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Production, Classification, Properties and Application of Chitosan

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***Abstract* – Chitosan was initially discovered in the mid-18th century, but remained little-known until preliminary clarification of its crystalline structure in 1934. Discovered in molds, and commercially produced from crustacean shells, chitosan is now used in diverse applications including Food industries. Due to the seasonal lulls in fishery industries and the still-growing demand for high quality chitosan, sources like mushrooms and other fungi are being re-evaluated. However, the crab shells currently used to make chitosan are waste materials of the fishery industry. Hence, chitosan production from fungi can only be economically competitive if waste mycelia from the industrial use of fungi as bio-catalysts in "white biotechnology", or waste carbon sources, e.g. from food processing industries, are used as substrates for cultivating high chitosan-yielding fungi. Chitosan have the property to get modified or make the complex with other excipients leading to enhanced properties. This article reviews different aspects of Chitosan with their applicability in different areas especially in food industry.**

***Keywords* – Chitosan, Crustacean Shells, Mushrooms, Fungi, Production, Properties, Applications of Chitosan.**

I. INTRODUCTION

Chitosan is a partially derivative of chitin, a natural polysaccharide extracted from crustaceans, insects and certain fungi [1, 2]. Chitin, which is the second more abundant biopolymer after cellulose, is a white polysaccharide, harsh and with an inelastic structure, presents in the cellular walls of fungi and in the exoskeletons of crustaceans. Chitosan and its derivatives were identified as safe with the good characteristics such as high biodegradability and biocompatibility, non-toxicity and low cost [3, 4].

Chitosan obtained by N-deacetylation of chitin is mainly formed of 2-amino-2-deoxy-D-glucopyranose (GlcN) units bonded in β (1→4): its typical degree of acetylation is lower than 35% [5]. Chitosan is mainly known and used for its properties of cationic polyelectrolyte. However, its high viscosity and its low solubility at neutral pH make difficult its use in the food and pharmaceutical fields. Generally, the substance becomes soluble in dilute acids when the degree of deacetylation is more than 50% [6, 7]. Derivatization [8] by introducing small functional groups such as, alkyl or carboxymethyl groups can increase the solubility of chitosan at neutral and alkaline pH without affecting its cationic character. Hence, during the last decade, new classes of compounds with different physico-chemical and biological properties had been developed: oligomers coming from the hydrolysis of chitosan [9, 11].

Chitosan oligomers are oligosaccharides composed of β-(1-4) linked glucosamine residues [12]. They exhibit good properties when integrated with food materials and also exhibit pharmaceutical and biological properties such as antimicrobial activity, antitumor and immunostimulating activity, and a protecting effect against phytopathogens [13]. Several methods were proposed for preparing chitosan oligomers, including chemical hydrolysis with inorganic acid and enzymatic hydrolysis with chitosanolytic enzymes, particularly chitosanase [14, 15]. However, addition of salts occurs with chitosan acidification because of the use of chemical acid, resulting in a decrease of purity of the oligomers**.** Furthermore, it has also been demonstrated that the main physiological activities and nutraceutical properties of chitosan oligomers depend clearly on their molecular weight and chain length. However, in the two main methods usually used in the industry for chitosan oligomer production, the

final product is a mixture of molecules of different molecular weights and contains minerals [16].

Chitosan as natural polymers belonging to amino polysaccharides having interesting structural features for chemical modifications generate novel properties, functions and applications. They provide improved solubility and enhanced functions and properties which give them innumerable applications. Besides being safe they possess antimicrobial activity where they are effective against the gram negative bacteria and also possess antifungal activities. This review covers the classification, developed processing and purification methods, physicochemical properties, functionalization of chitosan, and applications of chitosan and their derivatives.

II. Production, Extraction and Purification of Chitosan

Most of the commercially available chitin and chitosan is produced in Asian countries [17]. There are more than 200 suppliers and manufacturers of chitosan globally, and about 50 % of total capacity is in China [18]. Conventionally, chitosan is derived from chitin in seafood waste [13]. The development that has had the most positive effect on chitosan production has been its use as a dieting aid, since it promises a convenient way to lose weight via its fat-absorbing effect. Currently,there are more than 40 different dietary supplements on the market that include chitosan as a fat-absorbing agent, with end-user prices ranging from 180-370 EUR/kg. In 2009 the market price of chitosan was about 16$/kg, but it is decreasing with increases in the number of factories producing it [18].

