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Chitin and Chitosan

Part I. PROPERTIES AND PRODUCTION

Summary — A review covering 115 refs. presents an introduction into the chitin and chitosan chemistry. Properties of chitin and chitosan, the methods of their preparation (by means of enzymatic and chemical treatment) have been described.
Key words: chitin, chitosan, properties, production, application.

Chitosan is a polysaccharide obtained mainly as a result of *N*-deacetylation of chitin, which is one of the most abundant renewable natural resources, second in quantity produced annually by biosynthesis to cellulose [1]. Chitin occurs in animals, particularly in marine invertebrates such as: crustacean and molluscs or in insects, where it is a major constituent of the exoskeleton, and in certain fungi where it is a component of cell walls as the principal fibrillar polymer (Table 1.) [2].

Table 1. Occurrence of chitin in living organisms [2]

Organism	Structure	Chitin	
		% Organic fractions	Crystal type
1	2	3	4
FUNGI			
Ascomyceta	Cell walls and structural membranes of mycelia stalks and spores	Traces — 45	
Basidiomyceta			
ALGAE			
Chlorophyceae	Cell wall components	+	—
PROTOZOA			
Rhizopoda	Cyst wall, shell	+	—
Ciliata	Cyst wall	+	—
CINDARIA		3.2—30.3	α
Hydrozoa	Perisarc, coenosteum	+	α
Anthozoa	Skeleton	+	—
Scyphozoa	Podocyst	+	—
BRYOZOA	Ecocyst	1.6—6.4	—
PHORONIDA	Tubes	13.5	—
BRACHIPODA			
Articulata	Stalk cuticle	3.8	—
Inarticulata	Stalk cuticle, shell	29.0	β, γ
ANNELIDA	Chactae	20.0—38.0	β
Polychaeta	Jaws	0.28	—
MOLLUSCA			
Polyplacophora	Shell plates, mantle bristles redula	12.0	—
Gastropoda	Shell, redula, jaws, stomacal plates	3.0—36.8	α
Cephlopoda	Calcified shell, pen, jaws, redula	3.5—19.5	α, β, γ
Lamellibranchia	Shells	0.1—17.3	α, β, γ
ARTHOPODA			
Crustacea	Calcified cuticles, intersegments membranes	48.0—85.0	α
Insecta			α
	Hardened cuticle	20.0—60.0	—
POGONOPHORA	Tubes	33.0	β

Chitin was first discovered in 1811 by Branconnot as an alkali-resistant fraction from higher fungi. In 1823, Odier isolated an insoluble residue, which he named chitin (in Greek *chitin* means tunic or covering) [3]. Chitosan was first reported by Rouget in 1859, who obtained the acid soluble fraction of chitin during boiling in a concentrated potassium hydroxide solution [1]. Previously, it was used in the Orient for the treatment of skin abrasions and in America for the healing of machete gashes [1]. The copolymer is a principal derivative manufactured from chitinous wastes. It occurs naturally in some fungi, but its content is much lower than that of chitin [4, 5].

STRUCTURE OF CHITIN AND CHITOSAN

Chitin is a copolymer — poly[β-(1, 4)-2-acetamido-2--deoxy-D-glucopyranose] — containing low percentage of 2-amino-2-deoxy-β-glucopyranose residual units. Its idealized structure is shown in Scheme 1, where some structural similarity to cellulose is visible, except that the C(2)-hydroxyl group of this biopolymer is transposed by an acetamide group in chitin.

Chitin is also related structurally to murein, which is main structural polymer creating the cell walls of bacteria. This correspondence in structure is reflected in the identical roles played by these three polymers in nature, *i.e.* as structural and defensive material [3].

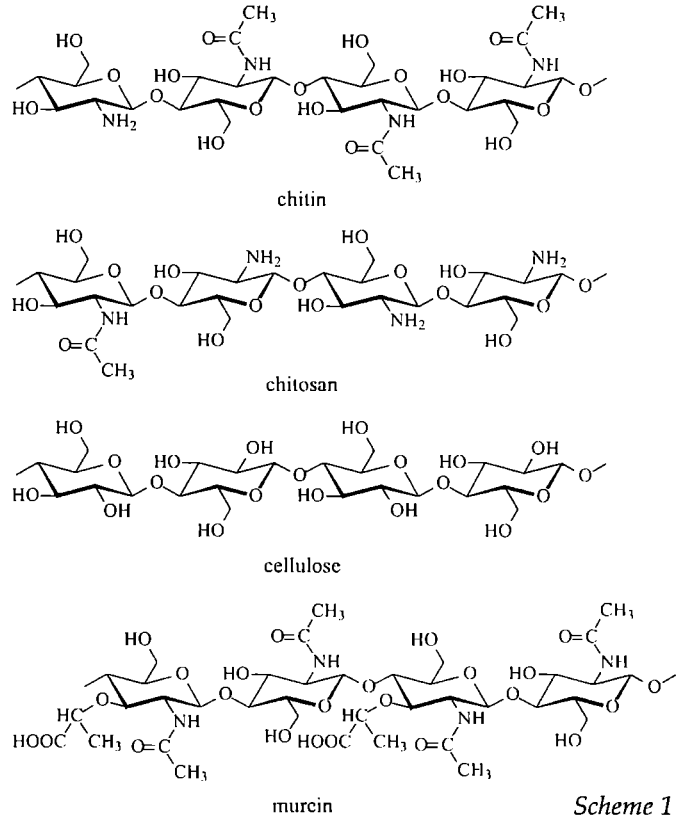
Chitin shows a highly ordered structure with an excess of crystalline regions and appears in three polymorphic forms: α, β and γ-chitin, which differ in the arrangement of the chains within their crystalline regions. In α-chitin, the chains are anti-parallel, in β-chitin, they are parallel, and in γ-chitin two of three chains are parallel and the third is anti-parallel.

