International Journal of Biotechnology and Microbiology
www.biotechnologyjournals.com
Online ISSN: 2664-7680
Received: 07-05-2026, Accepted: 07-06-2026, Published: 22-07-2026
Volume 8, Issue 3, 2026, Page No. 6-18

Chitosan- A versatile biopolymer: Insights into source, extraction process, characterization, properties and its use across fields

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DOI: https://doi.org/10.66856/ijbm.2026.8.3.8049

Abstract
Chitosan, a naturally occurring cationic polymer substance obtained as a result of deacetylation of chitin, a parental form, is the second most commonly used natural biopolymer next to cellulose. The main source of chitin is crustaceans like shrimp, lobster, crab, etc., followed by insects and fungi. Its non-toxic, biodegradable and biocompatible properties have extended its application in various areas. It has antimicrobial, antioxidant, anti-inflammatory, antiviral, and wound-healing properties. These properties were determined by their molecular weight and deacetylation degree. Chitosan is soluble in acidic solutions. It is non-soluble in neutral and alkaline solvents, making its application limited in many fields. The chitosan was extracted through chemical and biological methods. It typically comprises deproteination, demineralization and deacetylation. The pure chitosan was obtained through a reprecipitation process. Various techniques such as XRD, SEM and FT-IR are employed for the characterization of chitosan, which provide insights into its crystallinity, structure and purity. The different forms of chitosan have been used, which include powder, solution, flake, fiber, hydrogels, sponges, scaffolds, membranes, films and nanoparticles. Due to its diverse properties, it has a significant application in various sectors. It can be used as an antimicrobial agent, wound dressing, drug delivery, wastewater treatment, dye removal, plant regulator, bio stimulant, food packaging, animal feed, functional textile, deodorant, personal care, bioimaging and biosensor, which can be applied in biomedical, environment, agriculture, food, textile, cosmetics, pharmaceuticals, etc.

Keywords: Chitin, chitosan, degree of deacetylation, antimicrobial agent, hydrogel

Introduction

The initial discovery of chitosan was made by Rouget in 1859. Chitosan is the most abundant biopolymer globally, next to cellulose. The structure of chitosan is similar to that of cellulose, which is made up of several repeating units of β-(1-4)-linked-D-glucosamine units (Abo Elsoud & El Kady, 2019) [2]. Chitosan is a cationic polymer produced after the deacetylation of chitin. Chitin is present primarily in the exoskeletons of crustaceans, insects, arthropods, molluscs, green algae, and fungi cell walls (Iber *et al*., 2022) [34]. These organisms have varying amounts of chitin: 15-30% in crab cuticles, 20-30% in exoskeletons of crustaceans, 30-40% in prawn cuticles and 2-44% in fungal cell walls. Nowadays, the majority of chitosan utilised in industry is extracted from crustaceans, whose exoskeletons can be found easily as waste products from the food processing industries (Pellis *et al*., 2022) [76]. Annually, around 6-8 million tons of crustacean shell waste are produced (Ngasotter *et al*. 2023) [67]. Chitin may be converted into a deacetylated form, chitosan, a usable product. The conversion method comprises biological and chemical methods. For the mass production of chitosan, a low-cost chemical conversion method contributes to its dominance (Yan *et al*. 2021) [96]. The chemical conversion method comprises four processes: demineralization (removing inorganic $CaCO_3$), deproteination (removing proteins), decolouration (removing colours) and deacetylation (removing the acetyl group). Additionally, a few pre-treatments, such as grinding, washing, etc., and some post-treatment methods, such as purifying methods, may be applied to improve the quality of the final product chitosan (Pakizeh *et al*. 2021) [19]. The biological conversion techniques are fermentation and enzymatic methods. As the names suggest, enzymes break down the crustacean shell, while the other breaks it down using bacteria until only chitin remains. Biological conversions often yield chitin with a low DD and a high molecular weight, but they have difficulty in demineralization and deproteination of chitin (Kozma *et al*. 2022) [48]. Chitosan can interact with a wide range of negatively charged molecules, such as phospholipids, bile acids, fatty acids, anionic polysaccharides and proteins, as well as diluted aqueous acetic acid, lactic acid, malic acid, formic acid and succinic acid (Naghib *et al*. 2024) [66]. Chitosan has gained a lot of attention due to its potential properties. It includes mucoadhesive, anti-inflammatory, antioxidant, antimicrobial, antitumoral and wound healing (Aranaz *et al*., 2021) [8].

In addition, chitosan also possesses biodegradable, biocompatible, bioactive, nontoxic, nonallergenic and good absorption properties (Alemu *et al*., 2023) [5]. Chitosan has been applied in the field of medicine, pharmaceuticals, environment, food, cosmetics, agriculture, paper and textile industries, etc. (Thambiliyagodage *et al*. 2023) [86]. Additionally, chitosan has attracted a lot of interest from the fields of dentistry, bioimaging, veterinary medicine, cosmetotextiles and nutraceuticals (Morin-Crini *et al*., 2019) [62]. Over the past few years, chitosan production and demand have kept on increasing across a wide range of global markets. The market for chitin and its derivatives was calculated to be worth 7.1 billion USD and 7.9 billion USD in the years 2021 and 2022[19, 93], respectively, and it is expected to increase to a revised USD 24.9 billion by 2030 (Weng *et al*. 2023) [90].

The present review provides an overview of the biopolymer chitosan with its extraction process and characterisation. It also provides remarkable properties of chitosan in association with broad applications in various fields.

Occurrence of chitosan in nature and their sources

Chitosan, a naturally occurring polysaccharide, was obtained through the deacetylation of chitin, which is present in the exoskeletons of crustaceans, insect cuticles, and cell walls of some fungi (Sharkawy *et al*. 2020) [83]. However, chitin is absent in animals and higher plants (Alemu *et al*., 2023) [5]. Currently, shrimp and crab shells are used for the major production of chitin in large-scale industries (Abo Elsoud & El Kady, 2019) [2]. About 69–70% of chitin was produced from the shrimp and crab shells (Kaur and Dhillon 2014) [42]. Under X-ray diffraction studies, chitin exists in three crystalline forms: α-, β-, and γ-chitin based on their orientation, degree of hydration, unit size, and number of chains. The α-chitin is the most abundant structure in the exoskeletons of krill, lobster, crab, and insect cuticles. β-chitin will be present in diatoms and squid. γ-chitin will be present in fungi, yeast, and insect cocoons (Pellis *et al*., 2022) [76]. α-chitin was found to be the only extractable and common type of chitin among them (Alemu *et al*., 2023) [5].

