Chitosan

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Contents

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K.G. Ramawat, J.-M. Mérillon (eds.), *Polysaccharides*,
DOI 10.1007/978-3-319-16298-0_13

Abstract

Chitin is the most abundant natural amino polysaccharide and is next to cellulose in abundance on the planet. Chitosan is obtained by deacetylation of chitin. Chitosan is being researched by academic and industrial scientists as an underutilized resource and as a new functional material of high potential in various fields. The purpose of this chapter is to give an overview of chitosan production, characterization, modification, and applications.

Keywords

Chitin • Chitosan • Polysaccharide • Deacetylation • Antimicrobial • Gel formation • Biopolymer • Active packaging • Metal chelation • Food additive • Biomedical • Biosorbent • Biodegradability

1 Introduction

Polysaccharides are polymeric carbohydrate molecules composed of long chains of monosaccharide units bound together by glycosidic linkages and on hydrolysis give the constituent monosaccharides or oligosaccharides. They have linear to highly branched structure. Their major role in organism is to store energy or to give structural support. Starch and glycogen are examples of storage polysaccharide and cellulose and chitin are examples of structural polysaccharides.

2 Chitin

Henri Braconnot, a French professor, discovered chitin in 1811 and named it fungine. In 1823, Odier found the same material in insects and plants and named it chitin. After cellulose, chitin is the most abundant natural polysaccharide available on the planet. Chitin is similar to cellulose in chemical structure (Fig. 1) and in biological function. Both polymers mainly serve as structural components supporting cell and body surfaces: cellulose strengthens the cell wall of plant cells, whereas chitin contributes to the mechanical strength of fungal cell walls and exoskeletons of arthropods (Rudall and Kenchington 1973).

It has been estimated that at least 1.1×10^{13} kg of chitin is present in the biosphere. However, its use has been limited because it is insoluble in most solvents and relatively difficult to isolate from natural sources in pure form under economically viable conditions. Chitin is a white, hard, inelastic, nitrogenous polysaccharide found in the exoskeleton as well as in the internal structures of invertebrates. It is a linear cationic polymer of *N*-acetylglucosamine residues with β-1,4-linkage. Chitin occurs in three polymorphic forms, α, β, and γ, but α-chitin is the most abundant (Khoushab and Yamabhai 2010). The arrangement of the chains is found to depend on the origin of the chitin. α-Chitin is present in fungal and yeast cell walls, insect cuticles, egg shells of nematodes and rotifers,

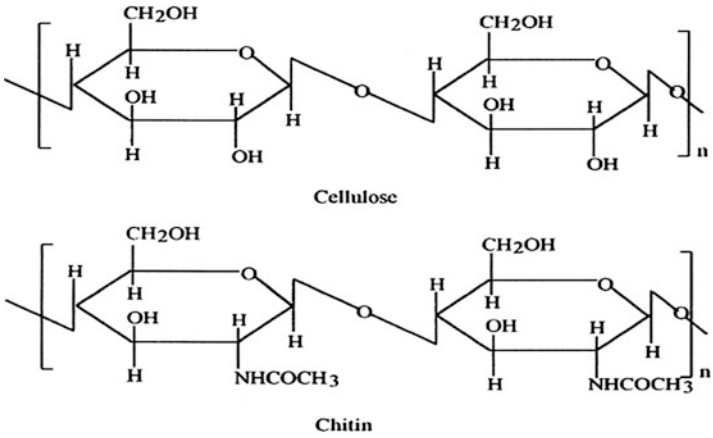

Fig. 1 Chemical structure of cellulose and chitin

the radulae of mollusks, and cuticles of arthropods. This form of chitin is also present in krill, lobster, crab tendons and shells, and shrimp shells, as well as in other marine organisms such as the harpoons of cone snails and the filaments ejected by *Phacocystis* seaweed. β-Chitin is found in the pen and cuticle of squid and the diatom *Thalassiosira fluviatilis*. In α-chitin, sheets are formed by intermolecular hydrogen bonding in parallel chains. Interchain hydrogen bonding occurs between sheets in different directions. There is also intermolecular hydrogen bonding between CH_2OH groups, which is believed to be the cause for the lack of swelling of α-chitin in water. β-Chitin has a monoclinic unit cell with polysaccharide chains attached in a parallel manner (Gardner and Blackwell 1975). In β-chitin, hydrogen bonding occurs only within sheets, not between sheets as in α-chitin. This is thought to be responsible for the swelling of β-chitin, as water can be included between the sheets. γ-Chitin is said to be a combination of α and β structure rather than a third polymorph (Robert 1992). Chitin has <10 % degree of acetylation, 7 % nitrogen content, nitrogen/carbon ratio of 0.146, and molecular weight of 1–2.5 $\times$ 10^6 Da corresponding to a degree of polymerization of ca. 5,000–10,000, which differ in the arrangement of their molecular chains.

During biosynthesis of chitin, monomers of *N*-acetylglucosamine are joined in a reaction catalyzed by the membrane-integral enzyme chitin synthase, a member of the family of glycosyltransferases. The polymerization requires UDP–*N*-acetylglucosamine as a substrate and divalent cations as cofactors. Chitin formation can be divided into three distinct steps. In the first step, the catalytic domain of chitin synthase facing the cytoplasmic site forms the polymer. The second step involves the translocation of the nascent polymer across the membrane and its release into the extracellular space. The third step completes the process as single polymers spontaneously assemble to form crystalline microfibrils of varying diameter and length (Merzendorfer 2006).

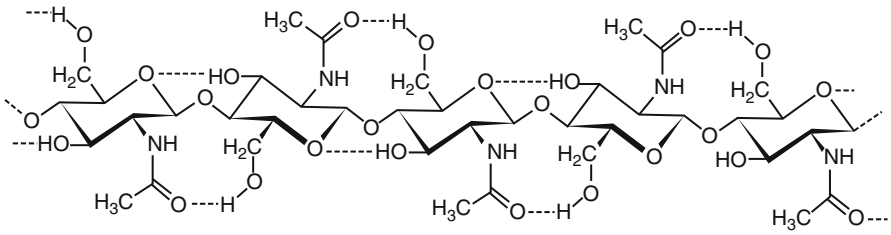

Fig. 2 Chemical structure of chitin shown with its intramolecular hydrogen bonds (*dotted lines*)

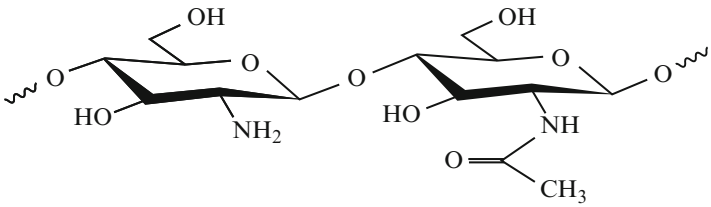

Fig. 3 Chemical structure of chitosan

In the chitin crystal structure, the chains form hydrogen-bonded sheets linked by C=O and H-N-groups. In addition, each chain has intramolecular hydrogen bonds between the neighboring sugar rings: the carbonyl group bonds to the hydroxyl group on C6. There is also a second hydrogen bond between the OH-group on C3 and the ring oxygen, similar to that in cellulose (Minke and Blackwel 1978). This extensive hydrogen bonding shown in Fig. 2 enhances the stiffness of the chitin chain.

