Egyptian Journal of Veterinary Sciences

https://ejvs.journals.ekb.eg/

Extraction, Characterization and Conversion of Chitin to Chitosan in Basidiomycetes *Phellinus igniarius* and *Coriolopsis trogii*

Maha Ebrahim Irzoqy [1,2]* and Amjad Abdul-Hadi Mohammed [2]

[1]*Ministry of Education, Mosul, Iraq.*

[2]*Department of Biology, College of Science, University of Mosul, Mosul, Iraq.*

Abstract

CHITIN is alow degradability and insolubility in several common solvents limit its use in food systems. Therefore, it was deacetylated to produce chitosan, a multifunctional biopolymer. Mushrooms are macro fungi that contain a large amounts of chitin. This study discussed the chitin extracted from *Phellinus igniarius* and *Coriolopsis trogii* (basidomycetes fungi type) where its yield were 15.43 and 11.37%, respectively. This chitin was converted to chitosan by the process of deacetylation with yield were 24.73 and 22.51%, respectively. Characterization of chitosan by Fourier Transform Infrared Spectroscopy (FT-IR), where the bands associated with the stretching and vibration of O-H, N-H, and CO bonds were apparent in the FTIR patterns. Scanning Electron Microscopy (SEM) images validated the chitosan's purity.

Keywords: chitin, chitosan, basidiomycetes, *Phellinus igniarius, Coriolopsis trogii.*

Introduction

Basidiomycetes, also known as Basidiomycota, are a varied and intriguing category of fungi that contain a broad variety of recognizable and iconic species, including mushrooms, puffballs, stinkhorns, bracket fungi and jelly fungi. These fungi serve vital functions in diverse ecosystems, contributing to nutrient cycle, decomposition, and symbiotic relationships with other species[1]. It is worth to be mentioned that mushrooms contain large amounts of chitin in its cell wall.

Chitin, a β (1,4)-linked homopolymer is a polysaccharide (simple) that is represented in the component of the cell walls of insects, fungus, crustaceans, microbes, and some invertebrate creatures [2]. After cellulose, chitin is the second most prevalent natural non-toxic biopolymer, chitosan is produced by the process of chitin deacetylation. Because of its reactive amino and hydroxyl groups, chitosan has several functional characteristics, such as being a polyelectrolyte, antibacterial, antioxidant, gel-forming, biocompatible, copper chelating agent, and easy processability [3,4]. Chitosan has a wide antibacterial activity against *Bacillus subtilis, Escherichia coli, Psuedomonas aeruginosa, Listeria monocytogenes* [5], antifungal activity against Fusarium, Alternaria and Helminthosporium as well as an antiviral activity [6].

Chitosans amino groups probably bind to microorganisms anionic groups and causing membrane disturbance and cell death, also can bind with DNA and inhibit DNA transcription and mRNA synthesis (7)(8).

Blending chitosan matrices with gelatin or collagen, for example, could enhance their biological and mechanical characteristics. chitosan nanoparticles have been developed for use in the pharmaceutical industry (9)(10). Chitosan hydrogel was shown to be effective in treating dermal burns in rats by creating full-thickness transcutaneous dermal wounds, as shown by macroscopic and histological examinations(11).

Hence, the aim of this study was extraction of chitin from the fruiting bodies of *Phellinus igniarius* and *Coriolopsis trogii* mushrooms and to the convertion of chitin into chitosan with diagnosis it by Fourier transform infrared spectroscopy(FTIR) and scanning electron microscopy (SEM) techniques.

*Corresponding authors: Maha Ebrahim Irzoqy, E-mail: mmnm9867@gmail.com. Tel.: 009647730773955
(Received 22/03/2024, accepted 13/06/2024)
DOI: 10.21608/EJVS.2024.278668.1951

Material and Methods

Source of fungi

The fruiting bodies of the fungi *Phellinus igniarius* and *Coriolopsis trogii* were supplied from Irzooqi and Mohammed [12], which previously collected from wild trees found in the forest of Chamanki resort in Duhok city/Iraq , morphological and microscopic diagnosis was achieved then genetically wasconfirmed by the amplification of 18s rRNA by polymerase chain reaction (PCR) technique and registered for the first time in the National Center of Biotechnology Information (NCBI) with the accession number OR436898 and was identified as *Phellinus igniarius* AmMa9 and the accession number OR432582 identified as *Coriolopsis trogii* MaAm2.

