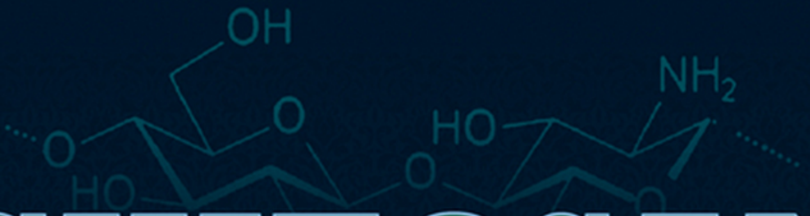

CHITOSAN

Derivatives, Composites and Applications

Edited by

Shakeel Ahmed

Saiqa Ikram

Scrivener Publishing

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Chitosan

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Preface

Many books are devoted to the description of polymers that are not of biopolymers or the polymers which are originating from the natural resources; the techniques for their mass/industrial production, their art, value, and their distribution reflecting the wealth of the information existing in the field of science and engineering to therapeutics. On the other hand, there is no book exclusively devoted to the fascinating topic of biopolymers especially to "Chitosan" and its unique properties and applications. A few books contain scattered chapters and details on chitosan emphasizing the possibilities of locating fragments of information elsewhere. However, again there is no book that is solely devoted to chitosan and its versatile applications. In the meantime, the expanded applications of chitosan are on the rise in many fields, making a book that covers both past and novel applications of this "Biopolymer for the Future" as well as its properties and ways in which to manipulate them, crucial.

The main aim in compiling this book is to present, in easy-to-follow sequence, a description of chitosan production methods and of techniques which can be used to estimate and modify its physical and chemical properties. This book offers a full description not only of the traditional and recent developments following applications of chitosan in the fields of biotechnology, environmental studies, food, medicine, water treatments, drug delivery including all therapeutically usages, but also topics which have never been covered in the literature, making it of utmost importance to industry and academia.

We hope that this book will assist all levels of readers. It is dedicated not only to academic community but also to the researchers and industrialist who will find this book to be a source of knowledge as well as a launching pad for novel ideas and inventions. In particular, this book is expected to be of interest to the people involved in formulations, water-treating methods, food scientists, technologists, industrial chemists and engineers. Potential readers also include both professional and dedicated non-professional environmentalists, agriculturists, and those working on the development of novel chitosan-based matrices and their applications.

Finally, it is expected that this book will find a prominent place in the traditional universities and research institutions libraries where chemistry, biotechnology, medicine as well as environmental studies, and other practical and theoretical mechanized topics are taught, studied, and implemented.

Shakeel Ahmed and Saiqa Ikram
Delhi, India
June 2017

Section I

PRODUCTION AND DERIVATIVES OF CHITOSAN

1

Chitin and Chitosan: History, Composition and Properties

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Abstract

Chitin and chitosan are most abundant naturally occurring polymers, ranked second after cellulose. Chitin is generally extracted from natural sources viz. terrestrial organisms, marine organisms, microorganisms like fungi and enzymatically from crustaceans shell waste materials. On the other hand, chitosan is obtained from the deacetylation of the former, chitin.

Nowadays, chitin and chitosan are commercially manufactured from biowastes obtained from aquatic organisms. But due to the seasonal and capricious availability of raw materials, terrestrial crustaceans and mushrooms are the alternative source for their production. Structurally, chitin and chitosan are *N*-acetyl-D-glucosamine units and D-glucosamine units, respectively, with only difference in hydroxyl group of cellulose. Both the biopolymers are biodegradable and possess many beneficial properties such as biocompatibility, antimicrobian, hemostatic, anti-inflammatory, antioxidant, mucoadhesion, analgesic, non-toxicity, adsorption enhancing, antihypertensive, anticholesterolemic, anticancer, and antidiabetic. Due to having such respectable properties chitin, chitosan, and their corresponding derivatives are greatly exploiting by the scientists and also getting tremendously better results in medical and engineering fields.

