Plant Archives Vol. 20 No. 1, 2020 pp. 109-114 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

ANTIMICROBIAL ACTIVITIES OF CHITOSAN PRODUCED FROM *AGARICUS BISPORUS* STALKS

Abbas Fadhil[1] and Ebtisam F. Mous[2]

[1]Baghdad Governorate, Directorate Agriculture Governorate of Baghdad Alkarkh, Iraq.
[2]Departement of Food Science, College of Agricultural Engineering Sciences,
University of Baghdad, Baghdad, Iraq.

Abstract

Chitosan was prepared from Chitin of *Agaricus bisporus* fungus stalks, where the final outcome of Chitin was 22.5% based on dry weight of mushroom stalks powder, and the final outcome of the Chitosan was 44.4% based on the dry weight of the Chitin. The Inhibitory effectiveness of Chitosan under study was estimated at 10 mg / ml against a group of microorganisms, which included five types of pathogenic bacteria and type of yeast, The largest diameter of inhibition was 25 mm that found against *Salmonella typhimurium* bacteria, followed by *Bacillus cereus* where the diameter of inhibition zone reached 21 mm. While *Staphylococcus aureus*, *Psedumonas aeuroginosa*, *Escherichia coli* bacteria had a diameter of inhibition zone are 18, 17 and 15 mm, respectively, after 24 hours of incubation at 37°C, the diameter of the inhibition zone against *Candida albicans* yeast was 14 mm after 24 hours of incubation at a temperature of 25°C.

Key worsds: chitin, chitosan, *Agaricus bisporus*, stalks, antimicrobial activities, FTIR.

Introduction

Chitin is the second most active polymers in nature after cellulose (Hajji *et al.*, 2015), which is the main component of the external structure of insects, sea crustaceans, and fungal cell walls, which it forms 1-40% of the cellular walls of the fungi (Mohammed *et al.*, 2013; Yan and Chen, 2015). Chitosan is one of the Chitin derivatives, which is obtained after removal acetyl group from Chitin by treating it with an alkaline solution of sodium hydroxide. The main commercial sources of Chitin are crustaceans such as shrimp and crab, recently, an interest in researching of Chitosan production sources has increased, where modern research indicates that fungi may be used as an alternative source of Chitin and Chitosan production (Kleekayai 2011; Cheng *et al.*, 2014). The process of the removal acetyl group from Chitin for the purpose of Chitosan production were very important process for obtaining Chitosan, as the degree of acetyl removal (DA%) determines the qualities of the produced Chitosan such as solubility, antimicrobial efficacy and others of associated application properties (Elsabee and Abdou, 2013). The fungus contains a small percentage of minerals compared to sea crustaceans, thus, the extracted Chitin from an fungal source contain a small percentage of the minerals associated with it. As a result, the process of extracting the Chitin from the fungal sources was less expensive compared to the Chitin extracted from crustacean sources, in addition to the possibility of controlling the Chitosan production with high quality (Di Mario *et al.*, 2008). The cellular wall of edible fungi contains Chitin, which can be used as a non-animal source for the Chitosan production (Kalac, 2013), as mentioned in Several studies that pointed out that Chitosan can be produced from edible fungi such as (Bilbao-Sainz *et al.*, 2017; Yen and Mau, 2007; Wu *et al.*, 2004). *Agaricus bisporus* fungus is one of the edible abundant mushrooms with fast-growing cycle, and thus could be a successful alternative to the commercial Chitosan production. wall in fungi of taxonomic groups of Zygomycetes, Ascomycetes Chitin is the structural component of the cellular, Basidiomycetes and Deuteromycetes (Vetter, 2007). *Agaricus bisporus* belongs to the Basidiomycetes group, which is the most widely used mushroom species around the world (Muzzarelli *et al.*, 2012). Cultivation of mushrooms in the world produces millions of tons of wastes, which are difficult to get rid of, therefore, it can be used in the fungal

***Author for correspondence :** E-mail : ebtisamfadel12@gmail.com

Chitosan production. Furthermore, the production of Chitosan does not require a high quality fungus, where can take advantage of mushroom production farm wastes, which often contains mushroom stalks, in addition to the mushroom that is exposed to mechanical damage or mushrooms are irregular in shape (Wu *et al*., 2004; Zhang *et al*., 2012). The aim of this study was to estimate the counter activities of pathogenic microbial of the Chitosan that extracted from the Agaricus bisporus stalks, and thus can take advantage from the wastes (stalks) of the Al-Wadaq farm for mushroom development in the Chitosan production as shown in Fig. 1.

