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CHITOSAN: AN IN-DEPTH ANALYSIS OF ITS EXTRACTION, APPLICATIONS, CONSTRAINTS, AND FUTURE PROSPECTS

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Review

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ABSTRACT

The primary focus of this review is an eco-friendly biopolymer chitosan which is mainly produced by the conversion of environmental wastes into useful applications. Chitosan has drawn much interest due to its unusual characteristics, including anti-microbial, biodegradability, and non-toxicity. This study explores numerous approaches available for the production and extraction of chitosan from various sources, including crustaceans, fungi, and insects. The available techniques are classified into chemical and biological methods and further divided into enzymatic and fermentation methods. This review also covers the steps in the upstream and downstream processes that are involved in the production of chitosan. Additionally, a few techniques available for the characterization of chitosan are covered, including Fourier transform infrared spectroscopy, potentiometric titration, differential scanning calorimetry, thermogravimetric analysis, X-ray powder diffraction, and emission scanning electron microscopy. Comparisons are also made between the characteristics of chitosan derived from sources like insects and fungal strains. Characteristics of chitosan obtained from sources such as fungal strains and insects are compared with those of commercial chitosan. This study also focuses on the most recent applications of chitosan in the medical sector, wastewater treatment plant, agricultural sector, food packaging industry, and cosmetic industry. A few patents related to chitosan in the health sector are also discussed. Finally, it concludes that further research on chitosan and its derivatives is necessary to fully understand the advantages of this polymer. It also emphasizes the difficulties involved in extracting chitosan from crustaceans, insects, and fungi. To fully explore the advantages of this polymer, it is concluded that further research into chitosan and its derivatives is necessary.

Keywords: Applications, Biodegradation, Biopolymer, Bioremediation, Characterization, Chitosan, Patents

INTRODUCTION

Chitosan is an environmentally beneficial biopolymer that has received a lot of interest since it transforms wastes from the environment, including crab and shrimp shells, into useful applications **(Omar *et al.*, 2021; Said Al Hoqani *et al.*, 2020)**. Chitosan was first discovered in 1859 by Professor C. Rouget. It is a natural polycationic linear amino polysaccharide, that is composed of β-(1-4)-linked glucosamine and N-acetylglucosamine and is biocompatible and biodegradable (figure 1) **(Wang *et al.*, 2020)**. Chitin is a nitrogen-containing polysaccharide, found abundantly in insect cuticles, mollusks, crustaceans, and fungi **(Omar *et al.*, 2021; Islam *et al.*, 2017)**. Chitosan is produced by the partial deacetylation of chitin **(Islam *et al.*, 2017)**. Conventionally, chitin was produced by demineralization, deproteinization, and deacetylation **(Kumar, 2000)**. **Tolaimate *et al.* (2003)** proposed a new approach using consecutive baths of lower HCl (0.55M) and NaOH (0.3 M) concentrations. Currently, enzymatic, acid-based, oxidative degradation, and mechanical processing methods are used **(Yin *et al.*, 2021)**.

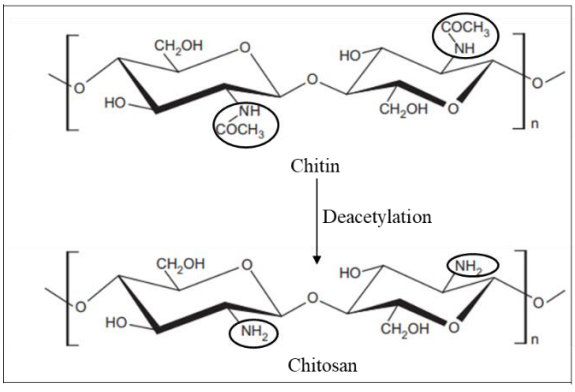

Figure 1 Structure of chitin and its deacetylated derivative chitosan **(Shirvan *et al.*, 2018; Kaur and Dhillon, 2014)**

The chitin and chitosan resemble cellulose in their chemical structure, which consists of 100's -1000's D-glucose units which are β (1→4) linked **(Islam *et al.*, 2017)**. They occupy the top position exhibiting a wide variety of applications as they possess properties that include biocompatibility, biodegradability, non-toxicity, antifungal, antibacterial, and antiviral properties **(Wang *et al.*, 2020; Islam *et al.*, 2017)**. The physical, mechanical, and biological properties of chitosan depend on its molecular weight and degree of deacetylation. Chitosan has low crystallinity compared to chitin. The crystallinity of the polymer is directly proportional to the deacetylation degree (when >50 %). The characteristics of chitosan can be enhanced increased degree of deacetylation and higher molecular weights **(Jiang *et al.*, 2014)**.

The main limitation is the insolubility of chitosan in most organic solvents as well as in water which limits its application. Its solubility is determined by pH and is soluble only in an acidic medium of pH = 6.5, and pK_a = 6.0 **(Vikas *et al.*, 2021; Rinaudo *et al.*, 1999)**. Hence the reactive functional groups that are present in it are subjected to chemical reactions. The quaternization, carboxylation, acylation, and alkylation of chitosan helps to improve sensitivity to pH and water solubility **(Wang *et al.*, 2020)**.

The addition of specific agents such as phenolic compounds, essential oils, and fruit extracts improves the functional properties of chitosan-based materials and in turn applications **(Florez *et al.*, 2022)**. Sometimes molecules such as cyclodextrin, crown ethers, dioxime, and thiosemicarbazones may be linked to Chitosan. These functionalized biopolymers have a significant role in gene delivery, drug delivery, antimicrobial activity against pathogens, and antifungal activity. By chelating the amine group in chitosan, Such Chitosan has enhanced water retention and metal uptake capacity through the chelation of the amine group in chitosan **(Negm *et al.*, 2020; Guibal, 2004)**.

Chitosan nanoparticles are mostly prepared by ionic cross-linking, making use of anionic cross-linkers. Chitosan can also be modified into anionic amphiphilic chitosan with different hydrophobic tails which exhibits the controlled size, aggregation, and stability of synthesized nanoparticles **(Badr *et al.*, 2020)**. Chitosan can interact with mucous membranes and can also exhibit bio-adhesion properties due to its cationic nature **(Vikas *et al.*, 2021)**.

Chitosan can also be extracted from renewable and sustainable sources of chitosan such as eggshells and silkworm pupae. Silkworm chitosan has slower thermal degradation than commercially available chitosan, and higher antimicrobial activity compared to amoxicillin. The eggshell and silkworm pupae chitosan have similar or better antibacterial and antifungal activity. These properties qualify them for medical and food applications (**Battampara *et al.*, 2020**). Recently, chitosan has also been isolated from *Procambarus clarkii*, which is a novel source. *P. clarkii* chitosan showed high purity, high solubility, and average molecular weight when compared to shrimp chitosan. The color, viscosity, and antioxidant activity *P. clarkii* chitosan make it suitable for food applications (**Omar *et al.*, 2021**).

Chitosan has a wide variety of applications. The mechanical and water sorption properties of certain substances used for agricultural and packaging applications can be enhanced by using chitosan as a filler as in thermoplastic starch. The elongation break, tensile strength, and Young's modulus of the composite are the function of chitosan content. The addition of chitosan results in a reinforcing effect which increases Young's modulus and the tensile strength of the substance. It also reduces the maximum uptake of water, shrinkage, and growth of mold on the surface (**Balla *et al.*, 2021**). The film-forming, antimicrobial, antifungal, and non-toxicity properties of chitosan make it useful in the packaging of food (**Florez *et al.*, 2022**). Various inert materials can be used to immobilize enzymes to reuse them and maintain their stability. One such material is chitosan. This is mainly because of the abundance of their functional groups, availability, and ability to withstand chemical degradation. Chitosan beads activated by glutaraldehyde, in which the peroxidase enzyme is immobilized, find their application in textile dye decolorization (**Neto *et al.*, 2021**).

The main aim of this study is to present an outline of the extraction, production, purification, and characterization of chitosan and its recent use in various fields. This review also emphasizes patents related to chitosan, the challenges in the upstream and downstream processing of chitosan, and their future scopes.

METHODS OF CHITOSAN PRODUCTION

Chitosan can either be extracted directly from fungal sources or chitin can be first extracted and then deacetylated to chitosan from crustacean or insect sources (**Korma, 2016**). The production and extraction of chitosan from various sources can be classified into chemical and biological methods.

Chemical method

This method includes three stages. Firstly, the demineralization step where HCl (0.5-2M) reacts with calcium carbonate ($CaCO_3$) to form carbon dioxide for a few hours under agitation to get rid of $CaCO_3$ in the crustacean shell. Secondly, the deproteinization step where heated NaOH (0.5-3 M) reacts for several hours with protein and organic components other than chitin, and finally the deacetylation step where the insoluble fraction (chitin) is converted into chitosan by treating with heated NaOH (3M) for several hours. The deproteinization step always follows the demineralization step as this can speed up the deproteinization by increasing the surface area in the shell (**Kou *et al.*, 2021; Younes and Rinaudo, 2015**).