***Keywords*:** Chitin, chitosan, history, structure, properties, solubility, viscosity, biomedical, anticancer

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1.1 Chitin

1.1.1 History

French scientist Prof. Henri Braconnot for the very first time discovered chitin in 1811 in mushroom. After that Odier found the same compound in the cuticles of insects in 1823 and called it "Chitin" from the Greek word meaning tunic, covering or envelope [1].

This was how it begins the research in a new direction of polymers present in our nature. Gradually as the time passed away in 1859, Prof. C. Rouget coined another noval biopolymer, actually derived from previous chitin, and it was known as "Chitosan." In 1878, Ledderhose revealed that the chitin consists of glucosamine and acetic acid. Thereafter, in 1930s and 1940s, both former and latter attract considerable attention as evidenced by about 50 patents. Chitin is the most abundant naturally occurring polymer, ranked second after cellulose and also most abundant naturally occurring polysaccharide possesses amino acid and sugars. Chemically, chitin is composed of *N*-acetyl-D-glucosamine units with β-(1-4) glycosidic linkage [2].

1.1.2 Sources of Chitin

Chitin is generally produced from natural sources viz. terrestrial organisms, marine organisms, microorganisms like fungi and enzymatically from crustaceans shell waste materials. On the other hand, chitosan is obtained from the deacetylation of the former, chitin. Nowadays, chitin and chitosan are commercially manufactured from biowastes obtained from aquatic organisms. But due to the seasonal and capricious availability of raw materials terrestrial crustaceans and mushrooms are the alternative source for their production [2]. The industrial manufacturing of synthetic polymers was restricted by the time because of the inadequate facilities as well as the cutthroat competition in synthetic polymers. Therefore, again the shellfish shells, crustaceans and shrimps revitalized the interest in late 1970s (Global industry analysis since 2004). Various sources for extraction and production of chitin can be categorized as follows:

i. Terrestrial organisms
ii. Marine or Aquatic organisms
iii. Microorganisms (e.g., Fungi)

1.1.2.1 Terrestrial Organisms

From commercial point of view, these organisms are mainly used for the extraction of chitin, due to their easy availability and processability.

Table 1.1 Percentage of chitin produced from different sources [3].

Source	**% Chitin produced**
Cockroach	30–37% (abdomen, legs, and head) 25–29% (genitalia, antennae, thorax, and cerci) 19% (fore and hind wings)
Extatosoma tiaratum	24% (exuviae) 3.4% (eggs) 3.8% (eggshells)
Sipyloidea sipylus	14%
Honey bee	23–32%
Silkworms	20%

Terrestrial species generally includes crustaceans such as *Porcellio scaber*, *Armadillidium vulgare;* arthropods, nematodes, insects, silkworms, mosquitoes, honeybee, *Sipyloidea sipylus*, *Drosophila melanogaster*, *Extatosoma tiaratum*, and many more [3]. As the composition of these organisms is quite different, there is a variation in the contribution to the percentage of chitin produced as depicted in Table 1.1.

1.1.2.2 Marine or Aquatic Organisms

Chitin produced from aquatic species includes diatoms, algae, crabs, shrimps, lobster, squids, and krill. The hazardous waste materials generated from head, thorax, shells, and claws of shellfish are utilized as raw materials for processing, containing 15–40% chitin, 20–40% proteins, and 20–50% $CaCO_3$. The waste streams of molluscs and crustaceans are the main source of chitin. Also they constitute a rich source of proteins, flavor compounds, and various pigments and hence are of great attention for different research fields and industries as well. Actually, if they are disposed of in the open environment of the water bodies, such as sea or river, then they are problematic due to the higher biochemical oxygen demand and eutrophication [4].

1.1.2.3 Microorganisms (e.g., Fungi or Mushroom)

Chitin can be obtained from microorganisms either by fermentative methods or biotechnological methods. Utilization of various microbes makes it easier for industries to produce chitin widely and hence microbes are considered as the economic source of extracting chitin as well as

Table 1.2 Examples of sources of extraction of chitin [5].

