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CHITOSAN SOURCES AND EXTRACTION: A REVIEW

Ruaa A. Salman[1], and Naser K. Zedain[2]

[1] Biomedical Engineering Department, University of Technology-Iraq, Alsinaa Street 52, Baghdad, Iraq, Email: 70153@uotechnology.edu.iq.

[2] Production and Metallurgy Engineering Department, University of Technology-Iraq, Alsinaa Street 52, Baghdad, Iraq, Email: 70113@uotechnology.edu.iq.

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ABSTRACT

Chitosan is a biopolymer that is obtained by deacetylating the chitin that is recovered from different sources, such as marine and animal. Also, the waste of agriculture and the seawater industry are considered good sources for extraction. This waste may be an environmental pollution source as a result of burning and dumping or a good source for producing important materials such as chitosan which is considered a biocompatible, biodegradable, biopolymer that has many industrial, biomedical, and agricultural applications. Chitosan material, extracted from chitin, has many attractive biomedical properties. This review focuses on the extraction methods of chitosan from different production sources, taking into account industrial considerations, and summarizes them, taking into account their environmental impacts and marketing potential. Also, this article reviews—based on existing publications—the classes of chemical methods and reaction parameters used in chitosan preparation and biological methods.

KEYWORDS

Extraction, Chitosan, Chitin, Polysaccharide, Biopolymer, Shrimp waste, Biotechnology.

1. INTRODUCTION

Chitosan is a component of N-acetyl glucosamine and glucosamine, a copolymer derived from chitin. Chitin is a biopolymer that has a linear form consisting of linked units of N-acetyl-D-glucosamine with glycosidic bonds (Khayria et al., 2017; Dai et al., 2020; Duan et al., 2019; Fatehi et al., 2010). Also, however, chitosan and chitin are defined as cationic polymers and can be obtained from the deacetylation of chitin material (Mao et al., 2017). Chitin material is a biopolymer abundant in many organisms, including the exoskeletons of mollusks and the cell walls of fungi (e.g., snails, clams, squid, oysters, and octopus); crustaceans (e.g., shrimp, lobsters, and crayfish); algae (e.g., green algae and brown algae); and insects (house flies, ants, silkworms, spiders, cockroaches, scorpions, and beetles); Biopolymers are used in the world in various ways, one of which is crustaceans (Younes and Rinaudo, 2015). Also, chitin is considered the origin compound of chitosan (Shen et al., 2014; Saeed et al., 2011; Yu et al., 2021). In general, based on significant studies in this direction, the percentage of chitin in the skin of most of the aforementioned organisms (e.g., crab skin) ranges between 12% and 28%, and it also ranges between 2% and 44% in the composition of fungal cells; calcium carbonate and chitin are found in more than 38% of the waste produced by crustaceans (Kadhim et al., 2019). Chitosan is emerging as a versatile raw material for the synthesis and manufacturing of a wide range of products with applications ranging from food, pharmaceuticals, healthcare, industry, agriculture, and environmental pollution. In general, most chitosan used in industrial applications, which are called "synthetic polymers," is derived primarily from crustaceans and fungi (Ali et al., 2021; Ahmed et al., 2021). These polymers are characterized by their complete stability, especially under biological conditions, and their biodegradability is minimal (Huq et al., 2022; Ali et al., 2021). On the other hand, around the world, approximately more than 141 tonnes of synthetic polymers are produced annually worldwide (Ali et al., 2021; Nwe et al., 2010). Since chitosan and chitin are important biopolymers that are biodegradable, environmentally friendly, biocompatible with organ cells, and nonpoisonous, they have attracted significant attention from industrialists and researchers, leading to their use in a wide variety of applications, particularly in bioengineering, textile products, agriculture, the food industry, and the paper industry (Santos,et al., 2020; Khan et al., 2020; Khan et al. 2019). On the other hand, other synthetic polymers, such as Teflon, polyethylene, polyester, and nylon, due to their different chemical and physical properties (e.g., their lack of biodegradability), have limited uses, especially in biological applications, and therefore may not attract much interest (Chai et al., 2020). The synthetic polymers are produced around 140.106 tonnes with minimal biodegradation (Saood and Sulaiman, 2016). These require using environmentally friendly biodegradable polymers (Zedin et al., 2020; Salih; Jawad, 2015).

