density depends on the degree of acetylation and pH of the media (Aranaz *et al.*, 2021). Chitosan has some advantageous properties, such as biocompatibility, biodegradable polymer of high molecular weight, nontoxic, and antimicrobial activity, that encourage its applications in many fields such as agriculture, paper industry, food and textile industries, pharmaceutics, biochemistry, biotechnology, cosmetics, biomedical applications, environment, and water treatment (Das *et al.*, 2024). Studies abound on the extraction of chitosan from shrimps and molluscs (Okafor *et al.*, 2020; 2023, 2024) but literature is scarce on the use of naturally sourced alkali especially *M. oleifera* as agent of deproteination and deacetylation. The aim of this study therefore was to utilize local waste, periwinkle shell and alkali extracted from an agrowaste, the petiole of drumstick (*M. oleifera*), as an alternative source of chitosan and compare the physicochemical properties with the commercial procured chitosan.

Materials and Methods

Sample collection and preparation:

The fresh periwinkle shells were collected from Esuk Itsu river bank, in Urua ato, Ikot Ekpene of Akwa Ibom state, Nigeria. The periwinkle samples were washed, cleaned to remove dirt and other extraneous materials; sundried for 120 hours, after which it was ground to fine powder and stored in a clean dry plastic bag at room temperature.

Extraction of Chitosan

Method of extraction described by (Okafor *et al.*, 2024]) was adopted in the extraction of chitin. Two hundred gram (200 g) of the ground periwinkle shell was soaked in 4 % HCl at room temperature in the ratio of 1:14 (w/v) for 24 hours. It was washed in the acid solution until no bubbles was seen and no colour change was observed. The sample was washed with distilled water until a relatively neutral pH is obtained. The demineralized powder was dried to constant weight. A 100 g of the demineralized powder was treated with 2.0 % caustic alkali extracted from the *M. oleifera* in the ratio of 1:20 (w/v). The mixture was heated at 90 °C with constant stirring for 2 hours. This was proceeded by filtration in a vacuum and the residue was washed thoroughly with distilled water until a pH of 7 to obtain the chitin. Deacetylation of the chitin was achieved by employing the method adopted by Novikov *et al.* (2023). The ratio of 1:15 (w/v) chitin to 40 % standardized caustic alkali solution was adopted. The mixture was allowed to stand for 2 hours. The filtration of the mixture was carried out through pump filter while washing of the residue was achieved with de-ionized water until a neutral pH was obtained. The residue dried in an oven at 105 ^{o}C for 24 hours was the chitosan.

Physicochemical Analysis

Proximate analysis including moisture, ash, protein, fat contents of the chitosan particles was conducted following the methods adopted by Ganogpichayagrai and Suksaard (2020) with some modifications. Percentage yield, solubility in 1 % acetic acid, water and fat binding capacities and degree of deacetylation were determined as described by El-Araby *et al.* (2023).

FTIR Analysis

The samples of prepared chitosan were characterized in KBr pellets by using an infrared spectrophotometer model (4100 Jasco, Japan) in the range of 400- 4,000 cm^{-1}.

Results and Discussion

Physicochemical Composition

The percentage moisture of the chitosan products showed that the moisture content of CM is lower than that of CC attributed to the low moisture content of the petiole of *M. oleifera*. This is an excellent property that can be utilized in the food and beverage industry. Low moisture content indicates longer shelf life as low moisture prevents contamination by microorganisms. Furthermore, the results were low when compared to the findings of Amor *et al.* (2024) and Sabon *et al.* (2015) in their studies where the moisture content of their extracted chitosan ranged from 7.8 to 17.2 % and 9.48 to 9.59 % respectively, and higher than the values reported by Nessa *et al.* (2010) who obtained moisture content in the range of 0.3 and 0.4 % from prawn shell.

