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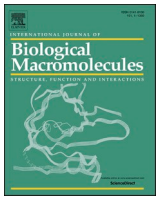

Review

Extraction, quantification, characterization, and application in food packaging of chitin and chitosan from mushrooms: A review

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ABSTRACT

The application of chitin in food systems is limited by its insolubility in some common solvents and poor degradability. Hence, it is deacetylated to obtain chitosan, an industrially important derivative with excellent biological properties. Fungal-sourced chitosan is gaining prominence and industrial attraction because of its superior functional and biological properties, and vegan appeal. Further, the absence of such compounds as tropomyosin, myosin light chain, and arginine kinase, which are known to trigger allergic reactions, gives it an edge over marine-sourced chitosan in food and pharmaceutical applications. Mushrooms are macro-fungi with a significant content of chitin, with many authors reporting the highest content to be in the mushroom stalks. This indicates a great potential for the valorisation of a hitherto waste product. Hence, this review was written to provide a global summary of literature reports on the extraction and yield of chitin and chitosan from different fruiting parts of some species of mushrooms, different methods used to quantify extracted chitin, as well as physicochemical properties of chitin and chitosan from some mushroom species are presented. Critical comparisons of reports on chitin and chitosan from mushrooms and other sources are made. This report concludes with an exposition of the potential application of mushroom-sourced chitosan for food packaging application. The reports from this review provide a very positive outlook regarding the use of mushrooms as a sustainable source of chitin and chitosan and the subsequent application of chitosan as a functional component in food packaging.

1. Introduction

Chitin is second only to cellulose in ubiquity among biopolymers. Chitin is a natural component of the exoskeletons of most marine animals, and the cell walls of fungi and yeasts. Glucosamine, a prominent precursor of proteins and lipids in a biological system, is the building block of chitin. The glucosamine units are linked together by β 1–4 glycosidic bonds [1] and are structurally similar to cellulose except that the hydroxyl (OH) group in cellulose is replaced by acetyl amine (NHCOCH3) group (Fig. 1) [2]. The functionality of chitin greatly depends on the content and percentage of *n*-acetylated units. The number of *n*-acetylated units in the macromolecule is termed the degree of acetylation (DA), while the percentage of the units in the biopolymer is called the degree of deacetylation (DD) [3].

Its abundance in nature notwithstanding, the application of chitin in foods presents some challenges. The challenges are primarily due to its insolubility in some common solvents and poor biodegradability. These limitations are imposed by some physicochemical properties of chitin which include its high crystallinity, its content of acetamido and the hydrogen bonding between its hydroxyl and carbonyl groups [4]. Hence, chitin is deacetylated to produce chitosan (Fig. 1). Chitosan is a biopolymer known for better solubility than chitin, high bacteriostatic, antimicrobial, biocompatibility, and biodegradability. These functional qualities of chitosan place it as an important biological material for applications in the food industry [5,6].

The exoskeleton of some marine organisms, a by-product of the

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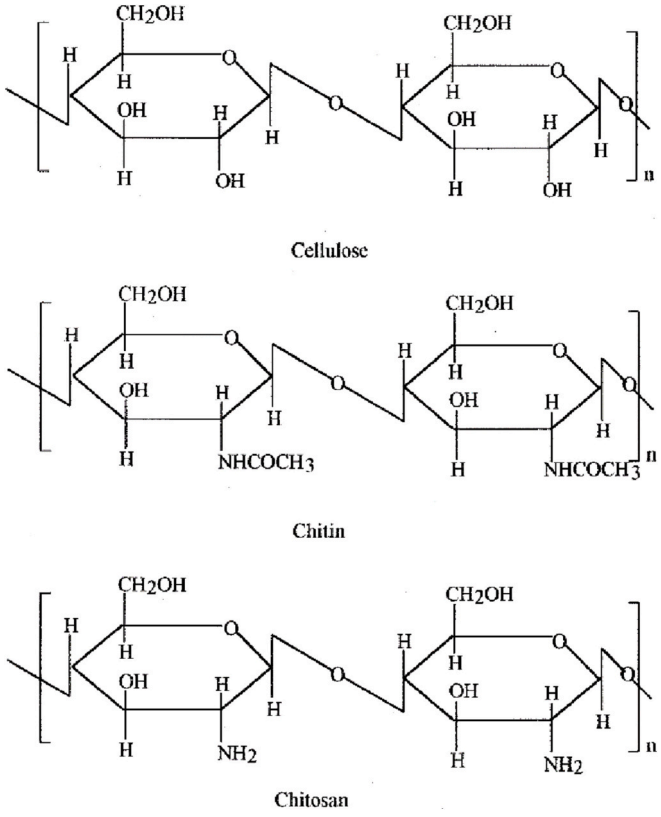

