1

Sources of Chitin and Chitosan and their Isolation

Leen Bastiaens, Lise Soetemans, Els D'Hondt, and Kathy Elst
VITO – (Flemish Institute for Technological Research), Mol, Belgium

COPYRIGHTED MATERIAL

Chitin is a natural biomolecule that was reported for the first time in 1811 by the French professor Henri Braconnot as *fungine* [1] and in 1823 by Antoine Odier as *chitin*. Chitin consists of large, crystalline nitrogen-containing polysaccharides made of chains of a modified glucose monosaccharide, being *N*-acetylglucosamine. It is ubiquitously present in the world and has even been reported to be one of the most abundant biomolecules on earth, with an estimated annual production of 10^{11}–10^{14} tons [2, 3]. Chitin serves as template for biomineralization such as calcification and silicification, providing preferential sites for nucleation, and controlling the location and orientation of mineral phases [4, 5]. This phenomenon explains the presence of chitin in solid structures in a variety of biomass such as cell walls of fungi and diatoms and in exoskeletons of Crustaceans. Chitin is present in diverse structures in at least 19 animal phyla besides its presence in bacteria, fungi, and algae [5].

Chitosan is mainly known as a partially deacetylated derivative of chitin that is more water soluble than chitin, and as such is easier to process. For this reason, chitosan—and, in some cases, even more preferably, the relatively small sized (1–10 kDa) chitosan oligomers—are the molecules that are envisioned for multiple applications such as agriculture; water and wastewater treatment; food and beverages; chemicals; feed; cosmetics; and personal care [6, 7]. In addition, chitosan oligomers have been reported as being bioactive [8], offering potential for application in, for instance, wound dressing and cosmetics. Although chitin and chitosan are versatile and promising biomaterials [9], the extraction

Chitin and Chitosan: Properties and Applications, First Edition.
Edited by Lambertus A.M. van den Broek and Carmen G. Boeriu.
© 2020 John Wiley & Sons Ltd. Published 2020 by John Wiley & Sons Ltd.

and purification of chitin and its conversion to chitosan (oligomers) require several process steps, and these have been mentioned as bottlenecks that hinder the wider use of the underspent chitin in the world.

This chapter intends to provide more information related to (1) the structure of chitin, (2) sources of chitin and chitosan, and (3) their extraction and purification, as well as (4) the conversion of chitin into chitosan. The further conversion of chitosan to chitosan oligomers is the subject of Chapter 3.

1.1 Chitin and Chitosan

1.1.1 Chemical Structure

Chitin, and its derivate chitosan, are natural polysaccharides consisting of 2 monosaccharides, *N*-acetyl-D-glucosamine and D-glucosamine, connected by β-1,4- glycoside bonds. Depending on the frequency of the latter monosaccharides, the molecule is defined as chitin or chitosan. Chitin contains mainly *N*-acetyl-D-glucosamine and can be transformed to chitosan by partial deacetylation of the monomer *N*-acetyl-D-glucosamine to D-glucosamine (see Figure 1.1) [7]. Diverse definitions of chitin and chitosan circulate in literature. Most sources mention a deacetylation degree of at least 50% [7, 10] as a criterion to define the molecule as chitosan. Others report a deacetylation degree of at least 60% or 75% for chitosan, implying that, respectively, more than 60% or 75% of the monosaccharides are D-glucosamine moieties [11–13]. Chitin in its natural appearance is usually already a heteropolymer, with a deacetylation degree ranging between 5% and 20% [14]. The structure of chitin is very similar to that of cellulose and shares generally the same function of providing structure integrity and protection of the organism.

1.1.2 Different Crystalline Forms of Chitin

Chitin usually functions as a supporting material and is composed of layers of polysaccharide sheets. Each individual sheet consists of multiple parallel-positioned chitin chains [17], as schematically presented in Figure 1.2. Highly crystalline fibers are formed when the polymer sheets are placed next to each other and form interactions [12]. Depending on their orientation, three crystalline forms have been reported (α, β, and γ).

The most abundant form is α-chitin, which is present in almost all crustaceans, insects, fungi, and yeast cell walls [7]. In this formation, the chitin sheets (three sheets as example in Figure 1.2a), consisting of parallel chitin chains (for each sheet, two chains are presented in Figure 1.2a), are positioned in an anti-parallel way, allowing a maximum formation of hydrogen bonding. More specifically, two intramolecular and two intermolecular bondings are formed: a first intermolecular bonding with a vertical neighbor chain (in the same sheet), and another with a horizontal neighbor chain form a different sheet [15]. These hydrogen bounds create a remarkably high crystallinity, resulting in a more stiff and stable material. Therefore, α-chitin is characterized as a non-reactive and insoluble product [16]. Since this form is the most common polymorphic, α-chitin has been extensively studied [12].

On the other hand, in β-chitin, the chitin sheets are ordered in parallel (Figure 1.2b) with weaker intermolecular forces. This results in a softer molecule with a higher affinity

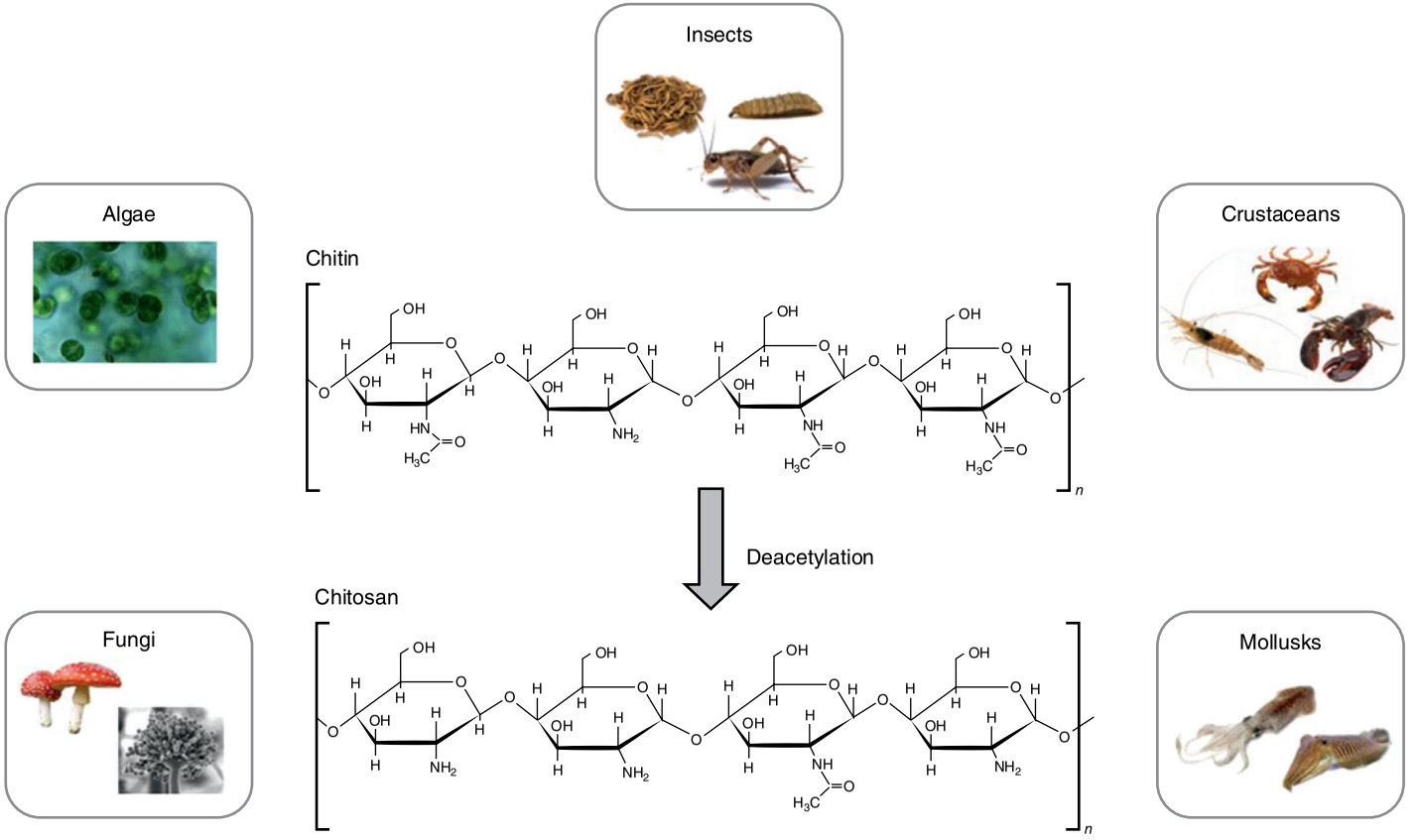


Figure 1.1 *Chemical structure of chitin and chitosan and some examples of species that contain chitin.*

