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# A review of fungal chitosan for bioactive and biodegradable food packaging: green extraction, properties, structure–function relationships, and applications

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The increasing global demand for sustainable food packaging has encouraged extensive research into biodegradable and functional biopolymers. Among these, chitosan, produced through the deacetylation of chitin, demonstrates outstanding antimicrobial, antioxidant, film-forming, and biocompatible properties that make it suitable for food preservation. Although crustacean shells remain the predominant industrial source, fungal-derived chitosan has emerged as a promising alternative due to its non-allergenic characteristics, consistent yield, and environmentally friendly extraction methods. This review provides a comprehensive overview of the chemistry, extraction techniques, and physicochemical characteristics of fungal chitosan, emphasizing its potential in bioactive and biodegradable food packaging. Both conventional acid-alkali extraction and novel green approaches, including microwave-assisted, enzyme-assisted, and deep eutectic solvent methods, are examined with respect to efficiency, purity, and environmental sustainability. Furthermore, the physicochemical, structural, thermal, biochemical, and biological properties of fungal chitosan are analyzed in relation to their functional relationships for packaging performance. The review also discusses the mechanical, barrier, thermal, optical, antimicrobial, antioxidant, and biodegradability properties of edible films and coatings formulated with fungal chitosan and their effectiveness in food preservation. Finally, potential challenges and future perspectives for advancing fungal chitosan-based packaging systems in the food industry are highlighted. In particular, this review underscores the significance of fungal chitosan as a sustainable and multifunctional biopolymer for next-generation food packaging solutions.

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### Sustainability spotlight

This review supports global efforts to develop sustainable alternatives to conventional plastic packaging by highlighting fungal chitosan as a biodegradable and bioactive material for food packaging. Fungal chitosan-based active films and coatings enhance food preservation through their antimicrobial and antioxidant properties, thereby reducing post-harvest losses and food waste, and contributing to zero hunger. The use of non-allergenic, renewable fungal biomass and eco-friendly extraction technologies promotes safer food systems and supports good health and well-being. Replacing petroleum-based plastics with biodegradable fungal chitosan-based packaging aligns with responsible consumption and production by reducing plastic waste and environmental pollution. Furthermore, this review emphasizes the potential of fungal chitosan to advance sustainable food packaging solutions that protect ecosystems, contributing to life below water and life on land.

## 1 Introduction

Food packaging is essential to maintain food hygiene and quality by protecting products from microbial, chemical, physical, and environmental contaminants and extending shelf life. Petroleum-based plastics, such as polyethylene (PE), polypropylene (PP), and polyethylene terephthalate (PET), are the most widely used due to their preservative properties.[1,2] However, these plastics are unsustainable, causing environmental damage, greenhouse gas emissions, and plastic waste

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accumulation, projected to reach 12 000 million tons by 2050.[3] This waste disrupts terrestrial and aquatic ecosystems, contaminates soil, reduces fertility, and threatens agriculture. Microplastics in water, air, and food chains pose long-term health risks, including inflammation and toxicity.[3,4] Furthermore, reliance on fossil resources for plastic production increases greenhouse gas emissions, intensifying climate change and its impacts on food security and public health.[1,5] Consequently, the drive to develop sustainable packaging materials is essential as an alternative. The 2023 United Nations Environment Programme (UNEP) report, "Turning off the Tap: How the World Can End Plastic Pollution and Create a Circular Economy," emphasizes goals aligned with ending plastic pollution and fostering a circular economy.[6]

Biopolymers, such as cellulose, chitin, starch, and collagen derived from natural sources, are promising alternatives to petroleum-based packaging materials.[2] These biopolymers are biocompatible, biodegradable, and non-toxic to the environment and human health.[7–9] Biocompatible materials can interact with living tissues without causing harmful immune responses, making them suitable for biomedical and food applications such as food packaging.[9,10] Their biodegradability allows them to decompose into harmless byproducts, while their non-toxic nature ensures safe use, as demonstrated by their use in edible packaging for food preservation.[2] Among these biopolymers, chitin and its deacetylated derivative, chitosan, have been widely studied for food packaging development due to their excellent film-forming ability, antimicrobial and antioxidant activities, and potential to replace conventional petroleum-based plastics.[7–9]

Chitin is the second most abundant linear polysaccharide, after cellulose, and consists of β-(1–4)-linked *N*-acetyl-D-glucosamine (GlcNAc) units. It is mainly present in crustacean and insect exoskeletons and in yeast and fungal cell walls.[11–13] Chitin exists in three allomorphs: α-, β-, and γ-, with α-chitin being the most prevalent in nature.[14] These allomorphs are highly crystalline, insoluble in most organic solvents, and resistant to biodegradation, limiting their industrial uses.[15] However, they are preferred as raw materials for producing chitosan through deacetylation.[16] The conventional chitosan extraction from insect and crustacean sources, such as shellfish, mollusks, crabs, and shrimp waste, involves three steps: demineralization, deproteination, and deacetylation.[15,17] Chitosan is a copolymer of chitin and is composed of β-(1–4)-D-glucosamine linked to GlcNAc residue, exhibiting improved solubility compared to chitin.[14,18] Chitosan possesses numerous desirable properties, including antimicrobial activity, biocompatibility, and biodegradability, making it a suitable candidate for food packaging applications.[19,20] While crustacean-derived chitin is the primary source for producing chitosan, several challenges persist with the traditional method. The extraction process relies heavily on the raw material availability, seasonal factors, and regional differences.[21] Moreover, the presence of specific compounds like tropomyosin, myosin light chain, and arginine kinase can trigger allergic reactions, such as shellfish allergy symptoms, limiting the use of crustacean-based chitin and chitosan.[22]

The alternative non-crustacean-based chitin sources, such as fungi, where chitin is the major cell wall component, have been explored.[14] Mycelium is the nutritional part of fungi that is built from slender cells and composed of natural polymers, such as chitin, dextran, *etc.*[23,24] On the other hand, mushrooms and their byproducts, such as stems and deformed mushrooms, contain chitin within their cell walls, are suitable for chitosan production.[25] Various fungal sources, including mycelium-based fungal biomass generated as a waste of industrial output, fungal species having a considerable amount of chitin, and macro-fungi from Basidiomycetes (*i.e.*, *Agaricus bisporus*, *Pleurotus ostreatus*) and Ascomycetes (*i.e.*, *Aspergillus* sp., *Penicillium* sp.) families and their byproducts, can be identified as potential sources for chitosan extraction.[23,25,26] As fungal biomass and mushroom varieties can be easily cultivated using inexpensive substrates, continuous production can be ensured without seasonal fluctuations.[21,22] Fungal chitosan production is also simpler than that of crustacean chitosan, as it does not require a demineralization step owing to the low inorganic matter content in fungal sources.[17,19] In crustacean shells, demineralization is crucial, which involves strong acids, such as hydrochloric acid treatments, to remove calcium carbonate and other minerals.[17] This process increases chemical use and environmental burden, while making the extraction more time-consuming compared to fungal sources, which naturally contain very low inorganic matter content.[19] Furthermore, unlike crustacean chitosan, which carries a risk of containing heavy metals, chitosan of good quality, such as low molecular weight ($M_w$), low viscosity, and a higher degree of deacetylation (DD), can be obtained from fungal sources.[17,27–29] The excellent antimicrobial and antioxidant properties of fungal chitosan help inhibit food spoilage and extend shelf life, while possessing negligible cytotoxicity, suggesting it is safe for direct contact with food.[27,30] However, it is important to note that not all fungi are antimicrobial, as some species are pathogenic or cause food spoilage, highlighting the need for careful selection of fungal strains for chitosan production.[15,18] Moreover, fungal chitosan shows strong film-forming and barrier properties that help protect food from moisture, oxygen, and microbial spoilage.[31,32] Its compatibility with natural polymers and bioactive compounds also enables the development of multifunctional packaging systems, making it a sustainable option for diverse food applications.[7,9,14]

However, fungal chitosan production remains largely underexplored, yet it holds significant potential to transform the food packaging industry by promoting eco-friendly solutions through the development of biodegradable polymers. Recently, limited studies have reviewed and compared conventional and eco-friendly extraction methods for fungal chitosan, its functional properties, and potential applications. For instance, Hussain *et al.*[33] compared different physicochemical properties (solubility, DD, and $M_w$) of fungal chitosan with crustacean chitosan along with eco-friendly approaches for chitosan extraction, and potential biomedical applications. Huq *et al.*[17] discussed potential sources for chitosan extraction (*i.e.*, crustacean waste, fungi, and insects), biosynthesis of fungal chitin, conventional extraction, and its effects on the

properties of fungal chitosan, as well as a market overview and potential biomedical and food applications as an antimicrobial. Additionally, Alimi *et al.*[14] summarized the effects of various extraction methods on chitin yield, chitin quantification, and the functional, biochemical, and biological properties of chitin and chitosan derived from mushroom sources, as well as their potential packaging applications. However, their work predominantly emphasized mushroom-derived chitin rather than chitosan. To date, there has been no comprehensive review that specifically addresses fungal chitosan as a raw material for bioactive food packaging and examines the properties of the developed packaging materials. Furthermore, the detailed account that consolidates the extraction methods, physico-chemical and functional properties of fungal chitosan, structure–function relationships, and comparative performance of fungal chitosan-based bioactive films and coatings remains lacking. Therefore, this review aims to fill this gap by providing an in-depth analysis of the current state of knowledge on fungal chitosan and its potential as a bioactive packaging material in the food industry.

## 2 Chemistry, molecular structure, and characteristics of fungal chitin and chitosan

Chitin ($[C_8H_{13}O_5N]_n$) is a linear polysaccharide composed of β-(1–4)-linked *N*-acetyl-D-glucosamine units (GlcNAc).[11,34] It is structurally similar to cellulose, but with an acetamido ($NHCOCH_3$) group replacing the hydroxy (OH) group at C-2 (Fig. 1).[35] This structural difference provides rigidity and

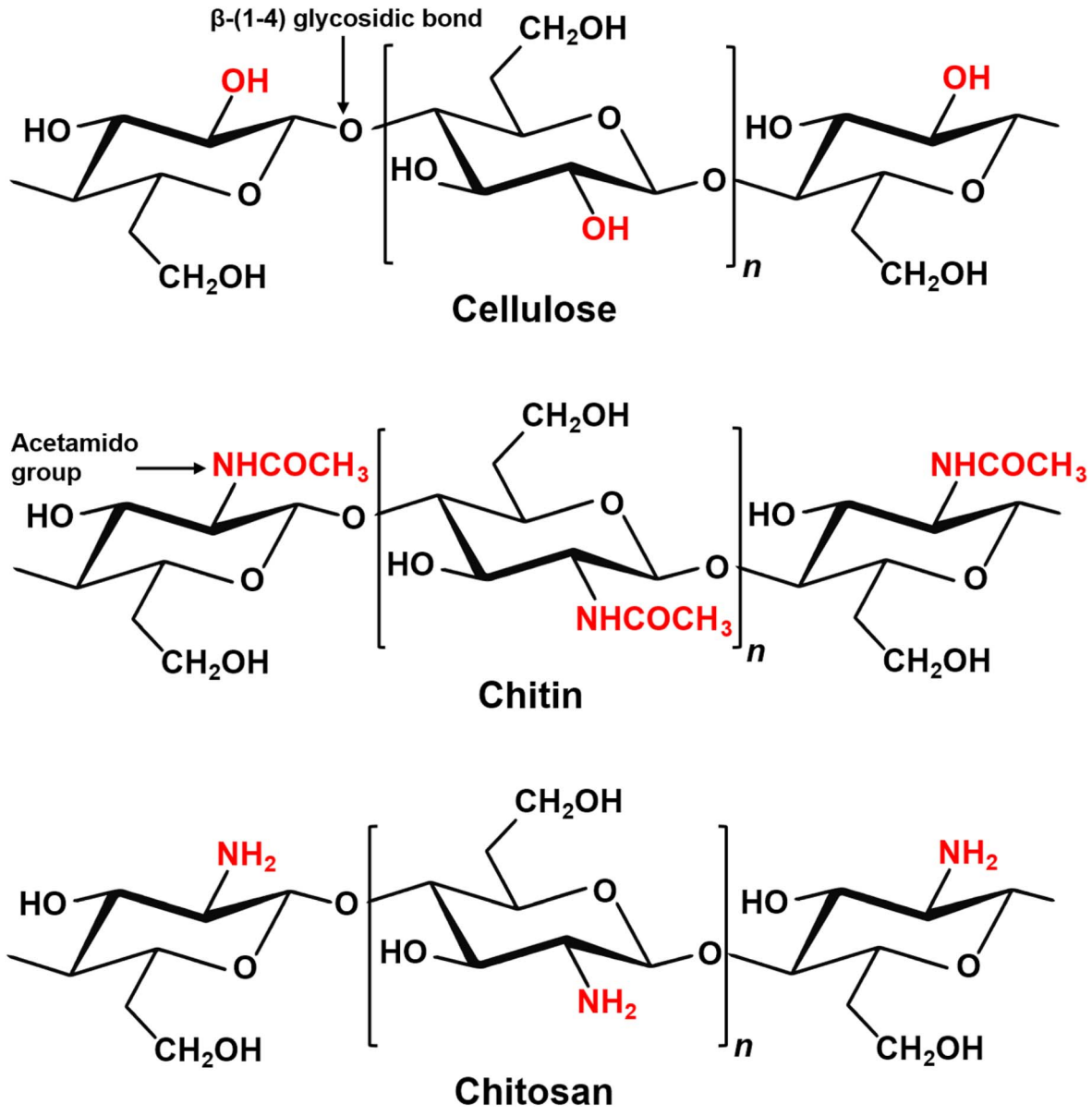


Fig. 1 Chemical structure comparison of cellulose, chitin, and chitosan, highlighting the substitution of hydroxy groups with acetamido and amino groups across the polysaccharide backbone.