Fig. 1. Chemical structure of cellulose, chitin and chitosan [14].

fishing industry, remains the principal source of chitin and chitosan [7]. Sourcing chitosan from insects is also showing great promise [8]. However, commercial extraction of chitin from fungal sources is currently gaining prominence. Fungal chitin and chitosan sources have been reported to provide a vegan-friendly alternative. Also, they do not contain tropomyosin, myosin light chain, and arginine kinase, which are known to trigger allergic reactions. Further, they were reported to offer the possibility of greater control of physicochemical properties [9]. Fungal organisms have low inorganic matter; hence, demineralisation may not be necessary [10]. In addition, the availability of marine animals' by-products may not be guaranteed all year round, whereas filamentous fungi are cultivated under controlled conditions throughout the year and their by-product sourcing is not seasonal [11].

Ghormade et al. [7] classified fungal sources with potential for commercial chitin/chitosan production into:

1. Biotechnology industry generated waste fungal biomass
2. Fungi with a significant content of chitin/chitosan
3. Innovative value-enhancement of mycotech products

Mushrooms are a group of fungi visible to the naked eye. They are macrofungi in the families of *basidiomycetes* and *ascomycetes* [12]. Mushrooms are known to offer different appeals (nutritional, organoleptic, medicinal, eco-balancing and cultural) to man since ancient times [13]. Current awareness of the different benefits of mushrooms to human health has further increased their production. Mushrooms are replete with bioactive compounds with potential health-improving capabilities. Also, the significant content of chitin in mushrooms, which can be further processed into industrially relevant derivatives such as chitosan, is of huge commercial interest [7].

Chitin was first discovered in mushrooms (*Agaricus*, *Hydnum*, and *Boletus* species) by Henri Braconnot in the 19th century [14]. Subsequent studies had shown the presence of chitin in different parts of edible and non-edible mushrooms [9,11,15,16]. Hence, it would be useful to provide a global summary of the recent progress in the research on chitin and chitosan from mushrooms. This summary was written to provide a useful guide for the prospective applications of mushroom-sourced chitin and chitosan and expose gaps for further research efforts to promote the macromolecules. Therefore, in this review, extraction, quantification, characterization, and application of chitin and chitosan from mushrooms in food packaging are highlighted.

2. Chitin from mushrooms: chemistry

2.1. Occurrence

Fungal cell walls are connected by layers of branched β-1, 3, and 1, 6 glucans. Beltrame et al. [17] reported that the cell wall of mushrooms is generally made up of three layers: an exterior layer made of proteins and heterosaccharides (this layer is heavily influenced by carbohydrates); a middle layer composed of β-glucan networks; and an interior layer of chitin covalently bonded with β-glucan. The amorphous α-1, 3 glucans interlink with the inner part of β-1, 3 glucan layer. Chitin exists in the cell wall as a complex with β-1, 3 glucan [18]. This intimate entwining between chitin and β-1, 3 glucan has major significance in the extraction, yield, and functional properties of chitin [19]. It is also responsible for the marked difference between chitin from mushrooms and crustaceans.

Generally, chitin is organized as crystalline microfibrils in three isomeric forms (α-chitin, β-chitin and γ-chitin) [20]. The α-chitin is the most prevalent form. It is also the strongest and the most resistant and is found in insects, crustaceans and fungi [21]. Hassania et al. [9] reported a predominance of α-chitin in *Agaricus bisporus*. The dominance of α-chitin isomer was also reported for *Boletus bovinus* and *Laccaria laccata* by Oberemko et al. [22]. However, Ospina Álvarez et al. [11] reported the presence of α-chitin and γ-chitin in *Ganoderma lucidum* mushrooms as evidenced by the presence of their characteristic peak bands at 9–10 and 19–20 degree (2θ) in X-ray diffractogram.

3. Extraction and yield of chitin

3.1. Conventional extraction

There are several studies on the extraction of chitin from edible and wild mushrooms [9,11,19,22–26]. These studies aimed at developing a scalable extraction process for chitin and subsequent chitosan production from mushrooms. Chitin was extracted from the fruiting body of mushrooms [9,23], stipes [9,24,25], and residues after the extraction of polysaccharides [26]. Chemical procedures, involving the use of acid and alkali, are the most common methods of extracting chitin from mushrooms [19]. These methods are often employed for the extraction of chitin from marine sources and insects [21]. However, unlike its extraction from crustaceans and insects, the demineralisation stage is mostly not necessary in the case of mushrooms. This is an advantage conferred by the low inorganic content of mushrooms [9]. The procedures essentially involve the pulverization of biomass, deproteinization, centrifugation, neutralization (washing with distilled water), and drying of purified chitin [9].