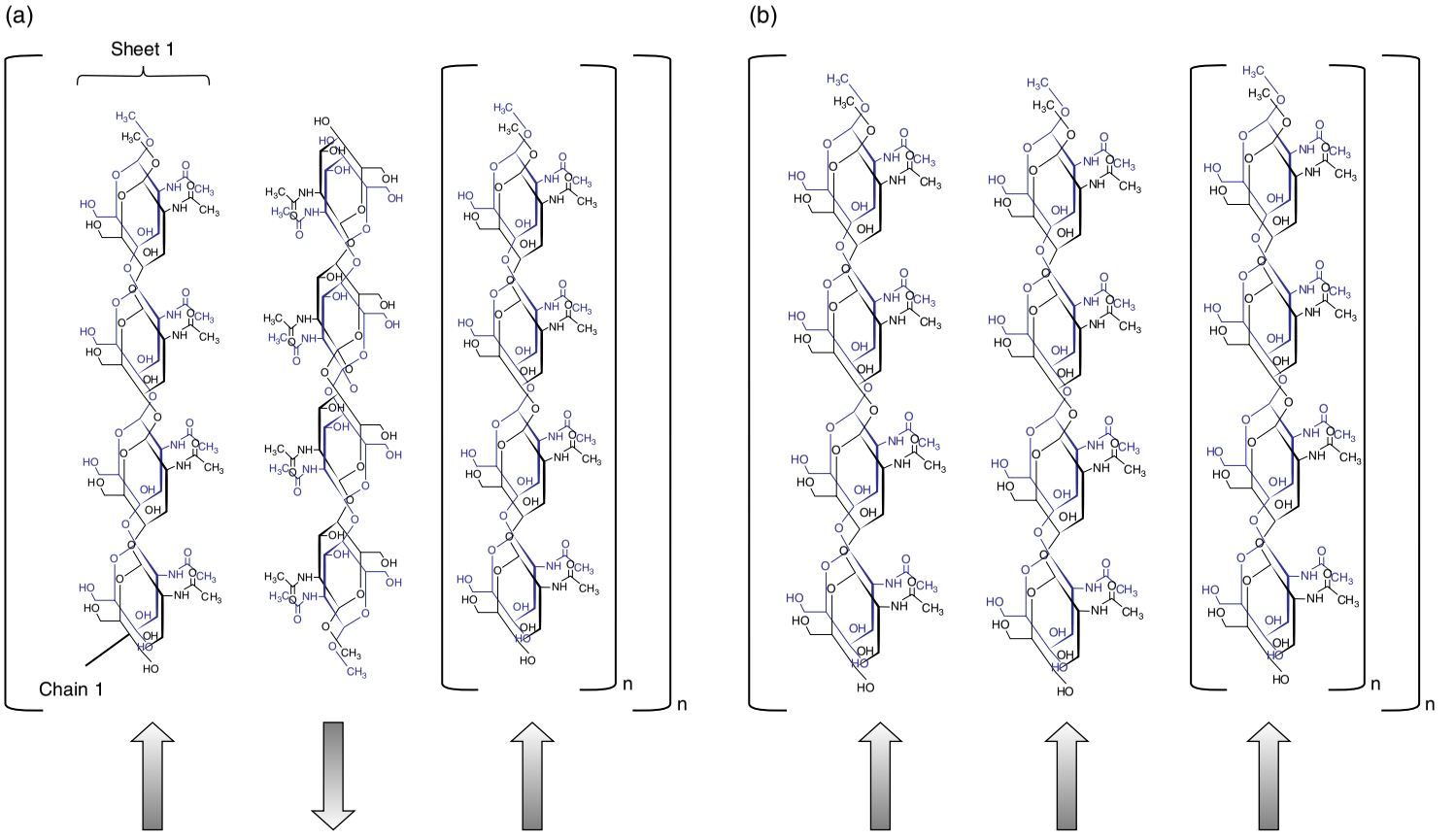


Figure 1.2 *Schematic representation of (a) α-form and (b) β-form of chitin.*

for solvents and a higher reactivity. It is proven to be soluble in formic acid and can be swollen in water [15]. This chitin form is present in the squid pen, in the tubes of pogonophoran and vestimentiferan worms, and in monocrystalline spines excreted by diatoms such as *Thalassiosira fluviatilis* [7]. Although squid and tubes of *Tevnia jerichonana* both contain β-chitin, their crystallinity differs. This implies that the crystallinity also depends on the source. Chitin obtained from squid pens is semi-crystalline, and chitin from *T. jerichonana* is almost complete crystalline [7, 8, 16].

The third formation, γ-chitin, is less common. It is considered to be a mixture or intermediate form of α- and β-chitin with both parallel and antiparallel arrangements [16]. More specifically, every third chitin chain has the opposite direction to the two preceding chitin sheets [13, 15]. Very few studies have been carried out on γ-chitin, and it has been suggested that γ-chitin may be a distorted version of the other two instead of a true third polymorphic form.

1.2 Sources of Chitin and Chitosan

1.2.1 Sources of Chitin

For more than a century, scientists reported chitin to be present in a variety of organisms. Initially, zoologists named all hard yellow–brownish structures chitin, without chemical analysis, sometimes generating misleading data. Later on, it was accepted that the presence of chitin could only be demonstrated after chemical tests. Hymann (1958), for instance, used an iodine-based color test to study the presence of chitin in different sea animals. Later on, more sophisticated techniques such as Fourier transform infrared spectroscopy (FTIR), nuclear magnetic resonance (NMR), mass spectroscopy (MS), X-ray diffraction (XRD), and Raman spectroscopy were used [18]. Quantification of chitin is challenging and only reported in more recent publications. Currently, quantitative data on chitin contents are still incomplete, and available numbers need to be interpreted with care. Not only are different quantification methods used, but also varying parts of the biomass are considered (whole organism versus chitin-rich part of the organisms).

Nowadays, it is estimated that a large portion of chitin produced in the biosphere is present in the oceans [19, 20]. It can be found in aquatic species belonging to phyla such as Cnidaria (corals [21, 22]), Entoprocta [23], Phoronida (horseshoe worms [18]), Ectoprocta [18], Brachiopoda (lamp shells [18]), Bryozoa [19], Porifera (sponges [5, 24]), and Mollusca (squid [8, 23], cuttlefish [26], and clams [8]). Further, chitin has also been detected in fungi (mushrooms and yeasts [1]), algae (diatoms [27], coralline algae [28], green algae [29, 30]), Onychophora (velvet worms), and protozoa [31]. The most easily accessible sources of chitin, however, are the exoskeletons of Arthropoda, which includes insects [32–35], arachnids (spiders [36] and scorpions [37]), myriapods (millipedes and centipedes [38]), as well as Crustaceans (shrimp, krill, crab, and lobster [8, 9, 18, 37]).

Table 1.1 lists examples of chitin-containing sources, along with available compositional data. The amount of chitin varies with species type, the biomass part considered, and even with seasons and growth stages [40]. Values ranging from <1% to 72% (w/w) on dry matter basis on the biomass type have been reported.

Table 1.1 *Sources of chitin.*

Origin	Species	Biomass type	N (%)	$CaCO_3$ (%)	% protein	% (w/w) chitin in biomass type of dry weight	Ref.
Crustaceans (*Arthropoda* phylum)							
Crab cuticle				40–50		15–30*	[8]
Blue swimming crab (male)	*Portunus pelagicus*	Shells		68.87	10.33	20.8	[26]
Blue swimming crab (female)	*Portunus pelagicus*	Shells		65.5	14.36	20.14	[26]
Crabs		Shells		66.58	16.68	16.73	[25]
Marbled crab	*Grapsus marmoratus*					10	[41]
Red crab	*Portunus puber*					10	[41]
Spider crab	*Maja squinado*					16	[41]
Cancer crab		Cuticula				72.1	[40]
Carcinus crab		Whole body				64.2	[40]
King crab	*Paralithodes*	Whole body				35	[40]
Shrimp cuticle				20–30		30–40*	[8]
Jinga shrimp	*Metapenaeus affinis*	Shells		45.66	37.59	16.75	[26]
Brown shrimp	*Penaeus aztecus*	Shells		48.97	29.5	21.53	[25]
Pink shrimp	*Penaeus duorarum*	Shells		42.26	34.02	23.72	[25]
Shrimp	*Palaemon Fabricius*					22	[41]
Shrimp	*Penaeus monodon*	Shells	5.74			10	[33]
Grooved tiger prawn	*Penaeus semisulcatus*	Shells		52.03	28.84	19.13	[26]
Scyllarid lobster	*Thenus orientalis*	Shells		61.81	16.93	21.26	[26]
Locust lobster	*Scyllarus arctus*					25	[41]
Spiny lobster	*Palinurus vulgaris*					32	[41]
European lobster	*Homarus vulgaris*					17	[41]
Crayfish	*Procambarus clarkii*	Shells		63.94	15.56	20.6	[25]
Crayfish	*Astacus fluviatilis*					36	[41]
Krill cuticle				20–25		20–30*	[8]
Barnacle	*Lepas anatifera*					7	[41]
Squilla	*Squilla mantis*					24	[41]
Isopoda	*Oniscus asellus*	Dried adult	4.7			6–7*	[32]

