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Valorization of african giant land snail shell waste (*Archachatinamarginata*) by extraction of chitosan from it

ABSTRACT

The African giant land snail (*Archachatinamarginata*) is one of the most common mollusks in southern Nigeria. It has a shell that constitutes more than half of its body mass and it is usually discarded as waste. The goal of the present study is to valorize snail shell wastes by extracting chitosan, a versatile and top-demanding biopolymer from it. Chitosan was extracted by chemical methods and characterized by Fourier transformed infrared spectroscopy, and powdered X-ray diffraction. Quality parameters such as solubility, moisture content, ash value and degree of deacetylation were determined for snail shell-chitosan. The results show that snail shell chitosan is of comparable quality to commercially marketed shrimp chitosan. Snail shell chitosan has a DD value of 78.4 %, solubility of 95.35 %, moisture of 1.42 %, and ash value of 1.61 % which affirms snail shell-chitosan as a sustainable and suitable feedstock for commercial production of chitosan. The yield of chitosan based on snail shells was 14.83 % which is comparable to yield from crustacean shell wastes.

Keywords: *Archachatinamarginata*, shell waste, chitosan, quality parameters, valorization

1. INTRODUCTION

The increasing awareness of the nutritional benefits of snail meat has continued to drive its consumption by humans of all classes. Snail meat, popularly known as 'Congo meat' is a rich source of protein, calcium, iron, and other essential minerals. Snail meat is edible for persons of all ages and nationalities and there is no cultural and religious restriction on the use and consumption of snail meat and its parts.

Snail is a member of the phylum *Mollusca*, with its muscular and soft parts partly covered by a protective shell. The African giant land snail (*Archachatinamarginata*) is one of the most common mollusks in southern Nigeria. It has a shell that constitutes more than half of its body mass and it is usually discarded as waste. As snail meat consumption increases, the mass of snail shell wastes is expected to increase and may constitute a nuisance in the environment. This is because snail shell waste is highly underutilized and its biodegradability rate is very low.

However, snail shell wastes can be harnessed as feed-stock for the production of industrial-grade chemicals such as chitin, chitosan, oligochitosan

and so on. Chitin and chitosan are essential biopolymers due to their bioactivity, biodegradability, non-toxicity, sorbent strength, and antimicrobial activities.

Although chitosan is not found free, it is derived from chitinous-based biomaterials such as exoskeletons of crustaceans, insects, mollusks, and cell walls of some fungi [1-3]. Commercial-grade chitosan is extracted from shrimp chitin [4] and used in many biomedical applications. Chitosan has been extracted from the shells of crabs, crayfish, squillas, turtles, and external parts of millipedes and mosquitoes but the shrimp chitosan is of superior grade [5].

The quality of chitin and chitosan is defined by parameters such as moisture content, viscosity, ash value, solubility, molecular weight, and degree of deacetylation (DD). These quality parameters vary for different chitinous-based sources. Each chitinous-based source yield chitin of different properties [2, 6]. The quality of chitin extracted from shrimp and crabs at different growth stages by the same method differs significantly [7]. Quality parameters also depend on the extraction methods and conditions adopted to derive chitin and chitosan from feedstocks [3, 8].

Chitin and chitosan have a similar structural framework as cellulose but with unique functional groups that make them distinct as shown in Figure 1. Chitosan is a derivative of chitin but chitin is not

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a derivative of cellulose. Also, chitin and chitosan are not obtained from cellulosic-based biomass.

Chitosan is a family of polymers with at least 51 % degree of deacetylation. It is a copolymer of N-acetyl-D-glucosamine linked to D-glucosamine units by β -1, 4 glycosidic bonds, as its monomeric

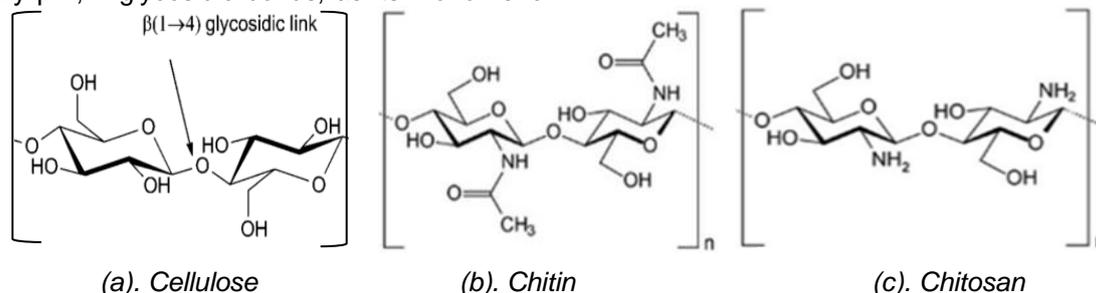


Figure 1. Chemical structure of cellulose [10], chitin (completely acetylated chitin) [11,12]; and chitosan (completely deacetylated chitosan) [13]

