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Production, Properties, and Some New Applications of Chitin and Its Derivatives

Józef Synowiecki and Nadia Ali Al-Khateeb***

Department of Food Chemistry and Technology, Technical University, Politechnika Gdańska, Ul. Gabriela Narutowicza 11/12, 80–952 Gdańsk, Poland

Referee: Dr. Norman F. Haard, Professor, Institute of Marine Resources, Department of Food Science & Technology, University of California, Davis, CA 95616

* To whom correspondence should be addressed.
** On leave of absence from the University of Aden, Republic of Yemen.

ABSTRACT: Chitin is a polysaccharide composed from *N*-acetyl-D-glucosamine units. It is the second most abundant biopolymer on Earth and found mainly in invertebrates, insects, marine diatoms, algae, fungi, and yeasts. Recent investigations confirm the suitability of chitin and its derivatives in chemistry, biotechnology, medicine, veterinary, dentistry, agriculture, food processing, environmental protection, and textile production. The development of technologies based on the utilization of chitin derivatives is caused by their polyelectrolite properties, the presence of reactive functional groups, gel-forming ability, high adsorption capacity, biodegradability and bacteriostatic, and fungistatic and antitumour influence. Resources of chitin for industrial processing are crustacean shells and fungal mycelia. Fungi contain also chitosan, the product of *N*-deacetylation of chitin. Traditionally, chitin is isolated from crustacean shells by demineralization with diluted acid and deproteinization in a hot base solution. Furthermore, chitin is converted to chitosan by deacetylation in concentrated NaOH solution. It causes changes in molecular weight and a degree of deacetylation of the product and degradation of nutritionally valuable proteins. Thus, enzymatic procedures for deproteinization of the shells or mold mycelia and for chitin deacetylation were investigated. These studies show that chitin is resistant to enzymatic deacetylation. However, chitin deacetylated partially by chemical treatment can be processed further by deacetylase. Efficiency of enzymatic deproteinization depends on the source of crustacean offal and the process conditions. Mild enzymatic treatment removes about 90% of the protein and carotenoids from shrimp-processing waste, and the carotenoprotein produced is useful for feed supplementation. In contrast, deproteinization of shrimp shells by Alcalase led to the isolation of chitin containing about 4.5% of protein impurities and recovery of protein hydrolysate.

KEY WORDS: chitin, chitosan, crustacean shells, *Mucor rouxii*, mold mycelia.

I. INTRODUCTION

With the exception of cellulose, chitin is the most abundant natural polysaccharide on Earth. It is synthesized by different crustaceans, molluscs, marine diatoms, insects, algae, fungi, and yeasts. Offals obtained during the processing of crab, lobster, shrimp, Antarctic krill, clams, and oysters consists in some cases of up to 75% shellfish total weight. At the present time only a small quantity of shell waste is utilized for animal

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feed or for chitin isolation. Thus, the processing of shellfish leads to environmental pollution. Recently, the commercial value of chitin has increased because of the beneficial properties of its soluble derivatives, which are suitable in chemistry, biotechnology, agriculture, food processing, cosmetics, veterinary, medicine, dentistry, environment protection, and paper or textile production. The industrial production of chitin is limited by season of crustacean harvesting and a limited supply of the shell waste in some countries, and environmental pollution caused by alkali deproteinization producing waste liquid containing base, proteins, and protein degradation products. Because chitin and its soluble derivative chitosan are the principial components of the cell walls of several *Zygomycetes*, attention has been drawn to fungi for use as an alternative resource of both polysaccharides available in a desirable amount using microorganism cultivation on an inexpensive media. In this review the data concerning the utilization of insect's cuticles for are excluded. The resources of insect biomass are huge; however, the availability of this material for industrial processing is limited only to silkwarm pupa (*Bombyx mori*) accumulated in a relatively small amount in the silk reeling industry. Recent information concerning this area is reported by Haga and Shirata.[24]

II. AVAILABILITY AND PROXIMATE COMPOSITION OF SOME RESOURCES OF CHITIN AND CHITOSAN

A. Shellfish Waste

Crustaceans are currently the major producers of chitin available for industrial processing. Annual synthesis of this polysaccharide in freshwater and marine ecosystems is estimated as about 600 and 1600 milion tons, respectively.[15] Among the best characterized sources of chitin are shellfish (including shrimp, crab, lobster, and krill), oyster, and squid, harvested in quantities of about 29.9, 1.4, and 0.7 million tons by year.[90] However, recently published calculations of chitin resources are still based on approximate and not complete data and need further verification. The chitin contents in crustaceans usually range from 2% to 12% of the whole body mass, and this proportion is estimated only in a limited number of crustacean orders (Table 1). The contents of chitin, protein, minerals, and carotenoids in the shell waste varies widely depending on peeling conditions during processing, as well as the species, the part of the organism, state of their nutrition, and stage of reproductive cycle. The crustacean shell consists mainly of protein (30 to 40%), mineral salts (30 to 50%), and chitin (13 to 42%).[31] Proteins are sourced from retained residue of the flesh

TABLE 1
Proportion of Chitin to the Whole Body Mass of Some Crustacean Orders from Fresh Water and Sea Water

Crustacean order	Chitin content, as % body mass of crustacean from:	
	Fresh water	Sea water
Cladocera	4.9	12.2
Anostraca	2,2	1.5
Copepoda	12.4	5.8
Amphipoda	-	7.3
Decapoda	-	8.8

Elaborated according to: Cauchie (1997)

and connective tissue, and from complexes with chitin and mineral salts forming shellfish exoskeleton. The contents of mineral salts influencing on hardness and shell permeability changes significantly with age and the reproductive cycle of the animals. Older specimens have more calcified exoskeleton and a relatively lower percentage of chitin. The mineral fraction of the shells contains mostly phosphates and carbonates of calcium and magnesium.

Small amount of lipids in the shell waste is sourced from retained viscera or muscle residue (Table 2). The distribution of fatty acids in crustacean lipids is seldom reported because it is difficult to study all variations of these data caused by the influence of freshwater and marine water ecosystems, temperature, crustacean species and its maturation, feeding conditions, harvesting season, as well as storage and processing history. Independently of aforementioned influences, crustacean lipids always have a nutritionally valuable proportion between saturated and unsaturated fatty acids. For example, the lipid fraction of the shell waste from snow crab harvested in cold waters consists of saturated (17.0 to 18.1%), monoenes (50.0 to 55.8%), and polyene (28.2 to 32.0%) compounds.[74] Low lipid content limits its influence on nutritional value of animal feed suplemented by shell waste. Other valuable compounds of the shells are carotenoids, which are associated mostly with proteins in the epithelial layer of exoskeleton. The carotenoid level in crustaceans is very low and changes depending on dietary pigment availability, organism size, its maturation, and genetic differences. For instance, the average values of pigment concentration determined in the offals from shrimp (*Pandalus borealis*) and crab (*Chinoecetes opilio*) were estimated as 14.7 mg% and 13.9 mg%, respecively.[74] However, recently elaborated were different, not expensive methods of shell carotenoids utilization and is presented in another part of this article. The main components of the carotenoid fraction in crustacean exoskeleton are astaxanthin, and its esters are present in the total amount of about 25 μg/g on a dry basis of the shells.[74]

The most exploited sources of chitin are crab and shrimp offals (Table 2). The shell

TABLE 2
Proximate Composition on a Percent (%) of Dry Basis of Crustacean Shell Wastes

Chitin source		Protein	Chitin	Ash	Lipids
Crab:	*Collinectes sapidus*	25.1	13.5	58.6	2.1
	Chinoecetes opilio	29.2	26.6	40.6	1.3
Shrimp:	*Pandalus borealis*	41.9	17.0	34.2	5.2
	Crangon crangon	40.6	17.8	27.5	9.9
	Penaeus monodon	47.4	40.4	23.0	1.3
Crawfish:	*Procamborus clarkii*	29.8	13.2	46.6	5.6
Krill:	*Euphausia superba*	41.0	24.0	23.0	11.6
Prawn		61.6	33.0	29.4	1.4