Insects (*Arthropoda* phylum)							
Honey bees	*Apis mellifera*	Exoskeletons	5.56			2.5	[33]
Grasshopper	*Aiolopus simulatrix*	Fully dried adult				5.3*	[42]
	Aiolopus strepens	Fully dried adult				7.4*	[42]
	Duroniella fracta	Fully dried adult				5.7*	[42]
	Duroniella laticornis	Fully dried adult				6.5*	[42]
	Oedipoda miniata	Fully dried adult				8.1*	[42]
	Oedipoda caerulescens					8.9*	[42]
	Pyrgomorpha cognata					6.6*	[42]
	Schistocerca gregaria	Exoskeletons	2.92			12.2	[33]
Black soldier fly	*Hermetia illucens*	Fully dried prepupae		2.7–19.7 [2]	37.7–40.7	5.6–6.7	[34]
	Hermetia illucens	Fully dried larvae			17.5	2.10 [3]	[43]
	Hermetia illucens	Whole larvae				6.4	Own work
Lesser mealworm	*Alphitobius diaperinus*	Whole worm				5.6	Own work
Beetle	*Melolontha melolontha*	Fully dried adult	6.72			13–14*	[35]
Beetle	*Calosoma rugosa*	Exoskeletons				5	[33]
Cockroach	*Blattella*					18.4 ^	[40]
Cockroach	*Periplaneta*	Cuticle				54.8^	[40]
	Blatta lateralis	Fully dried nymphs			19.0	0.67 [3]	[43]
Silkworm	*Bombyx*					44.2^	[40]
	Bombyx mori L.	Cuticle			23–52	36–62	[44]
Waxworm	*Galleria*					33.7^	[40]
Tebo worms	*Chilecomadia moorei*	Fully dried larvae			15.5	1.11 [3]	[43]
Tobacco hornworm	*Manduca sexta*	Exoskeleton of the adult (organic part)			60	20	[21]
Ladybug	Coleoptera					27–35^	[40]
Shield bug	*Palomena prasina*	Fully dried adult				10.8*	[45]
Butterfly	*Pieris*					2	[40]
Housefly	*Musca domestica*	Fully dried adults			19.7	1.19 [3]	[43]
Mollusks (Mollusca phylum)							
Squid (Cephalopoda)		Pen		Negligible		20–40*	[8]
		Pen		4.74	46.23	49	[25]
	Loligo vulgaris					40	[41]

(*Continued*)

Table 1.1 *(Continued)*

Origin	Species	Biomass type	N (%)	$CaCO_3$ (%)	% protein	% (w/w) chitin in biomass type of dry weight	Ref.
Cuttlefish (Cephalopoda)	Sepia spp.	Shells		91.25	1.35	7.4	[26]
		Pens		88.48	6.12	5.4	[25]
	Sepia officinalis					20	[41]
Clam/oyster (Bivalvia)		Shell		85–90		3–6*	[8]
Other animals							
Bryozoa	*Plumatella repens*	Dried				13.3*	[32]
Black coral (Cnidaria)	*Antipathella fiordensis*	Skeleton (organic part)			70	10	[21]
Horseshoe worms (Phoronida)		Tube					[18]
Sponges (Porifera)							[28]
Spiders (Arachnids)	*Geolycosa vultuosa*		6.42			8–8.5	[36]
	Hogna radiata		6.41			5.5–7	[36]
Fungi							
Basidiomycota (yeast)	*Fomes fomentarius*				2.4*		[45]
	Lactarius vellereus					19	[40]
		Full biomass				11	[46]
Basidiomycota (mushroom)	*Agaricus bisporus*	Cell wall				43.8	[47]
Zygomycota	*Mucor rouxii*	Cell wall				50.1	[48]
						44.5	[40]
	Rhizopus oryzae	Full biomass				14.6	[49]
Ascomycota (yeast)	*Aspergillus niger*	Cell wall				42	[40, 48]
	Penicillium chrysogenum	Cell wall				20.1	[40]
	Penicillium notatum	Cell wall				18.5	[40]
	Saccharomyces cerevisiae	Cell wall				2.9	[40]
Algae							
Diatoms	*Thalassiosira fluviatilis*	Ropes					[50]
Green algae	*Pithophora oedogonia*	Cell wall					[30]
	Chlorella vulgaris	Cell wall					[29]

Note: *not provided how it is measured; ^based on the weight of the organic cuticle, others were measured based on weight differences of the raw materials and that of the sample obtained after acid and alkaline treatments[2], crude ash[3] based on acid detergent fiber, minus present amino acids.

1.2.1.1 Crustaceans (Part of Arthropoda)

Chitin is located in the exoskeletons of Arthropoda. The skeleton is a tough and hard material designed for mechanical support to the body and functions as an armor against predators. These characteristics can be dedicated to the presence of highly crystalline α-chitin that, combined with proteins, forms a hybrid material with high stiffness (at least 150 GPa). These chitin–protein complexes, together with minerals for strength, form a fibrous structure that is among the most resistant organic materials [38, 52]. The shells of crustaceans mainly contain chitin (20–30%), proteins (20–40%), minerals (30–60%), pigments, and sometimes also lipids (0–14%) [8, 38]. Based on Table 1.1, it can be said that the chitin content ranges from 6% to 72% in crustacean shells. This large variation can be explained by the origin of the biomass (e.g., differences in species such as gray shrimp versus pink shrimp, or in growth phase), the part of the biomass considered for chitin analysis (e.g., shells as such or stripped of remaining flesh), or the varying pretreatment or analysis method used. Crabs and shrimp are mostly used at an industrial level and contain 10–72% chitin. As mentioned previously, the differences in species impacts the chitin content—for example, the Cancer crab contains 72% chitin as compared to 64% chitin in the Carcinus crab and 35% chitin in the king crab.

1.2.1.2 Insects (Part of Arthropoda)

Chitin is found in the exoskeleton of insects, but also in internal structures such as the inner cuticular linings of the alimentary canal and the tracheal system. In contrast with the exoskeletons of crustaceans, insect cuticles contain also catecholamines besides chitin, proteins, lipids, and minerals. The catecholamines are cross-linked by *o*-quinones with proteins and possibly also with chitin [14]. α-Chitin in the exoskeleton of insects serves the same function as for crustaceans. It increases the strength of the skeleton, gives structure, prevents physical and chemical damages, and protects against infectious diseases [14]. The chitin content differs significantly between different insect species. In addition, Kaya et al. found that the chitin content is also significantly dependent on the life stage of the insect. The larvae of *Vespa crabro* (wasp) had a chitin content of 2.2% dry weight (DW) in comparison with 6.2% for pupa and 10.3% of the adult. This phenomenon can be explained by the different chitin functions in different body parts during different stages [52]. Similar results were obtained by Kaya et al. with the larvae of the potato beetle (7% chitin) and the adult beetle (20% chitin) [53].

1.2.1.3 Other Sources

Spiders (also part of the Arthropoda) contain α-chitin (5–8.5%) with a high acetylation degree. Kaya et al. characterized physicochemically the chitin structure of two spider species (*Geolycosa vultuosa* and *Hogna radiata*), for which acetylation degrees of 97% and 99% were found, respectively [36]. Within the Mollusca, squids receive major attention because they are the prototype for β-chitin. In addition, high chitin content (up to 49%) in the pen have been reported [25], which may be the basis to conclude that squid pen can become increasingly common as another potentially important chitin source [54]. However, it should be kept in mind that these high percentages are related to the composition of the

chitin-rich pen that represents a very minor fraction of the whole squid. Chitin may be involved in the formation of skeletons in calcifying marine sponges [28]. Sponges are described more in detail in Chapter 4. Within the Cnidarian taxa, skeletons often contain, besides chitin, calcium-based minerals. Black corals form an exception and have a unique halogenated scleroprotein named *antipathin* associated with chitin [22]. Lophophorates (marine and freshwater Octopoda, Phoronida, Brachiopoda) have exoskeletons, named *tubes*, that consists of chitin [18]. Chitin is the most important ultrastructural compound of fungal cell walls, where it is embedded in the amorphous matrix and provides the framework of the cell wall morphology [55]. It exists in the spores, mycelia, and stalks, and has only been detected as α-chitin [55]. Its amount ranges from 2% to 50% (w/w) on dry cell wall base, whereby the lowest value corresponds to yeasts [56] and the highest to Euascomycetes [55]. Depending on the class of fungi, the cell wall can also contain glucans, mannans, as well as chitosan. As the cell wall is only a part of the fungal biomass, the overall chitin (plus chitosan) yield is lower, and values have been reported (glucosamine on dry matter base) of 8–16% (w/w) for *Aspergillus niger* and *Mucor rouxii* mycelia [48] and 12% (w/w) for *Agaricus bisporus* stalks [47].

In the case of algae, since 1965, diatoms such as *Thalassiosira* were reported to secrete β-chitin ropes that span between two recently divided daughter cells to keep them together, creating flexible cell chains that float in the water [27, 50]. However, chitin has also been shown to be present in diatoms in other forms—for instance, in the siliceous shell. In calcified coralline algae such as *Clathromorphum compactum*, chitin has been reported to be present that strengthens the skeleton and protects the algae from ocean acidification and grazing in shallow waters [28]. The presence of chitin was also demonstrated in the cell walls of the green algae *Pithophora oedogonia* [30] and *Chlorella vulgaris* [29]. Quantitative data on the chitin content in the algae, however, are scarce. The fact that chitin in algae is plant-based can be an advantage for some applications.

1.2.2 Sources for Chitosan

Chitosan is mainly known as a partially deacetylated derivative of chitin, but has also been found to be naturally present in some types of biomass. Some fungi contain chitosan as an important constituent of their cell wall at various stages their life cycle. The class of *Zygomycetes* (e.g., the *Mucor*, *Absidia*, *Benjaminiella*, *Cunninghamella*, *Gongronella*, and *Rhizopus* genera) has especially been recognized as a valuable source of chitosan [59, 60]. Chitosan content of 1–10% on dry biomass base have been found with a reported degree of deacetylation of 83–94%. Chitosan is not directly synthesized, but is rather the result of an efficient conversion of chitin to chitosan by the presence of a deacetylase enzyme [57]. The deacetylation enzymes are thought to be in close proximity to the regions where the chitin transverses the plasma membrane [59]. As chitin is synthesized, the deacetylase enzyme converts it to chitosan [57]. Since chitosan can be isolated with less extreme procedures, fungi may become an interesting source of chitosan in the future [26].