Biological methods

The biological method can be further divided into enzymatic and fermentation methods.

Enzymatic method

The enzymatic method is similar to the chemical method except that the high reaction temperature and alkali solution are replaced by enzymes and moderate temperature (25-59ºC) for deproteinization and deacetylation steps. Enzymes such as proteinases and deacetylases are used for deproteinization and deacetylation respectively (**Kou *et al.*, 2021**). These enzymes are extracted from microbes or fishes. The drawback of this method is that the large-scale production of chitosan becomes expensive. To overcome the problem of the usage of expensive enzymes, fermentation methods were used as the microbes multiply at a faster rate and continuously secrete enzymes.

Fermentation methods

Fermentation methods can be further divided into i) lactic acid fermentation and ii) non-lactic acid fermentation. The lactic acid culture is first inoculated into ground shell waste, in lactic acid fermentation. This results in lactic acid production which decreases pH and dissolves $CaCO_3$. Proteases produce proteolyzed proteins. Deproteinization and demineralization require fungi and bacteria in non-lactic acid fermentation such as *Mucor* species, *Streptococcus faecium*, *Pediococcus acidilactic*, etc. Enzymes that fungi release are used in these processes. The extra protein and minerals present at the end of this process are removed by treating with mild chemicals which yields pure chitin (**Kou *et al.*, 2021; Younes and Rinaudo, 2015**). Finally, the chitin produced is converted into chitosan by deacetylation.

PRODUCTION AND EXTRACTION OF CHITOSAN FROM VARIOUS SOURCES

Fungal source

Chitosan is generally produced from crustacean waste by chemical deacetylation which is environment-friendly to a certain extent, beyond which they result in environmental pollution due to the release of a toxic substance (**Sebastian *et al.*, 2020**), and the shellfish waste may not be available throughout the year. Chitin is also present in the cell walls of certain groups of fungi especially Zygomycetes (**Korma, 2016**). In *A. niger* a low degree of deacetylation of chitin has been observed due to the strong association of chitin with (1-3)-β-D glucans through a carbonyl linkage (**Heux *et al.*, 2000**). The detailed production and extraction process of fungal chitosan is depicted in figure 2. The fungal chitosan is consistent in its quality, and physical and chemical properties when compared to commercially available chitosan (**Aili *et al.*, 2019**). Also, the duration of the extraction process is shorter in the case of fungal chitosan. Foods and drugs that contain chitosan produced from crustacean shells are not generally consumed by vegetarians and vegans since it is an animal source (**Ban *et al.*, 2018**). Hence chitosan production by a chemical method is being switched over to green synthesis.

Unlike chemical methods, the production of fungal chitosan does not require deproteinization, or demineralization steps instead the chitin present in the cell walls of fungi is converted to chitosan by an enzyme chitin deacetylase (**Sebastian *et al.*, 2020; Namboodiri and Pakshirajan, 2019**). The production and extraction of fungal chitosan are represented schematically in Figure 2. Fungal chitosan can be produced by solid-state (**Vendruscolo and Ninow, 2014**) or submerged fermentation (**Aili *et al.*, 2019; Lin *et al.*, 2019**). Cheap substrates such as dairy wastewater, paper mill wastewater (**Namboodiri and Pakshirajan, 2019**), and apple pomace (**Vendruscolo and Ninow, 2014**) were used for the chitosan production from *Penicillium citrinum*, *Cunninghamella elegans*, and *Gongronella butleri* CCT 4274 respectively.

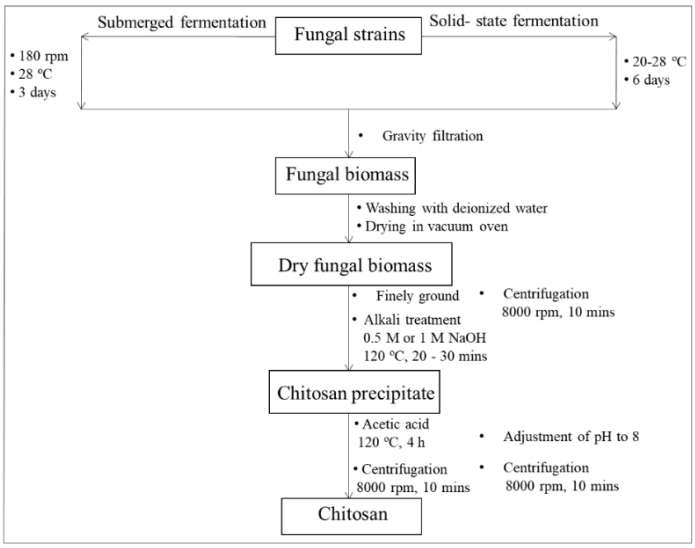

Figure 2 Production of fungal chitosan under Submerged and Solid-state fermentation followed by its extraction

Crustacean source

Crustaceans account for a major component of the biomass discarded by the fisheries. The by-product of seafood consumption contributes to 40-50% of the total waste which causes environmental pollution. The presence of polysaccharide (chitin) in the exoskeleton makes it useful for the production of chitosan (**Bernabe *et al.*, 2020**). 14-27 % of the dry shell weight accounts for chitin, of which 60-80 % can be converted into chitosan (**Balla *et al.*, 2021**). These crustacean shells contain lipids, carbonates, proteins, and pigments which have to be removed by demineralization using HCl (0.5-2 M), deproteinization using NaOH (0.5-3 M), and decoloration using H_2O_2 (30%) respectively to produce chitin which is then deacetylated using NaOH (3 M) to form chitosan (**Said Al Hoqani *et al.*, 2020; Bernabe *et al.*, 2020**). The production and extraction of crustacean chitosan by biological and chemical methods are represented schematically in figure 3. Biological and Chemical methods are mainly used for the synthesis and extraction of chitosan. Chemical methods exhibit a negative influence on the physical and chemical characteristics of chitosan. Higher ash content in chitosan and degradation of a small amount of chitin is mainly due to the use of HCl and NaOH for chitin hydrolysis. Biological extraction methods have several advantages such

as less energy consumption, less degradation of chitin structure, and being eco-friendly. However, they are expensive and less efficient than the chemical method of extraction (**Kou *et al.*, 2021**).

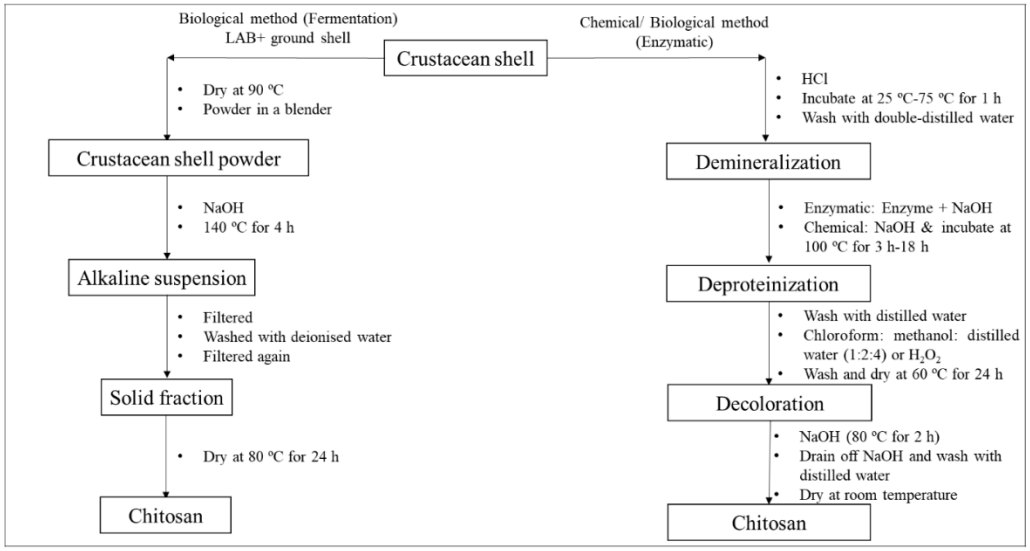

Figure 3 Production and extraction of crustacean chitosan by Biological (Fermentation and Enzymatic) and Chemical method

Insect source

Insect farming is rapidly increasing for the sustainable production of animal feed as some insects such as *Hermetia illucens* larvae can digest agricultural wastes, making the animal feed highly nutritional. Hence the insect biomass which is rich in chitin such as exuviae and cuticle is increasingly produced (**Hahn *et al.*, 2020**). Similar to the extraction of chitosan from crustaceans, insect chitosan also requires demineralization, deproteinization, and deacetylation steps (**Hahn *et al.*, 2020; Marei *et al.*, 2019**). The chitosan production from insects does not always require a demineralization step because of their low mineral content which makes them better than crustacean sources (**Jantzen da Silva Lucas *et al.*, 2021**). Generally, the chitosan from insects results in a lesser yield when compared to the yield of chitosan from crustaceans, hence the deacetylation process needs to be optimized (**Hahn *et al.*, 2020**). The production and extraction processes of chitosan from insects are represented schematically in figure 4.