Elaborated according to: Muzzarelli (1997), Naczk *et al.*(1981), Shahidi and Synowiecki (1991), Synowiecki and Al.-Khateeb (2000)

discards from crab (*Cancer magister*) and Pacific shrimp (*Pandalus borealis*) are available in the U.S. in amounts up to 39,000 tons yearly.[36] Only in the Atlantic regions of Canada does the harvesting of crab (*Chinoecetes opilio*) reach nearly 50,000 tons per year, and the resulting shells byproducts account for up to 80% of the original weight of this material. The chitin content on a dry basis of crab processing waste (13 to 26%) is lower that in the case of shrimp (14 to 42%) and krill (34 to 49%) offals.[6,57] The best sections of the crab for chitin isolation are legs, shoulders, and tips. These parts of crab the contain chitin in an amount similar to that in Louisiana crawfish (*Procambarus clarkii*). Furthermore, mineral content on a dry basis of shrimp and crab shells is up to 33% and 66%, respectively. The mineral fraction of Snow crab (*Chinoecetes opilio*) shells is of calcium (14.9%) and phosphorus (2.9%). The contents of Na, K, Mn, and Sr do not exceed 1% of the shell mass, whereas Mn, Fe, Cu, Zn, As, and Ba are present in trace amounts.[74]

Another promising source of chitin is the Antarctic krill (*Euphausia superba*). The potential annual catch of this crustacean which could not affect the Antarctic ecosystem, has been estimated to be as high as 100 million tons.[38] Such an amount of krill contains about 2.0 million tons of chitin, as well as 0.3 million tons of other sugars.[11] Krill meat is considered a rich source of nutrients for farm animals and for human consumption. However, the economy of krill fisheries depend not only on the efficiency of catching and edible parts processing, but also on the full utilization of all inedible constituents, such as chitin and carotenoids. Shells from Antarctic krill contain on a dry basis nutritionally not desirable amount, up to 9.0% of fluorine.[11] Fortunately, its content in the meat is about 50 times lower.[11]

The clams and oyster shells contain on dry weight up to 90% of mineral salts, and for this reason are deficient in chitin. Marine diatoms can be also considered as an attractive source of pure chitin not associated with protein. However, the low availability of these organisms limits its possible utilization.

B. Microbial Sources

Mycelia of variious lower fungi are also suitable for chitin isolation: *Allomyces, Aspergillus, Penicillium, Fusarium, Mucor, Rhisopus, Choanephora, Tamnidium, Zygorrhynchus,* and *Phy-comyces.*[55] Their chitin contents depend on the microbial species (Table 3). Apart from chitin, cell walls of the mold mycelia contain significant quantities of chitosan and different acidic polysaccharides.[3,8,19,34,35,89] Although fungi are practically unused as a source of these polysaccharides at the present time, several methods for its utilization in chitin and chitosan production were elaborated. Recently, the availability of mold mycelia and microbial cells has increased as a consequence of the enhanced use of microorganisms in the industrial production of citric acid, enzymes, vitamins, antibiotics, hormones, and many other pharmaceuticals or food additives. The development of chitin and chitosan utilization from fungal cell walls is advantageous, because they are readily available. All groups of these microorganisms possess a high growth rate, and under optimal cultivation conditions the time of fungal biomass doubling usually ranges from 1 to 3 h. The cultivation of fungi can be inexpensive with substrates such as cellulose containing byproducts from the paper and food industry. Moreover, fungi do not contain appreciable quantities of calcium carbonate and other mineral salts. Thus, the cost of acid treatment during manufacturing of chitin is lower, compared with processing of shellfish waste. Furthermore, the production yield of these polysaccharides from fungal sources can be effectively adjusted through the control of fermentation and pro-

TABLE 3
Chitin Content on a Dry Basis of the Mycelium from Different Fungi

Microorganism species	Chitin content (%)
Aspergillus niger	42.0
Aspergillus phoenicis	23.7
Mucor rouxii	9,4
Neurospora crassa	8.0 – 11.9
Penicilium chrysogenum	19.5 – 42.0
Trichoderma viridis	12.0 – 22.0
Saccharomyces gutulata	2.3
Blastomyces dermatidis	13.0
Histoplasma capsulatum	25.8 – 26.4
Tremeliamesenterica	3.7
Paracoccidioides brasiliensis	11.0

From: Knorr (1991), Ruiz (1978)

cessing conditions and through beneficial genetic manipulation. The contents of chitin and chitosan reported for dry basis of the fungall cell walls range from 2% up to 60%.[1] In deproteinized cell walls of *Basidiomycetes* and *Ascomycetes* the chitin and glucans contents varied from 26% to 65% and from 22% to 67%, respectively. The *Agaricus bisporus* cell walls consist of 22% proteins, 72% chitin, 3% cellulose, glucosamine, and mineral salts. The contents of proteins, nucleic acids, and lipids on a dry basis of the mold mycelia are 10 to 25%, 1 to 3% and 10 to 15%, respectively.[1,52,89] *Mucor rouxii* is the most commonly studied fungal sources of chitin, and its chitosan contents reported ranges from 8.9 to 35% of dry cell wall weight.[3,8,35,89,106] Higher *Basidiomycetes* surpass significantly lower fungi in chitin productivity. The contents of this polysaccharide in the cell wall in almost half of the studied species is higher than 50%, and it reaches 65% in *Fomes endopulus*. The total amount of chitin, glucans, and melanins on a dry basis of chitinous fibers isolated from cell walls of *Aphyllophorales* was 60 to 95%, 5 to 35%, and 0 to 10%, respectively.[21] For the effective utilization of the fungi mycelia it is necessary to establish optimal composition of the growth media, as well as time and other conditions of cultivation that influence chitin, chitosan, and protein contents. The amount of these compounds significantly changes during the growth of *Mucor rouxii* (Table 4). Furthermore, the chitosan synthesis by this microorganism reached a maximum in 2-day-old cultures.

III. STRUCTURE OF CHITIN AND ITS COMPLEXES

Chitin, a polysaccharide composed of β(1-4)-linked *N*-acetyl-D-glucosamine residues, occurs in exoskeletons or the cuticles of many invertebrates and in the cell walls of green algae, some fungi, and yeasts. Chitin exhibits structural similarity to cellulose and differs from it with the replacement of C-2 hydroxyl residues by acetamide groups (Figure 1). Depending on the polysacchride source and isolation conditions, chitin has a different degree of acetylation. In native chitin as many as one in six *N*-acetyl-D-glucosamine residues is deacetylated.[97] The

TABLE 4
Changes of the Main Components of the Mycelia During Growth of *Mucor rouxii*

Component	Growth time (h)			
	12	24	48	96
Water (%)	81,1	82.9	84.0	81.0
Proteins (% dry basis)	63.7	61.7	60.1	55.5
Deproteinized mycelia (% dry basis)	11.8	13.8	16.4	17.1
Crude chitin (% dry basis)	7.0	7.7	8.9	9.6
Chitosan (% dry basis)	4.4	6.1	7.3	7.0

From: Synowiecki and Al.-Khateeb (1997)

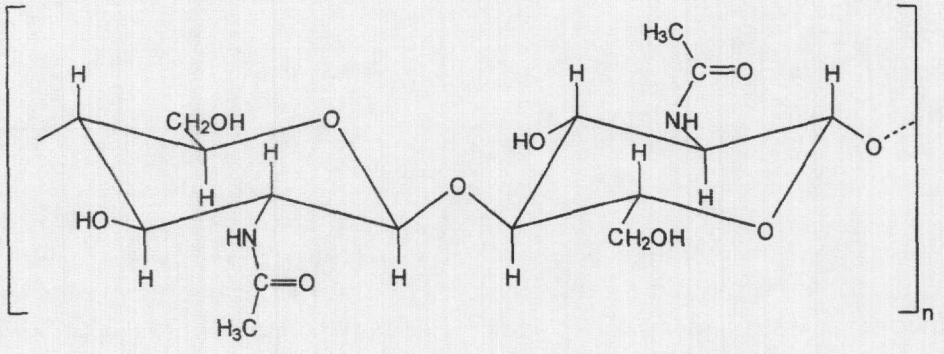

FIGURE 1. Formula of chitin molecule.

