CHITOSAN: A VERSATILE BIO POLYSACCHARIDE WITH POTENTIAL APPLICATIONS IN THE FOOD INDUSTRY

Abstract:

Chitin is the first identified, renewable and 2nd most prevalent polysaccharide after the cellulose on the earth. It serves as a primary component of fungi cell walls, exoskeletons of arthropods, insects, molluscs, scales of fish and cephalopod beaks. Chitosan is linear deacetylated polysaccharide form of chitin, composed of glucosamine and N-acetyl glucosamine residues linked by β-1,4 bonds. Network of molecules involved rather than just one polymer, which differ in their size, composition, monomer distribution and these properties influence the physicochemical, technical and biological performance of the polymer. Since 1970, chitosan has attracted various scientific and industrial attention because of its unique macromolecular structure, biodegradability, biocompatibility and its potential intrinsic functional characteristics such as antiviral, active element of the diet for weight loss, anticancer, antifungal activity, antimicrobial properties. Chitosan and its derivatives have a wider spectrum of applications in medicine, pharmacy, agriculture, textiles and food industries. Within the health sector, chitin and its derivatives are employed as functional fibers since they possess prebiotic properties, lower blood LDL cholesterol, improve (ameliorate, enhance) glucose intolerance, boost insulin production, alleviate dyslipidemia, and safeguard gut integrity. These unique and excellent structural properties (qualities, attributes) of chitosan and its derivatives permits chitosan for unlimited food applications.

Keywords: Chitosan, antimicrobial, antioxidant, prebiotic, edible films, crustaceans

Introduction:

According to UN predictions, the world population will rise to 9.8 billion by around 2050 and 11.2 billion in 2100 (World Population Prospects, 2019) and it is expected to increase food production by about 60%."Providing enough food that is safe and sufficient for such a rapidly growing population will be extremely difficult to be achieved with the scarcity of resources and the problems posed by climate change, desertification, and the generation of greenhouse gases. Therefore, food produced from non-traditional sources is expected to increase in the next decades" (Lopez-Santamarina, 2020).

"Over past 30 years, there has been increased interest in natural polymers due to adverse effects of synthetic polymers on the health and environment (Kaczmarek et al., 2019). Chitin and its derivative like chitosan are two biological polymers have found enormous utilisation in the recent times. Chitin is the first polysaccharide identified by man" (Harkin et al., 2019) and it is the 2nd most prevalent biopolymer followed by cellulose on the earth obtained from exoskeleton of insect's cuticles (cockroach, beetle, true fly, ants, brachiopods, scorpions, and worm), invertebrates, marine crustacean shell debris, algae (green and brown algae) and in cell wall of fungi (*Aspergillus niger, Mucor rouxii, Penicillium notatum,* yeast) (Aranaz et al., 2021; Kaczmarek et al., 2019; Muthu et al., 2021; Cosme and Vilela, 2021; Jiménez-Gómez and Cecilia, 2020; Sandeep et al., 2013; Lárez-Velásquez, 2023; Lopez-Santamarina, 2020). "The term chitin comes from the Greek word "chiton" which means tunic or envelope" (Hamed et al., 2016). "It is a non-toxic, biodegradable polymer with high molecular weight which exhibits outstanding biological and chemical properties, such as biodegradability, biocompatibility, bioactivity, non-toxicity and exceptional adsorption properties. considering these unique properties, chitin can be used in different industrial and biomedical applications" (Muthu et al., 2021; Rodrigues et al., 2012; Vedula and Yadav, 2021; Gutiérrez, 2017).

Chitin and chitosan

Chitosan is a deacetylated derivative of chitin (Tajdini et al., 2010), found less frequently in nature such as in Mucoraceae fungi (Aranaz et al., 2021). It has a highly organised crystalline structure, nitrogenous, hard, colourless to off-white and inelastic (Santos et al., 2020; Cheba, 2011). Chitosan is a non-toxic, biodegradable, renewable, and biocompatible polymer made of β-1,4-glucosamine and N-acetyl glucosamine units, with the degree of polymerization (DP) level (polymerisation level) ranging from about 1000 to 3000. (Maluin et al., 2020; Tajdini et al., 2010; Kaczmarek et al., 2019; Manigandan et al., 2018; Honarkar and Barikani, 2009; Kumari and Kishor, 2020; Alvarenga, 2014; Baptista et al., 2020; Liutkevicius et al., 2015; Cheba, 2011; Guan and Feng, 2022; Kurita, 2006). "As degree of deacetylation is defined as the ratio between glucosamine/N-acetyl glucosamine. Therefore, when the percentage of glucosamine exceeds N-acetyl glucosamine the compound is called chitosan and when N-acetyl glucosamine is higher than glucosamine, where the biopolymer is called chitin" (Hamed et al., 2016). "The level of deacetylation in both chitin and chitosan ranges between 5-15% and 70-95% respectively, the higher degree of acetylation (DA) of chitin lowers solubility in common solvents" (Cheba, 2011; Guan and Feng, 2022) and limits its applications. The overall nitrogen content of both chitin and chitosan is ~7% because the deacetylation process does not eliminate the nitrogen from the polysaccharide (Pal et al., 2021).