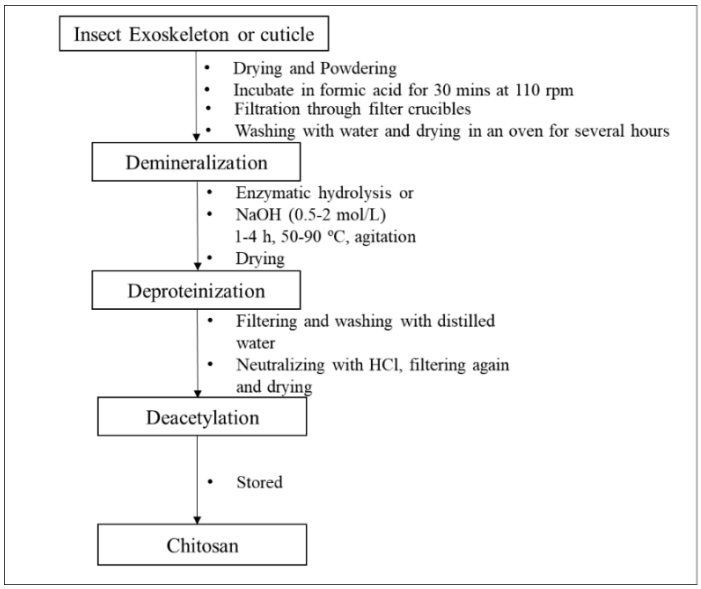

Figure 4 Production and extraction of chitosan from insects by Biological (Enzymatic) and Chemical methods

Tab 1 indicates the type of extraction method used to produce chitosan by different sources such as crustaceans, insects, and fungi, and also gives the comparison between the chitosan yield produced by these sources. It can also be observed that the chemical method of extraction produces more chitosan yield when compared to the biological method. Tab 2 gives a comprehensive comparison of the marine and non-marine sources to produce chitosan.

Table 1 Comparison of the yield of chitosan produced by crustaceans, insects, and fungal strain

Source	Extraction method	Yield	References
Zygomycetes	Enzymatic	>95 %	**Islam *et al.*, 2023**
G. butleri	Fermentation	70 %	**Huq *et al.*, 2022**
Omani shrimp shell	Chemical	53.313 %	**Said Al Hoqani *et al.*, 2020**
Penicillium citrinum	Chemical	13.8 %	**Namboodiri and Pakshirajan, 2019**
Gongronella butleri	Chemical	138.7 ± 0.4 mg/g of dry biomass	**Vendruscolo and Ninow, 2014**
Paecilomyces saturatus	Chemical	68.4 mg/g of dry biomass	**Lin *et al.*, 2019**
Hermetia illucens	Chemical	43 %	**Hahn *et al.*, 2020**
Tenebrio molitor	Enzymatic	31.9 %	**Jantzen da Silva Lucas *et al.*,2021**
Parapeneopsis stylifera	Chemical	4.41 ± 1.22 %	**Renuka *et al.*, 2019**
Scylla serrata	Chemical	44.57 ± 3.44 %	**Sarbon *et al.*, 2015**
Cunninghamella elegans	Chemical	57.82 mg/g of dry biomass	**Berger *et al.*, 2013**
Chyrysomya megacephala	Chemical	26.2 %	**Song *et al.*, 2013**

Table 2 A comprehensive comparison of the marine and non-marine sources to produce chitosan (**Sebastian *et al.*, 2020; Huq *et al.*, 2022**)

Sources	Advantages	Disadvantages
Non-marine sources (Bacteria, Fungi and Insects)	1. Consistent in its quality physical and chemical properties 2. Shorter duration of extraction 3. Suitable for vegetarians and vegans 4. Does not require demineralization and deproteinization steps	1. Fungal raw materials are neither available nor as plentiful as those from marine or animal sources 2. Production cost is higher 3. Low yield
Marine sources (Crustacean)	1. High yield 2. They are commercially available	1. Not available throughout the year 2. Environmental pollution caused by the use of concentrated acids and alkali

PURIFICATION OF CHITOSAN

The chitin deacetylation to form chitosan does not exclude the contaminants present in the starting material. Hence the purification of chitosan is very important if it is to be used in medical applications. However, its purification is not very easy as it is a viscous substance. Chitosan may contain materials such as gums, proteinaceous substances, salts, etc. as contaminants that need to be purified (**Dutta, 2015**). Figure 5 gives the schematic representation of various steps involved in the purification of obtained chitosan. Purification with the use of method I as shown in Figure 5 resulted in the increased nitrogen content of chitosan from 7.65 % to 8.30 % which can be further used as anticoagulants (**Doczi *et al.*, 1957**). With method II, the initial protein content of 50 % was reduced to 0.05 % wt. of chitosan after complete purification (**Nasti *et al.*, 2009**) (Figure 5). Whereas, with method III shown in Figure 5, the overall yield of chitosan was 67 % (**Paul *et al.*, 2014**).

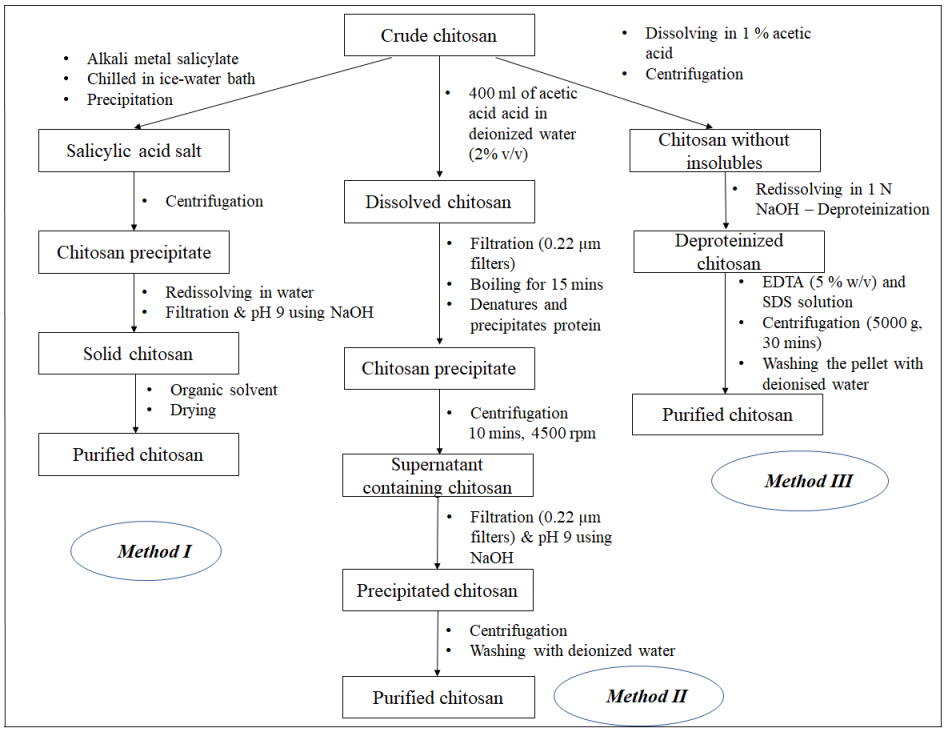

Figure 5 Different purification methods of chitosan by using alkali metal salicylate, acetic acid in deionized water, and 1% acetic acid

CHARACTERIZATION OF CHITOSAN

Fourier transform infrared spectroscopy (FT-IR)

FT-IR is the technique applied to check the presence of IR bands specific to chitosan. It is generally used to differentiate between α, β, and γ forms of chitosan. The dried and powdered chitosan sample is mixed with dried KBr, in the ratio of 5 mg: 100 mg or 1 mg: 100 mg, to form a disc. An average of 42-100 scans with a spectral region between 4000-6000 cm^{-1} and 4 cm^{-1} of resolution spectra are obtained (**Ban *et al.*, 2018; Renuka *et al.*, 2019**)

FT-IR facilitates to validation of the structure of chitosan obtained from different sources by identifying IR bands. The chitosan obtained from fungi and insect sources is confirmed by the similar bands in the commercial chitosan spectrum. The region that differentiates chitin from chitosan is in the range of 1500 and 1700 cm^{-1} as shown in figure 6A. The bands obtained from the stretching vibration of the C=O bond at 1550 cm^{-1} and N-H bond at 1650 cm^{-1} showed similar intensities in the chitin spectrum but in the chitosan spectrum, the band obtained stretching vibration of C=O is less intense compared to N-H which indicates the deacetylation of chitin. A similar profile is observed in the commercial chitosan spectrum. It can be used to differentiate between the α and β forms of chitosan. α form is mainly found in insects, especially in their cuticles (**Jantzen da Silva Lucas *et al.*,** 2021). In α form, the amide I band appears to overlap at around 1663 and 1618 cm^{-1} respectively. In β form amide, I appear as one band at around 1656 cm^{-1} (**Marei *et al.*, 2016; Mohan *et al.*, 2020**).