length of the chitin molecules varies widely from 5000 to 8000 of *N*-acetyl-D-glucosamine residues in crab to only of about 100 residues in some yeast. However, this value is often decreased as a consequence of chitin degradation during its deproteinization in hot alkali solutions. The chitin molecules after synthesis associate with one another by hydrogen bonds between >NH groups of the one molecule and >C=O groups of the adjacent chain. These hydrogen bonds account for the formation of fibrils occurring in polymorphic forms designated as α- and β-chitin. In *Arthropods* and *Crustacea*, the α-form is prevalent with the antiparallel arrangement of the adjacent chains. The β-chitin fibrils composed from two parallel chains are synthesized in marine diatoms. The third form, γ-chitin, in which two of three chains are parallel and the third antiparallel (↑↑↓) has been reported in the past. However, its existence appears to be controversial today.[14] The highest stability has α-chitin, because the β-chitin may be converted irreversibly to the α-form by treatment with lithium thiocyanate or by precipitation from solution in formic acid.[23] The proportion of α-chitin and β-chitin in the shell influenced its hardness, permeability, and flexibility. The chitinous microfibrils are arranged in sheets, which in the case of α-chitin are strongly fixed together by hydrogen bonds. It almost eliminates swelling in water and limits permeability of this polysaccharide form. The β-chitin, with a lower content of intersheet hydrogen bonds, swells readily and is more permeable. Due to the high density of hydrogen bonds in the solid state, chitin does not show a melting temperature and is completely insoluble in water, most organic solvents

and diluted acid and base solutions. Chitinous fibrils can be dissolved in concentrated HCl, H_2SO_4, and H_3PO_4, as well as in dichloroacetic, trichloroacetic, and formic acids.[69] The rate of solubilization of β-chitin is greater than that of α-chitin. Furthermore, this polysaccharide is also soluble in hot, concentrated solutions of some neutral salts or in hexafluoroisopropanole or hexafluoroacetone. The formation of hydrogen bonds is responsible for the resistance of chitin microfibrils against deacetylases, which can act on chitin molecules still not formed in microfibrils just after synthesis in microbial chitosomes.[39] In the fungal cell walls chitin microfibrils are surround by β-glucan matrix to form alkali-insoluble complexes associated with other polysaccharides and proteins. However, the chitinous structures in crustacea and insects are built in a glycoprotein framework, in which chitin form complexes with proteins tanned by phenolic derivatives. They are impregnated with mineral salts, waxes, carotenoids, and lipoproteins, which influence permeability, hardness, elasticity, and tensile strength of the structures. The hardness of the cuticles is usually enahanced by the deposition of calcium carbonate and to a lesser extent calcium phosphate. The shells of some *Rhizopods* and the teeth of the radula of some *Mollusks* are hardened by the deposition of silica or iron oxide.[69] Usually, proteinous matrix is stabilized through the cross-linking of polypeptide chains by quinones reacting with available amino groups of lysine and *N*-terminal amino groups. The quinones necessary for this reaction are formed by the action of diphenyl oxidase on diphenols available in the tissues. Insect cuticles and wings contain different glycoprotein complexes of chitin with arthropodins, sclerotins, or resilin to form microfibrils reinforcing the exoskeleton. Athropodins are characterized by a low content of glycine, the absence of sulfur-containing amino acids, and a high amount of tyrosine.

IV. SOLUBLE CHITIN DERIVATIVES

Various chemical modifications that can disrupt inter- and intramolecular hydrogen bonds without the cleave of glucosidic linkages are effective in making chitin soluble in water or other solvents. The simplest modification being *N*-deacetylation, which transforms chitin to chitosan, occurring naturally mainly in the cell walls of some *Zygomycetes* species. Its name concerns a group of chitin derivatives with various but higher than 50% contents of nonacetylated D-glucosamine units that are formed in an amount dependent on the conditions of alkaline or enzymatic deacetylation. In acid solution the free amino groups are protonated, and after solubilization chitosan exhibit polyelectrolyte properties. Naturally occurring chitosan and other polysaccharides containing *N*-deacetylated amino-sugar residues are produced in the cells through enzymatic deacetylation subsequent to the formation of their polymer chain.

Other products are formed during the reaction of chitinous hydroxyl groups with alkyl- and acyl-halides or isocyanates to yield ethers, esters or carbamate derivatives. These compounds are generally more polar, and consequently more soluble than the original polysaccharide.[102] Solubility improvement and physico-chemical properties of acylated chitin derivatives depend on the amount of substituted hydroxyl groups. Long residues introduced into chitin molecules through acylation improve the solubility of modified polysaccharide.[69] Butyryl-chitin as example has a good solubility in acetone, alcohols, dimethyl-formamide, tetrahydrofurane, and methylene chloride.[91] The solubility of this derivative in organic solvents made their formation easy into fibers not swelling in the water. Among different water-soluble chitin derivatives is carboxymethyl-chitin obtained during the reaction of monochloroacetic acid with alkali-chitin prepared previously by soaking the chitin flakes in 40% aqueous

sodium hydroxide solution at low temperature (0 to 15°C) to limit deacetylation and degradation of the polysaccharide. The β-chitin with relatively weak intramolecular bonds has a higher chemical reactivity for modifications than the α-polymorphic form.[42] Dihydroxypropyl-chitin is also soluble chitin derivative. Chitosan and partially deacetylated chitin are the good substrate for *N*-substitution, which led to the synthesis of branched derivatives with antitumor and immunoadjuvant activities.

V. VALUABLE PRODUCTS FROM CHITIN SOURCES

A. Feed Ingredients

The crustacean offals have been widely investigated because of its protein and carotenoid contents. The incorporation of carotenoid pigments to salmonids feed influences the red coloration of the flesh and favors consumer acceptability of the product.[16,71,74,81] Moreover, carotenoids in salmonids serve different biological functions and possibly influence the nutritional value of fish meat.[87,95] Wild salmonids assimilate carotenoids from zooplankton or small fish that have zooplankton in their digestive track. However, in farmed animals carotenoids should be added to the feed, because only plants and prostists are able to synthesize such compounds.[95] Improvement fish flesh color is achieved with the addition per ton salmonid feed of about 5 kg of the synthetic pigment. The high prices for astaxanthin and canthaxanthin preparations used for pigmentation increases the cost of farmed fish. Thus, the utilization of natural resources of these carotenoids has great economical importance. It is limited by the oxidative degradation of carotenoids on drying and storage, as well as by expensive, solvent-consuming and sometimes not effective extraction of the pigment. Although protein level on a dry basis of shell waste varies from 25 to 40%, the high calcium content in nondemineralized offals causes problems with nutrition and pelleting for animal feed. However, valuable additives to fish and poultry feed are demineralized crustacean meal, carotenoproteins recovered from the offals by enzymatic digestion, or edible oil enriched in carotenoids extracted from the shells. Almost decalcified protein concentrate can be produced by the mechanical separation of the shells from soft tissue fraction occurring in the shrimp processing waste. Separated protein slurry after acidification with acetic acid to the isoelectric point and the addition of antioxidant is then coagulated at 80°C. Obtained coagulate contains on a dry basis about 74% of proteins and 7% of minerals.[75] The amount of carotenoids in this product is sufficient for a satisfactory level of salmonids pigmentation after about 4 weeks of feeding.

A valuable dietary source of pigment and protein for cultured salmonids is the product called carotenoprotein.[44] It is prepared by mild enzymatic treatment of the shell waste, followed by precipitation of solubilized material according to a procedure outlined in Figure 2.[78,79] The residue after the extraction of carotenoproteins may serve as a source for further recovery of chitin. The best enzyme for carotenoproteins isolation is trypsin.[13,77] Specifity of other investigated proteases is broader, and their action may result in undesirable excessive hydrolysis of the product. The enzymic process removes about 90% of the protein and carotenoids from shrimp processing waste.[76] However, recoveries are lower for other crustacea crab, lobster, and langostilla (*Pleuroncondes planipes*).[20] The product obtained from snow crab (*Chinoecetes opilio*) processing waste contains on a dry basis 239 mg% carotenoids and 65% proteins, and include 22% of the proteins, 66% of total carotenoid, as well as 74% of total lipid