"Various degrees of the deacetylation and molecular weights of chitosan have been widely applied in different fields such as potential food preservative of natural origin, bactericidal, antifungal, antitumor agents, drug delivery systems, carriers in DNA, enzyme inhibiting activity, adjuvant for vaccine delivery. It is also applied in food, medicine, agriculture and pharmaceutical industries. The level of extent of deacetylation and functional groups influence the effect of chitosan on bacterial activity" (Tajdini et al., 2010). The global market size of chitosan is anticipated to grow at a CAGR (Compound Annual Growth Rate) of 24.7% between 2020 and 2027. "The market for this sort of polymer is expanding in terms of practical/ commercial applications like biomedical, pharmaceutical, food and cosmetics. Recently, there has been an increased interest in the modification of polymers to extend their applicability; knowledge of the mechanisms involved in the biological activity of chitosan and its derivatives had a great interest"(Aranaz et al., 2021). "Currently, chitosan and its derivatives have practical applications in the form of solutions, suspensions, particles such as nanoparticles and sponges, beads, resins, spheres, gels/hydrogels, foams, membranes and films, fibres, microscopic threads in many fields" (Morin-Crini et al., 2019).

History of chitosan:

Charles Rouget was the first person to work on extracted chitosan from mushrooms in the year 1850 (Harkin et al., 2019). When chitin treated with a concentrated alkaline solution under reflux, he obtained a novel chitine modifiee. After many years in 1890's, Felix Hoppe-Seyler treated the shells of crabs, spiders and scorpions with a solution of KOH at $180^{\circ}C$. He obtained a soluble acid solution product named chitosan. Chitosan chemical structure was determined in 1950s. "The first record of chitosan production date back to 1970 in both countries like Japan and united states, these are the two nations dominate worlds chitosan production. The First identification of the presence of chitosan in fungal species was discovered in the year 1983. Craveiro, Craveiro, and Queiroz established the first records for manufacturing and sales of chitosan in the year1999. As a result of the increased use of natural polymers, there was a rise in interest in chitin and chitosan" (Teixeira-Costa and Andrade, 2021; Jiménez-Gómez and Cecilia, 2020; Santos et al., 2020; Lizardi-Mendoza et al., 2016).

Sources of chitosan

The average chitin content of sources is cockroaches (18–38%), krill (34–39%), lobster (16–23%), crabs (25–30%), shrimps (30–40%), butterflies (22–64%), mushroom cell walls (8–43%), yeast (1-3%), *Mucor rouxii* (30%), silkworms (20–44%), mold cell walls (8-27%), and *Choanephora cucurbitarum* fungi (28%) (Vedula and Yadav, 2021; Jones et al., 2020; Lopez-Santamarina, 2020; Yadav et al., 2019). "During processing of shrimps for human consumption, about 40 and 50% of the total mass is waste and 40% of this waste is chitin. It is uncommon for fishing industry waste to be recycled, and a significant portion of waste biomass is dumped into the environment without any prior processing. A significant portion of the biomass generated from fishing-related activities is thrown into the environment untreated, and recycling it is not a usual practice. The majority of the waste is often disposed of in seawater, which is one of the biggest sources of pollution in coastal areas, with a minor portion being dried and used as fertilizer and animal feed. As a substitute for traditional waste disposal methods, the use of shellfish waste has thus been able to address environmental issues"(Santos et al., 2020; Muthu et al., 2021). "This is an effort towards achieving a zero-waste food industry, hence benefiting both the economy and the environment" (Maluin et al., 2020; Wong et al., 2020).

"The major source of industrial level production of chitin comes from the crustacean shells, which are composed of 30–40% proteins, 30–50% mineral salts and 13–42% of chitin (α-, β-and γ-forms). The α, β forms of chitin are usually found in nature" (Santos et al., 2020; Muthu et al., 2021). In contrast to β-chitin, which has parallel sheets, α -chitin has an antiparallel crystalline structure with extensive hydrogen bonding. The third type of chitin is composed of both α and β-chitin, with α-chitin being the most prevalent type in nature.

"In α-chitin, the crystalline structure is composed of antiparallel sheets with widespread hydrogen bonding, while β-chitin consists of parallel sheets. The third form, γ-chitin is a combination of α and β-chitin with α-chitin being the most abundant in nature" (Jardine and Sayed, 2017; Hajji et al., 2014; Yadav et al., 2019).

Chitosan production

"Chitosan is the only natural cationic alkaline polysaccharide (Liu et al., 2022) made of glucosamine and N-acetylglucosamine monomeric units. Chitin is produced by various chemicals (demineralisation followed by deproteinization) and biological treatments (using enzymes and microorganisms). Decolourisation is most commonly performed to produce colourless pure chitin. A chemical procedure (use of both acids and bases) or partial deacetylation of chitin through hydrolysis by a chitin deacetylase is used to produce chitosan" (Cosme and Vilela, 2021; Harkin et al., 2019; Premasudha et al., 2015). "Certain physicochemical flaws, like protein contamination, uneven deacetylation levels, and molecular weight, affect chemical extraction. These days, chitosan is prepared using microwave irradiation in conjunction with chemical and enzymatic techniques, which speeds up chitosan production" (Guan and Feng, 2022). Chitosan can also be obtained directly from the fungi cell walls (Santos et al., 2020). "Compared to crustaceans' chitosan, fungal mycelium has no allergenic marine crustacean protein. It has low inorganic content, for which demineralization is not required when processing" (Guan and Feng, 2022).