The degree of deacetylation can also be quantified by IR spectroscopic methods. The spectrum for chitosan samples in the form of KBr disks is obtained by an IR instrument. The absorbance is recorded at 1655 cm^{-1} and 3450 cm^{-1} for the amide-I and OH groups respectively. The degree of deacetylation is calculated by:

$$Degree\ of\ deactylation\ (\%) = \frac{[1 - (\frac{A1655}{A3450})]}{1.33 * 100}$$

where A_{1655} and A_{3450} are the absorbances of chitosan, $\frac{A1655}{A3450}$ = 1.33 for fully N-acetylated chitosan (**Antonino *et al.*, 2017; Brugnerotto *et al.*, 2001**).

The polymer obtained from various sources can be differentiated as chitin or chitosan depending on the degree of deacetylation. For a polymer to be chitosan, the deacetylation degree needs to be above 50 %, and for chitin it needs to be below 50 %. The deacetylation degree of chitosan from mealworm's cuticle was found to be around 53.9 % which is above 50 % but it was around 88.9 % for commercial chitosan. The deacetylation degree of insect chitosan is lower than that of crustaceans (**Jantzen da Silva Lucas *et al.*, 2021; Mohan *et al.*, 2020**).

Potentiometric titration

Various methods such as potentiometric titration, infrared spectroscopy, nuclear magnetic resonance spectroscopy, UV spectrophotometry, etc. are used for the determination of the degree of deacetylation. Two of these methods are discussed. The DD of the chitosan sample can be quantified by potentiometric titration and the infrared spectroscopic method discussed previously. In a potentiometric titration, 0.1-0.5 g of chitosan is dissolved in 0.1-0.3 M HCl which forms the chitosan solution after stirring continuously. Deionized water is added for the solubilization of chitosan. The titration is carried out using 0.1 M NaOH. This results in the pH versus volume titration curve of NaOH consumed. At each transition, inflection points are found. The degree of deacetylation is calculated by:

$$Degree\ of\ deacetylation\ (\%) = 16.1 \times \frac{(y - x)}{W}$$

where, w- is the weight of the chitosan sample; x and y- are the volume of NaOH solution at the first and second inflection points respectively (**Marei *et al.*, 2016; Dimzon and Knepper, 2015**).

Differential scanning calorimetry (DSC)

The DSC can be used to determine the thermal stability of obtained chitosan. To evaluate the thermal stability, a small amount of chitosan is weighed in a capsule made of aluminum and sealed hermetically. The heating index is maintained at around 10 °C/min and the temperature is around 25-500 °C (**Jantzen da Silva Lucas *et al.*, 2021**).

The maximum temperature (T_{max}) required for the chitosan prepared from the mealworm's cuticle to be decomposed (111.96 °C) was similar to that of commercial chitosan (110.18 °C), whereas chitin decomposes between 307 – 412.4 °C (**Jantzen da Silva Lucas *et al.*, 2021; Mohan *et al.*, 2020**). This is the temperature at which the aggregation state of the sample changes and the sample begins to burn. The thermal stability of chitosan also depends on the NaOH concentration used in the deproteinization and deacetylation steps. As the concentration of NaOH increases the thermal stability of chitosan slightly increases (**Jantzen da Silva Lucas *et al.*, 2021**).

Thermogravimetric analysis (TGA)

Thermobalance is usually used for the thermogravimetric analysis of chitosan. Here the small amount of chitosan sample was heated over a temperature between 20-600 °C under nitrogen (**Jantzen da Silva Lucase*t al.*, 2021**). The thermogravimetric analysis is performed to obtain weight loss spectra patterns. The thermal stability of insect chitosan is usually measured in two steps: first, the weight loss due to the evaporation of water, and the weight loss due to the degradation of chitosan. The insect chitin usually disintegrates at a temperature higher than chitosan as the maximum degradation temperature of insect chitin is in the range of 307-412.40 °C (**Mohan *et al.*, 2020**). The chitosan obtained from fungal sources showed nearly a similar pattern as commercial chitosan which indicates the complete extraction of chitosan. The pattern obtained from thermogravimetric analysis of commercial chitosan showed no weight residues at 520 °C but the chitosan obtained from mushrooms showed weight residues at this temperature which indicates the presence of a strong bond in it (**Ban *et al.*, 2019**). The thermal stability of chitin is greater than that of chitosan as shown in figure 6B (**Jantzen da Silva Lucas *et al.*, 2021**).

X-ray powder diffraction (XRD)

XRD is carried out to evaluate the crystallinity of chitosan. The crystallinity index value depends on the amorphous and crystalline nature of chitosan, which in turn helps in determining its applications. The instrument is operated at 4 kV and 30 mA using Cu Kα as a common source of X-ray. The crystallinity index is given by:

$$CI(\%) = (\frac{I-Iad}{I})*100$$

Where I_{ad}, is the maximum intensity of amorphous diffraction at 16 °C; and I, the maximum intensity at 20 °C (**Marei *et al.*, 2016; Antonino *et al.*, 2017**).
It can also be used to distinguish chitosan from chitin since chitosan is less orderly arranged. The value of the crystallinity index for commercial chitosan is around 17.9 %. The crystallinity is usually reduced by the deacetylation of a sample (**Jantzen da Silva Lucas *et al.*, 2021**). This analysis showed different patterns for commercial chitosan and the chitosan obtained from various sources such as fungal strains (**Ban *et al.*, 2019**), insects, etc. which could be due to low deacetylation degree as shown in figure 6C. The peaks observed in X-ray diffraction analysis of chitosan from sources such as fungi and insects are similar to those described elsewhere (**Marei *et al.*, 2016**).

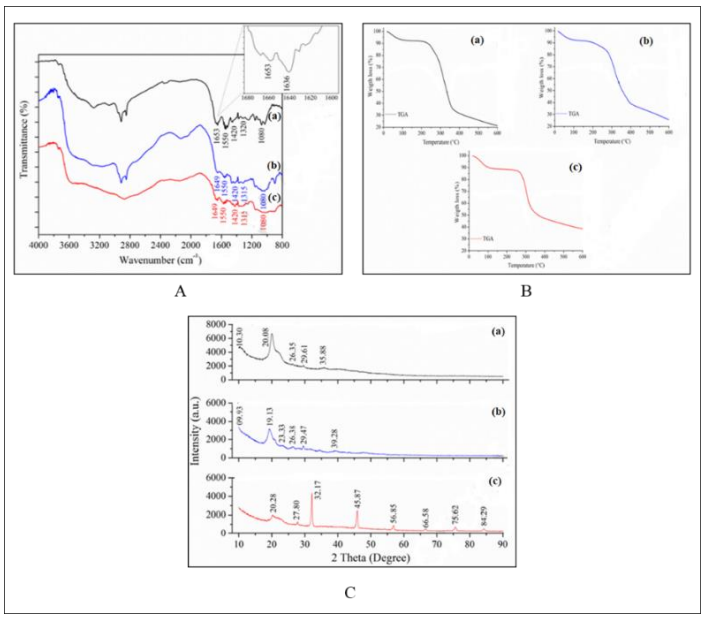

Figure 6 Characterization of chitosan: Fourier transform infrared spectroscopy (A); Thermogravimetric analysis (B); and X-ray powder diffraction (C). Where (a), chitin; (b) extracted chitosan; and (c) commercial chitosan (**Jantzen da Silva Lucas *et al.*, 2021**)

Field emission scanning electron microscopy (FE-SEM) and scanning electron microscopy (SEM)