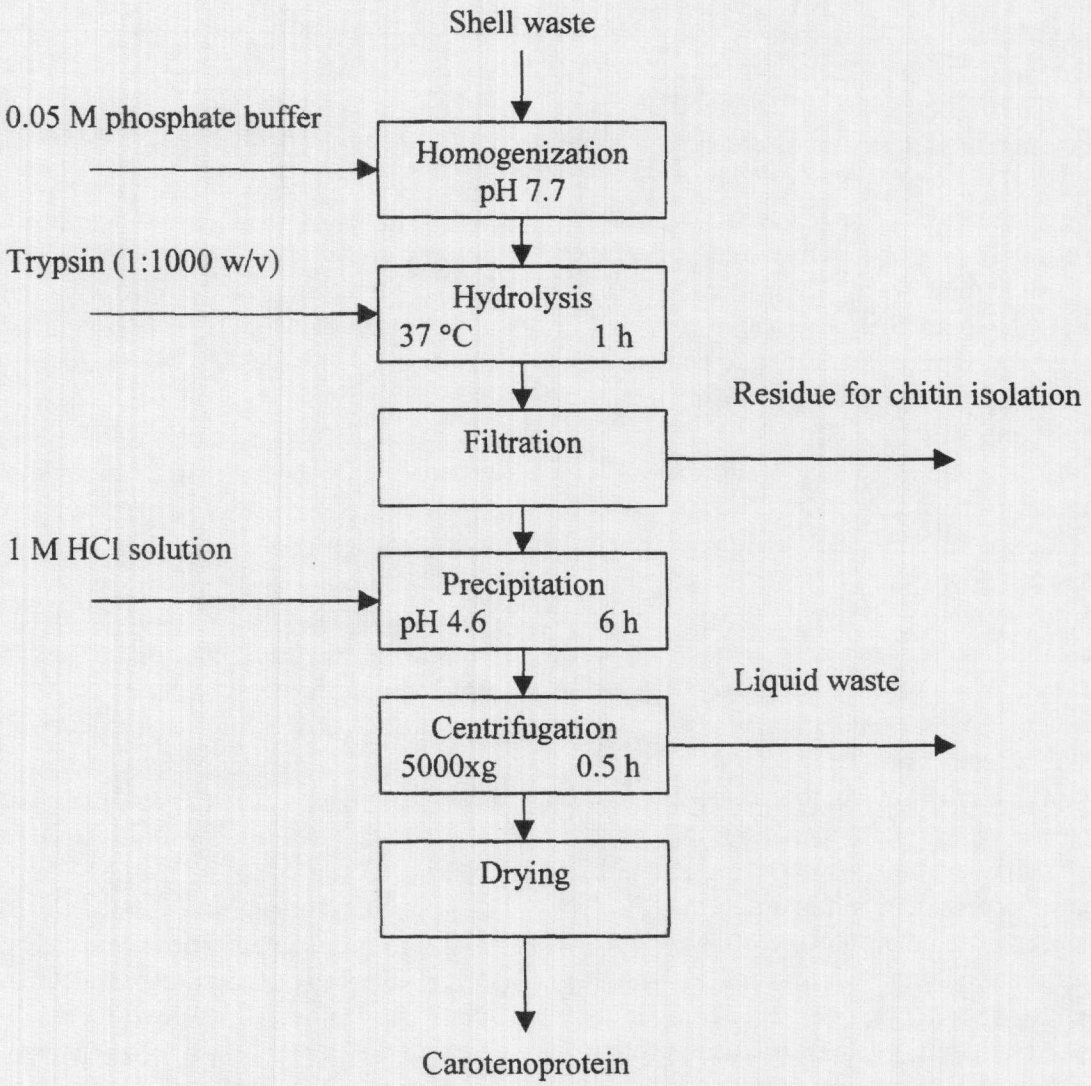

FIGURE 2. Recovery of carotenoprotein from crustacean shell waste. (According to: Simpson and Haard [1991] and Manu-Tawiah and Haard [1987].)

originally present in the shells.[45] Endogenous proteases adapted to low temperatures are useful for the processing of krill shell waste in a process based on controlled autoproteolysis. The product obtained contains about 4.5 mg% carotenoids is also a good source of these compounds for salmonid fish and chicken egg yolks pigmentation.[38]

Other methods of carotenoid utilization are based on its extraction from the crustacean meal with edible oil. The yield of the process can be increased by extraction at eleveated temperature (about 90°C), the addition of antioxidant protecting against pigment degradation, or by preliminary digestion of the shells with a commercial proteases, which increases the carotenoids release by about 58%.[50] Carotenoids should be isolated before deproteinization of the shells, because astaxanthin is converted by alkali to astacene, which is unable to color salmonids flesh. At an extraction time longer than 0.5 h the protective effect of antioxidant (ethoxyquin) is exhausted, and it considerably enhances pigment degradation.

B. Chitin

Chitin available on the market is at present produced from crustacean shell waste, and the other sources of this polysaccharide are almost not utilized. Today several companies are producing chitin and chitosan on a commercial scale. The majority of them are located in the U.S. and Japan, where large amounts of chitin and chitosan are manufactured each year from the shells of crabs and shrimps.[99] The methods used for chitin isolation and purification should assure the effective removal and if possible utilization of other shell components. Thus, various procedures have been adapted for chitin recovery. Moreover, the usefulness of different chitin resources depend on their availability, simplicity of processing, chitin content, and the suitability for generation of other valuable products.

Usually chitin isolation consists of demineralization, deproteinization, and bleaching. The first two steps can be used in reverse order, depending on the method of carotenoids and protein recovery and further chitin application. Chitin used as an adsorbent or enzyme support should be demineralized in first order, because the removing of salts impregnating and protecting the chitinous structures assure polysaccharide deacetylation at mild alkali treatment used for deproteinization. An enhanced level of deacetylation increases the amount of free amino groups participating in adsorption and protein attachment. The beneficial high chitin porosity depends on the polysaccharide source. However, deproteinization in the first step should be used during processing of the shells previously extracted by oil for the purpose of carotenoid recovery. In this step the oil residue still remaining after centrifugation is easily soaped and removed with the liquor after alkali deproteinization.

Demineralization can usually be achieved in 1 to 3 h extraction of the shells with diluted (1 to 8%) hydrochloric acid at room temperature.[23,59] The exception to the above investigations demineralization was accomplished with 90% formic acid, 22% HCl, 6 N HCl, or 37% HCl, respectively.[60,85] Full demineralization of the shells is possible when the amount of acid is stoichiometrically greater than the mineral content. To avoid chitin depolymerization ethylenediaminetetra-acetic acid (EDTA) can be used for removal of mineral salts.[7,70] The demineralization of the shells using acetic acid or sulfuric acid has been also reported.[60] Prolonged reaction time, up to 24 h, results only in a minimal drop in the ash content, but can cause chitin degradation.

Traditionally, shell wastes are deproteinized using aqueous sodium or potassium hydroxide solutions. The effectivness of alkali deproteinization depends on the temperature of processing, base concentration, and the ratio of its solution to the shells. Crustacean shell wastes are usually treated with dilute sodium hydroxide solution at concentrations ranging from 1% to 10% (w/v) and an elevated temperature (65 to 100°C). Optimal deproteinization can be also achieved through digestion with KOH solution.[74,85] Almost all proteins contained in shrimp and crab offals were removed by extraction at 90°C and shells to base ratio of 1:20 (w/v) using 1.0% or 2.0% KOH solutions, respectively.[74] An increase in the shell-to-base volume ratio above 1:4 (w/v) has only a minor effect on the efficiency of deproteinization. Reaction time usually ranges from 0.5 to 6 h. Prolonged alkaline digestion causes depolymerization and deacetylation of the polysaccharide. Furthermore, the production costs of chitin and chitosan derived from both crustacean and microbial cell walls can be limited by the recovery of proteins for use as feed and food component. However, the biological value of alkali-extracted proteins is reduced through the formation of lysinoalanine and other amino acids derivatives. Thus, enzymatic procedures, including digestion of shell pro-

teins by proteases or proteolytic bacteria should be investigated as an alternative to alkaline processing. Outlined in Figure 3 is the enzymatic deproteinzation of demineralized shrimp shells by commercial Alcalase from *Bacillus licheniformis* that led to the isolation of chitin containing only 4.5 ± 0.5% protein impurities.[90] The protein hydrolysate obtained has good essential amino acid index (46%) and protein efficiency ratio PER (2.74) values.[90] In contrast to alkali treatment, the enzymatic hydrolysis does not