In order to further consider chitosan production we need to differentiate between sources and discuss possible extraction and processing methods. Chitosan can be extracted directly from various, specific fungi or chitin can be extracted and then deacetylated [19]. Chitin is a more widely synthesized polymer in the fungi kingdom than chitosan, being produced by Zygomycetes, Ascomycetes, Basidomycetes, Deuteromycetes and Phycomycetes. In contrast, chitosan is found only in the cell walls of certain groups of fungi, especially Zygomycetes [20]. The great advantage of producing chitosan from fungi is that it can be directly extracted from fungal biomass at any time, avoiding seasonal fluctuations [21].

Table1. Composition of the crustacean shells based on dry weight [18].

	Crawfish /Crabs	Shrimp %	Fungi %
Chitin	25-30	30-40	15-40
Protein	15	35	5-10
$CaCo_3$	55	30	Glycan
Lipids	2-5	5-10	5-10

The cell wall provides cells mechanical and chemical stability and possibilities to interact with their environment via exchanges of nutrition and metabolites. It is a complex structure of proteins, lipids and polysaccharides beside many other minor components. Polysaccharides, proteins and lipids account for up to 80%, 3-20% and up to 5% of the cell walls' dry weight, respectively [22]. Depending on the fungal species, chitin, chitosan and glycan are the most abundant carbohydrates.

Different stepwise procedures are required to extract chitosan and chitin from the cell wall since different compounds are associated with them, and they have different chemical properties, *inter alia* differences in solubility and other characteristics in acidic and basic medium.

The most resistant compounds are the polymers chitin, cellulose, chitosan and glycan [23]. Hence for a successful extraction and/or purification, one has to remove all other compounds like proteins and lipids. Various protocols have been used for this in published studies, which can be divided into chemical and enzymatic procedures, as illustrated in Figure below (where the enzymaticmethod is on the right). The chemical procedures can be further divided into alkali- and acid based methods. The extraction of chitin from fungi is similar to its extraction from crustacean shells. The main difference is the higher fraction of CaCO3 in the latter, which is a major component of crustacean waste. For chitin 5% NaOH is usually used in the first step to remove proteins and lipids, while 30% HCl is used to remove the CaCO3, which dissolves in acid [24]. The insoluble fraction remaining after these treatments is chitin. To obtain chitosan from shells 5% of HCl or EDTA is initially used to dissolve or bind the calcium carbonate, then 40% NaOH at 110 °C to remove the proteins and lipids, with parallel deacetylation of chitin to chitosan. Chitin normally accounts for 14-27 % of the dry weight of shells, of which 60- 80 % can be converted to chitosan. A great advantage of fungal chitosan is its very low contents of inorganic material. Hence, demineralization is not required [25].

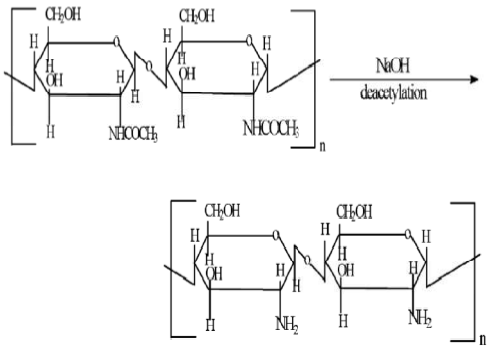

Fig. 1. Chitosan production through diacetylation [17]

Many protocols have been proposed and applied for fungal chitosan extraction in published studies. The most common general procedure (for which there are many variations in process parameters, especially in concentrations of NaOH and acetic acid, temperature and treatment times, depending on the biomass source) is shown on the left in Figure below. For deproteination, NaOH is the caustic agent of choice although other

hydroxides could also be used. Chitosan is soluble in many acids, but not sulphuric acid [26].

Extraction experiments have shown that use of hydrochloric acid instead of acetic acid has advantages in terms of the final yield**,** but in most protocols acetic acid is used as extraction agent, due to the higher degree of deacetylation of the extracts and the lower rate of depolymerization. Of the three main extraction options the filtration protocol is simplest, since the others require numerous steps, including washing, neutralisation and centrifugation. The filtration protocol requires fewer steps by exploiting the solubility of chitosan in sulphuric acid at temperatures above 90°C (like protein and lipids) and its insolubility at lower temperatures. Chitin is not soluble at either above or below 90°C, so it can be removed by hot filtration [27].