However, chitosan is more exploited for industrial applications compared to chitin due to its high pH sensitivity [14], biodegradability, physiological compatibility, biodigestibility, adsorption, and chelating capacity [5, 15]. Industries such as food, pharmaceutical, cosmetics and medicine are the main areas of applications of chitosan but the food and nutrition industry are the most important users of chitosan [15-17].

Chitosan is used in drug formulation as a carrier [18]; in wound dressing materials due to its adsorptive and antimicrobial properties [18-21]. It is also used in wastewater treatment due to its antimicrobial, adsorptive capacity, and eco-friendliness [22]. Chitosan's application in plastic production is due to its biodegradability [23]. It has been used as filler in paper production [3, 24,25]. This is why the global chitosan market has continued to grow in a positive trend. In 2019, the global transactions of chitosan were valued at USD 6.8 billion and the average return on investment in chitosan is expected to grow at a rate of 24.7 % annually by 2027 [18].

The isolation of chitosan from biomass comprises the removal of minerals (particularly, calcium carbonate), proteins and lipids, and acetyl groups [26]. However, the quality and properties of chitosan are affected by the extraction procedure, the nature of the chitinous feedstock, and the conditions under which chitin was extracted [27].

The extraction of chitosan of good quality from shrimp shell materials was executed using 3 % hydrochloric acid (as demineralized), 4 % sodium hydroxide (as deproteinized) and 50 % NaOH as deacetylating agent [28].

Therefore, the extraction of chitosan from African giant land snail shell waste is expected to valorize it as there are many existing and new

units [2, 9]. The ratio of N-acetyl-D-glucosamine to D-glucosamine subunits of chitosan monomeric units is far less than unity hence; the D-glucosamine unit is the dominant structural component of chitosan.

potential markets for chitosan. Further still, valorization of snail shell waste will mitigate or eliminate potential negative environmental impact of shell wastes on the long run. Therefore, the present study is designed to extract chitosan from snail shell wastes and ascertain the potentiality for its exploitation as commercial feedstock for chitosan production.

2. MATERIALS AND METHODS

2.1. Materials

Snail shell from African giant land snail, *Archachatina marginata*, represented in figure 2, is indigenous to West Africa [29]. The shells of about 2 years old snail were collected by the authors from Facha Integrated farms within Obiaruku in Delta State, Nigeria on the 12th April, 2022. The place is geographically situated between latitudes 5°2 and 5°3 and longitude 5°4 and 5°4. Chemical reagents were of analytical grade, used without further purification.



Figure 2. African giant land snail (*Archachatina marginata*)

2.2. Methods

2.2.1. Preparation of snail shell powder from snail shell wastes

The snail shells were processed for chitosan extraction by approaches used by Oyekunle and co [30] and Thillai and co [1] with some modifications.

The snail shells were washed over running tap water to remove debris and then broken into smaller particles with a cleaned metallic hammer, to loosen tissue and organics. The tissue-free particles were further washed in hot tap water (90 °C) and then rinsed with warm deionized water to remove any foreign matter before it was sun-dried for 14 days in a dust-free environment.

The sun-dried snail shells were further downsized in a mortar and then pulverized using an industrial-grade blender into fine particles as shown in Figure 3. The residual fine particles were sieved using an industrial sieve to obtain fine particles of sizes below 600 microns. Finally, the shell powder was stored in cleaned air-tight containers at ambient temperature.

