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(REVIEW ARTICLE)

Chitin – A structural polysaccharide for health and environmental alimentation

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Abstract

Ever increasing physiological and environmental abnormalities warrant natural, safe, easy to extract, less expensive and eco-friendly bio-components of versatile usage. Chitin, the second most abundant polysaccharide in the world, seems promising in fighting diseases, boosting immunity up and maintaining environment fresh. Chitin is a polysaccharide of N-acetyl-D-glucosamine forms the exoskeletons of arthropods, mollusks, and insects, cell walls of fungi and scales of fishes. Based on extraction, purification and integration enhancement, chitin could be of greater importance for the maintenance of human health, agriculture and environment. Present article compares and contrasts the methods utilized for extraction and purification of chitin from different sources and review its current fields and suggest the future aspects.

Keywords: Chitin; Chitin derivatives; Chitosan; Bio-medical applications of chitin; Environmental usage of chitin; Future aspects of chitin

1. Introduction

Chitin is a large, structural linear polysaccharide made from chains of *N*-acetyl-D-glucosamine [2-(acetylamino)-2-deoxy-D-glucose)] [Fig. 1]. It was first identified in 1811 [1]. After cellulose, chitin is the most abundant aminopolysaccharide in nature [2]. It is the building material that provides strength to the exoskeletons of crustaceans, insects, and the cell walls of fungi [2]. Chitin occurs in nature as ordered macro fibrils that can be converted to its most well-known derivative, chitosan. Chitosan is produced commercially by enzymatic or chemical deacetylation of chitin [3]. Chitin is not soluble in water, but chitosan is [3]. Based on sources, chitin has two allomorphs: α and β [3]. These can be differentiated by infrared and solid-state NMR spectroscopy, together with X-ray diffraction. Chitin synthase is the key enzyme in the biosynthesis of chitin [3]. However, chitin has some excellent chemical and biological properties that open the chances to use it efficiently in different fields. The biological properties of chitin includes biocompatibility, biodegradable to normal body constituents, safe and non-toxic, haemostatic, fungistatic, antitumor and antimicrobial, spermicidal and antihypercholesterolemic [4]. This excellent biological property widens its uses in food industry, textile industry, agriculture, bioengineering, biotechnology, biomedical and pharmaceutical fields, waste water treatment, pollutant removal, cosmetics and toiletries [4]. Besides, some new aspects of chitin are also in consideration. This article reviews the already explored and probable future aspects of chitin in health and environmental alimentation.

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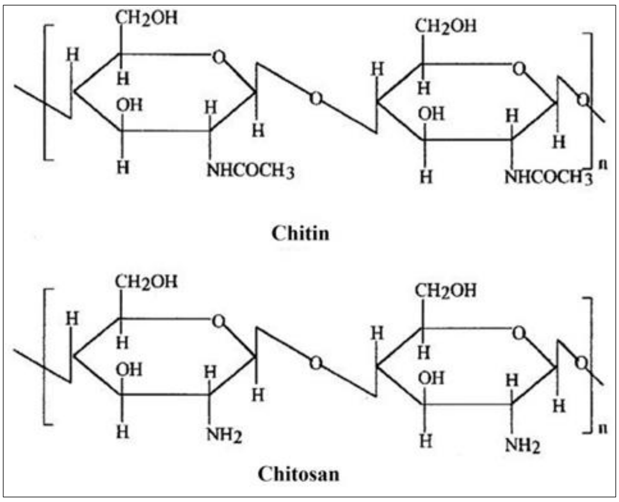

Figure 1 Structure of Chitin and Chitosan

2. Sources of chitin

Chitin is found in both animal and plant kingdom [4-5]. Chitin is a crucial structural component of various marine organisms including the crustaceans, coelenterate, annelids, cnidarians and mollusks [4-5]. Although the main commercial sources of chitin are crabs and shrimps, insects serve as an alternative source of chitin, which forms up to 40% of their exoskeleton [4-5]. Insects containing chitin are ant, scorpion, brachiopod, cockroach, spider and beetle. [4-5]. Some microorganisms are also a potential source of chitin that includes fungal cell wall and mycelia, brown algae, green algae and yeast [4-5]. If it is utilized properly from its sources, it can be big boost for new applications in various new fields.