“Largescale production of chitosan at the industrial level usually follows nonenzymatic way of deacetylation process where R-NHCOCH$_3$ residue is removed by treating chitin with strong alkali (40-50% NaOH solution) at high temperatures (100°C). When the degree of deacetylation is higher than 50 mol% (expressed as a molar percentage), the biopolymer becomes soluble in acidic aqueous solutions and is called chitosan and behaves as a cationic polyelectrolyte due to the protonation of amine groups in the presence of H$^+$ ions. Commercial chitosan has a DD of up to 90%. Molecular weight, free amino groups and degree of deacetylation significantly influences the physical, chemical properties and wider application of chitosan. Various degrees of deacetylated and molecular weight chitosanes are commercially available in the market” (Cosme and Vilela, 2021; Harkin et al., 2019; Guan and Feng, 2022). Countries like Japan, Norway, Australia, the USA, Korea, China, Canada, France, the UK, Poland and Germany are the markets for chitin and chitosan. Among all the countries, Japan has the highest market for chitosan (Hauzoukim et al., 2021; Morin-Crini et al., 2019). Chitosan is extracted from bio-waste by a variety of cost-effective techniques, making it significantly less expensive than other biopolymers. (Santos et al., 2020**).**

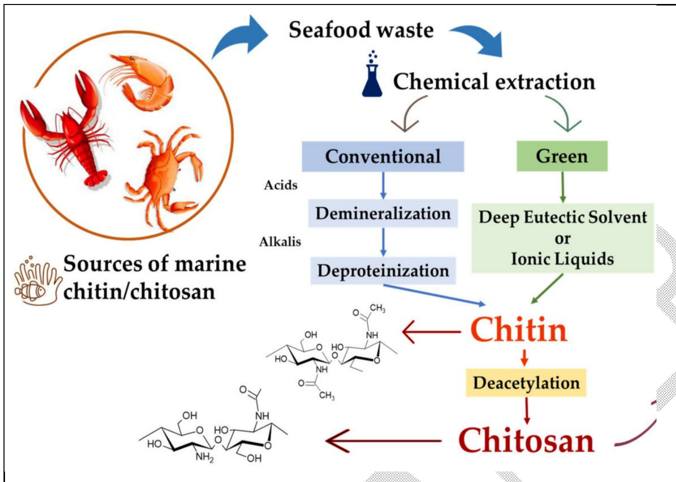

Fig 1a. Production of chitosan from marine waste (Teixeira-Costa and Andrade, 2021)

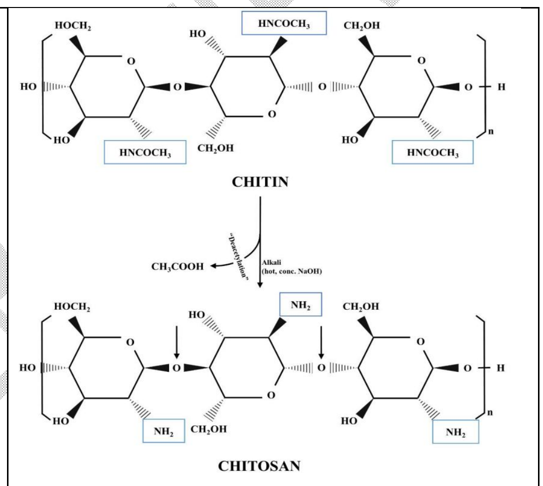

Fig 1b. Deacetylation of chitin to produce chitosan (Martau et al., 2019)

Properties of chitosan

“Various factors influence the solubility of chitosan such as molecular weight, degree of acetylation, pH, temperature, and polymer crystallinity. Chitosan becomes soluble when around 50% of all amino groups are protonated. Chitosan oligomers are soluble over a wide pH range, from acidic to basic ones. Even at high deacetylation, chitosan with high molecular weight is soluble only in the acidic aqueous media. Lack of solubility at neutral and basic pH has hindered the use of chitosan in some applications under neutral physiological conditions (i.e., pH 7.4). Therefore, a great number of chitosan derivatives with enhanced solubility have been synthesized. Viscosity can be used to determine the stability of the polymer in solution, which is also influenced by the molecular weight of the polymer and the deacetylation degree” (Aranaz et al., 2021). “The presence of free amino and hydroxyl groups improves the functionality of chitosan and so, it can be modified by various methods such as crosslinking, phosphorylation, Sulphatation, graft copolymerisation, etherification, esterification, and carboxymethylation” (Jiménez-Gómez and Cecilia, 2020; Chawla et al., 2014). Chitin derivatives have high economic value due to their biological activities and applications (santos et al., 2020). “Chitosan is an amino polysaccharide with high nitrogen content, hydrophilicity, crystallinity and high viscosity contains reactive groups for cross-linking and chemical inactivation. It is insoluble in water and organic solvents but soluble in dilute acid solvents, forms salts with organic and inorganic acids, chelating properties, ionic conductivity, film forming ability, adsorption properties and flocculating agent" (Jiménez-Gómez and Cecilia, 2020).

Regulations on chitosan

Food Drug Administration (FDA) declared chitosan to be generally recognized as safe (GRAS) (Cosme and Vilela, 2021; Morin-Crini et al., 2019). In Japan, it was approved as a food additive in 1983 and in Korea in 1995. In 2011, FDA approved chitosan from *A. niger* can be used in alcoholic beverages as a direct secondary ingredient and in 2013 it was approved by the Food Standards Agency of Australia and New Zealand. FDA also added chitosan from white button mushrooms as a "generally recognized as safe" (GRAS) material for use in foods and beverages (Lárez-Velásquez, 2023; Cheba, 2020). Chitosan is used as a food quality enhancer in several countries (Chawla et al., 2014). In the United States, Environmental Protection Agency classified chitosan as a preservative and antioxidant substance. National Health Surveillance Agency of Brazil, Agencia Nacional de Vigilância Sanitária approved chitosan as a functional health ingredient in food products (Chitosan-fortified fruit juices and chocolates) (Teixeira-Costa and Andrade, 2021). It is also approved by the European authorities as safe for consumption and sold in the form of dietary capsules to assist in weight loss (Martau et al., 2019; Chawla et al., 2014). Since 1990's chitosan has also been approved as a food additive (e.g., noodles, potato crisps, biscuits) in Japan and Korea (Hauzoukim, 2021).