With only one known exception, the chitin of diatoms, chitin is found in nature cross-linked to other structural components. The chitin microfibrils combine with other sugars, proteins, glycoproteins, and proteoglycans to form fungal septa and cell walls as well as arthropod cuticles and peritrophic matrices, notably in crustaceans and insects (Kozloff 1990). In animals, chitin is associated with proteins, while in fungal cell wall it is associated with glucans, mannans, or other polysaccharides. In fungal walls, it is found covalently bound to glucans, either directly or via peptide bridges (Roberts 1992). In insects and other invertebrates, the chitin is always associated with specific proteins, with both covalent and non-covalent bonding, to produce the observed ordered structures.

3 Chitosan

Chitosan is obtained by deacetylation of chitin. It consists of D-glucosamine linked to *N*-acetyl D-glucosamine by β-1,4-glycosidic bond (Fig. 3). The distribution of these subunits depends on the method of preparation of chitosan. In chitosan, degree of deacetylation ranges from 40 % to 98 % and the molecular weight ranges between 5×10^4 Da and 2×10^6 Da.

Intense research and development work is being carried out on chitosan as it is considered to be a material of great futuristic potential with immense possibilities for structural modifications to impart desired properties and functions. The presence of reactive amino groups at C2 atom and the hydroxyl group at atom C3 and C6 on chitosan is useful in a wide application in various industries. The positive attributes of excellent biocompatibility and admirable biodegradability with ecological safety and low toxicity with versatile biological activities such as antimicrobial activity and low immunogenicity have provided ample opportunities for further development.

4 Sources

Chitosan can be extracted from insects, yeast, mushroom, cell wall of fungi, and marine shellfish such as crab, lobster, krill, cuttlefish, shrimp, and squid pens (Table 1). In shellfish, chitin forms the outer protective coating as a covalently bound network with proteins and some metals and carotenoids. Shrimps are in general sold headless and

Table 1 Contents of chitin in different commercially important organism

Organism	W (chitin)%
Cancer (crab)	72.1[a]
Carcinus (crab)	64.2[b]
Paralithodes (king crab)	35.0[b]
Callinectes (blue crab)	14.0[c]
Crangon and *Pandalus* (shrimp)	17–40
Alaska shrimp	28.0[d]
Nephro (lobster)	69.8[a]
Homarus (lobster)	60–75[a]
Lepas (goose barnacle)	58.3[a]
Bombyx (silk worm)	44.2[a]
Mollusks	
Clam	6.1
Shell oysters	3.6
Squid pen	41.0
Krill, deproteinized shells	40.2
Fungi	
Penicillium notatum	18.5[e]
Penicillium chrysogenum	20.1[e]
Mucor rouxii	44.5
Lactarius vellereus	19.0

Adapted from Jo et al. (2011) and Kurita (2006)
[a]Based on the mass of the organic cuticle
[b]With respect to the body dry mass
[c]Compared to the body fresh mass
[d]Compared to the total mass of the cuticle
[e]Relative to the dry mass of the cell wall

often peeled of the outer shells and tail. Crustacean shells consist of 30–40 % proteins, 30–50 % calcium carbonate, and 20–30 % chitin and also contain pigments (astaxanthin, canthaxanthin, lutein, and β-carotene). These proportions vary with species and with seasons. Shrimp, prawn, and crab wastes are the principal source of commercial chitin and chitosan production. The increase in consumption of shellfish and the expansion of aquaculture have led to a tremendous increase in the quantity of shrimp and prawn being processed and hence in the amount of waste available for chitin/chitosan production. Using mycelium waste from fermentation processes as a source of chitin and chitosan still remains a vast and as yet untapped potential source.

5 Production of Chitosan

Majority of chitosan available globally is produced by the USA, Japan, Norway, Thailand, India, Australia, and Poland. The production of chitosan involves various steps such as preparation of the chitin from the biological material followed by the deacetylation that would result in chitosan. Thus, typical production of chitosan from crustacean shell generally consists of four basic steps: demineralization, deproteinization, decoloration, and deacetylation. Demineralization and deproteinization steps are interchangeable in terms of order. The exoskeleton of crustacean is a major starting material used for commercial production of chitosan. Typical flow chart for manufacture of chitosan is given below (Fig. 4).

5.1 Demineralization

The mineral content in the exoskeleton of crustacean is not the same for all species of crustaceans. Demineralization is generally carried out using acids such as hydrochloric acid, nitric acid, acetic acid, or formic acid (up to 10 %) at room temperature with agitation to dissolve calcium carbonate as calcium chloride. However, hydrochloric acid is the preferred acid and is used at a concentration of 0.2–2 M for 1–48 h at temperatures varying from 0 °C to 100 °C. Demineralization for 1–3 h using dilute (1–8 %) hydrochloric acid at room temperature produces appreciable amounts of calcium chloride. A solid-to-solvent ratio of 1:15 (w/v) is usually used. The ash content of the demineralized shell is an indicator of the effectiveness of the demineralization process.

5.2 Deproteinization

Chitin occurs naturally in association with protein. The protein is bound by covalent bonds to chitin through aspartyl or histidyl residues, or both, thus forming stable complexes (Attwood and Zola 1967). Deproteinization of chitin is usually carried out by alkaline treatment. The shells are treated with sodium or potassium hydroxide at 65–100 °C at a minimum shell-to-alkali ratio of 1:4 for periods ranging from 1 to 12 h. Under these conditions, the protein becomes detached from the solid

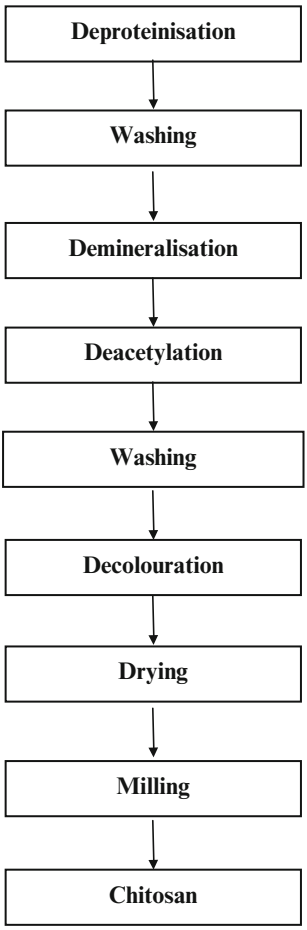

Fig. 4 Flow chart for chitosan production

component of the shrimp waste. Relatively high ratios of solid-to-alkali solution of 1/10 or 1/20 with proper agitation are used to increase the deproteinization efficiency. To prevent oxidation of the products, the process is usually carried out in a nitrogen atmosphere and in the presence of sodium borohydride ($NaBH_4$). After completion of deproteinization step, the protein hydrolysate is removed easily by separation of the solids from the protein slurry by filtration. Prolonged alkaline treatment under severe conditions causes depolymerization and deacetylation.