In addition to fungi, bacteria too have been reported to be able to convert chitin into chitosan using enzymatic deacetylation. Kaur et al. isolated, from soil, bacteria (*Bacillus* sp. and *Serritia* sp.) that produce chitin deacetylase and release chitosan. Although the efficiency of this process is limited due the insolubility of chitin [13], it contributes to the

degradation of chitin in nature. Further, these fast-growing bacteria, or the isolated enzymes, offer a tool for biological chitosan production [3] (see Section 1.4).

1.3 Isolation of Chitin

Although large quantities of chitin are available and future applications look promising, chitin is still an underutilized resource. Limited attention has been given to chitin, in contrast to the extensive studies on cellulose, because of the relatively laborious isolation process. Chitin present in biomass is strongly connected to other biomolecules such as proteins and minerals. These impurities need to be removed to generate a high-purity product for application development [60]. Traditional extraction processes are long, involve high consumption of hazardous chemicals, are energy consuming, and are environmentally polluting due to the need for high amounts of mineral acids and alkali [8, 40, 62].

1.3.1 Technology Principles

The process steps that have been reported to isolate and purify chitin from biomass are summarized in Figure 1.3, and explained in more detail in the subsequent subsections. It concerns biomass pre-treatment, deproteination, demineralization, decoloration, and post-treatment processes. Depending on the biomass type, the individual steps can change order, or may even not be required. The degree of crystallinity and the deacetylation degree of chitin depend on the source, and may also alter during the purification process, as was shown for crustaceans [62].

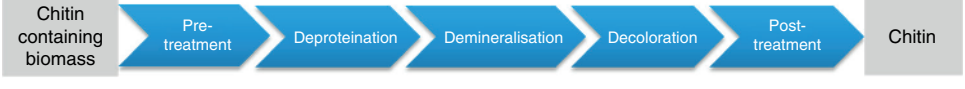


Figure 1.3 *Processes involved in the isolation and purification of chitin from biomass.*

1.3.1.1 Pre-Treatment

The pretreatment step groups all activities that are required to prepare the biomass for chitin extraction. This may comprise the removal of soft tissues by scraping, boiling, and pressing. For other organisms, boiling can be a part of a hygienization step. The biomass is mostly dried and reduced in size. However, for fermentation purposes, for example, drying is not necessary. Examples of pre-treatments are given in Table 1.2.

1.3.1.2 Demineralization

When a high amount of minerals is present, demineralization is advisable. For example, the exoskeleton of crustaceans can contain more than 50% (w/w) of $CaCO_3$ to enhance its strength [38, 52]. Two approaches have been reported—chemical and biological demineralization. Chemical demineralization uses acids such as HCl, HNO_3, H_2SO_4, CH_3COOH, and HCOOH [60]. Among these acids, HCl is the most commonly used reagent for removing the mineral constituents [40]. Biological demineralization is based on acid-producing biological processes using bacteria [3, 39, 40] or enzymes such as Alcalase® [40]. The acids

Table 1.2 *Examples of pre-treatment steps.*

Origin	Biomass type	Pretreatment step(s)	Ref.
Crustaceans	Shells	Washed with tap water or boiled; drying, mechanical crushing to reduce size; sieving aiming at 60–120 μm fraction	[63]
	Shrimp shells	Pressing and washing to remove residual meat and connective tissue	[64]
	Shrimp waste	Shells were dried at 50°C for 24 h and homogenized in a laboratory mixer	[65]
	Shrimp waste	Washed with tap water, dehydrated at room temperature, and shred into pieces smaller than 2 mm	[66]
	Shrimp shells	Scraped to remove loose tissue, washed and dried, and finally grounded to pass through a 250 μm sieve	[33]
Insects	Grasshopper	Cleaned by washing with distilled water, dried at room temperature and pulverization	[42]
	Crickets, waxworms, mealworm, silkworms, beetles	Starved for 24 hours to clear the gastrointestinal tract of any residual food, killed by freezing	[67]
	Beetles	Starved for 48 hours to clear the gastrointestinal tract of any residual food, washed with water, and killed by freezing. Thawed at room temperature and air-dried at 50°C for 2 days. The dried beetles were milled to a powder to pass through a 20-mesh screen.	[68]
Mollusks	Squid pen	The squid pens were washed with water, dried, and cut in small pieces (sieved from 2 to 5 mm).	[69]
	Squid pen	Samples were ground into about 18 mesh (ASTM) size	[70]

formed (such as lactic acid) react with calcium carbonate, resulting in a precipitate that can be removed by washing. Khanafari et al. stated that fermentation is less likely to change the physicochemical characteristics of the chitin chain [65]. Table 1.3 summarizes some demineralization procedures as examples.

1.3.1.3 Deproteination

Chitin is traditionally viewed as a chain that is embedded in a protein-matrix. During the purification step, complete removal of proteins is advisable when applications in medical, food, or feed are envisioned since proteins can be allergenic [10]. The proteins are bound by multiple hydrogen bridges, and the 'free' amino groups of the chitin may covalently be bound to the proteins [8, 51]. Therefore, extreme process parameters are often required for the deproteination. Chemical deproteination is commonly performed with sodium hydroxide as a preferential reagent. The effectiveness of alkali deproteination depends on the process temperature, the alkali concentration, as well as the alkali/biomass ratio [26]. During deproteination, chitin may undergo some changes such as partial deacetylation and hydrolysis of the polymer [10]. Also, biological deproteination using (1) proteases (such as pepsin, papain, and trypsin) secreted by proteolytic bacteria in the fermentation medium or (2) isolated proteases (crude or purified) has been reported [39, 40]. As the conditions are less harsh as compared to chemical deproteination, small peptides and amino acids remain attached to the chitin chain after enzymatic hydrolysis [10], and the deacetylation degree is altered less (less increase) [72]. Some examples are summarized in Table 1.4.

Table 1.3 *Examples of demineralization steps.*

Origin	Biomass type	Demineralization approach (scale)	Ref.
Crustaceans	Crustacean shell waste	Chemical: 0.2–2 M HCl; 1–48 hours; 0–100°C	[39]
	Prawn shells	Chemical: 0.5–1% (v/v) HCl; 24 hours; 25°C; 4:1 or 10:1 (v/w)	[63]
	Shrimp shells	Chemical: 0.25 M HCl; 0.25 hours; 25°C; 40:1 (v/w); 2–5 repeats	[60]
	Shrimp waste	Biological: *Lactobacillus plantarum* (PTTC 1058), *Lactobacillus acidophilus* (PTTC 1643), and *Lactobacillus rhamnosus* (PTCC 1637) were added (OD_{600} =0.8–1) to 50 mL water + 5 g of shrimp waste. Incubation for 7 days at 30°C and 5% CO_2	[65]
	Shrimp shells	Biological: *Lactobacillus plantarum* PTCC 1058—5% w/v shrimp shell powder—addition of peptone, yeast extract, meat extract, K_2HPO_4	[40]
Crustaceans/ mollusks	Barnacles, crab, lobster, crayfish, shrimp, cuttlefish, squid	Chemical: 0.5–2 M HCl; 0.5–1.5 hours; 4–50°C; 10:1 (v/w): 1–3 repeats	[71]
	Prawn, shrimp, crab, lobster, cuttlefish	Chemical: 0.25 M HCl; 0.25–3 hours; 40 mL/g	[26]
	Marine shell waste	Chemical: 0.55 M HCl; 2 hours; 25°C; 11.25: 1 (v/w)	[41]
Crustaceans/ insects	Shrimp shells, Desert locus, honey bee, beetles	Chemical: 1 M HCl; 25°C; 15 mL/g	[33]
Insects	*Palomena prasina*	Chemical: 2 M HCl; 2 hours; 100°C	[45]
	Grasshopper	Chemical: 4 M HCl; 1 hour; 75°C	[42]
	Beetle	Chemical: 1 M HCl; 30 min; 100°C	[68]

1.3.1.4 Decoloration and Other Post-Treatment Processes

When desired, a decoloration step can be introduced to remove pigments (such as pink colors for crustaceans). A mild oxidizing treatment with, for example, hydrogen peroxide [74] or potassium permanganate [75], or an extraction with solvents such as acetone [63], ethanol, and chloroform [42], have been reported. Finally, some post-treatment steps such as neutralization, drying, and milling may be required to finalize the chitin production process (Table 1.5).

1.3.2 Isolation of Chitin from Crustaceans

Isolation of chitin from crustaceans is well studied and documented. Generally, chitin is produced from crustacean waste (shells) through washing and crushing of the raw material in a pretreatment step (Table 1.2). The shells are washed with tap water or boiled before drying [64]. After drying, the shells are mechanically crushed. Different particle sizes are obtained and reported, such as, for example, 60–120 μm [63], less than 250 μm [26], 0.5–1 mm [76], or 0.5–10 mm [77].