Table 1. COMMERCIAL SOURCES OF CHITOSAN PRODUCTS

Company	Products
Chitosanlab	Crustacean shells and squid pens are used to produce Micronized chitin and chitosan, Chitosan nanoparticles, Chitosan quaternary ammonium
Alpha Chitin	Hermetia illucens larvae, fungi, or krill are the sources of chitosan
ChitoLytic	Crustacean, Mushroom, Chitosan lactate, Trimethyl chitosan and Chitin
Chibio Biotech	Agaricus bisporus and niger chitosan, Chitin-glucan, Oyster mushroom chitosan, Carboxymethyl chitosan
Polymar	Crustacean chitosan, Poly Floc (flocculant), Poly Protec (fungicide)
ISF Chitin & Marine Products LLP	Chitin and chitosan from marine wastes, Carboxymethyl chitosan

(Lárez-Velásquez, 2023)

Applications of chitosan in food sector

Food safety and quality are fundamental concerns for the food industry and consumers (Hu and Ganzle, 2018). Chitosan is a safe, non-toxic food additive that has been approved by the US Food and Drug Administration (FDA) for use in human food. It is also halal and kosher certified. The use of chitin and chitosan as ingredients in foods or pharmaceutical products, however, requires standardization of identity, purity, and stability (Chawla et al., 2014). "In the food and nutrition field, there is an increased demand for chitosan due to the utilisation of chitosan as a nutraceutical/functional ingredient in dietary supplements and as a natural additive with a wide range of applications in food products. Many studies have documented the countless biological activities of chitosan such as antioxidant, antimicrobial, antifungal, anti-inflammatory, wound healing, immunostimulant, and prebiotic benefits" (Teixeira-Costa and Andrade, 2021). "In the beverage industry, chitosan is used for clarification, coagulation, deacidification, stabilization, filtration membrane and removal of unwanted substances during the processing of alcoholic and non-alcoholic beverages" (Hauzoukim et al., 2021).

"Chitin and chitosan products fall within the lowest level of concern for toxicological testing. Being naturally present in living organisms, chitin and its deacetylated derivative chitosan are considered safe. The available literature on chitin and chitosan suggests a low order of toxicity, based on chemical structure and animal studies. Like several high-molecular-weight food polymers of natural origin such as cellulose and carrageenan, chitin and chitosan are not expected to be digested or absorbed from the human gastrointestinal tract. To date, chitosan appears to be clinically well tolerated" (Chawla et al., 2014).

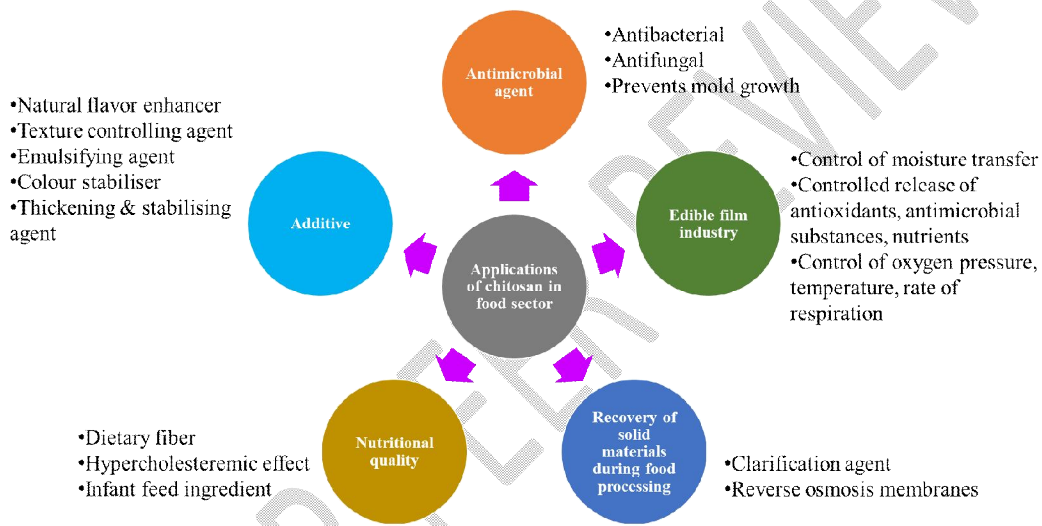

Fig 2. Applications of chitosan (Ahmed and Ikram, 2015)

Table 2. Applications of chitosan in food sector

S. No.	Applications	Chitosan concentration (g/kg)
1.	Antimicrobial edible coating for fruits and vegetables	2-10
2.	Antimicrobial packaging films	5-15
3.	Fat replacer	5-20
4.	Cholesterol reducer	1-5
5.	Food thickener and stabilizer	1-5
6.	Encapsulation carrier for food additives and flavours	2-10
7.	Dietary fibre supplement	1-5
8.	Antimicrobial agent	0.1-1

9.	Food preservation and shelf-life extension	1-5
10.	Nutrient delivery system	2-10
11.	Antioxidant delivery system	1-5
12.	Clarifying agent for juices and wines	1-5
13.	Texture enhancement	1-3
14.	Allergen control	1-3
15.	Water purification	1-5

Nutritional uses

As chitosan is a non-digestible polysaccharide in the upper gastrointestinal tract, which inhibits the absorption of carbohydrates, fats, cholesterol and promotes faster intestinal transit time. It helps to reduce the risk of chronic diseases by increasing the excretion of saturated fatty acids. Prebiotic potential of chitosan improves the gut microbiota. It is also used as a dietary ingredient to control body weight (Teixeira-Costa and Andrade, 2021).