1. Crustacean as a source of chitin

Exoskeletons of crustaceans from marine organism waste (e.g., shrimp, crab, or krill shells) are the most common source for the production of chitosan (Iber *et al*., 2022) [23]. These sources are less expensive, renewable, and easily available as a by-product of food processing (Hisham *et al*., 2024) [31]. Shells of crustaceans are mainly composed of chitin (20-30%), proteins (30-40%), lipids (0-14%), and minerals (30-50%) (Pellis *et al*., 2022) [76]. Chitin content in dried exoskeletons varies depending on the crustacean species (HEMMAMI *et al*., 2024) [30]. Crabs and shrimp contain 10–72% chitin and are widely used in industrial areas for chitin production (Leen *et al*.,). The rigid nature of the crustacean shell is due to the mineral-protein complex meshing with chitin. During the extraction of chitin, this mineral-protein complex will be removed by demineralization and deproteination. The final product, chitosan, was obtained by deacetylation of chitin, which involves the removal of the acetyl group from chitin (Hisham *et al*., 2024) [31].

2. Fungi as a source of chitin

Fungi are the second most commonly used source of chitin, next to crustaceans. Fungi account for 1- 15% of chitin in their cell wall and their structure is similar to that of crustaceans. Fungal chitin content will vary according to the species, seasonal conditions, and the age of the fungi (Iber *et al*., 2022) [34]. The major components of the fungal cell wall are chitin, glucans, glycoproteins, and mannans (HEMMAMI *et al*., 2024) [84]. Chitosan can be directly extracted from the fungal cell wall without a deacetylation process. *Absidia* spp., *A*. niger, *Rouxii*, *Rhizophusoryzae*, and *Lentinus edodes* are the most commonly used fungal species for direct chitosan extraction (Iber *et al*., 2022) [48]. The mycelium of the fungal cell wall was considered to have chitin. A large amount of mycelium waste was produced by biotechnological industries (Kaur and Dhillon 2014) [42]. In addition, the availability of chitin is not influenced by seasonal and geographical conditions (HEMMAMI *et al*., 2024) [30]. Chitin and chitosan are not present in all fungal species.

3. Insects as a source of chitin

In addition to crustaceans and fungi, the possible source of chitin is from insects (Pellis *et al*., 2022) [23]. Increasing the demand for chitin has increased the demand for sources. The availability of marine crustacean sources was constrained due to their seasonal and geographical obstacles (Hisham *et al*., 2024) [31]. Insect sources have some advantages over marine crustacean sources, as they are not seasonal, readily reproduce, and are highly fertile (Elsoud *et al*., 2022) [23]. Insect cuticle contains 30-45% of protein, 25-40% of fat, and 10-15% of chitin (Iber *et al*., 2022) [34]. Mineral content in insect cuticles is lower when compared with crustacean sources. This makes the demineralization process easier (HEMMAMI *et al*., 2024) [30]. Chitin content will vary in contrast to the life cycle of the insect (Leen *et al*.,). Chitosan derived from insect cuticles, is used in agricultural applications such as seed coating, plant protection, gene transferring, etc., and biomedical applications such as drug delivery (Pellis *et al*., 2022) [71].

4. Other sources of chitin

The cell walls of certain species of microalgae possess chitin and chitosan. Additionally, the investigation was conducted on green microalgae, yeast cell walls, marine diatoms, coralline algae, and mollusks, and the results were positive in chitin yield. Fish scales acquired recognition as the source of chitin (Iber *et al*., 2022) [23]. The chitin presence was shown in the cell walls of the green algae *Pithophora oedogonia* and *Chlorell*a *vulgaris.* In addition, spider contains 5-8.5% of chitin (α-chitin). In Mollusca, squid attains major consideration because of its β-chitin structure. High chitin has been reported in the squid pen (49%) (Leen *et al*.,). Wu *et al*., (2022) [53] attempted the first study on the extraction of chitosan from plant sources. It was found that leaves and peel waste of *L. cylindrica* can be used as a sustainable source of chitosan.

Table 1: Summary on source, treatment method and yield of chitin and chitosan

Source	Organism	Extraction Method	Chitin Yield (%)	Chitosan Yield (%)	Degree of Deacetylation (%)	Reference
Crustaceans	Shrimp	Microwave Radiation	36.43	90.12	95.19	(Mahdy Samar *et al*. 2013) [57]
	Shrimp	Chemical	Not specified	20.45	69.25	(Saleh *et al*. 2016) [82]
	Crab	Chemical	26.23	73.73	Not specified	(Lalit Mahatma 2021) [52]
	Crayfish	Chemical	23.72	17.50	92.06	(Kadak *et al*. 2023) [41]
Insect	Beetle	Chemical	17.7	78.26	95.09	(Nafary *et al*. 2023) [65]
	Cockroach	Chemical	Not specified	5.80	36.8	(Cheraghi *et al*. 2023) [14]
	Mealworm	Biological	85	31.9	53.9	(Jantzen da Silva Lucas *et al*. 2021) [37]

	Black soldier fly	Chemical	5.00 ± 0.002	93 ± 0.16	85.71±0.24	(Lee *et al*. 2022) [53]
Fungi	*M. pseudolusitanicus*	Biological (fermentation)	Not specified	40.59 mg/g	88.5	(Almeida *et al*. 2023) [7]
	Saccharomyces cerevisiae	Chemical	Not specified	20.85±0.35 mg/g	63.4	(Afroz *et al*. 2021) [3]
	Aspergillus niger	Chemical	16.0± 0.8	30.5 %	90.5	(Krake *et al*. 2024) [49]
Molluscs	Oyster shell	Chemical	Not specified	61.1	89.14	(Handayani *et al*. 2018) [27]
	Asian green mussel	Chemical	41.6	39.5	79.8	(Danarto and Distantina 2016) [16]