Terrestrial	**Marine or aquatic**	**Microorganisms**
Porcellio scaber	*Euphausia* sp. (Krill)	*Mucor rouxii*
Armadillidium vulgare	*Paralithodes* sp. (King crab)	*Mucor racemosus*
Sipyloidea sipylus	*Chionoecetes* sp. and *Carcinus* sp. (Crab)	*Cunninghamella Echinulate*
Drosophila melanogaster	*Callinectes* sp. (Blue crab)	*Aspergillus niger*
Extatosoma tiaratum	*Loliginidae* sp. (Squid)	*Rhizopus oryzae*
Bombyx mori	*Pandalus* sp. (Shrimp)	*Mucor circinelloides*
Apis mellifera	*Nephrops* sp. (Lobster)	*Pleurotus ostreatus*
Periplaneta americana	*Lepas* sp. (Goose Barnacle) *Penaeus* sp. (Prawn)	*Absidia. Coerulea*
	Crassostrea sp. (Oyster)	*Lentinus edodes*
	Mytilus sp.	*Auricularia auricula-judae*

chitosan. Microbial world mainly includes fungi (cell wall, mycelia, septa) molds, chrysophyte algae, yeasts, prosthecate bacteria, spores of streptomycete and ciliates. Except Oomycetes, remaining Ascomycetes, Basidiomycetes, Deuteromycetes, and Zygomycetes consists of 2–65% chitin/chitosan. Especially, Mucoralean strains viz. *Syncephalastrum racemosum* and *Cunninghamella echinulata* cell wall exhibited maximum chitin/chitosan yield of 7% per mycelia dry weight under optimum conditions [5–7]. Some of the examples of extraction of chitin from terrestrial, marine and microorganisms are listed in Table 1.2.

1.1.3 Extraction of Chitin

Chitin can be extracted from insect cuticle, tracheae and peritrophic matrix [25], shellfish waste such as shrimps, crabs, krill, lobster, fishes and microorganisms such as fungi or mushroom mycelia and some bacteria as discussed above. Generally, extraction of chitin involves the following steps:

i. Demineralization
ii. Deproteination
iii. Decolorization

There may be difference in the sequence of these steps [3, 8]. For instance, Kumari *et al.* described the extraction of chitin from fish scales of *Labeo rohita* but after demineralization they performed decolorization followed by deproteination [9].

1.1.3.1 Demineralization

It can be performed by using strong acids such as HCl, H_2SO_4, HNO_3 and weak acids such as CH_3COOH and HCOOH. But generally HCl seems to be best one. Previous researchers showed that the concentration of HCl was about 1N or 2N for 0.3–96 h from 25–100 °C (for insect cuticles). But from last decade, it has been found that only 0.25M to 2M concentration of HCl for 1–36 or 48 h is sufficient at temperature 0–100 °C for just 15 min [8]. This step particularly performed to remove the minerals, especially $CaCO_3$.

1.1.3.2 Deproteination

It is generally performed under alkaline medium of 0.75N–2.5N or 1M NaOH aqueous solution for 2–42 or 1–72 h at temperature 40 or 65–100 °C in crustaceans and marine shrimp shells, respectively. NaOH, KOH, $NaHCO_3$, Na_2CO_3, K_2CO_3, $Ca(OH)_2$, $NaHSO_3$, and Na_2SO_3 are the reagent that can be used for deproteination of the crustaceans. Due to the adverse effect of these perilous chemicals on environment, nowadays, proteolytic enzymes such as trypsin, pepsin, or papain have been used to raise the efficiency of deproteination [10, 11].

1.1.3.2.1 Enzymatic Deproteination

Proteolytic enzymes can be extracted from various sources such as *Bacillus mojavensis* A21, *B. subtilis* A26, *B. licheniformis* NH1, *B. licheniformis* MP1, *Vibrio metschnikovii* J1, alkaline protease from Sardinelle (*Sardinella aurita*), Goby (*Zosterisessor ophiocephalus*), *Aspergillus clavatus* ES1, and Grey triggerfish (*Balistes capriscus).* One unit of protease activity can be defined as the amount of enzyme required to liberate 1 μg of tyrosine per minute [12]. Degree of Deproteination can be calculated as follows:

$$\%\ DDP = \frac{[(Pi \times S) - (Pf \times R)]}{Pi \times S} \times 100 \tag{1.1}$$

where *Pi* and *Pf* are the concentrations of protein at initial and final hydrolysis; while *S* and *R* are the mass of original sample and hydrolysed residue, respectively [12].