3.2. Eco-friendly approaches

Due to the effect of chemical waste on the environment, and the adverse effect of acid and alkali on the quality of chitin from the process, studies have been carried out to explore more environmentally friendly approaches that would also give a better yield of chitin. Deproteinization with chemicals takes several hours and occurs at high temperatures. This affects the molecular weight and degree of acetylation of chitin, and subsequently, the quality of chitosan obtained [27].

Several green chemistry techniques have been used for the extraction of chitin from marine sources. They include microwave aided [28,29],

ultrasound-assisted extraction [30], subcritical water [31], ionic solvent [32], deep eutectic solvents [33], alkali/urea aqueous solution [34], microbial fermentation [35] and enzymatic extraction [36,37] methods. Further, Kaur and Dhillon [38] extracted chitin using chemical and biobased processes and reported that a compact chitin structure was obtained with a biological extraction process. These techniques showed higher yields and improved technological properties of chitin and chitosan compared to conventional methods [27]. Also, among the potential advantages of green technology are the minimal use of chemical and process reproducibility [2]. Some of these techniques have been applied to extract chitin from mushrooms and were reported to offer some promising benefits. For instance, Liao and Huang [26] reported that pure chitin (without any non-chitin component) was obtained in their study using an alkali/urea solution for the extraction of chitin from *Hericium erinaceus* with the absence of impurities/non-chitin. Kim et al. [19] also obtained high-purity chitin from *A. bisporus* using deep eutectic solvents.

Enzymatic extraction of chitin and chitosan from marine sources was reported to offer greater advantages such as yielding chitin of pure, reproducible, higher molecular weight structures with a controlled degree of acetylation [36]. This may present a good platform for the complete enzymatic extraction of chitosan from mushrooms.

3.3. Yield of chitin

Reported chitin contents from some edible and wild species of mushrooms are shown in Table 1. In a comparative study on the chitin contents of pileus and stipes of fruiting bodies during different growth stages of *A. bisporus*, *Pleurotus ostreatus*, and *Lentinula edodes* as affected by the stage of growth, Vetter [23] reported that *A. bisporus* had highest chitin. However, Di Mario et al. [39] showed that *P. ostreatus* had a significantly higher chitin content than *A. bisporus*. Hassania et al. [9] also reported that the stipe had higher chitin than the pileus of *A. bisporus*. Differences in the values reported may be due to various factors including differences in agricultural practices and processing (especially, the deproteinization agents). Vetter [23] used acid (HCl) for extraction, whereas Di Mario et al. [39] and Hassania et al. [8] used alkali (NaOH).

Table 1
Yield of chitin from some edible and wild mushroom species.

Sample	Treatment	Yield (wt %)	Author
Agrocybe aegerita	Acid hydrolysis using HCl	0.8	Manzi et al. [40]
Pleurotus eryngii	,,	0.5	
A. bisporus (pileus)	Acid (HCl) hydrolysis	6.17–7.23	Vetter [23]
A. bisporus (stipe)	,,	6.94–7.84	
A. bisporus (whole fruit body)	,,	8.68	
L. edodes (pileus)	,,	6.55	
L. edodes (stipe)	,,	5.46	
L. edodes (whole fruit body)	,,	5.36	
P. ostreatus (pileus)	,,	2.93–5.46	
P. ostreatus (stipe)	,,	2.16–3.93	
P. ostreatus (whole fruit body)	,,	4.77–4.95	
A. bisporus	Alkaline (NaOH) hydrolysis	8.5	Di Mario et al. [39]
Armillaria mellea	,,	11.1	
Auricularia auricula-judae	,,	19.6	
Lentinula edodes	,,	10.1	
P. ostreatus	,,	15.3	
P. eryngii	,,	8.7	
Trametes versicolor	,,	13.1	
G. lucidum	Alkaline (NaOH) hydrolysis	34–40	Ospina Álvarez et al. [11]
A. bisporus (pileus)	Alkaline (NaOH) hydrolysis	6.4	Hassainia et al. [9]
A. bisporus (stipe)	,,	7.4	
A. bisporus (gills)	,,	5.9	
A. bisporus (stalk)	Acid (acetic) hydrolysis	7	Boureghda et al. [41]

4. Quantification of chitin

Methods used to quantify chitin from mushrooms are essentially the same as those employed for chitin from marine sources. The major challenge to the direct quantification of chitin is its insolubility in most conventional solvents. It is, however, quantified indirectly, through the determination of the quantity of any of its derivatives, such as chitosan and *N*-acetylglucosamine [42]. For instance, an indirect method developed by Tsuji et al. [43] and modified by Ride and Drysdale [44] involved the use of concentrated KOH to convert chitin to chitosan and further hydrolysis of chitosan to glucosamine. This method was successfully used to quantify the chitin content of *Lentinus edodes*, *Lycophyllum shimeji*, *Pleurotus sajor-caju*, *Volvariella volvacea* [45], *A. bisporus*, *P. ostreatus*, and *Boletus* group [46]. Also, Vetter [23] quantified chitin from *A. bisporus*, *P. ostreatus*, and L. *edodes* through the complete hydrolysis of chitin to *N*-acetylglucosamine. Chen and Johnson [47] improved the colorimetric method of Svennerholm to quantify the chitin content of some varieties of mushrooms by quantifying the released glucosamine after acid hydrolysis of chitin.