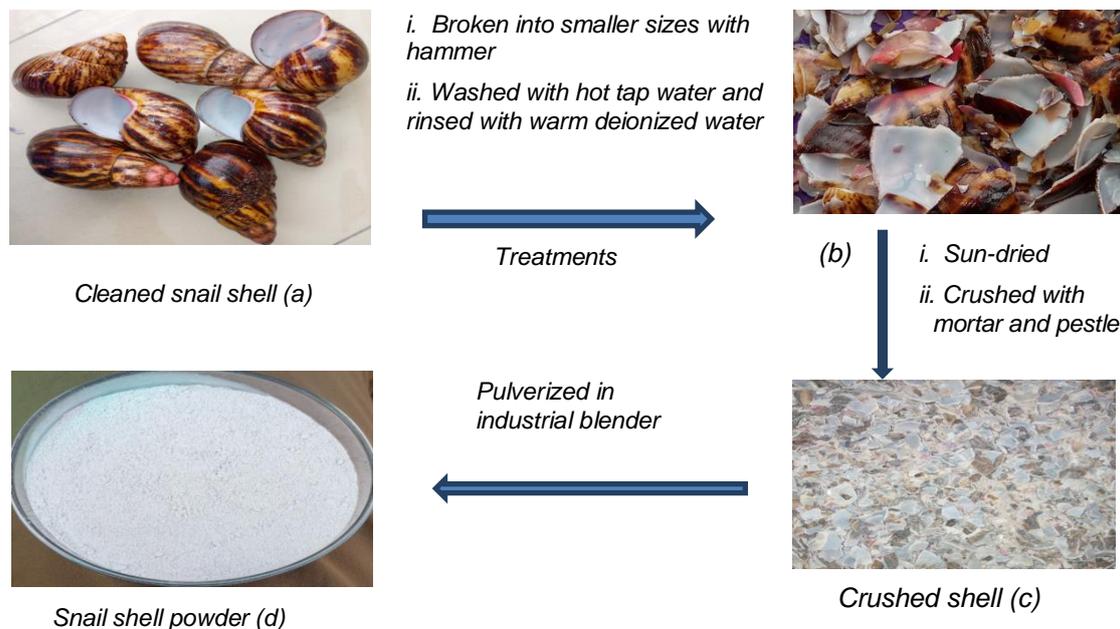


Figure 3. Fresh Snail shell powder from snail shell

2.2.2. Extraction of chitin from the snail shell powder

Chitin was extracted from the snail shell powder by consecutive acid and alkaline treatments of the shell powder under different experimental conditions of temperature, concentration, and retention time. The extraction entails demineralization and deproteinization using hydrochloric acid and sodium hydroxide as demineralizer and deproteinizer respectively. For optimal deproteinization efficiency, shell powder was demineralized first [31].

Demineralization of snail shell powder

The demineralization was performed according to the method used by Thillai and Co to demineralized shrimp shells [1] with slight modifications to suit the present biomass. About 18 g snail shell powder was demineralized in 420 mL of 7 % HCl, in the ratio of 3:70 g shell powder to mL HCl solution. The shell powder was slowly added to 7 % (v/v) HCl solution in 500 mL flask to avoid effervescence. Thereafter, the reaction flask was stir-heated at 150 rpm and 80 °C for 4 hours over a magnetic stirrer, to remove carbonates and phosphates of calcium. The demineralized sample

was filtered and washed repeatedly with deionized water to remove any trace of HCl and soluble impurities before it was oven-dried at 70 °C for 6 hours to achieve constant weight and then stored in an air-tight container.

Deproteinization of demineralized shell sample

The deproteinization was performed according to the procedure used by Thillai et al [1] with some modifications. The demineralized snail shell powder was deproteinized in 5 % (w/v) NaOH solution in the ratio of 3:70 g dried demineralized snail shell powder to mL NaOH solution (w/v) at 80 °C with constant stirring at 200 rpm for 3 hours. The resulting solid was filtered and the residue bathed in 5 % NaOH to obtain a clear solution, which marked the end of deproteinization. The deproteinized sample (residue) was washed to neutrality with hot deionized water (100-120 °C). The pure deproteinized sample (chitin) was then oven-dried at 70 °C for 6 hours to achieve constant weight.