Material and Methods

Preparation of chitin and chitosan

The *Agaricus bisporus* mushrooms, (Sylvan A 15) line was obtained from the Al-Wadaq mushroom farm located in Baghdad, Iraq, where the Stalks washed with distilled water and cut into small slices, then dried at 50°C for 40-48 hours and the dry material has been crushed by electrical mill to obtain the fungus powder (He *et al*., 2014). Chitin was prepared from the stalks of *Agaricus bisporus* fungus, According to the method that mentioned by (Wu *et al*., 2004), the fungus stalks powder was treated with sodium hydroxide solution (1 M) with a ratio of 1: 40 (W / V), and the sample was placed in the reflux condenser for 30 minutes and at a temperature of 95°C for the purpose of removing the protein. Alkaline Insoluble Materials (AIM) were isolated with a speed of 12000 Xg for 20 minutes at a temperature of 22°C, and washed with distilled water and ethanol at a concentration of 95% until reaching to the neutral pH, where the AIM was dried and then crushed into a fine powder. Then it treated by Acetic acid with a concentration of 2% and by 1:100 (W/V), and the sample was placed in the reflux condenser for 6 hours and at a temperature of 95°C. The Centrifugal process was performed at speed of 12000 Xg for 20 minutes at a temperature of 22°C, then the obtained deposit was washed with distilled water and ethanol at a concentration of 95%, then dried and crushed into a fine powder which was the Chitin. Chitosan was prepared from the Chitin of *Agaricus bisporus* fungus stalks accordance to the method that described by (Vairamuthu *et al*., 2018), by treating the Chitin with a concentration of 50% sodium hydroxide solution by heating at 100°C for 2 hours and stirring. After heating, the sample was left for 30 minutes at a temperature of 25°C for the purpose of cooling, the sample was filtered using the filter paper Whatman N0. 1, the leachate was neglected and the deposit was dried at 60°C for 24 hours until the weight was firm.

Characterization of Chitosan using FTIR technology

The prepared Chitosan was diagnosed from *Agaricus bisporus* fungus stalks using a Fourier Transform InfraRed Spectrophotometer (FTIR) device, using a frequency of 400-4000 cm^{-1} (Vaingankar and Juvekar, 2014).

Microorganisms used

Five types of bacteria were used, which are*: Escherichia coli, Salmonella typhimurium, Bacillus cereus, Psedumonas aeuroginosa,* and *Staphylococcus aureus*. As well as, one type of pathogenic yeast *Candida albicans* was used to detect the antimicrobial activities of producing Chitosan from *Agaricus bisporus* stalks, which obtained from the College of Agricultural Engineering Sciences / University of Baghdad/ Food Science Department. The medium Nutrient broth was used to activate the types of test bacteria, where all types of bacteria incubated at 37°C for 24 hours, while Candida albicans yeast was activated in the medium of Potato Dextrose broth and incubated at 28°C for 48 hours.

Fig. 1: Mushroom development wastes (stalks) for Al-wadaq farm.

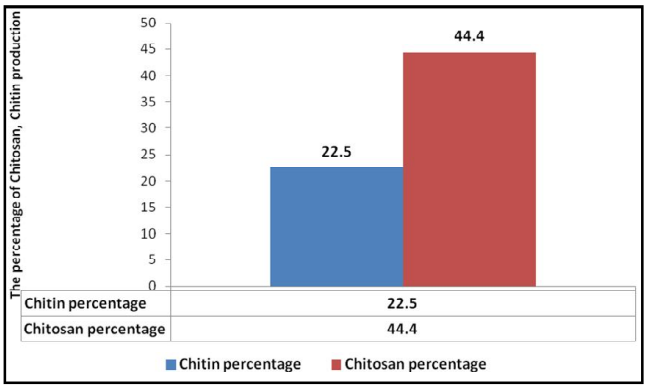

Fig. 2: The Percentage of Chitin, Chitosan production from *Agaricus bisporus* stalks.