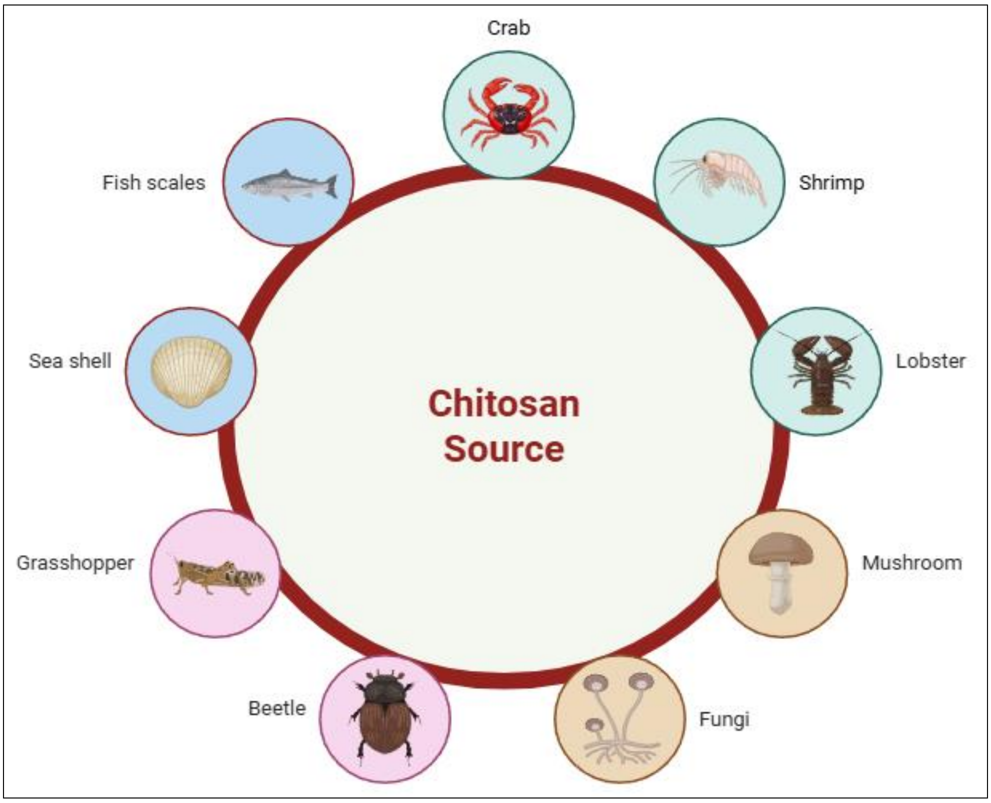

Fig 1: Various sources of chitosan including crustacean, fungi and insects.

Chitosan: Structure and Chemistry

1. Structure and Chemistry

Chitosan is a linear biopolymer made up of D-glucosamine and N-acetyl-D-glucosamine units linked by β-(1→4) glycosidic bonds. It contains amino, primary hydroxyl, and secondary hydroxyl functional groups which can be chemically modified and impact its physicochemical properties. The chitosan is easily dissolved in acidic water because the amino groups become protonated and form a positively charged polyelectrolyte configuration (Román-Doval *et al*. 2023) [80]. However, chitin is more acetylated, which means less protons can be released and makes it less soluble in acidic medium. This is one of the important properties that make chitosan attractive for various biomedical and industrial applications (Aranaz *et al*., 2021) [19].

2. Chitin vs Chitosan

Chitin is a naturally occurring polysaccharide and is the second most abundant natural polymer, after cellulose, found in many different living organisms. The polysaccharide backbone of chitin is similar to that of cellulose, but chitin contains acetamide (–$NHCOCH_3$) groups at the C2 position instead of hydroxyl groups. Chitin is mainly composed of β-(1→4)-linked N-acetyl-2-amino-2-deoxy-D-glucose (N-acetyl-D-glucosamine, GlcNAc) monomers, with a minor number of 2-amino-2-deoxy-D-glucose units (Casadidio *et al*. 2019) [11]. Chitosan has three important functional groups namely amino group (-NH_2) at C2, secondary hydroxyl group (-OH) at C3 and primary hydroxyl group (-OH) at C6 (El-Araby *et al*. 2024) [22]. Unlike cellulose, chitin and chitosan have approximately 5–8% nitrogen in their molecular structure. The nitrogen present in chitin is primarily present in the form of N-acetylated amino groups (-$NHCOCH_3$), whereas in chitosan nitrogen is mostly present in the form of primary aliphatic amino groups (-NH_2) during deacetylation. Both polymers contain these functional groups with nitrogen and are capable of undergoing several chemical reactions involving amines. However, chitosan has greater chemical reactivity than chitin because there are more free amino groups and primary and secondary hydroxyl groups in the repeating unit (Bhandari *et al*. 2020) [10].

Extraction of chitosan

Typically, chitosan is extracted by chemical and biological methods. Biological methods involve the use of microorganisms and enzymes. The crustacean source of chitin naturally contains minerals, proteins, and pigments, while the fungal and insect source has fewer contaminants, so certain stages of the extraction process were excluded (Elsoud *et al*., 2022) [23]. The main steps involved in chitosan extraction were demineralization, deproteination, discolouration and deacetylation.

1. Pre-treatment process

The pre-treatment process involves a group of steps needed to prepare for the extraction of chitin. It mainly comprises the removal of soft tissue by scraping, boiling, washing, grinding and drying (Leen Bastiaens, n.d.). Usually, shells of the crustacean were put into boiling water for 1-3 hours, then followed by drying in the oven at 160°C. Next, the dried shells were ground into fine powder (Al-Hasan Hamdan *et al*. 2020) [6].

2. Demineralization

The process of demineralization is advised to be used when the mineral content is high (Leen Bastiaens, n.d.). The demineralization process involves the use of dilute hydrochloric acid to remove calcium carbonate, calcium chloride, and other minerals (Hisham *et al*., 2024) [31]. The demineralization is essential for both crustacean and insect sources of chitin, whereas the demineralization is not necessary for fungal sources (Pellis *et al*., 2022) [76]. Biological demineralization involves the use of acid-producing bacteria or enzymes such as alcalase. The resultant acid will react with calcium carbonate and produce a precipitate, which can be removed during washing (Leen Bastiaens). Generally, lactic acid is mediated in enzymatic demineralization (Elsoud *et al*., 2022) [23].

3. Deproteinization

The deproteinization process involves the breakdown of a chemical bond between protein and chitin (Hisham *et al*., 2024) [31]. Deproteinization is typically carried out by sodium hydroxide treatment (Leen Bastiaens, n.d.). The process is completed by separating the protein from the sample through filtration (HEMMAMI *et al*., 2024) [30]. In the enzymatic method, proteolytic bacteria are used to produce proteases such as pepsin, papain, and trypsin (Leen Bastiaens).

4. Decolouration

Chitin produced during demineralization and deproteinization is a coloured substance; it must be bleached or decoloured before commercial use (HEMMAMI *et al*., 2024) [30]. Decolouration was achieved by using solvents such as acetone, sodium hypochlorite, and hydrogen peroxide (Joseph *et al*. 2021) [39].

5. Deacetylation

The deacetylation process involves the removal of an acetyl group from chitin and replacing it with reactive amino groups. The percentage of unbound amino groups in the structure can be determined with the Degree of Deacetylation (DD), which is used to distinguish chitin from chitosan (Hisham *et al*., 2024) [13]. The most effective deacetylation condition should produce chitosan without any damage and be easily soluble in diluted acetic acid quickly. This process can be repeated to obtain a higher degree of deacetylation up to 98%, but complete deacetylation cannot be attained. The degree of deacetylation depends on the alkali concentration, temperature and time (Abo Elsoud & El Kady, 2019) [2]. A polymer is considered to be chitosan when its DD value is said to be more than 60% (HEMMAMI *et al*., 2024) [30]. A good soluble chitosan should attain a degree of deacetylation of at least 85% (Abo Elsoud & El Kady, 2019) [2]. In the biological method, the deacetylase enzyme was used for the deacetylation of the chitin (Elsoud *et al*., 2022) [23].