FE-SEM and SEM facilitate the observation of the physical state, microstructure, and morphology of the chitosan sample. A thin layer of gold is coated over the samples and to ensure a high-resolution image the acceleration voltage is kept at a range of 10 kV (**Jantzen da Silva Lucas *et al.*, 2021**). But in scanning electron microscopy, the chitosan sample is used without coating on its surface and the acceleration voltage is kept at a range of 15 kV (**Marei *et al.*, 2016; Mohan *et al.*, 2020**). It helps to visually confirm the surface morphology of the chitosan obtained from various sources. The surface morphology of chitosan obtained from fungi, insects, and crustaceans is different as shown in figure 7. It can be classified as surfaces with nanofibres and pores, with pores only, with nanofibers only, with a rough and hard surface (**Marei *et al.*, 2016**). The surface of the insect's chitosan appears to be a nanofiber, nanopore, smooth surface, and rough surface under SEM. In a few crustaceans such as krill and shrimp, the fibers appear to be tightly arranged on the surface. The physical nature of the surface plays a significant role in defining the applications of chitosan. Nanofiber and nanopore forms of chitosan mainly find their application in food, therapeutics, and textiles (**Marei *et al.*, 2016; Mohan *et al.*, 2020**)

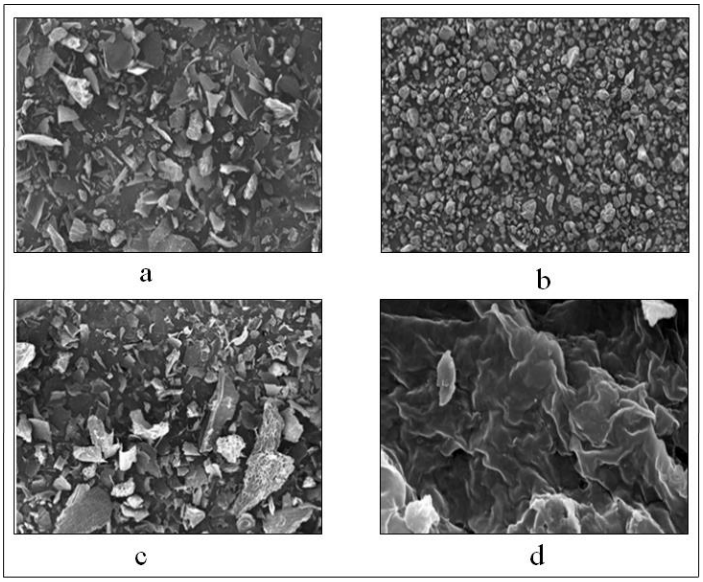

Figure 7 Characterization of chitosan-based surface morphology: chitin (a); commercial chitosan (b); insect chitosan (c); and fungal chitosan (d) (**Jantzen da Silva Lucas *et al.*, 2021**).

APPLICATIONS OF CHITOSAN IN VARIOUS SECTORS

Chitosan is non-toxic and possesses superior bioavailability, biodegradability properties, and adsorption qualities. Due to these characteristics, it can be explored in a wide range of sectors, including the treatment of wounds, wastewater treatment, agriculture, food packaging, cosmetics, and gene and drug delivery (**Wang *et al.*, 2020; Islam *et al.*, 2021**). The recent applications of chitosan in different sectors are discussed in this section.

Medical sector - Wound healing

Skin acts as a barrier that prevents pathogens from entering the body. When damaged, it is prone to infections. A wound occurs as a result of chemical, mechanical, physical, and thermal damage to the skin (**Ahmed and Ikram, 2016**). Conventional methods of wound healing are now being replaced by antimicrobial polymers which are inexpensive, stable, biodegradable, and non-toxic. Chitosan is one such cationic polysaccharide, which possesses these properties (**Matica *et al.*, 2019**).
Nowadays, chitosan is being used in wound dressings because of its above-mentioned properties. It prevents complex wound formation by accelerating the healing process of the wound as shown in figure 8 (**Feng et al., 2021**) (https://www.shutterstock.com/search/wound-healing). The following steps are involved in the inhibition of microbial growth by chitosan: i) Disruption of cell wall through electrostatic interaction between chitosan (cationic) and microbial cell surface (anionic) ii) Chitosan being low molecular weight penetrates the microbial cell and interferes with the protein synthesis process by interacting with microbial DNA iii) It will cause chelation of nutrients and metals essential for the cell stability of microbes. Once it enters the human body, it is disintegrated by enzymes and hence enhances the healing process without any toxicity (**Ahmed and Ikram, 2016**). Functional wound dressings that release drugs and other substances are used for treating chronic wounds. One such material is a chitosan-based hydrogel which can release growth factors, stem cells, peptides, etc. in a sustained manner (**Liu *et al.*, 2018**). These are widely used in the treatment of wounds as they provide a moist and cool environment to the wound surface. Alginate/chitosan hydrogels combine the hemostatic ability of the alginate and drug loading ability of chitosan in various physical forms making them highly efficient for wound healing. In a study, these hydrogels are loaded with anti-inflammatory, antibacterial agents such as hesperidin to accelerate wound healing (**Bagher *et al.*, 2020**).

Chitosan membranes are generated by electrospinning of chitosan, with a suitable degree of deacetylation and molecular weight. These membranes rupture the negatively charged cell membranes of the bacteria, exhibiting antibacterial activity and wound healing capacity. They can also be loaded with wound-healing agents such as curcumin and released in a controlled and sustained manner (**Augustine *et al.*, 2020**). Chitoseal® Abbot and Tegasorb® 3M are a few commercially available chitosan-based wound plasters for the treatment of bleeding wounds and chronic wounds respectively (**Liu *et al.*, 2018**).

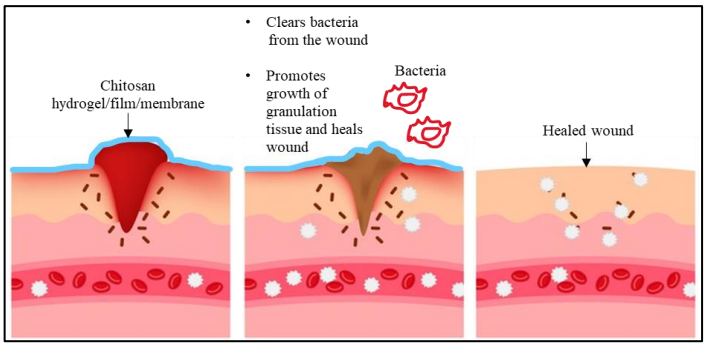

Figure 8 Wound healing by chitosan-based wound dressings which involves cell wall disruption, interaction with the microbial DNA, and chelation of nutrients to kill the bacteria

Wastewater Treatment Plant

The presence of colloids and other substances deteriorates the water quality. An important phenomenon in industries for the disposal of these substances is flocculation and coagulation (**Nechita, 2017; Iber *et al.*, 2021**). To coagulate particles in wastewater, lime, aluminum, and iron are commonly used as coagulants and they also help in the removal of turbidity. Inorganic coagulants have certain disadvantages such as toxic effects on the aquatic environment due to metal coagulants and also the use of aluminum in water treatment is associated with Alzheimer's disease (**Marey *et al.*, 2019**). Inorganic coagulants are now being replaced by organic polymeric flocculants which can flocculate pollutants at low dosages (**Desbrieres *et al.*, 2018; Nechita, 2017**).

Based on the properties of chitosan, it is considered an eco-friendly adsorbent for wastewater pollutants. The properties of chitosan such as cationic surface charge can be exploited to get rid of negatively charged impurities such as organic colloids, inorganic substances, and bacteria from wastewater (**Nechita, 2017**). The binding of chitosan to microbial cells modifies the cell permeability which in turn results in the leakage of intracellular constituents and finally the death of microbe (**Chopra and Ruhi, 2016**) as shown in Figure 9. The acid-base characteristics of chitosan also allow protein binding. Chitosan can retain the dye molecule and metal ions present in wastewater as the amine groups in chitosan get protonated at acidic pH (**Nechita, 2017; Chopra and Ruhi, 2016**) as shown in figure 9. This protonation also creates competition for ternary complex formation and the binding of metal cations in neutral solution (**Desbrieres *et al.*, 2018; Rhazi *et al.*, 2001**). Amine groups of chitosan can also bind to halides and metal ions present in drinking water, thus eliminating the by-products formed by chlorine disinfection and toxic heavy metals respectively (**Chopra and Ruhi, 2016**).

In a study, nano-chitosan is also being used to remove organic dyes and heavy metals. The incorporation of chitosan solution with tripolyphosphate resulted in the formation of nano chitosan. Better extraction of metals was observed in acetophenone-modified nano chitosan when compared to only nano chitosan-based adsorbent (**Zubair *et al.*, 2020; Desbrieres *et al.*, 2018**).

