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SYNTHESIS AND CHARACTERIZATION OF CHITOSAN FROM WASTE SHELLS OF *Tagelus Plebeiu* OBTAINED FROM OYOROKOTOR, RIVERS STATE, NIGERIA

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ABSTRACT

The investigation was carried out to synthesize chitosan using waste shell of *Tagelus plebeiu* collected from Oyorokotor as an alternative to other kinds of shells. Standard procedures such as demineralization, deproteinization and deacetylation were used. Methods such as fourier transformed infrared spectroscopy (FTIR), X-Ray diffraction (XRD) and scanning electron microscope (SEM) were used for characterization to obtain results. Results reveals FTIR spectra bands at 3637.9 cm^{-1} for hydroxyl and 3384.4 cm^{-1} for amino functional group as well as 18.2^0 at 2 titha from XRD pattern. The degree of deacetylation was recorded as 80.48 %, moisture content as 0.101 %, yield was 53.24 % and ash content was recorded as 25 %. It is therefore concluded that the waste shell of Tagelus plebeiu can be used in producing quality chitosan as an alternative to other kinds of shells.

Keywords: Chitosan, Shell-waste, Tagelus plebeiu, Oyorokortor

INTRODUCTION

Tagelus plebeius is a sturdy razor clam. Mollusca species known as the sturdy razor clam (*Tagelus plebeius*) live in cohesive sandy silt sediment-covered tidal flats (1). They are a species of deep burrower that lives in permanent burrows up to 0.70 m deep. They have a suspension-feeding pattern that shows vertical movements during each semidiurnal tidal cycle, but they do not exhibit intertidal migration. This clam improves habitat complexity by raising the amount of organic matter in the sediment through siphon holes and surrounding depressions. They inhabit both marine and blackish water and are distributed throughout Argentina, Brazil, and Spain on the American Atlantic coast as well as the Atlantic Ocean that cut-across Ogoni, Andoni and Bonny in Rivers State, Nigeria. They are found between 30 cm below mean low water and 90 cm above mean low water, with a density that has been reported to range from 200 clams m^{-2} (2).

The stout razor clam is one of the important food products across the globe including Oyorokotor and other parts of Rivers State, Nigeria.

Particularly, in Oyoroktor and its adjoining areas, people rely greatly on *Tagelus plebeius* for food thereby making the waste shells to be littered at specific locations within our locality and constituting environmental pollution when unused. Nevertheless, the waste product of *Tagelus plebeiu* has economic value for the construction sector because its shells are widely

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used as building materials and to extract chitosan for a variety of applications (3). According to (4), shell waste typically contains 30–40% protein, 30–50% calcium carbonate, and 20–30% chitin. The most plentiful renewable natural resource is chitin, which is a homopolymer of N-acetyl-D-glucosamine and is mostly derived from the waste of crustaceans. Chitin is white, hard inelastic, nitrogenous polysaccharides found in the exoskeleton as well as in the internal structure of invertebrates and serves as the raw material for chitosan (5). Chitosan is a naturally occurring polymer made up of copolymers of glucosamine and N-acetylglucosamine. It can be produced using a conventional process that involves deproteinizing, demineralizing, and deacetylating the waste from the shell (6). According to (6), there are an estimated 200 novel families of biological macromolecules with potential applications, including chitosan. In previous studies, (7) has proposed that chitosan with high solubility (97.65%), degree of deacetylation (81.24%), moisture content (69.30%), ash content (32.27%), and yield (45.0%) can be extracted from shrimp shell waste. It has also been shown that 85% degree of deacetylation, 46% yield, moisture content of 5%, and a pH of 8 can be obtained from chitosan synthesized from waste shells of *Penaeus monodon* (8). Many other researchers have as well extracted chitosan from lots of materials; fish scales (9); freshwater shrimp and saltwater crabs (10); prawn shells (11); insects (12). Therefore, as an alternative, this study is intended to synthesize and characterized chitosan extracted from the shell waste of *tagelus plebeiu* obtained from Oyorokotor, Andoni LGA, Rivers State, Nigeria.

MATERIALS AND METHODS

Sample collection and preparation

The shell waste of razor clam (*tagelus plebeiu*) was obtained from Oyorokotor community, Rivers State. It was washed with warm water and sun-dried for one week. Thereafter, it was ground, sieved to 250µm size and stored in an air-tight container for use.