A concern in chitosan production is the presence of protein in the cell walls of mushrooms. The cross-reaction of the amino groups of amino acids or protein may present complications in chitosan detection. To circumvent this concern, Nitschke et al. [48] developed a direct method for the quantification of chitin and chitosan in some edible mushroom varieties (*L. edodes* (Shiitake), *P. ostreatus* (Oyster Mushroom), *Pleurotus eryngii* (King Trumpet Mushroom), *Hypsizygus tessulatus* (Shimeji Mushroom), *Flammulina velutipes* (Enokitake), *A. bisporus* (Button Mushroom), *G. frondosa* (Maitake), *Pleurotus pulmonarius* (Lung Oyster Mushroom), *Trametes versicolor* (Turkey Tail), *Morchella esculenta* (True Morel)). In the procedure, the optical density of the insoluble polyiodide–chitosan complex, which was based on the interaction between polyiodide anions and chitosan, was measured. The approach relied on the formation of a coloured complex between chitosan and Lugol's solution. The authors concluded that the method was reliable and specific because no cross-reactions were found.

5. Functional properties of chitin and chitosan from mushrooms

5.1. FTIR spectra of chitin and chitosan

FTIR is a convenient method for rapid comparison of the properties of chitin [39]. Absorption bands in the regions 890, 1155, and 1370 cm^{-1} were reported for chitin-glucan complex extracted from some mushroom varieties [19]. Liao and Huang [26] also reported a peak at 1371 cm^{-1} and a weak band at 891 cm^{-1} in chitin derived from *H. erinaceus* residue. These peaks were reported to be due to C—H bending and C—H deformation of the glycosidic bond, respectively [49]. The presence of peak(s) in these regions is suggestive of the presence of β-glucans due to the β-1,3 linkage indicating traces of glucan in a chitin sample [50]. The presence of a peak at 1540 cm^{-1}, in a chitin sample, suggested the presence of residual protein due to an incomplete deproteinization process. This band was reported absent in chitin extracted from *B. bovinus* and *L laccata* [20], and *A. bisporus* [9]. Hassainia et al. [9] and Boureghda et al. [41] reported the absence of absorbance in the region 1700–1740 cm^{-1} in chitin extracted from *A. bisporus* and *P. ostreatus*, respectively. Bands in this region point to the presence of ester groups. Their absence is a confirmation that the chitin is fat-free. The band at 3100 cm^{-1} is a distinguishing feature of chitin from fungal and marine sources [19,25]. It is present in chitin from marine

sources and absent in fungal-sourced chitin [19].

FTIR analysis can be used to identify chitin isomers (α-, β- or γ-) present in a chitinous material. While α-chitin has characteristic two bands in the 1620–1660 cm^{-1} spectra region, representing CO stretching of amide1, β- and γ-chitin isomers have one and semi-double bands, respectively in the region [51]. The presence of a doublet in the amide region, an indication of the presence of α-chitin isomer, is generally shown by chitin extracted from *A. bisporus* [9,25], *Boletus bovinus* and *Laccaria laccata* [22]. Conversely, Ospina Álvarez et al. [11] reported the detection of a single band in the region (1657 cm^{-1}) for chitin extracted from *Ganoderma lucidum* mushroom variety, indicative of β-chitin. Kim et al. [19] reported the same doublet feature in chitin extracted from *A. bisporus*. The occurrence of the doublet was reported to be the result of the interference of β-glucan complex with chitin. Boureghda et al. [41] also reported low spectral resolution for chitinous materials from *P. ostreatus* mushroom species. The presence of water molecules and high content of β-glucan in the sample was mentioned to be responsible for the low resolution [52].

Analysis of reports on FTIR spectra of chitosan from mushrooms showed that they are similar to spectra from marine and other fungal sources [22,39,53,54]. Chitosan from mushrooms has a characteristic band in the region of 1600 cm^{-1} which is linked to amine absorption, a band around 3450 cm^{-1} representing OH wagging, and a CH stretching band in the 2875 cm^{-1} region [22]. Infrared spectra are found useful in the rapid comparison of the properties of chitin and chitosan from different species of mushrooms, and in the identification of functional groups present in them. However, the possible interference of extraneous materials (which may be present in the chitin or chitosan sample) may cause a significant change in the FTIR spectra. This may make an accurate baseline setting and definite conclusion impossible. Hahn et al. [8] mentioned this as the greatest limitation of the FTIR report.