Extraction of chitin from Phellinus igniarius and *Coriolopsis trogii*

In order to obtained chitin the alkali and acid treatment depended by a modified procedure adapted from Rane and Hoover [13], the fruiting bodies were dried in oven at 50°C for 24hour then grinded well to obtain fine powder. The powder of the fruiting bodies was stirred with 1MNaOH and refluxed for 1h at 95 °C. The slurry was centrifuged (5000 xg for 10 min) to obtain the alkali insoluble material (AIM). AIM was rinsed (7-10) times with distilled water) until Ph= 7 and ethanol 95%. The insoluble residue from the alkali was freeze-dried after the last centrifugation and grinded as a powder. The crude fungal chitin that is insoluble was obtained by refluxing AIM in a solution of acetic acid 2% (1:100 w\v) for 6 h at 95 °C. Then centrifuged (5000 xgfor 10 min) and rinsed with deionized water till neutral. The slurry was bleached with sodium hypochlorite 5% (1:10 w\v) then dried after being rinsed with DW in oven at 50°C till a constant weight was obtained then the yield of chitin was determined using the aquation :

$$\text{chitin yield (\%)} = \frac{mf}{mi} \times 100$$

Where mf is the final mass (g) of the dried chitin.

mi is the initial mass (g) of fungal powder.

Conversion of chitin into chitosan

Chitosan was obtained by chitin deacetylation were refluxed with 50% (1:50/w:v) NaOH at 100 °C for 4 h. Then centrifuged and rinsed [7-10] times to a pH of neutral using distilled water. The obtained chitosan dried in oven for 24 h at 50 °C and till a constant weight was obtained, then chitosan yield was determined by the aquation:[14]

$$\text{Chitosan yield (\%)} = \frac{mc}{mf} \times 100$$

Where mc is the final mass (g) of the dried chitosan.

mf is the initial mass (g) of dried chitin.

Characterization of chitosan

Fourier Transform Infrared Spectroscopy (FT-IR)

Structure of chitosan extracted from the fruiting bodies for basidiomycetes was confirmed by Fourier transform infrared spectroscopy (FTIR) at the Central Laboratory of Science Collage/ Mosul University, record of FTIR spectra was between 400-4000 cm^{-1} at 25 °C.

Scanning Electron Microscopy (SEM)

To determine the morphological and surface characteristics samples were examined using PHILIPS SEM Scanning Electron Microscopy at a voltage of 10 to 25 KV at the Collage of Pharmacy/ Nineveh University/ Mosul.

Results and Discussion

Chitin extraction

The extraction of chitin was performed from dry fruiting bodies of the Bsidiomycetes *Phellinus igniarius* and *Coriolopsis trogii*. Chitin was recovered after basic and acidic treatment, the removal of proteins and lipids was in basic treatment, while the elements removed by acidic treatment.

Chitin yield isolated from dry fruiting bodies of *Phellinus igniarius* was about 15.43% DW (dry weight biomass), while Chitin yield isolated from dry fruiting bodies of *Coriolopsis trogii* was 11.37% . *Phellinus igniarius* polysaccharides (PLP) extraction rate reached a maximum of 12.98%. (15). Novak and others[16] found that the complex of chitin-glucan, which forms with aromatic polymers together, the solid part basis of the fruiting bodies for *Phellinus* sp. Chitin and other polysaccharides are responsible for the propagation of high amounts of insoluble fibers, this can explain the hardness in the fruiting bodies of *Phellinus* sp. (17).

Chitosan production

The chitin deacetylation process, which involves the hydrolysis of acetamide groups, provides the basis for the creation of chitosan(18), the yield of chitosan extracted by deacetylation process of *Phellinus igniarius* was 24.73%, while chitosan extracted from *Coriolopsis trogii* was 22.51%. Because of their cheap cost and adaptability for mass production, chemical techniques to generate chitosan are extensively employed for industrial reasons (19).

Chitosan Characterization

Fourier Transform Infrared Spectroscopy (FT-IR)

Chitosan FTIR spectrum (figure,1) extracted from *Phellinus igniarius*, (Fig. 2) an FTIR spectrum extracted from *Coriolopsis trogii*, and (Fig. 3) FTIR spectrum for commercial chitosan (Sigma USA).

on the results above chitosan had been characterized depending on determination of its active organic compounds.

Scanning Electron Microscopy (SEM)

Chitosan surface morphologies from mushrooms *Phellinus igniarius* and *Coriolopsis trogii* were analyzed by SEM. The results revealed the absence of nanofibers or nanopores (Fig. 4).

The chitosan from shitake stipes showed a solid structure devoid of nanopores and nanofibers, according to a related research (23). In contrast to the fibrillar and porous chitosan seen in crustaceans and insects, the surface morphologies of chitosan from these two kinds of mushrooms showed a smooth and solid structure (24). The study's observation of smooth surface morphologies suggested that chemical methods were ineffective in successfully removing components of β-1,3-glucan from structure of chitin.