Chitosan can also remove kaolinite turbidity at a low dosage. 1 g/L of chitosan can remove 96.9 % of turbidity from wastewater. A higher dose of chitosan results in destabilization of dispersion and hence removes less amount of turbidity (**Marey *et al.*, 2019**). To boost the adsorption performances of chitosan, it is modified by crosslinking, functionalizing, and grafting. The adsorption property of chitosan is mainly due to its cationic nature (**Nechita, 2017**).

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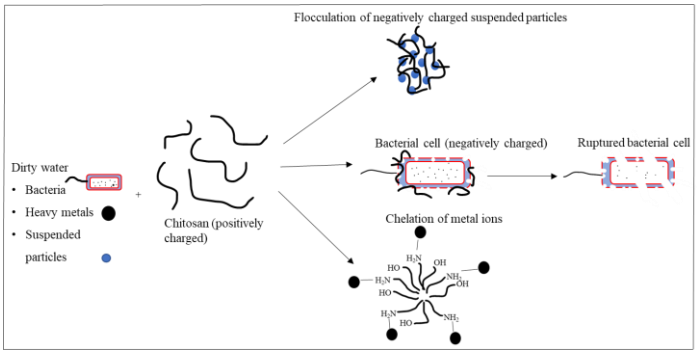

Figure 9 Wastewater treatment using chitosan by flocculation of suspended particles, microbial cell death, and chelation of metal ions

Agricultural Sector

Excessive application of chemicals in agriculture to increase crop yield and to get rid of insect pests has created serious environmental and health concerns. It has also created resistance in pathogens to chemical pesticides which necessitates new chemicals or even increased dosage of chemicals, which becomes expensive and a threat to the atmosphere (**Faqir *et al.*, 2021; Malerba and Cerana, 2019**). Hence chitosan finds its application in agriculture due to its antimicrobial, biodegradability, anti-insecticidal, and non-toxicity properties. Chitosan not only helps in the regulation of plant growth but also the protection against pathogens and pests. Chitosan can help plants protect themselves from pathogens by various mechanisms of action such as metal chelation and interaction with an anionic microbial surface which results in the inhibition of protein synthesis and finally, death of the microbe as shown in figure 10. Chitosan with procyanidin can be used as a coating to preserve the quality of fruits (Faqir ***et al.***, 2021). It can also be used as an herbicide due to its ability to cause electrophysiological modification (**Faqir *et al.*, 2021**). Chitosan nanoparticles (CHNP) are more beneficial than chitosan and their derivatives as chitosan nanoparticles can release plant hormones, pesticides, and fertilizers in a controlled manner (**Reshad *et al.*, 2021; Malerba and Cerana, 2019**). Chitosan nanoparticles have a combination of properties exhibited by chitosan and nanoparticles such as small size, quantum effects, etc. It can be used in the entrapment of potassium (K), phosphorus (P), and nitrogen (N) which increases the rate of photosynthesis and rate of nutrient uptake when released into the soil (**Faqir *et al.*, 2021; Malerba and Cerana, 2019**). It can also be used to create a defense response, reduce pest infection, and increase the shelf life of plants (**Malerba and Cerana, 2019**). The various applications of chitosan in the agricultural sector are shown in Tab 3.

Table 3 Applications of chitosan nanoparticles and their derivatives in the agricultural sector

Plant species	Chitosan (CH) type	Significance	References
Triticumaestivum	CHNP	Improved growth and longevity	**Riseh *et al.*, 2023**
Maize	CHNP	Antimicrobial activity	**Sangwan et al., 2023**
Rice	CHNP + sodium tripolyphosphate	suppression of rice blast fungus (*Pyricularia grisea)*	**Hassan *et al.*, 2022**
Zea mays	CHNP + Zn	Promotes crop yield	**Choudhary *et al.*, 2019**
Allium cepa	CHNP + Zn / Cu	Increases plant growth	**Abd-Elsalem *et al.*, 2018**
Capsicum annuum	CH + Tripolyphosphate	Stimulates plant growth	**Asgari-Targhi *et al.*, 2018**
Zea mays	CH + Salicylic acid	Improves disease resistance and plant growth	**Kumaraswamy *et al.*, 2019**
Musa acuminate AAA group	CHNP	Increases shelf life and maintains the quality of fruit	**Lustriane *et al.*, 2018**

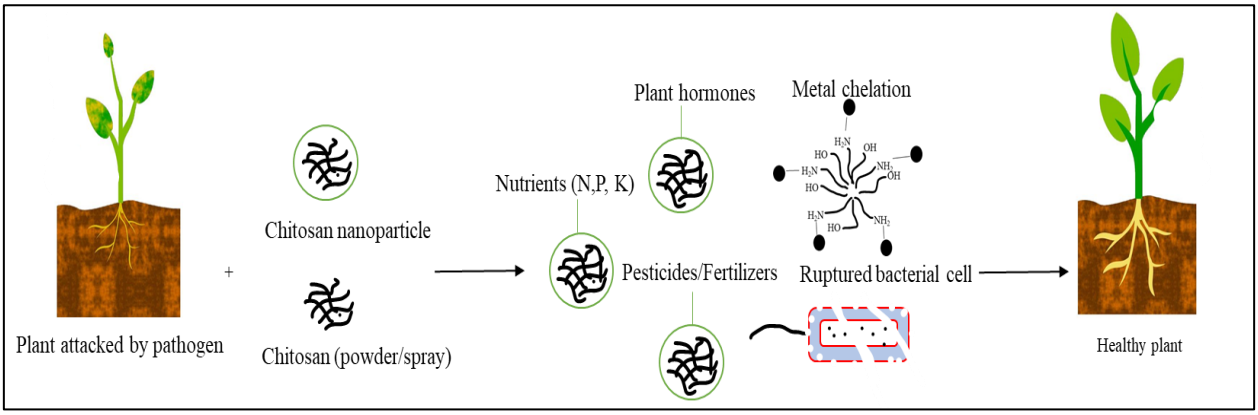

Figure 10 Promotion of plant growth and protection against pathogens by the application of chitosan-based derivatives which involves entrapment of growth-promoting substances and microbial cell death respectively

Food packaging Sector

The use of petroleum-based plastic materials for packaging causes environmental pollution due to the accumulation of these materials. Hence alternative packaging materials are now being explored as a substitute for plastic materials. One such material is chitosan (**Florez *et al.*, 2022; Souza *et al.*, 2020**). The combination of chitosan with additional constituents such as lipids, proteins, and plasticizers improves the barrier properties in addition to its mechanical properties, which are essential for food packaging (**Florez *et al.*, 2022**). As they exhibit antioxidant, antimicrobial, and film-forming properties, they are used as a coating in food packaging. The shelf life of perishable foods can be increased by using chitosan or a combination of chitosan with other materials for packaging (**Cazon and Vazquez, 2019**).

Chitosan is used in active food packaging as an edible coating that is applied to food products by dipping or spraying them in chitosan solution (**Souza *et al.*, 2020**) as shown in figure 11. Chitosan films that are semipermeable can be used to extend the shelf life of vegetables and fruits. This is mainly due to the control of the ethylene production rate and gas exchange rate by chitosan. Chitosan as a coating on food products retains the water content, solid content, and color of the fruits and vegetables when stored. They are also used to control microbial growth and reduce the oxidation of lipids in meat which causes spoilage by their antimicrobial and oxygen barrier properties (**Cazon and Vazquez, 2019; Souza *et al.*, 2020**).

Chitosan nanocoatings are also being explored which further enhances the barrier properties of chitosan (**Souza *et al.*, 2020**). The properties of chitosan can be further increased by the addition of fruit extracts, essential oils, and phenolic compounds (**Florez *et al.*, 2022**). Tab 4 shows the applications of chitosan-based films.