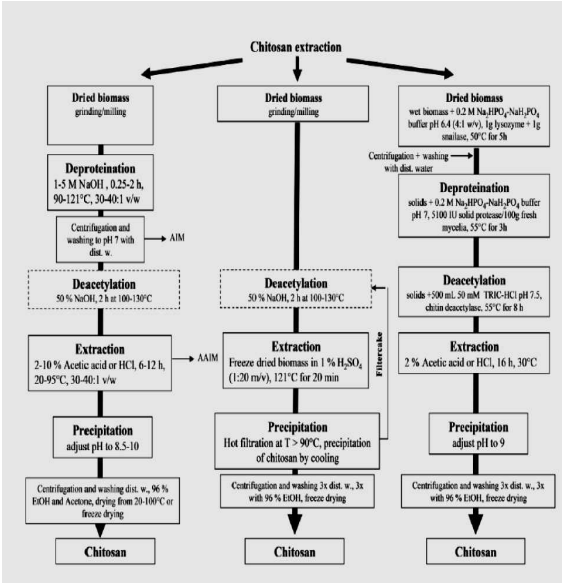

Fig. 2. Comparison of different protocols for extracting chitosan from fungi. [18]

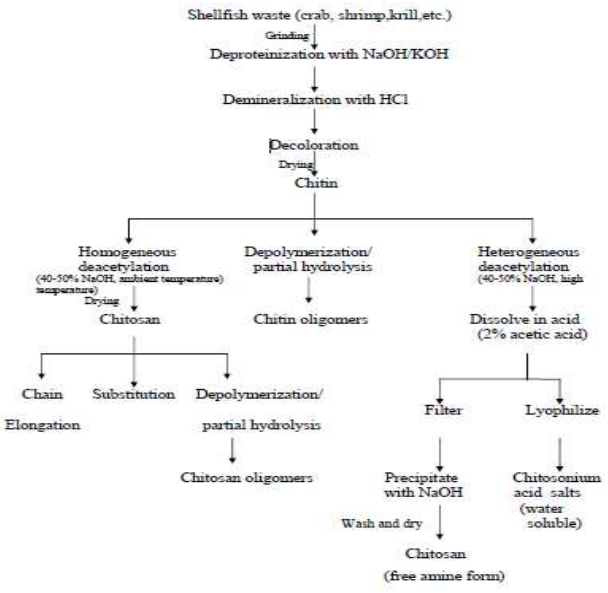

Fig. 3. Industrial processes of chitosan obtaining from shellfish wastes by means of Chemical extraction [25].

III. Characterization Methods

Despite the large numbers of commercial manufacturers who offer chitosan of various quantities and qualities, and increasing interest in potential applications of chitosan in food industry and other fields of interest, it is still very difficult to obtain chitosan that is fully standardized with respect to molecular weight and degree of deacetylation for any research. Hence, for any successful application key characteristics - e.g. degree of deacetylation (DD), molecular weight (MW), polydispersity and crystallinity - of the material must be thoroughly classified and validated to ensure it is of sufficient quality. The degree of deacetylation also provides important information about the solubility of chitosan, which is essential for optimising the manufacturing process and the scope for modifying the reactive amino group. The molecular weight determines the viscosity of solutions and increases with increasing MW. Hence, this is a parameter that influences procedures such as spraying chitosan coatings. The crystallinity influences the stiffness of the polymer and knowledge of the crystal structure provides further information about incorporated metal ions and salts, which correlate with the ash content. Basically, high ash contents interfere with further processing and modification. The protein content is especially important in medical uses since proteins may cause inflammatory reactions [28, 29].

As summarized in Table 2, various methods have been used to determine of chitosan characteristics.

Table 2. Methods for determining physico-chemical characteristics of chitosan [18]

Physico-chemical characteristics	Determinations methods
Degree of deacetylation	Infrared spectroscopy First derivative UV-spectroscopy Nuclear magnetic resonance spectroscopy Titration (alkalimetric, conductometric, potentiometric) Differential scanning calorimetry
Molecular weight /Mw distribution	Viscosimetry Gel permeation chromatography Light scanning Electrophoresis
Crystallinity	X-ray diffraction
Moisture content	Gravimetric analysis
Ash content	Gravimetric analysis
Protein content	Bradford method

IV. General Properties of Chitosan

Chitosan is a non-toxic, biodegradable polymer of high molecular weight, and is very much similar to cellulose, a plant fiber.