6. Purification of chitosan

The chitosan obtained needs to be purified to make it compatible for biological use. The reprecipitation process was used to purify chitosan (Omar *et al*. 2022) [53]. During deacetylation process, impurities in the material cannot be completely removed. Therefore, purification is a key process, especially for medical and biomedical applications. However, purification is often a problem due to the high viscosity of chitosan solutions. Common contaminants include proteins, gums, salts, pigments and other residual impurities that must be removed to ensure the quality, purity and biocompatibility of the final chitosan product (Hegde and Subbalaxmi Selvaraj 2024) [29].

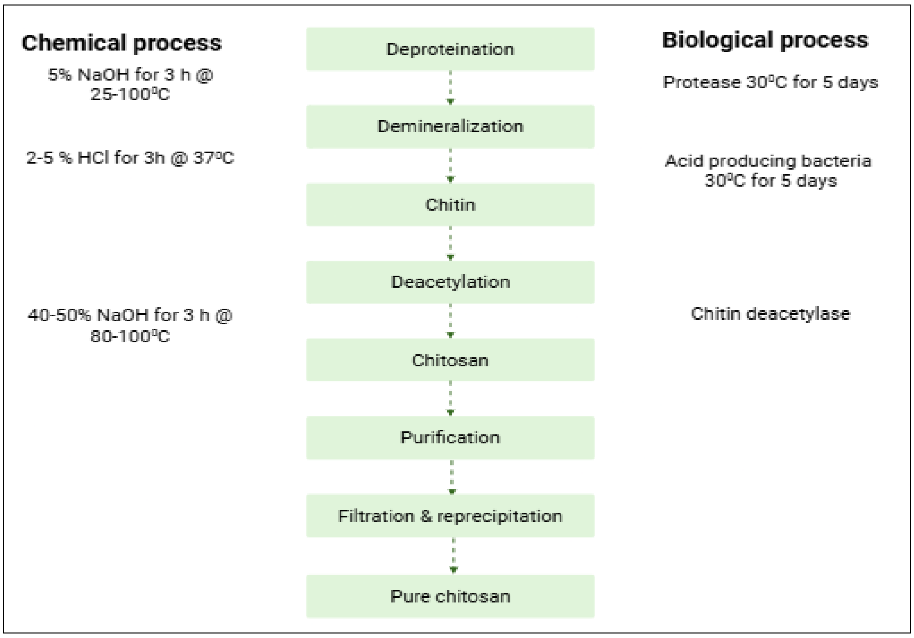

Fig 2: Chemical and biological processes of chitosan production and purification

Characterization of chitosan

Applying chitosan in different areas, such as agriculture, biomedical, and food, highly depends on its physicochemical characteristics. These characteristics are closely related to the extraction process of the chitosan and its source. The final utilization of the chitosan was determined by its physicochemical characteristics (Elsoud *et al*., 2022) [23].

1. Molecular weight

Capillary viscometry is the most extensive and simple method used to determine the molecular weight of chitosan. Molecular weight can be used as a standard parameter to determine the higher quality of chitosan (Elsoud *et al*., 2022) [23]. Measuring the molecular weight of chitosan constitutes an essential factor since it dramatically impacts the physicochemical properties (EL Knidri *et al*. 2019) [20]. Lower molecular weight chitosan is used in the food sector as an anti-tumour, antioxidant, and antibacterial substance. The lower molecular weight chitosan will readily penetrate the microbial cells and bind to RNA to stop transcription and cause cell death, while chitosan with a medium molecular weight has a higher anti-cholesterol property than a higher molecular weight chitosan (Elsoud *et al*., 2022) [23]. The relationship between chitosan molecular weight and its water solubility follows: the lower the molecular weight, the more soluble it is (Omar *et al*. 2022) [70].

2. Degree of deacetylation

The degree of deacetylation or Deacetylation degree (DD) is defined as the distribution of amino groups in the chitosan polymer chain. The activity of chitosan will differ according to its molecular weight and degree of deacetylation (Iber *et al*., 2022) [34]. Based on the degree of deacetylation, chitosan can be categorised as a higher degree of deacetylation (70-99%) and a lower degree of deacetylation (55-70%) (Joseph *et al*. 2021) [39]. Chitosan having a higher deacetylation degree will have a higher positive charge density when compared with a lower deacetylation degree, which affords strong antibacterial activity (Egorov *et al*. 2023) [18]

3. Solubility

The solubility of the chitosan was determined during the deacetylation process by removing the acetyl group (Elsoud *et al*., 2022) [23]. Chitosan is readily soluble in diluted acids such as acetic, formic, and lactic acid. Mostly 1% of acetic acid is used (pH 4) (Abo Elsoud & El Kady, 2019) [2]. The amines become protonated and positively charged when chitosan is dissolved in acidic solutions, producing soluble chitosan. It becomes insoluble and loses its charge when the pH rises to 6 or greater (Alemu *et al*., 2023) [5]. The pKa value of chitosan is approximately 6.5, and about 50% protonation of the chitosan amino groups renders the molecules soluble in acidic environments (Edo *et al*. 2025) [17]. Chitosan having a higher degree of deacetylation, will be more hydrophilic (Sharkawy *et al*. 2020) [83].

4. Zeta potential

The net charge of the particles or zeta potential reveals the level of electrostatic attraction or repulsion between charged particles (Warsito and Agustiani 2021). Ramanery *et al*., 2013[77, 89] reported that the surface charge decreased as the pH level increased, from +65 mV at pH 3.5 to around 0 mV near pH 6.0.

5. Crystallinity

The functionality of chitosan is determined by its crystallinity (HEMMAMI *et al*., 2024) [30]. The crystalline structure of the chitosan can be examined by X-ray diffraction (XRD). It is also used to determine the crystallinity index (CI), which gives the degree of purity of chitosan (Elsoud *et al*., 2022) [23]. The crystallinity index was calculated using the following equation:

$CI = I_{110} - I_{am} / I_{110} \times 100$

Where I_{am} is the intensity of amorphous diffraction and I_{110} is the maximum intensity (EL Knidri *et al*. 2019) [20]. Chitosan with a higher CI will have a rough and uneven surface, whereas lower CI chitosan will have an amorphous area and a porous surface (HEMMAMI *et al*., 2024) [30].

