Some Characteristics and Functional Properties of Chitin Produced From Local Mushroom Agaricus bisporus

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Abstract. The physiochemical characteristics include: density, solubility, viscosity, molecular weight, degree of deacetylation and functional properties like fat and water binding capacity were studied for chitosan that prepared from chitin of Agaricus bisporus mushroom, the chitosan prepared by treatment of chitin with 47% alkaline solution at 60°C remove 4 hour. The yield of chitin extract from chitosan showed that the chitin extraction rate was 16% based on the dry weight of the fungus. Chitosan, which was produced in this study, was diagnosed by Fourier Transform Infrared Spectrophotometer (FTIR) method. The degree of deacetylation (DD%) of the chitosan produced from the fungus it was 71.5% chitosan showed viscosity of 5.5 centipoise the viscosity has been estimated when chitosan dissolved in 1% acetic acid solution. The molecular weight of the fungus chitosan was 604,610 Dalton. The chitosan was characterized by high solubility (72%) in 1% acetic acid solution and showed high water binding capacity and fat binding capacity were 674%, 257% respectively of the fungal chitosan.

1. Introduction
Chitosan was discovered by Rouget in 1895 from chitin, which is N-acetyl D-glucose amine, by boiling it with a base solution of sodium hydroxide, which led to the removal of acetylcholine groups from chitin in order to obtain chitosan. The main commercial sources of chitin are crustaceans such as shrimp and crabs. Recently, interest in research on sources for chitosan production has increased, as recent research indicates the possibility of using fungi as an alternative source for chitin and chitosan production[4]. The process of removing acetyl groups from chitin to produce chitosan is a very important process, because the degree of elimination of acetylated groups (DA%) identifies the qualities of the produced chitosan such as solubility, antimicrobial activity, and other associated application properties[8].

Fungi contain a lower percentage of minerals compared to marine crustaceans, and therefore the chitin extracted from the fungi contains a small percentage of associated minerals, and as a result, the process of extracting chitin is less expensive compared to the chitin extracted from crustaceans and the possibility of producing high quality chitosan[5]. Many studies have indicated the possibility of producing chitosan from edible fungi, including Agaricus bisporus fungus, which belongs to the Basidiomycetes group. Fungi are characterized by a rapid growth cycle, and thus could be a successful alternative to commercial chitosan production [24, 23]. Chitosan can be prepared from chitin of Agaricus bisporus fungus stalks, which reached 44.4% on the basis of the dry weight of the chitin, and the latter was 22.5% based on dry weight of mushroom stalks [10].

Chitin and Chitosan derivative have been considerable interest in as bio-functional materials in a wide range of different applications in the fields of food, agriculture, medicine, pharmaceuticals, cosmetics, paper industry and water purification [19], For example, chitosan is...
used in the food industry as a binding, thickening, stabilizing and emulsifying agent. Chitosan is used in wastewater treatment, as a chelating agent and as a heavy bulk fluid [2]. The aim of this study was to prepare chitosan from the chitin of the *A. bisporus* fruiting body and then studied some physiochemical and functional properties of the produced chitosan.

2. Materials and Methods

2.1. Preparation of chitin and chitosan

The *Agaricus bisporus* fungus was obtained from the Al-Wadaq mushroom farm located in Baghdad, Iraq, where the fruit bodies of fungus washed with distilled water and cut into small slices, then dried at 50 °C for 40-48 h and the dry material has been crushed by electrical mill to obtain the fungus powder [13]. Chitin was prepared from the fruit bodies of *Agaricus bisporus* fungus according to the method that mentioned by Wu et al., the fungus powder was treated with sodium hydroxide solution (1M) with a ratio of 1: 40 (W/V), and the sample was placed in the reflux condenser for 30 min at 95 °C for the purpose of removing the protein. Alkaline Insoluble Materials (AIM) were isolated with a speed of 12000 Xg at 22 °C for 20 min, and then washed with distilled water and ethanol 95% until reaching to the neutral pH, where the AIM was dried and then crushed into a fine powder. Then it treated with acetic acid (2%) by 1:100 (W/V) and the sample was placed in the reflux condenser at 95 °C for 6 h. The centrifugal process was performed (12000 Xg) for 20 min at 22 °C, then they obtained deposit was washed with distilled water and ethanol 95%, dried and crushed into a fine powder which was the Chitin[24]. The Chitin was treated with 50% sodium hydroxide solution at 100 °C for 2 h with stirring. After heating, the sample was left for 30 min at 25 °C for the purpose of cooling, the sample was filtered using the filter paper What man No.1 the leachate was neglected and the deposit was dried at 60 °C for 24 h until the weight was firm[22].