The chains associate to one another by very strong hydrogen bonding between the amide groups and carbonyl groups of the adjuncted chain. Hydrogen linkages account for the great insolubility of chains in water and for the formation of fibrils. The units cell of α-chitin contains disaccharide sections of two chains with full intramolecular C(3)-OH...O(5) and intermolecular C(2)N-H...O=C(7) hydrogen bonding and O(6')H...O(7)/O(6)H...O(6') intra-/intermolecular hydrogen bonds [6].

Compared with the ordinary α-chitin, β-chitin is distinguished by its loose packing of molecules caused by their parallel arrangement. Therefore, β-polymorph shows higher susceptibility to lysozyme [E.C. 3.2.1.17] than α-chitin, a property, which could be useful for developing materials with desirable biodegradability [7].

Chitosan is chemically defined as a copolymer consisting of two residues: 2-acetamido-2-deoxy-β-D-glucopyranos and 2-amino-2-deoxy-β-glucopyranose, as shown in Scheme 1. The proportion of glucosamine is higher than *N*-acetylglucosamine, producing much better solubility in an aqueous solution of organic and numerous inorganic acids.

Five crystalline polymorphs have been found by X-ray diffraction measurement — four hydrated and one anhydrous. Hydrated polymers show extended two-fold helical structure including so-called: Tendon (major component), Form II, L-2. The "eight-fold" is the last of these hydrated polymorphs, predicted to take up less-extended 8/3 or 8/5 helical structures. The "Eight-fold" polymorph is unstable and is easily converted into crystals of the extended two-fold helices [8]. The hydrated form of chitosan is stabilized, as in chitin, by C(3)-OH...O(5) intramolecular hydrogen bonds. Four tetramers of glucosamine residues pass through the unit cell. Two adjacent chains along the bc-plane are arranged in an anti-parallel manner and linked by two sets of



Scheme 1

N(2)....O(6) inter-chained and O(6)....O(5) intra-chained hydrogen bonds. However, there is a space between the chain sheets for three water molecules that stabilize the chains through their hydrogen bonds [9]. Preparation of anhydrous chitosan ("Annealed") by annealing at high temperature resulted in removal of water molecules and rearrangement of chains yielding a structure parallel to the ac-plane, stabilized by series of N(2)...O(6) inter-chained, weak O(6)....O(3) and O(6)....O(5) intra-chained hydrogen bonds [9]. This transformation is irreversible and involves a drastic change in the molecular conformation of each chitosan chain [10].

Anhydrous crystalline chitosan does not dissolve in any aqueous acid solution, and does not form any complexes with transition metals [10].

The difference between chitin and chitosan lies in the degree of deacetylation (*DD*), usually defined as the ratio of the number of glucosamine groups to the overall number of *N*-acetylglucosamine (GlcNAc) and glucosamine (GlcN) groups. This measure is the most important descriptive parameter of chitosan and chitin. The quality and properties of chitosan products such as purity, viscosity, molecular weight, polymorphous structure, and *DD* may vary significantly because of the multitude of factors affecting the characteristics of the final product during the manufacturing process. Importantly, properties of the biopolymer are affected not just by the total content of deacetylated amino groups but also by distribution pattern of the minor component substitution: *i.e.* GlcN contents in chitin, GlcNAc contents in chitosan.

PROPERTIES OF CHITIN AND CHITOSAN

The degree of deacetylation is one of the more important chemical parameters distinguishing chitosan and chitin, being a statistic interpretation of the product obtained after the deacetylation process and characterizing the macromolecular composition of biopolymer chains. The value of *DD* determines properties of this natural polymer, such as its solubility in aqueous acid solutions, extent of swelling in water, susceptibility to biodegradation, bioactivity, biocompatibility, *etc.*

Degree of deacetylation

The methods most widely used for DD determination are as follows: ^{1}H-NMR [11—15], ^{13}C-NMR [14, 16—18], IR spectroscopy [19-26], titration methods [14, 26—30], dyes absorption [14, 26], UV spectroscopy [14, 26, 31, 32], hydrolytic technique [15, 33, 34], gas chromatography [14, 24], circular dichroism [14], periodate oxidation [14], residual salicylaldehyde analysis [14, 35], reaction with 2,4-dinitrofluorobenzene [14, 35], *etc.*

Average molecular weight

The weight average molecular weight ($\overline{M}_w$) of native chitin exceeds one million, whereas commercial chitosan products fall in the range from 100 000 to 1 200 000. Molecular weight of chitin and chitosan can be determined by methods such as chromatography [36—37], light scattering [33, 38], and viscometry [14, 37, 39—41].