Conclusion

Chitin yield from the fruiting bodies of *Phellinus igniarius* and *Coriolopsis trogii* was 15.43 and 11.37 % DW, and chitosan yield for the same basidiomycetes was 24.73 and 22.51 %, respectively.

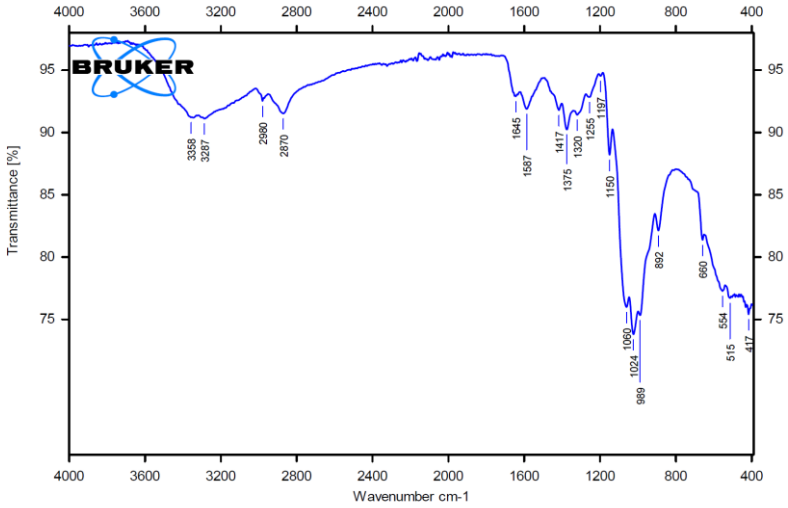


Fig.1. FTIR spectrum for chitosan extracted from *Phellinus igniarius*

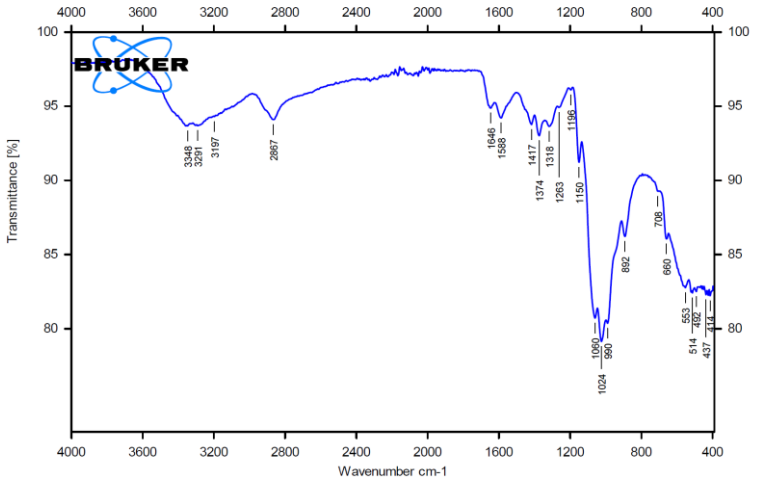


Fig.2. FTIR spectrum for chitosan extracted from *Coriolopsis trogii*

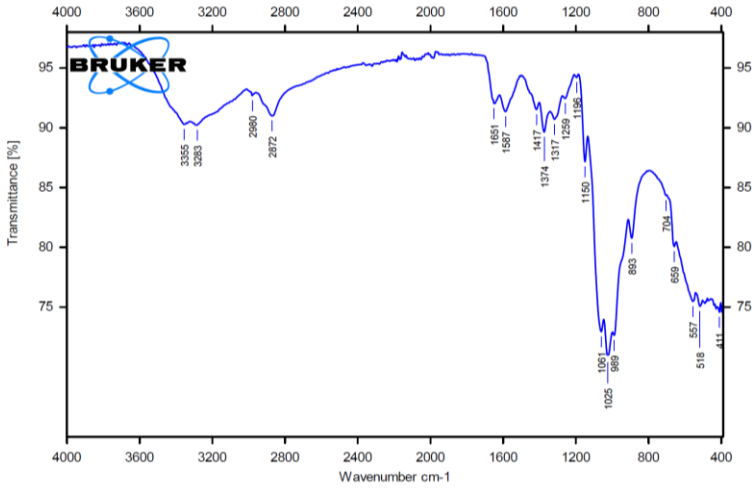
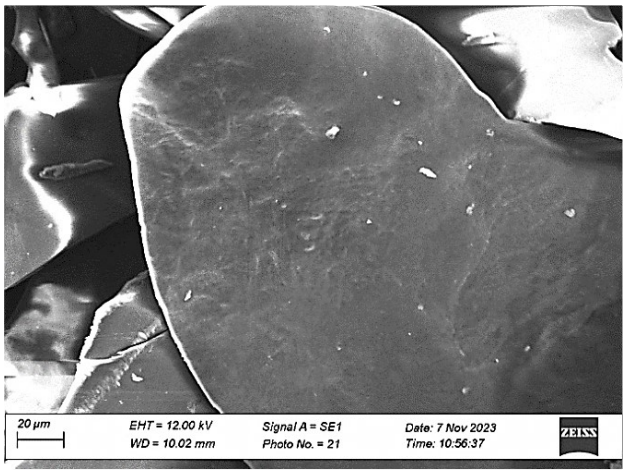
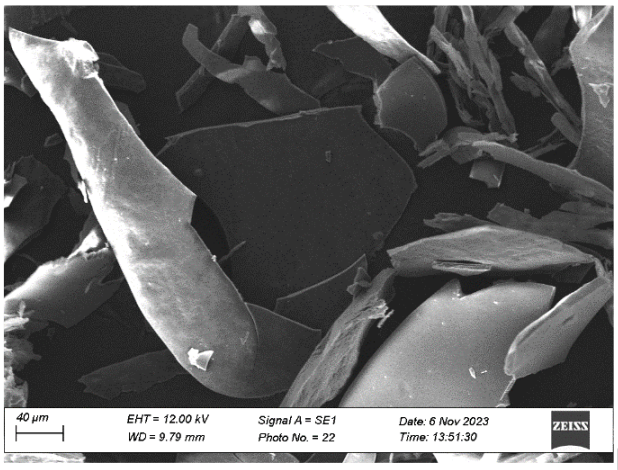


Fig.3. FTIR spectrum for commercial chitosan



[A]



[B]

Fig.4. SEM images for chitosan extracted of basidiomycetes spices: a) chitosan from *Phellinus igniarius*. b) chitosan from *Coriolopsis trogii*

References

1. Vallavan, V., Krishnasamy, G., Zin, N. M. and Abdul Latif, M. A review on antistaphylococcal secondary metabolites from basidiomycetes. *Molecules,* **25**(24), 5848(2020).

2. Tsurkan, M.V., Voronkina, A., Khrunyk, Y., Wysokowski, M., Petrenko, I. and Ehrlich, H. Progress in chitin analytics. *Carbohydrate Polymers,* **252**, 117204(2021).

3. Pellis, A., Guebitz, G.M. and Nyanhongo, G.S. Chitosan: Sources, processing and modification techniques. *Gels,* **8**(7), 393(2022).

4. Behr, M. and Ganesan, K. Improving polysaccharide-Based Chitin/Chitosan-Aerogel Materials by Learning from Genetics and Molecular Biology. *Materials,* **15**(3),1041(2022).

