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Physicochemical properties of Chitosan from SevenDifferent Wild Edible Nigerian Mushrooms

Elem, R. C[1]., Uraku, A. J[2]

[1]Department of Biochemistry, Federal University of Technology, Minna, Niger State, Nigeria
[2]Department of Biochemistry, Ebonyi State University, Abakaliki, Nigeria

ABSTRACT

Melon Background: Chitosan are characteristically free powders consisting of polymers and copolymers derived from the deacetylation of chitin, biodegradable in nature. Chitosan has unique properties which make it useful as adhesives, flocculating aid, viscosity control agent and in paper-strengthening agent. The industrial production of chitosan from crustacean shells, lobsters, shellfish waste and shrimps, is associated with several drawbacks; the problems with limited and seasonal supply, product variability and confined production locations, and the high cost associated with the chemical conversion of chitin to chitosan shows the limited potency in industrial acceptance of those polymers. The search for an alternative source becomes necessary. **Materials and Methods:** This study evaluated quality and quantity of chitosan from different wild edible Nigerian mushrooms. Chitosan were prepared from seven wild edible Nigerian mushrooms by deproteinisation and deacetylation of chitin. **Results** The results of physicochemical properties assayed included: Yield (7.18 - 61.11%),), Degree of deacetylation (89.60 - 91.10%), Acetylation degree (1.94 - 10.40%), Molecular weight (6039.70 - 6914.18g/mol, Viscosity (5.03 - 5.62cPs), Solubility (30.00 - 60.00%), Moisture (9.00 - 34.00%) and Ash content (5 - 28%). **Conclusions**: The production of chitosan from wild mushrooms could be a cheap alternative to that of shrimps.

KEYWORDS:
mushroom meal, nutrition, chitosan, polymer, copolymer

***Correspondence to Author:**
Uraku, A. J
Department of Biochemistry, Ebonyi State University, Abakaliki, Nigeria
Email: urakuaj @yahoo.com, urakuaju @gmail.com,
Phone: +234-8068073037

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INTRODUCTION

The usefulness and importance of chitosan applications cannot be overemphasized in this present age. Therefore, the improvement and transformation of industries can be met by an intense development in this area of study, although just a handful of industries have currently ventured into the technology. The potentials of nanotechnology have been harnessed, however, in the development of different chitosan sources for enzyme immobilization as well as drug delivery [1]. A spontaneous transformation has been observed in the last twenty years as regard the improvement in drug delivery as a result of increased length of residential time at the target site of absorption as well as in enzyme immobilization [2].

Chitosans are polymer or co-polymer derivatives of the deacetylation of chitin which are biodegradable with particle size less than 200μm[3]. Thus, carrier nanobiotechnology offers an intelligent approach for enzyme immobilization and drug delivery by coupling the drug and enzyme to a carrier particle such as nanocomposites, microspheres, nanoparticles, liposomes, chitosan etc, which modulates the release and absorption characteristics of the enzymes and drug [4].

Chitosans are famous natural cationic polyelectrolytes and this has placed them as vital materials for nanoparticles[5]. This polymer is a partially deacetylated product of chitin, which is a basic structural polysaccharide available in crustacea, insects, as well as some fungi. It has recieved much interest as a biocompatible, biodegradable, mucoadhesive, and nontoxic material which may be largely utilized in biomedical applications [6]. Moreso, chitosan has a basic attribute of sticking on to target organs (e.g. mucosal surfaces) as well as loosening junction between epithelial cells which are tight in short time frame[7]. Accordingly, chitosan nanoparticles have the capacity to be utilized as delivery system for hydrophilic drugs as a result of its outstanding physicochemical and biological characteristics.

There is a major setback of present industrial production of chitosan from crustacean chitin [8] as it is poorly ecofriendly. This is because crustacean chitin possess high amount of $CaCO_3$ which therefore release CO_2 and consequently pollutes the environment as extraction is being done. Another setback is the availability of raw material in chitosan production as crustacean shells are basically seasonal and therefore resulting to product variability [9]. It is therefore necessary to venture into other more economical friendly approach for the production of chitinous polysaccharides. This research work was therefore triggered so as to use alternative source such as edible wild Nigerian mushrooms where chitin is present as a cell wall component to produce chitosan. Certain characteristic features exist in mushroom chitin and chitosan different from those isolated from crustaceans. They possess more functional properties as well as enhanced bioactivity. Another edge they have to other sources is the fact that mushrooms can be harvested all round the year [10]**.** This informed their selection for the present work. The present study was therefore aimed at producing and characterizing chitosan from seven wild edible Nigerian mushrooms.