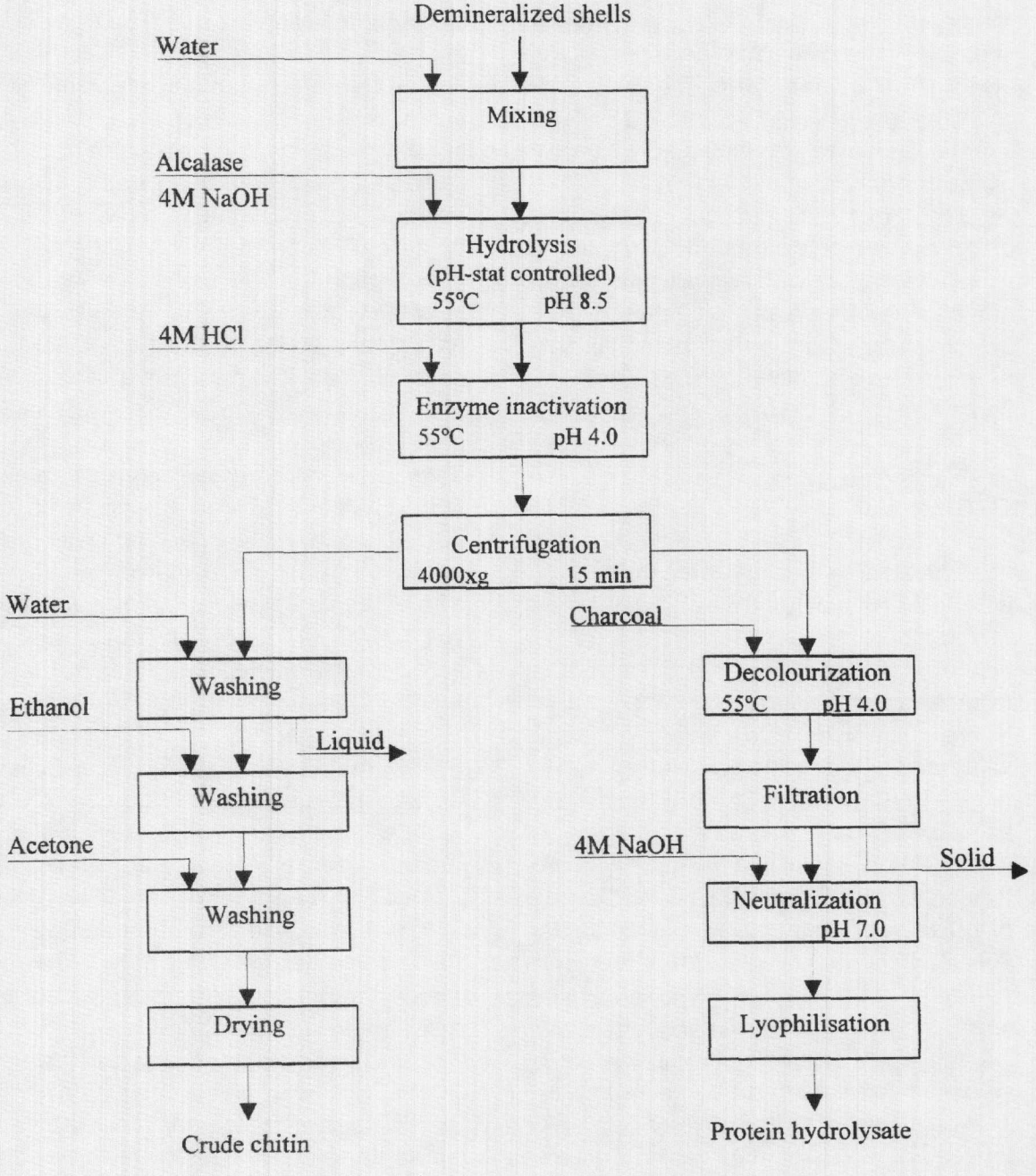

FIGURE 3. Flowsheet for production of chitin from crustacean shell waste. (From Synowiecki and Al.-Khateeb [2000].)

assure complete removal of proteins and protein degradation products and their residue on chitin depends on the selectivity of the enzyme used and process conditions. However, when chitin is used for chitosan production, the protein residue can be easily removed by concentrated alkali solutions used in the next step of the process, is not important. For the enzymatic recovery of proteins, the preliminary demineralization seems to be more beneficial.[90] It increases the tissue permeability for enzyme penetration and removes the minerals, which can act as enzyme inhibitors.

The removal of pigment residue from chitin can be achieved by the extraction at room temperature with acetone, chloroform, ethyl acetate, or ethanol and ether mixture. Decolourization is usually carried out by a bleaching treatment with NaOCl or H_2O_2 solutions.[69]

C. Chitosan

Chitosan is usually prepared by *N*-deacetylation of chitin from crab and shrimp offals. It is performed at different combinations of temperature (80 to 140°C) (up to 10 h) using concentrated (30 to 60% w/v) sodium or potassium hydroxide solution.[52,69] Alkali concentration and time and temperature of the process should be strictly controlled, because of its influences on degree of deacetylation, molecular weight, and molecular weight distribution, as well as the distribution of deacetylated units along the polysaccharide chain.[70,80] These properties reflect on the usefulness of chitosan for many applications, especially in the pharmaceutical industry. The preparation of chitosan at moderate concentration of sodium hydroxide, relatively low temperature, and prolonged deacetylation time cause a random distribution of deacetylated residues in chitosan molecules and in consequence diminish the ability to form aggregates and other supramolecullar structures.[80] High temperature in chitin processing increases the degree of deacetylation but also reduces the size of the molecules. According to Bough at an NaOH concentration of 35% and at 100°C, an acid-soluble product was obtained after 27 h of the process.[9] The rate of deacetylation is highest during the first hour of chitin incubation in 50% NaOH solution at 100°C. The degree of deacetylation achieved at these conditions was about 68%, and after the next 1 h of the process was enhanced only to 78%. Thus, prolonged alkali treatment does not significantly increase in chitin deacetylation and is not beneficial, because it causes depolymerization of the polysaccharide. The molecular weight of chitosan is also affected by deproteinization conditions used for the isolation of the chitinous substrate. The presence of oxygen during chitin deacetylation also influences polysaccharide degradation, and as a result decreases the viscosity and molecular weight of the product. Such changes can be limited through the processing of chitin under nitrogen.[10] It is difficult to prepare a chitosan with a degree of deacetylation higher than 90% without significant degradation of polysaccharide molecules. Shortened alkali exposure caused by the separation of the deacetylation process on a few stages decrease changes of the molecular weight of the final product. An alternative method of chitosan production, which assured almost complete deacetylation, consists of the prior incubation of chitin at 4°C in 50% NaOH for 24 h, followed by its separation, mixing with 10% NaOH, heating up to 230°C, and sudden decompression of polysaccharide and base solution mixture.[56] The degree of chitosan deacetylation and molecular weight changes caused by process conditions influence the properties important for many applications, such as solubility of the product in dilute acids, viscosity of the obtained solutions, as well as on their biological activity. It shows the necessity of chitin and

chitosan standarization. Alkali treatment of chitin during chitosan preparation led to the product with a broad range of molecular weights and a heterogenous extension of deacetylation of particular polysaccharide molecules in the product. Moreover, the generation of a large amount of harmful, concentrated alkaline solution results in an increase in the level of environmental pollution. An alternative to alkali deacetylation of chitin is the separation of chitosan occurring naturally in some fungal mycelia or enzymatic process exploiting chitin deacetylases.

A low-molecular-weight chitosan that is isolated from some fungi is especially useful for number of medical purposes and also in agriculture, because it is more effective in inducing plant growth than chitosan originating from shellfish chitin.[35] The isolation of fungal chitosan can be realized by its extraction from the mycelia deproteinized previously with diluted alkali solution or by enzymatic digestion. Polysaccharide extracted with diluted acetic acid is then precipitated by the dropwise addition of NaOH solution. The conditions of this process outlined in Figure 4 minimize the undesirable degradation and deacetylation of the product. In alternative procedures, the mold mycelia are treated with boiling concentrated NaOH solution. It causes both deproteinization of the substrate and also deacetylation of chitin occurring in the cells. This process results in an enhanced yield of chitosan caused by its formation from chitin residue.[4,48,55,106] However, the treatment of fungal mycelia with a concentrated base during deproteinization causes uncontrolled deacetylation and degradation of chitosan, as well as a loss of valuable single cell proteins. Furthermore, the product obtained is contaminated with glucan residue and is not useful for many applications.[55] The reported results suggest the cultivation of selected microorganisms with a high productivity of chitosan, which eliminates the necessity of alkaline deacetylation of chitin residue. For example, a satisfactory yield of extractable chitosan (6.2 g/kg of medium) was achieved during the solid-state fermentation of *Lentinus edodes*.[18] The efficiency of chitosan synthesis by this microorganism is about 50 times higher than in the case of chitosan production from other fungi.

The use of chitin deacetylase for the preparation of chitosan and its oligomers was widely investigated. These studies show that insoluble crystalline chitin is resistant to enzymatic deacetylation.[40] However, chitin deacetylated partially by chemical treatment can be further deacetylated by chitin deacetylase. The final degree of enzymatic processing of the substrate is related to the initial content of the *N*-acetyl groups in the polysaccharide (Table 5). The enzymatic process is more efficient in the case of deacetylation of chitin oligomers, which are soluble in water and therefore are more accessible for enzyme action. In contrast to chemical processing, the enzymatic deacetylation produces chitosan oligomers with well-defined range of molecular weight and the content of *N*-acetylated residues. Deacetylase activity was found in *Zygomycetes* species, which can be used as a source for commercial enzyme preparations. Furthermore, the biomass of these fungi after enzyme isolation is a valuable material for the extraction of chitosan.