5.3 Decoloration

Chitin obtained after the demineralization and deproteinization of shell waste is a colored product. For commercial acceptability, the chitin needs to be decolorized or bleached to yield cream white chitin powder (No et al. 1989). The pigment in the

crustacean shells forms complexes with chitin. Fox (1973) found one 4-keto-ß-carotene and three 4, 4′-diketo-ß-carotene derivatives firmly bound to the exoskeletal chitin of red kelp crab. The level of association of chitin and pigments varies from species to species among crustacean. The residues are decolorized using solvents and/or oxidants (Acosta et al. 1993). During the process of decoloration, the chemical used should not affect the physicochemical or functional properties of chitin and chitosan. No et al. (1989) were able to prepare a near-white-colored crawfish chitin by extraction with acetone, which was dried for 2 h at ambient temperature, followed by bleaching with 0.315 % (v/v) sodium hypochloride solution (containing 5.25 % available chlorine) for 5 min with a solid-to-solvent ratio of 1:10 (w/v), based on dry shell.

5.4 Deacetylation

Deacetylation is the process to convert chitin to chitosan by removal of acetyl group. There are several critical factors that affect the extent of deacetylation including temperature and time of deacetylation, alkali concentration, prior treatments applied to chitin isolation, atmosphere (air or nitrogen), ratio of chitin to alkali solution, density of chitin, and the particle size. Considering all these as necessary conditions, the ideal process condition of deacetylation should yield a chitosan that is not degraded and is soluble in dilute acetic acid in minimal time (Muzzarelli et al. 1980). The *N*-acetyl groups cannot be removed by acidic reagents without hydrolysis of the polysaccharide, thus, alkaline methods must be employed for *N*-deacetylation (Muzzarelli 1977). Severe alkaline hydrolysis treatments are required due to the resistance of groups imposed by the trans arrangement of the C2-C3 substituents in the sugar ring. It is generally achieved by treatment with concentrated sodium or potassium hydroxide solution (40–60 %) usually at 80–140 °C for 30 min or longer using a solid-to-solvent ratio of 1:10 (w/v) to remove some or all of the acetyl groups from the polymer (No and Meyers 1989). Sodium hydroxide is the preferred alkali. After deacetylation, the chitosan is washed to completely remove alkali and is dried to give flakes. The material should be low in protein and ash. Production of chitosan by chemical processes has several disadvantages such as environmental pollution, inconsistent molecular weights, and degree of acetylation.

6 Novel Methods for Preparation of Chitosan

The conventional harsh conditions used for extraction could adversely affect the quality of the chitin. Novel methods are being developed to replace conventional demineralization and deproteinization to extract chitin from crustacean waste. The use of enzymes in the deproteinization step has been extensively studied. Shrimp waste deproteinized using *Aspergillus niger*, washed, dried, and then demineralized using acetic or lactic acid produced by fermentation from low cost biomass such as

cheese whey, has been reported (Rinaudo 2006). A number of microorganisms such as *Bacillus subtilis, Lactobacillus helveticus, Pseudomonas aeruginosa, Lactobacillus paracasei, Lecanicillium fungicola*, and *Penicillium chrysogenum* have been utilized for demineralization (Choorit et al. 2008; Oh et al. 2008). These microorganisms are responsible for the precipitation of organic salts such as calcium lactate, which is easily removed from media by wash out. Deproteinization is also carried out with the aid of proteolytic activities of some microorganisms. The calcium, magnesium, and potassium acetates obtained as by-products are suggested as possible de-iceing agents, while the calcium and potassium lactates could find applications as food preservatives. Enzymatic deacetylation by using fungal chitin deacetylase also has commercial potential.

7 Characterization of Prepared Chitosan and Its Properties

7.1 Molecular Weight

One of the most fundamental parameters characterizing a macromolecule is its molecular weight. Knowledge of the molecular weight of polysaccharides is of fundamental importance for the understanding of their applications and their role in living systems. The molecular weight of chitosan depends largely on the conditions of deacetylation and can be determined by methods such as chromatography (Bough et al. 1978), light scattering (Muzzarelli 1977), and viscometry (Maghami and Roberts 1988). Viscometry is the simplest and most popular method to determine molecular weight of chitosan. The method however has the disadvantage of not being absolute because it relies on the correlation between the values of intrinsic viscosity with those of molecular weight. Chitosan is available commercially with molecular weight ranging from 10,000 to 1,000,000 Da.

7.2 Viscosity

Viscosity of chitosan increases with increase in its molecular weight and concentration. Increasing the degree of deacetylation also increases the viscosity (Skaugrud 1991). This can be explained by the fact that high and low deacetylated chitosan have different conformations in aqueous solution. Chitosan has an extended conformation with a more flexible chain when it is highly deacetylated because of the charge repulsion in the molecule. However, the chitosan molecule has a rod-like shape or coiled shape at low degree of deacetylation due to the low charge density in polymer chain. The viscosity of chitosan solution is also affected by factors such as concentration and temperature. As the chitosan concentration increases and the temperature decreases, the viscosity increases. Chitosan viscosity decreases with an increased time of demineralization due to depolymerization (Moorjani et al. 1975). Similarly, No et al. (1999) demonstrated that chitosan viscosity is considerably affected by physical (grinding, heating, autoclaving,

ultrasonication) and chemical (ozone) treatments. Viscosity of chitosan solution stored at 4 °C is found to be relatively stable.

7.3 Solubility

Solubility characteristics of chitosan are based on its degree of deacetylation. High degree of deacetylation shows higher solubility, and low degree of deacetylation shows poor solubility (Heux et al. 2000). It has swelling characteristics due to much weaker intermolecular hydrogen bonding ascribable to the parallel arrangement of the main chains. Chitosan solubility depends on the amount of protonated amino groups in the polymeric chain and, therefore, on the proportion of acetylated and non-acetylated D-glucosamine units. Its cationic nature is unique relative to other neutral or negatively charged polysaccharides. Chitosan is a strong base possessing primary amino group with a pKa value of 6.3. The pH of solution substantially alters the charged state and properties of chitosan (Yi et al. 2005). At low pH, the amines get protonated and become positively charged and that makes chitosan a water-soluble cationic polyelectrolyte. On the other hand, as the pH increases above 6, chitosan amines become deprotonated, and the polymer loses its charge and becomes insoluble. At higher pH, precipitation or gelation tends to occur, and the chitosan solution forms poly-ion complex with anionic hydrocolloid resulting in gel formation (Kurita 1998). The soluble–insoluble transition occurs at its pKa value around pH between 6 and 6.5. Chitosan can easily form quaternary nitrogen salts at low pH values. So, organic acids such as acetic, formic, and lactic acids can dissolve chitosan. The most commonly used solvent for chitosan is 1 % acetic acid at about pH 4.0 (Rinaudo et al. 1999). Chitosan is also soluble in 1 % hydrochloric acid and dilute nitric acid but insoluble in sulfuric and phosphoric acids. Thus, solubility of chitosan is related to the degree of deacetylation, the ionic concentration, pH, the nature of the acid used for protonation, and the distribution of acetyl groups along the chain, as well as the conditions of isolation and drying of the polysaccharide. The high molecular weight of chitosan, which results in poor solubility at neutral pH and its high solution viscosity, limits its use in the food, cosmetics, agriculture, and health industry (Xia et al. 2011).