3. Extraction of chitin

Extraction of chitin begins with the selection of sources, especially the shells of crabs and shrimps, and ends with the cleaning; drying and grounding into tiny shell pieces [3]. This selection has major significance on the subsequent quality of the final isolated material [3]. More importantly, shells of the same size and species are needed to be chosen in case of lobsters and crabs, but the thinness of the shell wall often makes the isolation process very much simple which can be seen in case of shrimps [6]. In case of industrial and laboratory processing, chitin is extracted by acid treatment to dissolve the calcium carbonate followed by an alkaline solution to dissolve proteins [7]. Residual pigments can be removed by an extra step known as decolorization step [7]. The aim of chitin extraction is to eliminate proteins, minerals, lipids and pigments until only chitin is obtained [8]. A large number of methods have been developed and used for the preparation of pure chitin. Generally, two types of methods are utilized for the isolation of chitin: chemical and biological [5, 8]. Sometimes, the combination of both methods makes the isolation much efficient [5, 8].

3.1. Chemical extraction

Through different chemical bonding, chitin forms “chitin-protein matrix” that becomes further calcified (mostly through deposition of calcium carbonate and calcium phosphate) in the skeletal muscles of the sources [3, 6-8]. Chemical extraction process involves removal of proteins (deproteination, dp) and minerals (demineralization, dm) [3, 6-8]. Sometimes, removal of residual lipids (delipidation, dl) and carotenoids becomes inevitable for isolation of pure chitin.

3.1.1. Chemical deproteinization

Main concern in chemical deproteinization is the disruption of chemical bonding between chitin and protein through alkali (especially NaOH, at 95 °C) treatment.

3.1.2. Chemical demineralization

Acidic treatment at high temperature is performed for chemical demineralization of chitin. Hydrochloric acid (HCl) is the mostly used demineralizing agent for commercial production of chitin.

3.1.3. Chemical decomposition

Demineralization follows the decomposition of the calcium carbonate into the water soluble calcium salts with the release of carbon dioxide.

$2\ HCl + CaCO3 \rightarrow CaCl2 + H2O + CO2 \uparrow$

Finally, the soluble salt is separated through filtration followed by washing with deionized water. However, the colorless product can be achieved through decolorization and bleaching with “chloroform: methanol: water (1:2:4)” at 25 °C.

Purity of chitin, assessed through degree of acetylation, molecular mass and polydispersity, is an important concern of chitin extraction. Harsh acidic and alkaline treatments as well as increased temperature range badly affect the quality of chitin structure and function. In addition, excess usage of mineral acids and alkali cause environmental nuisance and the process is time and energy consuming.

3.2. Biological Extraction

Hazards associated with the chemical extraction process can be overcome through biological extraction process [9]. Biological extraction process utilizes the combination of chemical process along with biological methods such as usage of microorganisms to the system. Enzymatic deproteinization and fermentation (lactic or non-lactic) are the two most common biological methods.

3.2.1. Enzymatic deproteinization of chitin

Proteolytic enzymes (proteases), obtained from plant, microbial and animal sources are used for enzymatic deproteinization of chitin [10]. The mostly used proteases for chitin extraction are chymotrypsin, papain, trypsin, alkalase, devolvase, pepsin, and pancreatin [10]. Depending on the source of chitin and the enzyme utilized, deproteinization yield varies. Some of these enzymes also aid in deacetylation process [10]. One drawback of this method is that the enzymes are expensive.

3.2.2. Bacterial fermentation of chitin

This is relatively an inexpensive method where the cost of using enzymes is decreased. This method is mainly performed when chitin is extracted from sea food wastes [11-12]. The fermentation is attained by adding selected strains of microorganisms or endogenous microorganisms [11-12]. It can be with or without lactic acid bacteria capable of following one-stage and two-stage fermentation, co-fermentation/subsequent fermentation, or from endogenous microorganisms (auto-fermentation) [11-12].