Table 4 Applications of chitosan-based films in food packing sectors

Chitosan-based films with components	Uses	Food product	References
Citric acid	Improves antioxidant property	Green chilli	**Priyadarshi et al., 2018**
TiO_2	Absorbs ethylene	Tomato	**Kaewklin et al., 2018**
Aloe Vera gel	Absorbs ethylene	Mango	**Shah and Hashmi, 2020**
Spice extracts	Improves antioxidant property	Pork	**Liu et al., 2021**

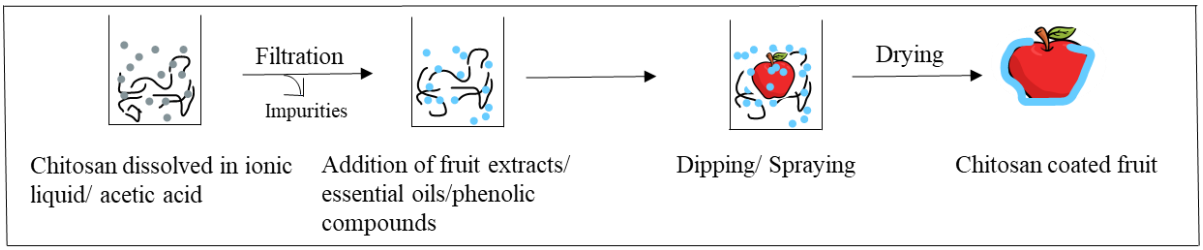

Figure 11 Role of chitosan as an edible coating in food packaging to increase the shelf life of fruits and vegetables

Cosmetic Industry

Chitosan is used as a basal material for cosmetics (**Hirano *et al.*, 1991**). The antifungal property of chitosan and its ability to become viscous in the presence of acid has been exploited for its use in cosmetic applications. The resulting cationic gum is used in lotions, nail polish, creams, and shampoo (**Kianirad *et al.*, 2021**). Chitosan having a high molecular weight is useful in moisturizing skin due to its film-forming properties that can reduce water loss and increase smoothness. It is also used in protective creams as it shows adhesion properties and absorbs ultraviolet rays. Because of the ionic interaction between the negatively charged surface of the skin and hair and the positively charged surface of chitosan, it is being used in formulations for skin and hair care (**Guzman *et al.*, 2022**). as shown in figure 12. The amino acid groups of chitosan can assist in crosslinking keratin molecules and restore the broken disulfide bonds by being added to the structure of keratin (**Ta *et al.*, 2021**).

Nanoparticles of chitosan are used as skin delivery systems for the release of cosmetic products. In a study, chitosan nanoparticles were synthesized by ionic gelation technique sodium tripolyphosphate and acacia as crosslinkers. This resulted in the formation of positively charged nanoparticles that depolarises negatively charged cell membrane to deliver cosmetic ingredient to the skin (**Ta *et al.*, 2021**). Hydroxypropyl chitosan protects the nail structure by acting as a film and also maintains hydration which in turn prevents psoriatic nails (**Casadidio *et al.* 2019**).

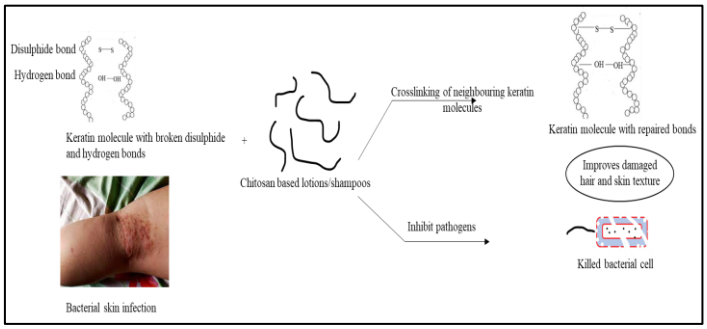

Figure 12 Role of chitosan in improving damaged hair and skin texture by crosslinking keratin molecules and inhibiting pathogens respectively

Biomedical Sector - Gene and drug delivery

Gene therapy is one of the most important therapies for the treatment of diseases. The high molecular weight and polyanionic nature of free plasmid DNA prevent it from crossing the membrane. Hence carriers such as viral or non-viral vectors are used. But the problem with viral vectors is the toxicity and immunogenicity which can be overcome by using nonviral gene delivery vectors. One such vector is chitosan based (**Chuan *et al.*, 2019; dos Santos Rodrigues *et al.*, 2019**).

Chitosan can be used to fabricate gene vectors due to its cationic nature. This property can make the surface of inorganic materials (iron oxide, gold nanoparticles) positively charged which enhances the insertion of vectors as the positive charges interact with the negatively charged nucleic acid and biomembrane. In an acidic environment, chitosan coating prevents the degradation of nucleic acid, enhances the biodegradation of inorganic materials, and prevents phagocytosis by macrophages (**Chuan *et al.*, 2019**).

High transfection efficiency can be achieved by chitosan grafted with amine-rich polymers. It has a critical role in cell transfection by the lysosomal escape of DNA and adhesion to the membrane. It protects the DNA from DNAse degradation by forming polyelectrolyte complexes with it (**dos Santos Rodrigues *et al.*, 2019**). It can also act as a controlled gene delivery system because of its sensitivity to pH. Chitosan and iron-oxide-based vectors enhance the cancer treatment by accumulating at the tumor site, with the application of an external magnetic field (**Chuan *et al.*, 2019**). Double-stranded small interference RNAs (siRNA) are assembled into endoribonuclease associates to create an RNA-induced silencing complex (RISC) interference, in RNA interference therapy (RNAi). The naked siRNAs have a very low ability to enter cells. Therefore, a healthy carrier like chitosan facilitates gene delivery as shown in figure 13 (**Nandgude and Pagar, 2021**).

Chitosan plays a critical role in the controlled release of drugs due to its bio-absorbable and non-toxic nature. They are mainly used for the release of growth factors, antibiotics, vaccines, etc. The interactions between the negatively charged acid groups of mucus and the positively charged amino groups on chitosan exhibit mucoadhesive properties and are hence used in nasal formulations. Chitosan is also sensitive to pH and hence targets a low-pH tumor environment. pH-dependent drug-loaded chitosan nanoparticles release the drug effectively without harming the healthy cells. Receptor-mediated delivery is one of the promising mechanisms of drug delivery. Ligands also enhance the overall therapeutic response by binding to the clustered receptors over-expressed on unhealthy tissues and the surface of cells (**Bakshi *et al.*, 2020**). Chitosan-based drug delivery systems are mainly used to deliver cytotoxic and insoluble drugs as shown in figure 13 to the target site which in turn reduces cytotoxicity and increases the drug efficacy. The administration of drugs with chitosan orally increased the absorption of drugs from the intestine to the blood when compared to the one without chitosan (**Hirano *et al.*, 1991**).

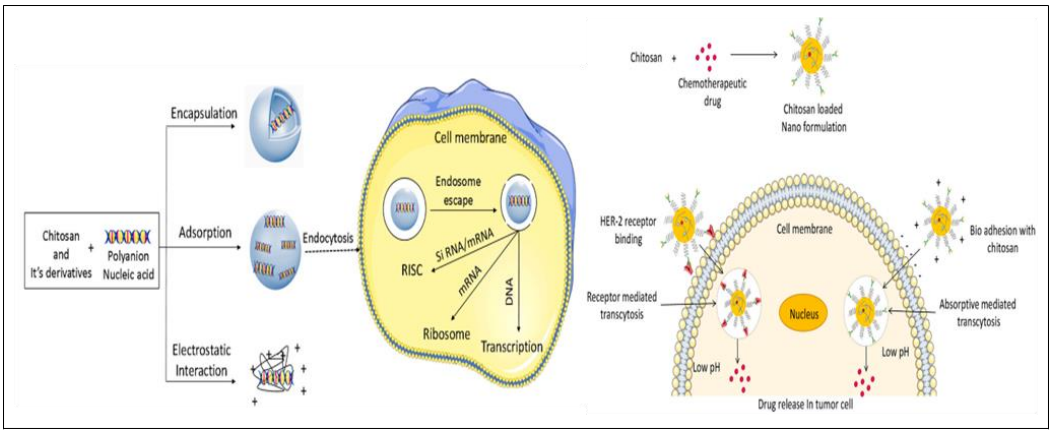

Figure 13 Role of chitosan in gene delivery as vectors and drug delivery for the controlled release of drugs (**Nandgude and Pagar, 2021**)

PATENTS RELATED TO CHITOSAN IN HEALTHCARE AND ENVIRONMENTAL HEALTH

Chitosan and its derivatives have demonstrated notable effects in various sectors. One such application is in the healthcare sector. Patents granted for the use of chitosan in the treatment and prevention of coronavirus infections, as an antibacterial coating agent, as a tissue dressing material