6. SEM

The surface morphology of the chitosan was examined using a Scanning Electron Microscope (SEM). It also reveals their microstructure (Elsoud *et al*., 2022). Kumari *et al*., 2017[23, 51] have reported that chitin from fish scales was not in a regular form, but shrimp and crab shell chitin were fibrillar and granular on the surface. Their chitosan was found to have a smooth surface. The structure of chitosan has been revealed through SEM to be far from uniform depending on the parameters of extraction and processing, the degree of deacetylation, and the origin. In general, chitosan has rough and heterogeneous surface to smooth and uniform surface structures. Some chitosan are granular or flaky-like and have irregular edges, and others are solid and continuous surfaces. (Khairi and Al-Aubadi 2023) [44]. The chitosan may have nanofibre, nanopores without nanofibers, nanopores, nanofibers without nanopores, smooth and rough surfaces (HEMMAMI *et al*., 2024) [30].

7. FT-IR

As per the literature, chitin is composed of three forms of crystalline, alpha, beta and gamma. The amide I band is used to differentiate between the alpha and beta forms. In alpha form, the amide I band will break into two bands between 1650 and 1620 cm^{-1}, whereas in beta form, the amide I band has one area at 1656 cm^{-1} (HEMMAMI *et al*., 2024) [30]. Alpha chitin is found in prawns, crabs and lobsters and also in beetle shells and fungi cell walls, while beta chitin is found in diatoms, molluscs and insects (Hou *et al*. 2021) [32]

Properties

The effective use of chitosan in different fields such as agriculture, biomedicine, food industry and textile industry depends on its properties (Elsoud *et al*., 2022) [23]. Physicochemical properties of chitosan will vary according to their source and extraction process (Iber *et al*., 2022) [53]. Along with chitosan, its derivatives have expanded their importance in various fields (HEMMAMI *et al*., 2024) [30]. The physicochemical properties of chitosan mainly depend on several factors such as Degree of Deacetylation (DD), Molecular weight, solubility and Degree of crystallinity (El-Araby *et al*., 2024) [22]. Some of the biological properties of chitosan, including nontoxicity, biodegradability, antioxidant and antimicrobial activity, make it very useful for biomedical applications (Zhao *et al*. 2018) [99].

1. Solubility

Chitin is insoluble in solvents, and deacetylation of chitin results in chitosan solubility (Jiménez-Gómez and Cecilia 2020) [38]. Chitosan is readily soluble in diluted acids such as acetic, formic, and lactic acid. Mostly 1% of acetic acid is used (pH 4) (Abo Elsoud & El Kady, 2019) [2]. The amines become protonated and positively charged when chitosan is dissolved in acidic solutions, producing soluble chitosan. It becomes insoluble and loses its charge when the pH rises to 6 or greater. The solubility of the chitosan is greatly affected by its DD, molecular weight, temperature and crystallinity (Alemu *et al*., 2023) [80]. The degree of protonation of the -NH_2 groups in the linear molecular chain increases with chitosan's DD, facilitating its dissolution in acidic aqueous conditions (El-Araby *et al*., 2024) [22]. For better solubility of chitosan, the degree of deacetylation should exceed 85%. The solubility of the chitosan can be improved by Carboxymethylation. Similarly, the solubility can be increased by quaternization of chitosan (Jiménez-Gómez and Cecilia 2020) [38].

2. Viscosity

Viscosity is the determining factor for the polymer's stability in solution. It depends on the DD and molecular weight of chitosan. Viscosity increases with an increase in DD and a decrease in molecular weight. It is also particle size and chitosan storage time dependent (Alemu *et al*., 2023) [80].

3. Biocompatibility

Chitosan is a biocompatible polymer because of its structural and functional resemblance to glycosaminoglycans found in the body's extracellular matrix (Harugade *et al*. 2023) [28]. Biocompatibility of chitosan is a vital property needed in biomedical applications. Biocompatibility is correlated with chitosan's DD. Chitosan with higher DD shows higher inflammatory responses than lower DD chitosan, revealing a slower degradation time (Kołodziejska *et al*. 2021). It makes chitosan an ideal compound for the application in anti-inflammatory treatment (Zosangpuii and Sudheer 2024) [101].

4. Biodegradability

Chitosan is a biodegradable polymer that produces nontoxic byproducts. Its degradation rate is vastly dependent on its molecular weight and DD. These factors make chitosan a suitable material for biomedical use. Chitosan degradation is mainly *in vivo* by enzymatic reaction, usually lysozyme is used (Matica *et al*. 2017) [58]. Biodegradable chitosan is emerging as a sustainable alternative to non-biodegradable polymers across various applications (Yarahmadi *et al*. 2024) [31]. The rate of biodegradation increases as the crystallinity decreases. Chitosan with smaller chains degrades more effectively than the high-mass chitosan (Kołodziejska *et al*. 2021).

5. Antibacterial activity

Chitosan is known for its antibacterial properties. It is effective against both Gram-positive and Gram-negative bacteria. The antibacterial activity of the chitosan can be determined by various factors such as DD, molecular weight, concentration, source, pH and storage temperature. The mode of action of the antimicrobial activity of chitosan is its interaction with the cell wall, cell membrane and cytoplasmic constituents of bacteria (El-Araby *et al*., 2024) [22]. It is based on chitosan's cationic nature, where the chitosan binds and interacts with DNA transcription and mRNA synthesis (Cheung *et al*. 2015) [15]. A better antibacterial activity was seen with chitosan with a low deacetylation degree and low pH. Reduction in molecular weight will boost the activity against gram-negative bacteria and lower the activity against gram-positive bacteria (Zhao *et al*. 2018) [99]. The interaction of chitosan with gram-negative bacteria will enhance the cell wall permeability, causing cell membrane rupture and cell lysis. The absence of an outer membrane for gram-positive bacteria makes the chitosan diffuse directly to the bacterial cell wall. Thus, the antibacterial activity is more effective in gram-positive bacteria when compared with gram-negative bacteria (El-Araby *et al*., 2024) [22]. Chitosan's antibacterial activity has extended its application in various fields such as textiles, food, the cosmetic industry, and others (Aranaz *et al*., 2021) [8].

6. Antifungal property

Similar to bacteria, chitosan exhibits antifungal activity against various fungi. The antifungal activity of chitosan can be controlled by the DD and the molecular weight. Chitosan can penetrate cell walls and inhibit DNA/RNA and protein synthesis (El-Araby *et al*., 2024) [22]. Chitosan shows fungistatic activity. It has been reported that chitosan is very effective in preventing spore germination, germ tube elongation and radial growth (Goy *et al*. 2009) [25]. It also interferes with ribosome synthesis and glycerophospholipid metabolism (Alemu *et al*., 2023) [5]. Usually, 1-5% of chitosan has been more effective for antifungal activity (El-Araby *et al*., 2024) [22].