Demineralization of shells: In this step, 50g of finely powdered shell waste was weighed into a 1000 ml beaker. 5% HCl was poured onto the shell waste at room temperature and demineralized for 6 hours with a solid to liquid ratio of 1:5 (w/v) at 50^0C on a magnetic stirrer. After 6 hours of stirring, the shell waste was filtered and rinsed with distill water to remove acid and impurities and then dried in an oven to 100°C for 2 hours (9). This test was carried out to ensure complete demineralization of the shell waste.

Deproteinization: The demineralized shell waste was deproteinized in a constant stirring with 5% NaOH solution for 6 hours at 60 – 70°C at a solid to solvent ratio of 1:10 (w/v). At the end of the required duration of stirring, the residue was washed with distill water to remove NaOH. Then it was dried for 2 hours and the snow-white product formed was called chitin.

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Deacetylation: The deproteinized shell waste (chitin) was transferred to a 60% sodium hydroxide (NaOH) solution at a solid to solvent ratio of 1:15 (w/v). The solution was stirred at 30^0C for 4 hours for deacetylation and filtered. After rinsing the white residue which is now chitosan with distilled water, it was then dried at 60ºC and ready for use.

Properties of chitosan

***Determination of Yield of Chitosan*:**

The yield of chitosan was determined by dividing the mass of the synthesized chitosan by the mass of the ground shell waste.

$$\% \text{ Yield} = \frac{Mass\ of\ Chitosan}{Mass\ of\ Shell} \text{ x } 100$$

***Degree of Deacetylation (DD)*:** The degree of deacetylation was carried out by the process of titration (13). In the process, 0.3 g of chitosan was dissolved in 30 ml of 0.1M HCl, stirred for 20 minutes at room temperature and titrated against 0.1 NaOH in the presence of methyl orange indicator. The DD was clculated as follows;

$$\%DD = \frac{(C_1V_1 - C_2V_2)0.016}{M\ x\ 0.0994} \text{ x } 100$$

Where;
C_1 = Conc. Of Standard HCl aqueous Solution

C_2 = Conc. Of Standard NaOH Solution

V_1 = Vol. of Standard HCl used to dissolve Chitosan

V_2 = Vol. of Standard NaOH Solution Consumed during Titration

M = Weight of Chitosan

0.016g = Equivalent Weight of NH_2 group in 1ml of standard 1mol/l HCl solution

0.0994g = Proportion of NH_2 group by weight in Chitosan

***Moisture Content*:** The moisture content was measured by weighing 0.5 g of chitosan into a porcelain boat and heated in an oven at 60^0C for 1 hour and reweighed after cooling until a constant weight was obtained. The moisture content was calculated as shown below;

$$\% \text{ Moisture Content} = \frac{W_b - Wa}{Wb} \text{ x } 100$$

Where;
W_b = Weight of Chitosan before heating
W_a = Weight of Chitosan after heating

***Ash Content*:** The ash content was determined according to methods described earlier (7). In the process, 2 g of the synthesized chitosan was placed in a crucible and heated in a furnace to 550^0C for 2 hours. The sample was allowed to cool and the weight of ash residue weighed using the expression below.

$$\% \text{ Ash Content} = \frac{Weight\ of\ ash\ residue}{Weight\ of\ the\ sample} \text{ x } 100$$

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RESULTS AND DISCUSSION

Characterization: Fourier transform infrared spectroscopy (FT-IR) was used to characterize the chitosan sample and determine its functional groups. X-ray diffractometry (XRD) was used to examine the product's crystallinity, and scanning electron microscopy was used to show the product's morphology.

FTIR Analysis of chitosan:

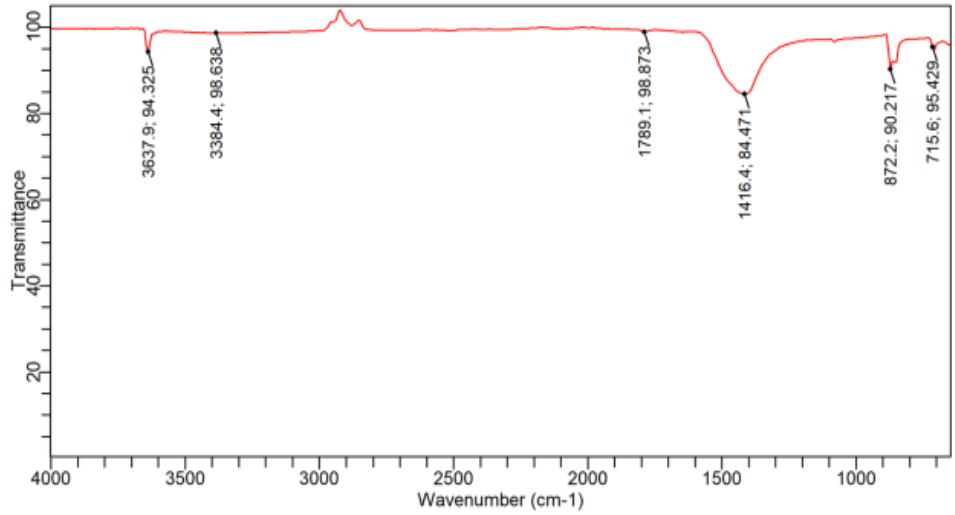

Fig.1: FTIR Spectra for Chitosan

The FT-IR bands for the chitosan separated from the chitin of *Tagelus plebieu* waste shells is displayed in figure 1. According to (14), the FTIR spectra band 3637.9 cm^{-1} corresponds to O-H stretching vibration due to alcohol, 3384.4 cm^{-1} for N-H stretching due to aliphatic primary amine, 1789.1 cm^{-1} for C=O bending vibration due to acid halide, 872.2 cm^{-1} for C=C bending vibration due to alkene and N-H wagging vibration in primary amine, and 715.6 cm^{-1} for N-H wagging vibration due to primary amine and C=C bending vibration due to alkene. The bands obtained in this study agree with findings 3425-2881 cm^{-1} for ν (N-H) in ν (NH_2) due to primary amines and bands at 3425-3422 cm^{-1} due to ν (N-H), ν (O-H), and ν (NH_2) earlier reported (9) in chitosan from (*Labeo rohit*) fish scales. This was comparable to a report on chitosan derived from prawns (15). Additionally, results about 3450 cm^{-1} (O-H stretching), 1870–2880 cm^{-1} (CH-stretching), 1655 cm^{-1} (Amide I), 1580 cm^{-1} (–NH_2 bending), and 1320 cm^{-1} (Amide III) in chitosan derived from shrimp waste shells were not consistent with the results of the present study because amide bands were not taken into account.

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XRD Pattern of chitosan:

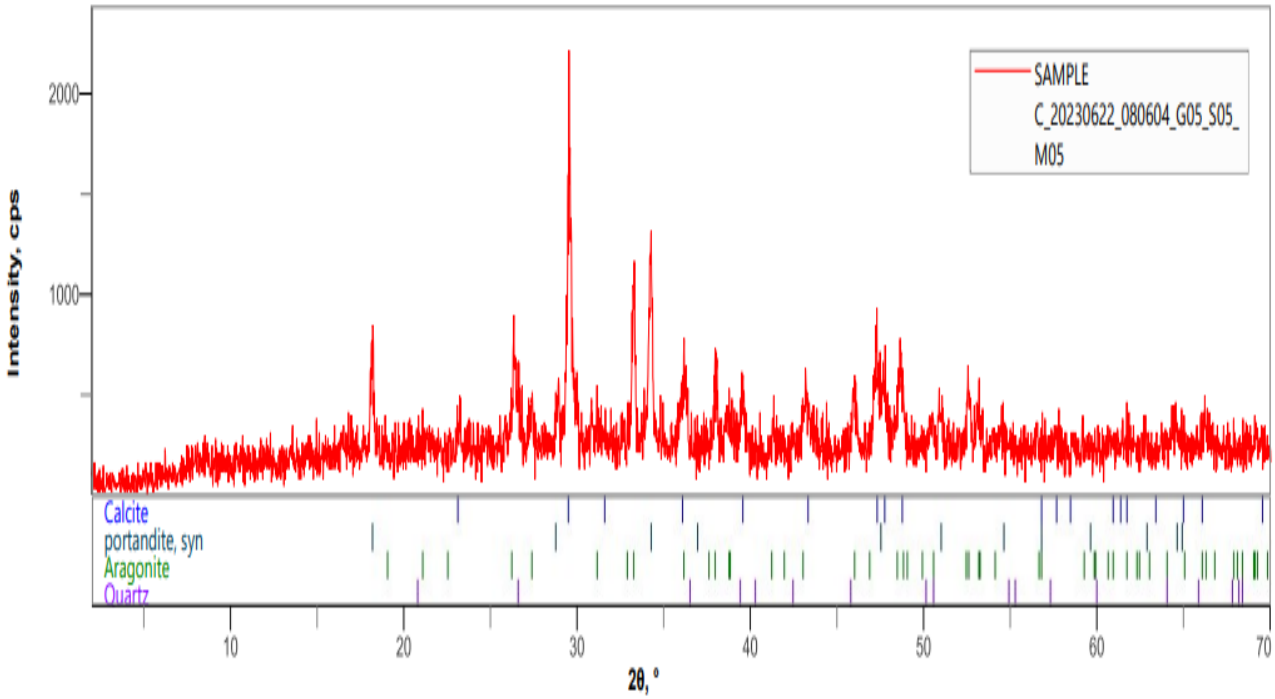

Fig. 2: XRD pattern of chitosan

The XRD pattern of chitosan extracted from waste shell of *Tagelus plebieu* is shown in figure 2. The peak produced at 2Θ = 18.202^0 corresponding to the crystallographic plane of 101 correspond to the range 18.8 to 20.7^0 for a typical chitosan (8,14). The sharp peaks produced indicated that the chitosan is crystalline in nature as also showed by the particle size 397.2 nm. Other larger peaks (26.349^0, 29.534^0, 33.282^0, 34.247^0, 36.17^0, 37.938^0, 39.51^0, 45.95^0, 47.23^0, 47.66^0, 48.67^0, 52.532^0 and 53.07^0) may be attributed to impurities. Earlier Findings (9.4 and 20.2°) and (22.0 and 26.8°) for the shrimp chitosan, (9.3, 20.2 and 24.4°) locust chitosan, (9.7 and 20.3°) for honey bee chitosan and (9.7, 20.3 and 22.6^0) for beetles' chitosan (12) were in agreement with results obtained from the current study. Also, report (2θ = 20◦, 35◦, 45◦, 65◦ and 75◦) obtained earlier (16) were in agreement with the results recorded in the present study.

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Scanning electron microscope (SEM) Analysis:

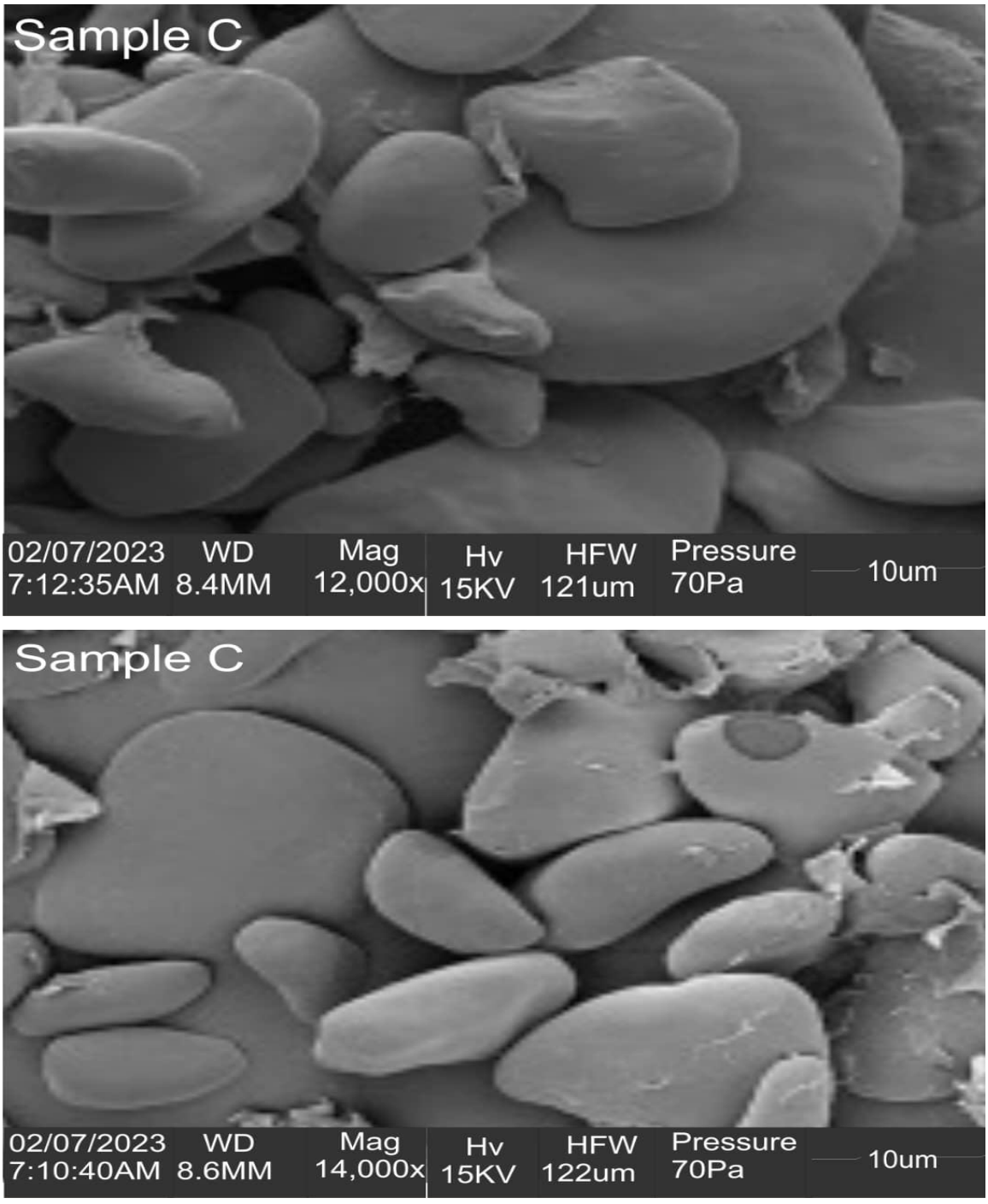

Fig. 3: SEM of Chitosan obtained from *Tagelus plebieu*

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The result of scanning electron microscope (SEM) of chitosan synthesized from the chitin of *Tagelus plebieu* is presented in figure 3. The smooth and spherical-shaped crystalline structure with a well-arranged distinctive surface showed high diversity and low porosity (17). As reported (15), result obtained for chitosan extracted from shrimp were in agreement with findings recorded in this study. Elsewhere, SEM results (13) obtained for chitosan from shells of two oyster shells revealed rougher surface in disagreement with those recorded in the current study.

Physical Properties of Chitosan:

Table 1: Physical Properties of Chitosan

Parameters	% Values
Degree of Deacetylation	80.48
Moisture Content	0.101
Yield	53.24
Ash Content	25

The result for physical properties of chitosan were recorded in Table 1. The degree of deacetylation (DD) recorded at 84.8% was within the range of 75% to 95% that is considered a good chitosan (18). The number of acetyl groups on the chitosan molecule that have been eliminated is measured by the degree of deacetylation. As stated, (7), the higher degree of deacetylation observed suggests that the chitosan is more deacetylated and contains more amine groups. The amount of water in the chitosan sample is indicated by the moisture content. The low moisture content of 0.0101% indicates a low water content, which can increase the metal ions' ability to adsorb (18). The efficiency of the chitosan production process is indicated by the comparatively higher yield of 53.24%. The low ash content of 25% indicates an increased adsorption capacity of the chitosan, and the yield is a measure of the amount of chitosan produced from the starting material (8). The amount of non-chitosan material in the sample is indicated by the ash content. It was found that the chitosan derived from shrimp shell waste had an 87% degree of deacetylation, which was higher than the value found in this study (14). However, the yield of 19% was lower than the value found in this study. In other studies, (7) reported higher results (degree of deacetylation-81.24%, moisture content-69.30%, ash content-32.27%, and yield-45.00%) for chitosan extracted from shrimp waste than the results of this study. In addition, (12) found that the degree of deacetylation (DD) and ash content were higher than what the current study found.

CONCLUSION

Based on the result obtained from the study, the shell waste of *Tagelus plebeiu* yielded a significant amount of chitosan like other shell wastes. It is therefore concluded that the waste shell of *Tagelus plebeiu* can serve as an

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alternative to other sources of shell waste for the synthesis of chitosan.

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