Materials and Methods

Collection of Mushroom Samples

Seven different edible wild Nigerian mushrooms *Pleurotus ostreatus, Lactarus deliciousus (Milk mushroom), Laccaria amethysta, Cantharelles cibarius, Laccaria laccata, Hericium erinaceus* and *Pleurotus tueragium* were collected from different areas within the base of a thick forest located in Ugu–Uleri in Biladebia Ntezi in Ishielu Local Government Area of Ebonyi State, Nigeria and were identified by their spore prints and by comparing their morphological, anatomical and physiological characteristics with the standard description in the Department of Applied Biology, Ebonyi State University, Abakaliki, Nigeria.

Preparation of Mushroom Samples

The mushrooms were uprooted, destalked, washed and air-dried under room temperature for 2-6 days while turning the mushroom to avoid fungal growth. The mushrooms were later milled to obtain the mushroom meals (MRMS) using mortar and pestle and stored in an ovum at 110^0 C in a container for analysis.

Chitosan Extraction

Chitosan extraction was carried out by a modified method of Rane and Hoover [11] and Crestini et al

[12]. The mushrooms powder was suspended with 1 M NaOH solution (1: 30 w/v) and autoclaved at 126°C for 15 min. Alkali-insoluble fractions (AIF) were collected after centrifugation at 12 000 X 9.8m/sec^2 for 15 min, washed with distilled water to neutrality. The residues were further extracted using 2% acetic acid (1:40 w/v) at 95 °C for 8 h. The extracted slurry was centrifuged at 12 000 9.8m/sec^2 for 15 min. The pH of the supernatant fluids was adjusted to 10 with 2 ml of 1M NaOH, the solution centrifuged at 12 000 x 9.8m/sec^2 for 15 min and the precipitated chitosan was washed with distilled water, 95% ethanol (1 : 20 w/v) and acetone (1 : 20 w/v), respectively and dried at 60 °C to a constant weight.

Determination of the Percentage Yield of Chitosan

The yield from each prepared chitosan was calculated from the following equation:

$$\% \text{ Yield} = \frac{Wo - Wc}{Wc}$$

Where, W_o is the mass of the sample before production, Wc is the mass of the chitosan material after production.

Determination of the degree of deacetylation by Titrimetric Method

A known amount of chitosan was hydrolyzed with sodium hydroxide and acidified with phosphoric acid to convert the salt to acetic acid. The aqueous acetic acid was distilled, and when the distilling flask began to go dry, 15ml of hot distilled water was added to the flask. Aliquots of 25ml were titrated with 0.01M sodium hydroxide using phenolphthalein as indicator. The volume of base was multiplied by ten to give the total volume of the distillate (250ml) [13]. The DA was determined from formula;

%DA = (V x 0.04305)/m

Where V is the volume of sodium hydroxide multiplied by ten and m the mass of the chitosan

Determination of Molecular Weight

The molecular weight average was determined by viscosity-average molecular weight (Dalton), the mushroom chitosan was dissolved in a mixture of 0.1M acetic acid with 0.2M NaCl, then, the U tube viscometer was used to measure the intrinsic viscosity in Centipoise (η). The Mark-Houwink equation relating intrinsic viscosity with the empirical viscometric constants K = 1.81 X 10^{3}cm^3/g and a = 0.93; were used to calculate the molecular weight using the equation below: [η] = Kma [14].

Determination of Viscosity of Chitosan

The viscosity of wild edible mushroom chitosan was determined with the Brookfield Viscometer (Gallenkamp U tube viscometer model 072845). Chitosan solution was prepared in 1% acetic acid at a 1% concentration on a dry mass basis. The measurement was made in duplicate using a No.5 spindle at 50 X 9.8m/sec^2 on solutions at 25°C with values reported in centipoises (cPs) units.

