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Characterization of chitosan extracted from three mushroom species from Edo State, Nigeria

Famous OSSAMULU[*],[1,2] Sarah EVBOUAN,[1] Helmina AKANYA,[1,2] Evans EGWIM,[1,2,3] and Stella LEH-TOGI ZOBEASHIA[4]

[1]*Department of Biochemistry, Federal University of Technology, P.M.B 65, Minna, Nigeria*
[2]*Africa Centre for Food and Mycotoxin and Food Safety, Federal University of Technology, Minna, Nigeria*
[3]*Center for Biotechnology and Genetic Engineering (CBGE), Federal University of Technology, Minna Nigeria*
[4]*National Biotechnology Development Agency, Abuja Nigeria*

Abstract. Chitosan, a biodegradable and nontoxic biopolymer, has applications in a wide range of fields. This study aimed to produce and characterize chitosan from three mushroom species obtained from Edo State, Nigeria. Standard protocols were used to extract and characterize chitosan. Chitosan yield from all three samples differed significantly ($p<0.05$) with the highest chitosan yield (19.00±0.03 %) from *Lenzites betulina*. There was no significant difference in the degree of deacetylation of *T. versicolor* and *L. betulina* extracted chitosan (82.71 and 83.54 % respectively). Chitosan from *Lenzites betulina* had significantly higher solubility (79 %), viscosity (1.04×10^{-1} centipoise) and molecular weight (4.70×10^4 Da) than those from the others. The bands of the spectra indicate the presence of NH_2, OH, C-O, CH, C-N functional groups. It was observed that the particle distribution was non-homogenous, irregular with the presence of pore for all spectra. The characteristics of chitosan obtained indicate that mushrooms from this locality could serve as an alternate source of chitosan to crustaceans with *Lenzites betulina* possessing the most promising features.

***Keywords*:** *Pleurotus ostreatus*; *Trametes versicolor*; *Lenzites betulina*; mushroom; degree of deacetylation; viscosity; solubility.

1. Introduction

Chitosan is a very important polymer of carbohydrate that is modified from chitin. This polymer is obtainable from different sources in nature such as fungi, insects, certain algae and crustaceans which are the major source [1]. Chitin is made up of units of N-acetyl-D-glucosamine and D-glucosamine linked through β-1,4-glycosidic bond. Availability of chitosan from chitin polymer is on the basis of deacetylation reaction. Chitosan is a polymer made up of 2-acetamino-2-deoxy—D-glucopyranose and 2-amino-2-deoxy—D-glucopyranose connected by β-1,4 links [2]. The proportion of deacetylation to acetylation determines whether the polymer is chitosan or chitin (where the number of N-acetyl-D-glucosamine units is greater than 40 % and the number of D-glucosamine units is less than 60 %) (Figure 1).

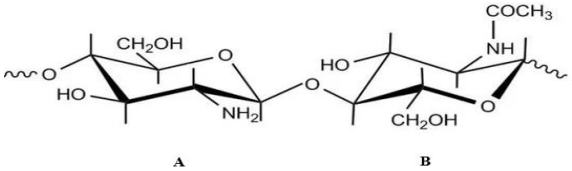

Figure 1. Structural units of chitin and chitosan. (A) glucosamine unit; (B) N-acetylglucosamine unit. In chitosan, (B) > (A); in chitin, (A) > (B).

One basic difference between chitin and chitosan is their solubility in diluted acid. In weak and diluted acids, chitin is not soluble whereas in most situations, chitosan polymer is very soluble in diluted acids [3].

Biocompatibility, biodegradability, and non-toxicity, among other qualities, have led to a wide range of applications for chitosan and other polysaccharide polymers. Film forming ability, chelation, absorption characteristics, and antibacterial feature are all distinct qualities of chitosan [4]. Chitosan has a variety of commercial and biological applications. Chitosan has been utilized effectively in agriculture in recent years due to its antifungal, antibacterial, and antiviral properties: in soil correction, enhancement of secondary metabolites synthesis, and activation of defense mechanisms, assisting plants in fighting fungal infections [5]. Chitosan can be used as a fining agent in winemaking, as well as a preservative [6]. It may be utilized in industries to make a self-healing polyurethane paint layer that can mend scratches on its own when exposed to sunshine [7].