7.4 Degree of Deacetylation

Degree of deacetylation (DD) has often been cited as an important parameter that determines many physiochemical and biological properties of chitosans such as crystallinity, hydrophilicity, degradation, and cell response. Degree of deacetylation of chitosan is generally controlled by processing of the native polymer with alkali and with increasing time and temperature to obtain the highest degree of deacetylation (>90) materials. During the deacetylation reaction, the acetyl group of the chitin reacts with NaOH and produces an amine group. This is a reversible reaction, and when NaOH concentration is increased, the reaction is biased toward the forward

direction by producing more chitosan. As a result, deacetylation will increase. In the deacetylation process, acetyl groups are removed from the polymers randomly, resulting in a final polymer that has a random distribution of acetyl glucosamine and glucosamine units. The biopolymer is characterized as either chitin or chitosan according to the deacetylation which is determined by the proportion of D-glucosamine and *N*-acetyl D-glucosamine. Various methods have been reported for the determination of the degree of deacetylation of chitosan such as (1) spectroscopy (infrared, ultraviolet, or 1H, 13C, 15N nuclear magnetic resonance), (2) conventional methods (various types of titration, conductometry, potentiometry, ninhydrin assay, adsorption of free amino groups of chitosan by picric acid), and (3) destructive methods (elemental analysis or acid or enzymatic hydrolysis of chitin or chitosan) followed by colorimetric methods or high-performance liquid chromatography, pyrolysis gas chromatography, and thermal analysis using differential scanning calorimetry. Of these, 1H NMR has been found to be simple, rapid, and more precise than many of the other methods (Rinaudo 2006).

7.5 Crystallinity

One of the major physical characteristics that determine the functional properties of chitosan is the crystallinity (Trang et al. 2006). Crystallinity has been found to have an effect on metal sorption. Piron et al. (1997) found that the crystallinity of chitosan controlled the sorption rate and total uptake of uranyl, concluding that sorption was only possible in the amorphous domains and not in the crystalline domains. The crystallinity of the polymer can also control the accessibility of the amine groups (Guibal 2004). The crystallinity of chitosan is determined by X-ray diffraction (XRD) in which the pattern produced by the diffraction of X-rays through the closely spaced lattice of atoms in a crystal is recorded and then analyzed to reveal the nature of the lattice.

7.6 Complex Formation with Metals

Chitosan exhibits superior metal ion sequestering ability than chitin. It has reactive amino group and hydroxyl group and chelates many transition metal ions. Chelation is related to the amino content as well as to the distribution of the amino group. The nature of the cation is very important in the mechanism of interaction (Rhazi et al. 2002). Various processes such as adsorption, ion exchange, and chelation have been considered as the mechanisms responsible for complex formation between metal ions and chitosan. The type of interaction prevailing depends on the metal, its chemistry, and the pH. Under heterogenous conditions, at pH less than 6, chitosan acts as a poly(monodentate) ligand, while at a higher pH, it behaves as a poly(bidentate) ligand forming chelates. However, in solution, the formation of complexes in which two amino groups belonging to the same chain or different chains coordinated to the same metal ion can also take place.

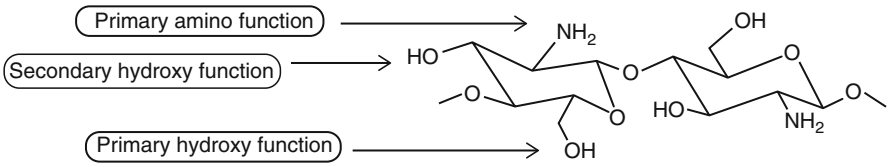

Fig. 5 Functional groups in chitosan that can be modified

8 Modifications of Chitosan

Chitosan can be modified to improve its physicochemical properties to suit various applications. Modification of chitosan is possible due to the presence of several functional groups in the polymer (Fig. 5). It has both reactive amino and hydroxyl groups that can be used to chemically alter its properties under mild reaction conditions The main goals of modifying chitosan are to provide derivatives that are soluble at neutral and basic pH values; to control hydrophobic, cationic, and anionic properties; as well as to attach various functional groups and ligands (Mourya and Inamdar 2008). Strong intramolecular and intermolecular hydrogen bonds exist in chitosan to form random orientations. The dissociation and reorganization of these hydrogen bonds by chemical modification facilitate the production of novel molecular conformations in the forms of solutions, hydrogels, fibers, films, and sponges (Tokura et al. 1996).

8.1 Acylation

A variety of acylation reactions are possible with chitosan. Acylation with long chain aliphatic carboxylic acid chlorides such as hexanoyl, dodecanoyl, and tetradecanoyl chlorides give derivatives with a high degree of acylation. *N*-acylation of chitosan with fatty acid (C6–C16) chlorides increased its hydrophobic character. Such acylated products are soluble in chloroform (Fujii et al. 1980). Chitosan with a higher degree of deacetylation is more susceptible for acylation owing to a decrease in hydrogen bonding. *N*-acyl chitosan has the ability for longer retention in body and resistance to digestible enzymes like lysozyme and chitinase and is more biocompatible than native chitosan (Hirano and Yagi 1980).

8.2 Graft Copolymerization

Graft copolymerization reaction introduces side chains and makes various molecular designs possible, thus affording novel types of tailored hybrid materials composed of chitosan and synthetic polymers. The properties of the graft copolymers can be controlled by molecular structure, length, and number of side chains attached. Grafting of chitosan allows the formation of functional derivatives by

covalent binding of a molecule, the graft, onto the chitosan backbone. The swelling behavior of chitosan at different pH has been improved by graft polymerization of vinylic monomers such as acrylic acid, acrylamide, and acrylonitrile onto chitosan (Borzacchiello et al. 2001; Mahdavinia et al. 2004). Super absorbents (absorb aqueous solutions up to hundreds of times their own dry weight) have been prepared by grafting these resins with chitosan (Nge et al. 2004) and have possible applications in infant diapers, feminine hygiene products, agriculture, and other specialized areas (Dutkiewicz 2002). Different types of chitosan graft copolymers have been prepared for use as flocculants, paper-binder strengtheners, and slow-release drug carrier. Polyethylene glycol (PEG) has been grafted onto chitosan to prepare water-soluble chitosan derivatives that have been used as carrier of anticancer drugs. Phosphorylated chitosan synthesized by grafting mono(2-methacryloyl oxyethyl) acid phosphate onto chitosan improved antimicrobial activities (Jung et al. 1999).

8.3 Carboxymethyl Chitosans

It is an amphoteric polymer, is a derivative of chitosan, and is prepared under controlled reaction conditions. It can be synthesized by reductive alkylation wherein the amino group of chitosan is reacted with the carbonyl group of aldehyde glyoxylic acid and then hydrogenated by reaction with $NaBH_4$ or $NaCNBH_3$ to give carboxymethyl chitosans. It can also be prepared by direct alkylation using monohalocarboxylic acids such as monochloroacetic acid in alkaline medium. Carboxymethyl chitosans have enhanced biological and physicochemical properties compared to chitosan and hence have promising biomedical applications (Mohan et al. 2012).