1.1.3.3 Decolorization

In order to remove pigmentation and melanin, the mixture has been treated either with hydrogen peroxide or potassium permanganate solution [3].

According to Mohammed *et al.* approximately 35% of chitin (dry weight) were obtained from prawn shell after deproteination, decolorization, and demineralization [13]. Chitin can be extracted either via using biotechnological method or via chemical method as depicted in Figures 1.1 and 1.2.

1.1.4 Structure and Composition

Chitin is a linear copolymer of β-(1-4)-linked 2-amino-2-deoxy-D-glucan and 2-acetamido-2-deoxy-D-glucan. Structurally, chitin is composed of β-(1-4)-linked *D*-glucosamine units. Unlike cellulose, the hydroxyl group of cellulose is replaced by *N*-acetyl group ($-NHCOCH_3$) as shown in Figure 1.3. Chitin has been existed mainly in three solid state polymorphic forms viz. α, β, and γ. Out of these allomorphs, α-chitin is the most abundant one

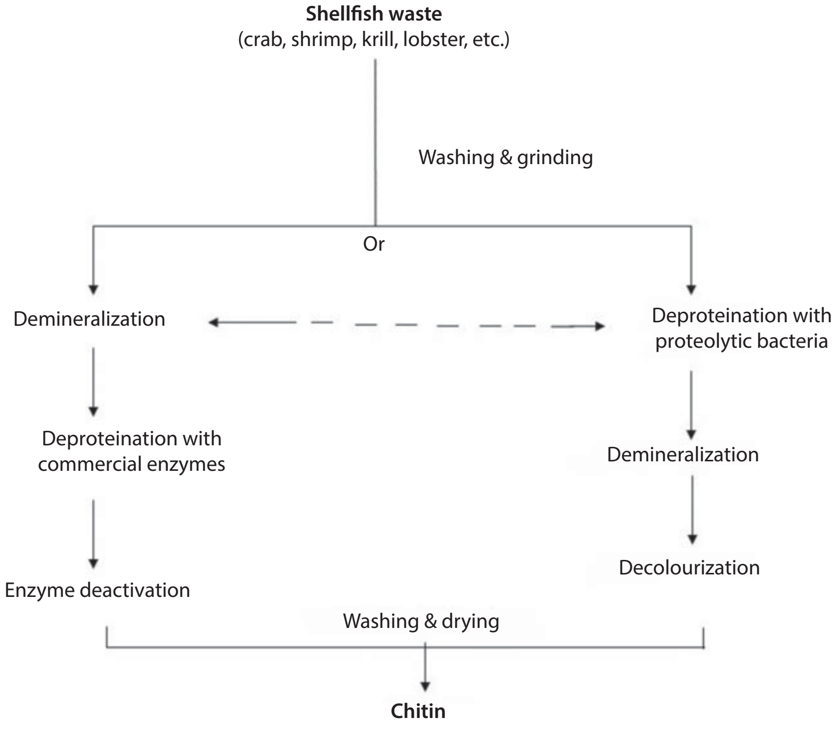


Figure 1.1 Extraction of chitin.