The use of strong alkalis and acids in chemical deacetylation and demineralization as well as extended durations of high temperatures treatments, are used to commercially produce chitin and chitosan from crustacean shells. Because of the variety and challenges of the process conditions, as well as the seasonal and fluctuating availability of raw materials, the

[*] Corresponding author. *E-mail address*: i.ossamulu@futminna.edu.ng (Famous Ossamulu)

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physicochemical characteristics of chitosan generated by this approach may vary [2]. When compared to crustacean chitosan, fungal chitosan has better stable characteristics (degree of acetylation, molecular weight, viscosity, and charge distribution); fungal chitosan does not include heavy metals such as nickel and copper [8]. In comparison to high values derived from crustacean sources (approximately 1.5 ×10^6 Da), chitosan produced from fungal mycelia has a medium-low molecular weight (1–12 × 10^4 Da). Chitosan has the property of absorbing cholesterol [8] as well as being used in diverse medical applications. For these reasons, fungal based chitosan is increasingly gaining ground for use.

2. Experimental

2.1. Sample collection

The mushroom *P. ostreatus*, *T. versicolor*, and *L. betulina* were obtained from a farm in Edo State Nigeria, precisely Owan West Local Government Area (Figure 2). The mushroom species were taken to the Department of Biological Sciences, Federal University of Technology, Minna, for identification. The analyzed spore prints, morphology, and physiological aspects were comparable to the standard description.

Figure 2. Sampled mushroom types: *P. ostreatus* (A), *T. versicolor* (B), *L. betulina* (C).

2.2. Extraction of chitosan

Chitosan was extracted using a modified Rane and Hoover technique [9]. Each sample (50 g) of mushroom was weighed using an analytical weighing balance (MXBAOHENG, HQ-2183 China) into a conical flask containing 500 ml of 1 M NaOH and autoclaved at 126 ^oC for 30 minutes. The alkaline insoluble part of the fractions were obtained, cooled and washed with distilled water until it became neutral. To the neutral portion, 300 ml of 10 % acetic acid was added and allowed to stand for 12 hours inside an oven of 95 ^oC. The resulting slurry was centrifuged (70BL JAPSON, India) for 30 minutes at a speed of 12, 000 rpm. By changing the pH with drops of 5 M NaOH, the supernatant was collected and precipitated. Whatman filter paper was used to filter precipitated chitosan from the solution, which was then dried at 50 ^oC.

2.3. Estimation of chitosan yield

The equation below was used to calculate the percentage (%) yield of chitosan.

$$\text{Yield } = \frac{Wo - Wc}{Wo} \times 100$$

where Wo = initial weight of sample, Wc = final sample weight.

2.4. Degree of deacetylation estimation using infrared spectroscopy

The FTIR method reported by Sanuja *et al*. [10] was adopted in determining the degree of deacetylation. To a known weight of chitosan (dried at 60 ^oC) was added 200 mg potassium bromide tablet. The resulting mixture was then ground into a fine powder and subsequently placed in the spectrometer (IRAffinity-1S – Compact, Shimadzu Japan), in other to measure the intensity of the highest absorption bands by using the baseline approach. The degree of deacetylation in chitosan was measured using absorbance at 1655 cm^{-1} for amide-I and 3540 cm^{-1} for the OH group, and the equation below was used to calculate it:

$$\text{Degree of deacetylation } = \frac{A_{1655}}{A_{3450}} \times \frac{100}{1.33}$$

where "1.33" represents the value of the ratio of A_{1655}/A_{3450} for fully N-acetylated chitosan.

2.5. Determination of percentage solubility

Chitosan powder (0.1 g) was added to the tube containing acetic acid (1 %) and agitated for 30 minutes. The resulting solution was then boiled for 10 minutes in water bath. It was then allowed to cool to 25 ^oC before being centrifuged (Eppendorf™ Centrifuge 5425, Thermo Fisher Scientific, USA) for 10 minutes at 7000 x 9.8 m/sec^2. The residue was obtained by decanting the supernatant, it was rinsed in distilled water (25 ml) and centrifuged for 10 minutes at 7000 x 9.8 m/sec^2. The undissolved pellet was dried at 60 ^oC for 24 hours after the supernatant was decanted [11]. Finally, the pellet was weighed and the % solubility was estimated using the following equation:

$$\text{Solubility (\%) } = \frac{Wi - Wf}{Wi} \times 100$$

where: Wi = initial weight of chitosan, Wf = final weight chitosan.

