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Recent Trends in Production, Characterization, and Utilization of Fungal Chitosan

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Abstract

Chitosan is the second most abundant biopolymer in nature after cellulose that can be extracted from marine sources, as well as from agricultural waste products such as mushrooms or other fungal sources following chitin deacetylation. The consistent production of these glycans by fungi is unaffected by seasonal changes, allowing for precise process control. This results in a more consistent quality of the final product. Currently, crustacean shells and other marine species are the most important sources of chitin/chitosan production globally. However, extraction from marine sources involves a number of problems, including an unreliable raw material supply chain. Large-scale chitosan extraction from crustaceans degrades the environment by requiring severe processing procedures such as alkali deproteinization. Many researches have recently been conducted to investigate alternate sources or environmentally friendly techniques for producing chitosan. Fungal biomass can be good alternate as well as environmentally friendly source for the extraction fungal chitosan. Due to its steady property and low molecular weight fungal chitosan is more suitable in application like pharma and biomedical. Chitosan can be used for an extensive range of applications due to its macromolecular structure and distinct physiological and biological features, such as biodegradability, solubility, biocompatibility, solubility, and reactivity. Chitosan can be used for a different range of applications that include in agricultural, biomedical, pharmaceutical, nutraceuticals, cosmetics and cosmeceuticals, and wastewater treatment. This review highlights the diverse applications of commercial chitosan products across various fields, alongside its primary focus on fungal chitosan extraction. It underscores the broad utility of chitosan beyond its extraction from fungi.

Keywords: Biopolymer, Fungal Chitosan, Chitosan, Chitin, Application, Production

INTRODUCTION

Chitin is a linear biopolymer composed of N-acetyl-D-glucosamine units connected by glycosidic β-(1,4 links) (Figure 1). Chitin, which is found in the cuticles of insects and the exoskeletons of crabs and other mollusks, is the second most common polysaccharide in nature after cellulose. Another significant source of chitin comes from fungal biomass [1]. The latest research in fermentation technology for the extraction and characterization of biopolymers from fungal sources has attracted many researchers. The fungal biomass used in chitosan production is a low-cost bio-waste from an abundant and affordable source, and it can be used for that purpose. These bio-wastes may be beneficial in the form of extremely valuable products like chitosan that can be

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extracted from it whereas chitosan is deacetylated form of chitin with the degree of deacetylation >40% [2].

Solid-state fermentation (SSF) and submerged fermentation (SmF) have the potential to produce fungal biomass. The SmF has a distinct benefit in that it allows for more precise control over fermentation factors, including temperature, pH, and nutrient concentration in the process of fermentation media [3]. However, the SSF is known to create more biomass than the SmF, indicating a higher potential for chitosan production. Chitosan synthesis from fungi involves several types of components, including peptone, D-glucose, and yeast extract. Lately, the main focus of research has been on growing fungus for the synthesis of chitosan using inexpensive carbon sources, like biowastes [4].

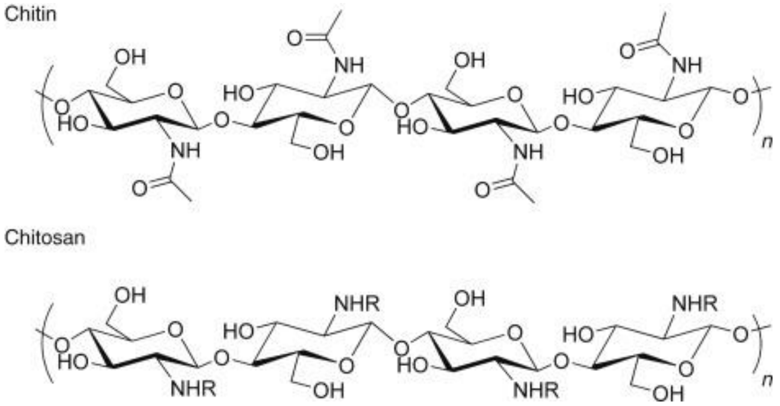

Figure 1. Chemical Structure of Chitin and Chitosan.