As dietary fibre

"Prebiotics are a group of nutrients (non-digestible food ingredient) that are degraded by gut microbiota and stimulates the growth of gut microbes and thus improves host health (Davani-Davari et al., 2019). In recent years, human gut microbiota plays an important role in the maintenance of human health and control development of many non-communicable diseases. Diet has a great influence on the modulation of microbiota (Lopez-Santamarina et al., 2020). Chitin and chitosan are also considered as dietary fibres and are also naturally present in the fibrous part of tempeh" (Harkin et al., 2019).

"Chitosan extracted from crickets was evaluated for its prebiotic potential and its impact on probiotic and pathogenic gut microbes was studied by in-vitro methods. The different concentrations of (1%, 5%, 10% or 20%) chitosan supplanted media on the growth of probiotic and pathogenic bacteria were evaluated after 6, 12, 24 and 48 h upon incubation at 37°C. it was found that all chitosan concentrations significantly decreased the populations of pathogenic bacteria and increased the populations of probiotic bacteria" (Kipkoech et al., 2021).

The prebiotic potential of chito-oligosaccharide and its nano/microencapsulation for the production of functional yoghurt was done by Ismail et al. (2020). *Bacillus cereus* chitosanase was used to produce chito-oligosaccharide by enzyme hydrolysis. The developed Chito-oligosaccharide has a high degree of acetylation, solubility, prebiotic potential, good antioxidant activity but least stable in the yoghurt. So, encapsulation of chito-oligosaccharide into nanoparticles (100nm) and microparticle ((850µm) have maintained the chito-oligosaccharide 96.83±1.2 % and 45.09 ± 2.5 % respectively in yoghurt.

Hypo-cholesterolemic effect

Chitosan is used as an additive to reduce blood, liver triglycerides and cholesterol by binding with lipids. It interferes with the digestion, absorption of fat, lipid metabolism, bile acid synthesis, pancreatic lipase activity and anti-inflammatory effect, binds with micelle and bile acids in the small intestine and forms an insoluble gel-like substance which is excreted in the faeces. It may also chelate cholesterol from food and inhibits food absorption (Ahn et al., 2021; Preuss and Kaats, 2006; Lopez-Santamarina et al., 2020). One animal study found that faecal fat excretion was approximately 7.5 times higher in the chitosan fed group compared to that of a cellulose-fed group. (Gallaher et al., 2000).

"An edible low-calorie pseudo-fatty-rich meal was prepared by different formulations of chitosan and pectin to standardise a stable preparation with acceptable physical characteristics. A formulation containing 1% chitosan and 6% pectin was best acceptable and when fed to rats resulted in reduced body weight, food and water intake and reduced faecal excretion in the emulsion-administered rats" (Qinna et al., 2013).

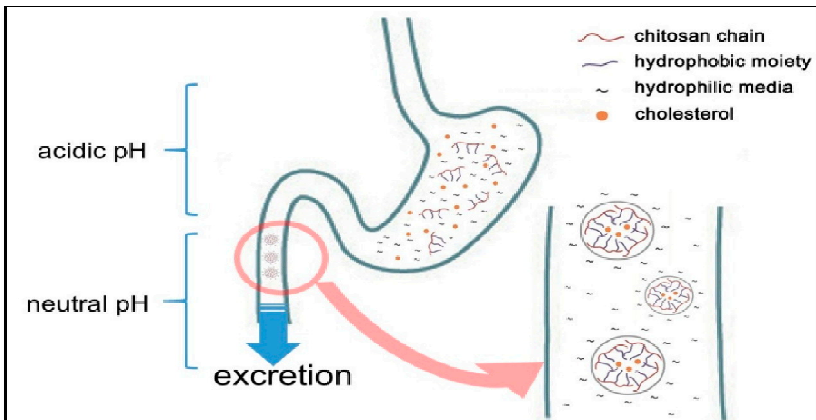

Fig 3. Cholesterol adsorption of chitosan in gastrointestinal tracts (Gallaher et al., 2000)

Applications in food industry

As an additive in bakery products

Water-soluble chitosan extracted from green mussel by H_2O_2 was used as a fat binder in cookies by Permadi et al. (2022). The study found that the addition of this chitosan found no effect on the sensory, protein, moisture or carbohydrate content of cookies, but it showed a significant on the ash and fat content. In all the cookies chitosan binds to fat in liquified butter and peanut oil used during the preparation of cookies.

The chitosan derivative such as succinyl chitosan (2g/100g) was used to replace different levels of fat in cakes. The study found that chitosan replaced cakes have the same density, crumb structure and texture to that of full fat cakes. During staling, chitosan derivative reduced the hardening rate of cakes containing half fat, although drying rate was accelerated (Rios et al., 2018).

As a functional additive in meat and meat products

"Due to antimicrobial and antioxidant properties, chitosan is used as an additive in flesh foods to control microbial growth, flavour loss, oxidation and extended shelf life. N-carboxymethyl chitosan (NCMC) and its acetate and lactate derivatives are effective in controlling oxidation and off-flavour development in cooked meat stored at refrigerated temperatures. A study conducted by the US Department of Agriculture found that NCMC is useful as a preservative in flesh foods. The use of chitosan is advantageous, as it is nontoxic, colourless and non-allergic. Prior to flash freezing it is used as a glazing compound to prevent oxidation and extends the shelf life of the product. In combination with other additives, it can be used as a preservative and to improve the textural properties of meat" (Chawla et al., 2014).