D. *N*-acetyl-D-glucosamine

An alternative application of chitin is the production of *N*-acetyl-D-glucosamine, which is currently used as a food supplement and for the healing of ulcerative colitis and other gastrointestinal inflammations.[2] This compound can be obtained during the digestion of chitin, for instance, in cold 70% H_2SO_4 solution or by enzymatic hydrolysis. In the biodegradation of chitin endochitinases are involved, producing high-molecular-weight chitooligosaccharides and exochitinases,

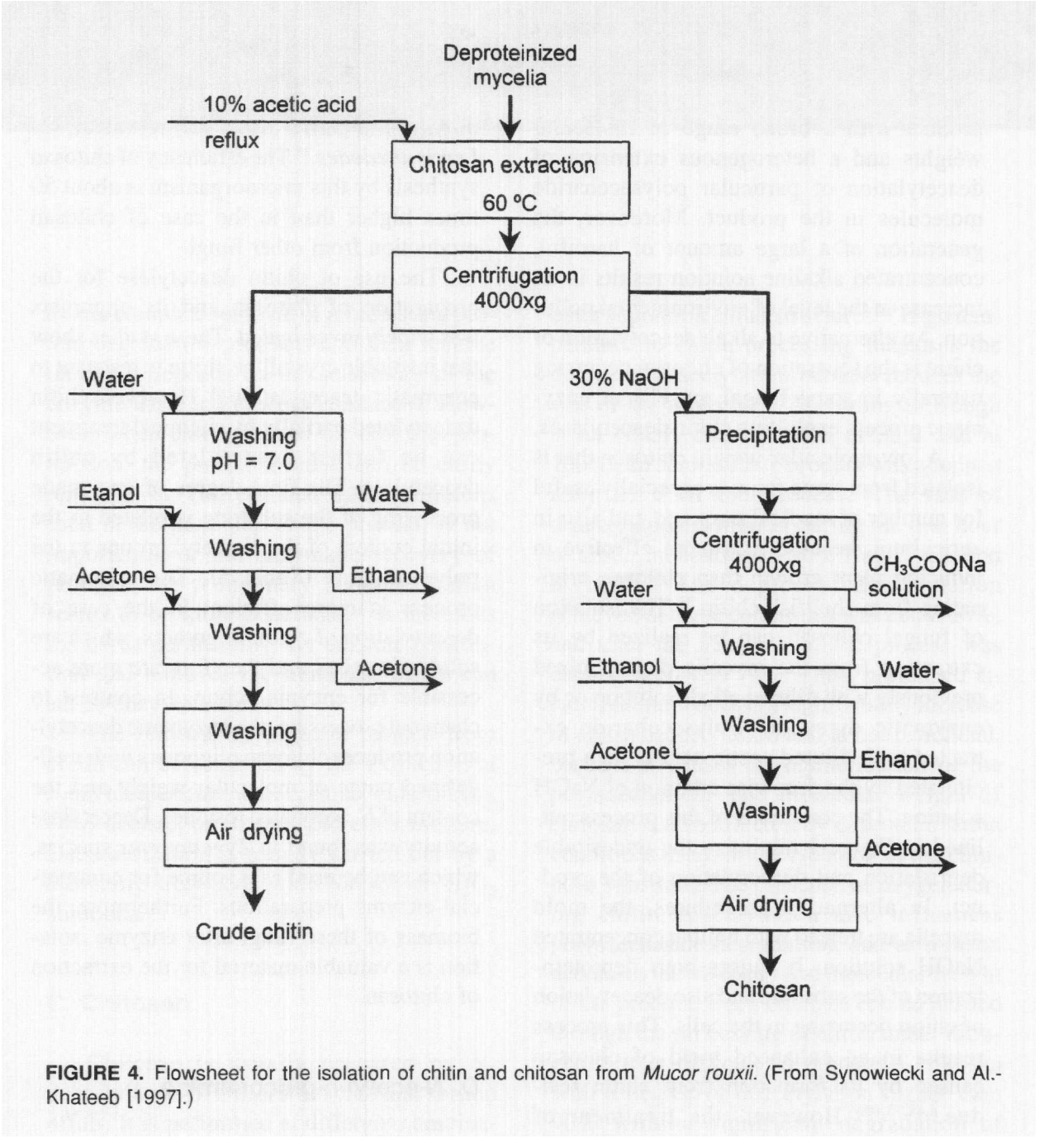

FIGURE 4. Flowsheet for the isolation of chitin and chitosan from *Mucor rouxii*. (From Synowiecki and Al.-Khateeb [1997].)

TABLE 5
Susceptibility of Chitosans with Different Degree of Deacetylation on Action of Chitin Deacetylase

Chitosan:	Degree od deacetylation (%)						
Before enzymatic deacetylation	55	68	73	82	87	95	98
After deacetylase treatment	33	26	16	14	7	2	1

Elaborated according to: Kołodziejska *et al.*(2000)

which release mostly dimers and some trimers from the nonreducing ends of high-molecular-weight polymers. Finally, chitobiase hydrolyses dimers to produce *N*-acetyl-D-glucosamine. All these enzymes are produced by many microorganisms, which are able to assimilate chitin from the shell offals.[51] Among them, the Gram-negative bacteria *Serratia marcescens* has been selected as one of the best producers of both chitosanases and chitobiase activities.[2] An efficient method for producing *N*-acetyl-D-glucosamine using the extracellular chitynolytic enzymes separated by ultrafiltration from chitin-based cultures of *Serratia marcescens* was developed by Aloise and co-workers.[2] The chitinases and chitobiase mixture obtained has sufficient activity for the effective hydrolysis of chitin particles in packed-bed reactor. The final product containing about 85% of *N*-acetyl-D-glucosamine and 15% of chitobiose is separated from reaction media using membrane ultrafiltration.[2]

VI. APPLICATIONS OF CHITIN AND CHITOSAN

A. Preface

Specific properties of chitin and its derivatives provide numerous industrial applications listed in Table 6, for example, for the preparation of dietary fibers, bandages, cosmetics, and toiletries. The most useful chitin derivative is chitosan. It is due to high molecular weight, polyelectrolyte properties, the presence of reactive functional groups, gel-forming ability, and adsorption capacity. Furthermore, chitosan can be chemically or enzymatically modified and is biodegradable and biocompatibile with animal and human cells and tissues. For many applications the molecular weight and degree of *N*-acetylation is important, because both these parameters influence not only on solubility and other physicochemical properties but also their biocompatibility and immunological activity.[99] It was also found that the adsorption capacity of chitin and chitosan enhances with increased free amino group content.

B. Medicine and Veterinary

Chitin is a nontoxic and biodegradable polysaccharide that can find many medical and veterinary applications such as antifectious, antiviral, antitumor, bacteriostatic, fungistatic, and antisordes agent.[47,54,67] In many cases the introduction of chitin derivatives to the human or animal body is helpful for the healing of different diseases or for preventing sickness (Table 7). However, chemical modifications are regarded to improve chitin solubility and for the development of biological functions, such as macrophages activation, antithrombogenic properties, stimulation of lyzosome secretion, inhibition of pathogens growth and antimethastatic, immunoadjuvant, antiurucemic, as well as antiosteoporotic activities.[108] Chitin derivatives are usually incorporated into the human body in the form of dressings for wounded soft and bone tissues, anticholesterolemic dietary foods, and items for the controlled delivery of drugs. The effectivness of biodegradation can be controlled by changes in the chemical structure of *N*-substituents and the degree of substitution of free amino groups in polysaccharide molecules.[25,26] Chitin and its derivatives are biodegradable in animal and human tissues by the action of lysozyme and chitinases. Thus, chitin, chitosan, and its derivatives are a good carrier for the controlled release of drugs during an extended period of time. It assures almost a constant level of drugs in the blood serum. The *N*-acylated chitosan derivatives are more readily digested than natural chitin because of the lower amount intra- and intermolecular hydrogen bonds. In contrast, cationic *N,N,N*-trimethyl chitosan chloride with a high degree of substitution is