resistance to degradation by microorganisms, in contrast to cellulose.[14] Chitin occurs in three allomorphic forms: α, β, and γ, distinguished by the arrangement of polymer chains in their crystalline structure.[36] The α-chitin is the most common and stable form, composed of antiparallel chains of β-(1–4)-linked GlcNAc units, with strong intermolecular hydrogen bonding that results in high crystallinity and low solubility, making it mechanically robust and chemically resistant.[36,37] In contrast, β-chitin has parallel chains with weaker hydrogen bonds, resulting in a more open and flexible structure, higher swelling capacity, and better reactivity, making it suitable for chemical modification and bioactive applications.[36,38] The γ-chitin, a less common form, consists of parallel and antiparallel chains, posing intermediate properties between the α and β forms.[19,36]

Chitin, a major polysaccharide in the fungal skeletal structure, provides rigidity and mechanical strength to the cell wall.[39] It occurs either as free amino glucoside chains or covalently bound to β-glucans, differing from the crystalline allomorphs (α, β, γ) classified by chain orientation.[15] The pathway of chitin biosynthesis in fungi has been well documented by Nwe *et al.*[40] Chitin and β-glucan are first synthesized separately, then cross-linked into a rigid chitin-glucan complex that reinforces cell wall integrity.[39,40] In the inner wall, chitin forms microfibrils that maintain turgor pressure.[15,19] Chitin is synthesized in both vegetative and sporulating cells, mainly at polarized growth sites.[40] During the cell cycle, isotropic growth deposits wall material across the bud, followed by repolarization to the mother-bud neck for cytokinesis.[17,23,40] In filamentous fungi, apical growth drives continuous hyphal extension and morphological development.[41]

On the other hand, chitosan ($[C_6H_{11}O_4N]_n$) is the partially or fully deacetylated derivative of chitin, composed mainly of D-glucosamine units along with varying amounts of GlcNAc.[42] The DD (*i.e.*, the percentage of acetyl groups ($-COCH_3$) that have been removed from chitin to produce chitosan) significantly affects the physicochemical properties of chitosan, such as solubility, since higher DD increases the availability of free amino groups that improve dissolution in acidic solutions.[43] Furthermore, it also influences the biological activities of chitosan, such as antimicrobial and antioxidant functions, which depend on the density of protonated amino groups interacting with microbial cell membranes or free radicals.[44,45]

# 3 Extraction of fungal chitin and chitosan

Fungal chitin is typically extracted by isolating the alkali-insoluble fraction of fungal biomass, followed by chemical treatments to remove proteins and other polysaccharides. To enhance yield and purity while reducing environmental impact, extraction methods are generally classified as conventional acid-alkali processes or novel green chemistry-based approaches, each suggesting distinct advantages for specific applications.

## 3.1 Conventional acid-alkali extraction process

As illustrated in Fig. 2a, extracting chitosan from fungal sources mainly uses conventional chemical methods, with acid and alkali treatment applied to isolate chitin and subsequently convert it into chitosan.[46] In general, acid-alkaline extraction follows three steps: demineralization, deproteination, and deacetylation.[47] Demineralization removes inorganic materials such as calcium carbonate and calcium chloride using diluted hydrochloric acid (HCl) in the case of crustacean chitosan.[15] Although some studies have followed, demineralization is not required for fungal chitosan, as the inorganic matter content in fungi is low.[17] For instance, *Pleurotus ostreatus* mushroom waste was demineralized using 2% acetic acid at 90 °C for 12 h, to eliminate residual inorganic matter.[48] In contrast, Irbe *et al.*[21] extracted chitosan from the fruiting bodies of *P. ostreatus* and *Agaricus bisporus* without demineralization. However, both studies reported no significant differences in the properties of chitosan, such as semi-crystallinity, moderate DD, and the yield ranging from 1.15–1.70%.[21,48] Subsequently, an optional decolorization can remove pigments (*i.e.*, Astaxanthin and β-carotene) using organic or inorganic solvents, such as ethanol, sodium hypochlorite, acetone, and hydrogen peroxide.[48,49]

In deproteination, the protein–chitin polymer complex undergoes depolymerization by breaking the chemical bonds to remove protein from chitin.[36] It is typically carried out under alkaline conditions, using 0.125–5 M sodium hydroxide (NaOH) at 90–121 °C for 2–12 h.[15] This process yields alkali-insoluble materials comprising chitin and partially deacetylated chitin, which must be further converted into chitosan through deacetylation.[48] Deacetylation involves removing acetyl groups from chitin and replacing them with reactive amino groups. The proportion of these free amino groups, expressed as DD, distinguishes chitin from chitosan.[42] For this process, alkaline treatment is preferred, as acid conditions can degrade glycosidic bonds. Thus, NaOH at 12–15 M is commonly used for deacetylation.[17] Yadav *et al.*[48] applied demineralization using acetic acid at 90 °C for 12 h, decolorization with ethanol reflux at room temperature for 6 h, and deproteination with 1 M NaOH to extract chitin from *P. ostreatus* mushroom waste. Chitin was deacetylated using 50% NaOH at 100 °C for 8 h, yielding 1.15% chitosan.[48] Similarly, Lam *et al.*[22] extracted chitosan from wild fungal species, including *Auricularia auricula-judae*, *Hericium erinaceus*, *P. ostreatus*, *Tremella fuciformis*, and *Lentinula edodes*. Mycelial raw materials underwent demineralization at 60 °C under constant shaking with 2 M HCl, followed by deproteination at 80 °C for 12 h with 2 M NaOH. The resulting material was then soaked in ethanol for 24 h to decolorize and produce chitin. Deacetylation was done by shaking at 100 °C for 6 h in 15 M NaOH, yielding 2.69–15.67% depending on species.[22] In contrast, some studies combined alkaline and acid treatments for the deacetylation. For instance, Irbe *et al.*[21] converted chitin from mycelial biomass into chitosan by deacetylation at 90 °C for 3 h in 10 M NaOH, followed by 2% acetic acid under the same conditions. The resulting chitosan was purified by filtration or centrifugation, yielding 0.03–0.38% chitosan, washed to neutral pH, and dried before subsequent applications.[21]

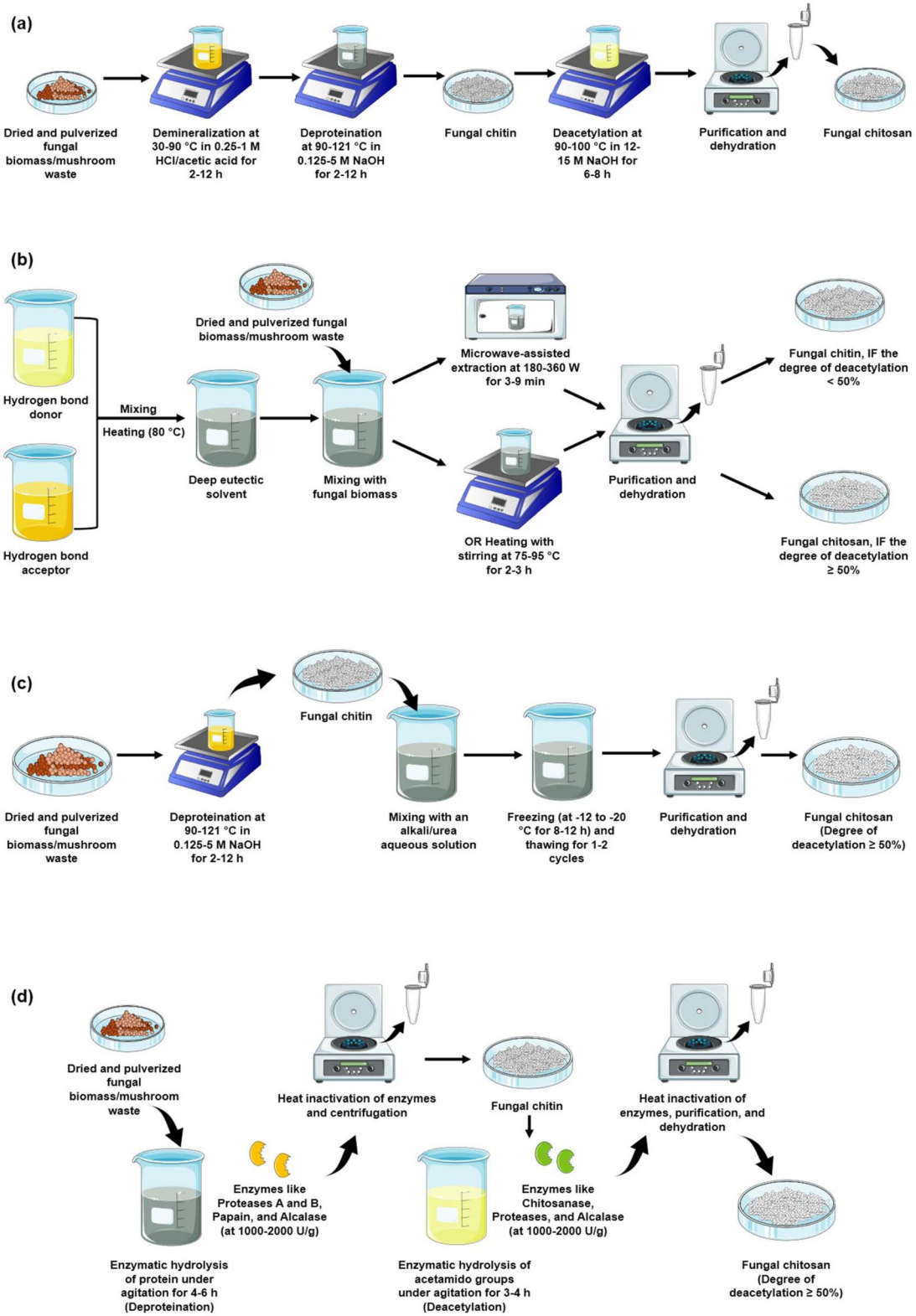


Fig. 2 Schematic illustration of (a) the acid/alkali extraction process, (b) deep eutectic solvent (DES) extraction, showing a single-step deproteination and deacetylation *via* microwave-assisted extraction or heating under stirring in the presence of DES, (c) alkali/urea aqueous extraction through deproteination followed by deacetylation *via* freezing and thawing for several cycles in the presence of alkali/urea aqueous solution, and (d) enzyme-assisted extraction process for isolating chitosan from fungal sources.

Conventional acid-alkaline extraction is widely practiced for its simplicity, cost-effectiveness, and scalability.[21,22,36] It effectively removes proteins and other impurities without the need for demineralization in fungal chitosan production.[15,17] However, it requires prolonged heating, making the process lengthy, energy-intensive, and unsustainable.[21,50] Therefore, novel extraction methods based on green chemistry principles have emerged as promising alternatives.

### 3.2 Novel green chemistry techniques

Due to environmental concerns and the adverse effects of chemical methods on chitosan quality ($M_w$ and DD), research has shifted toward eco-friendly alternatives for fungal chitosan extraction.[19,36] Green chemistry approaches aim to minimize hazardous chemicals, energy use, and waste generation.[36] Recent studies have explored microwave-assisted extraction, deep eutectic solvents, alkali/urea aqueous solutions, and enzyme-assisted methods.

**3.2.1 Microwave-assisted extraction.** Microwave irradiation induces dielectric heating within food systems.[51] Electric dipole moments are generated when passing this electromagnetic radiation through food systems (*i.e.*, water, sugar, or lipid molecules), indicating the presence of distinct positive and negative charges within the molecules.[36] These molecules rapidly align with the alternating electric fields of microwaves, and as the field oscillates, the molecules rotate and vibrate. This rapid molecular motion creates friction, which in turn generates heat.[51,52] Microwave-assisted extraction significantly reduces the reaction time while enhancing the yield and quality of fungal chitosan, in contrast to the conventional acid-alkali extraction.[50] A few studies have demonstrated the effectiveness of microwave-assisted extraction for fungal chitosan production as a function of reduced extraction time, high yield, and DD. For instance, Bahndral *et al.*[50] extracted chitin from *A. bisporus* mushroom waste using microwaves: demineralization at 540 W for 8 min in 3 M HCl, deproteination at 180 W for 8 min in 10% NaOH, and deacetylation at 360 W for 8 min in 50% NaOH to yield chitosan by converting acetyl to $-NH_2$ groups. In contrast, the conventional acid-alkali extraction involved demineralization at 30 °C for 2 h in 1 M HCl, deproteination at 90 °C for 2 h in 1 M NaOH under reflux, and subsequently deacetylation at 100 °C for 8 h in 60% NaOH under constant agitation. Microwave-assisted extraction achieved a higher yield (6.98%) and DD (79.94%) than conventional extraction (yield 6.09%), suggesting the effectiveness of microwave-assisted extraction.[50] Therefore, microwave-assisted extraction is a rapid, energy-efficient method for chitosan production compared to the conventional acid/alkali extraction.