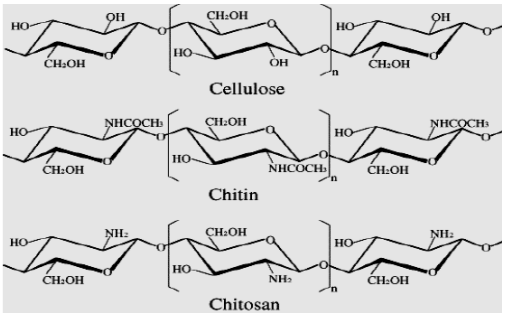

Fig. 5. Structure of Cellulose, Chitin, and Chitosan[17]

As seen in Figure 3, the only difference between chitosan and cellulose is the amine (-NH2) group in the position C-2 of chitosan instead of the hydroxyl (-OH) group found in cellulose. However, unlike plant fiber, chitosan possesses positive ionic charges, which give it the ability to chemically bind with negatively charged fats, lipids, cholesterol, metal ions, proteins, and macromolecules. Hence, chitin and chitosan have attained increasing commercial interest as suitable resource materials due to their excellent properties including biocompatibility, biodegradability, adsorption, and ability to form films, and to chelate metal ions [17].

4.1. Degree of Deacetylation (DD)

The process of deacetylation involves the removal of acetyl groups from the molecular chain of chitin, leaving behind a compound (chitosan) with a high degree chemical reactive amino group (-NH2). This makes the degree of deacetylation (DD) an important property in chitosan production as it affects the physicochemical properties, hence determines its appropriate applications. Deacetylation also affects the biodegradability and immunological activity. The degree of deacetylation of chitosan ranges from 56% to 99% with an average of 80%, depending on the crustacean species and the preparation methods. Chitin with a degree of deacetylation of 75% or above is generally known as chitosan [30, 31]. Various methods have been reported for the determination of the degree of deacetylation of chitosan. These included ninhydrin test, linear potentiometric titration, near-infrared spectroscopy, nuclear magnetic resonance spectroscopy, hydrogen bromide titrimetry, infrared spectroscopy, and first derivative UV-spectrophotometry [32].

4.2. *Molecular Weight*

Chitosan is a biopolymer of high molecular weight. Like its composition, the molecular weight of chitosan varies with the raw material sources and the method of preparation. Molecular weight of native chitin is usually larger than one million Daltons while commercial chitosan products have the molecular weight range of 100,000 – 1,200,000 Daltons, depending on the process and grades of the product. In general, high temperature, dissolved oxygen, and shear stress can cause degradation of chitosan. For instance at a temperature over 280oC, thermal degradation of chotosan occurs and polymer chains rapidly break down, thereby lowering molecular weight. Also, maximal depolymerization caused by utilization of high temperature or concentrated acids, such as hydrochloric acid followed by acetic acid and sulfurous acid, results in molecular weight changes with minimal degradation with the use of EDTA. The molecular weight of chitosan can be determined by methods such as chromatography, light scattering, and viscometry [33, 34].

4.3. *Viscosity*

Viscosity is an important factor in the conventional determination of molecular weight of chitosan and in determining its commercial applications in complex biological environments such as in the food system [35]. Higher molecular weight chitosans often render highly viscous solutions, which may not be desirable for industrial handling. But, a lower viscosity chitosan facilitate easy handling.

Some factors during processing such as the degree of deacetylation, molecular weight, concentration of solution, ionic strength, pH, and temperature affect the production of chitosan and its properties. For instance, chitosan viscosity decreases with an increased time of demineralization. It also increases with increase in concentration of the chitosan and degree of deacetylation, but decrease in temperature and pH.

Chitosan is known to possessed good complexion capacity. The presence of C-NH2 group involved in specific interaction with metal. A higher degree of chitosan is attained with chitosan characterized by greater degree of deacetylation. The affinity of chitosan for divalent and trivalent cation of chloride salts as Cu^{2+}>> Hg^{2+}>> Zn^{2+}>>Cd^{2+}> Ni^{2+}> Co^{2+} - Ca^{2+}> Eur^{3+}> Nd^{3+}> Cr^{3+} - Pr^{3+} [7].

