CHITOSAN PRODUCTION OF *PLEUROTUS OSTREATUS* (JACQ.EX FR.) P. KUMM CULTIVATED ON DIFFERENT SUBSTRATES

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ABSTRACT

Pleurotus ostreatus possesses numerous potentials and resources. The substance chitosan is one of the numerous resources of this mushroom which are widely used in industries such as pharmaceutical industries, food industries, agricultural and cosmetic industries. This work is centered on the extraction of chitosan and study of its physicochemical properties. The result of physicochemical properties of chitosan extracted *P. ostreatus* mushrooms cultivated on six different substrates was investigated. The highest chitosan yield was obtained from substrate A.G (14.77 ± 1.25^{a} g) while the lowest was from substrate P.P (12.48 ± 2.14^{f} g), the highest degree of deacetylation was obtained from substrate A.G + D.C (85.78 ± 1.89^{a}%) while the lowest was from A.G + P.P (84.20 ± 1.43^{d} %), the highest percentage of acetylation was obtained from substrate with (13.39 ± 2.13^{a} %) while the lowest was from A.G (10.31 ± 2.33^{a}%), the highest molecular weight was obtained from A.G + P.P (6482.49 ± 4.71^{a} g/mol) while the least was from A.G+ D.C (6255.19 ± 3.27^{d} g/mol), and the highest viscosity was obtained from P.P (5.24 ± 2.47^{a} ml/g) while the least was from P.P + D.C (4.13 ± 2.57). The physicochemical properties of chitosan result showed that quality chitosan can be obtained quantitatively from mushroom at low cost, simple, and devoid of limitations of site and season. The result of this study also indicates that chitosan has significant potentials for industrial and agricultural uses.

Keywords: mushroom, *Pleurotus*, chitosan, physicochemical, alkalinization

INTRODUCTION

Mushrooms are higher fungi which belong to the division Basidiomycetes, with few members in the division Ascomycotina. They are fleshy and noticeable and possess mycelia which are networks of hyphae. Sometimes, the mycelia are enshrouding in the tree trunk tissues, on a fallen log of wood or in other nourishing substrates (Ingold, 1993). Mushrooms are saprophytes which mean they can strive in an extensive environment and substrates. However some substrates are ideal for the growth of mushroom than others (Adesina *et al.,* 2011). Mushrooms have been valued as a significant food for generations due to its high protein content and delicious flavor (Gbolagade *et al,* 2005; Vieira *et al,* 2014). Aside from that, several mushroom species have been utilized medically (Tochikura *et al.,* 1988).

Pleurotus ostreatus (Oyster mushroom) are the most prospective and second largest cultivated mushroom in Nigeria. The viability, proliferation and nutritional content of this mushroom depend on the type of substrates (Khare *et al.,* 2010). The importance of mushroom especially *P. ostreatus* has drawn lots of interest. The cell wall which contains the biodegradable polymer known as chitosan has attracted the attentions of both agriculturists and industrialists globally. Globally there are roughly 140 × 106 tons of synthetic polymers produced yearly. Nevertheless, these synthetic polymers are somewhat stable and their biodegradation is limited. This necessitates the need for biodegradable polymers that are compatible with the ecosystem. Among these biopolymers, chitin and chitosan have attracted the attention of both scientists and industrialists due to their numerous potential applications in biomedicine, agriculture, paper making, food industry, and textile industry (Akila, 2014). The wider applications of chitosan are not only due to their abundance but also due to their non-toxicity and biodegradability (Islam *et al.,* 2017). Production of chitosan from fungal mycelium has recently received increased attention due to significant advantages. For example, while crustacean waste supplies are limited by seasons and sites of fishing industry; fungal mycelium can be obtained by convenient fermentation process that does not have geographic or seasonal limitations. Due to its biodegradability, nontoxicity and intrinsic antibacterial effects, chitosan has been widely used as an antimicrobial agent to improve food quality and extend shelf life. This could be achieved by using chitosan alone or with its derivatives or blended with other ingredients like biocontrol yeast and calcium chloride (Yu *et al.,* 2012).

This study also was designed for assessment of chitosan constituent of *P. ostreatus* quantitatively and to analyze it in order to ascertain the physicochemical properties of this chitosan for industrial and agricultural use which are simple, cheap and compatible with ecosystem compared to synthetic chitosan and chitosan produced from crustaceans and crabs.

MATERIALS AND METHODS

The study was carried out in the mushroom house and laboratory of the Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture Umudike in Umuahia, Abia State.

SOURCE OF MATERIALS

Pleurotus ostreatus were obtained from Mushroom house in the Department of Plant Science And Biotechnology, Michael Okpara University Of Agriculture, Umudike.