5. Sachan, R.S.K, Das, S. and Singh, S. Extraction of chitosan from Basidiomycetes species and analysis of its antimicrobial property. *Think India Journal*, **22**(17), 4248-4257(2019)..

6. Malerba, M. and Cerana, R. Recent applications of chitin-and chitosan-based polymers in plants. *Polymers,* **11**(5), 839 (2019).

7. Verma, M.L., Kumar, S., Das, A., Randhawa, J.S. and Chamundeeswari, M. Chitin and chitosan-based support materials for enzyme immobilization and biotechnological applications. *Environmental Chemistry Letters,* **18**(2), 315-323 (2020).

8. Al-Rooqi, M.M., Hassan, M.M., Moussa, Z., Obaid, R.J., Suman, N.H., Wagner, M.H. and Ahmed, S.A. Advancement of chitin and chitosan as promising biomaterials. *Journal of Saudi Chemical Society*, 101561(2022).

9. Crognale, S., Russo, C., Petruccioli, M. and D'annibale, A. Chitosan production by fungi: current state of knowledge, Future opportunities and constraints. *Fermentation*, **8**(2), 76(2022)..

10. Fatullayeva, S., Tagiyev, D., Zeynalov, N., Mammadova, S. and Aliyeva, E. Recent advances of chitosan-based polymers in biomedical applications and environmental protection. *Journal of Polymer Research*, **29**(7), 1-19(2022).

11. Baharlouei, P. and Rahman, A. Chitin and chitosan: prospective piomedical applications in drug delivery, cancer treatment, and wound healing. *Marine Drugs,* **20**(7), 460(2022).