Table 5 Patents related to the application of chitosan in environmental health

Sl. No.	Patent no	Title	Description	References
1	US 9732164B2	Chitosan derivative compounds and methods of controlling population	Methods of making chitosan-derivative compounds such as chitosan arginine compounds, chitosan guanidine compounds, chitosan-acid amine compounds, co-derivatives of the chitosan derivative compounds, salts of the chitosan derivative compounds, and chitosan-guanidine compounds. Inhibiting and enhancing microbial population in animals	**Shenda *et al.*, 2017**
2	US 20030101521A1	Method of adsorbing dye in aqueous solution by chemical cross-linked chitosan beads	Chitosan solution mixed with tripolyphosphate (TPP) solution to form ionic cross-linked chitosan beads. Crosslinking of ionic cross-linked beads to form cross-linked chitosan beads adding NaOH and cross-linking agent	**Ming-Shen *et al.*, 2003**
3	US 20020089080A1	Method of manufacturing chitosan micro flakes	Chitosan is dissolved in a weak acidic solution to form a chitosan solution which is incubated for 1-30 days followed by freeze-drying, thermal drying or vacuum drying, and pulverization to obtain chitosan micro flake	**Won *et al.*, 2002**
4	WO 2020234643A1	Chitosan-based coating system	Consists of a carrier agent (cleaning agent, polishing agent, or finishing agent) and a chitosan-based agent (chitosan acetate, chitosan citrate acetate). Chitosan-based agent increases the shelf life of the coating system, prevents microbial attack, and also acts as a chelating agent to reduce soap scum	**Schierlmann *et al.*, 2020**
5	US 4278696A	Deacidifying coffee extract with chitosan	Coffee extract is contacted with chitosan for 5-30 min at 10-80 ºC and the deacidified extract is collected	**Danièle *et al.*, 1981**
6	WO 2019227848	Method for preparing chitosan and derivative nanofiber thereof by mechanical means	Chitosan fiber or chitosan derivative fiber is pretreated with water, acid, and alkali. Microsized chitosan fiber is obtained by refining or beating. Microsized fiber is then homogenized under high pressure to nanofiber chitosan	**Zhao *et al.*, 2020**

The application of chitosan in the treatment and prevention of coronavirus has been patented which covers the use of chitosan polymer to inhibit viral replication in the treatment of coronavirus infection+ns. Chitosan polymer N-(2-hydroxypropyl)-3-trimethylammonium chitosan chloride (HTCC) was synthesized through a reaction

with glycidyl trimethyl ammonium chloride. It was further modified by substituting the hydrophobic groups. The resultant polymer was used as a solution (aerosol or liquid) for intravenous, oral, and topical administration to treat and prevent coronavirus infections (**Pyrc *et al.*, 2017**). A US patent was granted for the use of chitosan hydrogel as an antibacterial coating agent. Due to its antimicrobial properties, a chitosan hydrogel matrix is used to coat and protect biomedical devices (contact lenses) and bio-implants (artificial hearts, pacemakers, urinary catheters, etc.). This was obtained by grafting the quaternized chitosan with polymerizable groups, such as polyethylene glycol derivatives & methacrylate by UV or thermal polymerization, resulting in a water shield coating agent. The quaternized chitosan and the native chitosan were grafted with polyethylene glycol monoacrylate (PEGMA) using sodium hydroxide as a base, resulting in chitosan hydrogels such as chitosan grafted-poly (ethylene glycol) methacrylate (chitosan-g-PEGMA) and quaternized chitosan-g-PEGMA respectively (**Mary *et al.*, 2014**). Another US patent obtained was related to the crosslinked core and core-shell nanoparticle polymer preparation method from chitosan. The chitosan was chemically modified by the reaction of its amine group with one or more mono, di, tri, or polycarboxylic acids forming an intramolecular bridge and amide linkage.
This resulted in the conversion of the coiled chitosan structure to the globular nanoparticle. These crosslinked nanoparticles had amine groups on the surface and hydroxyl groups on the shell, which then reacted with the functional carboxylic acid. Functional groups were predominantly vinyl groups. The resulting chitosan is crosslinked, nanosized, biocompatible, and biodegradable and finds its applications in drug delivery, enzyme immobilization, and as additives to pharmaceutical products (**Borbely *et al.*, 2004**). A patent was obtained for chitosan tissue dressing. The solid, gel-like, or liquid tissue dressing material was composed of two layers, the first layer was made of chitosan (solid or aqueous) supported by a second layer which is used for the application on patient tissue. Chitosan (deacetylated, native, or deacetylated native chitosan) accounts for 50 % of the weight of tissue dressing material. The improved method for the treatment of tissue includes contacting the tissue with tissue dressing material and then applying detachment solvent to avoid irritation or damage to the tissue. This tissue dressing material accelerates wound healing, maintains humidity at the site of a wound, allows gas exchange acts as a thermal insulator, and is partly or fully dissolvable (**Montenegro and Freier, 2009**). A patent has been granted for chitosan-based implants for tissue repair. The implant is composed of many layers. It has a porous layer made of freeze-dried chitosan containing an aqueous solution with a pH lower than 5. Chitosan with a degree of deacetylation ranging from approximately 0-60% is present in the aqueous solution containing chitosan. The non-porous layer is a biodegradable film containing collagen or collagen derivatives. This chitosan-based implant is biocompatible and has improved mechanical properties such as higher tensile and suture anchoring strength. Improved mechanical properties are achieved by regulating the pH without the need for cross-linking agents. These matrices can be applied to several medical applications, such as surgical implants (**Claret *et al.*, 2019**). Tab 5 depicts patents related to applications of chitosan in the field of environmental health.

LIMITATIONS

Although chitosan has gained great attention in various fields be it biomedical, environmental remediation, food packaging, agriculture, etc. due to its unusual properties, there are various challenges in obtaining the raw material, in production, extraction, and purification of chitosan.
Currently, chitosan production is mostly from crustacean shells and it shows certain disadvantages such as the availability of shellfish waste throughout the year and the availability being restricted to a particular area. Hence the quality of chitosan produced may be inconsistent whereas the production of chitosan from microbial sources can be controlled to produce pure chitosan and the quality can be maintained. The problem with the production of chitosan from fungal sources is the variation in the composition of the fungal wall and it depends on growth conditions, type of media (solid or liquid) and carbon and nitrogen sources used, age of the culture, etc. Fermentation parameters can be easily controlled in liquid media when compared to solid media.
The chemical method used for the production and extraction of chitosan from crustacean shells is not environmentally friendly due to the use of a large amount of alkali and acid solution and this method requires high temperature in the demineralization step which can result in depolymerization of the polymer. The continuous hydrolysis of chitosan by alkali reduces the essential properties (mechanical properties and molecular weight) of chitosan. The enzymatic method may be thought of as an alternative to the extraction of chitosan from crustacean shells but the problems associated with this method are the high cost and less efficiency in the conversion of chitin to chitosan, which limits its use in laboratory scale. Pre-treatment of chitin by grinding, heating, etc. can cause an increase in enzymatic activity but is still not sufficient for efficient conversion. Limitations of the chemical method can also be overcome by using a freeze-thawing method which enhances the chitosan yield and quality without increasing the temperature or amount of alkali solution. The presence of small amounts of impurities in purified chitosan can result in severe allergies in people sensitive to those impurities. Because of the high viscosity of chitosan, purification becomes difficult and requires sophisticated instruments such as molecular sieves, and ultrafiltration as the techniques which are in current use become expensive.

FUTURE SCOPE

Presently, the hydrolysis of alkali and acid used in the production of chitosan has toxic effects on plants which limits its extensive use in the agricultural sector. Hence chitosan and its derivatives which are non-toxic need to be developed. Although there are many applications of chitosan in various fields, its application in the medical field is still limited especially because of the concerns about its purity. Further research needs to be done on chitosan to know if it can have any impact on the environment and human health and also on the combination of chitosan with other materials so that they can enter the market as useful products. Improvement in the mechanical, thermal, water, and oxygen barrier properties of chitosan for applications in food packaging.
Since the demand for chitosan is increasing due to its extraordinary properties and diverse applications, the current production methods need to be expanded which leads to the overexploitation of crustacean shells and hence a large amount of waste. This necessitates alternative chitosan sources such as fungal strains and insects to meet this huge demand. Currently, fungal sources such as mushrooms and *Aspergillus niger* are used to produce chitosan commercially, and more such fungal strains need to be investigated for the production of commercial chitosan. Presently, insect as a chitosan source is the area of focus as it is an alternative to crustaceans. When compared to the chitosan yield obtained from crustacean chitin, the yield of chitosan from insect chitin is less and hence the process of deacetylation needs to be optimized in the future to increase the yield.

CONCLUSION

Chitosan is an eco-friendly natural biopolymer that is produced by the deacetylation of chitin. It is synthesized by two methods: chemical and enzymatic. The method which yields chitosan with a lower degree of deacetylation and molecular weight must be considered. An alternative to the chemical method of chitosan production is the biological method which does not make use of high temperatures and high concentrations of acid and alkali. Replacing the synthesis of chitosan with fungal and insect sources instead of crustacean sources can reduce the environmental impact. The characteristics of chitosan obtained from fungi and insect sources are nearly similar to that of commercial chitosan maybe with slight variations. Limitations of chitosan concerning solubility can be overcome by alkylation, acylation, and carboxylation. The presence of reactive functional groups helps in the functionalization of chitosan. Chitosan nanoparticles can be synthesized by ionic cross-linking which has more advanced properties than chitosan. The antimicrobial, non-toxic, biocompatibility properties of chitosan make it useful in various fields such as healthcare, especially during the COVID-19 pandemic, food packing, agriculture, etc.

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