EXTRACTION OF CHITIN AND PRODUCTION OF CHITOSAN

Extraction of chitin and production of chitosan were carried out by alkalization method (Johney *et al.,* 2017; yang *et al.,* 2017) with some modifications. Twenty grams (20g) fruit bodies of the fungus were dried in an oven set at 60°C for 2 days and the dried materials were grinded to make powder. The powder was treated with 1N NaOH solution and kept at 100°C for 3h. The alkali insoluble materials (AIM) were collected by filtering the slurry. The AIM was dried in an oven at 40°C for 4 days. The dried

AIM were then dissolved in 2% acetic acid and kept in a water bath set at 100°C for 5h. The sample solution was centrifuged at 6,000 rpm for 10 minutes and the supernatant was decanted into a beaker. The solution was adjusted at pH 12.0 by 2N NaOH solution for precipitation. The precipitate was collected through centrifugation at 6000 rpm for 5 min. The precipitate was held 4-5 times with distilled water by using the same centrifugation condition and dried in the oven at 45°C.

DETERMINATION OF PHYSIOCHEMICAL PROPERTIES OF CHITOSAN EXTRACT

Determination of intrinsic viscosity and molecular weight (MW) of chitosan

The molecular weight of chitosan was determined by Ostwald viscometer described by Tolaimate *et al.,* (2000). In this method, the viscosity of a liquid was measured by comparing the viscosity of an unknown liquid (sample solution) with the viscosity of a known solvent (0.3 M acetic acid + 0.2 M sodium acetate). The viscosity of the liquid was measured by comparing the flow times of two liquids (t solvent = flow time of solvent and t sample = flow time of sample) of equal volume using the same viscometer. On the basis of these flow times, average runtime, specific viscosity (*spe) and reduced viscosity (*red) werecalculated. A graph was prepared using concentration and reduced viscosity of chitosan at X and Y coordinates, respectively. Then an extrapolation plot of reduced viscosity against chitosan concentration was made using trend line to find out the intrinsic viscosity [*], which is equal to Y intercept. The intrinsic viscosity of the sample were calculated using the following formula: Average runtime = (Runtime A + Runtime B + Runtime C)/3. Specific viscosity (*spe) = (Sample runtime- Solvent runtime)/ Solvent runtime. Reduced viscosity (•red) = Specific viscosity/ Sample concentration. Intrinsic viscosity = Y-intercept of the plot. The molecular weight was calculated by Mark-Houwink equation described by Wang *et al.,* (1991). [*] = KM± where, {[*] = Intrinsic viscosity, M= Molecular weight, K and ± = Constant (K= 0.078 and ±= 0.76)}.

Deacetylation

The extent of chitosan deacetylation was determined by titration with $0{\cdot}01^{-1}$ NaOH Donald *et al.,* (1988). The method involved hydrolysing the acetyl groups in chitosan with a strong alkali and converting the salt to acetate, which was evaporated as an azeotrope with water and titrated. The acetyl percentages were determined from the equation:

% of acetyl = V x 0.04305/w

Where *V* is the corrected volume of NaOH and *w* is the weight of the sample. The degrees of deacetylation were calculated using the equation:

%deacetylation=1–acetyl

Acid hydrolysis, distillation and titration

This is described by (Davies & Hayes, 1988). Twenty grams (20g) of chitosan were hydrolyzed with sodium hydroxide and acidified with phosphoric acid to convert the salt to acetic acid. The aqueous acetic

acid is distilled, and when the distilling flask begins to go dry, 15 ml of hot distilled water was added to the flask. Aliquots of 25 ml were titrated with 0.01 N sodium hydroxide using phenolphthalein as indicator. The volumes of base were multiplied by ten to give the total volume of the distillate (250 ml). The DA were determined from formula:

$$\% DA = V \times \frac{0.04305}{m}$$

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

STATISTICS

The values obtained from the various parameters were statistically analyzed by analysis of variance (ANOVA) using statistical package for social scientist (SPSS) software version 2.0.0.0 Mean separation were done using Duncan's Multiple Range Test at probability level less than 0.05($P<0.05$).