**3.2.2 Deep eutectic solvent extraction.** As shown in Fig. 2b, deep eutectic solvents (DESs) are mixtures of two or three safe solvents that typically interact through hydrogen bonding between a hydrogen bond donor and acceptor, forming a eutectic system with a lower melting point than each component.[53,54] Few studies have applied DESs for fungal chitosan extraction. Ozel and Elibol[55] compared microwave-assisted, ultrasound-assisted, and shaking water bath methods using choline chloride and acetic acid DESs. Microwave-assisted extraction showed higher deproteination efficiency (38.7%) than ultrasound (10%) or shaking water bath at 95 °C (5.6%) for chitosan extraction from *A. bisporus*. Furthermore, synergistic application of microwave-assisted extraction with DESs prepared from choline chloride and acetic acid (at a molar ratio of 1 : 2) produced chitosan with a higher DD (69%) in a single-step deproteination and deacetylation compared to conventional acid-alkali extraction. Kim *et al.*[56] also reported a higher chitin-glucan recovery (30.4%) from *A. bisporus* mushroom using choline chloride: lactic acid DESs compared to 17% from chemical extraction, suggesting the applicability of DESs for chitosan extraction in future studies.[56] DESs accelerate protein–chitin fiber swelling, thus facilitating protein separation from the fiber complex owing to the strong intra- and intermolecular hydrogen bonding.[55] As protein contains fewer active functional groups, such as carboxy, amino, and hydroxy, compared to chitin, DESs interact with proteins *via* hydrogen bond acceptors.[54] These interactions disrupt the internal and external energy bonds within the chitin-protein fiber complex, leading to the removal of protein, resulting in deproteination.[55,57] As a greener and milder alternative to conventional acid/alkali extraction, DESs reduce the use of harsh acids and bases while maintaining good extraction efficiency for fungal chitosan.[55] It allows for selective solubilization of non-chitosan components, such as protein, preserving the structural integrity of the polymer.[55,57] Nonetheless, the complex interactions of the components of DESs with extracted materials often require additional steps for the regeneration and reuse of solvents, especially at an industrial scale.[53,54]

**3.2.3 Alkali/urea aqueous extraction.** Alkali/urea aqueous systems are promising green solvents for fungal chitin/chitosan extraction (Fig. 2c). Alkali, usually NaOH, helps break down hydrogen bonds in the polymer matrix, thereby accelerating swelling and partial dissolution of chitin.[12,36] Urea acts as a hydrogen bond disruptor and stabilizer, preventing polymer aggregation and improving solubility.[16] The solution is usually frozen to sub-zero temperatures (*i.e.*, −12 to −20 °C) to maintain stability and improve dissolution of polymers for processing into biodegradable films, hydrogels, or fibers.[36,58] Chitin is inherently insoluble in water and other common organic solvents owing to the presence of strong intra- and intermolecular hydrogen bonding.[12,42] However, chitin can be dissolved in alkali/urea aqueous systems coupled with freeze-thawing by breaking down the existing hydrogen bonds as proposed by Liao and Huang.[16] They dissolved chitin extracted from *Hericium erinaceus*, a traditional edible mushroom, in an aqueous system of NaOH/urea (11%/4%) at −20 °C to form hydrogels.[16] Although very limited studies on the use of alkali/urea aqueous systems for chitosan extraction from fungal sources have been published, research on crustacean feedstocks demonstrated that alkali/urea pretreatment and alkali/urea dissolution can produce chitosan of high purity and DD, suggesting the advantages of this method over harsh, high-temperature, concentrated-alkali deacetylation.[59–61] For instance, Huang *et al.*[62] reported that NaOH/urea freeze-thaw pretreatment of crab shell chitin yielded chitosan with very low ash content

 

(0.052%), high DD (86.02%), high solubility (99.44%), and improved antibacterial activity compared with conventionally extracted chitosan. Therefore, alkali/urea aqueous extraction presents a promising and eco-friendly alternative approach to extract chitosan from fungal sources with high purity.

**3.2.4 Enzyme-assisted extraction.** Enzyme-assisted extraction is an eco-friendly green chemistry approach providing higher specificity, rapid reactions, and lower energy use than conventional extraction for fungal chitosan.[36] Unlike acid-alkali treatment, it produces high-quality chitin by avoiding irregular deacetylation and $M_w$ reduction.[44] The extraction of chitin and chitosan from their natural origin involves specific enzymes, such as chitinases and chitosanases, as well as non-specific enzymes, including carbohydrases and proteases, as shown in Fig. 2d.[15] Lee *et al.*[63] reported no structural disparity between hydrolysis of high-$M_w$ crab exoskeleton- and mushroom-derived chitosan in terms of glucosamine and GlcNAc composition, after hydrolysis by chitosanase enzyme, suggesting the similarity of chitosanase activity on both crustacean- and mushroom-derived chitosan, and proposed the potential substitution of crustacean chitosan by fungal chitosan.[63] Deng *et al.*[64] reported a higher chitin yield of 88.9% from shrimp shells using enzymatic hydrolysis with protease A and B for deproteination and chitinase for chitin hydrolysis, which was more efficient than conventional acid-alkali extraction. Thus, enzyme-assisted extraction shows promise for high-purity fungal chitosan under mild and eco-friendly conditions.[44,64] However, the applicability of this technique on an industrial scale may be limited due to its inadequacy in deproteination, as some enzymes, such as papain, yield lower deproteination rates, and the high cost of specific enzymes.[36] Therefore, applying enzyme-assisted extraction in combination with other physical treatments, such as microwave irradiation for fungal chitosan synthesis, would minimize the current drawbacks. For instance, microwave irradiation facilitates rapid heating and improves cell wall disruption, thus enhancing the accessibility of enzymes to their substrates to break down proteins and polysaccharides in fungal cell walls under mild conditions.[36,51,52]

# 4 Conventional and emerging analytical methods for quantifying fungal chitosan

Quantification of chitin and chitosan in fungal sources is essential for evaluating their potential in chitosan production and optimizing processing for higher yields.[17] However, the direct determination of chitin remains a challenge owing to its insolubility in most solvents.[48] Therefore, chitin quantification generally relies on indirect measurement of derivatives like chitosan and GlcNAc.[65] The quantification of chitosan is easier than that of chitin because of its solubility in acidic solutions. In this regard, several attempts were conducted to quantify chitosan using colorimetric methods. For instance, Larionova *et al.*[66] developed a colorimetric assay based on the reaction between amino groups and *o*-phthalaldehyde and thiol-*N*-acetyl-L-cysteine. However, as discussed by Nitschke *et al.*,[65] the possible cross-reaction with amino acids or proteins limited its applicability. Therefore, Nitschke *et al.*[65] developed a reliable and specific colorimetric method without such cross-reactions. The assay was based on the formation of an insoluble polyiodide-chitosan complex between chitosan and polyiodide anions and utilized Lugol's iodine solution to form a colored complex, quantified by optical density. The authors quantified chitin in mycelia of *A. bisporus* (9.60%), *Pleurotus eryngii* (3.56%), *Lentinula edodes* (2.49%), *Morchella esculenta* (1.70%), *Grifola frondosa* (1.67%), *Pleurotus pulmonarius* (1.64%), *Hypsizygus tessulatus* (1.57%), *Trametes versicolor* (1.35%), *Flammulina velutipes* (1.21%), and *P. ostreatus* (0.82%). However, chitosan was not detected in any of the cases, suggesting that the glucosamine units have been predominantly acetylated.[65]

In contrast to those chemical methods, Urs *et al.*[67] utilized a combination of enzymatic hydrolysis and mass spectrometric analysis to quantify GlcNAc, followed by calculating total and average acetylation fractions. Fungal cell wall glucosamine residues were *N*-acetylated using isotopically labeled ($^2H_3$) acetic anhydride, followed by enzymatic hydrolysis into GlcNAc and $^2H_3$ GlcNAc monomeric units using chitinases and chitosanases. An internal standard ($^{13}C_2$, $^2H_3$)-labeled GlcNAc monomers were introduced to quantify the amounts of GlcNAc and D-glucosamine units in fungal cell walls or mycelia through ultra-high-performance liquid chromatography + electrospray ionization mass spectrometry (UHPLC-ESI-MS). The absolute chitin + chitosan amounts in whole mycelia ranged from <1% (in *Ustilago maydis*) to >10% (in *Fusarium graminearum*) and were found within 3% (in *Ustilago maydis*) to 9% (in *Puccinia graminis*) in the case of purified fungal cell walls.[67] More recently, Barroso-Solares *et al.*[68] introduced a Raman spectroscopy-based method to quantify both relative and absolute chitin and chitosan contents in fungal cell walls. Spectral analysis targeted Raman bands in the region of 1500–1750 $cm^{-1}$, corresponding to the vibrational bands of amide and $NH_2$ of chitin and chitosan, which are absent in cellulose and β-glucan. The authors reported relative chitosan contents of five *Pochonia chlamydosporia* and *Akanthomyces lecanii* strains, ranging from 19.3–28.3% of total chitin and chitosan.[68]

# 5 Factors influencing the yield of fungal chitosan

Table 1 presents the yield of fungal chitosan obtained from different fungal species, showing wide variations across studies. This variation can be attributed not only to the fungal source but also to factors such as the growth stage of the mycelial biomass or the specific part of the mushroom fruiting bodies (*i.e.*, pileus, stipes, gills, and stalk).[48,69] In addition, cultivation conditions (submerged or solid-state fermentation) and the extraction approach employed significantly influence the yield.[21,22] For instance, some species, such as *Rhizopus oryzae* and *Mucor rouxii*, consistently produce higher yields in submerged fermentation, whereas yields from mushroom fruiting bodies vary depending on the anatomical part used.[14,21] Thus, the data summarized in Table 1 highlights the

Table 1 Yield, molecular weight, and the degree of deacetylation of chitosan extracted from different fungal species using different extraction methods[a]

| Fungal species | Fungal source/ growth stage | Extraction method | Yield (%, w/w) | Molecular weight (kDa) | Degree of deacetylation (%) | References |
|---|---|---|---|---|---|---|
| *Pleurotus ostreatus* | Fruiting bodies | Conventional acid ($CH_3COOH$)/alkali (NaOH) extraction | 1.15 | 47.00 | 79.69–84.14 | 48 |
| *Pleurotus ostreatus* | Fruiting bodies | Conventional acid ($CH_3COOH$)/alkali (NaOH) extraction | 8.70 | 26.80 | 78.64 | 69 |
| *Lenzites betulina* | | | 19.00 | 47.00 | 83.54 | |
| *Trametes versicolor* | | | 16.00 | 26.80 | 82.71 | |
| *Lentinula edodes* | Fruiting bodies | Conventional acid ($CH_3COOH$)/alkali (NaOH) extraction | — | 58.36 | 95.60 | 73 |
| *Pleurotus ostreatus* | Fruiting bodies | Conventional acid (HCl)/alkali (NaOH) extraction | — | 153.88 | 71.86 | 74 |
| *Hericium erinaceus* | | | | 61.16 | 54.00 | |
| *Lentinula edodes* | | | | 165.89 | 74.55 | |
| *Auricularia polytricha* | | | | 68.06 | 61.31 | |
| *Tremella fuciformis* | | | | 54.26 | 50.64 | |
| *Ganoderma lucidum* | | | | 191.19 | 77.69 | |
| *Schizophyllum commune* | | | | 215.26 | 81.94 | |
| *Penicillium* sp. IITISM-ANK1 | Mycelial biomass | Conventional acid ($CH_3COOH$)/alkali (NaOH) extraction | 3.16 | 53.76 | 74.55 | 70 |
| *Penicillium johnkrugii* IITISM-ANK2 | | | 3.09 | 59.82 | 75.17 | |
| *Rhizomucor miehei* | Mycelial biomass | Conventional acid ($CH_3COOH$)/alkali (NaOH) extraction | 7.06 | 14.00 | 78.00 | 72 |
| *Aspergillus brasiliensis* | Mycelial biomass | Conventional acid (HCl)/alkali (NaOH) extraction | — | 28.40 | 92.00 | 75 |
| *Aspergillus oryzae* | Mycelial biomass | Conventional acid ($CH_3COOH$)/alkali (NaOH) extraction | 1.10 | — | 50.43 | 46 |
| *Aspergillus niger* | Mycelial biomass | Conventional acid ($CH_3COOH$)/alkali (NaOH) extraction | 3.52 | 790 | 75.00 | 49 |
| *Ganoderma lucidum* | Fruiting bodies | Conventional acid (HCl)/alkali (NaOH) extraction | 0.59 | 65.68 | 85.00 | 44 |
| | | Enzyme-assisted extraction | 13.20 | 47.65 | 89.00 | |
| *Agaricus bisporus* | Stalks | Microwave-assisted acid (HCl)/alkali (NaOH) extraction | 6.18–6.98 | — | 75.09–79.94 | 50 |
| *Agaricus bisporus* | Fruiting bodies | Microwave-assisted deep eutectic solvent (choline chloride/acetic acid) extraction | — | 120.00 | 69.00 | 55 |