8.4 *N*-methylene Phosphonic Chitosans

These are anionic derivatives with amphoteric character and are synthesized under various conditions and proved to have good complexing efficiency for cations such as Ca^{2+} and those of transition metals (Cu (II), Cd (II), Zn (II), etc.) (Heras et al. 2001). The complexation provides corrosion protection for metal surfaces. These derivatives are also modified and grafted with alkyl chains to obtain amphiphilic properties that have potential applications in cosmetics.

8.5 Carbohydrate-Branched Chitosan

Carbohydrates can be grafted on the chitosan backbone at the C2 position by reductive alkylation: disaccharides such as cellobiose and lactose (having a reducing end group) are introduced, in the presence of a reductant, on chitosan in the open chain form. These derivatives are water soluble. Carbohydrates can also be

introduced without ring opening on the C6 position. These derivatives are important as they are recognized by the corresponding specific lectins and thus could be used for drug targeting (Morimoto et al. 2001).

8.6 Alkylated Chitosans

Alkylated chitosans are very important as amphiphilic polymers based on polysaccharides. They exhibit surface activity and increase considerably the viscosity of aqueous solution due to hydrophobic interchain interactions. Alkyl chitosans are compatible with neutral and cationic surfactants (Yang et al. 2002).

9 Chitosan Depolymerization

The main limitations in the use of chitosan in several applications are its high viscosity and low solubility at neutral pH. Low molecular weight chitosans and oligomers can be prepared by hydrolysis of the polymer chains. For some specific applications, these smaller molecules have been found to be much more useful (Rege and Block 1999). Chitosan depolymerization can be carried out chemically, enzymatically, or physically.

9.1 Chemical Depolymerization

It is mainly carried out by acid hydrolysis using HCl or by oxidative reaction using HNO_2 and H_2O_2 (Prashanth and Tharanathan 2007). It has been found to be specific in the sense that HNO_2 attacks the amino group of D-units, with subsequent cleavage of the adjacent glycosidic linkage.

9.2 Enzymatic Depolymerization

In the case of enzymatic depolymerization, low molecular weight chitosan with high water solubility is produced by several enzymes such as chitinase, chitosanase, gluconase, and some proteases (Cabrera and Cutsem 2005). Nonspecific enzymes including lysozyme, cellulase, lipase, amylase, and pectinase that are capable of depolymerizing chitosan are also used. Enzymatic methods for the hydrolysis of chitosan are performed in gentle conditions, and the molecular weight distribution of the product can be controlled (Jeon et al. 2001).

9.3 Physical Depolymerization

Physical depolymerization yielding dimers, trimers, and tetramers has been carried out by radiation (Co-60 gamma rays) but low yields have been achieved. High-pressure homogenization is a novel method employed for the depolymerization of

chitosan (Mistry et al. 2012). Chitosan has been physically modified in a variety of ways, resulting in conditioned forms such as powders, nanoparticles, gel beads, gels, fibers, and sponge (Denkbas 2006).

10 Applications of Chitosan

A lot of research is being carried out by both academic and industrial scientists on applications of chitosan. This can be seen by a number of relevant research papers and patents on the subject. Chitosan and its derivatives have varied applications in agriculture, food processing, biotechnology, chemistry, cosmetics, dentistry, medicine, textiles, veterinary medicine, and environmental sciences. The polyelectrolyte nature and the presence of reactive functional groups are responsible for the gel-forming ability, high adsorption capacity, biodegradability, and antimicrobial properties of chitosan which in turn are essential for its commercial applications.

11 Antimicrobial Activity

Chitosan displays a broad-spectrum antimicrobial activity against bacteria, molds, and yeasts. It is effective against both Gram-positive and Gram-negative foodborne microorganisms, including *Aeromonas hydrophila*, *Bacillus cereus*, *B. licheniformis*, *B. subtilis*, *Clostridium perfringens*, *Brochothrix* spp., *Enterobacter sakazakii*, *Lactobacillus* spp., *Listeria monocytogenes*, *Pseudomonas* spp., *Salmonella typhimurium*, *S. enteritidis*, *Serratia liquefaciens*, *Staphylococcus aureus*, and *Escherichia coli* O157H7; the yeasts *Candida*, *Saccharomyces*, and *Rhodotorula*; and the molds *Aspergillus*, *Penicillium*, and *Rhizopus*. 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* (Vishnukumar et al. 2005; Venugopal 2011).

The exact mechanism of antibacterial activity of chitosan is not fully understood and several factors contribute toward this. Three models have been proposed, to explain the antimicrobial action of chitosan. The most satisfactory model suggests that the antimicrobial effect of chitosan is due to its polycationic nature. In an acid environment, the NH_2 groups in the C2 position of chitosan protonates to yield NH_{3+}, which binds to negatively charged carboxylate (–COO–) groups located on the surface of the bacterial and fungal cell surfaces, causing disruption of the barrier properties of the outer membranes of the microorganisms followed by leakage of cell components (Tsai and Su 1999). This hypothesis is supported by electron microscopy studies that show binding of chitosan to outer membrane of bacteria (Raafat et al. 2008). The pH of the microenvironment in which chitosan functions determines the relative concentrations (ratios) of unprotonated and protonated amino groups. At a pH ~ pKa, 50 % of amino group are protonated. At pH 5.5, the positively charged amino group contributes 90 %, and at pH 4.5, 99 %.

The antimicrobial effectiveness of chitosan appears to be highest below pH 6.0, where the protonated form predominates and where chitosan is most soluble.

Second proposed mechanism is based on ability of chitosan to bind with microbial DNA, leading to inhibition of the mRNA and protein synthesis (Sebti et al. 2005). In this hypothesis, chitosan molecules are assumed to be able to pass through the bacterial cell wall, composed of multilayers of cross-linked murein, and reach the plasma membrane. This theory is supported by confocal laser scanning microscopy where the presence of chitosan oligomers (a chain with few number of monomer units) inside *E. coli* exposed to chitosan under different conditions has been demonstrated (Lui et al. 2001).

The third mechanism is based on ability of chitosan to chelate metals. It is well known that chitosan has excellent metal-binding capacities where the amine groups in the chitosan molecules are responsible for the uptake of metal cations by chelation; this results in reduced microbial growth and toxin synthesis (Goy et al. 2009). This mechanism is likely to be more efficient at high pH values where positive ions are bounded to chitosan, since the amine groups are unprotonated and the electron pair on the amine nitrogen is available for donation to metal ions.

The ability of chitosan to form gas-impermeable coating interferes with fungal growth. It inhibits different developmental stages such as mycelial growth, sporulation, spore viability and germination, and the production of fungal virulence factors (El- Ghaouth et al. 1992).