Preparation of Chitosan solution

Chitosan solution was prepared by dissolving 10 mg of Chitosan in 1 ml of acetic acid at 1% concentration with a continuous stirring until solubility., then the sterilized tablets were soaked in 24 hours in a Chitosan solution (Logesh *et al*., 2012).

The Antimicrobial activities Estimation

Antimicrobial bacteria activities were estimated towards a number of pathogens that previously mentioned, according to (Logesh *et al*., 2012) technique. Where the mediums of Nutrient broth and Potato Dextrose broth were poured into sterile Petri dishes and left it until hardening. The culture process was carried out by separating method by adding 100µl from the microorganisms farm on the surface of solid medium and separated using L-shape. The tablets were fixed on the medium surface with the use of acetic acid as a control treatment, then the dishes were incubated at a temperature of 37°C for 24 hours, except the yeast it was incubated at 25°C for 24 hours. The diameter of inhibition zone was estimated after the end of the incubation period (Goy *et al*., 2009).

Results and Discussion

The percentage of Chitin extract from *Agaricus bisporus* stalks was 22.5%, this percentage was less than what (Wu *et al*., 2004) found, when extracting Chitin from *Agaricus bisporus* stalks which amounted to (27%). The difference in the final outcome of Chitin was due to the fact that the latest study based on different storing conditions for the used fungus stalks during 5-15 days and at a temperature of 4-25°C. Thus, the content of *A. bisporus* cellular walls increased when stored after harvest at room temperature, (Hammond, 1979). Moreover, the percentage of Chitosan production from Chitin of *Agaricus bisporus* stalks was 44.4%, and this percentage was higher than what (Yen and Mau 2006) found, when producing Chitosan from Chitin of shiitake stalks which amounted (19.14%). The reason for this variation in the ratio was caused due to the difference in the preparation method, as well as the difference in type of Mushroom as shown in Fig. 2.

Fig. 3 shows the Fourier Transform Infrared Spectrophotometry (FTIR) of the Chitosan model