Viscometry is by far the most simple and rapid method for the determination of average molecular weight by measuring an intrinsic viscosity $[\eta_i]$ for several concentrations of chitosan or chitin solutions. Viscosity average molecular weight ($\overline{M}_v$) determined by viscometry is expressed by the Mark-Houwnik equation as:

$$\overline{M}_v = \sqrt[a]{\frac{[\eta_i]}{K}} \qquad (1)$$

where: *exponent a and constant K are specific for the solvent used [42].*

A solvent system containing urea, acetic acid, and sodium chloride is usually used for determining the viscosity of chitosan. In a case of chitin, a mixture of *N,N*-dimethylacetamide (DMAc) containing lithium chloride as a solvent is applied. Solubility of chitin does not depend on its molecular weight, but is related to the degree of *N*-acetylation described by the number of *N*-acetyl amino groups present [43, 44]. Chitin is soluble in concentrated inorganic acids such as HCl, H_2SO_4, and H_3PO_4 and its β-polymorph form dissolves in concentrated formic acid [45]. However, decrease of the average molecular weight of polymer influences, as it has been reported, on dissolution in HCOOH. A number of organic carboxylic acids, such as: dichloroacetic or trichloroacetic acids are also used as solvents [45].

Viscosity and behavior in solution

The viscosity of chitosan solution depends on a number of factors, such as: *DD* value [20], average molecular weight [24, 42], concentration [46], ionic strength [46], pH [14] and temperature [14]. Increase in the *DD* value with decrease in molecular weight strongly reduces the viscosity of a chitosan solution. Increase of temperature, ionization or ionic strength usually decreases viscosity of chitosan.

Chitosan molecules with different *DD* values, in various solutions may exist in the form of compact spheres (for Mark-Houwink exponent *a* equal 0), random coils (a = 0.5—0.8) or rods (a = 1.8). It was found that chitosans with ($\overline{M}_v$) less than 220 000 formed conformation of random coil ($a > 0.65$), whereas chitosans with ($\overline{M}_v$) higher than 220 000 adopt the shape of more compacted molecules (*a* is below 0.6) [47]. This phenomenon is attributed to the fact that high molecular chitosan possesses more intramolecular hydrogen bonds and/or more even charge distribution [48].

In low ionic strength media, chitosan adopts an extended conformation because of electrostatic repulsion between chain segments. Protonated groups are totally neutralized for ionic strength approaching infinity. Electrostatic repulsion forces disappear; and chitosan conformation becomes a compact sphere regardless of molecular weight differences. However, intermolecular hydrogen bonds are destroyed when solution contains 4M urea and chitosan conformation becomes more extended regardless of differences in molecular weight [48].

The chain flexibility also depends on the temperature, as illustrated by decreasing of the viscosity when temperature increased. Urea is useful in chitosan molecular weight determination methods due to the destruction of hydrogen bonds between chitosan chains.

Mucha [49] concluded that the rheological properties of semi-diluted chitosan solution (1.5 or 3 wt. %) in aqueous acetic acid (0.5, 1.0 or 5%) strongly depend on the concentration of chitosan in the solution, its *DD* value and shear rate applied, but weakly on pH value. Chitosan solution behaves as non-Newtonian shear thinning fluids in a broad range of concentrations, but Newtonian flow has been reported at a high degree of deacetylation and low concentrations. Apparent activation energy for viscous flow decreases with increase in shear rates, solution pH and chitosan concentrations. Presumably, this results from disruption of the network structure of molecules or from formation of aggregates in a thermodynamically poor solvent.

Solubility

Chitosan is insoluble in water, alkali and most organic solvents, and dissolves in most aqueous solutions of organic acids such as formic, acetic, lactic, citric, etc., acids, at pH values of less than 6.3. Moreover, a polysac-

charide precipitates at pH > 6.5 in aqueous solutions, in the form of a gelatinous-like flock [50]. Some diluted inorganic acids such as nitric acid, hydrochloric acid, perchloric acid and phosphoric acid can facilitate preparation of chitosan solutions, but only by prolonged agitation and frequent warming. However, under the conditions described above, significant reduction of molecular weight of chitosan takes place.

The preparation of water-soluble chitosan from alkali chitin dispersion was reported [51]. This chitosan is dissolved in the absence of acids, in contrast to chitosan salts, which are readily soluble in water.