Lactic acid bacterial fermentation

Lactic acid bacteria have the ability to ferment waste materials and simultaneously produce organic acids in situ (lactic and acetic acids) [13-14]. Lactic acid induces precipitation of chitin and production of calcium lactate after reaction with calcium carbonate [13-14]. This action of lactic acids separates the two fractions obtained during fermentation: the liquid fraction (containing high amount of proteins, minerals and pigments) and the solid phase (containing crude chitin) [13-14]. Crude chitin can be separated by filtration and washed with water. Lactic acid is obtained by its conversion into glucose (the lowered pH activates the proteases and suppresses the growth of spoilage microorganisms). However, the efficiency of lactic acid fermentation relies on many factors such as the species and quantity of inoculums, carbon source and its concentration, initial pH and pH evolution during fermentation, temperature and the duration of fermentation. The frequently used bacterial strains for fermentation are *Lactobacillus sp.* strain especially *L. plantarum, L. paracasei and L. helveticus* [11-14].

Non-lactic acid bacterial fermentation

Mostly used non-lactic acid bacteria for chitin extraction *are Bacillus sp., Aspergillus sp. and Pseudomonas sp., Bacillus sp.* and *Aspergillus sp* [5].

4. Chitin applications

Special characteristics of chitin including biodegradability, biocompatibility and non-toxicity has enabled chitin a wide range of applications in food industry, biomedicine, environmental management, textile industry and cosmetics etc. Some of the application areas have been described below.

4.1. Food industry

Antioxidant and antimicrobial activity of chitin allows it to be used in the food industry to improve nutritional quality of food, maintain food safety and to extend the shelf life of food [15-20]. Chitosan is used as a food additive to enhance food taste and flavor [15]. It is also a carrier film for the antimicrobial agents [16-20]. Chitosan has applications as an emulsifier, flocculant and dietary supplement [21]. It is beneficial in making stable emulsions without any other surfactant [21]. Chitin can be used as a weight loss agent as it is a good source of dietary fiber [22]. Cationic nature of chitosan enables it to bind to cholesterol and to reduce intestinal cholesterol absorption and act as cholesterol lowering agent [22-23]. In the same fashion, it lowers the level of saturated fatty acids that lowers the "saturated to unsaturated fatty acid ratio" and ameliorates the atherogenic lipid profile and hypertension [23-24]. As a prebiotic, chitosan aids in maintaining gut flora, viscosity and fermentative milieu [25-26]. Chitosan has a higher sedimentation rate, and thus, is a good flocculent in the purification system of drinking water and beverage production plants [27]. It is also used in cleansing steps of preserving fruit juices, fruits, grape wines and beer [28-29].

4.2. Agriculture

Chitin acts as soil modifier by enhancing soil quality, plant growth, and plant resilience [30].

Content of nitrogen in chitin and chitosan make them a rich bio-fertilizer [31]. As a bio-pesticide, chitin kills root-feeding nematodes and their eggs, as well as pathogenic fungi in the root zone [31-33]. As an elicitor of signal transduction, chitin takes part in the plant defense mechanism [34]. During fungal infection, chitin present in the fungal cell walls are degraded by plant cells' chitinase into chitin fragments (chito-oligosaccharides) [34-35]. Chito-oligosaccharides participate in further protective action of plants against invaders [36]. Chitin and chitosan has important roles in seed and leaf coating [37]. They increase the seed germination rates as chitosan coating changes the permeability of seed plasma membrane and increase the enzymatic activities [37].

4.3. Environmental hazard mitigation plants

As chitosan is a good adsorbent of heavy and radioactive material, its usage in waste water treatment plant, for detoxifying water bodies and removal of different dyes seem promising [38-41]. It is an efficient removal agent for metal ions such as copper, chromium, cadmium, lead, nickel, iron and arsenic [42-43].

4.4. Biomedical and Pharmaceutical Industries

Chitin and chitosan have been incorporated in numerous biomedical and pharmaceutical applications [44-45]. It acts as a homeostatic agent. It accelerates wound healing and dermal regeneration. As a hypocholesterolemic agent, chitosan has received seminal attention [23, 45]. Similar aspect had been noted for its anti-tumor effects [46]. Improved anti-cancer effects of some anti-cancer agents have been observed when chitin or chitosan derivatives had been used as conjugates or as adjuvants [46-48]. These combined therapies showed better anticancer effects with reduced side effects and gradual release of free drug in the cancer tissues [46-48]. As drug delivery agent, chitin and chitosan have received widespred applicability in the pharmaceutical industries [49-50]. Their muco-adhesive properties enable their carrier capacity as film, gel and powder. They are also of choice in nano-technological application [44]. High stability, low toxicity and simple preparation method have made the chitosan nanoparticles efficient drug carriers [51].

