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Commentary

Comparative study on chitin content of Bangladeshi edible and medicinal mushrooms

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Abstract

Chitin provides immense beneficial roles to the humanity and environment. Most of the chitin extracted worldwide are from the shell of the crustaceans. An alternative source of chitin has been observed as the number of crustaceans has been dilapidating. Here, extraction of chitin from the edible and medicinal macrofungi, mushrooms, have been described. This is a novel approach to meet the ever increasing chitin demand worldwide. Among four mushroom species *(Pleurotus ostreatus, Agaricus bisporus, Lentinula edodes* and *Ganoderma lucidum)*, the reishi mushroom or ling zhi (G. lucidum) had been found containing the highest amount of chitin on percentage basis of dried mushroom powder. Chitin content trend observed in this study was: *G. lucidum* (44%) > *L. edodes* (35%) > *A. bisporus* (18%) > *P. ostreatus* (10%). Thus, mushrooms could be considered as important source of chitin and its deacetylated product, chitosan. In this way, mushrooms could aid in biomedical and environmental intervention through suppliance of chitin and chitosan.

Keywords: Chitin, Chitosan, Macrofungi, Mushroom, Reishi, Shiitake

Introduction

Chitin is the most abundant, easily obtainable and renewable natural polymer in the world, second only to cellulose [1]. Its structure is similar to that of cellulose but with hydroxyl groups being replaced by N-acetyl groups [1]. Thus, chitin consists of N-acetyl-D-glucosamine moieties linked by β(1-4) glycosidic linkages [1]. The term "chitin" currently refers to a polymer of N-acetyl glucosamine where a minority of the acetyl groups has been lost [2]. Chitosan, a polysaccharide deacetylated from chitin, is a hard substance that constitutes the exoskeletons of shrimps, lobsters, crabs, and other crustaceans [3]. It consists of the polymers of D-glucosamine moieties of β(1-4) glycosidic linkages [4]. The term "chitosan" currently refers to a deacetylated product obtained from chitin, where most of the acetyl groups have been removed [5]. Experimentally, chitosan can be distinguished from chitin because of its solubility in dilute acetic acid or formic acid [6].

Advancement of knowledge about chitin and chitosan has been very uneven. This has stemmed from the erratic tests led erroneous data that ultimately misled towards faulty structural elucidation [7]. Real difficulty was also encountered because of the variety of the sources of chitin [8]. Few chemists, biochemists, structural biologists, and physicists have been involved in the study of chitin until recently [9]. Consequently, still today, there remains paucity of integrated knowledge and information regarding isolation and utilization of chitin as well as generation of chitosan from chitin for the sake of biomedical and therapeutic intervention [10]. During the first half of the last century, research on chitin was mostly directed towards the study of its occurrence in living animals especially in the exoskeletons of the crustaceans and arthropods such as the crabs, prawns, shrimps and the lobsters [10]. Recently, occurrence of chitin has been reported from the cell walls of the fungi [8-12]. Among plethora of fungal strains, the edible and medicinal macrofungi, mushrooms, contain chitin as an integral part of their cell walls [12-15]. Thus, mushrooms could be an important and easily reachable source of chitin.

Mushrooms of various sorts have been adored as culinary and medicinal items at different parts

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of the world [16]. The traditional Chinese medicine (TCM) hails mushroom since hoary past due to its health-giving and psychological as well as aesthetic attributes [17]. The reishi mushroom, *Ganoderma lucidum* has been attributed as "ling zhi", the elixir of life or panacea [18]. The western cuisine also welcomes mushrooms significantly and internationally reputed research institutions and faculties have been set up for the cultivation, study and development of mushroom and mushroom-based nutraceuticals, pharmaceuticals, cosmetics, nano-materials, bioremediation, biosorption and biotransformation [19,20].

Bangladesh is a south Asian, densely populated and developing country. Her economy is agriculture based and mostly dependent on natural support. Here, cultivable lands have been decreasing due to ever increasing populace, urbanization, and deforestation [21]. The rivers of Bangladesh are also drying out and the natural sources of protein and chitin, the crustaceans, have been declining [22]. In this context, the dwindling animal sources could hardly afford the source of chitin to the Bangladeshis. Thus, search for an alternative source of chitin seem crucial. Mushrooms, whose cell walls abound with chitin, seem to be the ultimate solution as Bangladeshis have become accustomed to mushroom-based culinary and medicinal practices [23]. Here, mushroom is easily available and the government of Bangladesh have been advocating toward mushroom-oriented feeding and supporting mushroom-industrialization [24]. In this context, extraction and purification and utilization of mushroom-based chitin, chitosan and relevant products would benefit this country highly and also aid in fulfilling the international market demand of chitin and chitin-based products. Thus, the present study has been designed to extract and purify chitin from both and edible mushrooms: oyster *(Pleurotus ostreatus),* button *(Agaricus bisporus),* shiitake *(Lentinula edodes)* and reishi *(G. lucidum)* and to demonstrate comparative chitin content in these mushroom species.

Materials and Methods

Collection of mushroom samples

Mushroom samples had been purchased from Bangladesh national mushroom development institute. Samples had been dried and grinded into powder. Mushroom powder had undergone sequential decalcification, deproteination, and deacetylation to yield chitin [4]. Later, acid-extraction of chitin yielded chitosan [4].