[a] $CH_3COOH$, acetic acid. HCl, hydrochloric acid. NaOH, sodium hydroxide.

importance of species selection, cultivation strategy, and extraction method in optimizing fungal chitosan production. Singh *et al.*[70] revealed that the mycelium of *Penicillium* sp. strains had the maximum chitosan yield at the late exponential phase (30.90–31.60 mg $g^{-1}$) compared to earlier growth phases. This variation could be attributed to nascent chitosan

molecules being produced in the active growth phase.[70] As the growth stage progresses, chitosan tends to bind to the cell wall and may be converted into chitin or degraded by endogenous enzymes, resulting in a reduced yield of extractable chitosan.[71] Irbe *et al.*[21] demonstrated that the amount of chitosan extracted from *P. ostreatus* mycelium cultivated under submerged fermentation (0.09%) was approximately three times higher than that from the fruiting body (0.032%). In contrast, similar yields were recorded from mycelium grown in solid-state fermentation and the fruiting body of the same species, suggesting that chitosan yield depends on growth stage (mycelium/fruiting body) and cultivation/fermentation method.[21]

Chitosan yield also varies depending on the extraction method. As discussed by Savin *et al.*,[44] enzymatic extraction yielded significantly higher chitosan content of 132 mg $g^{-1}$ compared to 5.90 mg $g^{-1}$ obtained through chemical extraction from *Ganoderma lucidum* mushroom. Furthermore, Bahndral *et al.*[50] found low chitosan yield (6.09%) from *A. bisporus*, through chemical extraction, while microwave-assisted extraction yielded 6.98%. As discussed by Atlı *et al.*,[72] yield also depends on extraction conditions such as NaOH strength, deproteination time/temperature, acetic acid strength, and deacetylation time/temperature. Accordingly, the chitosan yield from *Rhizomucor miehei* biomass ranged from 10.90 mg $g^{-1}$ (1 N NaOH, 20 min at 108 °C, 4% acetic acid, 6 h at 75 °C, respectively) to 70.60 mg $g^{-1}$ (under optimized conditions: 3 N NaOH, 20 min at 95 °C, 4% acetic acid, 6 h at 85 °C, respectively), depending on acid-alkali extraction conditions.[72]

# 6 Properties and structure–function relationships of fungal chitosan

A comprehensive evaluation of the properties of fungal chitosan, including physicochemical, structural, thermal, biochemical, and biological characteristics, is essential to understanding their functional relationships for food packaging.[14,21,76] Physicochemical and structural traits ($M_w$, DD, and crystallinity) affect solubility, film formation, and mechanical strength.[31,32,77] Thermal stability indicates processing suitability, while antioxidant and antimicrobial activities support bioactive applications.[32,78] Biocompatibility and cytotoxicity further determine its safety in food packaging.[78]

## 6.1 Physicochemical properties and their functional relationships for food packaging

**6.1.1 Molecular weight.** The $M_w$ is an important parameter that determines the quality of fungal chitosan (Table 1). It influences packaging performance, where higher $M_w$ improves strength and barrier properties, while lower $M_w$ enhances solubility, processability, and antimicrobial activity due to better microbial membrane interactions.[32,79,80] $M_w$ of fungal chitosan is a function of source/species, growth stage, extraction method, concentration of acids/alkali used, and even the growth media used for cultivation.[14] However, fungal chitosan is generally characterized by a low-$M_w$.[48,69,75] Singh *et al.*[70] characterized chitosan extracted from two *Penicillium* strains: IITISM-ANK1 and IITISM-ANK2 through acid-alkali extraction in terms of low-$M_w$, and were found to be 53.76 kDa and 59.82 kDa, respectively. Yadav *et al.*[48] also reported a low-$M_w$ of ~47 kDa for chitosan from *P. ostreatus* waste *via* acid-alkali extraction. Similarly, $M_w$ of 65.68 kDa was reported for chitosan from *G. lucidum* mushroom through chemical extraction.[44] Conversely, it was decreased to 47.65 kDa when extracted by the enzymatic method due to the selective action of enzymes on protein–chitin linkages and glycosidic bonds, shortening the polymer chain compared to chemical extraction.[44,81] Nevertheless, the $M_w$ reported for commercial shrimp chitosan (192 kDa) was significantly higher than that reported for fungal chitosan, suggesting better solubility, mechanical resistance, and antimicrobial activity of fungal chitosan.[17,44]

**6.1.2 Degree of deacetylation.** The deacetylation process removes acetyl groups from chitin, releasing free amino groups that enhance antimicrobial and antioxidant properties.[82] Therefore, the degree of deacetylation (DD) is defined as the percentage of deacetylated β-1,4-D-glucosamine units and directly reflects the purity of chitosan.[50] Theoretically, chitosan is considered as chitin with DD ≥ 50%.[22] The DD depends on fungal strain, extraction technique, composition of the production medium, and even the quantification method (Table 1).[22,48,50] Chitosan from *G. lucidum* mushroom exhibited a higher DD (89%) from the enzymatic extraction than that of 85% from the conventional extraction, as determined by $^1$H-nuclear magnetic resonance ($^1$H-NMR), implying the effect of the extraction method on the DD.[44] Furthermore, Yadav *et al.*[48] showed variation in the DD of chitosan (from *P. ostreatus* waste) depending on the quantification method: 84.14% (elemental analysis), 79.69% (acid–base titration), 82.55% (conductometric titration), and 81.52% (potentiometric titration).[48] Additionally, Atlı *et al.*[72] reported that fungal chitosan from the waste *R. miehei* under optimized conditions (NaOH concentration (3 N), deproteination time/temperature (20 min/95 °C), acetic acid concentration (4%), deacetylation time/temperature (6 h/85 °C)) had a higher DD (78%) compared to milder conditions (1 N, 20 min/108 °C, 4%, and 6 h/75 °C).[72] DD generally decreases with higher deproteination temperatures but increases with stronger alkali and higher deacetylation temperatures, although extreme conditions may cause excessive depolymerization and dark coloration.[17,50,72] Higher DD improves antimicrobial activity due to more reactive amino groups.[50] Furthermore, the gel strength of film-forming solutions containing chitosan with a higher DD increases due to free amino groups, which form stronger interactions with other components in the solution, such as proteins or polysaccharides, through hydrogen bonds and electrostatic interactions, resulting in films with improved mechanical integrity and stability.[14] For instance, Zheng *et al.*[80] reported that the tensile strength of collagen-chitosan films increased from 93.08 MPa to 107.99 MPa as the DD increased from 75% to 95%, along with reduced water vapor permeability (WVP) and UV transmittance. This was attributed to the formation of more hydrogen bonds between collagen molecules and chitosan, as higher DD provides more reactive groups for interactions.[80]

**6.1.3 Solubility.** Solubility is a key quality parameter of chitosan from both animal and fungal sources and is critical for food packaging development. It is influenced by the proportion of acetylated or non-acetylated D-glucosamine units in the polymer chain, protonated amino groups, and processing conditions such as extraction and deacetylation parameters.[9,30,83] For instance, chitosan from *A. bisporus via* microwave-assisted extraction showed 75.98% solubility, higher than 59.04% from conventional acid-alkali extraction (in 1% acetic acid).[50] Similarly, Ossamulu *et al.*[69] stated that the solubility of chitosan extracted *via* the acid-alkali method from *P. ostreatus*, *Trametes versicolor*, and *Lenzites betulina* was 75%, 76%, and 79%, respectively. As discussed by Lam *et al.*,[22] the solubility of fungal chitosan ranged from 20.76% (*Lentinula edodes*) to 91.61% (*Hericium erinaceus*), correlating with increased DD (79.36–79.86%), as chitosan solubility is directly linked to higher DD.[22] Nevertheless, Kalutharage and Rathnasinghe[84] observed very low solubilities of 3.41 and 7.38% for chitosan from *P. ostreatus* and *Schizophyllum commune*, respectively, indicating lower DD (53.10–60.68%) and the presence of inorganic materials that were not fully removed during demineralization, as discussed by Pellis *et al.*[85] In a separate study by Yadav *et al.*,[48] chitosan derived from *P. ostreatus* exhibited low solubility (~13%) in the sole aqueous solution and high solubility (~81%) in 1% acetic acid, suggesting the effect of solvent on solubility as a function of pH. When dissolved in acetic acid, chitosan becomes a positively charged molecule as a result of the protonation of its amino groups, converting into $NH^{+3}$, thus improving solubility. Conversely, when the pH increases up to 6.5, chitosan becomes insoluble owing to the deprotonation of amino groups, which is visually indicated by turning the solution cloudy.[83]

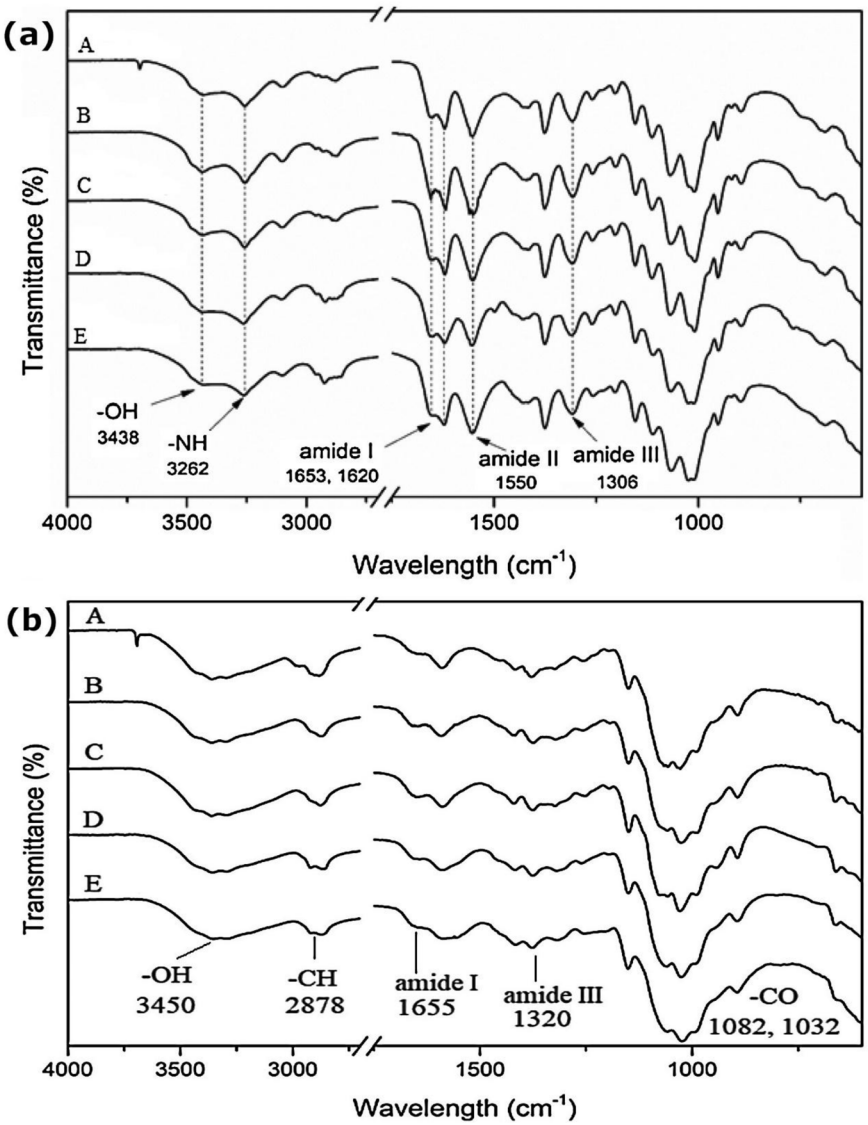


Fig. 3 (a) ATR-FTIR spectra of chitin from shrimp (commercial α-chitin) (A), lobster (B), insect (C), and mushroom (*B. bovinus* (D) and *L. laccata* (E)). (b) ATR-FTIR spectra of chitosan from shrimp (commercial chitosan) (A), lobster (B), insect (C), and mushroom (*B. bovinus* (D) and *L. laccata* (E)). (Reproduced from Oberemko *et al.*[86] with permission from Elsevier Ltd © 2019 Elsevier Ltd).