Chitosan as coating for eggs

It prevents the transfer of moisture and CO_2 from the albumin and improves the shelf life. It prevents weight loss and enhances yolk index values and Haugh unit, indicating improved yolk and albumin quality of eggs respectively. The coated eggs can be best preserved at $25^{\circ}C$ for up to 5 weeks, which is at least 3 weeks longer than that observed for uncoated eggs. Overall consumer acceptability of coated eggs did not differ from the commercial eggs (Bhale et al. 2003; Hu and Ganzle, 2018).

In a study, 1.5% of chitosan extracted from shrimp skin was tested for its preservative effectiveness in meatballs. The three treatments were given, meatballs without chitosan, meatballs coated with 1.5% of chitosan and meatballs mixed with 1.5% chitosan solution and all samples were tested using organoleptic properties during storage (1, 2, 3, and 4) in a room temperature. The study revealed that 1.5% of chitosan coating was found effective for preserving meatballs naturally (Gita et al., 2021).

Different concentration of chitosan (0%, 1%, 1.5%, 2%, and 2.5%) extracted from shrimp skin was studied for its effectiveness as preservative in sausages by Gita et al. (2022). All the five treatments were evaluated for sensory analysis and bacterial count test. The results showed that sausage with 1% chitosan edible film can maintain the quality of the sausages for three days.

"Chitosan glucose complex showed excellent antioxidant activity than glucose or chitosan alone. It has good scavenging hydroxyl and superoxide anion radicals were also very high. The antimicrobial activity of the chitosan glucose complex was similar to chitosan against common food spoilage and pathogenic bacteria such as *E. coli*, *Pseudomonas*, *Staphylococcus aureus* and *Bacillus cereus*. Incorporation of this modified chitosan into lamb meat increased its shelf life by more than 2 weeks during chilled storage and pork cocktail salami to 28 days. Due to its potential antioxidant and antimicrobial properties the chitosan glucose complex can be used as a promising novel preservative for various food formulations" (Kanatt et al., 2008).

As an emulsifying agent

Among the food-grade hydrocolloids, chitosan is an excellent emulsifier. It can be used to stabilise oil in water without the addition of any surfactant, which is due to protonated amino groups giving a positive surface charge to the chitosan under acidic conditions. This converts it into an amphiphilic substance that can adsorb bioactive substances at oil/water interfaces, thus facilitating the formation of an emulsion by lowering interfacial tension. The emulsifying capacity of chitosan, however, is highly dependent on its molecular weight and degree of deacetylation (Gutierrez, 2017).

Antimicrobial agent

Antimicrobial resistance is one of the biggest challenges. The World Health Organisation (WHO) recognises the emergence of resistance as a serious global problem. Resistance has developed to most antimicrobial agents and pesticides, thus placing an enormous burden on food security and health systems (Jardine and Sayed, 2017). Nowadays, there is an increased demand for healthy and safe food with minimal use of synthetic preservatives. Synthetic polymers are hazardous to the health and environment. And so, many studies are focussing on the development of biobased polymer as an alternative to plastic packaging (Kumar et al., 2020). "Several food antimicrobial assays and in vitro experiments have confirmed the bacteriostatic properties of chitosan. Covering the surface of food with chitosan coating or with films extended the shelf life of the food to a certain extent by resisting microbial attack and slowing the food spoilage. Chitosan with a high degree of deacetylation and low molecular weight had a better inhibition effect. The three generally accepted theories for the antibacterial properties of chitosan are due to electrostatic interaction between negatively charged groups on the bacterial surface and $R\text{-}NH^{3+}$ of chitosan which destabilizes the cell membrane structure, and the leakage of intracellular material leads to microbial death. The second idea pertains to chitosan's ability to chelate metal ions. Meanwhile, the third theory posits that chitosan penetrates cells through DNA binding, influencing RNA transcription and protein expression to provide the inhibitory impact on bacteria"(Liu et al., 2022; Luangapai et al., 2019).

"It is effective against both Gram-positive and Gram-negative foodborne microorganisms, including *Clostridium perfringens, Brochothrix spp., Aeromonas hydrophila, Bacillus cereus, B. licheniformis, B. subtilis, Enterobacter sakazakii, Lactobacillus spp., Listeria monocytogenes, Pseudomonas spp., Salmonella typhimurium, S. enteritidis, Serratia liquefaciens, Staphylococcus aureus*, and *Escherichia coli* O157H7; the molds *Aspergillus, Penicillium,* and *Rhizopus*; the yeasts *Candida, Saccharomyces,* and *Rhodotorula*. The chitosan and its derivatives are effective against plant pathogenic bacteria such as A. *tumefaciens, C. fascians, E. amylovora, E. carotovora, P. solanacearum,* and *S. lutea and fungi A. alternata, B. fabae, F. oxysporum, P. digitatum, P. debaryanum, and R. solani"*(Chawla et al., 2014).

In a study, whey protein isolates and chitosan edible films containing 4% Zataria multiflora Boiss essential oil exhibited good microbial activity against *Listeria monocytogenes, Bacillus cereus, Staphylococcus aureus, Escherichia coli* and *vibrio parahaemolyticus* (Gharehbagh et al., 2017).

Chitosan-fructose Maillard reaction products exhibited greater antibacterial activity compared to the original chitosan against S. aureus and E. coli. It resulted from the chitosan-Maillard products' disrupted the microorganism's membrane integrity, which successfully prevented bacterial cell inactivation because of their positively charged state. (Yang et al., 2020).

Edible films, coatings and packing

In the last 20 years, there has been an increase in the concentration on improving the safety and sanitation of food production. Packaging plays an important role in the protection of food from physical, chemical and biological hazards. Due to functional properties, convenience and price, plastic packing is widely used. But the use of petroleum-based packaging affects the environment and the health of consumers. So, biodegradable natural polymers widely studied for prospective applications in the food industry are chitosan (Florez et al., 2022; Manigandan et al., 2018).