This review shows the promising biopolymer (chitosan) with a specific structure and various applications. Also, we have highlighted various sources of chitosan with the different methods of extraction (Thornber et al., 2019; Mohsin, 2023). Therefore, the random or irregular disposal of these wastes causes pollution and negative effects on water and soil (Moss et al., 2010; Mahmood, 2024); the impacts will affect living creatures (Valenti and Kimpara, 2011; Kadhim et al., 2019). As a result, it is highly recommended for the work of biopolymer research. Biopolymers such as chitosan and chitin are used in the world in various ways, one of which is crustaceans (Younes and Rinaudo, 2015; Kadhim et al., 2019). Therefore, this review focuses on a brief overview of the history and structure of chitin and chitosan, as well as their chemical differences, identifying the most important crustacean species suitable for chitin and chitosan production, and exploring other sources that can be extracted and applied commercially.

2. CHITOSAN'S STRUCTURE

Chitosan is consisted of one amino (NH_2) group with two hydroxyl (OH) groups and N-acetyl-D-glucosamine with D-glucosamine (Moss et al., 2010) as shown in Fig. 1.

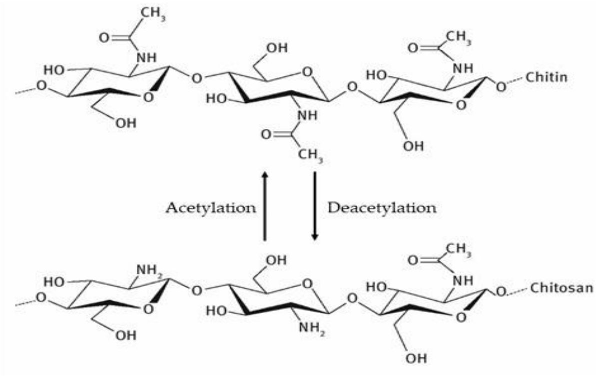

Fig. 1. Chitosan's structure (Hosney et al., 2022, Kiuchi et al 2008).

The chitosan stills acts as a polycationic species at low pH with increased soluble properties because of the amino group protonation (Ali and Ahmed, 2018; Jayakumar et al., 2007; Lv et al., 2014). Depending on the procedure for source extraction preparation, the specified molecular weight (Mw) can change from 300 and reach 1000 kD and the deacetylation degree varies from 30% until 95% (Gutiérrez, 2017). So, it is an important issue to choose the appropriate procedure and proper source to define the properties of extracted material. The deacetylation of chitin can be used to extract Dglucosamine (Rhim et al., 2006; Jin et al., 2004). Both chitin and chitosan have a high content of nitrogen (Kiuchi et al., 2008). The chemical composition of chitosan as a linear polysaccharide. Chitin has fewer applications compared with chitosan due to its immiscibility with most organic solvents and water (Abdel-Rahman et al., 2015; Revathi et al., 2012). Chitin's stiffness is returned to its strong intra-bonds of hydrogen (Iber et al., 2022).

3. OCCURRENCE OF CHITIN/CHITOSAN IN NATURE

Chitin is a highly organized crystalline structure that has a natural polymer that is low in chemical reactivity, hard, and white. It is characterized by a number of properties, including that it appears after purification as a yellowish powder. It is insoluble in water and organic solvents and has a high molecular weight (Aranaz et al., 2009; Al Sagheer et al., 2009; Duan et al., 2019). From a chemical point of view, it consists of N-acetyl-2-amino-2-deoxy-D-glucose units connected by glycosidic bonds, which form a linear chain with some deacetylated monomer units. Finally, it is considered the second most abundant sugar in nature (Santos et al., 2020). Fig. 2 presents the main sources of chitin production and its extraction. The extraction process of chitosan has many sources, the wastes of the sea food as the shells of shrimp or crustaceans are considered the traditional source for chitosan extraction. Another source is the insects' exoskeleton beside the kingdom of fungal as in Fig. 2 (Iber et al., 2022).