Cationic properties

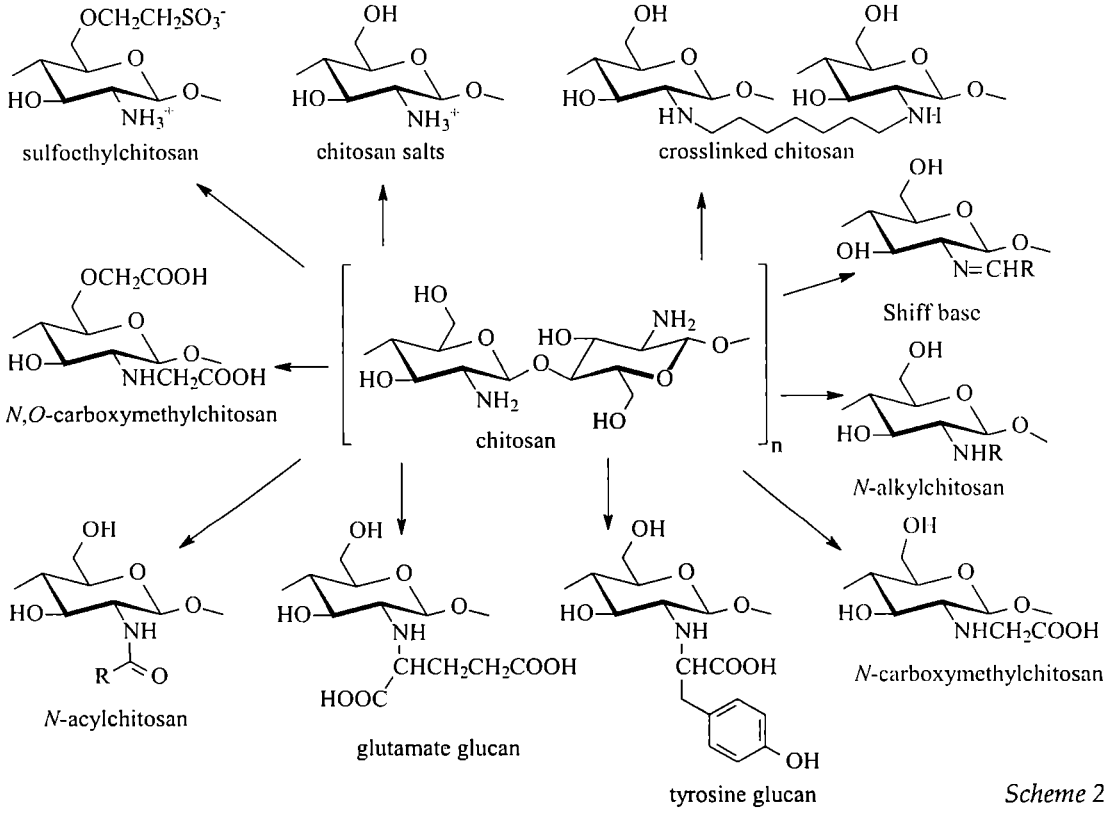
Polycationic behavior, resulting from the presence of free, protonated amino groups, enables to form complexes with derivatives carrying negative charges, such as polymers, proteins, dyes, *etc.* In addition, chitosan is capable to bind selectively with cholesterol, fat, tumor cells, or DNA and RNA [52].

Chitosan also forms a chelate with metal ions, requiring the involvement of -OH and $-O^-$ groups on the D-glucosamine residues as ligands, or else, two or more amino groups from a single chain, binding to the same metal ion. The free amino groups of chitosan are considered much more effective for complexing metal ions than acetyl groups in chitin. Nevertheless, the increase of free amino groups content does not directly increase the ability to form derivatives, due to the effect of other properties, such as crystallinity, affinity to water and/or distribution of residual units (GlcN or GlcNAc) [53]. The capacity for metal adsorption could be enhanced by crosslinking [54], controlled *N*-acetylation [1] or by cooperative bonding with other polymers like glucan [55].

Chemistry of chitin and chitosan

Chitin and its derivatives have shown biological activity such as antibacterial, antifungal and antiviral properties. The construction of definite molecular, supermolecular, and chemical structure (an alteration of charge, *etc.*) may enhance their susceptibility to degradation, wound healing, inducing organism defense reactions against pathogens [58—59] and releasing suitable quantities of oligomers, owing partly to their bioactivity [59].

Chemical modification of chitin and chitosan under mild conditions allowing protection of the glycosidic and acetamido linkage leads to chitin-like products with higher solubility, showing improvement in biodegradability, bioactivity, reactivity, *etc.* Chitin may be treated with 2-chloroethanol to obtain 2-hydroxyethyl chitin (glycochitin) [60]. Di-O-butyrylochitin [61] is a useful, biodegradable diester of chitin used in preparation of microsphere forms, strong transparent film and fibres [62] or chitin fibres, obtained by means of alkaline hydrolysis of di-butyrylochitin precursor filament [63]. The DMAc-LiCl system has been used for the production of fibres [64], by exposure to films [65] or in a homogenous



Scheme 2

system for synthesis of chitin derivatives [41]. Several possible reactions of chitin are shown in Scheme 2.