2.2.3. Extraction of chitosan from chitin by deacetylation

The snail shell chitin was deacetylated by heterogeneous alkaline hydrolysis at 120 °C. The

acetyl group was removed from the chitin based on the procedures used by Chang et al [32], Tsai et al [33], and Boukhelifi [34] with minor modifications. The chitin was deacetylated in 50 % (w/v) NaOH solution in the ratio of 1:20 g dried chitin to mL NaOH solution (w/v) at 120 °C with constant stirring at 250 rpm for 6 hours. The residual mixture was filtered and the residue (chitosan) was washed to neutrality with hot deionized water (100-120°C) and then dried in an oven at 120 °C for 3 hours to constant weight. The dried chitosan obtained was quantified and recorded as a percent of the original weight of the snail shell sample [35] and then stored in airtight containers.

Table 1 shows the process parameters for the extraction of chitosan from snail shell waste.

Table 1. Chitosan extraction parameters

S/N	Parameter	Level
1	Weight of shell powder	18 g
2	Particle size	0.3-0.5 mm
3	Temperature	80 °C
4	Concentration of demineralized	7 % HCl
5	Shell powder to acid ratio	3:70
6	Concentration of deproteinized	5 % NaOH
8	Concentration of deacetylating agent	50 % NaOH
9	Deacetylation temperature	120 °C

2.3. Determination of percentage yield

The yield percent was obtained based on the original weight of snail shell powder used in the extraction process as expressed in the equation, Eq. 1

$$Yield (\%) = \left(\frac{W_y}{W_{sp}} \right) \times 100 \quad (1)$$

where W_y = Weight yield of chitosan (g) and W_{sp} = Weight of snail shell powder used

2.4. Characterization of snail shell chitosan

The formation and purity of the snail shell chitosan were confirmed by Fourier transformed-infrared (FT-IR) spectroscopy and powdered x-ray diffraction (pXRD). The model and operating condition of the FT-IR: Thermo Nicolet iS10 Smart ITR spectrophotometer (Thermo Fisher Scientific, USA), equipped with an OMNIC-Software, a DTGS detector, and a Ge-on-KBr beam splitter) in the wavelength range of 500 cm^{-1} and 4000 cm^{-1} and the solid-state using potassium bromide (KBr) pellets.

The powdered x-ray diffractometer (a Bruker D2PHASER diffractometer, Karlsruhe, Germany) furnished with a Ni 24 filtered Cu $K\alpha$ -radiation ($\lambda =$

1.5406 Å), generated at voltage of 40 kV and intensity of 40 mA, and a diffracted beam monochromator. Each of the dried powdered samples was viewed in the diffraction angles (2θ) in the range of 5°–80° and a counting time of 5.0 s/angle step (that is step size of 0.05).

2.5. Determination of chitosan quality parameters

The quality of snail shell chitosan was ascertained by the following parameters: moisture content, solubility, ash value, and degree of deacetylation (DD).

2.5.1. Determination of moisture content

The moisture content was determined by gravimetric method according to the procedure used by Pereda and co-workers [36] and calculated by equation, Eq. 2. The process was repeated three times and the average moisture content was recorded.

$$Moisture\ content (\%) = \frac{W_1 - W_2}{W_1 - W_0} \times 100 \quad (2)$$

Where W_0 = Weight of empty crucible (g); W_1 = Weight of fresh sample plus crucible (g); W_2 = Weight of dried sample plus crucible (g).

2.5.2. Ash value

The ash value was determined by dry ashing 1.0 g of sample in muffle furnace according to the procedure used by Jiang [37] and Mohan and co-workers [38] with some modification. The sample was placed on a pre-weigh crucible and transferred into muffle furnace that was pre-heated to 550 °C. The sample was allowed to tar in the furnace for 6 hours and then allowed to cool to <200 °C in the furnace before it was transferred into a desiccator with a vented top to further cool to room temperature. The ash value was calculated by equation, Eq. 3. The process was repeated twice and the average value was recorded.

$$Ash\ value (\%) = \frac{W_{res}}{W_{sam}} \times 100 \quad (3)$$

Where W_{res} = Weight of residue (ash) (g) and W_{sam} = Weight of sample (g)

2.5.3. Determination of solubility of chitosan

The solubility test was performed according to the procedure used by Adekanmi and co-workers [31] with minor modifications. A sample of 1.0 g was dissolved in 100 mL of 1 % (v/v) acetic acid solution. The resulting mixture was stirred at 400 rpm for 2 hours at ambient temperature and subsequently made to pass through a pre-weighed filter paper, dried to constant weight at 80 °C in an oven. At the end of filtration, the filter paper was re-dried to constant weight and re-weighed.