Fungal mycelia cell walls are made up of glycoproteins and polysaccharides like glucan and chitin. In fungal cell walls, glycoproteins (xylomanno, galacto, mannos, and glucurono) are interstitial components; polysaccharides, on the other hand, are structural elements. *Aspergillus niger* and *Mucor rouxii* cell walls contain up to 45% chitin, while *Penicillium notatum* cell walls contain 20% [5]. Chitin can be deacetylated to produce chitosan. The degree of deacetylation (DD) and molecular weight of chitosan determine its solubility in aqueous acids like acetic and lactic acids; a high DD and low molecular weight increase solubility [6]. More recently, the concept of a “shell biorefinery” for fractionating and upgrading fungal cell walls and crustacean exoskeletons into valuable biomaterials has been put out in several studies [7]. Chitosan is being utilized in more and more applications due to its special properties. Chitosan is biocompatible with a variety of organs and tissues and has a chemical structure that is generally stable, polycationic, safe, non-toxic, and biodegradable [8].

Chitosan molecules are physiologically and physically active, and they can be chemically or enzymatically altered for a variety of applications. A wide range of applications can be achieved by processing chitosan to create membranes, flakes, gels, beads, and sponges. Chitosan finds diverse applications such as food additives, encapsulation of live cells, immobilization of enzymes, agents for wastewater treatment, anti-cholesterolemic agents, wound healing or dressing in biological settings, and excipients in pharmaceuticals. Additionally, fungal chitosan serves as an outstanding scaffolding material for the fabrication of biodegradable templates for tissue regeneration [9].

Why Fungal Chitosan?

Chitosan can be extracted from various sources. The most common source of chitosan remains crustacean seafood processing shells, like crab or prawn shells Another source is the exoskeletons of beetles. The fungal kingdom, which includes molds and macro-mushrooms, is another naturally occurring source that is becoming more and more popular. There are some disadvantages or limitations to using seafood waste these are [10]:

- Raw materials are available seasonally with limited availability.
- Alkalis and acids are required in large quantities which directly increase environmental pollution. The procedure is also time-consuming and demands a significant quantity of energy. It requires a temperature above 100°C alongside a substantial alkali concentration ranging from 30% to 50% (w/v).
- The difficulty in obtaining highly deacetylated chitosan limits its use in biological applications.

On the other hand, chitosan extracted from fungal sources has numerous advantages which are as follows:

- There are no seasonal variations and chitosan derived from fungal sources can be generated at any time of year.
- Fungal chitosan is free from heavy metals like copper and nickel, as well as from the allergic animal-sourced protein.
- Fungal chitosan production allows for the regulation of both the degree of deacetylation and the molecular weight, which is beneficial for highly specialized applications like drug delivery carriers and nanomedicine.
- A by-product of the fungal-based industry could be fungus-based chitosan.

Production of Fungal Chitosan

Fungi biomasses are a possible alternate source of chitosan, in addition to their use as a bio transformer of chitin from seafood waste into chitosan. Chitosan produced from fungus has attracted plenty of attention, mostly from Zygomycetes species, which are known to include chitosan as a natural component of their cell wall [11]. Fungus from the Ascomycetes, Basidiomycetes, and Deuteromycetes all have chitin as the primary structural component of their cell walls, however natural chitosan has not been found in these species. Treatment with concentrated sodium hydroxide can deacetylate chitin, dissolve proteins, remove soluble glucan, and hydrolyze lipids. As a result, this method can extract chitosan from fungi that are chitinous but lack native chitosan [12]. Figure 2 represents steps for extracting chitosan from fungal mycelia. Furthermore, they may be processed to produce chitosan with specified physicochemical qualities, unlike chitosan derived from crustaceans. Furthermore, advancements in fermentation technology provided us with an alternate approach to sustainably producing biopolymers [13].