Acid calcination is selected most often as a demineralization step (Table 1.3), with HCl as the preferred reagent [40]. A concentration below 1 M is usually applied at room temperature. For crustaceans, the mineral content differs significantly, resulting in the need for different treatments [25]. As a result, the time and number of repeats can differ, depending on the biomass. Percot et al. found that, for shrimp shells, the demineralization was

Table 1.4 *Examples of deproteination steps.*

Origin	Biomass type	Deproteination approach (scale)	Ref.
Crustaceans	Marine shell waste	Chemical: 30–50% (w/v) NaOH; 1–72 hours; 0–100°C	[40]
	Shell waste	Chemical: 1 M NaOH; 1–72 hours; 65–100°C	[39]
	Shrimp shells	Chemical: 1% (w/v) NaOH; 24 hours; 70°C	[60]
		Chemical: 0.3 M NaOH; 1 hour; 80–85°C; repeat till absence of color	[41]
	Shrimp shells	Biological: Protease production of *Bacillus cereus SV1* was used, shells with water (1:2 w/v) were cooked for 20 min and protease was added, incubation for 3 hours at 40°C	[72]
	Shrimp shells	Biological: *Bacillus mojavensis A21* and *Bacillus subtilis A26* were used to produce proteases, 15 g shrimp shells + 45 mL water was mixed with the protease	[71]
Crustaceans/ Mollusca	Shrimp waste	Chemical: 0.5–1.25 M NaOH; 0.5–24 hours; 60–100°C; 1:10 (w/v)Room temperature	[66]
	Fish scales	Chemical: 0.5 M NaOH; 18 hours; 25°C; repeated till absence of color	[73]
Crustaceans/ Mollusca	Shrimp shells, squid pens, crab shells, lobster shells	Chemical: 1 M NaOH; 105–110°C; repeated till absence of color	[25]
	Prawns shells	Chemical: 5% (w/v) NaOH; 2 hours; 60°C; 8:1 (v/w)	[63]
Bryozoan, insects, fungal		Chemical: 2 M NaOH; 20 hours; 140°C;	[45]
	Marine shell waste	Chemical: 0.3 M NaOH; 1 hour; 80–85°C; 2–7 repeats	[74]
Insects	Grasshopper	Chemical: 2 M NaOH; 18 hours; 175°C	[42]
	Beetle	Chemical: 1 M NaOH; 24 hours; 80°C	[68]

complete within 15 min at ambient temperature [60]. Younes et al. performed a two-level fractional factorial design and concluded that the number of batches and the concentration of HCl are the most influential parameters for the demineralization efficiency. As temperature was not found to be a significant parameter, lower temperatures are preferred to reduce chain degradation and chitin deacetylation. The best demineralization for shrimp was found when using lower temperatures and longer reaction times [71]. Tolaimate et al. confirmed that the number of repeats (a multi process) had a positive influence, but did not find a relation between higher acid concentration and longer reaction time in their settings [74]. Biological demineralization by the use of fermentation, for instance, solid-state fermentation with organic acid–producing bacteria, received more attention recently as being a more eco-friendly, safer, and more technologically flexible alternative to chemical demineralization. Arbia et al. and Kaur et al. gave an overview of the most common bacteria utilized [39, 40]. Lactic acid–producing bacteria were usually selected, for example, *Lactobacillus spp. B2* or *L. plantorum A3*. Other acid-producing microorganisms that were tested include *Bacillus subtilis*, *Pseudomonas aeruginosa K-187*, and *Serratia marcescens FS-3*. Since the acid also lowered the pH, which activated proteases [10], demineralization and deproteination occurred partially simultaneously. Khanafari et al. proved that demineralization using fermentation could be evenly effective if not better than chemical demineralization. They also stated that fermentation was less likely to change the physicochemical characteristics of the chitin chain [65].

Table 1.5 *Examples of decoloration and post-treatment steps.*

Origin	Biomass type	Post treatments approach	Ref.
Crustaceans	Shrimp waste	Sample filtrated under vacuum and washed with deionized water Decoloration: hydrogen peroxide (35% (v/v)) overnight at room temperature Post-treatment: filtered, washed, and dried at 50°C for 3 hours	[66]
	Shrimp waste	Post-treatment: filtered, washed to neutrality, freeze-dried	[72]
	Shrimp shells, crab shells, lobster shells	Sample washed with deionized water to neutrality and dried Decoloration: $KMnO_4$ + oxalic acid + H_2SO_4 or refluxing in ethanol for 6 hours Post-treatment: filtered, washed, and dried at 50°C for 3 hours	[25]
	Prawn, shrimp, crab, lobster, cuttlefish	Post-treatment: washed to neutrality and washed with hot ethanol (10 mL/g), and later boiled in acetone to remove any impurities; purified chitin then dried	[26]
Bryozoa, insect, fungi		Decoloration: refluxed with distilled water, methanol, and chloroform (4:2:1), respectively, room temperature, 2 hours Post-treatment: neutralized and dried (3 days, 50°C)	[45]
Insect	Grasshopper	Decoloration: chloroform, methanol, and distilled water (1:2:4), rinsed with distilled water Post-treatment: dried (60°C, 24 hours)	[42]
	Beetle	Decoloration: 1% (w/v) potassium permanganate solution, 1 hour, rinsed with distilled water Post-treatment: dried (50°C)	[68]

In respect to deproteination (Table 1.4), a chemical approach with sodium hydroxide is often used, even if reaction conditions are very variable. The concentration of NaOH ranges from 0.1 to 5 M, and the temperature can increase up to 160°C. Chang and Tsai found that 2.5 M NaOH at 75°C and a minimal solution-to-solid ratio of 5 mL/g was the most effective to clean shrimp shells [78]. In contrast, Tokatli and Demirdoven did a Box–Behnken experimental design and found that 0.95 M NaOH for 75.6 min at 60.5°C were the optimal conditions to deproteinate a similar biomass [66]. Both studies did not assess possible changes on the physicochemical properties of the polymer. During deproteination, partial deacetylation of chitin has often been reported [10]. Nevertheless, Percot et al. found that temperature and time had no influence on hydrolysis and deacetylation with NaOH concentrations below 1 M and temperatures below 70°C [60]. In summary, literature reported divided conclusions concerning optimal process parameters, which can be attributed to the different sources (species) used or the different pretreatment applied. For example, Younes et al. also found that the particle size obtained in the pretreatment step impacted the efficiency of the purification of chitin [71]. Biological deproteination with proteolytic enzymes and protease-producing bacteria is an alternative to remove proteins during chitin isolation [39, 40]. In comparison to chemical deproteination, the efficiency of the enzymatic method is lower, leaving approximately 5–11% residual protein [10, 72]. In addition, the use of crude proteolytic extracts obtained from different microorganisms or even from fish viscera

have been studied [61]. Although the deproteination levels achieved in such cases are generally lower than those obtained using alkaline treatments, this alternative has the advantage to produce nutritionally valuable protein hydrolysates in addition to chitin.

In conclusion, the intensity of the demineralization and deproteination steps depends on the source [74]. It is generally accepted that these steps significantly change the physicochemical properties of chitin, for example, the molecular weight and acetylation degree [40, 60]. Both the degree of crystallinity and the acetylation degree of chitin depend on the source and the method of purification [62]. Most researchers favor performing the demineralization first, followed by deproteination [74]. However, it is considered that the order of these two phases in interchangeable depending on the source [61, 79].

1.3.3 Isolation of Chitin from Insects

In recent years, the production of proteins from agri-waste using insects has increased rapidly, and numerous startup companies have emerged. This has led to a new chitin-rich side-stream composed of spent prepupae shell, residue after molting, and dead insects, which offers a tremendous potential for chitin and chitosan production as a by-product. The procedure to isolate chitin from insect cuticles is very similar to the one used in crustaceans. Nevertheless, insects have lower levels of inorganic material, allowing a more gentle demineralization step [2]. Some examples of chitin extractions from insects are given in Table 1.6. Only a few articles could be found, indicating that insect chitin is a relatively new field. For this reason, no standard or engineered procedure for insects was found.

1.3.4 Isolation of Chitin from Other Biomass Types

As mentioned in Section 1.2.1.3, fungi can contain considerable amounts of chitin, reaching up to 50% of the cell wall on a dry matter base. They are, however, one of the very few biomass sources in which chitosan can naturally be present in significant amounts, and many studies target the isolation of chitosan rather than that of chitin (see Section 1.4). Nevertheless, the isolation of (crude) chitin is regularly targeted on a lab scale, mostly to investigate its properties [29, 48, 63]. As the characteristics of the fungal biomass differ from the ones of the traditional and commercial source of chitin (i.e., crustaceans), the chitin isolation procedure deviates in some aspects from process steps as outlined in Figure 1.2. During the pretreatment, the filtered and washed fungal biomass is typically dried, which requires a more extensive dewatering/drying step, given the wet nature of the biomass. No demineralization step is performed, since the fungal biomass does not contain appreciable quantities of calcium carbonate and other mineral salts [88]. After pretreatment, a base extraction is applied, which not only aims to remove the proteins but also the alkali-soluble polysaccharides, leaving the alkali-insoluble cell wall behind. This chitin-rich cell fraction is characterized and used [89, 90] as such or further purified to remove the remaining glucans, for instance, by selective enzymatic hydrolysis [91] (or separate chitosan, if present).

Mollusks are generally processed following the standard procedure elaborated for crustaceans. Nevertheless, some differences are reported. For instance, it was noticed that the CO_2 emission was stronger in case of cuttlefish than other (crustacean) species [26].