 

### 6.2 Structural properties and their functional relationships for food packaging

**6.2.1 Characterization of functional groups *via* FTIR spectroscopy.** FTIR (Fourier Transform Infrared Spectroscopy) is widely used to characterize fungal chitosan. Oberemko *et al.*[86] compared ATR-FTIR spectra of commercial shrimp α-, lobster, insect, and fungal chitins (from *Boletus bovinus* and *Laccaria laccata*). Characteristic α-chitin peaks appeared at 1620 and 1653 $cm^{-1}$ (amide I, C═O stretching), while β- and γ-chitin exhibited distinct single or semi-double peaks, suggesting the applicability of FTIR to identify chitin isomers (Fig. 3a).[86] Similarly, Kim *et al.*[56] reported α-chitin in shrimp with split peaks at 1630 and 1657 $cm^{-1}$. Ssekatawa *et al.*[87] observed β-chitin, with bands at 1455 and 1374 $cm^{-1}$ ($CH_x$ deformation) and narrow C═O/C–O stretching peaks at 1200–950 $cm^{-1}$. Oberemko *et al.*[86] also noted the presence of amide II peak at 1550 $cm^{-1}$ corresponding to N–H and C–N stretching vibration, amide III peak ($CH_2$ wagging) at 1306 $cm^{-1}$, N–H stretching vibration at 3262 $cm^{-1}$, and band at 3438 $cm^{-1}$ attributed to the O–H stretching vibration. Interestingly, the spectral peaks of fungal chitin accurately corresponded to those of crustacean and insect chitin.[86] As noted by Bahndral *et al.*,[50] Oberemko *et al.*,[86] and Hassainia *et al.*,[88] the absence of absorption bands at 1540 $cm^{-1}$ and 1700–1740 $cm^{-1}$, attributed to the stretching vibration of protein, suggests that the protein molecules have been eliminated during the deproteination process of chitin from *B. bovinus*,*L. laccata*, and *A. bisporus*.

Like chitin, fungal chitosan presents characteristic absorption peaks based on the structure and composition. Similar to commercial crustacean chitosan, chitosan derived from *P. ostreatus*, *Hericium erinaceus*, *Lentinula edodes*, *Auricularia polytricha*, *Tremella fuciformis*,*G. lucidum*, and *Schizophyllum commune* exhibited amide I bands at 1625–1660 $cm^{-1}$ assigned to C═O stretching vibration of the amide groups. Unlike chitin, the amide II bands at 1530–1590 $cm^{-1}$ in chitosan are attributed to the C–N and N–H stretching vibrations, indicating the presence of proteins and other amide-containing compounds.[74] As previously reported, chitosan from *B. bovinus* and *L. laccata* exhibited an amide I band at 1655 $cm^{-1}$ (C═O stretch), amide II at 1580 $cm^{-1}$ (C–N and N–H stretching), and amide III at 1320 $cm^{-1}$ ($CH_2$ bending) and corresponded accurately to crustacean and insect chitosan (Fig. 3b).[86] As stated by Affandy *et al.*,[74] the $CH_3$ band (amide III) at 1380–1390 $cm^{-1}$ indicated the presence of GlcNAc units in chitosan with increased acetylation, as an increase in band intensity. Furthermore, absorption bands at 1070–1300 $cm^{-1}$ (C–O stretching) indicate the presence of ester functional groups, suggesting the improved antimicrobial activity.[74] Although FTIR provides information on the presence of various functional groups in chitin and chitosan, which is useful for comparing different fungal species, the presence of extraneous materials within the sample can interfere with FTIR spectral readings, thereby altering the key absorption bands and limiting its applicability in structural characterization.[14] However, FTIR identifies structural features of fungal chitosan that directly influence its functionality in

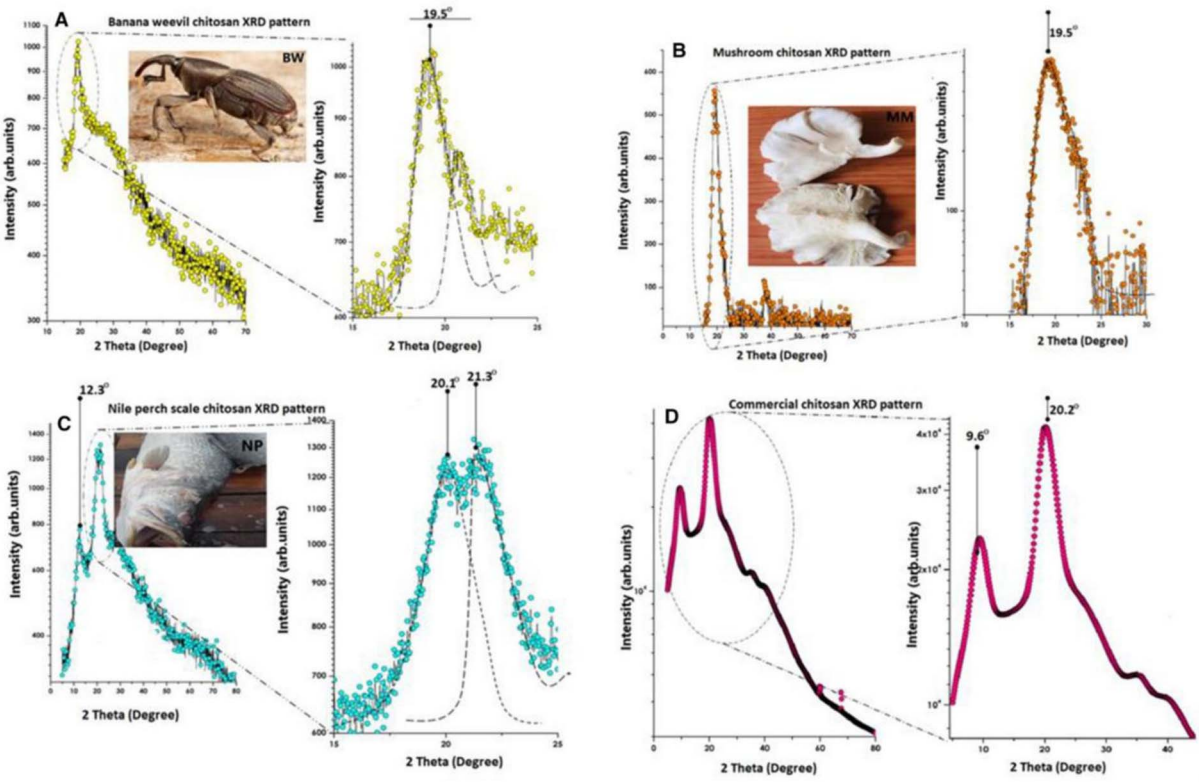


Fig. 4 XRD patterns for (A) banana weevil chitosan, (B) mushroom chitosan, (C) Nile perch scale chitosan, and (D) commercial chitosan (Reproduced from Ssekatawa *et al.*[87] with permission under Creative Commons Attribution (CC BY 4.0) license. © 2021 The Authors).

food packaging development. The identification of characteristic amide and hydroxy bands confirms the presence of reactive amino and carbonyl groups, which are essential for intermolecular bonding in the film formation.[33,72] Variations in these absorption peaks reflect differences in DD, affecting film flexibility, solubility, and bioactivity. Therefore, understanding structural properties helps correlate chemical composition with key packaging properties such as mechanical strength, barrier, and antimicrobial activity.[31,48]

**6.2.2 Characterization of crystal structure *via* X-ray diffraction (XRD).** In general, X-ray diffractograms of fungal chitosan show two main peaks near 10° and 20°, although their intensity and position vary with $M_w$ and DD.[50] The strong peak observed for chitosan from *P. ostreatus* at a diffractive angle of $2\theta = 20.19°$ corresponds to a lattice spacing of 4.39409 Å, suggesting a 'form II' polymorphic structure.[48] This form reflects a specific crystalline arrangement within the chitosan, indicating a semi-crystalline or hydrated polymorph configuration generally associated with fungal chitosan.[48,89] Accordingly, Yadav *et al.*[48] stated that the crystalline index (CrI) of *P. ostreatus*-derived chitosan was 43%. Ssekatawa *et al.*[87] reported a peak at $2\theta = 19.5°$ for mushroom chitosan, slightly shifted from 20° in commercial crustacean chitosan (Fig. 4B and D), suggesting greater crystallinity in fungal chitosan.[87]

Furthermore, Bahndral *et al.*[50] reported chitosan from *A. bisporus* as highly crystalline, with peaks at $2\theta = 29.4$, 32.3, 33.5, and 37.8°, typical of α-chitin. Hassainia *et al.*[88] found CrI values of 88.1% for commercial chitin and 63.2% for *A. bisporus*-derived chitin, showing structural differences. The DD strongly affects crystallinity: fully deacetylated chitosan (100% DD) is considered fully crystalline.[48,50] Higher $M_w$ also enhances crystallinity through chain entanglement and intermolecular interactions.[80] Greater crystallinity associated with increased DD and chain regularity improves mechanical strength, barrier properties, adsorption capacity, antimicrobial activity, biocompatibility, and biodegradability, making chitosan suitable for fabricating packaging materials.[74] In contrast, partially deacetylated chitosan exhibits a semi-crystalline structure due to residual acetyl groups disrupting chain regularity, thus improving flexibility but lowering the mechanical stability of packaging films.[21]

**6.2.3 Characterization of molecular structure *via* NMR spectra.** NMR (Nuclear Magnetic Resonance) spectroscopy is an important method for identifying the structure, crystallinity, purity, and DD of fungal chitin and chitosan. $^{13}$C NMR spectra revealed the α-crystalline form of fungal chitin.[88] Hassainia *et al.*[88] identified distinct peaks for C-3 and C-5 in the D-glucosamine split at 73.1 and 75.2 ppm, corresponding to the α-polymorph of chitin from *A. bisporus*, compared with commercial α- and β-chitin standards. The two resolved signals at C-3 and C-5 were characteristic of α-chitin, whereas β-chitin displayed only a single signal at 73 ppm (Fig. 5). Accordingly, NMR spectra are important to identify the native polymorphic form, which directly influences the mechanical and functional properties of chitin and chitosan.[88] Furthermore, Oberemko *et al.*[86] found that the degree of acetylation (DA) was significantly higher when determined by NMR than by elemental analysis, suggesting better sensitivity of NMR for structural identification. Interestingly, they reported that no *N*-deacetylation occurred during the extraction of α-chitin of different biological origins with higher DA above 90%.[86]

In contrast, the DD of fungal chitosan was lower in *B. bovinus* and *L. laccata* (70–74%) than in crustacean or insect sources (~100%), likely due to raw material composition. Nevertheless, $^{13}$C NMR spectra of commercial, lobster, and pine weevil chitosan showed similarities to chitosan derived from *B. bovinus* and *L. laccata*.[86] Yadav *et al.*[48] reported typical chitosan peaks in semi-purified *P. ostreatus* waste extracts, including signals for C-2/C-6 ($\delta$ 57.67/$\delta$ 61.19 ppm) and C-4/C-1 ($\delta$ 83.53/$\delta$ 105.13 ppm). However, minor peaks due to residual heteropolymeric glucans, other conjugates, and β-glucan confirmed the influence of fungal species and the efficiency of acid/alkali extraction on polymer composition.[48] For instance, saprotrophic basidiomycetes like *A. bisporus* contain less glucan than wood-decaying fungi.[90,91] Singh *et al.*[70] confirmed six sharp peaks from $^{13}$C NMR spectra of chitosan from *Penicillium* sp., confirming D-glucosamine repeat units. Based on $^{1}$H NMR, enzymatic extraction of *G. lucidum* chitosan yielded higher DD (89%) than chemical extraction (85%), with improved solubility and rheology.[44] Moreover, the $^{1}$H NMR profiles of shrimp and mushroom chitosan were comparable, suggesting similar physicochemical properties.[44] The identification of α- and β-polymorphs and variations DD helps predict mechanical strength, solubility, and film-forming ability. Higher DD, as revealed by NMR, is associated with improved solubility and antimicrobial activity of chitosan, suitable for developing bioactive films and coatings.[48,86]

### 6.3 Thermal properties and their functional relationships for food packaging

**6.3.1 Characterization of thermal stability *via* thermogravimetric analysis.** Thermal stability, as measured by TGA (thermogravimetric analysis), is crucial for fungal chitosan, as it indicates resistance to thermal degradation and determines its suitability for packaging.[48] Thermograms of fungal chitosan typically exhibit two or three degradation stages: moisture removal, polysaccharide depolymerization with major weight loss, and final breakdown of residual organics.[48,70,86] For instance, chitosan derived from *P. ostreatus* degraded in two steps within the range of 30–865 °C: moisture loss at 30–100 °C (12.58% weight reduction) and major decomposition at 247–450 °C (90.32% weight loss) due to glycosidic bond cleavage and residue breakdown.[48] Singh *et al.*[70] outlined a three-step decomposition of chitosan from *Penicillium* sp., with the first stage at 35–85 °C, the second at 210–320 °C, accompanied by 35–40% mass loss, and the third at 365–430 °C, indicating relatively high thermal stability. Oberemko *et al.*[86] reported maximum degradation of chitosan at 317 °C (from *B. bovinus*) and 309 °C (from *L. laccata*), compared to 294 °C for shrimp chitosan and 325 °C for lobster/insect chitosan, suggesting fungal chitosan has stability greater than commercial shrimp chitosan but lower than crustacean/insect sources.[86] However, the maximum degradation temperatures ranging from 289.53–373.21 °C reported for chitosan from *P. ostreatus*, *Hericium*