RESULTS AND, AND DISCUSSION

RESULTS

Table 1: Chitosan yield and its physico-chemical properties from *P. ostreatus* cultivated from different substrates

Treatment	Yield (g)	D of Deacety. (DD) (%)	Acety. (%)	Mol. Wt (g/mol)	Visc. (ml/g)
A.G	14.77 ± 1.25^{a}	85.69 ± 2.17^{a}	10.31 ± 2.33^{a}	6404.93 ± 3.21^{b}	4.78 ± 2.11^{c}
PP	12.48 ± 2.14^{f}	84.73 ± 1.86^{c}	13.39 ± 2.13^{a}	6312.83 ± 2.86^{cd}	5.24 ± 2.47^{a}
DC	13.38 ± 1.56^{d}	85.73 ± 2.77^{a}	10.69 ± 2.08^{c}	6342.80 ± 1.96^{bc}	4.53 ± 2.63^{d}
AG + PP	13.82 ± 1.95^{c}	84.20 ± 1.43^{d}	11.46 ± 1.24^{a}	6482.49 ± 4.71^{a}	4.35 ± 2.96^{e}
AG +DC	14.55 ± 0.98^{b}	85.78 ± 1.89^{a}	10.54 ± 1.27^{d}	6255.19 ± 3.27^{d}	5.05 ± 1.82^{b}
PP + DC	13.20 ± 0.79^{e}	85.29 ± 2.56^{b}	10.86 ± 0.25^{b}	6328.84 ± 3.21^{e}	4.13 ± 2.57^{f}

Values followed by the same superscript within the same column are not significantly different ($p > 0.05$) determined by one-way ANOVA.

D. of Acety. = Degree of deacetylation, Acety. = Acetylation, Mol. Wt = Molecular weight, Visc. = viscosity

The results of chitosan yield and its physical characteristics from *P. ostreatus* cultivated from different substrates were presented in Table 1 above.

The chitosan yield from *P. ostreatus* produced on different substrates and substrates combination results for each of the substrates are 14.77 mg/g, 12.48mg/g, 13.38mg/g, 13.82mg/g, 14.55mg/g and 13.20mg/g for A.G, P.P, D.C, A.G + P.P, A.G + D.C and P.P + D.C respectively. A.G produced mushrooms with the highest chitosan yield (14.77mg/g) followed by A.G + D.C (14.55mg/g) while

P.P had the mushrooms with the least amount of chitosan yield (12.48mg/g). Chitosan yield was significantly different for each substrate as shown in the table 1

The degree of deacetylation of chitosan from the *P. ostreatus* produced on different substrates and substrates combination results for each of the substrates are 85.69%, 84.73%, 85.73%, 84.20%, 85.78% and 85.29% for A.G, P.P, D.C, A.G + P.P, A.G + D.C and P.P + D.C respectively. A.G + D.C produced mushrooms with the highest degree of deacetylation of chitosan (85.78%) followed by A.G (85.69%) while A.G + P.P had the mushrooms with the least degree of deacetylation of chitosan (84.20%). The percentage degree of deacetylation of chitosan was significantly different for each substrate as shown in the table 1 except for those produced by A.G, D.C and A.G + D.C which are statistically similar.

Chitosan acetylation from the *P. ostreatus* produced on different substrates and substrates combination results for each of the substrates are 10.31%, 13.39%, 10.69%, 11.46%, 10.54% and 10.86% for A.G, P.P, D.C, A.G + P.P, A.G + D.C, P.P + D.C respectively. P.P produced mushrooms with the highest chitosan acetylation (13.39%) while A.G had the mushrooms with the least Chitosan acetylation (10.31%). The percentage chitosan acetylation was significantly different for each substrate as shown in the table 1 except for those produced by A.G, P.P and A.G + P.P which are statistically similar.

The molecular weight of chitosan from *P. ostreatus* produced on different substrates and substrates combination results for each of the substrates are 6404.93mg/kg, 6312.83mg/kg, 6342mg/kg, 6482.49mgkg, 6255.19mg/kg and 6328.84mg/mg for A.G, P.P, D.C, A.G + P.P, A.G + D.C, P.P + D.C respectively. A.G + P.P produced mushrooms with the highest molecular weight of chitosan (6482.49mg/kg) followed by A.G (6404.93mg/kg) while the lowest was produced in A.G + D.C (6255.19mg/kg). Molecular weight of chitosan was significantly different for each substrate as shown in the table 1

The viscosity of chitosan from the *P. ostreatus* produced on different substrates and substrates combination results for each of the substrates are 4.78%, 5.24%, 4.53%, 4.35%, 5.05% and 4.13% for A.G, P.P, D.C, A.G + P.P, A.G + D.C, P.P + D.C respectively. P.P produced mushrooms with the highest viscosity of chitosan (5.24%) followed by A.G + D.C (5.05%) while P.P + D.C had the mushrooms with the least viscosity of chitosan (4.13%). The percentage viscosity of chitosan produced was significantly different for each substrate as shown in the table 1

DISCUSSION

Chitosan has a great potential for a wide range of application due to its biodegradability, biocompatibility, antimicrobial activity, non-toxicity, and versatile chemical and physiological properties. Dutta *et al.,* (2009).