Due to rising awareness about the side effects of chemical preservatives in food, scientists are looking for new alternate food preservatives. Chitosan is considered as an effective natural preservative due to its functional properties like chelating agent, film forming ability, antimicrobial, antioxidant and binding (Siddique et al., 2020). Along with protective barrier properties, edible films can also be used as carriers of bioactive compounds to enhance the quality of the food (Hamed et al., 2016).

Chitosan based films are tough, flexible, durable to extension characteristics and exhibit gas (oxygen and carbon dioxide) and aroma barrier activities that are appropriate for food packaging (Wong et al., 2020; Luangapai et al., 2019). Chitosan has antimicrobial activity against a wide range of foodborne pathogens such as fungi, yeast, and bacteria, being more active against yeasts. It can be used as an alternative to synthetic antioxidant and antimicrobial agents (Cazón and Vázquez, 2019; Aranaz et al., 2021). Chitosan films are not applicable for aqueous medium containing small amounts of organic acids like acetic acid and humid conditions. Several studies have been going on to overcome these limitations by using surface modification techniques, combining chitosan with other polymers and adding active substances like essential oils and antimicrobial agents (Luangapai et al., 2019).

Carlim et al. (2022) worked on the production of chitosan-based biodegradable active films using bio-waste enriched with polyphenol propolis (PS) extract. The study found that the addition of PS extract enhanced the film's thermal stability, mechanical properties (tensile modulus, yield strength, and stress at break), antioxidant and antimicrobial activities. The incorporation of plant polyphenols improves the poor moisture barrier properties of chitosan film, as these antioxidant compounds interact and form bonds with reactive groups of chitosan. Chitosan is used in the production of edible, antimicrobial, intelligent, active, biodegradable films and coatings. The functionality of chitosan-based films and coatings is improved by blending it with other polymers such as polysaccharides, fats and proteins (Kumar et al., 2020).

Kumar et al. (2021) studied the effect of edible coating of chitosan-pullulan (50:50) blend enriched with pomegranate peel extract on the quality and shelf life of tomatoes stored at 23 °C and 4 °C for 18 days. The study found that the edible coating of tomatoes stored at both temperatures have significantly ($p \leq 0.05$) maintained TSS, colour, titratable acidity, pH and retained higher antioxidant activity and sensory quality than the uncoated tomatoes. Overall, the shelf life of tomatoes was increased by 9 days at 23 °C and 4 °C conditions.

The process of coating, which can be done by brushing, electrostatic spraying, or dip coating, involves applying a layer of chitosan solution to the food's surface. Food preservation research frequently makes use of it because of its exceptional operability and convenience benefits. (Liu et al., 2022).

The freshness of foodstuffs is monitored by the intelligent pH indicator films, the change in pH of the food changes the colour of the indicator. By this consumer can see the condition of food. Intelligent pH-indicator chitosan-based films have been extensively studied over the last few years (Florez et al., 2022).

Effect of coating different concentration (0.5, 1, 2 and 2.5%) of chitosan extracted from shrimp shells on tomato quality was evaluated by Sree et al. (2020). The TSS, pH, titratable acidity, shrinkage and lycopene content of tomatoes were investigated for a period of 30 days at ambient temperatures. Coated tomatoes were firmer, change in pH and TSS was less than the control sample. Control tomatoes deteriorated on the 20th day of storage whereas coated tomatoes delayed ripening and extended shelf life up to 30 days. Among all the treatments 2.5% chitosan resulted in better extension of the shelf life of tomatoes than other treatments.

The chitosan extracted from prawn shell waste was analysed for its preservative potential in vegetables such as brinjal, tomato and capsicum. The study found that chitosan sprayed vegetables caused delayed ripening and extended shelf life whereas softening of texture was observed in uncoated vegetables at the end 8th day of observation (Prabha and Sivakumar, 2015).

In a study, different grafting degrees of p-hydroxybenzoic acid-grafted chitosan (PA-g-CS) conjugates synthesized by a free radical-regulated grafting approach were developed into films by casting, and their characteristics and preservative effects on fresh-cut jackfruit were evaluated. Compared to the chitosan film, the PA-g-CS film exhibited excellent performance such as enhancements of water solubility, antioxidation, and antibacterial activity. When compared to the control, application of PA-g-CS films to fresh-cut jackfruit enhanced the quality of products by inhibiting the weight loss, softening, membrane damage and maintenance of TSS, ascorbic acids contents, reduced bacterial count and a higher sensory score. Among these PA-g-CS films, the highest degree of grafting posed the best preservation effect (Jiang et al., 2022).

In a study, nano-chitosan was made with various concentrations of 3:1, 4:1 and 5:1 using ionic gelation and sprayed on fishery products. These three concentrations showed bacterial inhibition and extended shelf life of fishery products. Among three concentrations, 3:1 nano-chitosan was the best treatment. Crab shell-based nano-chitosan spray has good antibacterial activity and preservative effectiveness (Saputra et al., 2022).

"The edible coating of chitosan–pullulan (50:50) composite was prepared with pomegranate peel extract (0.02 g/mL) was coating on the mango fruits stored for 18 days at room (23°C) and cold (4°C) temperature. The study found that application of composite coating extended the shelf life of mangoes at both storage temperatures. The coating reduced the physiological loss, maintained the sensory quality, freshness, TSS, pH, phenolic content and antioxidant activity of mangoes than the control sample" (Kumar et al., 2021).