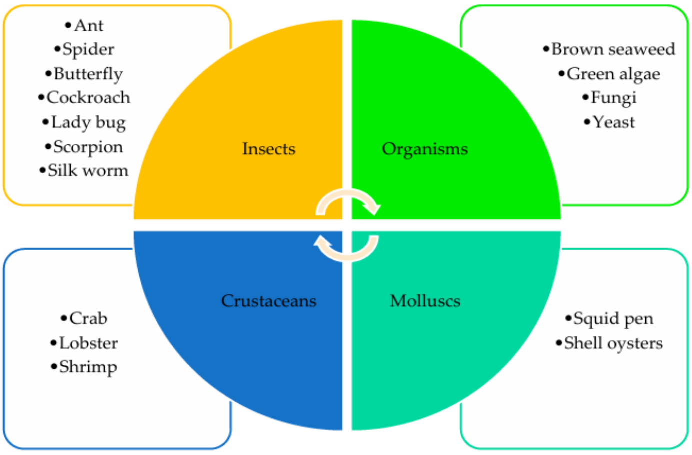

Fig. 2. Sources of chitin/chitosan production (Santos,et al., 2020).

The waste of seafood represents around 50% to 70% of the raw material for the extraction of chitosan. These wastes contain (20%–30%) of chitin, (30%–50%) of calcium carbonate, (30%–40%) of proteins, and besides the pigments (Aranaz et al., 2009; Al Sagheer et al., 2009; Duan et al., 2019). Chitin is found inside many microbes inner cell wall as a Mucor rouxii, Absidia glauca, Aspergillus niger, Gongronella butler, Trichoderma reesei, Rhizopus oryzae and Lentinus edodes (Merzendorfer, 2011; Dhillon et al., 2013). Both sporulating and vegetative cells can be used to synthesize the chitin (Lesage and Bussey, 2006). Table 1 lists the characteristics of chitosan extraction from the fungi and the shells of crustacean (Huq et al., 2022).

Table 1. The characteristics of chitosan extraction from the fungi and the shells of crustacean.

The material of source	The advantages	The limitations	References
Crustacean's shell	commercially and Industrially method.	1) limited supply for raw material effected by the seasonal variation.; (2) requires large chemical amounts, energy, and time-consuming. (4) hard to obtain chitosan with high deacetylation which leads to limit the biomedical utilizing.	(Aranaz et al., 2009; Ghormade et al., 2017; Kumari and Rath, 2014)
The Fungi	(1) There is no effect of the seasonal variation (2) the heavy metals are limited (3)without of animal allergenic (4) Molecular weight can be controlled (5) the polydispersity is low (6) high biocompatibility.	(1) The quantity is lower than the marine sources (2) the cost of production is higher (3) the commercial production is limited.	

4. THE EXTRACTION OF CHITOSAN.

The common way to extract a component from the matrix of materials is by dissolving the component in one or in a mixture of different solvents. After that, minor methods such as crystallization, concentration, and precipitation are used to remove the chosen material. But, chitin insolubility leads to many difficulties in extracting process due to unnecessary components dissolving (Rinaudo, 2006). Unnecessary material cannot be totally isolated due to the crustacean shells' complex structure (Younes and Rinaudo, 2015). The standard procedure for chitosan and chitin extraction are revealed in Fig. 3.

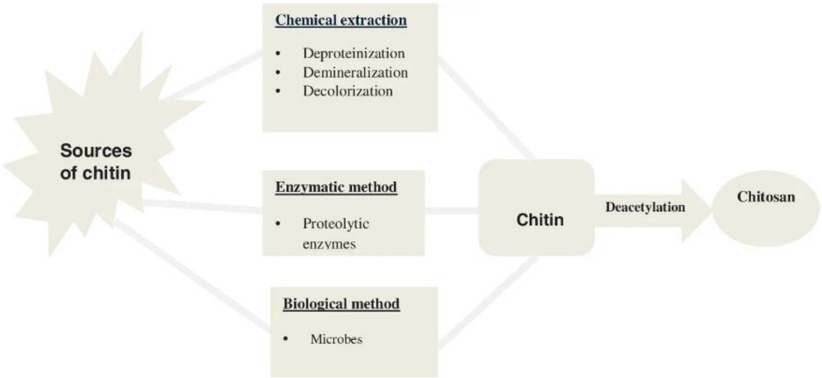

Fig. 3. The standard procedure for chitosan and chitin extraction (Iber et al., 2022).

4.1. Extraction by chemical method.

Fig. 4 shows a diagram for chitosan and chitin extractions from crustaceans' wastes. Other new methods for treatments depend on using hot water as a greener method (Margoutidis et al., 2018; Yang et al., 2019), In addition to mechanical and chemical processing (solid state) (Chen et al, 2017; Ruaa and Naser, 2021) and the glycerol method (Devi and Dhamodharan, 2018). All these methods reduce the consumption of chemical materials (Thornber et al., 2019).