4.5. Biotechnological sectors

In biotechnological plants, usage of chitin and chitosan in tissue engineering is widely performed nowadays [52]. Deaminated chitin affinity chromatography is used for the isolation, purification and concentration of lysozyme from food or biological tissues [53-54]. Productions of chitin based biosensors seem promising [55]. Chitin films and nanoparticles are ideal supports in enzyme immobilization [56]. Operational stability of chitosan-immobilized enzymes is high [57]. Bacteria-immobilized chitin flakes have been used for bioremediation of crude oil polluted sea water [58]. Chitin-based hydrogel is another biotechnological product of public importance [59].

5. Future aspects of chitin and chitosan

5.1. Chitin for neurodegenerative disease amelioration

Neurodegenerative diseases (NDs) like Alzheimer's disease (AD), Parkinson's disease (PD), and Huntington's disease (HD) have been causing immense sufferings to the humanity. No treatment is available to fully cure these diseases. Thus, search for their natural therapeutic agents have got momentum. Chitosan oligosaccharides (COs) have been found to be protective against amyloid beta (Aβ) 1-42 induced memory and learning deficits [60]. Aβ fibriliogenesis is an important reason in AD pathogenesis. COs have been implicated in the anti-fibriliogenesis and fibriliogenesis destabilizing effect [61-62]. Neuroprotective role of COs is accelerated through anti-oxidative mode [63]. Also, their role against the release of pro-inflammatory cytokines would aid in AD amelioration [64]. Besides, acetylcholine esterase inhibitory and anti-apoptic effects of chitin derivatives have been reported to be protective against AD [65]. Additionally, chitosan based nanoparticles seemed promising as AD drug delivery agents [66]. Dysregulated apoptosis, mitochondrial membrane potential disruption, increased lactate dehydrogenase activity and reactive oxygen species generation have been implicated in PD pathogenesis [67]. Antioxidative role of chitosan and CO enable their therapeutic usage against PD [64, 68]. Free-radical scavenging mode of action potentiates their usage in PD therapeutics [69].

5.2. Probable role of chitin in coronavirus diseases

Chitosan derivatives of low molecular weight (2-17 KDa), obtained through chemical hydrolysis, had been reported having anti-viral effects against tobacco mosaic virus (TMV), tomato yellow leaf curl virus, coliphages T2, T4 and T7 and HIV-1 [70-73]. Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), the causative agent of the coronavirus disease 2019 (COVID-19) binds through the receptor binding domain (RBD) of its spike protein with the angiotensin converting enzyme 2 (ACE2) receptor of the host cell [74]. Search for agents inhibiting this binding and entry into host cell have received momentum. Molecular dynamics study revealed strong binding affinity of chitosan with the RBD of SARS-CoV-2 [75]. More importantly, β-chitosan had been found effective in inhibiting the binding of SARS-CoV-2 with the ACE2 receptor. Even down regulated production of ACE2 receptor in vero E6 cells had been linked with chitosan [76]. Chitosan nanoparticles have been found effective anti-viral drug delivery agents [77]. Chitosan naoparticles and Nano fibers seem to be of utmost usage in delivering COVID-19 drugs [78]. Besides, innate immunity boosting effects of chitin micro particles substantiate their usage against SARS-CoV-2 [79]. Recently, possibility of chitosan-coated DNA vaccine against SARS-Co-V-2 has been conceptualized [80].

6. Conclusion

Chitin is a natural polysaccharide of immense importance. Food, bio-medicine, nanotechnology and environmental aspects of this natural bio-polymer beacons its extensive utilization for the betterment of the human being. Application of chitin in withstanding the progression of the pandemic COVID-19 would be of its most-immediate utilization. Thus, less expensive, eco-friendly and quick extraction process of chitin along with much extensive research is called for.

Compliance with ethical standards

Disclosure of conflict of interest

Authors declare no conflict of interest.

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