7. Antiviral property

Chitosan is well known for its antimicrobial activity, but its antiviral activity is not completely documented (Akbari *et al*. 2022) [4]. Chitosan reduces the infection process, likely by preventing replication (Lizardi-Mendoza *et al*. 2016) [55]. Jaber *et al*., 2022[35] reported that studies showing chitosan with antiviral activity increase with a decrease in molecular weight and chitosan with high molecular weight shows higher antiviral activity.

8. Antioxidant property

Chitosan is well known for its intense and potent antioxidant activity. Chitosan's antioxidant activity was recognized by its well-established hydrogen-donating capacity. The better antioxidant activity was found in chitosan with a low molecular weight (El-Araby *et al*., 2024) [22]. The antioxidant activity of chitosan was associated with its molecular weight, viscosity, and concentration (Yarahmadi *et al*. 2024) [31]. Chitosan plays a vital role in free radical scavenging by inhibiting the oxidative damage produced by free radicals. By interrupting the oxidation chain reaction, chitosan's antioxidant mechanism defends the host from oxidative damage (Muthu *et al*. 2021) [64].

9. Anti-inflammatory property

Chitosan exhibits a wide range of anti-inflammatory activity. It reduces inflammation through regulating cytokine expression, immune responses and scavenging free radicals (Zosangpuii and Sudheer 2024) [101]. Depending on the concentration, administration and dosage, chitosan may have either pro- or anti-inflammatory effects. Chitosan triggers the inflammatory response by activating the innate immune system, which contains macrophages, neutrophils, and dendritic cells. It also activates the pro-inflammatory cytokines such as interleukin-1 (IL-1), interleukin-6 (IL-6), and tumour necrosis factor. Pro-inflammatory response induces the growth of new blood cells and movement of immune cells to the damaged site, making it useful for wound healing (Mawazi *et al*., 2024) [59].

10. Wound healing

Generally, wound healing involves four stages: hemostasis, inflammation, proliferation and remodelling. It is a complex process because of the large wound area, high dehydration, long healing period, reoccurrence of wound site injury, and microbial contamination risk. Chitosan exhibits its wound-healing property through haemostasis, antimicrobial activity, free radical scavenging activity and regulation of inflammatory response. Chitosan's positive charge will promote the accumulation of negatively charged erythrocytes and platelets, as well as plasma proteins such as fibrinogen. These interactions are involved in blood aggregation and hemostasis (Loo *et al*. 2022) [56].

11. Anti-biofilm property

When compared with planktonic cells, microbes that form biofilms are more resistant to antibiotics (Zhang *et al*. 2013) [98]. Chitosan's distinct antibiofilm property is mostly due to its polycationic nature. It is expected that the positively charged chitosan will electrostatically interact with the negatively charged proteins, DNA and extracellular polymeric substance (EPS) in the biofilm to restrict the growth of microbes (Khan *et al*. 2020) [46].

Applications of Chitosan

Chitosan has received significant scientific and commercial consideration because of its non-toxicity, biocompatibility, biodegradability, and antimicrobial activity. These intrinsic properties make chitosan suitable for a vast application, especially in biomedical, textile, food, agriculture, pharmaceuticals, waste wastewater treatment (HEMMAMI *et al*., 2024) [30].

1. Biomedical

Chitosan is employed as a coating on traditional biological material and has a positive influence on the acceleration of wound healing (Yadav *et al*. 2024) [95]. Chitosan wound dressing has been developed in the form of hydrogel, films, membranes, and scaffolds. Chitosan hydrogels are mainly used for maintaining the moisture condition around the wound site and also provide antimicrobial activity (Ul-Islam *et al*. 2024) [88]. Chitosan hydrogel impregnated with silver nanoparticles can significantly increase the antimicrobial and antioxidant activity and also promote wound healing. The antimicrobial, antioxidant and immunomodulatory activities of chitosan inhibit wound infections and enhance healing through tissue regeneration, offering an effective wound dressing material (Xia *et al*. 2022) [93]. Chitosan films are placed on the wound site for the treatment process. It inhibits the infection and promotes wound healing by making the wound site wet, cell proliferation and migration (Mawazi *et al*., 2024) [59].

Chitosan is an excellent option for modifying biopolymers to regulate the release of lower and higher molecular weight drugs from different drug release systems, because its positive charge will bind with negatively charged drugs and excipients (Padhi *et al*. 2022) [71]. Chitosan nanocomposites are used for drug delivery to treat cancer and osteoarthritis (Kedir *et al*. 2022) [43]. Chitosan is an effective carrier of anti-infective drugs because it depends on mucosal membranes and can extend the therapeutic action by slowing down the biodegradation rate (Xia *et al*. 2022) [93].

In tissue engineering, chitosan serves as an ideal biomaterial for creating extracellular tissue matrices and has also shown interest in the treatment of haemodialysis and renal failure (Ul-Islam *et al*. 2024) [88]. Chitosan shows structural resemblance to glycosaminoglycans found in the extracellular matrix of various human organs (Yadav *et al*. 2024) [95]. Additionally, for cartilage tissue engineering, chitosan scaffolds are used as a substitute for synthetic cell scaffolds (Morin-Crini *et al*., 2019) [63].

In addition to initial anti-adhesive properties, a highly effective antimicrobial coating should have prolonged prophylactic and antimicrobial activity. The combination of chitosan with antimicrobial agents has been studied as an ideal option to increase the effectiveness of existing drugs (Teixeira-Santos *et al*. 2021) [85]. According to the findings of Tin *et al*., (2009) [87], using chitosan in combination with antibiotic agents can help to treat the multi-drug resistant strains. Chitosan has been used for protecting orthopaedic implants and catheters from *Streptococci sp., Pseudomonas aeruginosa* and from infectious biofilm-forming methicillin-resistant *Staphylococcus aureus* (MRSA). Additionally, chitosan coating on the implant surface made up of stainless steel, titanium alloy and pure titanium can significantly reduce the number of viable biofilms of *Staphylococcus aureus* and *Staphylococcus epidermidis* (Khan *et al*. 2020) [46].