4.4. *Solubility*

While chitin is insoluble in most organic solvents, chitosan is readily soluble in dilute acidic solutions below pH 6.0. Organic acids such as acetic, formic, and lactic acids are used for dissolving chitosan. The most commonly used is 1% acetic acid solution at about pH 4.0 as a reference. Chitosan is also soluble in 1% hydrochloric acid but insoluble in sulfuric and phosphoric acids. Solubility of chitosan in inorganic acids is quite limited [36]. Concentrated acetic acid solutions at high temperature can cause depolymerization of chitosan. Above pH 7.0 chitosan solubility's stability is poor. At higher pH, precipitation or gelation tends to occur and the chitosan solution forms poly-ion complex with anionic hydrocolloid resulting in the gel formation.

The concentration ratio between chitosan and acid is of great importance to impart desired functionality. At concentrations as high as 50 percent organic solvent, chitosan still works as a viscosifier causing the solution to remain smooth. There are several critical factors affecting chitosan solubility including temperature and time of deacetylation, alkali concentration, and prior treatments applied to chitin isolation, ratio of chitin to alkali solution, and particle size [37].

The solubility, however, is controlled by the degree of deacetylation and it is estimated that deacetylation must be at least 85% complete in order to achieve the desired solubility. The acid-soluble chitosans with >95% solubility in 1% acetic acid at a 0.5% concentration could be obtained by treatment of the original chitin with 45-50% NaOH for 10-30 min. Chitosans treated with 45% NaOH

for only 5 min, and/or with 40% NaOH for 30 min, were not deacetylated sufficiently to be soluble in 1% acetic acid. Insoluble particles were found in both solutions. According to Bough et al. (1978), a reaction time of 5 min with 45% NaOH may not be enough for chitin particles to be sufficiently swollen. A decrease of the NaOH concentration to 40% required increased time of >30 min to obtain a soluble chitosan [38].

4.5. *Bulk Density*

The bulk density of chitin from shrimp and crab is normally between 0.06 and 0.17 g/ml, respectively, indicating that shrimp chitin is more porous than crab chitin. Krill chitin was found to be 2.6 times more porous than crab chitin. In a study conducted by Rout (2001), the bulk densityof chitin and chitosan from crawfish shell, is very high (0.39 g/cm3); this was calculated as an unpacked bulk density of chitosan particles passed through a 0.5 mm mesh into a 25 ml measuring cylinder. This perhaps could be due to the porosity of the material before treatment. But once crawfish shell had been demineralized or deproteinized or both there seem to be very minor variations unpacked in bulk density between chitin and chitosan produced. A comparison of the bulk densities of crawfish and commercial chitin and chitosan indicated some variations, which can be attributed to crustacean species or sources of chitosan and the methods of preparation. Rout (2001) reported that increased degree of deacetylation (DD) decreased bulk density [34, 38].

4.6. *Color*

The pigment in the crustacean shells forms complexes with chitin (4-keto and three 4, 4'-diketo-ß-carotene derivatives). Chitosan powder is quite flabby in nature and its color varies from pale yellow to white whereas starch and cellulose powder have smooth texture and white color [18].

4.7. *Water Binding Capacity (WBC) and Fat Binding Capacity (FBC)*

Water uptake of chitosan was significantly greater than that of cellulose and even chitin. Basically, WBC for chitosan ranges between 581 to 1150% with an average of 702% [13]. The fat uptake of chitin and chitosan ranges from 315 to 170% with chitosan having the lowest and chitin the highest fat uptake [39]. In a study by Rout (2001) on this aspect, he reported that the average FBC of crawfish chitosan's and commercial crab chitosan's for soybean oil was 706% and 587%, respectively [40].

The inclusion of decoloration step during the production of chitosan was found to decrease the fat binding capacity of crawfish chitosan's, and decoloration (bleaching) had been shown to affect the viscosity of chitosan. The decreased viscosity as evidenced may be a cause for decrease in fat binding capacities among unbleached and bleached crawfish chitosan samples [30].