2.6. Measurement of viscosity

A viscometer (PSL-Rheotek, Indiana USA) was used to determine the viscosity of chitosan as stated by Li *et al.* [12], to 10 ml of 1 % acetic acid, 0.1 g of chitosan powder was dissolved. The formed solution was then passed through a U viscometer tube, and the eluting time was recorded to calculate viscosity of the chitosan solution. The measurements in centipoise (cPs) were taken at a temperature of 25 ^oC.

2.7. Molecular weight determination

To 10 ml of 1 % acetic acid, 0.1 g of chitosan powder was dissolved and the developed solution was passed through a viscometer; the eluting time was recorded to calculate the viscosity of the chitosan solution [12]. The Mark-Houwink equation as shown below was applied to deduce the molecular weight:

$$[\eta] = K \times M^{\alpha}$$

where: η = viscosity (cPs), M = molecular weight in Dalton, K and α are constants, K = 4.74 x 10^{-5} dl/g and α = 0.72.

2.8. Scanning electron microscopy

A weighed amount (0.5 g) of chitosan was placed on a gold-plated metallic stub while being observed at room temperature in scanning electron microscope (Verios 5 XHR SEM, ThermoFisher SCIENTIFIC) at a very high voltage of 10 Kv with a 1000 x magnification imaging as reported by Poerio *et al*. [13].

2.9. Analytical statistics

The data were analyzed with SPSS and evaluated statistically employing one-way. The obtained values were presented as mean ± standard error of mean at $p < 0.05$ which was used to determine if differences between means were significant.

3. Results and discussion

Chitosan yield from all three mushroom species are presented in Figure 3. The result showed a significant difference ($p < 0.05$) in the chitosan yield from all the mushroom samples. The mushroom *Lenzites betulina* had the highest yield of chitosan (19.00±0.03 %) while *Pleurotus ostreatus* had the lowest yield of chitosan (8.70±0.08 %). The report of Elem and Uraka [14] who obtained a yield of 7.18 % chitosan from *Laccaria laccata* and 18.8 % from *Laccaria amethysta* were similar to those in the present finding. However, the yield of chitosan extracted from oyster mushroom (*Pleurotus ostreatus*) in the work of Kabir *et al*. [15] was higher (16.6 %) than that obtained for the same mushroom species in the present study. The calculated yield as reported in this present investigation is also lower than the finding in the study of Majekodunmi *et al*. [16] for bivalved mollusk shell (*Mytilis edulis*). The observed chitosan yield variation, might have resulted from species variation, extraction protocol engaged as well as source of materials used for extraction [14, 15].

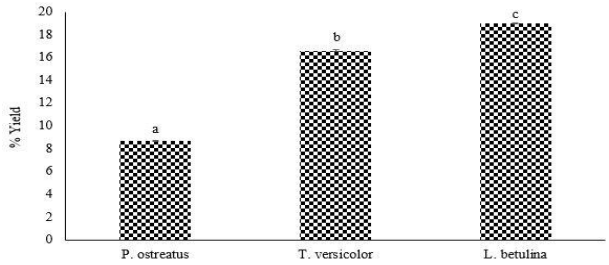


Figure 3. Percentage yield of chitosan derived from *P. ostreatu, T. versicolor* and *L. betulina*

Table 1 presents some properties of chitosan derived from the sampled mushroom species. The species *Trametes versicolor* and *Lenzites betulina* had similar levels of deacetylation, whereas *Pleurotus ostreatus* had a lower degree of deacetylation. In all the chitosan samples tested, there was no significant variation in solubility. The maximum molecular weight was found in chitosan derived from *Lenzites betulina*, which differed from values obtained for *Pleurotus ostreatus* and *Trametes versicolor* extracted chitosan. Chitosan obtained from *Pleurotus ostreatus* and *Trametes versicolor* had statistically similar viscosities while *Lenzites betulina* was significantly different. The degree of deacetylation of chitosan varies from 56 to 99 %, according to Jiménez-Gómez and Cecilia [17] and this is consistent with the present finding in this work. The % DD of chitosan derived from *Trametes versicolor* and *Lenzites betulina* are statistically similar although was significantly higher that was obtained for chitosan from *Pleurotus ostreatus* (Table 1). The % DD of chitosan extracted from *Pleurotus ostreatus* is in consonance with the value reported by Kannan *et al*. [18] for Ganoderma species (76.16 %). Chitosan from the fungi *Aspergillus niger* has also been reported with a 90 % DD [19], this value is higher than the values obtained in this study. However, chitosan from *Termitomyces titanicus* had degree of deacetylation (69.50 %) lower than was reported in the present work [20]. These discrepancies in degree of deacetylation might be due to variation in mushroom species. The degree of deacetylation is also dependent on the source of raw material as well as the employed extraction process [21].