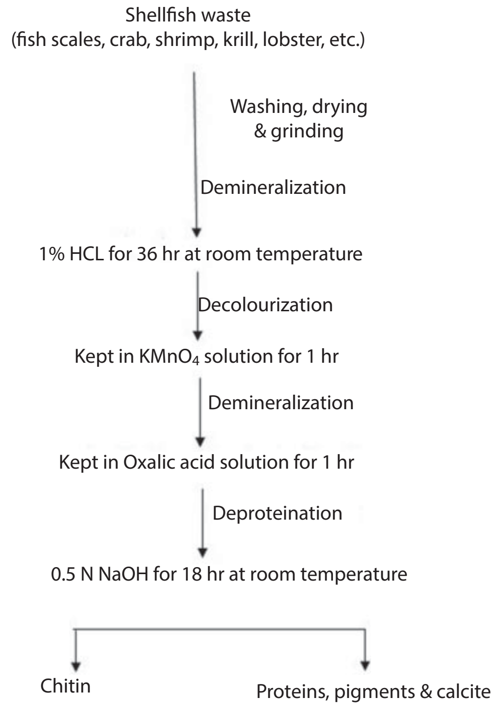
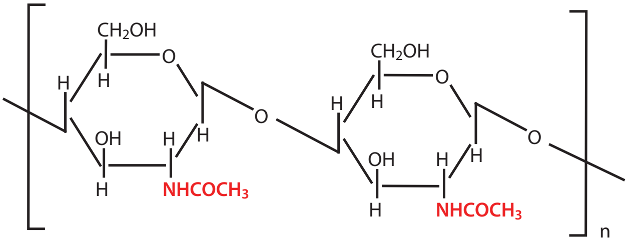
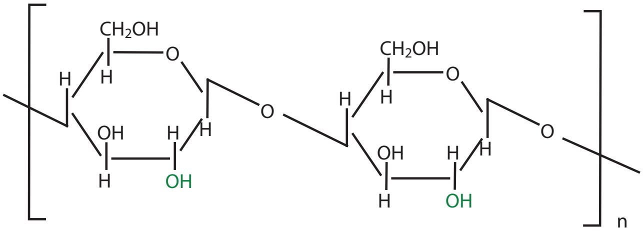


Figure 1.2 Extraction of chitin by alkaline solution.



Chitin



Cellulose

Figure 1.3 Structural comparison of chitin and cellulose.

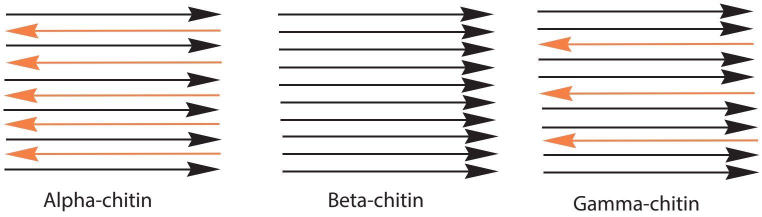


Figure 1.4 Antiparallel and parallel arrangements of different allomorphs of chitin.

followed by β and then rarest one is γ-chitin. The major source of α-form of chitin is generally shrimps, insect cuticle, crab, krill, lobster, cell wall of yeast and Zygomycetes. The abundancy of α-chitin favors the significant quality of chitin as high crystallinity and purity due to the absence of calcium carbonate, proteins, and pigments. Instead β-chitin is found in connotation with proteins in squid pens while γ-chitin is found in cuttlefish stomach lining. X-Ray diffraction revealed that the inner ring present in α-form of chitin is unaffected from hydration while the inner ring of β-chitin is sensitive to hydration. Moreover crystallographically, α-chitin exhibits two antiparallel molecules per unit cell, whereas β-chitin exhibits one parallel arrangement as depicted in Figure 1.4. As far as similarity is concerned, both the allomorphs have same moiety of *N*-acetylglycosamine [14–17].

1.1.5 Properties of Chitin

1.1.5.1 Solubility, Reactivity, and Viscosity

Like cellulose, chitin as biopolymer is highly crystalline materials having specific solvent behavior. As chitin is β-(1,4)-linked *N*-acetyl-D-glucosamine therefore, its solubility and reactivity are highly influenced by $-NH_2$ and $-OH$ functional groups. Being a typical natural polymer obtained from different sources, chitin possesses excessive hydrogen bonding which in turn degrade it before melting and thus making it essential to be dissolve in suitable solvent. Chitin is generally hydrophobic in nature and hence insoluble in water and organic solvents at room temperature but soluble in hexafluoroisopropanol, chloroalcohols, and hexafluoroacetone in association with mineral acid aqueous solution and dimethylacetamide having 5% lithium chloride [18]. In other words, water is a thermodynamically poor solvent for uncharged chitin unit due to strong interactions. Thus, charge on the polymeric chain can make the chitin soluble by releasing the attractive forces. It has also been reported that small amount of chitin can be dissolved in 1% acidic solution at 121 °C for 20 min and on reacting it