The ash content of chitosan is a key indicator of the effectiveness of calcium carbonate removal at the demineralization stage (Nessa *et al.*, 2010) The ash concentration of CM (3.92 %) is higher than that of CC (1.87 %) indicating that CM is of higher quality than CC. High ash content reduces viscosity and average molecular weight (Amor *et al.*, 2024). The ash contents of the chitosan particles are high when compared to those of Kadak *et al.* (2023) whose ash content ranged between 0.98 and 1.01 %.

Table 1: Proximate Analysis of the chitosan particles

Parameter	CM	CC
Moisture (%)	1.51	6.94
Ash (%)	3.92	1.87
Protein (%)	2.29	1.70
Fat (%)	0.05	0.66
Yield (%)	87%	-
Solubility in acetic acid	++	++
Water binding capacity (%)	1078	970
Fat binding capacity (%)	294.11	177.61
Degree of deacetylation (%)	78.30	*83.51

CM = chitosan extracted using *M. oleifera* extract, CC = commercial chitosan,
*manufacturer's information

From Table 1, CM has a greater protein concentration CM (2.29 %) than CC (1.70 %). The protein content of the chitosan sample was considered lower after deproteinating the chitin and this could be attributed to the high degree of deacetylation. This shows that *M. oleifera* is an effective deproteinating and deacetylating agent that can substitute the commercial caustic alkali. Protein content was relatively low when compared with results obtained by Vinusha and Vijaya (2019) in their extracted chitosan from shrimp waste. The result was however somewhat in agreement with that of Okafor *et al.* (2023) whose protein content in chitosan extracted from snail shell using sodium hydroxide as deproteination and deacetylation agent was 2.80 %.

The fat content of the extracted chitosan indicates that CM (0.05 %) < CC (0.66 %). The values obtained were slightly higher when compared to Byun *et al.* (2012) who reported fat content of 0.03% from crab shell but relatively lower when compared to results obtained by Vinusha

and Vijaya (2019). The percentage yield of CM as presented in Table 1 is 87.0 %. This result is higher than the percentage yield obtained by Sarbon, *et al.* (2015) that reported a value of 44.57 ± 3.44 % when they extracted chitosan from crab shell.

All the chitosan products were soluble in 1% acetic acid solution under the same conditions after 30 minutes. This is in concordant with the findings of Okafor, *et al.* (2023). Since the amino groups of chitosan are protonated ($–NH_3^+$) in an acidic medium, the protonation leads to a decrease in the electrostatic repulsion between the chitosan chains, making them more soluble in acetic acid and as well form soluble salts. Solubility of chitosan depends on the degree of deacetylation of chitin during the extraction of the chitosan. The higher the degree of deacetylation the higher the solubility of the chitosan (Akpan *et al.*, 2020). Furthermore, the solubility of the chitosan products in dilute acetic acid shows that they can be applied in pharmaceuticals as drug carriers or in the formulation of injectable solutions and saline solutions in the environment (Li *et al.*, 2017; Apetroaei *et al.*, 2019).

The water binding capacity is considered an essential attribute of food ingredients for the formulation of various value-added food products including bakery products to maintain their texture and structure during or after processing (Mortuza and Tzen, 2009). From Table 1, WBC of CM (1078%) is higher than that of CC (970%). These values were higher when compared to Kucukgulmez *et al.* (2011) whose WBC values ranged from 492.67 to 712.99 % when they extracted chitosan from *Metapenaeus stebbingi* shell. Again, the results were higher when compared to No, *et al.* (2000) whose WBC values ranged from 355 - 610%. The differences in water uptake between the two chitosan products could be as a result of differences in the crystallinity of the products, number of salt-forming groups, moisture and protein contents of the deacetylating and deproteinating materials (No, *et al.*, 2000).

Fat binding capacity is the ability of the chitosan to interact or adsorb fats, being a main characteristic in choosing this biopolymer as a food supplement (Apetroaei *et al.*, 2019). From the study, the FTB of CM (294.11 %) is greater than that of (177.61 %). These values were low when compared to the result reported by Islam *et al.* (2017). The authors reported a fat binding capacity of 370 % from shrimp shell waste using aqueous NaOH. These differences could be attributed to their ash content since ash content plays a major role in the fat binding capacity of chitosan (Younes *et al.*, 2015).