Chitosan solubility (%) as observed in the present work (Table 1) was significantly greater than that reported by Elem and Uraku for *Laccaria amethysta* (40 %) [14]. Solubility is a very important index that defines the usefulness or applicability of chitosan. Higher solubility values define the extent of its usefulness and applicability. Solubility of chitosan polymer, is influenced by its degree of deacetylation, which might explain the differences in solubility between the species investigated. Lower solubility values might imply inadequate protein and acetyl group removal [22, 23], which could explain the discrepancy in the results.

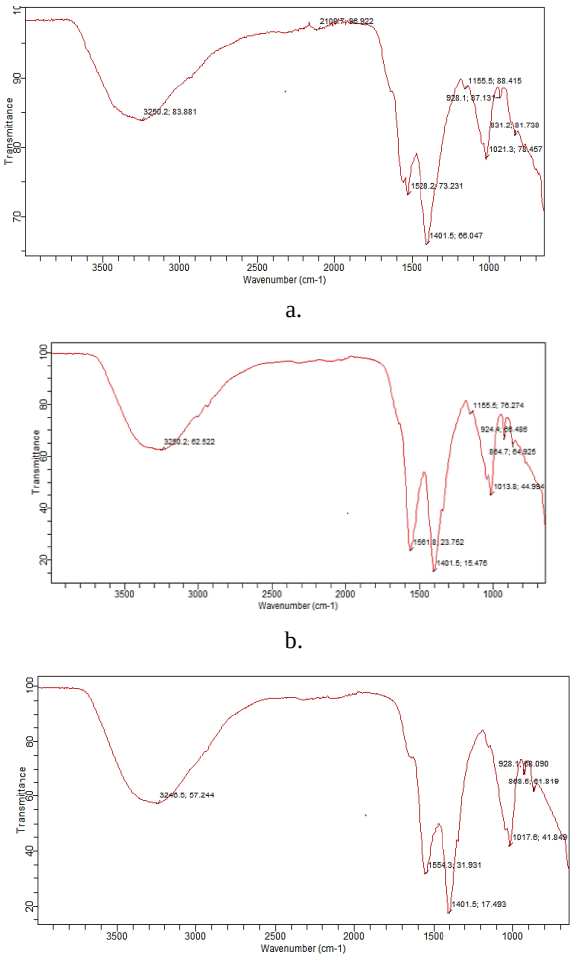
It was also observed from Table 1 that chitosan obtained from *Pleurotus osreatus* as well as *Trametes versicolor* were not significantly different in molecular weights although lower than the molecular weight observed for *Lenzites betulina*. However, all the obtained values in the present work for molecular weight were lower than that reported for shrimp chitosan (1.05×10^6) in the study of Hossain and Iqbal [24] and between 2.3-2.8 $\times 10^5$ Da [21]. The study of Chen *et al*. [25] achieved 5.9 x 104 Da for molecular weight of chitosan from *Ganoderma tsugae* which is somewhat higher than the values found in this investigation. Commercial chitosan has a molecular weight of 1.0 x 10^5 to 1.2 x 10^6 Da, according to Kabir *et al*. [15]. Chitosan tends to be more viscous when its molecular weight is high [26]. The species as well as source of chitosan may be the reason for differences in molecular weight.

Table 1. Physicochemical characteristics of chitosan derived from *P. ostreatus, T. versicolor* and *L. betulina*

Parameters	Mushroom Species		
	P. ostreatus	*T. versicolor*	*L. betulina*
DD (%)	78.64±0.32^a	82.71±0.37^b	83.54±0.79^b
% Solubility	75.00±0.58^b	76.00±0.58^a	79.00±0.58^a
Mwt (Da) x 10^4	2.68±0.03^a	2.68±0.11^a	4.70±0.40^b
Viscosity (cPs) x 10^{-2}	6.90±0.002^a	6.89±0.005^a	10.40 ±0.007^b

The values are presented as mean ± standard error of three determinations (n =3), values that have different alphabet as superscripts across the row are significantly different at $p < 0.05$ for each parameter.