Table 1.6 *Examples of chitin extraction procedures applied on insects.*

Insect	Insect stage	Pretreatment	Demineralization	Deproteination	Post-treatment	Ref.
Colorado potato beetle	Larvae/adult beetle	Drained in 70% (v/v) alcohol, washed, dried at room temperature, grounded, dried at 65°C for 3 hours	20 g + 100 mL 2 M HCl at 65–75°C for 2 hours	50 mL 2 M NaOH at 80–90°C for 16 hours	Filtered and washed, drained in chloroform (1), methanol (2), and water (4) for 1 hour, filtered and washed, dried at 60°C	[53]
Crickets: *Gryllus bimaculatus*		Dried crickets, grounded, washed, and dried at 60°C	(3) Oxalic acid, 3 hours at room temperature	(1) 40 g + 400 mL 1 M NaOH 95°C for 6 hours	(2) Drained in ammonium persulfate solution (50% (w/v)) at 50°C for 6 hours (4) Dried at 60°C overnight	[80]
Calliptamus barbarus and *Otopharynx decorus*		Grounded, dried at 70°C for 30 min	2 g + 100 mL 1 M HCl at 100°C for 30 min	1 M NaOH at 80–90°C for 21 hours	Washed and dried at 55°C, drained in chloroform (1), methanol (2), and water (4) for 1 hour	[81]
House fly: *Musca domestica*	Fourth instar larvae	Washed with 15% (w/v) aqueous NaCl, freeze-dried, grounded	(3) 10 mg/mL oxalic acid aqueous for 3 hours	(1) 10 g + 100 mL 1 M NaOH at 95°C for 6 hours	(2) 10 mg/mL potassium permanganate aqueous solution for 4 hours (4) Neutralized and freeze-dried	[82]
Silkworm	Chrysalides	Dried by lyophilization for 12 hours	1 M HCl at 100°C for 20 min (10 mL HCl/g)	1 M NaOH at 80°C for 24 hours (10 mL NaOH/g)	Washed with Na_2CO_3 0.4% (w/v), dried at 80°C	[83]
Blowfly: *Chrysomya megacephala*	Larvae	Washed with NaCl, dried at 50°C, grounded	(3) Oxalic acid (10 mg/mL) for 3 hours	(1) 10 g + 100 mL NaOH 1 M at 95°C for 6 hours	(2) Decolored with NaCl (0.5%, w/v) for 3 hours (4) Washed to neutral with distilled water and then freeze-dried in vacuum	[84]
Black soldier fly: *Hermetia illucens*	Pupal exuviae and dead imago	Cleaned, dried, grounded	1 M HCl for 1 hour	1 M NaOH at 80°C for 24 hours	1% $KMnO_4$ and excess of $KMnO_4$ was removed by 4% (w/v) oxalic acid	[85]
House cricket: *Brachytrupes portentosus*	8 weeks old	Starved for 48 hours, frozen-washed with water, dried at 60°C for 48 hours	(2) 200 mL 10 M oxalic acid at room temperature for 3 hours	(1) 20 g + 200 mL 1 M NaOH at 95°C for 6 hours	200 mL 1% NaCl (1%, w/v), at room temperature for 3 hours, filtered, washed to neutral pH, dried at 60°C overnight	[86]

(*Continued*)

Table 1.6 *(Continued)*

Insect	Insect stage	Pretreatment	Demineralization	Deproteination	Post-treatment	Ref.
Beetle: *Holotrichia parallela*	Adult beetles	Starved for 48 hours, washed, frozen, defrosted, dried at 50°C for 48 hours, grounded	5 g + 250 mL 1 M HCl at 100°C for 30 min	1 M NaOH at 80°C for 24 hours	Washed till neutral pH, 1% (w/v) potassium permanganate for 1 hour, washed and dried at 5°C	[68]
7 Orthoptera species	Grasshoppers	Washed, dried at room temperature, grounded	2 g + 100 mL 4 M HCl at 75°C for 1 hour	50 mL 2 M NaOH at 175°C for 18 hours	Filtered, washed, drained in chloroform (1), methanol (2), and water (4), washed with distilled water, dried at 60°C for 24 hours	[42]
Desert locus, beetles, honey bee	Exoskeletons	Scraped free from loose tissue, washed, dried, grounded	1 M HCl at room temperature (15 mL/g)	1 M NaOH at 100°C for 8 hours, repeated several times	Filtered, washed till neutral pH, washed with hot ethanol and boiled in acetone, dried in vacuum oven at 50°C	[33]
Melolontha melolontha	Adults	Killed using a killing agent, washed, dried at 60°C for 24 hours, grounded	2 g + 50 mL 4 M HCl at 75°C for 2 hours	4 M NaOH at 150°C for 18 hours	Filtered, washed till neutral pH, drained in chloroform (1), methanol (2), and water (4) for 20 min, washed, filtered, dried at 60°C for 24 hours	[35]

1.4 Production of Chitosan

Due to the high crystallinity, chitin is insoluble in many common solvents. Even if there are some exceptions, however, these solvents are usually toxic, corrosive, or degradative, and thus cannot be used in scaling up procedures for pharmaceutical applications [92]. Due to this insoluble nature of chitin, attention has been given to convert chitin into more soluble derivatives. Among the various reactions that can disrupt the intra- and intermolecular bonds causing the high crystallinity, *N*-deacetylation is the simplest modification, transforming chitin to chitosan [79]. Chitosan is generally insoluble in organic solvents and water, but soluble in diluted acid solutions with a pH below 6. This is because the chitosan amino groups have pKa values around 6.3, implying that, at low pH, the amines will be protonated and become positively charged. There is a positive correlation between the degree of deacetylation (DDA) and the number of positive charges potentially present in the chitosan chain. The charge density, as well as the exact pKa value, and in turn also the solubility of chitosan, are highly dependent on DDA [7, 87]. Other factors affecting the charge density of chitosan, such as the ionic strength and the distribution of acetyl groups along the chain, also affect the solubility of chitosan [7]. Chitosan is chitin's most important derivate in terms of application [7], since the amino group on chitosan makes easy chemical modification possible [11] and gives the molecule some specific functionalities.

The production of chitosan can be achieved via two approaches that start from a different biomass type (Figure 1.4). The most common approach is converting extracted and purified chitin into chitosan via a deacetylation step that may be linked to some pre-treatment (to decrease crystallinity) and post-treatment steps. A second option is to start from chitosan-containing fungi biomass and separate it from other biomass components via a cleaning and chitosan extraction step, potentially linked to some pre-treatment (drying, etc.) and post-treatment steps (drying, milling, etc.). Both approaches are detailed in the following sections.

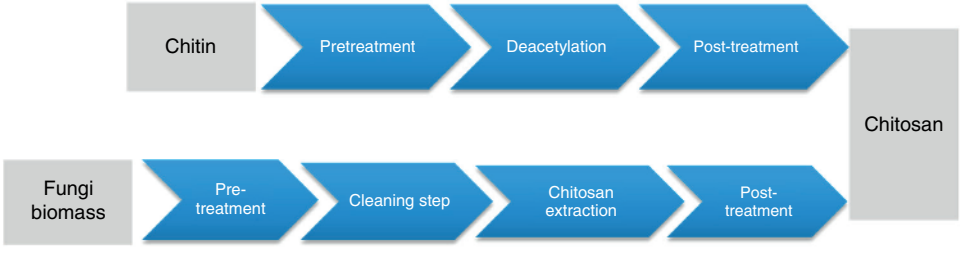


Figure 1.4 *Processes involved in the production of chitosan.*

1.4.1 Conversion of Chitin to Chitosan

1.4.1.1 Deacetylation Reaction Mechanism

Deacetylation is a two-step nucleophilic substitution reaction (Figure 1.5). The first step consists of a nucleophilic addition of an hydroxide on the carboxy groups [74], while, in the second step, an amine is formed (=chitosan) when acetic acid is split off. The reaction

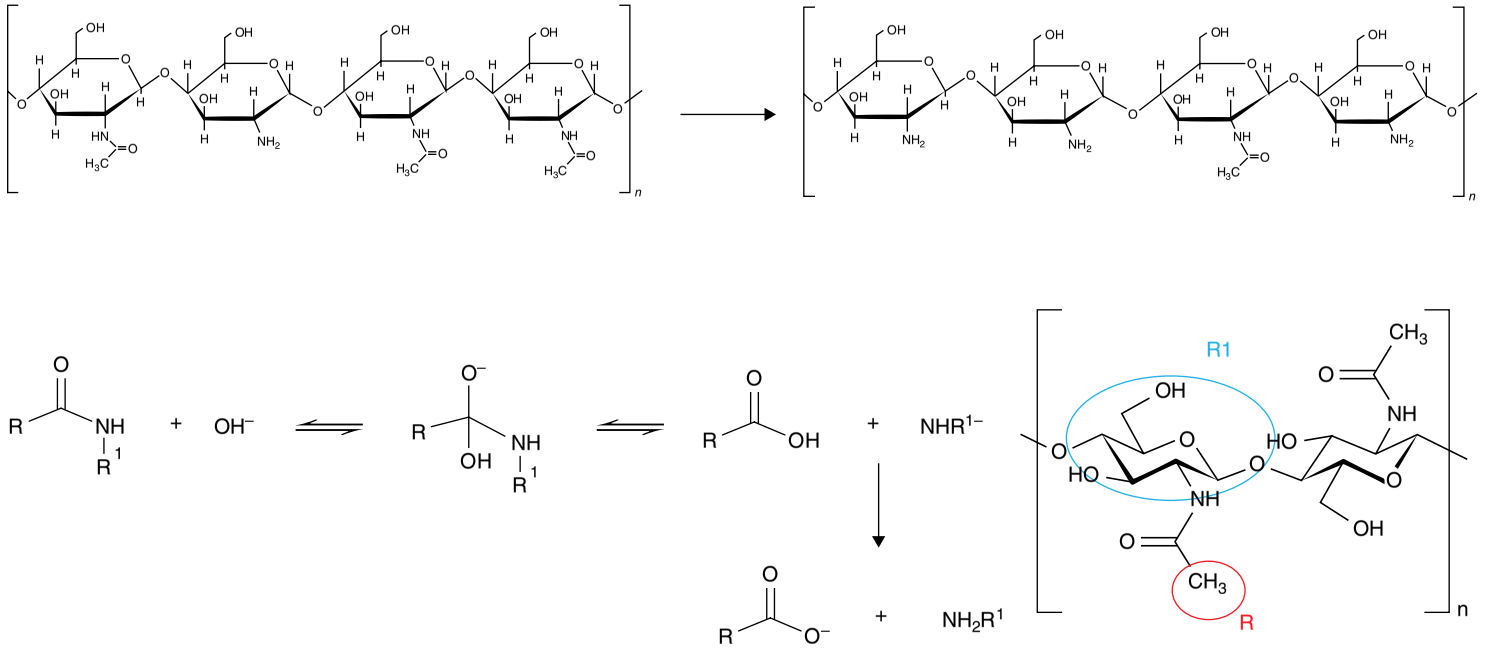


Figure 1.5 *Reaction mechanism of the deacetylation step.*

follows a pseudo-first-order kinetic during the initial period (first hour) when the concentration of alkaline is high [76, 93].