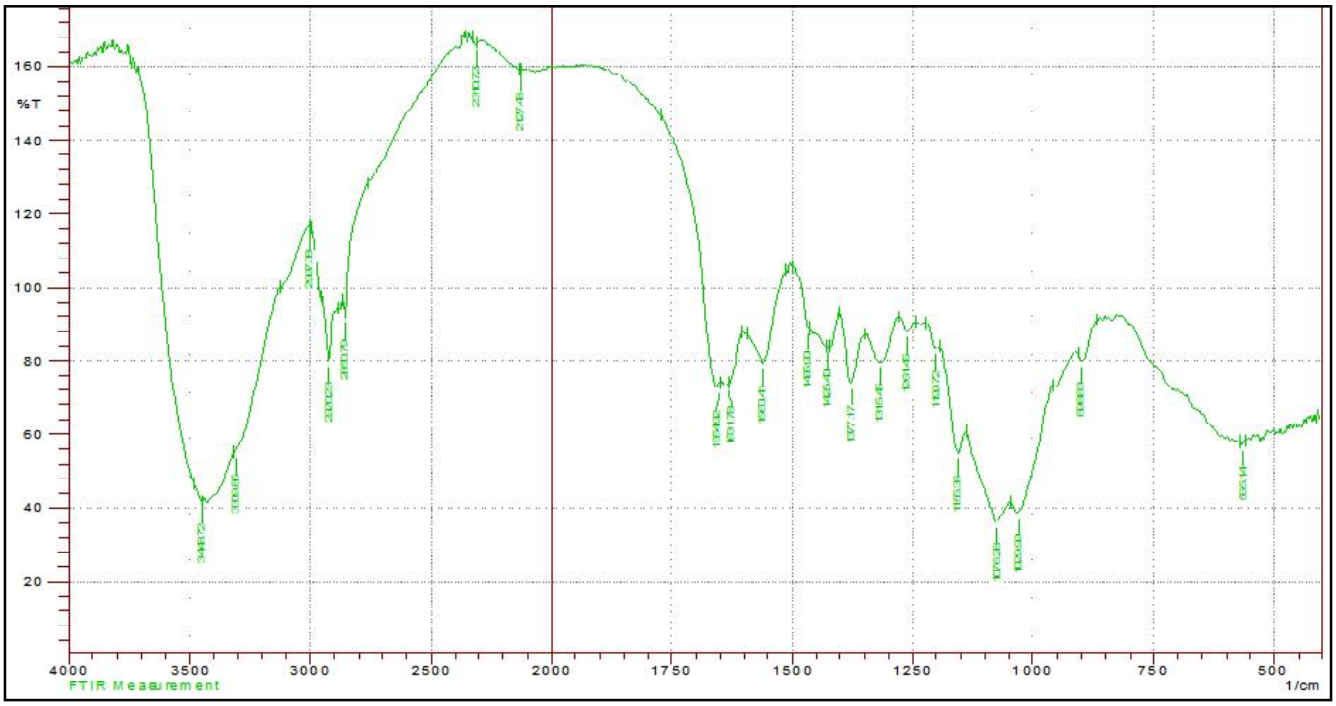

Fig. 3: The Fourier Transform Infrared Spectrophotometry (FTIR) for the prepared Chitosan from Chitin product from *Agaricus bisporus* fungus stalks.

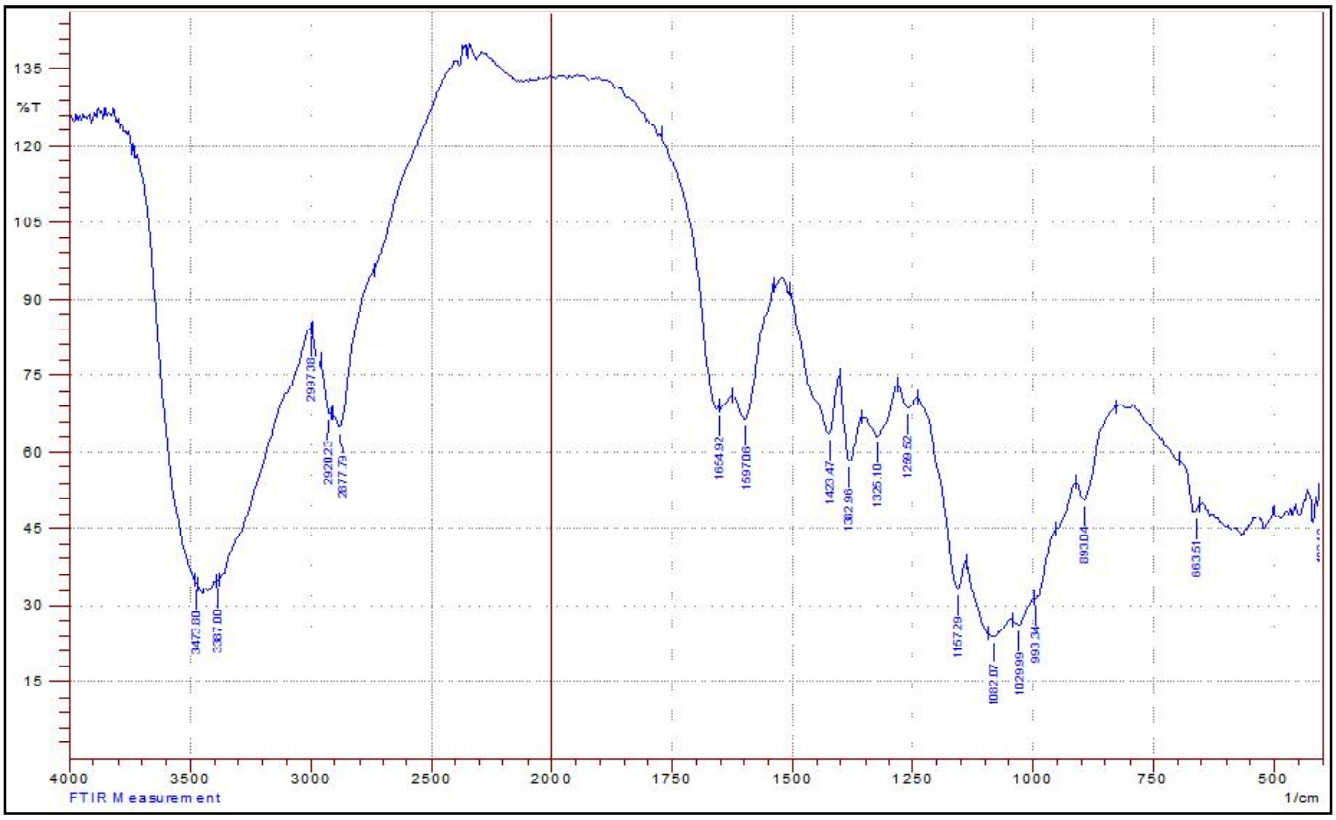

Fig. 4: The Fourier Transform Infrared Spectrophotometry(FTIR) of the commercial Chitosan.