5.2. Degree of acetylation

The degree of acetylation (DA), the number of *n*-acetylated units in a macromolecule, is an important determinant of the technological properties of chitin and chitosan [39]. It can be used to distinguish between chitin and chitosan samples. According to Pillai et al. [55], a value above 40 % indicates chitin, while below 40 % indicates chitosan. Different methods were reported in the literature for the determination of the DA of chitin from some mushroom varieties. Hassainia et al. [9], Kim et al. [19], Wu et al. [25], and Liao and Huang [26] used an equation (see Eq. (1) below) reported by Baxter et al. [56] to calculate DA from FTIR spectra of chitin extracted from *A. bisporus* and *H. erinaceus*.

$$DA = \left(\frac{A_{1655}}{A_{3450}}\right) \times 115 \quad (1)$$

where A_{1655} and A_{3450} are the absorbance of amide and hydroxyl groups at 1655 and 3450 cm^{-1}, respectively [9].

Di Mario et al. [39] used a method based on the reaction of picric acid and amino groups of chitin and chitosan to determine the DA of chitin from some *Basidiomycetes*. Nuclear magnetic resonance (NMR) spectroscopy was used for the determination by Hassainia et al. [9] and Oberemko et al. [22]. Results from all the studies mentioned above showed that chitin from the studied mushroom species had high DA (75.8–87.6 % (*A. bisporus*) [25], 91.0–98.7 % (*Auricularia auricula-judae*, *A. bisporus*, *L. edodes*, *Trametes versicolor*, *Armillaria mellea*, *P. ostreatus*, *P. eryngii*) [39], 70.0 % (*A. bisporus*) [9], 77.67 % (*H. erinaceus*) [26], 92–94 % (*B. bovinus*, *L. laccata*) [22], 77.3 % (*A. bisporus*) [19]).

5.3. Degree of deacetylation

The degree of deacetylation (DD) is the percentage of β–1,4-D-glucosamine in a biopolymer. A high DD was generally reported in the literature for chitosan from mushrooms: 85 and 90 % for *Ganoderma lucidum* [15]; 66.35 % for *A. bisporus* [57]; 70 and 74 % for *B. bovinus* and L. *Laccaria*, respectively [22]. Wu et al. [57] reported that chitosan from *A. bisporus* was similar to marine-sourced commercial chitosan in terms of its DD.

The duration of deacetylation was found to have a significant effect ($p < 0.05$) on the DD of chitosan from some mushroom varieties. Yen and Mau reported that the DD of chitosan from shitake stipes increased from 78.11 to 90.18 % when the extraction time was increased from 60 to 120 min [24]. Kannan et al. [16] used acid and alkaline treatment in the extraction of chitosan from *Agaricus*, *Pleutorius*, and *Ganoderma* species, and the DD was found to increase significantly ($p < 0.05$) with temperature, incubation period, and concentration of acid or alkaline. However, we have reservations to the report of Kannan et al. [16], because chitosan cannot be extracted directly from natural sources and the chitin extraction process was not stated in the report.

The DD has an important bearing on the physicochemical properties of chitosan, and chitosan with high DD is reported to have positive effects on the performance of biodegradable packaging film [58]. Different reports showed that the gel strength of film-forming solutions containing chitosan increased with the DD of chitosan. Increasing the DD made more reactive groups available for interaction, thus strengthening the interactive bonds in the solution [59,60].

High DD in chitosan was also reported to improve the mechanical properties (increased tensile strength and elongation at break) of manufactured films. Liu et al. [59] reported an increase in tensile strength from 26.5 MPa (control: film developed without chitosan) to 38.0 MPa (film containing chitosan of 76.5 % DD) and reached 63.2 MPa (film containing chitosan with 93.6 % DD). The improved mechanical strength was reported to be due to an enhanced network which resulted from the increased chain entanglement within and between the crystals [61].

5.4. Molecular weight of chitosan

Generally, chitosan from mushrooms is characterised by low molecular weight (MW) [62]. Oberemko et al. [22] reported a MW of 5–5.8 $\times 10^3$ Da for chitosan extracted from *B. bovinus* and L. *laccata*, compared to 1.62–6.96 $\times 10^4$ Da for insects and crustaceans. Some other studies also reported lower MW for chitosan from mushrooms compared to marine-sourced chitosan. Savin et al. [15] reported that the MW of chitosan extracted from shrimp was 3 and 4 times higher than enzymatically and chemically prepared chitosan, respectively, from *G. lucidum*. Wu et al. [62] obtained a much higher factor, a multiple of 40 (MW of crustacean chitosan: *A. bisporus* chitosan) in their study. Yen and Mau [24] reported that chitosan prepared from shiitake stipe, without further dialysis treatment, had higher MW (3.82–4.37 $\times 10^5$ Da) than chitosan from the same source that was subjected to dialysis treatment (5.423–6.093 $\times 10^4$ Da).