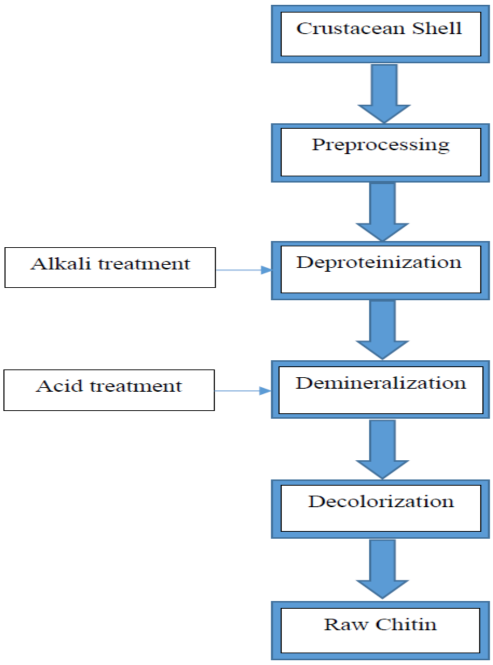

Fig. 4. The chemical extraction of chitin (Rinaudo, 2006; Younes and Rinaudo, 2015).

Proteins, minerals, and chitin are the main compounds in crustaceans' exoskeleton. The proteins and minerals are removed by using deproteinization and demineralization processes (Rinaudo, 2006). Generally, different kinds of raw materials have different amounts of their compounds (Younes and Rinaudo, 2015). The calcium carbonate is removed by the acid treatment, and then any color of the product is removed by the depigmentation process (Seenuvasan et al., 2020; Acosta et al., 1993). Acetamide group hydrolysis inside chitins is used to prepare the chitosan. To obtain this reaction, high-temperature treatment and hydrolysis with strong alkaline are adopted (Kumari and Rath, 2014).

4.1.1. The process of deproteinization

Deproteinization process' importance comes from the necessity of removing the proteins. The allergy of biomedical materials is caused by Proteins. Disrupting the chemical bonds within the proteins in chitin leads to the process of protein deproteinization (Younes and Rinaudo, 2015). Potassium hydroxide (KOH), sodium hydroxide (NaOH), and sodium carbonate (Na_2CO_3) are the usually using materials in deproteinization process of the waste crustacean. Sodium hydroxide (NaOH) considers the favorite reagent at room temperature til 160˚C with concentrations from 0.125M to 0.5M. The biopolymers are deproteinization by NaOH and this affects its molecular weight (Synowiecki and Al-Khateeb, 2003). Deproteinization process conditions show in Table 2.

Table 2. Conditions for chitin production from various sources.

	Deproteinization			**Demineralization**			
Source	**NaOH References Concentration**	**Temperature (°C)**	**Time (h)**	**HCl Concentration**	**Temperature (°C)**	**Time (h)**	**References**
Shrimp	1 M	100	24	1 M	-	24	(Kawada et al., 2001)
Shrimp	1.25 M	100	0.5	1.57 M	20–22	1-3	(Song et al., 2018)
Crab	40%	100	2	1N	100	6	(Guibal 2004)
Tenebrio molitor	50%	105	3	2N	Room	3	(Teli and Sheikh, 2012)
Crab	1 M	100	36	2 M	Room	48	(Younes and Rinaudo, 2015)
Crab	1 M	100	72	1 M	Room	-	(K.M. et al., 2017)
Leptinotar sa	3 M	80–90	3	2 M	65	2	(Casadidio et al., 2019)
Clanis bilineata	10%	60	24	7%	25	24	(Fournier et al., 2020)
Krill	0.875 M	90–95	2	0.6 M	Room	2	(Dai et al., 2020)
Bombyx mori	40%	80	24	1M	100	0.25	(Shin et al., 2019)
Catharsius molossus	8 M	80	24	1.3 M	80	0.5	(Bello, and Olafadehan 2021)
Lobster	10%	100	2.5	10% HCl, 90% formic	Room	18	Younes and Rinaudo, 2015
Krill	3.5%	25	2	3.5%	20	1.5	(Al Sagheer, et al, 2009)
Grasshopp er	1M	90	2	1 M	90	2	(Simionato et al., 2006)