The derivatives of chitosan, such as *N*-trimethyl, sulfonated chitosan, and chitose oligomers, have been reported to demonstrate antibacterial activities against *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *S. epidermidis*, *Klebsiella pneumoniae*, and *Proteus vulgaris* to different extents (Venugopal 2011).

11.1 Factors Affecting Antimicrobial Activity

The antimicrobial activity of chitosan depends on its molecular weight, degree of deacylation, pH of solution, and, of course, the target organism.

Molecular weight: The antimicrobial activity of chitosan increases as the molecular weight increases. However, it is difficult to find a clear correlation between molecular weight and antimicrobial activity of chitosan when comparisons are between different studies. This is mainly attributed to the fact that many investigators have used an uncertain term for low MW (LMW) and high MW (HMW) chitosan without indicating exactly its MW. There are reports that conclude positive, negative, and neutral effects of MW on antimicrobial activity of chitosan (Badawy and Rabea 2011).

Degree of deacetylation: The antimicrobial activity of chitosan is directly proportional to the degree of deacetylation of chitosan. The increase in degree of deacetylation means the increased number of amino groups on chitosan. As a result, chitosan has an increased number of protonated amino groups in an acidic condition

and dissolves in water completely, which leads to an increased chance of interaction between chitosan and negatively charged cell walls of microorganisms (Sekiguchi et al. 1994).

The pH: The antimicrobial activity of chitosan is strongly affected by the pH. At lower pH, there is an increase in the number of protonated amino groups on chitosan in addition to the "hurdle effect" of inflicting acid stress on the target organisms (Badawy and Rabea 2011).

Temperature: The incubation temperature also has an effect on the antimicrobial activity of chitosan. Higher temperature (37 °C) has been shown to enhance its antimicrobial activity compared to refrigeration temperatures (Kong et al. 2010).

Cations: Antimicrobial action of chitosan is inhibited by divalent cations in the order of $Ba^{+2} > Ca^{+2} > Mg^{+2.}$ It is proposed that the cations form complexes with chitosan and consequently the reduced available amino groups of chitosan lead to the reduced bactericidal effect (Badawy and Rabea 2011).

Chitosan posses a number of characteristics that make it a suitable antimicrobial polymer for various industrial applications. These include the following: (1) easy and abundant availability, (2) long-term storage stability at the temperature of its intended application, (3) it does not decompose to and/or emit toxic products, (4) it is not toxic or irritating to handlers, and (5) it is biocidal to a broad-spectrum of pathogenic microorganisms.

12 Antioxidant Activity

Chitosan and its derivatives have been reported to have strong antioxidant activity. They control lipid oxidation by scavenging free radicals, which can be attributed to their ability to chelate metals. The antioxidant effects of chitin and chitosan are dependent on their molecular weight, viscosity, and degree of deacetylation (Venugopal 2011).

13 Chitosan as Edible Coating for Fruits and Vegetables

The edible films and coatings are used to extend shelf life and improve quality of food products. At present, edible films based on cellulose and proteins are being used for the purpose. They provide good reduction of O_2 and CO_2 partial pressure but are not so good for moisture transfer between food and the surrounding environment. Chitosan forms tough, long-lasting, flexible, semipermeable films that can be used as food wraps for extending their shelf life.

Fruits and vegetables undergo a number of physiological changes during postharvest storage. These include tissue softening, increase in sugar levels, degradation of chlorophyll, and synthesis and degradation of volatile flavor compounds. Controlling respiration rate significantly improves the storability and shelf life of fresh produce, as a certain level of respiration activity is required to prevent plant tissues from senescing and dying. In minimally processed agricultural

products, the most important quality attributes contributing to marketability are appearance, color, texture, flavor, nutritional content, and microbial quality. The marketability of these products, therefore, demands efficient control of these quality changes. Due to its barrier properties, chitosan film can prevent moisture loss and drip formation, retain color and flavor attributes, and improve microbial quality, thereby extending the shelf life of a variety of fruits and vegetables. Rather than packaging produce within a chitosan film, dipping the produce in a dilute solution of chitosan and dilute acetic acid can be performed. The technique also allows the incorporation of additives such as vitamin E, rosemary, oleoresin, calcium, and potassium to enhance the efficiency of treatment (Aider 2010). The efficacy of treatment is demonstrated in strawberries, bell peppers, cucumbers, peaches, pears, and kiwifruit (Bautista-Banosa et al. 2006).

14 Chitosan as Functional Additive in Muscle Foods

Chitosan is used as an additive in flesh foods to control flavor loss, microbial growth, and oxidation resulting in extended shelf life. When cooked flesh foods are stored, a "warmed-over" flavor develops which is perceived as loss of freshness. Chitosan is capable of preventing this flavor deterioration due to its antioxidant activity (No et al. 2007; Venugopal 2011). *N*-carboxymethyl chitosan (NCMC) and its lactate and acetate derivatives are effective in controlling the oxidation and off-flavor development in cooked meat at refrigerated temperatures. Research by the US Department of Agriculture has revealed that NCMC is useful as preservative in flesh foods. It can be sprinkled on gravies or meat products. NCMC is very useful in preserving microwavable or quickly prepared foods as well as in preventing development of the "warmed-over" flavor of institutional foods. It is advantageous to use as it is itself tasteless, blends well with foods as a colorless ingredient, and is nontoxic and nonallergenic. It is used as a glazing compound prior to flash-freezing of many flesh foods to inhibit surface oxidation and enhance shelf life. Meat and poultry processors use NCMC as a post-slaughter perfusion and as a long-term flavor and storage preservative (Flick and Martin 2000). Textural properties of surimi products can also be improved by addition of chitosan in combination with other additives (Benjakul et al. 2001; Gomez-Guillien et al. 2005).

Chitosan as coating for eggs: Chitosan coating of eggs can provide a protective barrier against moisture and CO_2 transfer from the albumen through the egg shell, thus extending the shelf life of eggs. It prevents weight loss and enhances Haugh unit and yolk index values, indicating improved albumen and yolk quality of eggs, respectively. The coated eggs can be preserved for up to 5 weeks at 25 °C, which is at least 3 weeks longer than that observed for control, uncoated eggs. Overall consumer acceptability of coated eggs did not differ from that for control and commercial eggs (Bhale et al. 2003).

Chitosan as additive in bakery and dairy products: Chitosan and chitin can be used as food additives in cookies, noodles, and bread to improve their texture. These effects are due to the ability of chitosan to control starch retrogradation.

Microcrystalline chitin has a positive effect on emulsion stability, in addition to increasing the specific loaf volume of white bread and protein-fortified breads (No et al. 2007). Maillard reaction products (MRPs) prepared from chitosan and xylose extend the shelf life of fresh noodles (Huang et al. 2007). Chitosan–lysozyme (CL) film is reported to prevent growth of *Listeria monocytogenes*, *Escherichia coli*, or *Pseudomonas fluorescens* in pre-inoculated mozzarella cheese (Duan et al. 2007).

15 Chitosan as Clearing Agent in Wines and Vinegars

Browning due to oxidation is one of the most common defects affecting white wines. It can be minimized by using adsorbents to reduce phenolic compounds. Chitosan is useful for the clarification of wine and vinegars. It exhibits high affinity to a number of phenolic compounds, particularly cinnamic acid, and prevents browning in a variety of white wines (Spagna et al. 1996).