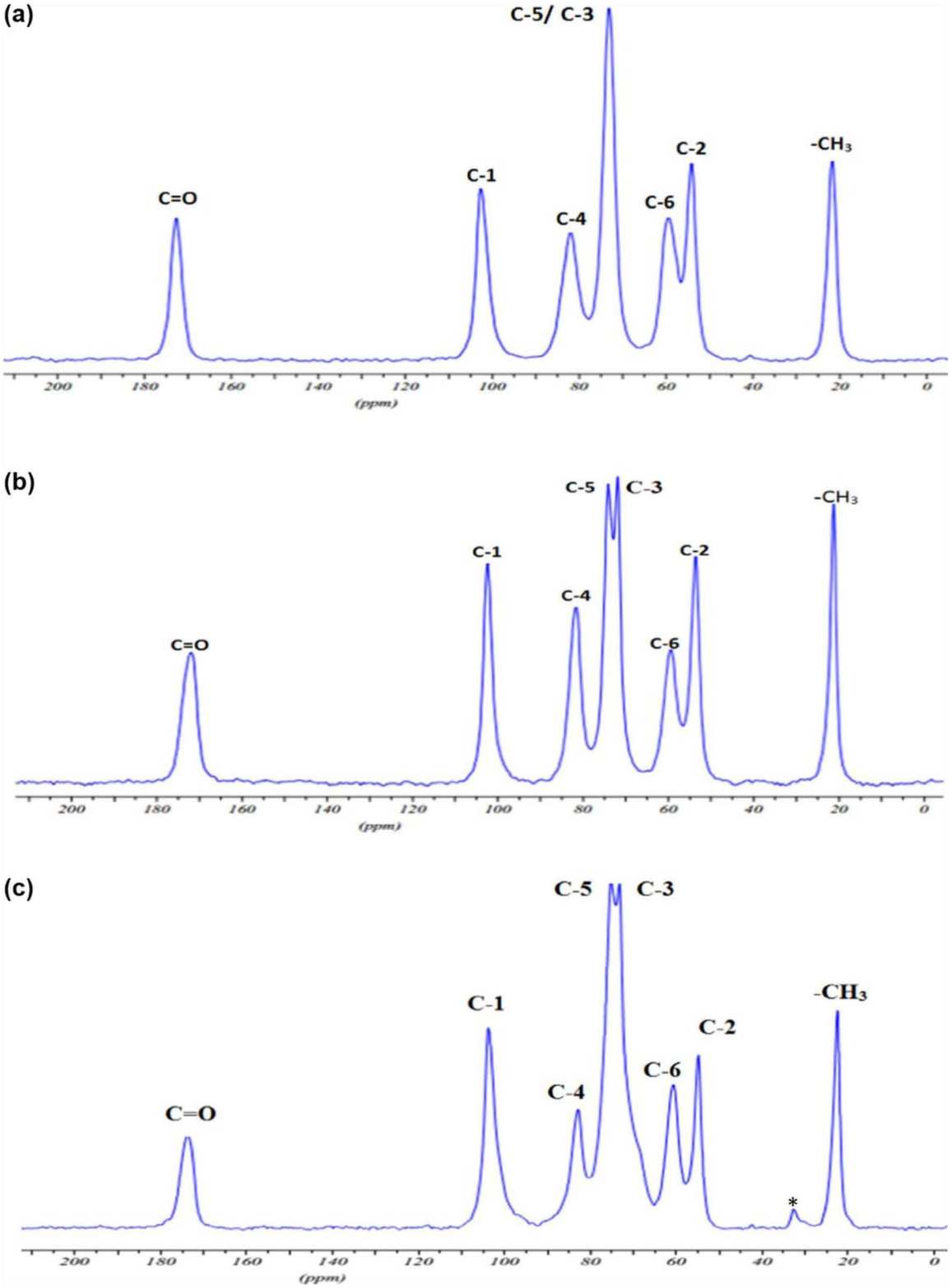


Fig. 5 CP/MAS $^{13}$C-NMR spectra of (a) commercial β-chitin, (b) commercial α-chitin, and (c) chitin extracted from the *A. bisporus* stipes. (Reproduced from Hassainia *et al.*[88] with permission from Elsevier Ltd © 2017 Elsevier Ltd).

*erinaceus*, *Lentinula edodes*, *Auricularia polytricha*,*Tremella fuciformis*, *G. lucidum*, and *Schizophyllum commune* were higher than those reported for commercial shrimp chitosan (220.98 °C), suggesting that the fungal chitosan can withstand high temperatures without undergoing significant structural breakdown or degradation compared to crustacean chitosan.[74]

**6.3.2 Characterization of thermal transitions *via* differential scanning calorimetry.** Differential scanning calorimetry (DSC) measures the heat transfer of a sample relative to a reference as temperature changes, indicating thermal transitions such as glass transition, where the material changes from an amorphous to a rubbery or viscous state, melting, and crystallization, indicating structural changes between different solid states, based on the purity and molecular configuration.[48,50,76] Generally, fungal chitosan exhibits a two-step DSC degradation pattern.[50] As stated by Singh *et al.*,[70] the DSC of *Penicillium* sp. derived chitosan exhibited endothermic peaks at 104–135 °C (bound water evaporation) and an exothermic peak at 240–250 °C (amino/carbohydrate decomposition).[70] Similarly, chitosan derived from *P. ostreatus* displayed mass loss at 100 °C, followed by decomposition at 257 °C with maximum breakdown at 295 °C.[48] The DSC of chitosan from *A. bisporus* revealed the onset temperature ($T_0$), melting temperature (*cc*), crystallization temperature ($T_c$), and enthalpy change ($\Delta H$) as 91, 99.10, 114.10 °C, and 0.791 J $g^{-1}$ for the sample deproteinated at a microwave power of 360 W compared to 180 W ($T_0 = 59.8$ °C, $T_m = 82.40$ °C, $T_c = 97.50$ °C, and $\Delta H = 0.046$ J $g^{-1}$), suggesting enhanced thermal stability *via* stronger intermolecular interactions as a result of deproteination process.[50] Affandy *et al.*[74] further reported higher glass transition temperature ($T_g$) (347.88–373.21 °C) for *Tremella fuciformis*, *Schizophyllum commune*, and *G. lucidum* chitosan compared to 220.98 °C for crustacean chitosan, suggesting fungal chitosan can withstand greater thermal stress, making it more suitable for high-temperature processing and food packaging applications.[74]

## 6.4 Biochemical and biological properties and their functional relationships for food packaging

**6.4.1 Antioxidant activity.** The antioxidant activity of fungal chitosan is essential in food packaging, as it helps to retard oxidation, extend shelf life, and preserve food quality.[77,78] As reported by several studies, antioxidant activity mainly depends on DD, where higher DD increases active amino groups on C-2, enhancing radical scavenging.[92,93] For instance, chitosan derived from *A. bisporus via* microwave-assisted extraction (DD of 79.94%) possessed higher antioxidant activity of 53.97% against DPPH (2,2-diphenyl-1-picrylhydrazyl) radicals and 3.58% reducing power, in contrast to the chitosan with a DD of 75.71%, which exhibited 42.06% $DPPH^{\bullet}$ scavenging and 2.41% reducing power.[50] Savin *et al.*[44] highlighted strong antioxidant activity of chitosan derived from *G. lucidum via* enzymatic extraction against $DPPH^{\bullet}$ (255.43 mM Trolox $g^{-1}$) and $ABTS^{\bullet+}$ (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) (129.46 mM Trolox $g^{-1}$) compared to those from acid-alkali extraction ($DPPH^{\bullet}$: 46.52 mM Trolox $g^{-1}$ and $ABTS^{\bullet+}$: 65.41 mM Trolox $g^{-1}$). Notably, its $IC_{50}$ was 8.5-fold lower than that of shrimp chitosan, confirming superior activity.[44] As suggested by these studies, the antioxidant activity of chitosan could vary depending on the source of origin and the extraction method.[94]

**6.4.2 Antimicrobial activity.** Antimicrobial activity of chitosan is a key property influencing bioactive food packaging performance.[73] It mainly depends on the polycationic nature of chitosan, where amino groups at C-2 of glucosamine units interact with negatively charged microbial cell surfaces, particularly under acidic conditions, causing membrane disruption, leakage, and cell death.[20] A higher DD increases positive charge density, thus enhancing electrostatic interactions with negatively charged bacterial cell membranes, possessing higher bactericidal effects than chitosan with moderate DD.[95] Chitosan from waste of *R. miehei* mycelium (78% of DD) exhibited strong antimicrobial activity with minimum inhibitory concentrations (MIC) of 125 μg $mL^{-1}$ for *Escherichia coli* and *Candida albicans*, and 250 μg $mL^{-1}$ for *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Aspergillus brasiliensis*, in contrast to the crustacean chitosan that had the MIC of 125 μg $mL^{-1}$ for *E. coli*, *P. aeruginosa*, and *C. albicans* and 250 μg $mL^{-1}$ for *S. aureus*, and 500 μg $mL^{-1}$ for *A. brasiliensis*.[72] Similarly, *P. ostreatus*-derived chitosan exhibited MIC of 125 μg $mL^{-1}$ for *E. coli* and 250 μg $mL^{-1}$ for *S. aureus*.[48] Both studies highlighted that fungal chitosan was more effective against Gram-negative (*E. coli*) than Gram-positive bacteria (*S. aureus*), due to structural differences in their cell walls.[48,72,95] As consistently discussed in most studies, chitosan with low $M_w$ and high DD possesses increased antibacterial activity.[44,48,72,94] Furthermore, chitosan with a higher inorganic matter content exhibited low solubility, affecting its bioavailability and resulting in reduced antimicrobial activity.[44,70]

**6.4.3 Cytotoxicity.** It is important to assess the cytotoxicity of fungal chitosan before food packaging applications, as they are in direct contact with food products. Savin *et al.*[44] reported dose-dependent but low toxicity of *G. lucidum* chitosan on L929 fibroblast cells, with >80% viability at 50–1000 μg $mL^{-1}$, indicating good cytocompatibility. The cytocompatibility of chitosan from *G. lucidum* mushroom on the same cell line was also noted by Ospina *et al.*[96] and suggested its suitability as a biomaterial. Some studies also highlighted selective cytotoxicity against cancer cells, suggesting therapeutic potential. For instance, Yadav *et al.*[48] recorded a decline in human colon cancer cell (Caco-2 cells from human colorectal adenocarcinoma) viability with the chitosan from *P. ostreatus*, showing $IC_{50}$ of 70 μg $mL^{-1}$ and potential in colon cancer treatment.[48] Oberemko *et al.*[86] observed >85% viability in both cancerous (MH-22A) and non-cancerous (Chines hamster ovary cells) cells treated with *B. bovinus* and *L. laccata* chitosan at 10 μg $mL^{-1}$, but at 1000 μg $mL^{-1}$, necrosis increased by 22% (cancerous) and 30% (non-cancerous).[86] Wei *et al.*[97] attributed this to interactions between positively charged chitosan and $Na^+/K^+$ ionic pump, leading to intercellular $Na^+$ overload and subsequent mitochondrial damage, resulting in cellular dysfunctions. Nevertheless, as recent studies emphasized that fungal chitosan was non- or less toxic to normal cells, there is great potential to use chitosan of fungal origin in food applications, including food packaging.[44,48]

# 7 Development of fungal chitosan-based active packaging films and coatings

As a biodegradable and polycationic polysaccharide, chitosan has attracted attention for bioactive packaging with minimal environmental impact.[7] Although only limited studies have explored fungal-sourced chitosan films for food applications, they show strong potential to replace petroleum-based plastics.[42] Chitosan exhibits unique physicochemical traits, including pH-dependent solubility, metal ion-chelation, antioxidant and antimicrobial activity, and negligible cytotoxicity owing to the presence of $NH_3^+$ and $OH^-$ groups in its structure.[8,98,99] Beyond film formation, fungal chitosan functions as a carrier for essential oils, organic acids, phenolic extracts, nanoparticles, and pH-sensitive pigments.[27,97,100,101] For instance, microencapsulation of peppermint essential oil (L-carvone) *via* complex coacervation and subsequent spray drying using fungal chitosan, gum Arabic, and maltodextrin, exhibited good shell permeability and diffusivity in aqueous ethanol, and with no unnecessary oil leakage, suggesting better controlled release ability.[89] Baiocco *et al.*[102] developed dual-shell microcapsules using fungal chitosan as the inner shell and silica as the outer layer, demonstrating improved mechanical strength and barrier properties. Furthermore, betulinic acid-loaded liposomes coated with low-$M_w$ fungal chitosan from *A. bisporus* showed improved solubility, encapsulation, and antioxidant activity compared to crab chitosan.[101] These findings highlighted fungal chitosan as a promising material for packaging films and a carrier for hydrophobic bioactives in the food and pharmaceutical sectors.