4.1.2. The demineralization process.

The waste of crustaceans has a high amount of minerals such as ($Ca_3(PO4)_2$) and $CaCO_3$ which must isolate (Bello et al., 2021). The minerals inside the chitin are removed by demineralization process. Usually, acid treatment is accomplished in this process. The dilute HCl is propered for the demineralization process because of its ability for decomposition of ($CaCO_3$) as revealed in this equation (Younes and Rinaudo, 2015):

$$2\ HCl^{+}\ CaCO_3 \rightarrow CaCl_2 + H_2O + CO_2 \uparrow \qquad (1)$$

After that, the filtration process may use to remove the solution of salt. At least, the diluted water is used for washing the residual and leaving the chitin . Many factors are effected on the process of deproteinization as the concentration of acid, temperatue, extration's time, the particales' size, and the mineral degree (Santos et al., 2020; Hemmami et al., 2024).

4.1.3. The process of decolorization

The final level in the extraction process of chitin is decolorization. It means removing the pigments from the extracted material. Crustaceans contained the carotenoid as one of the major groups. In this process, the organic solvents with an acetone mixture are preferred (Seenuvasan et al., 2020; Zedin et al., 2022). The steps of chemical extraction levels for shrimp waste are shown in Fig. 5.

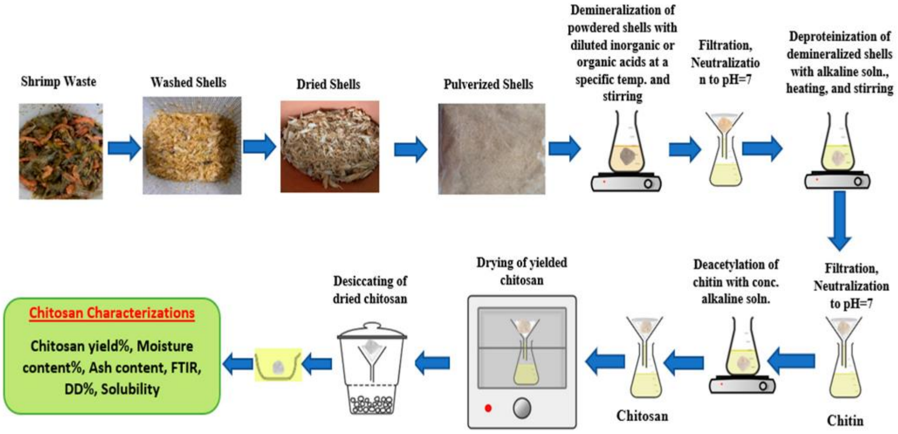

Fig. 5. Chemical extraction levels for shrimp waste (Al Sagheer, et al, 2009).

4.2. The biological extraction

Although the advantages of the chemical extraction process as the simplifying, large scales of production, and easy there are many disadvantages. Some of these problems are consuming of high energy and using high-concentration acids (Iber et al., 2022; Sami et al., 2020). The high concentration acids are effected on the environment. So, there is a need to apply other method as the biological extraction. It is applied as two methods including proteolytic enzymes for the deproteinization process and microorganisms that remove the minerals and proteins together (Younes and Rinaudo, 2015; Marwa, and Asmaa 2022; Seenuvasan et al., 2020).

4.3. The enzymatic deacetylation process

The deacetylation of chitin is worked on hydrolyses the chitin (N-acetamido) group to obtain the chitosan. chemical deacetylation process depends on the concentrated alkali which has large solubility. deacetylation can be applied by enzymes to overcome the chemical deacetylation process (Pareek et al., 2012; Yan et al., 2018; I Chai et al., 2020 and Zhao et al., 2010).