Deacetylation is the procedure of eliminating acetyl groups from the molecular structure of chitin, resulting in the retention of the amino group ($–NH_2$). From the results in Table 1, the degree of deacetylation of CC (83.51) > CM (78.30). The degree of deacetylation is important for its use in specific industries because it determines properties such as solubility; viscosity; ion-exchange capacity; flocculation ability; tensile strength; ability to chelate metal ions; immune-adjuvant activity and reaction with amino groups (Kucukgulmez *et al.*, 2011; Gbenebor *et al.*, 2017).

FTIR Characterization

The FTIR spectra shown in Figures 1 and 2 represent the absorption strength of several functional groups in CM and CC. The wavenumber quantifies the energy of the infrared radiation that is absorbed by the sample, whereas the absorption intensity gauges the magnitude of the absorption at each specific wavenumber (Dai *et al.*, 2023).

The most prominent peaks in the spectrum of CM occurred from 4040.00 cm^{-1} to 607.83 cm^{-1}. The broad band around 3470-3418 cm^{-1} corresponds to O-H stretching vibrations, indicating the presence of hydroxyl groups, likely from alcohols or phenols. The peak around 3112.66 cm^{-1} corresponds to N-H stretching vibrations, commonly found in amines or amides. This

indicates the presence of primary or secondary amines. The peaks at 2927 cm^{-1} and 2853 cm^{-1} are characteristic of C-H stretching vibrations, suggesting the presence of alkane or aliphatic groups (Norhidayah *et al.*, 2017; Odili *et al.*, 2021). The strong peak at 1629 cm^{-1} can be attributed to C=O stretching vibrations, indicating the presence of carbonyl groups, such as those found in ketones, aldehydes, or carboxylic acids (Wanule et al 2014). The peaks at 1382 cm^{-1} and 1317 cm^{-1} are likely due to C-H bending vibrations, further confirming the presence of aliphatic groups. The peak at 1259 cm^{-1} may be associated with C-O stretching vibrations, suggesting the presence of ether or ester functional groups. The peaks at 1076 cm^{-1} and 1026 cm^{-1} can be attributed to C-O stretching vibrations, indicating the presence of alcohols or ethers. The peak at 900 cm^{-1} can be assigned to out-of-plane C-H bending vibrations, typical of aromatic compounds (Ramya *et al.*, 2012) while the peak at 607 cm^{-1} may correspond to C-Cl stretching vibrations, suggesting the presence of chloro-substituted compounds.