produced from *A. bisporus* fungus stalks compared with the (FTIR) of the commercial Chitosan standard model. The results showed that the Chitosan model under investigation gave a similar pattern to the standard Chitosan (Di Mario *et al*., 2008; Ospina *et al*., 2014). The most important active groups were 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 stalks, the appearance of this group was an indication of the Chitosan existence (Vaingankar and Juvekar, 2014). The active group representing by the hydroxyl stretching band, which showed the highest absorption peak in fungus Chitosan, residue, commercial Chitosan of at frequency of 3433 cm^{-1}, 1 3448 cm^{-1}, 3473 cm^{-1} respectively. Where this group is shown in both Chitosan and Chitin because they are not affected by the process of 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 (Ebrahimzadeh *et al*., 2013). Furthermore, The peak absorption was appeared for the amine group bands of Chitosan produced from the *A. bisporus* at a frequency of 1654 cm^{-1}, 1560 cm^{-1} and 1377 cm^{-1}, which present good agreement with (Wu *et al*., 2019) findings, who indicated that the amino group bands of Chitosan produced from *A. bisporus* fungus its absorption peak at a frequency 1653 cm^{-1}, 1597 cm^{-1} and 1379 cm^{-1}. The special Glycosidic bond of β-anomer of Chitosan showed an absorption peak at frequency of 898 cm^{-1}, where this result is agreed with (Wu *et al*., 2019) findings, who stated that the Glycosidic bond appeared at a frequency of 897 cm^{-1}. While (Bilbao-Sainz *et al*., 2017) Noted that, the Glycosidic bond of Chitosan produced from the mushroom wastes appeared at 894 cm^{-1}. Finally, according to the results obtained above, the obtained product identity was detected as Chitosan, in addition to the detection of the active organic compounds that found in it.

Fig. 5 shows the antimicrobial activities of Chitosan

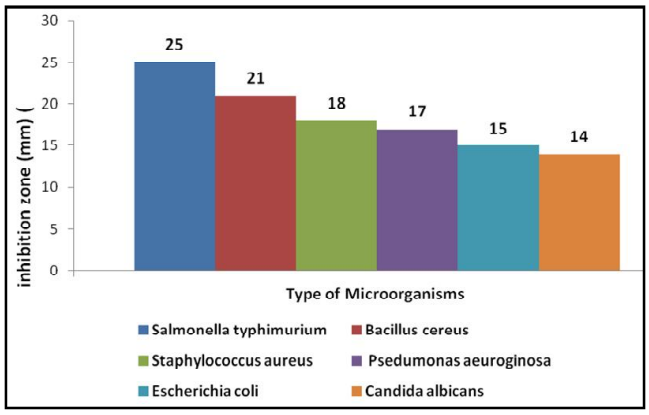

Fig. 5: Antimicrobial Activities of Chitosan produced from *Agaricus bisporus* fungus stalks with a concentration of 10 mg/ml against microorganisms.