Chitosan has a wider range of properties that are crucial in the development of superior contact lenses. These properties include anti-allergenicity, wetness, mechanical durability and transparency to light and air (Yadav *et al*. 2024) [95]

2. Environment

As an environmentally friendly biopolymer, chitosan is also suggested for water purification and clarification, wastewater treatment, remediation, sludge dewatering, and membrane filtration (Morin-Crini *et al*., 2019) [62]. Chitosan is used to eliminate numerous environmental contaminants, including both organic and inorganic. Furthermore, chitosan can be used to eliminate dyes like methylene blue and methylene violet (Reshad *et al*. 2021). Elzahar & Bassyouni, 2023[24, 78] reveal that chitosan showed effectiveness in removing direct blue 78 dye from wastewater. It achieves high removal efficiency (up to 94.2%). Chitosan is used as a chelating agent to remove heavy metals such as Cu, Pb, Cd, and Hg from wastewater to purify water. It can be used to recover rare and precious metals at a high economic rate (Niu and Hu 2024) [68]. Chitosan can be applied in flocculation and coagulation in water treatment and can also be used in pre-treating seawater for the desalination process (Pal *et al*. 2021) [73]. Oil spills may be removed more easily with chitosan because it keeps the oil mass together. Chitosan, a naturally occurring polyamine, is used as a substitute for amino-functionalized polymers in CO_2 fixation (Bakshi *et al*. 2020) [9]. Chitosan may also enhance soil's physical and chemical characteristics, which support soil's structural property and water retention. This can increase soil fertility and self-purification and provide a more ideal growing environment for plants (Niu and Hu 2024) [84].

3. Agriculture

Chitosan's antimicrobial, anti-insecticidal, non-toxic and biodegradable qualities make it suitable for use in agriculture (Reshad *et al*. 2021) [78]. Chitosan has been used as a plant regulator, fertilizer, antiviral agent and preservative for agricultural products. In recent years, chitosan has been effectively applied to soil erosion, plant protection, growth promotion and enhancement of secondary metabolites (Bakshi *et al*. 2020) [9]. Chitosan has been used to protect plants from plant-pathogenic bacteria. It has a great possibility for use as a biopesticide. It may be used as a spray, a root-applied agent. These actions play an ideal role in plant disease control and stress resistance (Morin-Crini *et al*., 2019) [62]. Applying chitosan to plant roots by mixing it with soil or adding it to the hydrophobic solution was likely to act as a bio-stimulant, promoting overall plant growth and also increasing crop production (Suwanchaikasem *et al*. 2024). According to Khairy *et al*. (2022) [45, 84], chitosan is an effective and eco-friendly agent to prevent bacterial wilt in potatoes and tomatoes. It pointedly reduces the severity of infection and promotes plant growth, making it a potential biopesticide alternative to chemical pesticides.

4. Food

The US Food and Drug Administration has approved chitosan as a food additive, dietary fibre and nutritional ingredient. Due to its bioactive properties, Chitosan is utilised as an antioxidant, antimicrobial agent and antimicrobial coating on fruit and vegetables, in anticholesterolemic diet products and nutraceuticals (Morin-Crini *et al*., 2019) [62]. To ensure food safety against a broad range of pathogenic microbes, chitosan can be combined with other natural antimicrobial agents. Chitosan can be used as an active food packaging agent by coating a thin edible film over the food (Cazón and Vázquez 2019) [12]. The most promising use of chitosan for increasing the shelf life of food is in edible film coating. Many research studies on the coating of chitosan blended with other polymers that create films have maintained the coating's non-toxicity, biodegradability, compatibility with food items and functional properties. Chitosan can be utilised to clarify drinks from solid suspended particles that include proteins, polysaccharides, minerals and polyphenols. Fruit juices, wines, beer and purification of drinking water can be successfully clarified using chitosan (Kabanov and Novinyuk 2020) [40]. The study on the effectiveness of preserving strawberries using chitosan-based edible coatings enhanced with apple peel polyphenols (APP) was carried out by Riaz *et al*., (2021) [79]. According to the studies, APP-enhanced chitosan-based coatings effectively increased the shelf life and preserved strawberry quality.

5. Textile

Chitosan is used in textiles because of its non-toxic, antibacterial, biocompatible and biodegradable properties. It improves functionality and environmental friendliness in textiles by acting as a sustainable substitute for synthetic polymers (Chandran *et al*. 2024) [13]. In the textile industry, chitosan has been used as an environmentally friendly finishing agent in producing valuable functional fabrics. It has been used in textile dyeing, antibacterial, anti-wrinkle and antistatic finishing (Xiao 2018) [94].

A large proportion of chitosan is made up of amino groups, which improves the number of dye sites for anionic dyes (such as direct, acid and reactive dyes) by the treatment of chitosan with fabrics through electrostatic forces and van der Waals forces (Zhou *et al*. 2019) [100]. Thereby, in salt-free dye baths, chitosan can enhance the textile tinctorial qualities, especially their affinity for anionic dyes, colour endurance and fastness (Elamri *et al*. 2023) [21]. As a finishing agent, chitosan is mainly focused on its antibacterial properties. Only a few chitosan-based antibacterial finishing products are on the market (Hahn *et al*. 2020) [26]. Chitosan is additionally utilised to provide a long-lasting textile finish that improves its qualities. The use of anti-odour fabrics has grown, probably as a result of increased consumer health concerns, particularly in textiles such as sportswear, underwear, socks and shoes (Elamri *et al*. 2023) [21]. The fabric's anti-wrinkle property was enhanced by being finished with chitosan. The fabric was finished using a dipping-rolling-baking method in the chitosan solution. The chitosan solution gets into the fabric to create a water-insoluble layer on the surface, making the fabric stronger and less deformable (Zhou *et al*. 2019) [100]. The most common use of chitosan in textiles is antimicrobial finishing. Chitosan is utilised, individually or in combination with other antimicrobial agents, to provide various textile products (Patankar *et al*. 2022) [75]. The antimicrobial property of chitosan-coated fabric was enhanced by binding it with another antimicrobial agent like nisin. This binding performs efficiently against various bacteria and is durable even after several washing cycles, making it appropriate for medical applications (Mirhosseini *et al*. 2023) [61]. Polyester fabric is prone to static electricity, which makes the fabric stick to the skin. The need for antistatic finishing fabric is an interesting topic in the textile industry. Chitosan-finished fabric shows antistatic properties due to the existence of NH_3^+ groups and stronger polar groups (hydroxyl groups and amino groups), which allow ion conduction and water absorption. As a result, a formation of stable water film forms on the surface of the fabric. The water film enhances the surface conductivity of the fibres by dissolving CO_2 and electrolytes, aiding in static charge (Xiao 2018) [94]. Chitosan with exceptional moisture content will have a strong antistatic property. Chitosan is fixed onto the fabric to produce an antistatic fabric (Roy *et al*. 2017) [81]. Textiles can be surface modified with chitosan to develop highly reactive surfaces with several features that include deodorizing, flame retardancy, antistatic property, shrink resistance, water repellency, UV protection and antimicrobial and antiviral activities (Elamri *et al*. 2023) [21].