4.8. *Emulsification*

Even though chitosan alone does not produce emulsions, Cho et al. (1998) reported that emulsifying capacity of egg yolk increased with the addition of chitosan compared with the control. At 0.5% chitosan concentration, better emulsifying capacity was observed compared with at 0.1 or 0.3% chitosan. In general, chitosan emulsions tend to be very stable under temperature changes and aging. With viscosity, the degree of deacetylation is reported to be a determining factor in the emulsification properties of chitosan. The protein solution containing chitosan with intermediate DD produces less effective emulsion compared with that containing chitosan with higher DD [31, 41].

4.9. *Antimicrobial Properties*

Recent studies in antibacterial activity of chitosan have revealed that chitosan is effective in inhibiting growth of bacteria. The antimicrobial properties of chitosan depend on its molecular weight and the type of bacterium. For gram-positive bacteria, chitosan with 470 KDa was the most effective, except for *Lactbacillus sp*., whereas for gram-negative bacteria, chitosan with 1,106 KDa was effective. Chitosan generally showed stronger bactericidal effects for grampositive bacteria (*Listeria monocytogenes, Bacillus megaterium, B. cereus, Staphylococcus aureus, Lactobacillus plantarum, L. brevis, and L. bulgaris*) than for gram-negative bacteria (*E.coli, Pseudomonas fluorescens, Salmonella typhymurium, and Vibrio parahaemolyticus*) in the presence of 0.1% chitosan [42].

Chitin and chitosan *in vitro* show antibacterial and anti-yeast activities. One of chitosan derivatives, i.e., N-carboxybutyl chitosan, was tested against 298 cultures of different pathogenic microorganisms that showed bacteriostatic and bactericidal activities, and there were marked morphological alterations in treated microorganisms when examined by electron microscopy [43, 44].

Conversely, growth inhibition and inactivation of mould and yeasts seem to depend on chitosan concentration, pH, and temperature. The antimicrobial action of chitosan is influenced by intrinsicand extrinsic factors such as the type of chitosan (e.g., plainor derivative), degree of chitosan polymerization, host nutrient constituency, substrate chemical and/ or nutrient composition, and environmental conditions such as substrate water activity [30].

Chitosan coating have been shown to significantly delay fruit spoilage or decaying of fruits and vegetables such as tomatoes, strawberries, etc., at different temperatures. Chitosan coated fruits were not only firmer and higher in titratable acidity, but were slow to decay and exhibited less pigmentation than control samples at the end of storage [45].

4.10. *Formation of Film*

Chitosan has an ability to form film which makes it suitable for use as food preservation for control of psychotropic pathogen in fresh/ processed meat and fish products packaged under modified atmosphere, the most potential application of chitosan is as a coating agent in the area of fruit preservation. The biodegradability of chitosan is one of the most advantageous features for concern of the environmental damage occurring by improper disposal of petrochemical based plastics [46].

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V. Functionalization of Chitosan

Chitosan itself shows some functional properties. To overcome its inherent limitation and generate desired properties or functions, chitosan is modified or functionalized by different strategies. The presence of hydroxyl especially amino groups provides chemical reaction or physical interaction sites for chitosan. Modifications will not change the fundamental skeleton of chitosan. As a consequence, the original physicochemical and biochemical properties are kept while new or improved properties are imparted.

Functionalizing chitosan can be carried out by chemical reactions or physical combination (Figure 6). It can be molecular-level modification or just happen on the surface/interface, which will construct sophisticated architecture having various interesting properties. Chemical reactions include oligomerization, alkylation, acylation, quternization, hydroxyalkylation, carboxyalkylation, thiolation, sulfation, phosphorylation, enzymatic modification, graft copolymerization, and covalently cross-linking. Physical modifications usually assume blend, ionic complex and composite [47].

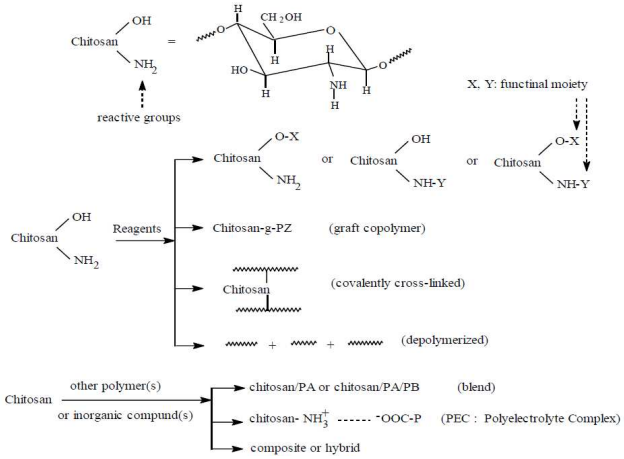

Fig. 6. Functionalizing ways of Chitosan [47]