16 Chitin as Feed Additive

Chitin has a growth-promoting effect on broiler chickens. It increases average live weight and dressed weight and decreases wastage during dressing in broiler chickens. The use of chitin as a source of dietary fiber in chicken feed promotes the growth of bifidobacteria in the guts (Hirano et al. 1990). Similarly, feeds containing chitin and glucosamine could also be used in aquaculture for improved growth of cultured fish (Kono et al. 1987). Chitin hydrolysates produced through the digestion of crustacean waste by chitinases are used as a carbon source for the cultivation of yeast that can convert chitin oligosaccharides into single-cell proteins (Carroad and Tom 1978). The yeast could be utilized as feed component.

17 Chitosan as Lipid-Lowering Agent

Chitosan is used as a dietary ingredient due to its ability to reduce serum cholesterol. It reduces lipid absorption by binding neutral lipids, such as cholesterol and other sterols, by means of hydrophobic interactions. Because of this inhibitory activity on fat absorption, chitosan acts as fat scavenger in the digestive tract and eliminates fat and cholesterol via excretion (Luo and Wang 2013). Chitosan satisfies the requirements of dietary fiber, including non-digestibility in the upper GI tract, high viscosity, and high water-binding ability in the lower GI tract. From a physiological standpoint, the prime function of a dietary fiber is to lower cholesterol levels and to promote the loss of body weight through a reduction of intestinal lipid absorption. It differs from other dietary fibers in that it possesses a positive ionic charge, which has the ability to bond chemically with the negatively charged lipids, fats, and bile acids. It is desirable that its prolonged use as fiber in diets should be

monitored to ensure that it does not disturb the intestinal flora or interfere in the absorption of micronutrients, particularly lipid-soluble vitamins and minerals, and that it does not have any other negative effects. Chitosan shows an LD_{50} (median lethal dose) of around 16 g/kg, comparable to the salt and glucose values ensuring safety for long-term use (Singla and Chawla 2001).

18 Biomedical Applications

Chitosan due its polyelectrolyte nature, gel-forming capability, biodegradability, biocompatibility, nontoxicity to living tissues, and antimicrobial and antitumor properties has extensive applications in medicine. It is used in hemodialysis membranes, artificial skin, hemostatic agents, and drug delivery systems. The property of chitosan to form gels at a slightly acid pH gives chitosan its antacid and antiulcer activities. Chitin and chitosan oligosaccharides, when intravenously injected, enhance antitumor activity by activating macrophages.

Chitosan as control release system: Chitosan has an advantage of forming covalent or ionic bonds with the cross-linking agents, building a sort of network, where the active substance is retained. Consequently, these bonds carry advantages in terms of controlled release (Estevinho et al. 2013). Depending on the cross-linker, the major interactions involved in the formation of the network are covalent or ionic bonds. Covalent cross-linking leads to the formation of hydrogels or microparticles with a permanent network structure, because irreversible chemical bonds are formed. This type of linkage allows absorption of water and/or bioactive compounds without dissolution and allows its release by diffusion. The addition of a second polymer as encapsulating agent makes possible the pH-controlled delivery (Berger et al. 2004). Cross-linking compounds used to create covalent bonds are molecules that have at least two reactive functional groups that allow the formation of linkage between polymeric chains. The most common cross-linkers used with chitosan are dialdehydes, such as glyoxal and in particular glutaraldehyde. But they are known to be toxic. For example, glutaraldehyde is known to be neurotoxic and glyoxal is known to be mutagenic. Hence, even if microparticles are purified before usage, the presence of free unreacted dialdehydes cannot be completely excluded and will induce toxic effects (Estevinho et al. 2013). Other covalent cross-linkers for chitosan such as diethyl squarate, oxalic acid, or genipin have been investigated to overcome this problem. Ionically cross-linked microparticles or hydrogels are more biocompatible and well tolerated. Ionically cross-linked chitosan hydrogels or microparticles exhibit a greater swelling sensitivity to pH changes compared to covalently cross-linked ones. This fact broadens their potential application, since dissolution can be regulated by pH conditions (Berger et al. 2004).

Chitosan with its positive charges reacts with polyanionic compounds forming polyelectrolytic complexes that can easily incorporate active substances. Tripolyphosphate, citrate, sulfate, and phosphate are used to prepare this kind of complexes. They are normally well tolerated and biocompatible with the human

organism, showing advantages in terms of applications for food and pharmaceutical industry (Berger et al. 2004; Gupta and Jabrail 2006).

Biotechnological application of chitosan: Chitin and chitosan have been found to be useful as a matrix for immobilization of various enzymes for the processing of such products as wine and sugar, the synthesis of organic compounds (Ravikumar 2000), and the construction of sophisticated biosensors for in situ measurements of environmental pollutants and metabolite control in artificial organs (Krajewska 2004).

Chitosan as drug delivery matrix: Chitosan is considered to be the drug carrier for the twenty-first century. For effective drug delivery, it is being used in the form of microspheres, microparticles, nanoparticles, granules, gels, or films. Chitosan microspheres are useful for the controlled release of antibodies, antihypertensive agents, anticancer agents, protein and peptide drugs, vaccines, and nutraceutical compounds (Dash et al. 2011).

Chitosan as wound healing agent: Due to bacteriostatic and fungistatic properties of chitosan, it is used as a wound healing agent in skin ointments. Chitosan implanted in animal tissues encourages wound healing and hemostatic activities. Biocompatible wound dressings derived from chitin are available in the form of hydrogels, xerogels, powders, composites, and films (Gavhane et al. 2013).

19 Chitosan in Water Treatment

Water gets polluted due to metal ions, inorganic anions, phenolic compounds, dyes, and radioactive isotopes. Many of these water pollutants are toxic and can enter the human food chain. The toxic heavy metal ions are discharged into the environment through different industrial activities. The high adsorption potential of chitosan is attributed to (1) high hydrophilicity due to a large number of hydroxyl groups of glucose units, (2) the presence of a large number of functional groups, (3) the high chemical reactivity of these groups, and (4) flexible structure of the polymer chain.

Chitosan and its derivatives are being successfully used in water treatment to remove lead, copper, and cadmium from drinking water, due to complex formation between the amino group and heavy metal ions (Bhatnagar and Sillanpää 2009).

Radionuclides are an important category of metals in terms of environmental impact and interest from nuclear industry. Chitosan is an excellent biosorbent to adsorb radionuclide from aqueous solution in an acid environment (Wang and Chen 2014).

Dyes are usually present in the effluents of textile, leather, paper, and dye manufacturing industries. These effluents are not only toxic to the aquatic biota but also disturb the natural equilibrium by reducing photosynthetic activity of water in streams. Some dyes are reported to cause allergy, dermatitis, skin irritation, and cancer in humans. The removal of dyes from effluents before they are released into natural water bodies is important. Chitosan-based biosorbents have an extremely high affinity for many classes of dyes (Crini and Badot 2008).