5. DIVERSION CHITIN INTO CHITOSAN

The deacetylation reaction of chitin with alkaline hydrolysis, followed by treatment with acidic solutions, converts it to chitosan, a polysaccharide obtained from 2-amino-2-deoxy-d-glycopyranose units linked by glycosidic bonds (Riyan et al., 2021; Hemmami et al., 2024). Chitosan units yield proportional ratios of distinct structural properties, such as molecular weight and degree of deacetylation, which are related to the biological, chemical, and physical properties of the polymer. The solubility of ion-exchange resins plays a significant role, particularly in organic acids and dilute metals, and this explains why polymers vary in their relative solubility ratios approximately; however, precipitation of these polymers occurs at pH values above 6.0 (Iber et al., 2022; Olusola et al., 2021). As previously mentioned, chitosan is derived from chitin and has a degree of deacetylation greater than 50%. Since chitosan is characterized by its ability to dissolve in acidic media due to the protonation of free amino groups, with a pH value above 6.0, these amino groups make it possible to bind to negatively charged materials and thus contribute to the extraction of chitosan from chitin (Pellis et al., 2022).

6. CHITIN AND CHITOSAN APPLICATIONS

What makes chitosan an industrially important material is its wide range of unique and important properties, such as its cationic behavior as a biopolymer, its insolubility in water and overall positive charge at biological pH, and its ease of gel formation. These properties are interesting in food, medical, and environmental applications, as shown in reviews compiled in Table 3 (Kaczmarek et al., 2019; Santos et al., 2020).

Table 3. The abridgement of the major applications of chitosan/chitin.

Field of application	Applications	References
Pharmaceutical and Biomedical applications	Bone, cornea, neural, cardiac, and skin regenerative technology (regenerative technology/tissue engineering). Antioxidant, Creating pores, Radical quencher. Antimicrobial agent. Drug transmission, where stuck properties increase drug penetration of various cells. Gene delivery and therapy. Chitosan-based drugs, and the lowering effect of cholesterol. Work macrophage activity, provides a tissue growth matrix with a three-dimensional structure, and activates cell proliferation. Stimulates precipitation Regular collagen and proliferation because it bit by bit degrades into N-acetyl-d-glucosamine. Homeostatic agents engage in the reform, activation, and replacement of the immune system as wounding administration and promote granulation.	(Iber et al., 2022; Salman et al., 2020; Olusola, et al., 2021)
Food Industry	Packaging, natural flavor extender, edible coatings, antioxidant agent, stabilizing agent, and food preservation.	(Riyan et al., 2021; Sami et al., 2021)

Field of application	Applications	References
Industrial application	Electrolyte: Chitosan is used in the production of solid-state batteries and can be used to extend ionic conductivity ; also, the combination of sulfuric acid and chitosan has the ability to high voltage discharge. Photography and using functional materials, tungsten carbide chitin whiskers are used in the production of microelectrochemical systems. Paper manufacture: it can be used in the production of filter papers and water-resistant papers.	(Santos et al., 2020; Hemmami et al., 2024; Synowiecki and Al-Khateeb, 2003; Namus et al., 2024).
Health care products	Cosmetics formulations: antifungal and antimicrobial, including in rinses, colorants, cleansing materials, sprays, hair lotions, toothpaste formulations, sunscreens, eyeshadow, shampoos, moisturizer foundation, mouthwashes, bath agents, and lipstick.	(Pellis et al., 2022)
Waste treatment	Used in whey, poultry, and dairy; also, negative charge, flocculating, and decontaminating heavy metal ion removal.	(Kaczmarek et al., 2019)

7. CONCLUSIONS

Chitin and chitosan are ideal renewable materials, whether engineered, natural, or as natural compounds, with multifunctional applications. They are considered important biopolymers. Their non-toxicity, abundance, and good biocompatibility contribute to their wide use in many important biological and industrial applications, including biomedicine, bioengineering, food and agriculture, papermaking, and textiles. Furthermore, the demand for alternative materials in various fields of biotechnology and industry has significantly promoted the increasing use of biopolymers. Chitosan is the most abundant and renewable of the polysaccharides, attracting increasing research interest. Chitin is easily obtained from marine organisms, crustacean remains, insects, and some plant species. This paper reviews the structure and sources of chitosan, as well as its extraction methods. It also demonstrates the various conditions for chitosan production. This review aims to provide background and diverse information on one of the most distinctive polymeric materials in important sectors. All of this may significantly contribute to improving future research in this field. Therefore, this polymer is highly attractive for applications in multiple fields due to its properties, giving this polysaccharide a very promising future as a biomaterial. These developments include important future applications, including the development of new smart future directions for chitosan-based materials (such as composites or advanced fibers), such as their promising effects on wound healing and the release of therapeutic molecules to accelerate tissue repair and wound healing processes in the pharmaceutical and biomedical industries.

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