Determination of Solubility of Chitosan

The wild edible mushroom chitosan powder (0.1g in triplicate) was placed in a centrifuge tube of known weight. It was then dissolved with 10ml of 1% acetic acid for 30minutes using an incubator shaker operating at 240 rpm and 25°C (C25KC, New Brunswick Scientific Co., Inc. NJ). The solution was then immersed in a boiling water bath for 10 minutes, cooled to room temperature (25°C) and centrifuged at 10,000 X 9.8 m/sec^2 for 10 minutes. The supernatant was decanted. The undissolved particles was washed in distilled water (25ml) and then centrifuged at 10,000 X 9.8m/sec^2. The supernatant was removed and undissolved pellet dried at 60°C for 24hours. Finally, it was weighed and the percentage solubility calculated:

solubility calculated:

$$\% \text{ Solubility} = \frac{(\text{Initial Weight of tube} + \text{Chitosan}) - (\text{Final Weight of tube} + \text{Chitosan})}{(\text{Initial Weight of tube,g} + \text{Chitosan}) - (\text{Initial Weight of tube})} \text{ X } 100$$

Determination of Moisture Content of Chitosan

The moisture content of the wild edible Nigerian mushroom chitosan was determined by the gravimetric method [15]. The weight was determined by drying the sample to a constant weight and measuring the sample after and before drying. The Weight was the difference between the weights of the wet and the oven dry samples. The Weighed and Recorded weight of dish with sample was then placed in the lid or filter paper to avoid contamination. The temperature of the oven was adjusted to 60°C and dried the samples

for 24 hours. The sample was evacuated from the oven using tongs and placed in desiccators until it cooled to room temperature. The weight of the sample was the recorded as weight of the dry sample. It was then calculated as:

$$\% \text{ Moisture content} = \frac{\text{Wet Weight, (g)} - \text{Dry Weight (g)}}{\text{Wet Weight(g)}} \text{ X } 100$$

Determination of Ash Content of Chitosan

The ash content of the wild edible Nigerian mushrooms was calculated according to the standard method [16]. The 3.0 g of the mushroom chitosan was placed in a tarred crucible in triplicate and was ignited into ash and was allowed to cool. The sample was then heated in a muffle furnace at 600°C for 6 hours. It was then allowed to cool and calculated as follows below;

$$\% \text{ Ash} = \frac{\text{Weight of residue}}{\text{Sample Weight}} \text{ X } 100$$

Statistical Analysis

The data was analyzed by ANOVA and results expressed as means and standard error. Differences between means were regarded significant at P<0.05.

RESULTS

The results of the physicochemical properties of chitosan produced from different Nigerian mushrooms are presented in Table 1. It was observed that *Laccaria laccata* had the lowest yield (%) of chitosan (7.18±0.01%) while *Cantharelle cibarius* had the highest chitosan yield 61.11±0.01 (%). The result showed that there was a significant (P<0.05) difference in the percentage yield of the chitosan of the various mushroom samples.The degree of deacetylation (DD) of the chitosan ranged from 89.60±0.005 in *Cantharelle cibarius* to 91.1067±0.01% in *Lactarus deliciousus*. The result also indicated that there is a significant (P<0.05) difference in the percentage degree of deacetylation of the chitosan samples.

The molecular weight (MW) of was observed to have the lowest value (6039.70±0.00 g/mole) in chitosan obtained from *Laccaria amethysta* while chitosan from *Lactarus deliciousus* was highest in M.W (6914.18±0.00 g/mole). The result indicates that there was no significant (P>0.05) difference in molecular weight of the different chitosan produced. The viscosity of the chitosan ranged from 5.03±0.01 in *Laccaria amethysta* to 5.92±0.01 (cPs) in *Lactarus deliciousus*. There was a significant difference in some species but *Hericum erinaecius, Pleurotus ostreatus* show no significant (P>0.05) difference. The solubility of the chitosans produced ranged from 30.00±0.58 - 60.00±0.58 (%). The result indicates that there a significant (P<0.05) difference occurred in the solubility levels of the various samples.

The moisture content ranged from 9.29±0.30 in *Laccaria amethysta* to 34.00±0.58% in *Cantharelle cibarius*. There was a significant (P<0.05) difference in the percentage moisture content of the chitosan samples. The ash content of the chitosans ranged from 5.00±0.57 - 22.50±0.06 (%). The result showed a significant (P<0.05) difference in the ash content of the different chitosan samples.

Discussion

In the present study an attempt has been made to develop and characterize chitosans produced from different wild edible Nigerian mushrooms. The results of the physicochemical and functional properties of the prepared chitosan are presented in the Table 1.