Table 1 shows that the viscosity of chitosan derived from *Lenzites betulina* is greater than that obtained from the other two species. This might have been influenced by their molecular weights. Majekodunmi *et al.* [16] reported a 3.86 cPs viscosity for chitosan derived from a mollusk (*Laevicardium attenuatum*), which was greater when compared to the results in this present investigation. Physical and chemical treatments have been shown to have a significant impact on chitosan viscosity. It diminishes as treatment duration and temperature rise [27]. Solutions that are high in viscosity have been reportedly difficult to manage [26]. According to Elem and Uraku [14], chitosan with a reduced viscosity may have more uses in medicine, pharmacy, and agriculture.



a.

b.

c.

Figure 4. FTIR spectrum of chitosan obtained from *Pleurotus ostreatus* (a), *Trametes versicolor* (b) and *Lenzites betulina* (c).

The FTIR spectra of chitosan derived from the three mushroom samples as shown in Figure 4. The peaks at 831.2 cm^{-1}, 864.7 cm^{-1}, as well as 863.07 cm^{-1} can be assigned to the C-N stretching. Peaks at 1021.3 cm^{-1}, 1013.8 cm^{-1}, and 1017.6 cm^{-1} for the three spectra may be assigned to the functional group of C-O stretching of the glucose molecule while the C-H bending of the side chain $–CH_2OH$ can be detected at 1401.5 cm^{-1} for the three spectra. The peak at 1528.2 cm^{-1}, 1561.8 cm^{-1}, and 1554.3 cm^{-1} in the relevant spectra is indicative of an amide, but the peak at 3250.2 cm^{-1} (for *Pleurotus ostreatus* and *Trametes versicolor* derived chitosan) and 3248.5 cm^{-1} (for *Lenzites betulina* derived chitosan) is indicative of free O-H groups. According to Ghannam *et al.* [28], the stretching of the amide group in chitosan obtained from crayfish and shrimp was 1660.41 cm^{-1} and 1658.48 cm^{-1} respectively. The peaks as shown on the FTIR spectra are typical of a chitosan molecule and the bands of the spectra reflect the presence of several functional groups in the chitosan biopolymer [29]. The spectra may also allow for a quantitative assessment of the degree of acetylation of the amines in the polysaccharide structure [30].

Figure 5 shows the morphological structure of the isolated chitosan using scanning electron microscopy. The micrographs revealed that distribution of the particles was non-homogeneous, irregular shaped as well as the existence of pore spaces. There is a variation in roughness and surface morphology in the different chitosan derived from the mushroom species. Chitosan derived from *Pleurotus ostreatus* had a rougher surface structure than that obtained from the other two mushroom derived chitosan, which is in consonance to Gupta and Jabrail's [31] report, who found that lower % degree of deacetylation (DD) is most likely to result to a rougher surface when observed from SEM. Of vital importance in regulated drug delivery are both the size as well as the shape of chitosan biopolymers particles. The surface charge and size of chitosan particles can also be adjusted to achieve delivery effectiveness, according to Quan and Paul [32].

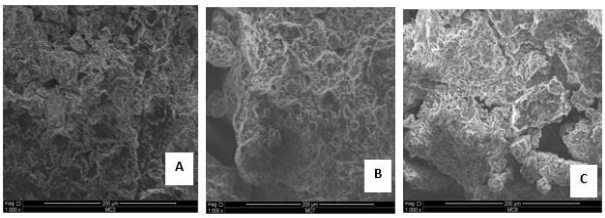


Figure 5. SEM images of chitosan from: *P. ostraetus* (A), *T. versicolor* (B) and *L. betulina* (C).

4. Conclusions

The three mushroom species namely; *P. ostreatus*, *T. versicolor*, and *L. betulina* generated appreciable yield of chitosan, although the later (*Lenzites betulina* which had the highest yield) if properly domesticated can become a reliable alternative source for chitosan production instead of majorly depending on crustacean shells. The physicochemical properties of the extracted chitosan presents them as viable biopolymers in biological system application.

Conflict of interest

There is no existing conflict of interest

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