N-carboxymethyl chitosan (NCMCh) is the most common derivative of chitosan, obtained by its reaction with glyoxylic acid. NCMCh has been used for the recovery and the separation of metal ions from various wastes [60, 66]. NCMCh cross-linking capabilities with epichlorohydrin could lead to production of chelates with insoluble and amorphous products. Several other reactions are shown in Scheme 3.

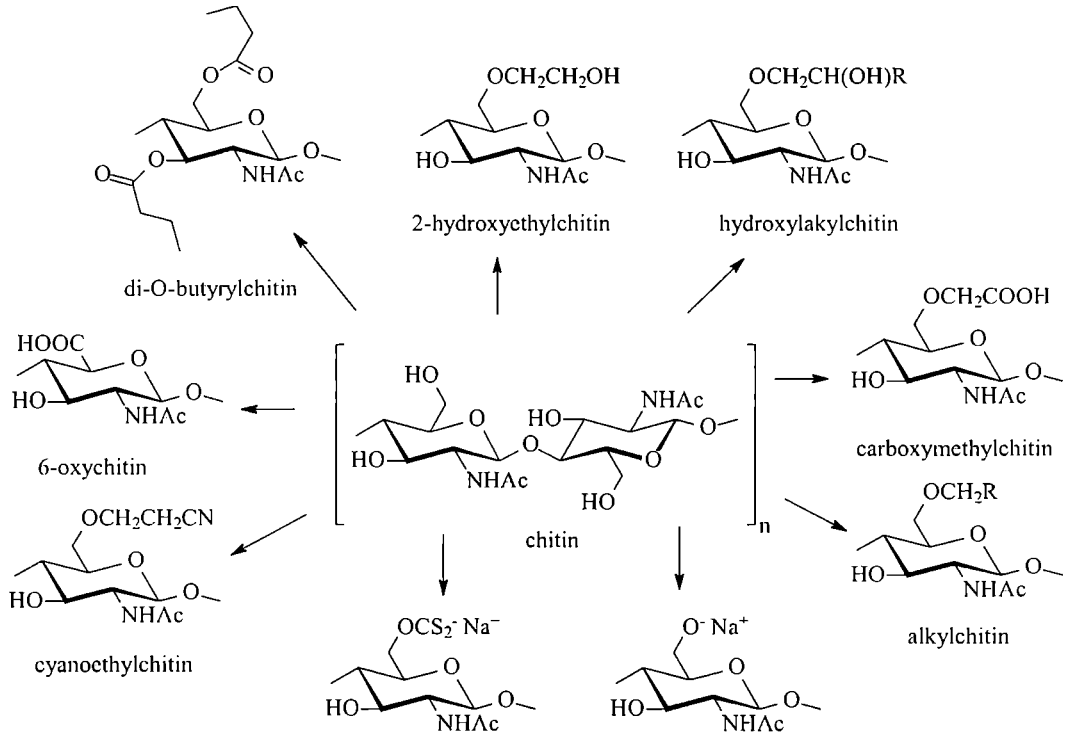
Cross-linking is facilitated by the presence of free amino groups, which increase the reactivity of chitosan and its other derivatives. Controlled *N*-acylation with acetic anhydride yields water-soluble, partially re-*N*-acetylated chitin [60].

— Inorganic materials (principally calcium carbonate and/or calcium phosphate) removal — demineralization,

— Elimination of carotenoid pigments — decoloration [68—70].

If chitin is separated from insect tissue, deproteinization and/or decoloration processes will be required, without removal of mineral compounds as their content in insect cuticles is low. It is also necessary to add phenylthiourea to inhibit tyrosinase activity and to prevent darkening [71].

The first two processes can be conducted in reverse order, *i.e.*, demineralization, followed by deproteinization. However, generally deproteinization is carried out prior to demineralization [69]. An aqueus sodium hydroxide solution at an elevated temperature is most preferred for the removal of protein (50—100°C, 0.5—6 h, concentration 0.25 M—2.5 M — depending on the preparation method) [36, 72], but use of Na_2CO_3, KOH, K_2CO_3, $Ca(OH)_2$, Na_2SO_3 solutions was also reported [69, 73]. The use of proteolytic enzymes (proteinase such as papain, trypsin and chymotrypsin) has been noted and examined [74—79]. Although such treatments do not modify the chitin, complete replacement of protein was not achieved.



Scheme 3

A similar reaction carried out with higher carboxylic acid anhydrides produces *N*-acyl-, *N*-arylidiene- or *N*-alkylidiene transparent gel-like dispersions [67]. Schiff's reaction with aldehydes or ketones leads to formation of the corresponding imines — converted to *N*-alkyl derivatives by a hydrogenation process.