TABLE 6
Appliance of Chitin and Its Derivatives

Specific properties	Main applications
Bioactivity	Prevention against microbial growth, antimicrobial additive to fibers and textile products, food packaging material, which act as inhibitor of microbial contamination, stimulation of immunological system, anticholesterolemic agent, lowering of body overweight, wound healig, blood anticoagulants
Biodegradability	Carbon source for single cell protein production, biodegradable radar countermeasure chaffs, biodegradable packaging materials, controled release of drugs, agrochemicals, drug and nutrients, cosmetics and toiletries production.
Reactivity of deacetylated amino groups	Enzyme immobilization, media for affinity chromatography and gel filtration, moisture retaining, antielectrostatic and hair protecting products, formation of polyelectrolites.
Selective permeability of chitosan membranes and film forming ability	Membrane separation in medicine, biotechnology and food processing, coated textile products with controlled water permeability, adjustment of internal atmosphere of fruit tissue, improvement surface affinity for acid dyes, prevention against microbial penetration of wounds, vegetables and fruits, production of dialysis membranes and dental fluids.
Chelation ability	Reduction of surface water and waste water pollutions by chelating of heavy metal ions and radionuclids, inactivation of metalloenzymes influenced on undesirable changes of food
Adsorption capacity	Removal of phenols from waste-water, efficient electrostatic painting, recovery or separation of protein and other by-products, clarification of juices and beverages.

effective as an enhancer of intestinal absorption of peptides and peptidomimetic drugs. Partially deacetylated and carboxymethylated chitin prevent against pathogen infections of the wound tissues of animal and human body.[72] The aforementioned chitin derivative also enhances the growth of the cells and causes activation of the macrophages.[25,26] The stimulation of extracellular secretion of lysozome results in an inhibition of pathogen growth through hydrolysis of their cell walls. The additional inhibitory effect causes the formation of chitosan complexes with anionic components of the cell walls, such as *N*-acetylmuramic, sialic and neuraminic acids, as well as an inactivation of microbial metaloenzymes through chelation of metal ions.[56] High-molecular-weight chitosans with high degree of *N*-deacetylation are more effective in inhibiting bacterial growth than chitosan with a lower molecular weight and a degree of deacetylation. Powdered chitin or chitosan has poor activity, but chitosan solutions inhibit the growth of many bacterial and fungal strains. Chitosan adipate and ascorbate administrated orally or intramus-

TABLE 7
Physiological Influence of Chitin Derivatives in THE Human Body

Significance:	Kind of activity:
Antimicrobial activity	Reaction with teichoic acid of polyelectrolite complexes, chelation of ions in metaloenzymes, changes in bacterial adhesion, inhibition of enzymes linking glucans to chitin.
Immunostimulation	Activation of macrophages secretion and synthesis of interferons and interleukin.
Chemotactic action	Stimulation of migration of fibroplasts and other stromal cells.
Action as a source of glucosamine and acetylglucosamine.	Rebuilding of the extracellular matrix
Enzymatic biodegradability	Depolymerization to oligomers and N-acetylglucosamine by lysozyme, cellular lipases and N-acetylglucosaminidase
Mucoadhesion and enhanced epithelial permeability	Interactions with membranes of the cells, molecular recognition and reaction with sialic acid residues in glycoproteins.
Enhanced reconstruction of connective tissue	Osteoinduction, healing of ulcers, meniscal lessions and other wounds, influence on assembly and orientation of collagen fibers.
Dietary significance	Anticholesterolemic and antiulcer activity, lowering of body overweight
Growth stimulation	Molecular recognition and entrapment of growth factor, stimulation of lectin type activity

Elaborated according to: Muzzarelli *et al.*(1997), Synowiecki (1997)

cularly to piglets stimulate immunity and decrease mortality of the animals through increased resistance against intestinal diseases.[67] The use of chitin as a source of dietary fiber in chicken feed enhance the growth of bifidobacteria in the guts, which eliminate other microorganisms and produce the β-galactosidase necessary for the digestion of feed supplemented by whey or other dairy byproducts.[7,82] Furthermore, the supplementation of feed for some farm animals and fish stimulates enzyme secretion in the stomach and the intestinal tract and consequently increases the growth rate of these organisms.[41] Chitin derivatives remarkably accelerate the healing of various types of wounds, including leg ulcers, which are common and very difficult for healing wounds developing in the course of venous, arterial, and lymphatic vessel disesases.[65] The improvement in the healing process is caused by hydrolytic activity of lysozyme and *N*-acetyl-β-D-glucosaminidase, which make available *N*-acetylglucosamine, a common aminosugar in the human and animal body. The released hydrolysis products are incorporated into glycoproteins or enter in different metabolic pathways. It causes macrophages and fibroplasts activation, enhances the hyaluronic acid synthesis, and its deposition in regenerated connective tissue, as well as influences on collagen deposition into extra-

cellular matrix during the rebuilding of the valid tissues. The effectiveness of these actions is increased, when low-molecular-weight chitosan, isolated from fungi, is applied. Methylpirrolidinone chitosan derivative shows osteoinductive properties and stimulates osteoblast formation and bone regeneration. The cationic nature and chelating ability of this derivative also improved the bone mineralization process.[88] Fungi are an advantageous source of chitin and chitosan applied to the burns and wound dressings in the form of films, bandages, cotton-like materials, and nonwouven napkins. These dressings have good hydroscopicity, show high bacteriostatic effect, and are completely biodegradable in the human body. A significant advantage also consists in the fact that repeated dressings is usually not needed. Fungal chitin has stronger sorption capacity when compared with that from crustacean shells and is more beneficial for the removal of toxins in the gastrointestinal tract. The high sorption properties are caused by a unique fiber structure. The beneficial properties of fungal chitin are improved by the presence of β-glucans and melanins. Chitin-glucan complexes isolated from the cell walls of *Aspergillus niger* and other fungi show after carboxymethylation an antimutagenic activity and can be used in the prevention of the mycotic infections caused, for example, by *Candida albicans*.[37] Mycoton produced from higher *Basidiomyces* used as the burns and wound dressing decrease bleeding removes pain and prevents inflammatory processes through growth inhibition of *Staphylococcus aureus, Escherichia coli, Proteus vulgaris,* and some other bacteria often resistant to antibiotics. Complete healing usually occurs in 5 to 7 days.[22] Mycoton is able to remove various food poisonings and allergic reactions, as well as negative impacts of intensive chemotherapy. It also decreases the influence of high radiation doses in the human body. Chitosan derivatives with different thrombogenic activity can find diverse applications. *N*-hexanoyl- and *N*-octanoyl chitosans are resistant to lysozome reaction, are blood compatibile, and are usable as antithrombo-genic material for artificial blood vessels and contact lens. However, unmodified chitosan, which is not antithrombogenic and is widely used as a homeostatic agent or material in the form of fibers, films and sponges.[94] Surface modification is a common technique for improving biomaterial hemocompatibility. Biologically active oligopeptides containing a sequence of Arg-Gly-Asp acid-Ser (RGDS) fixed on chitosan fibers are specific to endothelial cell attachment and can be used to coat vascular implants. The RGDS sequence is an active cell-binding site present in proteins such as fibrinogen, fibronectin, and vironectin.

Carboxymethylated at C-6 chitisan derivative is the substrate for synthesis of heparin-like blood anticoagulant.[94] Its inhibitory effect on thrombin activity depends on the degree of *N*-sulfatation of amino groups and reaches one-third of that for natural heparin, which acts as a strong anticoagulant reagent. Another biomedical application of chitosan is the utilization as an hypocholestrolemic agent also effective for the lowering of body overweight.[58] The acidic environment of the stomach causes chitosan solubilization. However, in the intestines at a pH above 6.3 chitosan precipitates in the form of complexes created mainly through ionic interactions between positively charged amino groups of chitosan and negatively charged groups of fatty acids, bile acids, cholesterol and lipids.[101] The decreased availability of bile acids limits intestinal emulsification and the absorption of lipids, which are excreted into the feces. Now available are chitosan preparations for the oral administration to people that are overweight and for the purpose of lowering serum cholesterol. Most effecive are a chitosan fraction having low molecular weight, about 8000 Da.[29] Chitin oligomers exhibit an inhibiting

effect on the growth of some tumors, which can be explained both by the oligosaccharide interactions with the surface of cancer cells and inhibition of their specific collagenases.[94] Observed immunenhancing effect depends strongly on molecular weight and the degree of *N*-deacetylation of chitinous oligosaccharides.