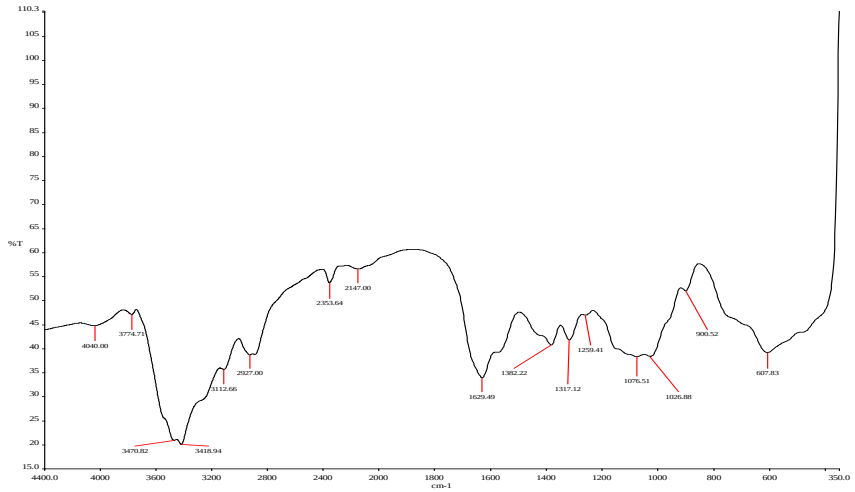


Figure 1: FTIR Spectrum of CM

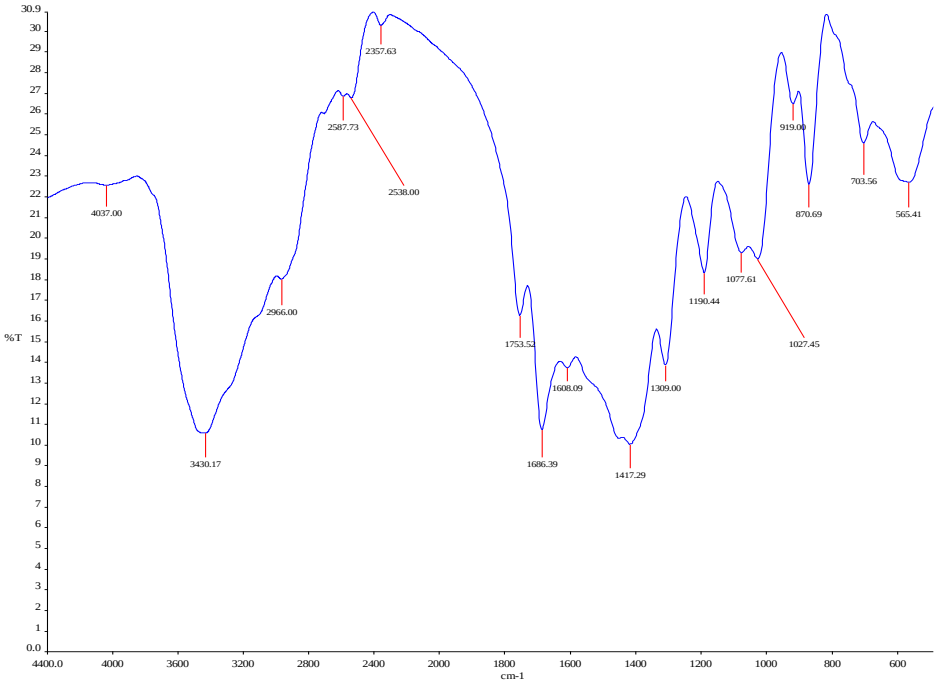


Figure 2: FTIR Spectrum of CC

The IR spectrum of CC ranged from 4037-565 cm-1 as seen in (Figure 2). Figure 1 showed a strong absorption band at 3430.17 cm^{-1} due to OH and amine N-H symmetrical stretching vibrations, a peak at 2966 cm^{-1} which indicates due to symmetric -CH_2 stretching vibration attributed to pyranose ring (Pawlak and Mucha, 2003). The intensive peak around 1,686.39 cm^{-1} corresponds to C=O and bending vibration of NH_2 which is represent amide I, which is a characteristic feature of chitosan polysaccharide and also indicates the occurrence of deacetylation (Zhang *et al.*, 2011). The peak at 1309.00 cm^{-1} was assigned to C-N stretching and NH bending vibrations present in Amide III, component of protein (Wang and Zhuang, 2022) while the bands at 1190.44 cm^{-1}, 1077.61 cm^{-1} and 1027.45 cm^{-1} were assigned to C–O–C group. Therefore, both CM and CC FTIR spectra exhibited similar bands, suggesting that both chitosan samples share similar chemical compositions.

Conclusion

Chitosan was successfully extracted from periwinkle shell, a biowaste, in this study. The two chitosan products' proximate compositions compared favorably, particularly in terms of the percentage yield and degree of deacetylation. Similar bands were seen in both chitosans' FTIR readings, indicating that their chemical compositions are comparable. As a result, periwinkle shell chitosan can be utilized for a variety of purposes, including wastewater treatment, biomedical applications, and other industrial uses like the commercial chitosan.

Conflict of Interest

The authors declare no conflicts of interest.

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