The solubility of chitosan was calculated by equation, Eq. 4.

$$\text{Solubility} = \frac{\text{Amount of sample that dissolved into solution}}{\text{Original amount of sample}} \times 100 \quad (4)$$

2.5.4. Determination of the degree of deacetylation (DD)

The DD value of chitosan was determined by the elemental method. The CHN elemental analyzer was used to determine the carbon-nitrogen ratio of chitosan and the degree of acetylation (DA) and DD were calculated by the equations, Eq. 5 and 6 respectively.

$$DA = \left\{ \frac{(C/N - 5.14)}{1.72} \right\} \times 100 \quad (5)$$

where 'C' and 'N' are the percent carbon and nitrogen content in the biopolymer respectively

$$DD (\%) = 100 - DA \quad (6)$$

3. RESULTS AND DISCUSSION

The formation and purity of snail shell chitosan were confirmed by the appearance of characteristic absorption bands in its Fourier transformed-infrared spectrum shown in Figure 4.

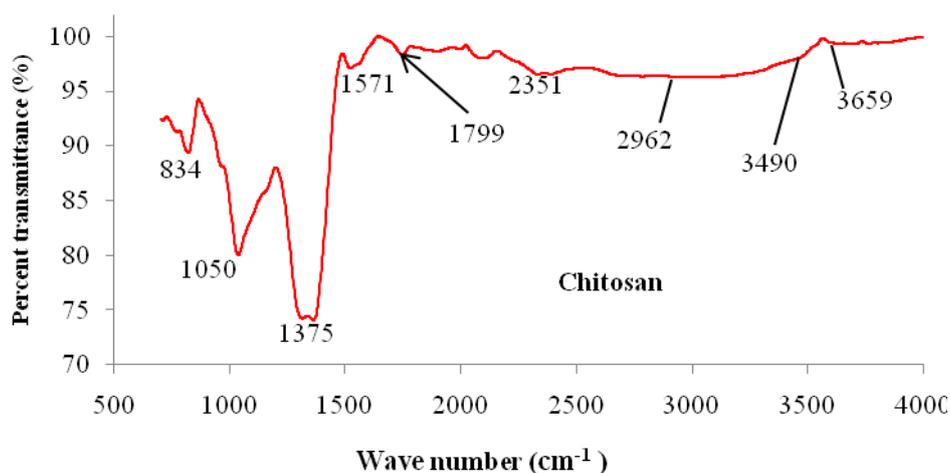


Figure 4. Fourier transformed-infrared spectrum of snail shell-chitosan

The broad peak at 3490 cm^{-1} corresponds to O-H stretching vibration [39, 40]. The peak around 2351 cm^{-1} is due to the absorption of atmospheric CO_2 by the chitosan [41].

Furthermore, the spectrum of snail shell-chitosan did not show a peak at 1500 cm^{-1} which is associated with amide band of protein [40]. This implies that snail-chitosan was sufficiently deproteinized and it is expected to manifest in the solubility of the biopolymer in acetic acid.

The intense peak at 1375 cm^{-1} indicates the CH_3 of the amide group [42]. Comparing the sharpness of this peak to that of the amino group at 1571 cm^{-1} indicates that snail shell-chitosan was insufficiently deacetylated as many molecules of acetyl-amide appeared to be in the sample. The polysaccharide component is represented by the band at 834 cm^{-1} [43].

Figure 5 is the powdered x-ray diffraction (XRD) pattern of snail shell chitosan. The formation of snail shell-chitosan was confirmed by the appearance of two characteristic peaks at $2\theta =$

18.19° and 29.50° which correspond to the (110) and (241) planes of orthorhombic crystalline structure based on JCPDS card no: 39-1894 and some literature [44-46].

The peak at $2\theta = 34.22^\circ$ is indicative of mineral contamination [47]. The multiple peaks in the XRD spectrum of chitosan show that the snail shell-chitosan is polycrystalline. Therefore, the snail shell-chitosan is orthorhombic in structure and polycrystalline material in nature.

The properties of chitosan from snail shell wastes as displayed in Table 2 was compared to the quality indices of standard chitosan in Table 3 to establish quality of snail shell-chitosan.