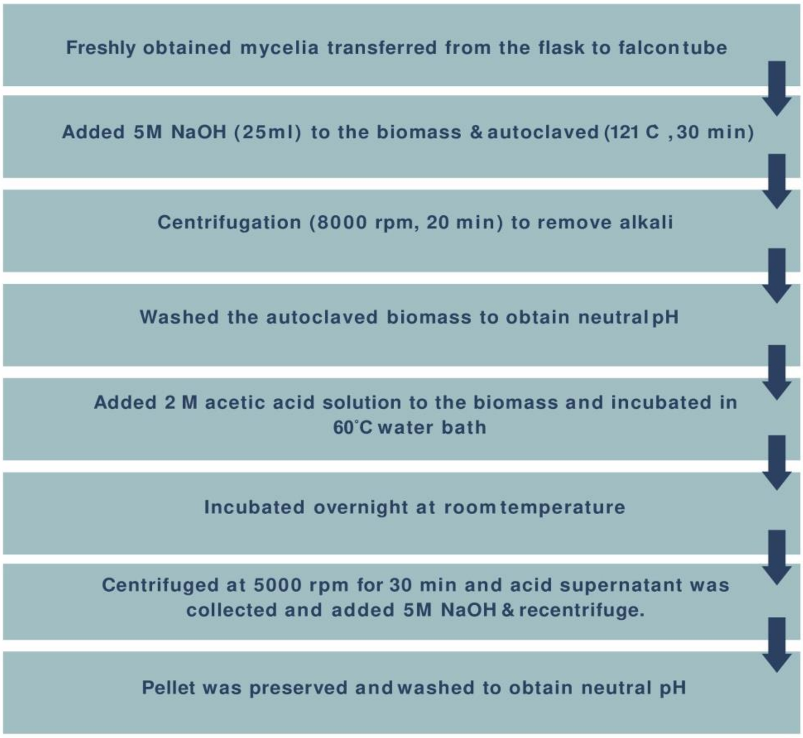

Figure 2. Flow chart for extracting chitosan from fungal mycelia [14].

Biological Properties of Fungal Chitosan

Chitin as well as chitosan has various unique properties which make them ideal for a variety of applications. These features include strong antibacterial activity, biodegradability, biocompatibility, nontoxicity, and high humidity absorption. Researchers also discovered anticancer, hypocholesterolemic, analgesic, hemostatic, antibacterial, and antioxidant properties.

Most of the biological characteristics of chitin and chitosan are associated with their physicochemical properties, encompassing factors such as molecular mass, degree of deacetylation, and moisture content [15]. For example, chitosan's functional groups and molecular weight determine its capacity to inhibit fungal and bacterial growth. Two explanations have been anticipated for chitosan's antibacterial activity. First, because smaller oligomeric chitosan may more easily cross the cellular membrane, it can impede cell development by preventing RNA transcription. This is because of its molecular weight [16].

The second emphasizes chitosan's polycationic nature, which alters cellular permeability and produces intracellular component leakage on contact with the cell membrane’s anionic components, ultimately leading to cell death. Additionally, by absorbing electronegative substrates like proteins, chitosan may impair the physiological functions of microbial cells, eventually resulting in cell death. The biodegradation kinetics of chitin and chitosan are dependent on the length of the polymer chain and the arrangement of acetyl groups. Research indicates that chitosan exhibiting a high degree of deacetylation (97.5%) possesses a higher positive charge than chitosan with a moderate degree of DD (83.7%), resulting in enhanced antibacterial activity [17].

The main thing that prevents chitin from being used more widely is its insoluble nature. Chitosan's free amino groups, which have dispersion-forming, polycationic, and chelating properties in addition to being soluble in diluted acetic acid, make it a promising candidate for bio polymerization. Chitosan's extraordinarily flexible biological, chemical, and physical qualities make it suitable for a varied range of uses, including medicinal, industrial, agricultural, and pharmaceutical [18].

APPLICATIONS OF CHITOSAN

Agricultural Applications of Chitosan

Chitin and chitosan are effective molecules that suppress the development of fungal and bacterial plant diseases, triggering defense responses in higher plants [19]. Chitosan has been shown to efficiently inhibit *Botrytis cinerea* polygalacturonases in bell pepper fruit pathogens, resulting in severe cytological damage to invading hyphae [20]. Likewise, applying chitosan or chitin to the cucumber plant's surface before *B. cinerea* inoculation increased the activity of chitosanase [21]. Additionally, a Brazilian patent held by Stamford and a colleague detailed the efficient use of fungus chitosan as a biofertilizer [22].

Applications of Chitosan in Biomedicine and Pharmaceuticals

Chitosan and Chitin biopolymers are regarded as beneficial, safe, and biocompatible substances for application in a variety of medical devices to cure, enhance, or substitute tissues, organs, or bodily functions. Furthermore, chitosan and its derivatives show promise as supportive materials in tissue engineering applications [23].