VI. Classification of Chitosan (Chitosan Derivatives)

The cationic nature of chitosan limits the versatility of aqueous solutions because addition of certain acids in excess quantity is required in order to form water-soluble chitosan salts. Many efforts have been reported to prepare functional derivatives of chitosan by chemical modifications, in order to increase the solubility in water [48, 49]. Removal of one or two hydrogen atoms of amino groups of chitosan and introduction of some hydrophilic substituent by chemical modification results in a solubility improvement in aqueous solvents [27].

6.1. *O-and N-Carboxymethlchitosans*

Carboxy-methylchitosan (CM-chitosan) is the most fully explored derivative of chitosan; it is an amphoteric polymer, whose solubility depends on pH. Under controlled reaction conditions (with sodium mono-chloracetate in the presence of NaOH), one gets O- and N-carboxymethylation. The yield of substituents on the three positions was determined by NMR. This reaction extends the range of pH (pH>7) in which chitosan is water-soluble, but a phase separation due to the balance between positive and negative charges on the polymer was observed at 2.5<pH <6.5[50].

6.2. *Chitosan 6-O-sulfate*

This derivative is an anticoagulant; it was first prepared as an O- sulfated derivative and more recently as N-sulfated chitosan [51].

6.3.*N-methylene Phosphonicchitosans*

N-methylene phosphonic chitosan is prepared from chitosan with different acetylation degrees and sources. The degree of substitution is not dependent on chitosan's source and degree of deacetylation but it depends on reaction time. These are anionic derivatives with some amphoteric character which were synthesized under various conditions and proved to have good complexing efficiency for cations such as Ca2+, and those of transition metals (Cu (II), Cd (II), and Zn (II) etc.)[52,53].

6.4. *Trimethylchitosan Ammonium (TMC)*

This cationic derivative, water soluble over all the practical pH range, is obtained by quaternization of chitosan with methyl iodide in sodium hydroxide under controlled conditions, and has been fully characterized by NMR. Methylation leads to increase solubility of chitosan in water at neutral and basic pH values. The increase in solubility is achieved by replacing the primary amino group on the C-2 position of chitosan with quaternary amino groups. TMC was investigated for permeation enhancing properties and toxicity, using the Caco-2 cells as a model for intestinal epithelium. Synthesis of TCM is shown in Figure below [54, 55].

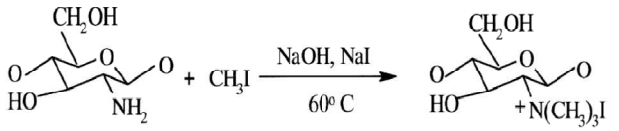

Fig. 7. Synthesis of N-Trimethyl chitosan chloride (TMC) [7]

6.5.*Carbohydrate Branchedchitosans*

Carbohydrates can be grafted on the chitosan backbone at the C-2 position by reductive alkylation: For that purpose, disaccharides (cellobiose, lactose, etc.) 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 C-6 position [56].

6.6. *Chitosan-grafted Copolymers*

One of the most explored derivatives is poly(ethylene glycol) grafted chitosan, which has the advantage of being water soluble, depending on the degree of grafting: higher molecular weight PEG at low DS gives higher solubility than low molecular weight PEG[57-58].

6.7. *Alkylated Chitosans*

Alkylated chitosans are very important as amphiphilic polymers based on polysaccharides. The first derivative having these characteristics was a C-10-alkyl glycoside

branched chitosan with a high degree of substitution (DS=1.5), which gelled when heated over 50oC. Another approach was used for selective N- and O-palmitoylation giving a derivative with two or three long alkyl chains per monomeric unit. This reaction involved protection and deprotection of the C-6 position [6].