Low molecular weight chitosan has physicochemical and biological properties associated with smaller phagocytose [64]. This could promote its application in biomedical and allied fields [63,65].

5.5. Thermogravimetry analysis of chitin and chitosan

Thermograms of chitin from mushrooms are characterised by either two or three decomposition steps (Table 2). Differences in the analysis procedures employed could be the reason for this variation. Generally, the first step, reported between 30 and 100 °C, was ascribed to water loss. The second step was associated with mass loss and occurred between 200 and 400 °C. The mass loss occurred due to the breakdown of the chitin chain. The third step, which occurred in the range of 400–600 °C, was ascribed to the complete thermal disintegration of the polymers, and breakdown of carbon remnant [66].

Maximum degradation (DTGmax) peaks (obtained from derivative curves of thermograms) give information on the thermal stability of

Table 2
Degradation steps and temperature of maximum degradation (DTGmax) of chitin from some mushroom varieties.

Chitin source	Degradation step	DTGmax (°C)	Analysis environment	Source
Agaricus bisporus	3	309	Helium	Hassainia et al. [9]
A. bisporus	2	287.9	Nitrogen	Kim et al. [19]
B. bovinus	2	320	Nitrogen	Oberemko et al. [22]
G. lucidum	3	313.57	Air	Ospina Álvarez et al. [11]
Hericium erinaceus	2	318	Nitrogen	Liao and Huang [26]
L. laccata	2	360	Nitrogen	Oberemko et al. [22]
Pleutorius ostreatus	3	ND	Nitrogen	Broughda et al. [41]

DTG: maximum degradation temperature; ND: not determined.

materials. Thermal stability is an important property that will establish the values and applications of materials, especially in extreme biomimicry. From the published results shown in Table 2, chitin from mushrooms generally has lower thermal stability than chitin from crustaceans (349–385 °C) [22,67].

Similarly, chitosan from mushroom sources (*B. bovinus*: 317 °C and L. *laccata*: 309 °C) showed lower thermal degradation peaks than insect-derived chitosan (325 °C) [22]. Wu et al. [63] reported a value of 310 °C for DTGmax of chitosan from *A. bisporus* and further mentioned that the fungal chitosan had a similar thermal degradation pattern with crab chitosan. Yen and Mau [24], in their study on the characterization of chitosan from shiitake (*Lentinula edodes*), observed that the DTG of chitosan was a function of its molecular weight. It was further reported, in the study, that precipitated chitosan (283.58–289.76 °C) had higher thermal stability than soluble chitosan (247.64–257.56 °C).

5.6. X-ray diffraction

Peaks from X-ray diffractograms of chitin from some mushrooms and other sources are presented in Table 3. Irrespective of sources, they all showed characteristic chitin peaks at 9-10° and 19-20°. There are divergent explanations for peaks at 5-6°. Some authors linked these to the presence of residual β-glucan attached to chitin [19,41]. While Yen and Mau [24] and Ospina Álvarez et al. [11] referred to it as a standard chitin peak. The fact that it was not detected for some chitinous materials may be due to the presence of β-glucan, which could have been removed by further hydrolytic reaction during the extraction of chitin.

All authors, except Oberemko et al. [22], reported lower crystallinity for chitin from mushrooms in comparison with chitin from other sources (Table 3). Lower comparative crystallinity of chitin from mushrooms seems to be accurate, considering the greater intensity of the peak observed for chitin from marine and other sources [63]. The main benefit of chitinous materials with low crystallinity is their ability to eliminate metallic compounds through the provision of low diffusion resistance [41,52]. This is an advantage in adsorption applications [41].

Chitosan from mushroom sources showed a similar diffraction pattern as chitin, with characteristic peaks at 10 and 20° [22,63]. However, a major difference in their patterns is the absence of a peak at 5-6° (2θ) in mushroom chitosan. The absence of this peak in chitosan was linked to the *N*-deacetylation process [24].

Reports from different studies also indicated that mushroom chitosan was characterised by low crystallinity [63]. Low crystallinity is known to impart some important functional characteristics to chitosan. For example, chitosan's sorption capacity, accessibility of its major free amino groups, and its solubility in acidic solutions are all facilitated by decreased crystallinity [68,69]. These are functionalities that could enhance the relevance of mushroom-sourced chitosan in some important applications.