Phenol and substituted phenols cause unpleasant taste and odor in drinking water and can exert negative effects on different biological processes. The ubiquitous nature of phenols, their toxicity even in trace amounts, and the stricter environmental regulations make it necessary to develop processes for the removal of phenols from wastewaters. Chitin and chitosan derivatives can remove phenol and substituted phenols from water (Bhatnagar and Sillanpää 2009). The pH primarily affected the degree of ionization of phenol and the surface properties of chitin. The functional groups of chitosan are protonated at low pH values and resulted in a stronger attraction for negatively charged ions in the adsorption medium. Phenol being weakly acidic is partially ionized in solution. These ions are negatively charged and are attracted due to electrostatic forces by the protonated amino groups of chitosan. As the pH increases, the overall surface charge of chitosan becomes negative and adsorption decreases. The equilibrium uptake of phenol is also affected by temperature due to the enlargement of pore size or creation of some new active sites on the adsorbent surface due to bond rupture. In comparison with activated charcoal, chitosan is more efficient in the removal of polychlorinated biphenyls from contaminated water (Venugopal 2011).

Inorganic anions are also an important class of aquatic pollutants, and various inorganic anions are found in potentially harmful concentrations in drinking water sources. The removal of these pollutants from drinking water supplies is an emerging issue. In recent years, chitin and chitosan derivatives have been successfully utilized for some anion removal from water (Bhatnagar and Sillanpää 2009).

Chitosan is currently employed in domestic sewage treatment systems in conjunction with other settling aids such as alum or bentonite clay to promote coagulation and settling of colloidal and other suspended solids. The polyelectrolyte is added at the rate of 1–2 ppm but can also be employed alone without alum when the concentration is raised to around 10 ppm. Being positively charged, it is very effective at agglomerating the negatively charged sludge particles (Venugopal 2011). Chitosan is also employed as a coagulant in the treatment of wastewater from food industries. The production of surimi generates a large amount of wash water that contains sizeable amounts of proteins, showing high turbidity. Chitosan treatment of surimi wash water results in the recovery of soluble proteins. The protein recovery is further increased by adding a complex of chitosan and alginate. It is also used as coagulant to treat wastewater from milk processing plants. Recovered proteins have application in food and feed industry (Wibowo et al. 2005).

20 Chitosan Application in Agriculture

Chitin and chitosan also have potential in agriculture with regard to controlling plant diseases. They are active against soil fungi, viruses, bacteria, and other pests. Addition of chitin and chitosan alters the environmental conditions in the rhizosphere and phyllosphere to shift the microbial balance in favor of beneficial

organisms and to the detriment of plant pathogens. Fragments from chitin and chitosan are known to have eliciting activities for a variety of defense responses in host plants, including the accumulation of phytoalexins, pathogen-related (PR) proteins, proteinase inhibitors, lignin synthesis, and callose formation (El Hadrami 2010).

21 Regulations and Commercial Applications

Chitosan is used as a food quality enhancer in a number of countries. Chitosan preparations in tablet, capsule, and powder form are being used in healthcare industry. In the European market, chitosan is sold in the form of dietary capsules to assist weight loss, and in some countries, such as Japan, it is added to various foods (e.g., noodles, potato crisps, biscuits). Chitosan-fortified fruit juices and chocolates are marketed in the USA. The role of chitosan as fiber is challenged by popular fiber products such as oats, soy, and bran. In spite of some limitations, chitosan promises to offer innovative applications in diverse areas of food processing and other fields (Venugopal 2011).

In the USA, the 1994 Dietary Supplement Health and Education Act permits use of chitosan as a food supplement without premarket approval as long as no health claims are made. The use of chitin and chitosan as ingredients in foods or pharmaceutical products, however, requires standardization of identity, purity, and stability. Manufacturers should consider filing petitions with agencies such as Food Chemical Codex, US Pharmacopoeia, European Pharmacopoeia, and Japan Pharmacopoeia. These organizations establish methods to identify specific products and standards of purity for pharmaceutical and drug use. Such standards will be necessary for future expansion of the use of chitin and chitosan (Heinze et al. 2005). Chitin and chitosan have been approved for pesticide and seed treatments, as fertilizer, and as animal feed additives. The US Environmental Protection Agency has approved the use of commercially available chitosan for wastewater treatment up to a maximum level of 10 mg/L.

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. The safety of chitooligomers prepared by the enzymatic depolymerization of chitosan has been reported in a short-term mice feeding study. No mutagenicity has been reported, as judged by the Ames test, mouse bone marrow cell micronucleus test, and mouse sperm abnormality test. A 30-day feeding studies did not show any abnormal symptoms and clinical signs or deaths in rats. No significant differences are reported in body weight, food

consumption, food availability, hematology values, clinical chemistry values, or organ/body weight ratios. No abnormality of any organ was found during histopathological examination (Qin et al. 2006).

22 Conclusion and Future Research Needs

Chitosan is a versatile biopolymer that has a variety of commercial applications. However, individual research reports have used chitosans from various sources with varying physicochemical properties. Hence, the question arises as to how to globally produce chitosans with consistent properties. Each batch of chitosan produced from the same manufacturer may differ in its quality. For proper quality control in the chitosan production, there is a critical need to establish less expensive and reliable analytical methods, especially for the evaluation of molecular weight and degree of deacetylation. Functional properties of chitosan vary with molecular weight and degree of deacetylation. With proper modification of chitosan, its functional properties and biological activities can be further enhanced, and more applications are being developed.

Chitosan with different structures shows different biological activities and not all the biological activities are found in one kind of chitosan. Each special type of bioactive chitosan should be developed for its potential application. Moreover, many studies carried out on chitosan and chitooligosaccharide bioactivity have not provided detailed molecular mechanisms. Hence, it is difficult to explain exactly how these molecules exert their activities. Therefore, future research should be directed toward understanding their molecular-level details, which may provide insights into the unknown biochemical functions of chitosan and chitooligosaccharide as well as help accelerate their future applications. The traditional chitosan production process is costly, thus limiting wider applications of chitosan. Simplification of chitosan production, for example, by elimination of deproteinization and/or demineralization or by reduction of reaction time required for deproteinization and demineralization, would considerably reduce production cost due to reduction in chemical usage, process time, and voluminous wastewater discharge. The typical astringent/bitter taste of chitosan limits its use as a food additive or preservative. Incorporation of L-arginine and adenosine monophosphate, both considered as GRAS, can be used to mask or minimize this effect and should be further investigated. Inherent antibacterial/antifungal properties and film forming ability of chitosan make it ideal for use as biodegradable antimicrobial packaging material. One major drawback of chitosan film is its high sensitivity to humidity, and thus, it may not be appropriate for use when it is in direct contact with moist foods. More research is needed to develop antimicrobial chitosan films that are less sensitive to humidity. Numerous researches conducted on food applications of chitosans have been done at a small or laboratory scale. Further research on quality and shelf life of foods, containing or coated with chitosan, should be conducted on scale-up with large volumes typical of commercial conditions.

This would provide a more realistic and practical information required for actual commercialization of food products containing or coated with chitosans.

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