METHODS OF PREPARATION OF CHITIN AND CHITOSAN

Various procedures have been reported and developed over the years for the separation and purification of chitin. Isolation of chitin from crustacean shell or armour wastes consists in principle of three steps:

— Protein removal — deproteinization,

The amount of residual inorganic contaminants in chitin is usually referred to ash content. Demineralization is conventionally accomplished by treatment with dilute hydrochloric acid at room temperature for 0.5—24 h with concentration of 0.275M—2.0M to dis-

solve the calcium carbonate as calcium chloride [36, 72, 80, 81]. The use of formic acid, nitric acid or sulfuric acid as demineralization agents with vigorous agitation at the temperature range 0—100°C for 2—4 h was reported as exceptions [82—84]. Temperatures not exceeding ambient are favored to minimize depolymerization of the biopolymer. Treatment with EDTA at pH 9.0—10.0 was used as a non-degradative method of extraction of minerals [75, 85].

Armour of crustacea contains coloring compounds (carotenoids), principally astacene, astaxanthin, canthaxanthin, lutein and β-carotene [86, 70]. Those compounds do not appear to be bonded with other compounds, such as protein or inorganic materials. However, the deproteinization or demineralization process does not extract above enumerated compounds (except upon treatment with highly-concentrated alkali — during the deacetylation process) [87]. Agitation with ethyl alcohol or acetone or their mixture with diethyl ether at temperatures of 20—60°C for 0.25—12h can be helpful for the removal of coloring derivatives [36, 78, 80—88]. Optionally, pigments could be destroyed with $KMnO_4$, NaOCl or H_2O_2 [68, 69].

Particle size of crustacean wastes is an important aspect. A decrease of the size of the armour usually increases deproteinization as well as decoloration yield [89].

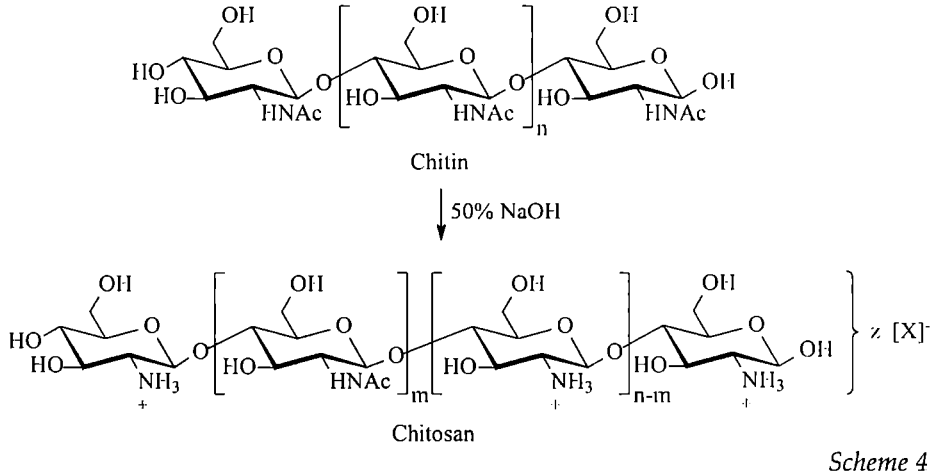
A new method employing lactic bacterial fermentation provides a most attractive approach to the production of chitin from crustacean shell. In this process, the ground shell wastes are inoculated with a lactic bacteria culture producing lactic acid and mixed with a carbohydrate (*e.g.* glucose). Acidification lowers the pH and dissolves calcium carbonate. At the same time, enzymes existing in shellfish viscera hydrolyze the residual proteins. After separation, solid chitin is purified by standard treatment using sodium hydroxide and hydrochloric acid; however with much lower consumption of chemicals [90].

Shrimp waste can be hydrolyzed using commercially available protease (Alcalase) and recovered as a protein hydrolyzate with a high content of essential amino acids, before preparation of chitosan from purified chitin [79]. Alcalase treatment had no adverse effect on either yield or quality of the produced chitosan. Nitrogen recovery yield was 68.5% as compared with only 13% while using conventional methods. Additionally, astaxanthin is more easy to be removed from the sediment by centrifugation of the crude protein hydrolyzate.

Another method proposed by Teng *et al.* [91], comprises the use of three proteolytic *Aspergillus niger* strains for the fermentation of shrimp shell powder. The hydrolyzed proteins were utilized in turn as source of nitrogen for the fungal growth, resulting in decreasing pH of the fermentation medium, thereby further improving demineralization of shrimp shells. Chitin isolated after extraction using 5% LiCl/DMAc solvent had a protein content less than 15%.

N-deacetylation of chitin is the main process for production of chitosan. Amide linkages are more difficult to cleave under basic conditions as compared with the ester group, thus it is necessary to apply vigorous conditions to remove *N*-acetamide groups. However, acetamide groups adjacent to *trans* hydroxyl groups are much more resistant to *N*-deacetylation than *cis*-related analogues. Chitin shows 2,3-*trans* arrangements of substituents in its monosaccharide units and is remarkably stable to most reagents, including aqueous alkali.

Generally, chitosan is produced by the treatment of purified chitin with highly-concentrated alkali (KOH or NaOH at concentration of 40—50 wt. %), usually at temperatures of 100°C or higher (Scheme 4).