Estimate the physicochemical properties of the chitosan

Viscosity estimate

Use the Ostwald viscometer device for measuring viscosity by (cP) units for both distilled water with acetic acid solution and the chitosan solution to concentration (0.5, 1, 1.5) % of both individuals with the intensity of density for these solutions to use the density base at 25°C.

Characterization of chitosan using FTIR technology

The prepared chitosan was diagnosed using a Fourier Transform Infra-Red Spectrophotometer (FTIR) device, using a frequency of 400-4000 cm⁻¹ [21].

Determination of molecular weight

The molecular weight of Chitosan has been evaluated depending on viscosity measurement according to the way mentioned by Kasaal *et al.*, as used Mark-Houwink-Sakurada Equation (MHS) [14].

Determination of solubility

Solubility was estimated by placing 0.1 g of the sample in a centrifugal tube well known weight, adding for the sample 10 ml of the acetic acid solution with 1% concentration, pipes were placed in a shaker incubator quickly was taken 240 rpm, at 25 °C for 30 min, then pipes were put in a boiled water bath for 10 min, Pipes were cooling to 25 °C and then Centrifugal at 10000 xg for 10 min the Insoluble parts are washed with distilled water (25 ml) and then celebrated central 1000 xg for 10 min and then dried at 60 °C for 24 h [11].

Functional properties of the chitosan

Water Binding Capacity (WBC) and Fat Binding Capacity (FBC)

The centrifugal tube containing 0.5 gm of the sample, adding it 10 ml of water or sun flower oil, pipes are well blended by a Vortex electric mix for one minute and left for 30 minutes by room temperature with blending pipe 5 sec. every 10 minutes. Pipes are centrifugal quickly at 6000 xg for 25 minutes, removal the part of the Running (flue and weight of the pipe again. The percentage of the water and fat binding were calculated by equations according to the method mentioned by No. *et al.* [18].
3. Results and Discussion

The results showed that the weight of the *A. bisporus* mushroom powder and the moisture rate has reached 7.9% , 92.1% respectively That result was in accordance with the results of other research, studies the chemical analysis of the mushrooms showed that the moisture in the mushroom ranges between 89.3 - 92.3%[20]. While Grube et al. found that, the moisture of mushroom was 85 - 90% [12]. This difference in the moisture proportion in mushrooms may be output from the effect of temperature and humidity during the growth and the storage period of mushroom. The percentage of chitin extract from *A. bisporus* fungus was 16% on the basis of dry weight , which was higher than what found by Vetter [23], this difference in the percentages may be due to the difference of the chitin extracting method, as well as the difference of the mushroom strains.

The chitosan product yield was 41% on the basis of dry weight of the chitin and this approach approved by the Vairamuthu et al. when the chitosan was extraction from the *A. bisporus* mushroom, which was 40% [22], while another study found that, the proportion of Chitosan from chitin of *Lactarius velereus* has reached 73.1% [9].

![Figure 1. A. bisporus mushroom for Al-Wadaq farm](image1)

![Figure 2. chitosan product of Agaricus bisporus mushroom](image2)