12. Irzooqi, M.E. and Mohammed, A.A. The First Record of Two Basidiomycetes collected from Chamanki Resort in Duhok City/ Iraq. Accept in Medical Journal of Babylon (2023).

13. Rane, K.D. and Hoover, D.G. Production of chitosan by fungi. *Food Biotechnology*, **7**(1), 11-33(1993).

14. Erdogan, S., Kaya, M. and Akata, I. (2017). Chitin extraction and chitosan production from cell wall of two mushroom species (*Lactarius vellereus* and *Phyllophora ribis*). In AIP Conference Proceedings (Vol. 1809, No. 1). AIP Publishing.

15. Gao, N., Zhang, W., Hu, D., Lin, G., Wang, J., Xue, F. and Zhang, L. Study on Extraction, Physicochemical Properties, and Bacterio-Static Activity of Polysaccharides from Phellinus linteus. *Molecules*, **28**(13),5102(2023)..

16. Novak, M., Synytsya, A., Veselá, A.N.E.Ž.K.A., Gomba, G.K. and Čopíková, J. Structural investigation of glucan complexes isolated from Phellinus and Inonotus wood fungi. *Chemické listy,* **104**(4), 236-242 (2010).

17. Kalač, P. Chemical composition and nutritional value of European species of wild growing mushrooms: A review. *Food Chemistry*, **113**(1), 9-16(2009).

18. Novikov, V.Y., Derkach, S.R., Konovalova, I.N., Dolgopyatova, N.V. and Kuchina, Y.A. Mechanism of Heterogeneous Alkaline Deacetylation of Chitin: A Review. *Polymers,* **15**(7),1729(2023).

19. Novikov, V., Derkach, S. and Konovalova, I. Chitosan technology from crustacean shells of the northern seas. *KnE Life Sci.,* **5**, 65–74(2020).

20. Ebrahimzadeh, M.A., Chabra, A., Gharaei-Fathabad, E. and Pourmorad, F. Preparation of chitosan from *Penicillium* spp. and determination of their degree of deacetylation. *Indian Journal of Biotechnology (IJBT),* **12**, 231-235(2013).

21. Mesa Ospina, N., Ospina Alvarez, S.P., Escobar Sierra, D.M., Rojas Vahos, D.F., Zapata Ocampo, P.A. and Ossa Orozco, C.P. Isolation of chitosan from Ganoderma lucidum mushroom for biomedical applications. *Journal of Materials Science: Materials in Medicine,* **26**, 1-9(2015).

22. Oberemko, A., Salaberria, A.M., Saule, R., Saulis, G., Kaya, M., Labidi, J. and Baublys, V. Physicochemical and *in vitro* cytotoxic properties of chitosan from mushroom species (Boletus bovinus and Laccaria laccata). *Carbohydrate Polymers,* **221**, 1-9(2019).

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

24. Kaya, M., Erdogan, S., Mol, A. and Baran, T. Comparison of chitin structures isolated from seven Orthoptera species. *International Journal of Biological Macromolecules*, **72**, 797-805(2015).

استخراج وتوصيف وتحويل الكايتين الى كايتوسان من الفطريات البازيدية *Phellinus igniarius* و *Coriolopsis trogii*

مها إبراهيم ارزوقي[1،2*] و أمجد عبدالهادي محمد[2]

[1] وزارة التربية - الموصل - العراق.
[2] قسم علوم الحياة - كلية العلوم - جامعة الموصل - الموصل - العراق.

الخلاصة

بسبب خواص الكايتين قليلة التحلل و عدم ذوبانه في العديد من المذيبات الشائعة تحدد استخدامه في الأنظمة الغذائية، لهذا السبب تتم إزالة مجاميع الاسيتايل منه و تحويله الى كايتوسان البوليمر الحيوي متعدد الوظائف. المشروم وهي من الفطريات الكبيرة التي تحتوي على كميات كبيرة من الكايتين.

نجحت هذه الدراسة في استخلاص الكايتين من الفطريات البازيدية *Phellinus igniarius* و *Coriolopsis trogii* وكانت حصيلة الكايتين 15.43 و 11.37% على التوالي ، كما تم تحويل الكايتين المستخلص الى كايتوسان بعملية إزالة مجاميع الاسيتايل حيث بلغت حصيلة الكايتوسان 24.73 و 22.51% على التوالي .و تشخيص الكايتوسان بجهاز طيف الاشعة الحمراء و المايكروسكوب الالكتروني الماسح.

الكلمات المفتاحية: الكايتين ، الكايتوسان ، الفطريات البازيدية ، *Phellinus igniarius, Coriolopsis trogii*