6. Antioxidant and biological properties of chitosan

6.1. Antioxidant properties of chitosan from mushrooms

The few reports on the antioxidant properties of mushroom-sourced chitosan established that chitosan has significant antioxidant activities [70]. Yen et al. [71] reported that fungal chitosan from shitake mushrooms possessed antioxidant activity (61.6–82.4 % at 1 mg/mL), reducing powers (0.42–0.57 at 10 mg/mL), radical scavenging ability on hydroxyl (61.9–77.2 % at 0.1 mg/mL), radical scavenging ability on DPPH (28.4–55.3 % at 10 mg/mL) and chelating ability on ferrous ions (88.7–90.3 %). Chitosan extracted enzymatically from *G. lucidum* showed higher antioxidant activity (129.26 mM Trolox/g dw) than chemically extracted chitosan (65.41 mM Trolox/g dw) from the same source [15]. In the same study, enzymatically extracted chitosan from the mushroom source also had higher antioxidant activity than shrimp chitosan (87.56 mM Trolox/g dw). Abd El-Hack et al. [70], concluded in their report, that the antioxidant activities of mushroom chitosan may be further enhanced through the synthesis of chitosan derivatives, and these could be tailored for different applications.

6.2. Cytotoxicity of chitosan from mushrooms

Assessment of the cytotoxicity of chitosan is important to determine its potential for biomedical applications [72]. Savin et al. [73] reported that chitosan from *G. lucidum* showed good cytocompatibility in the

Table 3
X-ray diffractograms of chitin from some mushroom species.

XRD (2θ)						Author
Mushroom source	Peaks	CI (%)	Other source	Peaks	CI (%)	
A. bisporus	9.1, 12.7, 19.3, 20.7, 23.2, 32, 39	63.2	Commercial alpha chitin	Same as mushroom source	88.18	Hassainia et al. [9]
A. bisporus	9, 20, 26	66.2	Shrimp shell	Same as mushroom source	85.0	Kim et al. [19]
A. bisporus stalk	6, 9.4, 19, 20.9,	65–70	Marine chitin	ND	~90	Boureghda et al. [41]
A. bisporus	10.5, 20	ND	Crab	Same as mushroom source. Greater intensity at 20°	ND	Wu et al. [61]
G. lucidum	5.7, 19.6	ND	Not reported			Ospina Álvarez et al. [11]
B. bovinus, L. laccata	9.5, 19.5, 20.9, 26.7	78–85	Commercial (shrimp), Lobster (*C. johni*), insect (*H. abietis*)	Same as mushroom sources	74	Oberemko et al. [22]
L. edodes (Shiitake) stipe	5.4, 9.1, 19.3	ND	Not reported			Yen and Mau [24]

ND: not determined; CI: crystallinity index; XRD: x-ray diffraction.

fibroblast cells of mice within the concentration range (0.05–1 mg/mL) tested. Similar results were reported by Mokhtari-Husseini et al. [74] for *G. lucidum* chitosan, which is known for medical applications in Iran [74]. Adequate compatibility was also reported for *G. lucidum* by Mesa Ospina et al. [72]. In a comparative study by Bierhalz et al. [75], it was reported that although chitosan membranes developed from white button mushrooms and shrimp were not toxic to fibroblasts, however, the lowest toxicity values were observed for membranes made from mushroom chitosan. This reported cytocompatibility presents a great outlook for the application of mushroom-sourced chitosan in biomedical and food sectors.

6.3. Antimicrobial activity of chitosan from mushrooms

Antimicrobial activity is one of the biological properties of chitosan [76]. Studies on the antibacterial activities of chitosan from *G. lucidum* showed it has inhibitory effects on Gram-positive (*S. aureus*) and Gram-negative (*E. coli*) bacteria (18 mm:12 mm). The inhibition was found to be dosage dependent (increased with chitosan content) and was more effective on Gram-positive bacteria [74,77]. Savin et al. [73] showed that enzymatically extracted chitosan from *G. lucidum* had higher inhibitory power than chemically (alkaline treated) extracted chitosan from the same source (2.5 mg/mL:0.625 mg/mL).

All comparative studies on the antimicrobial potency of chitosan from mushrooms and marine reported that chitosan from mushroom sources gave greater antimicrobial activity [15,70,78] Savin et al. [73] attributed these superior antimicrobial properties to the fact that chitosan from mushroom sources has lower molecular weight than crustacean-sourced chitosan. Indeed, the low molecular weight characteristic has been reported to be a favourable factor for the antimicrobial activity of chitosan [79,80].