Potential uses for chitosan include cancer treatment, wound-healing management products and wound dressings, nerve regeneration, burn treatment, drug delivery systems (e.g., as a carrier for gene therapy or vaccine delivery), bio-artificial liver (BAL), articular cartilage, artificial skin, blood anticoagulation, artificial tendon, bone damage, and antimicrobial applications. In addition, chitosan possesses antiulcer, anti-diabetic, anticancer, and antioxidant properties as well. It is offered as nutritional supplements under a variety of trademarked names, often in conjunction with other chemicals to aid with weight reduction [24].

In Japan and Europe, it is available as a prescription medicine to prevent fat absorption [25]. Patients with osteoarthritis can prevent structural abnormalities in their joints by using glucosamine (GlcN), commonly referred to as chitosan hydrolysate. Chitin is seen as a possible therapy for umbilical hernias [26].

Nutraceuticals

Nanoencapsulation, a cutting-edge approach utilizing nanostructures, can significantly increase the durability and bioavailability of bioactive food components. Vitamins, Phenolic chemicals, essential oils, and carotenoids, have all been successfully encapsulated in a range of nanostructures composed of chitosan, such as nanocomposites, nanoparticles, nanofibers, and nano hydrogels [27]. While cholesterol objectives may be met, there is still a high risk of cardiovascular disease, and lipid-lowering drugs can have negative side effects. It has been established that therapy with chemicals that imitate physiological proteins, including creatine-based medications, has the potential for this specific goal. Though, lifestyle adjustments are critical for decreasing cholesterol and minimizing cardiovascular risk. Nutraceuticals have been the subject of some research because they may lower cardiovascular risk and enhance metabolic indicators. Chitosan, a dietary fiber, helps to manage lipid levels and enhance anthropometric measures. Large, prospective clinical studies are necessary to confirm these benefits. Such interventions could be recommended when lipid-lowering drugs are neither necessary nor well tolerated, as well as to achieve therapeutic objectives and/or minimize long-term cardiovascular risk [28].

Cosmetics and Cosmeceuticals

The efficacy of various hydrating agents, sunscreens, and bioactive compounds can be increased by using water-soluble or granular forms of chitosan and its byproducts, for instance, chitosan powder. Chitosan's biocompatibility allows it to be utilized on the skin and with other substances such as fats, acids, oils, and glucose. It is an extremely effective hydrating agent that may form films, supplying water while avoiding dehydration [29]. Chitosan has several applications and can considerably enhance products for personal care by replacing undesirable compounds and increasing the bioavailability of the remaining active ingredients. Chitosan is often used in cosmetic and skincare products for its ability to maintain skin moisture, tone it, cure acne, strengthen the extracellular matrix, and enhance the skin's protective ability. Chitosan proves to be a superb component in skincare products targeting anti-aging and wound healing. Its natural properties stimulate skin regeneration and wound healing processes, fostering proper tissue organization and optimal collagen structure [30]. The electrostatic interaction of chitosan with substrates bearing negative charges (such as skin or damaged hair) generates polymeric films that provide conditioning and moisturizing effects on cosmetic substrates. This renders it a perfect component for formulations in skin and hair care products [31].

Removal of Dyes

Dyes produced by industry sectors such as textiles, paint, paper, food, and cosmetics have a negative influence on the environment, thus eliminating them is critical. Chitosan-based treatments have recently been produced for removing colors from water. In their investigation, Ragab et al. used nano-hydroxyapatite/chitosan to remove Brilliant green dye. They discovered that the dye was most adsorbent at neutral pH 7, where it exhibited the highest percentage of adsorption (99.5%). Adsorption was dissolved in a highly acidic medium, resulting in very low adsorption. The amount of adsorbent used and the length of contact both enhanced dye adsorption. The findings fit well with the first-order kinetics and Dubinin-Radushkevich isotherm and, with rising temperature indicating endothermic dye adsorption [32].

Graphene oxide-chitosan and amine-graphene oxide-chitosan hydrogels were similarly demonstrated to be efficient in eliminating diclofenac at 90.42% and 97.06%, accordingly [33].