1.4.1.2 Chemical Deacetylation Process

Deacetylation is traditionally performed in an alkaline solution using sodium or potassium hydroxide at concentrations of 30–50 w% [13]. A two-step homogeneous deacetylation process can be realized with water-soluble chitin, referring to chitin with a DDA between 45% and 55% that is soluble in neutral water [16]. Chitin can be made water-soluble by treating it with 10% (w/v) NaOH at room temperature for 70 hours (step 1). The subsequent deacetylation with soluble chitin is considered to take place randomly along the main chain and occurs under moderate alkaline conditions—for instance, with 13 w% alkali for 12–24 hours at 25–40°C (step 2) [13]. A heterogeneous deacetylation can be realized in a one-step process with solid chitin. Under these conditions, mainly the amorphous zones are impacted, leading to a heterogeneous, more block-like deacetylation pattern [13, 16]. Heterogeneous deacetylation is commonly performed with higher aqueous alkali concentrations (40–50% (w/v)) and at higher temperatures (100–160° C), and is preferred for industrial purposes [40]. Some examples of deacetylation conditions and linked pre- and post-treatment are summarized in Table 1.7 and Table 1.8, respectively.

Table 1.7 *Examples of chitin deacetylation.*

Origin	Biomass type	Deacetylation approach (DDA-reached)	Ref.
Not specified	Not specified	13 w% alkali for 12–24 hours at 25–40°C (homogeneous deacetylation)	[13]
Crustaceans and Mollusca	Crabs, lobsters, crayfish, shrimp, cuttlefish, squid	50 w/w% KOH, 25 w/w% 96° (v/v) ethanol, 25 w/w% monoethylene glycol process, 0.5 g scale	[74]
	Crabs, lobsters, crayfish, shrimp, cuttlefish, squid	50 w/w% NaOH, 80°C, 60 mL/g, under nitrogen atmosphere (Kurita process), 0.5 g scale	[74]
Crustaceans	Prawn shells	25–50% NaOH; 80–100°C; 2, 5, 10 hours; solid/liquid 1:5	[63]
	Lithodes antarcticus, Paralomis granulosa, Palinurus vulgaris	50 w% NaOH; 110°C; 4 hours; 10 mL/g, under N_2 atmosphere	[76]
	Shrimp waste	50 w% NaOH; 105°C; 1–4 hours; 20 mL/g	[94]
	Shrimp waste	421 g/L NaOH; 130°C; 16.5 g/L	[95]
	Shrimp shells	40–50 w% NaOH; 90°C; 1.5–2 hours	[70]
Insects	Larvae and adult beetles of the potato beetle	50% NaOH (w/v 1:20) at 100°C for 3 hours	[53]
	Calliptamus. barbarus and *Otopharynx. decorus*	50% NaOH (w/v 1:15) at 130°C for 2 hours	[81]
	Larvae of the blowfly	670 g/L NaOH at 90°C for 9 hours (with the renewal of alkali every 3 hours)	[84]
Mollusca	Squid (β-chitin)	40–50% NaOH; 60–90°C; 2–4–6 hours; solid/liquid 1:20; up to three treatment steps	[70]
	Squid (β-chitin)	20–60 w% NaOH; 60–110°C; 5–80 min; solid/liquid 5:100	[96]

Table 1.8 *Examples of pre- and post-treatment steps linked to chitin deacetylation.*

Origin	Biomass type	Deacetylation approach (scale)	Ref.
Not specified	Not specified	Pre: pre-swelling of chitin to increase the number of amorphous zones	[13]
Crustaceans	Krill shells	Pre: mechanical disintegration; repeated freezing and thawing of alkaline chitin solutions (13–24% (w/v) NaOH), increase the number of amorphous zones	[97]
	Lithodes antarcticus, Paralomis granulosa, Palinurus vulgaris	Pre: milling and sieving (0.5–1 mm fraction) Post: washed with distilled water until pH 7	[76]
Crustaceans and Mollusca	Crabs, lobsters, crayfish, shrimp, cuttlefish, squid	Post: wash step with water to neutral pH, wash step with methanol, wash step with acetone, drying 50°C for 12 hours	[74]

Two traditional deacetylation procedures described by Broussignac [98] and Kurita et al. [99] were compared by Tolaimate et al. using α-chitin and β-chitin from different sources. The Broussignac procedure is based on an anhydrous reaction medium composed of 50 w/w% potassium hydroxide, 25 w/w% 96% (v/v) ethanol, and 25 w/w% monoethylene glycol. For the Kurita procedure, an aqueous sodium hydroxide solution is used. Tolaimate et al. showed a benefit of the Kurita approach for the deacetylation of β-chitin, but not for α-chitin. For α-chitin, the method of Broussignac proved to be more efficient, explained by the higher solubility of NaOH in aqueous media as compared to the solubility of KOH in the anhydrous media. The dielectric constant of NaOH in aqueous solution is much higher and perhaps favors the development of charges (during the deacetylation reaction). They also found that α-chitin treated with the Kurita medium required a higher reaction time for reaching the same DDA as Broussignac, and this happened at the expense of the molecular weight of chitosan and its quality. They suggested that the difference between α and β chitin can be due to morphological changes over time leading to more accessible amine groups. For α-chitin, these changes may be quicker in time for the Broussignac media, and may thus lead to more efficient deacetylation [74]. Despite these results, the Kurita procedure is considered the most common process, as indicated in Table 1.7. In general, deacetylation is performed at high NaOH concentrations (30–60 w%), high temperatures (80–150°C), and with long reaction times (1–80 hours) [8, 13, 70, 100, 101].

1.4.1.3 Tailoring Deacetylation Degrees of Chitosan

The specific characteristics (DDA and molecular weight) of the resulting chitosan are not only influenced by the deacetylation process parameters (reagent type, reagent concentration, temperature, reaction time, atmosphere, repeating steps), but also by the nature of the chitin source, such as its physical structure (e.g., particle size) and the isolation process applied [10, 74, 102]. For example, the time needed to obtain a similar DDA with the method of Broussignac differed significantly. As such, a general procedure to obtain chitosan with specific characteristics does not exist.

- *Example 1*: The deacetylation procedure took 1 hour for squid chitin, 2 hours for grey shrimp and Squilla chitin, 3–4 hours for pink shrimp and prawn chitin, and 6–7 hours

for lobster or red crab chitin [74]. The intensity of the deproteination caused differences in partial deacetylation (5–20%) [16].

- *Example 2*: The optimal process parameters for α-chitin extracted from shrimp waste were 100°C for 12 hours with 50 w% NaOH in the study by Tokali et al., where a DDA of 80.06% was obtained [66]. For β-chitin, 50 w% NaOH at 90°C for 6 hours proved to be best and a DDA of 95.6% was achieved [70].
- *Example 3*: Younes et al. investigated the impact of seven factors on the deacetylation of shrimp chitin. A clear significant effect of temperature and the alkali reagent was detected (NaOH was much more efficient than KOH). Also, the DDA was significantly affected by the use of repeats, the reaction time, and the concentration of alkali. The atmospheric (nitrogen or air) condition and the use of reducing agents ($NaBH_4$), however, did not have a significant effect on the DDA, but influenced the molecular weight of chitosan.

1.4.1.4 Deacetylation Drawbacks and Alternative Methods

During the alkaline treatments, chain degradation occurs. Although the mechanism is not yet fully known, the high concentration and prolonged reaction time required to obtain a complete deacetylation seem to be disadvantageous [41]. Galed et al. studied the degradation during deacetylation for three different sea animals. Even when a nitrogen environment was applied, chain degradation occurred (±1000 kDa to ±500 kDa after 250 min at 110°C, 50 w% NaOH) [76]. Tolaimate et al. found that β-chitin, deacetylated by 40% (w/v) NaOH at 80°C for 6 hours, had a larger molecular weight than the one treated at the same conditions for 9 hours [69]. Methacanon et al. reported that a twofold increase in time and a 1.25-fold increase in temperature resulted in a chitosan molecular weight decrease of 20–30% and 60%, respectively [94]. A nitrogen environment or the presence of a reducing agent (such as $NaBH_4$ or thiophenol) could reduce the degradation of chitosan [10, 40].

From the preceding text, it is clear that chemical deacetylation results in high amounts of alkaline wastewater [61]. Therefore, alternative deacetylation procedures are studied to replace the environmental-unfriendly method of Kurita and Broussignac or to decrease the degrading effect.