7. Potential application of chitin/chitosan extracted from mushrooms for food packaging

The insolubility of chitin in some common solvents and its poor biodegradability are the major hindrances for its applications in food systems. However, some of its derivatives have been prospected for varying food applications. Nano-structural components of chitin have been utilized to produce chitin nanocrystals (or whiskers) and chitin nanofibers [81]. These nanoparticles combine the excellent properties of chitin with nano-impacted functionalities such as high aspect ratio, high surface area, and low density to form a stable and uniform dispersion in a system. Chitin nanomaterials were described as excellent materials with great potential to offer structural strength and improve mechanical properties in food systems, including food packaging applications [82].

Chitin nanomaterials have been successfully prepared from mushrooms [18,83–85]. Kaya et al. [86] developed active chitin nanocomposite films containing curcumin (film preparation without curcumin was used as a control). Chitin nanoparticles were extracted from *A. bisporus*. The incorporation of curcumin led to a drastic improvement in the film surface morphology and light transmission. The increasing smoothness of the composite films with curcumin concentration was reported to be due to the filling of the pores in the chitin structure by curcumin and the formation of complexes [19]. The improved structure was reported to be responsible for the enhanced mechanical properties of the chitin nanocomposite films. Importantly, all the developed films exhibited good biodegradability; they completely lost their integrity in the soil after 14 days. *A. bisporus* extracted chitin nanofilms with and without curcumin exhibited inhibition of *E-coli* but not to *S. aureus*. Furthermore, there was a 40 % extension of the shelf-life of a chicken breast wrapped with the developed film compared with one wrapped with a cling film. Results from this study showed the potential of chitin nanomaterials extracted from mushrooms in active food packaging.

The biocompatibility of chitosan is primarily due to its chemical structure, and this is responsible for its versatility and applications [2]. The biological activity of chitosan is dictated by three important factors:

1. Molecular weight
2. Degree of deacetylation; and
3. Modification to obtain derivatives such as chitooligosaccharides

These three factors confer functionality on chitosan and influence its application in various processes/systems [4]. The source of chitosan plays a significant role in the first two factors [2]. Generally, chitosan from fungal sources is characterised by low molecular weight, comparatively lower than from marine sources [15]. This is a positive attribute as low MW chitosan is known for higher antimicrobial properties and enhanced solubility [87,88].

Chitosan is gaining prominence as a candidate for food packaging applications because of its excellent functional and biological properties such as antioxidant, antimicrobial and antifungal activities [89]. Mushroom-sourced chitosan offers great potential because of its reported high antioxidant and antimicrobial properties.

Though, there are very few studies on the development of food packaging materials from mushroom-sourced chitosan. However, those available revealed great promises. Physical properties, such as density, porosity and water vapour properties of biofilms fabricated from chitosan extracted from the stalk of *A. bisporus* were not significantly different from those of films developed separately from high and low MW commercial chitosan (sourced from shrimp). However, *Agaricus* chitosan film showed more hydrophobic (as indicated by its lower water vapour permeability: 0.71 g.mm/kPa.h.m^2) and less flexibility (elongation at break: 30.2 %) than films developed from shrimp-sourced commercial chitosan (WVP: 0.74 g.mm/kPa.h.m^2; elongation at break: 65.7 %) [90]. In the same study of Bilbao-Sainz et al. [90], *Agaricus* chitosan film had lower tensile strength (4.31 MPa) (but higher than that of low MW chitosan film: 2.12 MPa) than high MW commercial chitosan (extracted from shrimp) film (5.71 MPa). Uragami et al. [91] reported a direct relationship between MW and the number of hydrogen bonds formed between hydroxyl and amino groups during the process of film forming. Therefore, modification of *A. bisporus* chitosan to achieve higher MW may improve the mechanical properties of films developed from it.

Melon seeds on which coats containing *A. bisporus* stipe-sourced chitosan was applied had similar morphology as those coated with commercial chitosan-sourced coatings [52]. Unlike commercial chitosan (from shrimp) coats, *Agaricus* chitosan coatings formed a relatively uniform layer on the melon seeds. It was further stated that fungal chitosan-based edible coatings improved the firmness of the fruit, enhanced fruit flavour through the generation of higher content of esters, limited off-flavour development and reduced microbial loads.

In conclusion, based on what is now known, it can be safely assumed that mushrooms will be a significant source of chitin and chitosan in no distant time ahead. The different contrasts presented on the physicochemical, functional and biological properties of mushroom-sourced chitin and chitosan, against marine-based chitin and chitosan presented great prospects for the industrial application of mushroom-sourced chitosan, especially in food and pharmaceutical sectors. It is recommended that future research effort focuses on providing innovations and bridging the knowledge gaps identified in this review and other relevant studies. This report showed that an eco-friendly approach to the extraction of chitin and chitosan from mushrooms holds great promise. We recommend that future research efforts explore this further.

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Declaration of competing interest

Authors declare no conflict of interest.

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