## 7.1 Role of biopolymer blends

Although chitosan has good film-forming ability, films made from pure chitosan exhibit limitations, including hygroscopicity, which weakens structural integrity, and poor flexibility and elongation.[9,10,103] To overcome these issues, plasticizers, cross-linking agents, and other biopolymers are often incorporated. For instance, plasticizers such as glycerol (20–25% w/w chitosan) improved flexibility and elongation but reduced tensile strength due to weaker hydrogen bonding.[104] Therefore, different polymers, including native or modified starch, protein (*i.e.*, gelatin, corn zein), lipids, or synthetic biopolymers, like polylactic acid (PLA), polyhydroxybutyrate (PHB), and poly-ε-caprolactone (PCL), can enhance the mechanical properties of chitosan films.[7–9,24] Specifically, fungal chitosan combined with potato starch[31] or sodium alginate,[105] has shown improved strength and reduced water vapor permeability through hydrogen bonding or electrostatic cross-linking.[7]

## 7.2 Role of bioactive compounds

As the only polycationic biopolymer in nature, chitosan exhibits strong antimicrobial and antioxidant properties desirable for food preservation.[31,103] However, these inherent properties may not fully meet active packaging requirements.[7] Therefore, to enhance functionality, chitosan films and coatings have been supplemented with bioactive agents such as plant-derived compounds (*i.e.*, eugenol, carvacrol, cinnamaldehyde), extracts, essential oils, organic acids (*i.e.*, citric, ascorbic, malic, rosmarinic, benzoic), and nanoparticles of metals (*i.e.*, Cu, Ag, Zn) or oxides (*i.e.*, ZnO, $TiO_2$).[8,106–109] Interestingly, incorporating additives like pomegranate peel extract,[110] fucoidan,[105] curcumin,[77] $TiO_2$,[75] vanillin, and gallic acid,[111] and cress (*Lepidium*

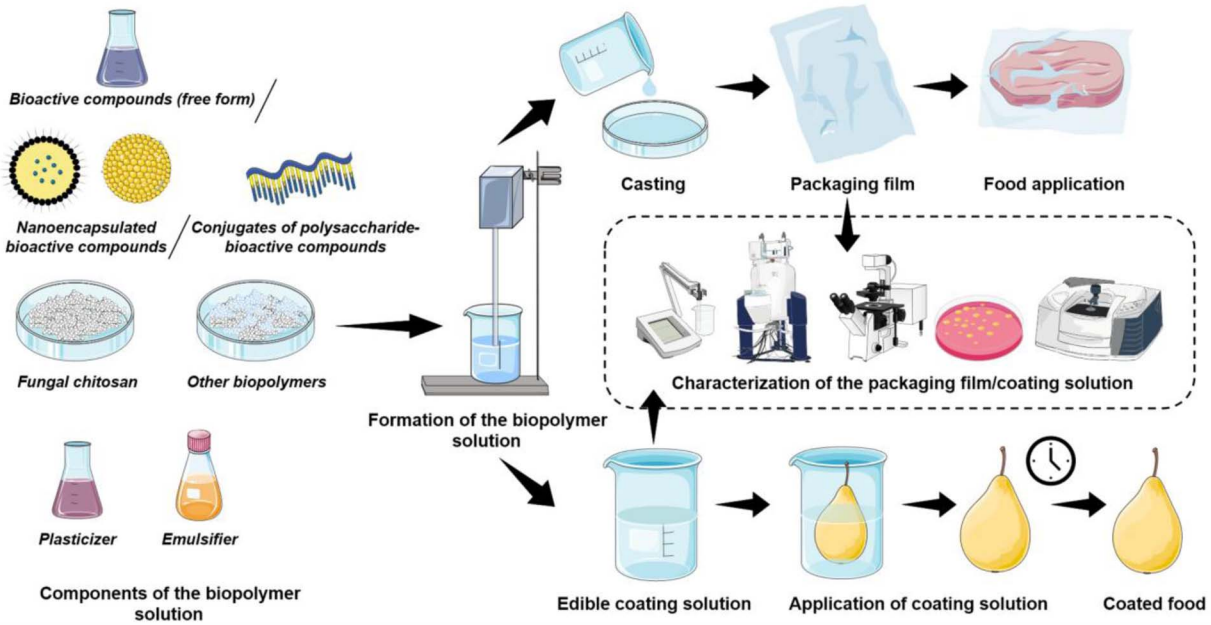


Fig. 6 The typical process involved in the fabrication of bioactive packaging films and coatings based on fungal chitosan, highlighting the components of biopolymer solution, formulation into packaging films or coatings, their characterization, and food applications.

*sativum*) seed extract[112] into fungal chitosan films has improved the shelf life of perishable foods by reducing microbial contamination.

Bioactive compounds can be incorporated into chitosan films and coatings in three main ways (Fig. 6). First, they may be directly mixed into the film-forming solution *via* homogenization before casting or coating.[77,105] Since many compounds (*i.e.*, extracts, essential oils) are hydrophobic, emulsifiers like Tween 20, Tween 80, or lecithin are used to create stable heterogeneous systems.[7,113,114] In the second method, active compounds are encapsulated within nano-scale carriers, including nanoemulsions,[115] nanoliposomes,[116] nanocapsules,[106] or other forms of nanoparticles,[117,118] before integrating into films or coatings. Nanoencapsulation enhances solubility, stability, and enables controlled release of antioxidant and antimicrobial agents.[108] Third, active compounds (*i.e.*, phenolics, flavonoids) can be grafted onto polysaccharides (*i.e.*, chitosan, starch) through modification methods such as 1-ethyl-3(3-dimethylaminopropyl)carbodiimide (EDC)-mediated, free-radical-induced, enzyme-catalyzed, bromide-mediated, or Schiff-base reactions to form conjugates.[119] These conjugates are added to film/coating solutions to improve biological and functional properties by covalently binding active compounds, reducing volatilization, and enhancing solubility and stability, while minimizing negative sensory effects.[7,98,119]

### 7.3 Preparation of bioactive packaging films and coatings

Fig. 6 illustrates the fabrication of fungal chitosan-based bioactive films and coatings. Solution casting is the most common method, where the film-forming solution is cast into molds and dried to form mono- or bilayer films.[7,78] Electrospinning has also been applied to produce chitosan nanofiber films, which improve barrier properties by limiting $O_2$ and water diffusion, reducing water solubility, and enabling encapsulation of active compounds within the nanofiber matrix.[120–122] For edible coatings, food products are typically immersed in the coating solution, drained, and dried to form a thin polymer layer around the product surface.[113] Although spraying and fluidized-bed processing have been proposed, immersing/dipping remains the preferred method due to its simplicity, layer-by-layer capability, and effectiveness for products with complex or rough surfaces.[5,108,123]

## 8 Properties of fungal chitosan-based packaging films

### 8.1 Mechanical properties

Mechanical properties such as tensile strength, elongation at break, and flexibility are critical as they reflect the resistance of a packaging material to damage during handling and storage.[10] These properties are strongly influenced by the $M_w$ and DD of chitosan due to differences in intermolecular interactions.[32] A film from *A. bisporus*-derived low-$M_w$ chitosan (21 kDa) with glycerol showed lower tensile strength (3.8 MPa) and elongation (36%) compared to a film from high-$M_w$ crustacean chitosan (250 kDa), which exhibited higher tensile strength (5.2 MPa) and elongation (66%).[124] This demonstrated that the tensile strength and elongation at break generally increase with the $M_w$ of chitosan used due to stronger hydrogen bonding and chain entanglement.[124,125] Westlake *et al.*[111] also reported improved tensile strength (10.4 MPa) in vanillin-cross-linked *A. bisporus*-derived chitosan films with glycerol and gallic acid, attributed to cross-linking and hydrogen bonding with bioactive compounds.[7,117] Similarly, *A. bisporus*-derived high-$M_w$ chitosan (~1490.4 kDa) with glycerol produced films with higher tensile strength (29.3 MPa), suggesting the direct proportionality of the tensile strength to the $M_w$ of chitosan utilized.[31] Moreover, blending fungal chitosan with other biopolymers, such as potato starch, yielded films with superior properties, such as breaking strain (71.5%), toughness (2.4 MJ $m^{-3}$), and tensile strength (24.7 MPa) due to intermolecular hydrogen bonding.[31]

### 8.2 Barrier properties

The shelf life of packaged foods largely depends on the barrier properties of packaging films against water vapor, $O_2$, and UV radiation.[78] Chitosan from fungal and crustacean sources is hydrophilic, resulting in higher WVP due to moisture migration and interactions with water molecules.[7,31] Alimi *et al.*[31] recorded higher WVP ($1.95 \times 10^{-13}$ g $m^{-2}$ $s^{-1}$ $Pa^{-1}$) from *A. bisporus*-derived pure chitosan film compared to the film reinforced with potato starch, which exhibited $0.99 \times 10^{-13}$ g $m^{-2}$ $s^{-1}$ $Pa^{-1}$ WVP, attributed to hydrogen bonding between chitosan and starch that reduced hydrophilic groups and formed a more rigid structure.[31,125] Although $M_w$ does not directly determine WVP, fungal chitosan films from low-$M_w$ *A. bisporus* exhibited lower WVP (0.63 g $m^{-2}$ $h^{-1}$ $kPa^{-1}$) than high-$M_w$ crustacean chitosan films (0.74 g $m^{-2}$ $h^{-1}$ $kPa^{-1}$), suggesting the lower water affinity of fungal chitosan, contributing to better moisture barrier properties.[124] Cross-linking further improves barrier properties; vanillin-cross-linked *A. bisporus*-derived chitosan films showed reduced WVP ($4.3 \times 10^{-10}$ g $m^{-2}$ $s^{-1}$ $Pa^{-1}$), comparable to conventional plastics such as LDPE (low-density polyethylene) ($6.67–8.70 \times 10^{-11}$ g $m^{-2}$ $s^{-1}$ $Pa^{-1}$) and PET (polyethylene terephthalate) ($5.8–22.9 \times 10^{-11}$ g $m^{-2}$ $s^{-1}$ $Pa^{-1}$).[111]

UV radiation (210–400 nm) induces photooxidation in foods, particularly fats, vitamins, and pigments, leading to rancidity, off-flavors, discoloration, and nutrient loss.[78,93] Hence, UV-barrier packaging films from fungal chitosan are valuable for food protection. *A. bisporus*-derived chitosan films cross-linked with vanillin showed 100% UV blocking (200–400 nm) due to their dense matrix.[111] In addition, the presence of aromatic polyphenolics such as gallic acid and the formation of imine interactions with the amino groups of chitosan can absorb UV light through the excitation of π-electrons within a conjugated molecular system.[126] Gomaa *et al.*[105] also reported 100% UV barrier properties with no light transmission at the UV region (200–280 nm) through the film produced from *A. niger*-derived chitosan and alginate, supplemented with fucoidan. Conversely, a pure crustacean chitosan film exhibited 60–80% UV transmittance compared to those containing curcumin grafted cellulose nanofibers, suggesting weak UV-blocking properties of pure chitosan films.[127] Therefore, fungal

 

chitosan films, especially when reinforced with other biopolymers or active compounds, can serve as effective UV barriers.

### 8.3 Thermal properties

The thermal properties of fungal chitosan-based films, typically assessed by TGA and DSC, provide information about their thermal stability.[34] These films generally show two to three degradation steps, consistent with the behavior of the fungal chitosan used in their preparation.[77] The first step corresponds to water loss, the second to polymer backbone decomposition, and the final step to further breakdown of organic components.[74,77] For instance, *A. bisporus*-derived chitosan films showed the first degradation at 46.8 °C with 9.1% mass loss, followed by 30.4% loss between 240–295 °C (maximum degradation temperature ($T_{max}$) 275 °C), and 19.1% loss between 295–356 °C ($T_{max}$ 325.8 °C). In contrast, high-$M_w$ crustacean chitosan films exhibited two peaks at 51.2 °C (9.6% loss) and 288.2 °C (40% loss), suggesting relatively lower thermal stability.[124] Fungal chitosan films also exhibited lower residue, indicating their reduced mineral content compared to crustacean counterparts.[85,124] Alimi *et al.*[31] and Westlake *et al.*[111] suggested that the thermal stability of fungal chitosan-based films could be further improved by combining other biopolymers, such as starch, and by utilizing cross-linking agents like vanillin to increase the inter- and intramolecular forces.