Scheme 4

The physico-chemical properties of resulting chitosan depends on several factors:

— Concentration of deacetylation agent: Reduction of alkali concentration increases the time required to obtain soluble polymer with a less viscous product [68, 69, 21, 92].

— Time of deacetylation: Degree of deacetylation increases rapidly during the first 1—2 h, whereas longer time of deacetylation causes smaller improvement in *DD*. Strong depolymerization of polymer chains during this time is observed. In summary, the prolongation of deacetylation increases DD with a reduction in average molecular weight [68, 69, 88, 89, 93, 94].

— Ratio of chitin to alkali: Low chitin to alkali ratio increases the solubility of deacetylated chitosan with a

decrease of deacetylation time. This affects the homogeneity of deacetylation media [68, 95].

— Temperature of deacetylation: A relatively high temperature and strong alkali lead to improvement of solubility with shorter reaction time [57, 59, 73, 80, 96].

— Atmosphere: Free access of oxygen to chitin during deacetylation has a substantial degrading effect on the chitosan. Chitin deacetylation in an atmosphere of nitrogen yields chitosan with a higher average molecular weight than that prepared under standard conditions, while differences in nitrogen and ash compositions reported [36, 68, 69, 95]. The degradation effect of air becomes more significant with the reduction of the deacetylation time [68].

— Type of the source: Chitin from the crustacea is much more difficult to deacetylate than chitin from any other sources [88, 97—99]. This could be due to different molecular structures of chitins, as well as to presence of accompanying compounds, such as minerals. β-Chitin is more reactive than the α-polymorph type [69],

— Particle size: Lower particle size distribution of chitin yields chitosans with lower average molecular weight. Deacetylation yield depends on the extent of swelling of chitin particles. Chitin with smaller particles requires a shorter swelling time, resulting in a higher deacetylation rate [36, 68, 88, 96],

— Type of deacetylation: Deacetylation process may be provided by two systems:

— homogeneous from alkali—chitin dispersion, yielding an amorphous product with an increased content of random position *N*-acetylated and free glucosamine units. This method results in a water-soluble chitosan with *DD* of 48—55% but low ($\overline{M}_v$),

— heterogeneous, characterized by acid-soluble and acid-insoluble fractions, and high crystallinity of highly-deacetylated chitosan [100].

In general, as was explained above, alkaline deacetylation of chitin proceeds rapidly until the polymer is deacetylated above 75—85%; after that time further treatment shows only very limited effect on the extent of deacetylation.

Gradual deacetylation is preferred to obtain a highly-deacetylated product with a low decrease of average molecular weight. This process involves the procedures shown below, which can be repeated several times:

— washing with water between successive deacetylation stages,

— dissolution/reprecipitation of chitosan between successive deacetylation treatments.

Two explanations of this phenomenon have been proposed. The first is based on the observed effect of sodium hydroxide concentration on the swelling of cellulose, suggesting that concentration of sodium hydroxide within the chitin/chitosan particles gradually rises to the maximum swelling concentration while washing. This augmentation of the swelling facilitates diffusion of alkali into the crystalline regions. The second suggests that chitin forms a complex with the alkaline medium. The chitin-medium complex exhibits much smaller constant rate of the deacetylation step than that for the deacetylation step of uncomplexed chitin. Washing and drying are considered to destroy the above-mentioned complex, thereby converting the remaining GlcNAc residues and yielding much more reactive forms for the subsequent deacetylation treatment [90]. It can be concluded that in order to obtain a highly deacetylated product it is necessary to carry out deacetylation procedure at 100°C for 1 h during multiple treatments. This can be more effective than a single treatment in a similar procedure over the total process time [68, 101].

The treatment of chitin with agents containing concentrated solutions of sodium hydroxide in the presence of an organic solvent such as 2-propanol, 2-methyl-2-propanol or acetone constitutes an alternative method of chitin deacetylation. However, deacetylation process yield (low *DD*, higher $\overline{M}_v$) is lower then obtained with aqueous sodium hydroxide alone [57, 102]. The application of enzymes such as fungal and bacterial deacetylases during this process seems to be an attractive technique, since more time is necessary to obtain a soluble product [103—106]. The commercial production of chitin and chitosan is additionally limited by the reagent cost economics, reagent recycling opportunities, costs of non-marketable wastes and their elimination charges.

The typical industrial process of chitosan production offers nearly complete conversion of shell wastes into marketable commodities and recovery of proteins, sodium acetate, carotenoid pigments and lime or calcium carbonate as by-products using sodium hydroxide as a deacetylation agent at concentrations from 30 wt. % to 50 wt. % at temperatures 120°C—150 °C [38].

Protein extraction is carried out with diluted sodium hydroxide obtained after washing of deacetylated and decarbonated chitin prior to deacetylation. Mineral residues are converted to calcium hydroxide and then to calcium carbonate in course of the deacetylation process. However, the process has several adverse effects, including consumption of considerable amounts of energy, is not environmentally safe and cannot be easily controlled due to the chemical deacetylation leading to a broad and heterogeneous range of products.