Table 2. Snail shell-chitosan quality parameters

Parameters	Snail shell-chitosan
Moisture content (%)	1.42
Ash value (%)	1.61
Solubility (%)	85.2
DD (%)	67.44

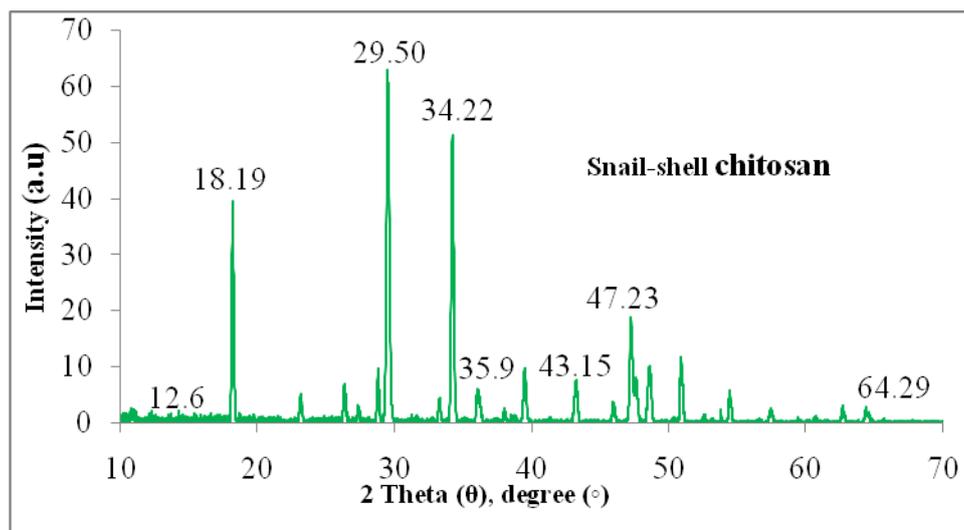


Figure 5. Powdered X-ray diffractogram of snail shell-chitosan

DD is the degree of deacetylation based on the carbon-to-nitrogen ratio.

Table 3. Quality parameters of commercial/standard chitosan

Parameters	Chitosan from crustacean (shrimp and crab) shell	Ref.
Moisture content (%)	<10	[48, 49]
Ash value (%)	< 1	[50, 51]
Solubility (%)	97.65	[5]
DD (%)	81.24	[5]
	80 (for high quality chitosan)	[2, 50]

3.1. Percent yields

Crustacean shells, especially shrimp and crab shells, contain 13-42 % of chitin and chitosan which is one of the reasons for their exploitation as main commercial sources of chitin and chitosan [52]. However, the extraction of the biopolymers from these feedstocks is strongly associated with the efficiencies of demineralization and deproteinization processes [4]. Most research groups had reported 15-25 % yield of chitosan from shrimp shell wastes [5, 53-54].

The chitosan yield of 2.67 g which corresponded to 14.83 % was obtained from snail shell waste. This yield percent is comparable to those of crustacean shell wastes as such snail shell is a potential feedstock for commercial production of chitosan.

3.2. Moisture content of snail shell chitosan

Chitosan is hygroscopic hence; it is stored in airtight container. Chitosan of good quality has low moisture content for prolonged self-stability and

storage [55-56]. Chitosan of excessive moisture content deteriorates quickly in quality as high moisture encourages fungal growth [31]. This is why the moisture content of most commercial chitosan is less than 10 % [48].

The moisture content of chitosan derived from snail shells is 1.42 % which is far less than the 10 % benchmark [48, 49] but above the range of 1.00-1.30 % reported for most shrimp shell wastes [57-59]. The high moisture content of the snail shell chitosan may be due to high atmospheric moisture (high humidity) at the time of extraction. Moreover, 7.56 % and 7.62 % moisture levels have been reported for shrimp and crab shells respectively by Parthiban and co [5].

3.3. Ash value of snail shell-chitosan

Ash value is a quality parameter that measures the extent to which a chitinous feedstock has been demineralized. Ash in chitosan is an indication that mineral salts were incompletely removed by demineralization [60]. The significance of ash value of chitosan is the effect it has on other quality parameters such as molecular weight, solubility, and viscosity [5, 55, 57]. As such, the ash value of chitosan of high quality is less than 1 % [61].