produced from *Agaricus bisporus* stalks against a number of microorganisms growth, it's found that the effect of Chitosan on *Salmonella typhimurium* was the highest compared with its effect on other microorganisms under study, where the diameter of inhibition zone against this bacteria was 25 mm, and in *Bacillus cereus* was 21 mm, while the diameter of inhibition zone against the growth of each *Staphylococcus aureus*, *Psedumonas aeuroginosa* and *Escherichia coli* was 18, 17 and 15 mm respectively, while the diameter of inhibition zone against *Candida albicans* yeast was 14 mm. (Vairamuthu *et al*., 2018) found that the inhibition zone of Chitosan produced from *Agaricus bisporus* against *Bacillus subtilis, Staphylococcus aureus* and *Pseudomonas aeruginosa* amounted as 14, 9 and 14 mm, respectively. (Chien *et al*., 2016) indicated that the diameter of inhibition zone for Chitosan produced from *shiitaka* stalks against *Salmonella typhimurium, Bacillus cereus, Staphylococcus aureus, Psedumonas aeuroginosa*, and *Escherichia coli* was 22, 18, 21, 23, and 22 mm, respectively. This difference in the antimicrobial activities can be attributed to the difference in fungus and to the different method of Chitosan preparation. As (Logesh *et al*., 2012) pointed out that the Chitosan efficacy that produced from some fungi types like endolichen fungi was a differential effect against the Gram stain positive and negative bacteria . However, increasing size of the Chitosan solution from 50 µl to 150 µl has led to increase the diameter of inhibition zone from 0 - 6 mm against the Gram stain positive *Staphylococcus aureus* bacteria growth, while this increase did not effect on the gram stain negative *Salmonella typhimurium* bacteria growth, but the effect of other types of Gram negative bacteria such as *Escherichia coli* from 0-7 mm and in the Vibro cholera bacteria from 0-10 mm. It was observed that the Chitosan had a differential effect in inhibiting the microorganism growth, based mainly on the microorganism type and on the source of Chitosan, and the consequent difference in its physiochemical characteristics such as viscosity, molecular weight, percentage of acetyl group removal DD%, as well as the effect of Chitosan concentration. The negative cells of a gram stain change their morphological characteristics further compared to the gram positive bacteria. This may be due to the high density of negative charges on the surface of these cells, thus allowing a greater quantity of Chitosan absorption, which has a positively charged. Thus, there are changes in permeability and the structure of cellular walls of these bacteria (Masson *et al*., 2008). The percentage of acetyl group removal degree DD% and the molecular weight of Chitosan has an important effect on the Chitosan properties and its ability to perform an inhibitory action against microorganisms, as well as increasing the concentration of Chitosan, leading to increase its effectiveness against the microorganisms growth (Omogbai and Ikenebomeh, 2013).

References

Bilbao-Sainz, C., B.S. Chiou, T. Williams, D. Wood, W.X. Du, I. Sedej, ... and T. McHugh (2017). Vitamin D-fortified chitosan films from mushroom waste. *Carbohydrate polymers*, **167:** 97-104.

Cheng, L.C., T.S. Wu, J.W. Wang, S.H. Wu, M.H. Chung, Y.M. Kuo and C.H. Tsai (2014). Production and isolation of chitosan from *Aspergillus terreus* and application in tin (II) adsorption. *Journal of Applied Polymer Science*, **131(12):**.

Chien, R.C., M.T. Yen and J.L. Mau (2016). Antimicrobial and antitumor activities of chitosan from shiitake stipes, compared to commercial chitosan from crab shells. *Carbohydrate polymers*, **138:** 259-264.

Di Mario, F., P. Rapana, U. Tomati and E. Galli (2008). Chitin and chitosan from Basidiomycetes. *International Journal of biological macromolecules*, **43(1):** 8-12.

Ebrahimzadeh, M.A., A. Chabra, E. Gharaei-Fathabad and F. Pourmorad (2013). Preparation of chitosan from *Penicillium* spp. and determination of their degree of deacetylation.

Elsabee, M.Z. and E.S. Abdou (2013). Chitosan based edible films and coatings: A review. *Materials Science and Engineering: C*, **33(4):** 1819-1841.

Goy, R.C., D.D. Britto and O.B. Assis (2009). A review of the antimicrobial activity of chitosan. *Polímeros*, **19(3):** 241-247.

Hajji, S., O. Ghorbel-Bellaaj, I. Younes, K. Jellouli and M. Nasri (2015). Chitin extraction from crab shells by Bacillus bacteria. Biological activities of fermented crab supernatants. *International Journal of Biological Macromolecules*, **79:** 167-173.

Hammond, J.B. (1979). Changes in composition of harvested mushrooms (*Agaricus bisporus*). *Phytochemistry*, **18(3):** 415-418.

He, J., A. Zhang, Q. Ru, D. Dong and P. Sun (2014). Structural characterization of a water-soluble polysaccharide from the fruiting bodies of *Agaricus bisporus*. *International Journal of Molecular Sciences*, **15(1):** 787-797.