Chan et al. observed that a DNA-chitosan hydrogel can remove various pharmaceuticals from water, including Paracetamol, Phenazone, 3,30-Dihydroxydiphenylamine, Clofibric, Ibuprofen, Ketoprofen, Carbamazepine, and Thymol, and. It also effectively adsorbs dyes like Congo red and methylene blue, as well as metal ions like Hg^{2+}, Pb^{2+}, $Cu^{2+,}$ and Cd^{2+} [34]. Cross-lined magnetic chitosan/activated biochar successfully eliminated 95.2% of naproxen, 96.4% of diclofenac, and 98.8% of ibuprofen [35].

Factors Influencing fungal Chitin and Chitosan Production

Depending on nutritional and climatic conditions, as well as the intrinsic characteristics of the generating species, the amount and/or quality of chitosan and chitin in the fungal cell wall can change. Numerous researchers strive to optimize the yield of fungal chitosan and chitin while minimizing production costs to keep up economically with those derived from crustacean shells. Several parameters have been suggested that could affect the production of chitosan and chitin via submerged culture based on the process of their creation in fungal cells [36]. These parameters are significant not just for manufacturing but also for controlling physicochemical properties. All of the above factors subsequently alter the fungi metabolism via catabolic inhibition. Many studies attempted to augment the standard fermentation medium with additional nutrition sources to increase the productivity of fungal mycelium and biopolymers [37].

Nwe and Stevens (2004) showed that urea increases total chitosan synthesis by the fungus species *Gongronella butleri*. Urea was introduced as a nitrogen source, resulting in both an augmentation of chitosan quantity and a modification of its molecular weight [38]. Mishra and Kumar (2007) investigated the impact of various N-sources on *Pleurotus ostreatus*, including urea, yeast extract, ammonium sulphate, and dry cyanobacterial biomass of *Anacystisnidulans*, and found that cyanobacterial biomass resulted in the greatest biopolymer recovery because of its rich nutritional content [39].

Benjamin and Pandey (1997) used coconut oil cake (COC) as a substrate and investigated the effect of various minerals, carbon or nitrogen sources on *Candida rugosa*. The optimal parameters are combined in this study, together with N- and C-source optimization [40]. Solís-Pereira et al. (1993) investigated the impact of several carbon sources (sucrose, glucose, or galacturonic acid) on *A. niger* fungal biomass production using pectin. The inclusion of these extra carbon sources resulted in increasing biomass concentrations [41].

In addition to using extra N and C sources, researchers have looked into how *A. niger* biomass content is affected by conditioning solid substrates at different moisture levels and applying ethanol, phosphate, or acid treatments. Xie and West (2009) observed a significant rise in biomass concentration of the solid substrate on treatment with acid [42].

Plant growth hormones were shown to have a considerable influence on fungus mycelium growth in SmF. Chatterjee et al. (2008) investigated the impact of auxin, kinetin, and gibberellic acid on the chitosan synthesis of *R. oryzae* and *M. rouxii* and in molasses-salt and whey mediums, accordingly [43]. In *R. oryzae* and *M. rouxii*, modest quantities of these plant hormones increased chitosan content as well as mycelium development. However, increasing amounts of plant hormones reduced development in both areas. Furthermore, the molecular weight of isolated chitosan was enhanced [44]. According to Akila (2014), harvesting the fungus during its late exponential development stage will optimize the production of chitin and chitosan [45].

CONCLUSION

Both chitin and chitosan are naturally occurring biopolymers found in crab shells, insect and mollusk exoskeletons, and fungal cell walls. They are generated industrially from crab shells and have several applications in a variety of sectors, including agriculture and medicine. However, because of the variable structure of chitosan and chitin from crustaceans, fungal sources constitute a viable alternative, particularly for biomedical and pharmacological applications. Chitin and chitosan extracted from fungi help to reduce a considerable amount of biological waste on the planet. The amount and quality of chitosan and chitin present in fungal cell walls can differ depending on nutritional and environmental conditions. Statistical approaches are preferable for optimizing these settings since the data provided from this approach is considerably more trustworthy, and intelligible, and can be utilized for prediction and simulation, as well as for evaluating the relationships between different factors.

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Declarations

Conflict of Interest

The authors declare that they have no conflict of interest in the publication.

Competing Interests

The authors have no financial and non-financial competing interests to declare that are relevant to the content of this article.

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