- Table 1 shows the yield and the physicochemical properties of chitosan extracted from *P. ostreatus*. These include the yield of chitosan extracted, the degree of deacetylation and chitosan acetylation, molecular weight and viscosity. The result from table 1 shows the yield of chitosan

was higher to what was reported by kabir *et al.,* (2020) on production of chitosan for oyster mushroom for α-amylase immobilization but lower than the yield reported by Pochanavanich and Suntornsuk (2002) when he worked on chitosan production from different fungi. The degree of deacetylation from this study was similar to what was reported by Pochanavanich and Suntornsuk (2002) where they stated that degree of deacetylation ranges from 83.8 to 97.9% in different fungi and a crab shell and as well similar to the report by Elem and Uraku (2017) but was greater to what was reported by kabir *et al.,* (2020). The degree of deacetylation of chitosan affects the physicochemical properties of chitosan. The large positive charge density due to high degree of deacetylation makes mushroom chitosan unique for biomedical, commercial and industrial applications, particularly as a carrier, supports for enzyme immobilization and drug delivery (Elem and Uraku, 2017). Molecular weight of the chitosan in this result is similar to what was reported by Elem and Uraku (2017) but lower than what was reported by Pochanavanich and Suntornsuk (2002). Several factors during production may influence the molecular weight of chitosan such as high temperature, concentration of alkali, reaction time and previous treatment of chitin, particle size, chitin concentration, dissolved oxygen concentration and shear stress as reported by Oh et *al.,* (2001). The result from this study shows low molecular weight chitosans (LMWCS) which is in line with the suggestion that low molecular weight chitosan (LMWCS) express higher bioactivity than medium molecular weight chitosans (MMWCS) or high molecular weight chitosans (HMWCS). Also, chitosan at low molecular weight acts as a potent biotic elicitor, able to induce plant defense responses and to activate different pathways that increase the crop resistance to diseases (Hadwinger, 2013; Katiyar *et al.,* 2014; Xing *et al.,* 2015; Malerba and Cerana, 2018). The result for viscosity shows to be similar to the report by Elem and Uraku (2017) and Pochanavanich and Suntornsuk (2002) but lower to what was reported by No and Meyers (1995) who reported that the viscosity of Chitosans varied considerably, from 60 to 5110 cP, depending on the species and methods of preparation used. Chitosan with lower viscosity could have better application in medicine, agriculture, food industries, pharmaceutical industries and waste water management (Shimahara *et al.,* 1989). Molecular weight tends to affect the viscosity level of chitosan (No and Meyers, 1995). Viscosity decreases when the molecular weight decrees (No and Meyers, 1995).

CONCLUSION AND SUGGESTION

Generally, this study supports that extraction of chitosan from mushroom is better than chitosan from crustaceans, crabs and shrimps. The processes of extraction of chitosan from chitin are simpler from mushroom compared to crustaceans. The revelation from this report shows that chitosan can be assess quantitatively from mushroom especially *P. ostreatus* and does not have seasonal or site limitations. It also indicates that chitosan from *P. ostreatus* in this study is soluble in 1% acetic acid solution, it is

biodegradable and non-toxic. The physicochemical properties of chitosan from the edible mushroom 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, crabs, crustaceans, lobsters and among others.

Physicochemical properties of chitosan from this report like degree of deacetylation, low molecular weight and viscosity shows that chitosan from oyster mushroom can be better used in biomedicals, pharmaceuticals, agricultural and industrial applications like food industries and cosmetic industries. It possesses antimicrobial and anti-oxidant properties and it's an excellent emulsifier in food grade hydrocolloids because of its amphiphilic substances that can absorb bioactive substances at oil/water interfaces, thus facilitating the formation of an emulsion by lowering interfacial tension. (Del Blanco *et al.,* 1999; Li *et al.,* 2011). Previous study also revealed that chitosan can be used to absorb heavy metal sludges and as well used in waste water management because of its highest sorption capacity for several metal ions (Deshpande, 1986).

Furthermore, this study revealed that chitosan can be assess quantitatively from mushroom and its process is simple, easy, less cost and consistent, and due to its biodegradability, intrinsic antimicrobial characteristics and other potentials possessed for pharmaceutical and other industrial use over other sources of chitosan like crustaceans, crabs, lobsters and shrimps, it is necessary to draw the attentions of the industrial men to be aware of these potentials and as well subscribe to use of the chitosan from mushroom than from other sources. Further studies should be carried out on other potentials of *P. ostreatus* in order to harness the resources possessed by this mushroom.

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