However, the ash value of snail shell-chitosan was 1.61 %. The implication is that 7 % of the demineralizer was insufficient for the complete removal of mineral salts from the snail shell powder. Hence, increasing the concentration of demineralizer as well as the retention time for demineralization might enable adequate removal of mineral salts.

Nevertheless, a high concentration of demineralizer could cause depolymerization via glycosidic cleavage (via hydrolysis), which might

reduce the polymer's molecular weight and chain length [5].

3.4. Solubility of snail shell-chitosan

Chitosan is insoluble in water. Chitosan becomes soluble when its amino groups are protonated at low pH conditions. The implication is that solubility is a function of the degree of deacetylation [47, 62]. Crystallinity, which is the order of arrangement of monomers in a polymer, is another factor that influences the solubility of chitosan [62].

Chitosan of high solubility, 97.65 % in 1 % acetic acid, is of excellent biological value [5]. However, solubility of the snail shell-chitosan in 1 % acetic acid is 85.2 %. Ahing and coworkers reported solubility of 99.0-99.7 % for shrimp shell chitosan in 1 % acetic acid solution [63]. The low solubility of snail shell chitosan relative to shrimp chitosan was due to its ash value as the FT-IR spectrum in Figure 4 had indicated no protein contamination because of the absence of absorption bands at 1500 cm^{-1} [30, 64]. Protein contamination and residual ash value are responsible for low solubility of chitosan [5,57]. Residual protein in chitosan results from incomplete deproteinization of its chitin, and it has tremendous effect on solubility.

3.5. Degree of deacetylation (DD)

The degree of deacetylation (DD) is the mole fraction of D-glucosamine in chitosan (N-acetyl-D-glucosamine and D-glucosamine) [65]. It is the mole fraction of deacetylated units of the chitosan chain [34, 66-68]. The DD is a functional parameter whose value affects other physico-chemical properties of chitosan. Properties such as solubility, chemical reactivity, antimicrobial and antioxidant activities, biodegradability, chelation, and mucoadhesive capacities of chitosan are affected by DD value [69-71].

The DD value of the snail shell-chitosan sample was determined by elemental analysis. The percent carbon and nitrogen in chitosan were 38.76 % and 6.80 % respectively hence, the DD value of snail shell chitosan was 67.44 %.

4. CONCLUSION

Chitosan was extracted from the shell waste of *Archachatina marginata* by demineralization, deproteinization, and deacetylation processes. The resultant chitosan was characterized by FT-IR and pXRD spectroscopy. Based on quality parameters, snail shell chitosan is of comparable quality to commercial/standard chitosan. Hence, snail shell waste is a potential feedstock for sustainable and commercial production of chitosan.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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IZVOD

VALORIZACIJA OTPADA LJUŠTURE AFRIČKOG DŽINOVSKOG KOPNENOG PUŽA (*ARCHACHATINAMARGINATA*) EKSTRAKCIJOM HITOZANA IZ NJEGA

Afrički džinovski kopneni puž (*Archachatinamarginata*) jedan je od najčešćih mekušaca u južnoj Nigeriji. Ima ljušturu koja čini više od polovine njegove telesne mase i obično se odbacuje kao otpad. Cilj ove studije je valorizacija otpada ljušture puža ekstrakcijom hitozana, svestranog i veoma zahtevnog biopolimera iz njega. Hitozan je ekstrahovan hemijskim metodama i okarakterisan Furijeovom transformisanom infracrvenom spektroskopijom i difrakcijom X-zraka praha. Parametri kvaliteta kao što su rastvorljivost, sadržaj vlage, vrednost pepela i stepen deacetilacije određeni su za hitozan iz ljušture puža. Rezultati pokazuju da je hitozan iz ljušture puža uporedivog kvaliteta sa komercijalno prodajnim hitozanom iz škampa. Hitozan iz ljuštura puževa ima DD vrednost od 78,4%, rastvorljivost od 95,35%, vlagu od 1,42% i vrednost pepela od 1,61%, što potvrđuje da je hitozan iz ljuštura puževa održiva i pogodna sirovina za komercijalnu proizvodnju hitozana. Prinosa hitozana na bazi ljuštura puževa bio je 14,83%, što je uporedivo sa prinomom iz otpada ljuštura rakova.

Ključne reči: *Archachatinamarginata*, otpad ljuštura, hitozan, parametri kvaliteta, valorizacija

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