"Effect of edible coating of chitosan: pullulan (50:50) composite enriched with pomegranate peel extract on the quality, sensory attributes and shelf life of litchi fruit stored period at room and cold temperature for 18 days. The results of the study showed that the application of coating significantly reduced the physiological weight loss, TSS, acidity and browning of litchi fruits during storage days as compared to control. Coating of the blend significantly increased the shelf life of litchi stored at both temperatures" (Kumar et al., 2020).

Antioxidant properties

The chelating activity, denser intramolecular hydrogen bonds of high molecular weight chitosan, -OH and -NH2 functional groups in chitosan prevent oxidation reactions and exhibit antioxidant activity (Liu et al., 2022; Chawla et al., 2014; Baptista et al., 2020). Amino and several hydroxyl groups of chitosan interact with free radicals and exhibit scavenging activity. Some chitosan derivatives such as chitosan sulphates or N-2 carboxyethyl chitosan and Chitooligosaccharides have improved antioxidant activity due modification of polymer with phenolic or gallic acid compounds (Aranaz et al., 2021). When compared to native chitosan, sulphated and sulfanilamide chitosans exhibited high antioxidant activity which is mainly due to the enhanced water solubility and hence increased availability to free radicals (Gutiérrez, 2017).

The chitin was extracted from *Parapenaeus longirostris* shrimp shell waste and chitosan was deacetylated by classical, ultrasound method and analysed for its antioxidant activity. The degree of deacetylation of chitin (CHI), classical deacetylated chitosan (CDC) and (ultrasound-assisted deacetylated chitosan (UDC) exhibited scavenging ability on DPPH radicals ranged from 4.71% to 21.25%, 11.45% to 32.78% and 18.27% to 44.17%, respectively, at the concentrations of 0.25 to 1 mg/mL. The inhibition of lipid peroxidation with TBARS ranged from 11.7% to 51.63%, 17.24% to 63.52% and 29.31% to 77.39%, respectively (0.25 to 1 mg/mL concentration) (Hafsa et al., 2016).

Fig 4. Effect of chitosan coating on fruits and vegetables (Duan et al., 2018)

As an encapsulation matrix: The material used as an encapsulation matrix must be food grade and should possess particular physical and chemical properties to protect the encapsulated compounds. Chitosan-based microcapsules exhibited a good positive influence on the survival rates of different probiotic bacteria under *in vitro* conditions (Calinoiu et al., 2019). Both *L. gasseri* and *B. bifidum* were microencapsulated using prebiotics quercetin and chitosan as coating material in alginate microparticles. It was found that microencapsulated organisms improved survival under *in vitro* conditions (Chavarri et al., 2010). Use of chitosan as coating material and resistant starch as prebiotics increased the viability of many probiotic bacteria up to 6 months at room conditions (Iyer et al., 2005). Encapsulation of bioactive compounds with chitosan protects from oxidation. Chitosan (CS) enriched with salicylic acid (SA) treatment significantly maintained texture and colour, inhibited moisture loss and acidity change (Cheba et al., 2020).

As a clarification agent

Chitosan is used as a membrane due to its good mechanical durability, chemical resistance, high selectivity and high permeate rates in membrane technology. Chitosan is used in the treatment of wastewater. As it is used for the removal of colour and metals. It is also used to precipitate/agglomerate compounds during the processing of food wastage (Jimenez-Gomez and Cecilia, 2020). Clarification is an important step to remove pectin and other carbohydrates present in the fruit juice during processing. It can be done by microfiltration or by the use of clarifying agents. Being nontoxic, biodegradable and as a coagulation agent, chitosan was successfully used as a clarifying aid for orange, apple, grape, lemon and bayberry juices, besides wine and green tea (Kahve and Duran, 2016).

One of the most common defects affecting white wines is browning due to oxidation. It can be reduced by using adsorbents to reduce phenolic compounds. The use of chitosan for clarification of wine and vinegar exhibits a high affinity to a number of phenolic compounds, particularly cinnamic acid which prevents browning in a variety of white wines (Chawla et al., 2014).

Other applications

Colour is the important parameter that indicates the freshness and quality of the product. Many foods contain colours naturally and some food colours are enhanced by various pigments and either natural or synthetic colourants. Nowadays there is an increased interest towards natural pigments but these are sensitive to the surrounding atmosphere. Chitosan natural pigment complexes can stabilise the pigments and prevent their decay during storage (Harkin et al., 2019). Depending upon the degree of deacetylation, chitosan is suitable to be used as a thickener and stabiliser in mayonnaise and peanut butter (Harkin et al., 2019).

The concept of sustainable catalysts has been increased recently. Chitosan-based preparations loaded with metal ions and complexes as well as metal nanoparticles can be successfully used to induce different reactions, enhancing the amount of available and increasing the availability of active sites (Jimenez-Gomez and Cecilia, 2020). Some of the potential health hazards of chitosan include: allergic reactions: gastrointestinal issues, nutrient absorption, interactions with medications, risk of contaminants and long-term safety.

Conclusion

Chitosan is one of the most emerging materials for various applications. A large amount of the crustacean shell waste as a major by-product of seafood waste can be used in the production of value-added chitin and chitosan. when compared to other biopolymers, the production of chitosan by energy-efficient methods is much cheaper. It has a prominent role in the protection of the plant due to the multiple biochemical activities and variety of inexpensive sources from which can be sustainably obtained. It is an abundantly available and versatile biopolymer with a broad range of applications with numerous merits. With the use of advanced technologies, it can be comfortably moulded into different derivatives according to the end use. Chitosan based degradable membranes in conjugation with other materials have enhanced their scope.

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