In fungal chitosan-based films, DSC thermograms showed endothermic and exothermic peaks corresponding to TGA degradation steps. Endothermic peaks relate to bound water loss and acetyl-glucosamine backbone disintegration, while exothermic peaks reflect the breakdown of hydrogen bonds and polymer interactions.[48,50] Alimi *et al.*[31] reported that increasing fungal chitosan content from 0.5–1% increased crystallinity and $T_g$, indicating a more ordered, tightly packed structure requiring higher thermal energy for decomposition.[31]

### 8.4 Optical properties

The optical properties of packaging films, including opacity, color, and light transmittance, are crucial for protecting food from light-induced damage and ensuring consumer acceptance.[32] *A. bisporus*-derived chitosan films combined with potato starch showed light transmittance of 86.99% and glossiness of 13.0 (at 20°), similar to pure chitosan films (84.16% and 14.5), suggesting the miscible compatibility of starch and chitosan and a homogeneous matrix with reduced surface roughness that limits light scattering.[31] Conversely, adding fucoidan or curcumin to *A. niger*-derived chitosan + alginate films or *A. bisporus*-derived chitin-glucan films reduced light transmittance due to phenolic and flavonoid interactions with the biopolymer matrix, which absorb the light in the visible spectrum.[7,77,105] In addition, a slight increment in opacity in fungal-sourced chitosan films was also noted by Kaya *et al.*[77] and Westlake *et al.*,[111] due to the incorporation of curcumin and the formation of Schiff base interactions between vanillin and chitosan, respectively.[127] Although film transparency and low opacity are crucial factors that affect the visualization of food products such as minimally processed fruits and vegetables, low light transmittance with high opacity could be beneficial for preserving light-sensitive food products, such as fat-based food.[78]

### 8.5 Antioxidant activity

As food products, especially those containing lipids, such as seafood, meat, and dairy products, are highly susceptible to oxidative degradation, the application of active packaging that possesses antioxidant activity is crucial to extend their shelf life by retarding oxidation.[78,128] While fungal chitosan shows inherent antioxidant activity due to amino groups, it is often insufficient for active packaging.[7] Therefore, to enhance antioxidant activity, bioactive compounds have been incorporated into fungal chitosan films.[77,105] For instance, incorporating gallic acid into *A. bisporus*-derived chitosan film matrix improved its antioxidant activity by scavenging 95.5% DPPH$^{\bullet}$.[111] Similarly, curcumin (0.2–2%) incorporated into *A. bisporus* chitin-glucan films increased total phenolic content (TPC) from 2.60–14.25 mg GAE $g^{-1}$ and DPPH$^{\bullet}$ scavenging from 9.66–70.96%.[77] Curcumin is a naturally occurring polyphenol characterized by a conjugated molecular structure that includes phenolic hydroxy groups and an enolic form of a β-diketone moiety, which possesses higher antioxidant activities.[45,129]

### 8.6 Antimicrobial activity

Both fungal and crustacean-derived chitosan possess antimicrobial activity due to their polycationic nature.[20,103] However, incorporating bioactive compounds can further enhance the antimicrobial performance of fungal chitosan films for food packaging.[10,20] For instance, *A. bisporus*-derived chitosan films supplemented with gallic acid and vanillin inhibited *E. coli* and *S. aureus* with inhibition zones of 26.0 and 19.3 mm, respectively.[111] Similarly, films from *A. bisporus* chitin-glucan showed antibacterial activity against *E. coli* (7.92 mm inhibition), which increased to 10.13 mm with 2% curcumin, as curcumin disrupts the polymerization of filamenting temperature-sensitive mutant Z (FtsZ) protein involved in cell division.[77,130] However, no activity was observed against Gram-positive *S. aureus*, aligning with reports by Yadav *et al.*[48] and Atlı *et al.*,[72] that fungal chitosan is generally more effective against Gram-negative bacteria due to differences in cell wall structure.

### 8.7 Biodegradability

"Biodegradable" refers to materials broken down by microorganisms.[78] Although biopolymers of natural origin are generally biodegradable, their derivatives, like chitosan, may resist degradation.[2] Therefore, it is important to analyze the biodegradability of packaging films to understand the rate of biodegradation as a function of time. The soil burial method under natural conditions is usually used to assess biodegradability.[77,94,111] Degradation occurs in three stages: fragmentation by enzymes and physical factors, depolymerization with $M_w$ reduction, microbial utilization of monomers, and final mineralization through oxidation.[131] Davis *et al.*[94] reported that *A. niger* biomass-derived chitosan films degraded rapidly between day 10 and 50 at 1.9–28.92%.[94] Westlake *et al.*[111] noted

Table 2 The applications of bioactive edible coatings based on fungal chitosan on food preservation[a]

| Polymer matrix | Active component | Food product | Effect of edible coating on food product | References |
|---|---|---|---|---|
| Chitosan derived from *Aspergillus niger* mycelium | Pomegranate peel extract | Nile tilapia fillets | Coating preserved fish fillets from microbial spoilage and oxidation with lower TVB-N (12.7 mg 100 $g^{-1}$), TBARS (0.21 mg MDA $kg^{-1}$), and PV (1.73) than those TVB-N (60.8 mg 100 $g^{-1}$), TBARS (0.32 mg MDA $kg^{-1}$), and PV (5.15) of the uncoated control sample after 30 days of storage at 4 °C<br>Coating decreased the entire microbial counts compared to the control<br>Coating preserved the sensory properties of samples for 30 days compared to the control, which was unaccepted by the 10th day | 110 |
| Chitosan derived from *Agaricus bisporus* stalk bases and sodium alginate | — | White grape fruit bar | Fungal chitosan + alginate bilayer coating retained ascorbic acid content, antioxidant activity, firmness, and moisture content of fruits compared to crustacean chitosan + alginate coating and uncoated control<br>Fungal chitosan + alginate bilayer coating preserved fruit bars from fungal contaminations for 34 days, compared to those with crustacean chitosan + alginate coating (31 days) and uncoated control (28 days) | 79 |
| Chitosan derived from *Aspergillus niger* mycelium | Fungal chitosan nanoparticles | Grapes | Edible coatings with fungal chitosan nanoparticles (FCNs) preserved grape quality by reducing weight loss and microbial decay, retaining moisture and acidity, slowing ripening (lower soluble solids and sugars), and maintaining sensory acceptability for 24 days at 12 °C compared to the control fruits, which were coated without FCNs<br>FCNs inhibited the growth of pathogenic bacteria such as *E. coli*, *S. aureus*, and *Salmonella* spp. with MIC ranging from 2–3 mg $mL^{-1}$ | 133 |
| Chitosan derived from stipe offcuts of mushroom | — | Fresh-cut melons | Coating improved the storability and quality of fresh-cut melons by preserving firmness (up to 1.66 N *vs.* 1.30 N in uncoated control), minimizing ethanol accumulation, and enhancing fruit flavor (ester content: 79.93% *vs.* 57.15% in uncoated control)<br>After 11 days at 6 °C, total aerobic bacterial count and total mold and yeast counts (log CFU $g^{-1}$) were recorded as 6.54 and 3.20 for fungal chitosan-coated, and as 7.00 and 3.67 for crustacean chitosan-coated fruits, respectively | 134 |
| Chitosan derived from *Aspergillus niger* mycelium | Microencapsulated *Lacticaseibacillus casei* LC03 probiotics | Strawberry | Coating preserved the quality of cold-stored strawberries by minimizing weight loss (below 6%), maintaining moisture (90.74%), titratable acidity (0.94%), and pH (3.16), while ensuring high probiotic viability compared to those uncoated fruits | 132 |

[a] TVB-N, total volatile basic nitrogen. TBARS, thiobarbituric acid reactive substances. PV, peroxide value. MDA, malondialdehyde. MIC, minimum inhibitory concentration.

faster degradation in fungal chitosan films with mass reduction of 89.7–100% in soil and 40.6–55.8% in seawater over 4–12 weeks due to lower $M_w$ and shorter polymer chain length, as they facilitate water permeation, thus accelerating microbial degradation.[111] In contrast, a film produced from the chitin-glucan complex extracted from *A. bisporus*, degraded from day 7–14 in soil by chitin-degrading microbes that can produce chitinase, such as *Streptomyces*, thus hydrolyzing glycosidic bonds in GlcNAc units.[77] However, some studies have claimed that reduced degradation of chitosan compared to chitin, due to a lack of enzymes capable of hydrolyzing chitosan, such as chitosanase in soil microorganisms.[81]

 

## 9 Applications of fungal chitosan-based active packaging films and coatings for food preservation

Fungal chitosan and its films or coatings exhibit strong potential as bioactive packaging for food preservation. As reported by Tayel *et al.*,[112] chitosan derived from *Mucor rouxii* mycelia inhibited *Penicillium digitatum* and *P. italicum* with MIC values of 65.0 and 57.5 μg $g^{-1}$, respectively. When combined with cress seed and pomegranate peel extracts, it completely suppressed fungal growth on citrus fruits for 14 days compared to uncoated and antifungal-free commercial coatings, suggesting their applicability as a potential antifungal packaging.[112] Similarly, Simões *et al.*[132] developed a coating from *A. niger*-derived chitosan and sodium alginate, incorporating *Lacticaseibacillus casei* encapsulated in an alginate-chitosan bilayer. On strawberries, the coating preserved pH, titratable acidity, moisture, and color, minimized weight loss (<6%), maintained >60% TPC, and ensured probiotic viability (>7 log CFU $mL^{-1}$) for 12 days, in contrast to controls that deteriorated rapidly, suggesting the potential of fungal chitosan for delivering functional probiotics to fresh produce.[132] Kaya *et al.*[77] also applied a curcumin-incorporated chitin-glucan film from *A. bisporus* to chicken breast. Unlike control samples wrapped in cling film, which exceeded the acceptable microbial limit (6.7 log CFU $g^{-1}$) by day 7, coated samples remained safe until day 10 (6.8 log CFU $g^{-1}$), extending shelf life and demonstrating fungal chitosan composites as effective, sustainable bioactive packaging.[77] Table 2 further summarizes the applications of bioactive edible coatings based on fungal chitosan on food preservation.

## 10 Present challenges and future perspectives

Despite the growing interest in fungal chitosan as a sustainable alternative to crustacean-derived chitosan in active food packaging, there are some challenges that must be overcome before its commercial and industrial scalability. One major obstacle lies in the variability of yield and quality of fungal chitosan, which is highly dependent on species, growth conditions, and extraction methods. While fungal biomass offers year-round availability, inconsistency in chitosan content among different fungal species and developmental stages can complicate standardization. Additionally, although fungal chitosan extraction avoids demineralization, conventional acid-alkali methods still involve lengthy processing times and generate chemical waste, raising concerns about sustainability and cost-effectiveness. Emerging green extraction technologies, such as microwave-assisted, enzyme-assisted, and deep eutectic solvent-based methods, offer improved yields and environmental compatibility; however, they require further optimization for scale-up and industrial adoption.

Another critical challenge is the limited understanding of structure–function relationships in fungal chitosan, particularly concerning how $M_w$, DD, and functional group interactions influence film performance and bioactivity. This gap restricts the ability to apply fungal chitosan films for specific food applications; for instance, they may be unsuitable for high-fat foods like fried products, high-moisture products such as fresh-cut fruits, strongly acidic foods like pickles, or highly aromatic products such as spices, where their hydrophilic nature, solubility at low pH, or limited aroma barrier properties reduce effectiveness. Furthermore, while the absence of allergenic compounds like tropomyosin, which trigger shellfish allergies and cause severe immune reactions in sensitive individuals, makes fungal chitosan more appealing for food applications, regulatory frameworks and consumer acceptance of fungal-derived packaging materials are still in early stages in many regions. Ensuring consistent antimicrobial efficacy, compatibility with various bioactive agents (*e.g.*, antimicrobials, antioxidants, and probiotics), and long-term food safety without compromising intended mechanical and optical properties, and biodegradability remains an ongoing challenge.

Looking forward, the future of fungal chitosan-based packaging lies in the integration of multi-functional properties, such as smart sensing capabilities, controlled release of bioactive compounds through different nanocarriers such as nanoemulsion, and synergistic combinations with other biopolymers or nanomaterials. Research into hybrid coatings that combine fungal chitosan with essential oils, polyphenols, or natural colorimetric indicators (*i.e.*, plant pigments) can enhance shelf life, safety, and consumer acceptability. Valorization of agro-industrial fungal waste and standardization of green extraction techniques will further advance circular economy goals. Therefore, fungal chitosan can be considered a promising bioresource for next-generation food packaging, provided that technological, regulatory, and scalability concerns are systematically addressed through interdisciplinary efforts.

## 11 Conclusions

This review highlighted that fungal chitosan possesses unique advantages, such as antimicrobial and antioxidant activity, film-forming capacity, and biodegradability, that make it a promising alternative to crustacean-derived chitosan for food packaging applications. Evidence from recent studies demonstrated its successful use in edible coatings and bioactive films, particularly for perishable foods like fresh fruits and meats, as well as its compatibility with other biopolymers and active agents to enhance food safety. However, several limitations remain, including variability in yield and quality across fungal species, limited understanding of structure–function relationships (*i.e.*, the influence of $M_w$ and DD on film properties), and regulatory as well as consumer acceptance challenges. Future work should focus on optimizing extraction methods, modifying physicochemical properties for specific food systems, scaling up production with sustainable technologies, and addressing safety and regulatory frameworks to fully realize the potential of fungal chitosan as a next-generation bioactive packaging material.

## Author contributions

Nimesh Dileesha Lakshan: conceptualization, investigation, software, visualization, writing – original draft, and writing –

review and editing. Amali U. Alahakoon, Rumesh Liyanage, Tharindra Weerakoon, Mayumi Silva, Arianna Dick, Lisa Newman, Benu Adhikari, and Stacey F. Y. Yong: supervision and writing – review and editing. Chathuri M. Senanayake: conceptualization, funding acquisition, supervision, and writing – review and editing. The authors read and approved the published version of the manuscript.

## Conflicts of interest

The authors have no conflicts of interest to declare that are relevant to the content of this article.

## Data availability

No datasets were generated or analyzed during the current study.

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