C. Cosmetics

Chitosan and its derivatives are used as a component of different cosmetics, toothpaste, hand and body creams, and hair-care products. These biopolymers were also investigated as ingredients of cosmetic formulations especially suitable for sensitive skin. Chitosan has a moisturizing effect on the skin that is dependent on molecular weight and degree of deacetylation, as well as offer protection from mechanical hair damage and exhibit an antielectrostatic effect on hair. High-molecular-weight chitosan increase the water resistance of emulsions protecting against sun irradiation and consequently enhances its film-forming ability.[27,73,103] A cosmetic cream supplemented with 0.1% chitosan influences an increased availability of bioactive, lipophilic ingredients such as vitamins, which better penetrate the outer layer of skin. A beneficial effect is also caused by the activation of fibroplasts and improved collagen deposition. Furthermore, the film forming capacity and antiseptic properties of chitosan protect the skin from possible microbial infections. Moreover, glucosamine from chitosan influences the desirable development of glycosaminoglycan and glycoprotein structures in the extracellular matrix of the skin. Dental fluids and toothpastes containing chitosan decrease dentin permeability. Chitosan introduced into such products form hydrogels, which can seal dentinal tubules and protect against microbial infection but maintain beneficial diffusion of ions and water.[61] This effect can be increased by the buffering capacities of chitosan.

D. Utilization in Agriculture and Food Preservation

Chitosans derived from the cell walls of some fungi or from crustacean shells show an inhibitory effect on the growth of phytopathogenic fungi and bacteria and induce resistance of plant to fungal, viral or viroid infections.[63,64,83] However, low-molecular-weight chitosan oligomers lost the ability to inhibit microorganism growth but still protected plant from pathogens.[46] That suggests the induction of the natural resistance of plants against infections by chitosan oligomers. The inhibitory effect develops in the chitosan-treated leaves almost immediately after application. Resistance against fungal infections is attributed to the hydrolytic destruction of their cell walls by plant chitinase and β-glucanase and the release of chitosan, which induces a synthesis of phytoalexin. This product is a potent supressor of fungal growth.[30] The antimicrobial activity of chitosan and its derivatives depend on their average molecular weight and the susceptibility to enzymatic degradation and the release of oligomers soluble in water.[83] Microcrystalline chitosan and its derivatives, especially salts, show large antiviral activity. Bean plants sprayed with aqueous chitosan dispersion were almost completely protected against virus infections.[104] The addition of chitin to soil is effective in elimination some plant diseases. The application of this polysaccharide promoted the growth of certain chitinolytic microorganisms and made them dominant in the soil. It limits the growth of plant pathogens both in soil and plant vascular system through the hydrolysis of fungal cell walls by chitinolytic enzymes secreted by antagonists.[96] Chitosan and its derivatives are also suitable for the intensi-

fication of seed germination observed as an example in the case of cucumber and pea seeds.[30,32]

Antimicrobial activities of chitosan and *N*-sulfobenzoylchitosan against various pathogens and food-spoilage microorganisms were also investigated for the purpose of application in food processing and preservation.[33,62] Sulfobenzoyl chitosan used as a natural preservative of oysters increases its storage life at temperatures of about 5°C through effective growth inhibition of *Pseudomonas, Salmonella, Aeromonas,* and *Vibrio* strains.[98] Treatment with chitosan solution protects against contamination of potatoes by pathogens, which cause tissue damage and soft rot.[68] Chitosan also inactivates polygalacturonase, pectate lyase, and pectin-methylesterase secreted by potato pathogens. Moreover, chitosan creates on the surface of the products semipermeable membranes. The addition of Ca^{2+} ions changes the rate of CO_2 and O_2 permeation through chitosan membranes, and it significantly increases the storage life of berries and other unstable fruits.[43] Coating with chitosan films by immersion in 1% polysaccharide solution containing 0.1% of Ca^{2+} limits the changes in sensory properties of stored fruits, tomatoes, and cucumbers. Another application beneficial for food preservation is the production of food packaging paper coated with chitosan, which acts as an inhibitor of microbial growth.

Chitosan decreases the undesirable changes of emulsifying properties, fat binding, as well as water-holding and gel-forming capacities caused by the denaturation of myofibrillar proteins during frozen storage.[5] This protective effect, depending on the degree of acetylation, is caused by the stabilization of the water structures surrounding protein molecules. The hydrolysates produced through the digestion of chitin containing byproducts from crustacean processing by chitinases were investigated as a carbon source for yeast *Pichia kudriavzevii* strans, which can converted chitooligo-saccharides into single cell proteins utilized as feed component.[12,17]

E. Textile Industry

Chitin and its derivatives can be used in the textile industry for the production of manmade fibers and as textile fiber finishes, coatings, and textile auxiliaries.[28,107] The fibers are prepared by a spinning process involving the extrusion of chitin, chitosan, or chitin formate and acetate solutions into coagulation bath. The solvents for these compounds are chloroethanol and sulfuric acids or trichloroacetic and methylene chloride mixtures for chitin and 10% acetic acid for chitosan dissolution. However, the fibers formed from chitin and its derivatives have relatively low tensile strength. Additionally, the chitosan fibers obtained by spinning from aqueous acetic acid solution through caustic coagulation are not resistant at pH below 5.5 and need further modification. From this reason, chitin and its derivatives are used only as a coating material for cellulosics, nylons, cotton, and wool fibers. The use of such modified fibers includes the production of wound dressings, medical textiles, sanitary absorbents and not allergenic, deodorizing, and antimicrobial underwear, sportwear, and socks. The addition of chitin into the coating of waterproof textiles causes a large increase in its water vapor permeability. Moreover, the finishing of wool fibers with chitin derivatives improves their dyeability and colorfastness.

F. Sorbents and Enzyme Supports

The high porosity and chemical properties of chitosan causes its high affinity for heavy metal ions such as cadmium, chromium, cooper, lead, mercury, and uranyl.[49,66] Chitosan has better sorption capacity and selectivity than zeolites, activated carbon, or

organic sorbents traditionally used for the reduction of the contamination of surface waters or waste-waters from industrial effluents.[55,59,100] Some studies were also performed on the interactions between chitosan and alkali-earth elements, but this polysaccharide seems to be ineffective toward alkaline or alkali-earth ions. The crosslinking of chitosan gel beads in glutaraldehyde or epichlorohydrin enhances the resistance of sorbent against dissolution in acid media. Chitosan can be also used as an effective coagulant of proteins from food processing liquid wastes.[9] Chitosan is more effective than activated carbon for the purification of polychlorinated biphenyl (PCB)-contaminated water.[93] The adsorption capacity of chitosan can be increased by cross-linking with glutaraldehyde and followed reduction with $NaBH_3CN$. Moreover, the combination of tyrosinase and chitosan is effective in removing of cancerogenic phenols from surface waters.[92] Tyrosinase in the presence of molecular oxygen catalyzes the transformation of monophenols to *o*-benzoquinones removed in the next step by adsorption and coagulation with chitosan. Chitosan may be applied as a thin coat crosslinked with glutaraldehyde coating of sand particles. The coated sand is an efficient adsorbent, for example, of anionic dyes, which can be easily regenerated by treatment with NaOH solution without appreciable activity loss.[105]

Many workers demonstrated the suitability of chitin and its derivatives as enzyme supports. Enzymes can be immobilized on partially deacetylated chitin by chemical linking or by adsorption.[53,86] Magnetic beads activated by chitosan coating are suitable as enzyme support, easily removed from reaction media.[84]

CONCLUSIONS

Recent investigations concerning the properties and applications of chitin and its derivatives show that the development of new technologies for the utilization of these polysaccharides will play a significant role in the creation of the new industrial field in the nearest future. The progress of industrial chitin and chitosan processing depends on the utilization microbial sources of these polysaccharides, which can be available in the desired amount from cultures cultivated on an inexpensive media. More study is also needed to select an enzyme with a high ability to cleveage glycoprotein complexes during deproteinization of crustacean shells and mold mycelia. Its application provides facilities for the isolation of chitin with a very low level of protein impurities. Furthermore, the enzymatic conversion of chitin to chitosan on an industrial scale should solve the problem of low efficiency of the process caused by the limited availability of acetyl groups in the interior of chitin molecules, which allow deacetylases to convert chitin to chitosan. A sufficient level of deacetylases production for this purpose can be achieved using recombinant microorganisms with a high productivity of the desired enzyme. Especially important seems to be the detailed study of the immunological and antimicrobial activities of chitin derivatives and their ability to stimulate connective tissue reconstruction. Future studies should also be focused further estimations of the chitin resources and chitin content on a whole body mass. Considering the high production of chitin by crustaceans, the importance of this polymer in biogeochemical cycles of carbon and nitrogen should be estimated.

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