The mean chitosan yield obtained in the study showed that *H. erinaecius (*61.11%*), P. tuberagium (*52.15%*), L. deliciousus (*35.78%*), L. amethysta (*18.80%*), P. ostreatus (*17.80%*), C. cibarius (*11.77%) and *L.laccata (*7.18%). The obtained results were higher than 14.00% and 18.60% gotten from krill and prawn respectively[17] and also higher than 23% reported by No and Meyers [18] except *L.laccata*. This observation indicates that mushroom could be a better source of raw material for chitosan production.

The degree of deacetylation of mushroom chitosan was in the range of 89.60-98.08%. The obtained results are in contrast with the report of Tajik et al. [19] where they stated that chitin content of Crustacean shells ranged from 13 to 42%, crab (13 to 26%), case of shrimp (14 to 42%) and krill (34 to 49%). In comparison to each other, *L. amethysta* and *P. ostreatus* had lowest values while thus *L. deliciousus* had highest *amounts.* The degree of deacetylation is an important parameter that affects the

physicochemical properties of chitosan. Though, the large positive charge density due to high degree of deacetylation makes mushroom chitosan unique for both biomedical, commercial and industrial applications, particularly as a carrier, support for enzyme immobilization and drug delivery.

The molecular weight of the mushroom chitosan samples ranged from 6089.49 to 6914.18 g/mole (Table 1.). This is in agreement with the report of Marianna *et al*. [20] who showed that the prepared chitosan is 165394 g/mole. Although, several factors during production such as high temperature, concentration of alkali, reaction time and previous treatment of chitin, particle size, chitin concentration, dissolved oxygen concentration and shear stress may influence the molecular weight of chitosans as reported by Oh *et al, [21]*. The obtained result showed low molecular weight chitosans (LMWCS) and thus conformed to the suggestion that low molecular weight chitosans (LMWCS) express higher bioactivity than medium molecular weight chitosans (MMWCS) and high molecular weight chitosans (HMWCS).

The result of the viscosity was 5.03-5.92 cPs. These results obtained from the present study were similar to reported by Shimahara *et al. [22]* but this was in contrast to the findings of No and Meyers [18], who demonstrated that the viscosity of Chitosans varied considerably, from 60 to 5110 cP, depending on the species and preparation methods used. Thus, mushroom chitosan could have potential medical and agricultural applications with lower viscosity; mushroom chitosan could have more applications in food industries, phamacuitical industries, medicine, agriculture, and waste water treatmement [22]. This is because Shimahara *et al, [22]* have reported that chitosan with lower viscosity could have better applications in medicine, pharmacy and agriculture. *Lactarius deliciousus* had the highest viscosity of 5.92 cP compare to other species. The two commercial crab chitosans showed higher viscosity values than our mushroom samples. The higher molecular weight of commercial chitosan may have resulted to the higher viscosity of commercial chitosan compared to our mushroom chitosan [23]. When molecular weight is lower, viscosity also tends to decrease [23]. On the basis of these composite observations, it is apparent that mushroom chitosan will have low viscosity. There are factors that affect viscosity during the production of chitosan such as the degree of deacetylation, molecular weight, ionic strength, pH, and temperature, etc. Moorjani *et al. [24]* reported that chitosan viscosity decreased with increased time of demineralization and deproteinization. The viscosity of chitosan in acetic acid tends to increase with decreasing pH. Intrinsic viscosity of chitosan is a function of the degree of ionization as well as ionic strength [25]. Deproteinization with 3% NaOH, and elimination of the demineralization step in chitin preparation, decreased the viscosities of the final chitosan samples[24] stated that it is not desirable to bleach the material at any stage since bleaching considerably reduces the viscosity of the final chitosan product.

Study affirmed that it could be argued that the molecular weight of chitosan depends on the degree of deactylation (DD). A hugher DD would therefore imply a smaller molecular weight. However, this relationship between MW and DD is not very predictable. It has been suggested that chitosan for gene delivery properties should be low (about 10KD) while that of drug delivery systems should have high molecular weight. It is very important to remember that, there are several factors during commercial production, including high temperature, concentration of alkali, reaction time, previous treatment of the chitin, and particle size may influence the MW of chitosans [23].