6. Other applications

Chitosan coatings are mostly employed in surgery; however, they can find use in the dental field due to their ability to enhance tissue healing and stabilise implants and membranes. In particular, chitosan coatings are used in dental implantation (Paradowska-Stolarz *et al*. 2023) [74]. Chitosan, being an effective biomimetic, can be used as a remineralising agent (Noaman *et al*. 2020) [69]. Using chitosan as an animal feed additive boosts the antibacterial, antioxidant and antistress properties, regulating the immunological response and improving the gut microbiota of animals (Abd El-Ghany 2023) [1]. In lithium-ion batteries, chitosan has been used as a polymeric binder for silicon graphite anodes and as an operational additive and binder for powerful lithium-sulfur batteries (Mayrén *et al*. 2024) [60]. Chitosan has also been found to be useful in wood protection, either as a single protective agent or as a multicomponent solution. Chitosan with high molecular weight reduces leaching and is a fungal resistance in treated wood (Woźniak *et al*. 2022) [91]. Chitosan's antibacterial activity has led to its function as an antifouling agent. On the other hand, chitosan paint considerably lowered the density of microfouling on a sea glider's surface (Kumar *et al*. 2019) [50]. Chitosan can be a strong, eco-friendly corrosion inhibitor by binding to metal surfaces, making it a high-potential anticorrosive agent. The corrosive property of chitosan can be enhanced by its functionalised form and composites in various corrosive conditions (El Ibrahimi *et al*. 2021) [19]. Chitosan is also quite useful in cosmetics due to its compatibility. Chitosan has been revalorising hair, lips, teeth and skin care by providing a natural and safe product for consumers' personal use (Morin-Crini *et al*., 2019) [62]

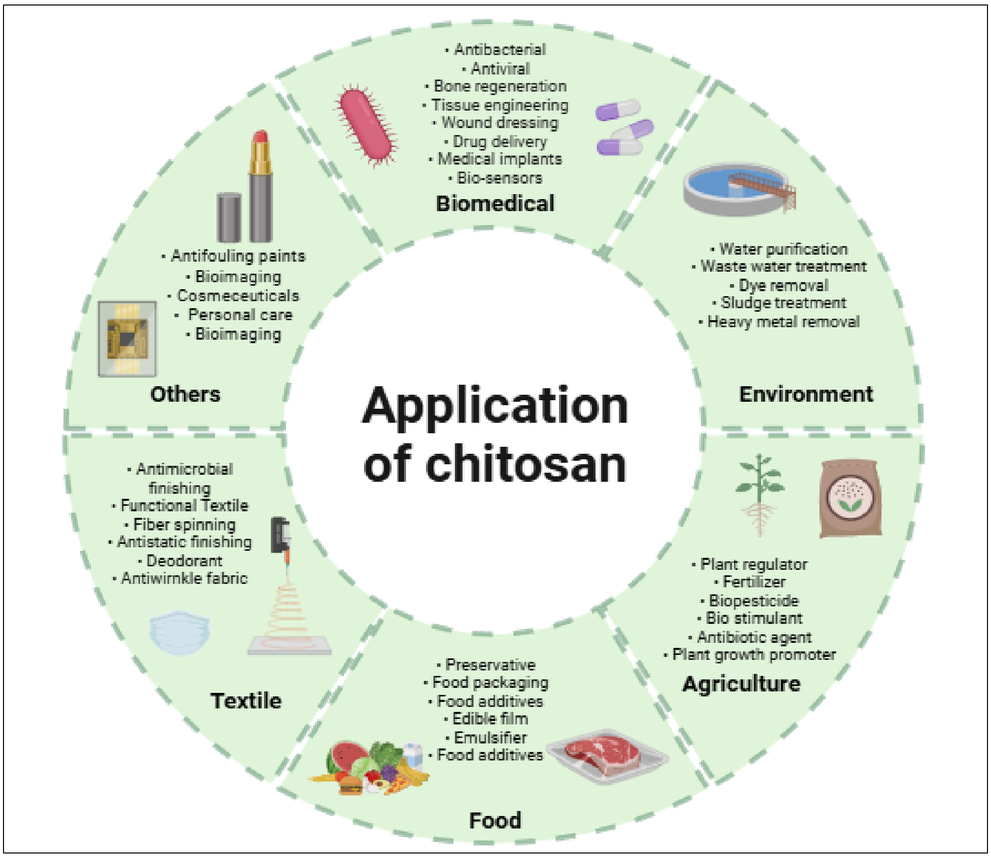

Fig 3: Various applications of chitosan and their uses

Limitations

Chitosan has gained much attention because of its characteristics and potential applications. However, chitosan is poorly soluble in basic and neutral solvents, which has restricted its use in these conditions (Aranaz *et al*. 2021) [8]. Chitosan's usage in drug delivery systems has been affected by its high hydrophilicity, thermal stability, ductility and swelling degree (Jafernik *et al*. 2023) [36]. The chitosan extraction from crustaceans itself has its limitations, such as the raw material supply, being seasonal and localized. In addition, the extraction process is time-consuming, expensive and requires caustic chemicals (Alemu *et al*., 2023) [5]. Mild to severe allergic reactions can occur from the crustacean chitosan source (Almeida *et al*. 2023) [74]. Chitosan's positive charge was affected by food components such as NaCl, proteins and starch, making chitosan neutralised. As a result, chitosan's antimicrobial efficacy in food is usually reduced (Hu and Gänzle 2019) [12].

Conclusion

Chitosan, a polycationic natural biopolymer extracted from chitin, is emerging as a promising biopolymer with a wide range of applications due to its potential physicochemical properties, including non-toxicity, biodegradability, biocompatibility and antimicrobial activity. This review highlighted its primary source, extraction process, characterisation, properties and broad application ranging from biomedical and environmental to agricultural and textile. Even though chitosan has its versatile properties and applications as mentioned in this review, there are still challenges in working, including solubility in neutral and basic pH, mechanical strength and purification methods. Future research on chitosan should attempt to develop more effective modification methods. Chitosan-based systems are needed to produce advanced 3D bioprinting, nanotechnology and targeted drug delivery systems. Additionally, chitosan can be combined with other natural or synthetic polymers to enhance its physicochemical properties. Furthermore, standardisation of extraction and purification methods is required for mass production in the industrial field. Chitosan is a sustainable and eco-friendly substance with potential properties that contribute to various scientific and industrial applications.

Credit Authorship Contribution Statement

Thangarajan and Vinitha were responsible for conceptualization, data curation, formal analysis, drafting communication, and reviewing and editing the manuscript. Muthukrishnan and Rajendran were accountable for conceptualization, data curation, formal analysis, drafting, supervision, validation, review, and editing of the manuscript.

Conflict of interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

Funding

No funding was received for conducting this study. The authors have no relevant financial or non-financial interests to disclose.

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