6.8. *Cyclodextrin-linked Chitosans*

The cyclic oligosaccharides, namely α-, β- and γ-cyclodextrins (CD), are important because of their ability to encapsulate hydrophobic molecules in their toroidalhydrophobic cavity, whose selectivity depends on the number of glucose units (respectively 6, 7,8D-glucose units). For various applications, it is interesting to graft the cyclodextrin on polymeric backbone such as a biocompatible polysaccharide. A synthesis of α- and β-cyclodextrinschitosans with relatively high degree of substitution has been described. The authors found that these new derivatives had the ability to differentially recognize and retain certain guest compounds based on their molecular shapes and structures. They proposed to use these polymers as supports for reverse-phase adsorption or as adsorbents in controlled release systems.

A β-cyclodextrins with a specific modification on one of the –OH groups on its small side was grafted to chitosan by reductive amination. At a DS lower than 10%, these derivatives are water soluble in acidic conditions with loose inter-chain interactions. The grafted cyclodextrin has the same association constant as the free CD with small hydrophobic molecules such as adamantine [6, 57].

VII. Applications of Chitosan

The poor solubility of chitin is the major limiting factor in its utilization. Chitosan is considered as a potential polysaccharide because of its free amino groups that contribute polycationic, chelating, and dispersion forming properties along with ready solubility in dilute acetic acid. Chitosan possesses exceptional chemical and biological qualities that can be used in a wide variety of industrial and medical applications. Some of these are listed in **Table** below.

Table 4.Field of application of chitosan [18].

Field of application	Function
Wastewater Treatment	Removal of metal ions, flocculant/coagulant, protein, dye, amino acids
Food Industry	Removal of dye, suspended solids, preservative, color stabilization, food stabilizer, thickener and gelling agent, animal feed additive, etc.
Medical	Wound and bone healing, blood cholesterol control, skin burn, contact lens, surgical sutures, dental plaque inhibition, clotting agent, etc.
Agriculture	Seed coating, fertilizer, controlled agrochemical release
Cosmetics	Moisturizer, face, hand, and body creams, bath lotion, etc.
Biotechnology	Enzyme immobilization, protein separation, cell recovery, chromatography

7.1. *In the Food Industry*

The food processing industry extensively uses polysaccharides in food product development and processing for the purpose of imparting desirable functional properties such as thickening, gelling, emulsifying, and whipping. Without exception, chitosan have been documented to possess several distinctive properties [59]. The good water uptake of chitosan has been found to be significantly higher than that of microcrystalline cellulose [39].

In 2016 researchers announced a chitosan-based plastic wrap that doubles the shelf life of some foods. The plastic also included grapefruit seed extract, which has antibacterial and antifungal properties, and is an antioxidant, antiseptic and anti-viral. The film blocked the transmission of ultraviolet light – slowing oxidation and photochemical deterioration. The plastic can use raw ingredients that would otherwise be discarded, and biodegrades once discarded [60, 61].

Several studies have also demonstrated the effectiveness of chitosan for coagulation and recovery of suspended solids in processing wastes from poultry [62], seafood[63] and vegetable operations [64]. These studies indicate that chitosan can reduce the suspended solids of various food processing wastes by 70 to 98%. Chitosan also is effective for dewatering activated sludge suspensions resulting from biological treatment of brewing and vegetable canning wastes [31].

7.2. *In the Wastewater Treatment*

The wastewater released from food processing plants typically seafood, dairy or meat processing industries contains appreciable amount of protein which can be recovered with the use of chitosan; this protein, after drying and sterilization, makes a great source of feed additives for farm animals [40]. Chitosan is effective for conditioning municipal and industrial sludge due mainly to their effectiveness in sludge conditioning, rapid biodegradability in soil environments, and economic advantages in centrifugal sludge dewatering [65].

7.3. In Medical

Chitosan has interesting biopharmaceutical characteristics such as pH sensitivity, biocompatibility and low toxicity. Moreover, chitosan is metabolised by certain human enzymes, especially lysozyme, and is biodegradable. Due to these favorable properties, the interest in chitosan and its derivatives in drug delivery applications have increased in recent years. It is used in different areas such as wound and bone healing, blood cholesterol control, skin burn, contact lens, surgical sutures, dental plaque inhibition, clotting agent, etc. [9, 66].

VIII. Conclusion

Chitosan is the second most abundant polysaccharide found in nature after cellulose. It has been found to be non-toxic, biodegradable, biofunctional, biocompatible and was reported by several researchers to have strong

antimicrobial and antifungal activities. It has been compared with other biomolecule used in food industry.

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