The results showed that the Chitosan model under investigation gave a similar pattern to the commercial Chitosan standard (Figure 3, 4).The amine group, which showed an absorption peak at the frequency of 1654.92 cm⁻¹ from this spectrum of the Chitosan model production from *Agaricus bisporus* fungus. These results are close to the data observed by Wu et al. which indicated that the amine group bands of Chitosan produced from the mushrooms *A. bisporus* show absorption peak at a frequency 1653 cm⁻¹ , 1597 cm⁻¹ and 1379 cm⁻¹[25],the appearance of this group was an indication of the Chitosan existence [21]. The effective group of Hydroxyl stretching band, the top of the absorption of the Chitosan mushrooms and commercial Chitosan at the frequency of 3433 and 3473 cm⁻¹ respectively, although this group is not affected by the process of removing the acetyl group removal or decomposition processes and thus they are considered as an internal standard reference to confirm the presence of Chitosan or Chitin [6].The special Glycoside bond of β-anomer of Chitosan showed an absorption peak at frequency of 898 cm⁻¹, where this result in agreement with Wu et al. findings the Glycoside bond appear when frequency 897 cm⁻¹[25]. Finally, according to these results, the obtained product identity was detected as Chitosan, based on determining the effective organic compounds in it.
The degree of deacetylation (DA %) has been estimated depending on FTIR technique [17]. The results shown in Fig. (5) that percentage of the degree of deacetylation for standards chitosan has reached 73.3%, while the percentage of the chitosan produced from A. bisporus mushroom was 71.5%, this result was higher than what was found in another study, and the degree of elimination of acetyl groups for A. bisporus chitosan was 64.08% [25].
The percentage of deacetylation for the Mushrooms and standard chitosan is shown in Figure 5. Figure 6 illustrates the viscosity (cP) of mushrooms chitosan and standard chitosan.

The viscosity of chitosan produced from the *A. bisporus* mushroom estimated, the 1% concentration was adopted because it gave the highest percentage of viscosity compared to standard chitosan. The viscosity of the chitosan prepared was 5.5 cP while the viscosity of standard chitosan was 8 cP (Fig. 6). This result was in accordance with the results of other research [7], in which the viscosity of chitosan from wild edible Nigerian mushrooms was 5.5 cP. The molecular weight of chitosan produced from *A. bisporus* was calculated based on the results of viscosity, reaching 604,610 Dalton, and the molecular weight of standard chitosan was 956,891 Dalton (Fig. 7). This result was higher than the molecular weight of chitosan produced from the mycelia of *A. bisporus* fungus, which was 37,300 Dalton [25]. In another study, it was found that the molecular weight of chitosan produced from *A. bisporus* brown fungus was 600,858 Dalton [3]. The results in Fig. (8) showed that the solubility of chitosan produced from *A. bisporus* was 72, which was higher than the solubility of chitosan produced from *Pleurotus tueragium* and *Pleurotus ostreatus* mushrooms, which reached 60 and 40 % respectively [7], this difference in solubility may be due to a difference in the genus and type of mushrooms and to the method of estimating solubility. The difference in solubility may also be attributed to the difference in molecular weight, since chitosan with higher molecular weight was less soluble than chitosan with lower molecular weight [1].
The density of chitosan at a concentration of 1% for *A. bisporus* was 0.99 g / cm$^3$, while the standard chitosan density was 1 g / cm$^3$ (Fig. 9).

Figure (10) shows that the water binding capacity of *A. bisporus* chitosan was about 674 %, while the fat binding capacity was 257 %. These percentages were less than the water and fat binding capacity of chitosan produced from shrimp, which amounted to 712.99% and 531.15% respectively [15]. In contrast, these were higher than that for chitosan produced from shrimp shells, which were 358 and 246 %, respectively [16].

![Figure 9. Density g / cm$^3$ for mushrooms and standard chitosan](image1)

![Figure 10. Water and fat binding capacity of *A. bisporus* chitosan](image2)

### 4. Conclusion
In this study, chitosan was prepared from *Agaricus bisporus* chitin, by treating mushroom chitin with 47% alkaline solution at 60ºC / 4hour. After that, some physiochemical properties were studied, including viscosity, solubility, molecular weight and density. Chitosan produced in this study was diagnosed by FTIR method, and the degree of deacetylation DD% was estimated. Mushroom chitosan showed a viscosity of 5.5 cPs and had a molecular weight of 604,610 Dalton. The mushroom chitosan was characterized by a high solubility in 1% acetic acid solution, reaching 72%. And he showed a high ability to bind water and fat, as it reached 674% and 257%, respectively.

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