The percentage solubility of seven wild edible Nigerian mushroom chitosan ranges from 30 - 60%. It is in order of *L. laccata* (30%), *L. amethysta* and *P. ostreatus* (40%), *C. cibarius* (50%) with *P tiberagium, L. deliciousus* and *H. erinaecus* 60%. The low solubilty of *L. laccata*, *L. amethysta* and *P. ostreatus* suggested incomplete removal of protein. This could be based on the chemical method used since it is based on the reaction with the amino group and the presence of protein contaminants remaining in the sample during the analysis process could adversely interfere with the obtained results. The result is in agreement with the report of Brine and Austin [26].

The chitosan from mushroom samples had a moisture contents from 9.29-34.00%. This

result is higher than the result of Tajik et al. [18] who reported percentage (%) moisture content between 1-1.30 %. Chitosan is hygroscopic in nature[27] hence it is very possible that the prepared chitosan samples were affected by moisture absorption during storage. According to Li *et al*. [28], commercial chitosan products contain less than 10% moisture content. The moisture adsorption may be important by affecting water holding capacity of CSs, when it comes to its processing and applications [18].

Ash measurement is an indicator of the effectiveness of the demineralization (DM) step for removal of minerals. High quality chitosans are suggested to have less than 1 % ash content. The result of this study showed that the chitosans prepared from mushrooms contained higher ash content with a range of 5.00-28.00%. Studies have shown that some residual ash in chitosans may affect final product qualities including solubility and thus consequently contributing to lower viscosity [29].

Conclusion

The present observations indicate that the prepared chitosan from edible Nigerian mushrooms in this study is soluble in 1% acetic acid solution. That the physiocochemical features of the chitosan from our mushrooms are better than that of others produced from other sources as reported by previous studies. Thus, preparation of chitosan from mushrooms can be a cheap alternative to that of shrimps, crustaceans and lobsters among others.

Authors' contributions

This work was carried out in collaboration between all Authors. Author ERC designed the Study and managed the analysis of the study and the bench work. Author UAJ wrote the protocol, performed the statistical Analysis as well as wrote the first draft of the manuscript and managed the literature search. All authors read and approved the final manuscript

Conflicting interest: the authors have decleared that there is no competing interest existing.

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Table 1 Physicochemical Properties of Chitosan Produced from Seven Edible Wild Nigerian Mushrooms

Species	Yield %	Degree of Deacetylation (%)	Acetylation (%)	Molecular Weight (g/mole)	Viscosity (cPs)	Solubility (%)	Moisture (%)	Ash (%)
P. tuberagium	52.15±0.01^{e}	98.09±0.01^{g}	1.94±0.006^{a}	6622.09±0.00^{a}	5.62±0.01^{f}	60.00±0.58^{d}	11.04±0.04^{b}	9.50±0.06^{b}
L. amethysta	18.80±0.05^{c}	89.7267±0.00^{c}	10.27±0.006d	6039.70±0.00^{a}	5.03±0.01^{a}	40.00±0.57^{b}	9.29±0.30^{a}	28.00±0.57^{e}
L. deliciousus	35.78±0.01^{d}	91.1067±0.01^{f}	8.90±0.006b	6914.18±0.00^{a}	5.92±0.01^{g}	60.00±0.58^{d}	25.00±0.58^{d}	12.00±0.57^{c}
L.laccata	7.18±0.01^{a}	90.7500±0.01^{e}	9.25±0.006c	6307.58±0.00^{a}	5.30±0.01^{c}	30.00±0.58^{a}	11.0367±0.04^{b}	5.00±0.57^{a}
C. cibarius	11.77±0.03^{b}	89.60±0.005^{a}	10.40±0.006f	6089.49±0.00^{a}	5.08±0.01^{b}	50.00±0.50^{c}	34.00±0.58^{e}	17.50±0.06^{d}
H. erinaecius	61.11±0.01^{f}	90.57±0.009^{d}	9.25±0.006c	6416.06±0.00^{a}	5.41±0.01^{e}	60.00±0.58^{d}	14.00±0.58^{c}	9.50±0.06^{b}
P. ostreatus	17.80±0.06^{c}	89.65±0.005^{b}	10.35±0.06e	6465.22±0.00^{a}	5.50±0.04^{e}	40.00±0.57^{b}	13.00±00.58^{c}	22.50±0.06^{d}

Values are expressed as mean±standard error of mean of two determinations.

Values Followed by different superscript on the same column alphabets are significantly different at P<0.05 level of significant.