Kalaè, P. (2013). A review of chemical composition and nutritional value of wild growing and cultivated mushrooms. *Journal of the Science of Food and Agriculture*, **93(2):** 209-218.

Kleekayai, T. and W. Suntornsuk (2011). Production and characterization of chitosan obtained from *Rhizopus oryzae* grown on potato chip processing waste. *World*

Journal of Microbiology and Biotechnology, **27(5):** 1145-1154.

Logesh, A.R., K.A. Thillaimaharani, K. Sharmila, M. Kalaiselvam and S.M. Raffi (2012). Production of chitosan from endolichenic fungi isolated from mangrove environment and its antagonistic activity. *Asian Pacific Journal of Tropical Biomedicine*, **2(2):** 140-143.

Másson, M., J. Holappa, M. Hjálmarsdóttir, O.V. Rúnarsson, T. Nevalainen and T. Järvinen (2008). Antimicrobial activity of piperazine derivatives of chitosan. *Carbohydrate Polymers*, **74(3):** 566-571.

Mohammed, M.H., P.A. Williams and O. Tverezovskaya (2013). Extraction of chitin from prawn shells and conversion to low molecular mass chitosan. *Food Hydrocolloids*, **31(2):** 166-171.

Muzzarelli, R.A., J. Boudrant, D. Meyer, N. Manno, M. DeMarchis and M.G. Paoletti (2012). Current views on fungal chitin/chitosan, human chitinases, food preservation, glucans, pectins and inulin: A tribute to Henri Braconnot, precursor of the carbohydrate polymers science, on the chitin bicentennial. *Carbohydrate Polymers*, **87(2):** 995-1012.

Omogbai, B.A. and M. Ikenebomeh (2013). Solid-state fermentative production and bioactivity of fungal chitosan. *The Journal of Microbiology, Biotechnology and Food Sciences*, **3(2):** 172.

Ospina Álvarez, S.P., D.A. Ramírez Cadavid, D.M. Escobar Sierra, C.P. Ossa Orozco, D.F. Rojas Vahos, P. Zapata Ocampo and L. Atehortúa (2014). Comparison of extraction methods of chitin from *Ganoderma lucidum* mushroom obtained in submerged culture. *Bio. Med. research international*, þ.

Vaingankar, P.N. & A.R. Juvekar (2014). Fermentative production of mycelial chitosan from zygomycetes: media optimization and physico-chemical characterization. *Advances in Bioscience and Biotechnology*, **5(12):** 940.

Vairamuthu, G.M., J.J.R. Peter, A. Jerley and S. Dhandapani (2018). Effect of Chitosan in Radical Scavenging and Bactericidal Activity Isolated from *Agaricus bisporus* Mushroom. *Int. J. Life. Sci. Scienti. Res. eISSN*, **2455(1716):** 1716.

Vetter, J. (2007). Chitin content of cultivated mushrooms Agaricus bisporus, Pleurotus ostreatus and Lentinula edodes. *Food Chemistry*, **102(1):** 6-9.

Wu, J., Y. Niu, Y. Jiao and Q. Chen (2019). Fungal chitosan from Agaricus bisporus (Lange) Sing. Chaidam increased the stability and antioxidant activity of liposomes modified with biosurfactants and loading betulinic acid. *International Journal of Biological Macromolecules*, **123:** 291-299.

Wu, T., S. Zivanovic, F.A. Draughon and C.E. Sams (2004). Chitin and chitosan value-added products from mushroom waste. *Journal of Agricultural and Food Chemistry*, **52(26):** 7905-7910.

Yan, N. and X. Chen (2015). Sustainability: Don't waste seafood waste. *Nature News*, **524(7564):** 155.

Yen, M.T. and J.L. Mau (2006). Preparation of fungal chitin and chitosan from shiitake stipes. *Fungal Science*, **21(1-2):** 1-11.

Yen, M.T. and J.L. Mau (2007). Physico-chemical characterization of fungal chitosan from shiitake stipes. *LWT-Food Science and Technology*, **40(3):** 472-479.

Zhang, Z., H. Song, Z. Peng, Q. Luo, J. Ming and G. Zhao (2012). Characterization of stipe and cap powders of mushroom (*Lentinus edodes*) prepared by different grinding methods. *Journal of Food Engineering*, **109(3):** 406-413.