Decalcification

100 gm of mushroom powder had been incubated with 0.1N HCl containing 0.1mM EDTA (final concentration) overnight for decalcification.

Deproteination

Decalcified mixture had been filtered through a mesh, air dried, and subjected to deproteination by treatment with 4% NaOH at 100°C for 2 hours.

Decoloration and extraction of chitin

The deproteinized mixture was filtered and subjected to docoloration by treatment with 0.01M oxalic acid and 0.00M potassium permanganate ($KMnO_4$) at 100°C for 2 hours.

The product up to this stage is chitin and it was subjected to extensive washing with water to neutralize the pH and then air dried.

Deacetylation

Extracted chitin had been deacetylated to chitosan by treatment with 40% NaOH at 100°C for 9 hours.

Extraction of chitosan

Chitosan had been washed to neutralize the pH and extracted from the remaining solids by treatment with 2M acetic acid, as chitosan is soluble in weak solutions of acid of pH <5.5. Finally, the solid chitosan powder had been washed with distilled water several times to remove any remaining acidity.

Determination of the degree of deacetylation of chitosan

The degree of deacetylation of the chitosan had been assessed by potentiometric titration following the method of Hossain et al. [4] with a little modification.

Results and Discussion

Among the four species of mushroom (*P. ostreatus, A. bisporus, L. edodes, and G. lucidum*) under the present study, the *G. lucidum* contained the highest amount of chitin (44% of the dry weight of the initial mushroom powder) (**Table 1**). *Lentinula edodes* scored second in position with 35% of the dry weight (DW) while *A. bisporus* stood third with 18% of DW of the initial mushroom powder (**Table 1**). *Pleurotus ostreatus* had been found containing the least amount of chitin (10% of DW) (**Table 1**). This value is intermediary of previously reported chitin content (6-10% of DW) of this mushroom species [25-31]. Similar trend had been observed for *A. bisporus* (**Table 1**). Variation in mushroom cultivation, processing, and extraction criteria influence the amount of extracted mushroom largely [25-31]. So far, solid state fermentation (SSF) had been found providing higher yield of chitin from mushroom [25-31].

Table 1: Chitin content in mushroom species.

Name of Mushroom species	Chitin content (% of dry weight of mushroom powder)
Pleurotus ostreatus	10
Agaricus bisporus	18
Lentinula edodes	35
Ganoderma lucidum	44

Besides, nutritive support in the growing media of the mushroom also affect chitin content. Mushroom culture media supplanted with urea aid in enhanced nitrogen content that ultimately leads towards enhanced chitin production of the mushroom [26,32]. Besides, increased carbon source also yields higher chitin content in mushroom. Mushroom growing media enriched with auxin, gibberellins, and cytokinin also showed better chitin yield [26,32]. Moreover, environmental condition would also impact the production and storage potentiality of chitin in mushroom [26,32]. Extraction process, the type of acid and alcohol used, their concentration and duration of action have substantial effect on chitin yield [26,32]. Interestingly, inter-organ difference in chitin content of the same species of mushroom had also been reported [26,32]. Higher concentration of chitin in pileus than that of stipes had been observed for both *P. ostreatus* and *L. edodes* [26,32]. Moreover, natural saprotrophic fungi contain higher level of chitin than those of the

wood-destroying ones [26,32]. Similar trend had been observed for the tissue culture generated and lab grown species of *A. bisporus, L. edodes,* and *P. ostreatus* [26,32].

Extraction and purification of chitin and chitosan as well as chitinous material from the fungi seem apt both economically and environmentally [33-35]. Edible and medicinal mushrooms are better choice of chitin extraction as these macrofungi provide quicker growth rate than the crustaceans; easy and less expensive cultivation process; less shell-waste producing and less hazardous to the environment and free from aquatic niche [33-35]. Also, mushrooms contain less minerals than those of the crustaceans and require less vigorous demineralization process [33-35]. Additionally, chitin from the edible and medicinal mushrooms would be less allergic than those of the crustaceans [33-35]. Moreover, consumers suffering from psychological shock of crustacean-based chitin would get relief of the woe once they have the mushroom-based chitin and chitosan at hand [33-35]. Thus, both edible and medicinal mushrooms beacon towards establishing mushroom-based chitin extraction, purification and biomedical intervention as well as therapeutic application of chitin and chitosan-oriented bio-materials for the greater benefit of the humanity [33-35].

Conclusion

Bangladeshi mushroom species of both edible *(P. ostreatus, A. bisporus, and L. edodes)* and medicinal types *(G. lucidum)* contain chitin at considerable rate. Mushrooms could meet chitin demand for clinical, biomedical, adsorption, and biotransformation purposes. As mushrooms are nutritionally and medicinally lucrative item worldwide, extra health and environmental benefits provided by the mushrooms through chitin and chitin-based biomaterials could help maintaining sound health and pollution-free environment. As the number of water bodies and crustaceans are declining, mushrooms could be alternative source of global chitin demand. Thus, mushrooms beacon to be the essential source of global chitin demand.

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Declaration of Competing Interest

Authors declare no conflict of interest.

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