The use of chitin deacetylase [EC 3.5.1.41] for producing of chitosan polymers and oligomers offers the possibility to develop an enzymatic process, which could potentially overcome most of these drawbacks [107]. Enzymatic deacetylation of chitin and its oligomers yields block type polymers, whereas chemical deacetylation usually produces random distributions of GlcNAc and GlcN residues. Therefore, enzymatic deacetylation offers a possibility to prepare of specific novel chitosan polymers [107]. The drawbacks are: high cost of the enzyme and necessity to use the pretreatment of the crystalline chitin substrate prior to enzyme injection to improve accessibility of the enzyme to acetyl residues [107]. The

advantages of the enzymatic deacetylation are more evident for processing of chitin oligomers, which are soluble in aqueous solutions and, therefore, more susceptible to enzyme action [107].

Industrial manufacturing of chitin and chitosan

Chitin and chitosan are presently industrially recovered from crustacean wastes in the United States of America, Japan, India, and with less extent in Russia, Norway, Chile, Korea and other European countries *e.g.* France, Germany, Poland, Ukraine [108].

Two essential facts require further attention:

— chitinous organisms, mainly crabs, shrimps, prawns, and krills are very abundant throughout the world and only limited portions of these resources are exploited by the marine food industry, producing canned or frozen meat, proteins, and shell wastes,

— production of chitin is carried out usually as a secondary activity related to the marine food industry.

The preparation of chitin and chitosan from *Insecta* does not involve the above-described limitations, except for the latter. Economic restrictions make necessary to combine their production with other usage. Thus, insect chitin and chitosan can be manufactured, similarly to crustacean biopolymers, as co-products [109, 110]. It has been found that the chitosan prepared from *Insecta* (*i.e.* from silkworm *Bombyx mori*, blue beetle fly larvae *Caliphora erythocephala* or beetle No Sato fly), shows similar properties as that obtained from crustacea, but the process is much cheaper due to the low crystallinity and the absence of the mineral residue in insect cuticles [109, 111]. Ash content (mineral residues) in chitin from prawns *Paenaeus indicus* is 26 wt. %, lobster *Panulirus ornatus* 36 wt. %, whereas in crab *Sylla cerrata* 45 wt. % [112]. The crystallinity index of crustacean chitin yields ca. 70%, whereas for blue beetle fly larvae *Caliphora erythocephala* is *ca.* 40% [111—113].

Chitin can also be obtained from sources other than marine wastes: large quantities of fungi grown currently in the fermentation systems producing organic acids, such as citric acid, antibiotics, and enzymes, constitute a potential source of chitin [38]. Krill is the richest source of crustacean chitin [114]. Estimates on the amounts of these shrimp-like crustacea, that are distributed in all oceans over the world, are in the 200—800 million tons range.

Economically, the Antarctic krill, *Euphausia superba*, is most important, with individual specimens measuring about 5—7 cm in length. It is generally accepted that ecological balance can be maintained at annual catches up to 50 million tons. The total allowable catch (TAC) is presently at only 3% of this figure, or 1.5 million tons per year. Factual figures are way under that limit: data from 1992 show that only 23% of TAC is taken out of the Antarctic Sea.

Of the total harvest of 350 000 tons per year, 200 000 tons is taken by Russian fleets, with a decreasing tendency, and 70 000 tons by Japan, with an increasing tendency [87]. Thus, concerns about the depletion of krill in the Antarctic Sea are not rational. Krill is a rich source not only of chitin but also of proteins and lipids, especially organic pigments (dyes) of the carotenoid group, and unsaturated fatty acids. The ability to recover the above valuable components from crustacean wastes, especially carotenoid pigments and chitin, plays an important role in environmental aspects of utilizing marine food wastes.

Deep-water shrimp, *Pandalus borealis*, which are caught in large quantities constitute another rich source of chitosan, at potential yield of about 700 tones chitosan per annum [87, 115]. These figures are very low when compared with the quantities of starch or cellulose that are utilized in the order of $>>10^8$ tons annually. However, a comparison of chitosan with polyglucose glucans is misleading in many respects, since the former is an ideal material for high value applications and chitosan is much too precious to be a competitor to cellulose or starch.

T a b l e 2. Chemical composition of various crustacean wastes [68] and of insect's larves of *Calliphora erythrocephala* [15]

	Chemical composition, %					
	Protein	Lipid	Pigment	Chitin	Ash	Water
Pandalus borealis *)	23,2	14.3	0.2	28,9	33.4	—
Euphausia superba *)	47.4	28.9	—	3.8	15.6	—
Euphausia superba	10.0	6.1	—	0.8	3.3	78.9
Calliphora erythrocephala *)	74.3**)			25,7	—	—

*) Dry basis.
**) Sum of protein, lipids and pigments.

Table 2 shows the chemical composition of various crustacean wastes produced from Antarctic krill *Euphausia superba*, from North Shrimp *Pandalus borealis*, as well as from the insect larval of *Calliphora erythrocephala*.

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