- Acid hydrolysis was not considered to be suitable, since the reaction was found to be limited due to steric effects, and it resulted in even more extensive hydrolysis of the backbone [54].
- Tsai et al. described a process of steam explosion for deacetylation. Flash treatment was also reported to give highly deacetylated chitosan over a shorter time [103].
- The use of enzymes such as deacetylases has been reported. The deacetylase was found to recognize a sequence of four *N*-acetyl glucosamine units, whereby one underwent deacetylation. The produced chitosan showed a more regular deacetylation pattern as compared to chemical deacetylation. However, it was concluded that deacetylase was not effective, as the DDA remained low (<10%) on natural, insoluble, crystalline chitin [10]. Pre-treatment to obtain better access to the acetyl groups was considered to be necessary. In this case, when chitin was soluble and more amorphous, deacetylase was able to perform deacetylation [104]. For example, Kim et al. were able to prepare

chitosan with a DDA of 71–88% with deacetylase [105]. Besides the necessary pretreatment, deacetylase has a high production cost and requires a long reaction time. This makes the enzymatic approach less ideal for commercial applications [100].

1.4.2 Chitosan Extracted from Fungi

Many fungal species have been investigated for the production of chitosan [47, 57, 106–109]. Typically, waste fungal biomass from the biotech industry as well as dedicated fungi are considered. More than 60% of the biotech industries use fungi in different processes such as brewing and baking, and for food, antibiotics, pharmaceuticals, organic acid, and enzyme production. For instance, citric acid, the most widely used organic acid in a wide array of food, beverage, and pharmaceutical applications, is produced with *Aspergillus niger*, which results in an annual waste of ~80 kTon mycelium [58]. As another example, the cultivation of *Agaricus bisporus*, the most popular mushroom worldwide, results in a waste of 5–20% in the form of stalks and mushrooms with irregular dimensions and shapes. This already corresponds to an annual mushroom waste of ~50 kTon in the United States [48]. These specific biotech wastes, among others, have been investigated for the extraction of chitin and chitosan. As a second approach, the direct fermentation of fungi for chitinous material production is also considered. *Mucor rouxii* has been the most commonly investigated [109–112] as the content of chitosan can reach 35% in the cell wall dry weight [112]. These fungi are usually harvested at their late exponential growth phase to obtain maximal yield [113].

As compared to the recovery of chitin from marine sources, differences can be noted in the isolation and production process. Whereas the isolation of chitin from marine sources is performed at an industrial scale, the recovery from fungi is still in its infancy, whereby procedures are applied that are more common to a lab environment [114]. The overall process can be separated into a pretreatment, an alkali-based "cleaning" step, an acid extraction step, followed by a posttreatment for chitosan separation, and purification.

1.4.2.1 Pretreatment

Fungi are a wet biomass and are typically extensively washed, dried, and refined before processing. Their low mineral content, on the other hand, does not require a demineralization step that is needed in the chitin production from marine resources. Moreover, as some fungi of particular interest are rich in the deacetylated chitosan, often the isolation of this particular compound is targeted, leaving the (crude) chitin behind. This not only alters the details of the extraction step to allow the specific isolation of chitosan, but also avoids the need for a post-deacetylation processing step.

1.4.2.2 Alkali-Based "Cleaning"

Even if the details of the extraction method vary among the different fungal sources/wastes used, the extraction of the fungal chitinous material always starts with a dilute alkaline treatment to remove proteins, glycoproteins, and alkali-soluble polysaccharides. This process resembles the alkaline process also performed to produce chitin from marine wastes. It is typically performed at low substrate loading (~1:30–1:40 w/v), high alkali concentration

(1–4 M), high temperature (80–121°C), and short contact times (15 min–3 hours) [48, 59, 60, 89, 106]. The alkali-insoluble residue, containing the chitin, chitosan, and beta-glucan, is separated and extensively washed till neutral pH before further processing.

1.4.2.3 Chitosan Extraction and Post-Treatment

In a next step, the residue is typically extracted with an acidic solution to dissolve the chitosan, leaving behind chitin and beta-glucan as insoluble residues. Acids such as HCl, H_2SO_4, and mostly acetic acid are used at a concentration of 0.5–2% (v/v), and the extraction is typically performed at a high temperature (60–95°C) over 6–14 hours [57]. The residual crude chitin is typically associated to the beta glucans through covalent bonds, and is difficult to recover in a chemical approach without degradation. The chitosan-loaded acid solution is recovered and brought to pH 10 to precipitate the chitosan. The precipitate is extensively washed, usually with a combination of water, ethanol, and acetone, and finally dried.

In a more industrial approach, recently described in patent literature, some modifications were suggested to improve the procedures described earlier. Rather than drying the biomass first, the alkali extraction was immediately performed on the wet biomass. Moreover, by optimizing the alkaline extraction to increase deacetylation of the chitin or, alternatively, to enzymatically hydrolyze the beta-glucans with specific enzymes to purify the crude chitin, increased extraction of chitosan/chitin was targeted [112, 113]. Some examples of chitosan extraction procedures from fungi are summarized in Table 1.9.

1.5 Towards Commercial Applications

Currently, the main sources for commercial chitin and chitosan production are crustaceans [58]. The annual worldwide production of crustacean shells amounts 1.2 million tons [118], and approximately 80000 tons of chitin is obtained from marine by-products [58]. Shells of crustaceans such as crabs and shrimp are mostly used to extract commercial chitin, for the simple reason that they are conveniently available as waste from seafood-processing industries [16]. About 35% of the captured marine organisms are handled as by-products and waste. This includes the skeletons, heads, and the viscera. This waste is mostly dumped back into the sea, causing environmental pollution [61]. Kurita noted that the most likely promising source in the future for chitin extraction will be krill (20–30%) [16]. Nevertheless, other sources such as fungi, squids, oyster, cuttlefish, and insects also offer potential [2]. Rhazi (2000) indicated that squid pen can become increasingly common as another potentially important chitin source [54]. An overview of advantage and disadvantages for three chitin sources is given in Table 1.10, being crustaceans, fungi, and insects.

The advantages associated with crustaceans are the existence of full-scale industrial processes, the availability of significant amounts of seafood waste, and extended data sets on chitin extraction and processing. The disadvantages reported comprise discontinuous supply due to seasonal variations of marine sources, heterogeneity of the biomass (different species), the high mineral contents that require long processing times and more stringent process conditions, and allergens.

Fungi have recently received increased attention as a source of chitin due some specific advantages as compared to crustaceans. The fungal mycelium can be obtained by convenient

Table 1.9 *Examples chitosan extraction procedures from fungi.*

Origin	Biomass type	Extraction approach (scale)	Ref.
Fungi (Zygomycota)	*Absidia coerulea*	Alkali "cleaning" with 1 N NaOH/121°C/30 min Chitosan extraction with 2% (v/v) HCl/95°C/12 h	[106]
Fungi (Zygomycota)	*Mucor rouxii* *Rhizopus oryzae*	Washing of mycelia and drying Alkali "cleaning": 1 N NaOH/121°C/15 min/substrate ratio 1:30 w/v Recovery of insoluble fraction and washing till neutral pH Chitosan extraction: 2% (v/v) acetic acid/95°C/8 hours/substrate ratio 1:40 w/v Recovery of soluble fraction and precipitation at pH 10 Recovery of chitosan precipitate and washing with water, ethanol, and acetone, and drying	[115, 116]
Fungi (Zygomycota)	*Rhizopus pusillus*	Concentrated mycelium Alkali "cleaning": 0.5 N NaOH/90°C/120 min/substrate ratio 1:30 w/v Recovery of insoluble fraction and washing till neutral pH Chitosan extraction: 1% (v/v) sulfuric acid/121°C/20 min/substrate ratio 1:100 w/v Recovery of soluble fraction and precipitation at pH 10 Recovery of chitosan precipitate and washing with water Scale: < 1 kg mycelium	[117]
Fungi (Basidiomycota)	Various Basidiomycetes	Washing of mycelia and drying Alkali "cleaning": 1 N NaOH/40°C/overnight/substrate ratio 1:33 w/v Recovery of insoluble fraction and water wash at 95°C Chitosan extraction: 5% (v/v) acetic acid/90°C/3 hours Recovery of soluble fraction and precipitation with 1 N NaOH Recovery of chitosan precipitate and water wash till neutral pH Scale: 3 g	[87]
Fungi (Zygomycota)	*Mucor rouxii*	Freeze-dried mycelia Alkali "cleaning": 1 N NaOH/95°C/30 min/substrate ratio 1:40 w/v Recovery of insoluble fraction and washing with water and ethanol Chitosan extraction: 2% (v/v) acetic acid/95°C/6 hours/substrate ratio 1:100 w/v Recovery of soluble fraction and precipitation at pH 10 Recovery of chitosan precipitate and washing with water till neutral pH and drying	[48]

fermentation, and its availability does not experience geographic or seasonal limitations. The better controllable fermentation guarantees a chitin/chitosan final product with more consistent properties [119]. Fungal mycelium also has some compositional advantages as compared to crustacean wastes. It contains less inorganic compounds, avoiding the need for a demineralization treatment in the chitin production [120]. Moreover, whereas chitosan

Table 1.10 *Advantages and disadvantages of three chitin sources (partially based on [58]).*

	Crustaceans	Insects	Fungi
Source	Main source for industrial purposes nowadays [19, 124]	Only lab scale [124]	Only lab scale [19]
	Side-stream of seafood industry [39], which is widely available, renewable, and zero cost [124]	Side-stream of breeding (cocoons, sheddings) and other industries (e.g., the silk industry) [84]	Side-stream from production of mushrooms [124]
		Possibly, a side-stream when proteins are extracted for food/feed industries	Controlled production all year round that results in better reproducibility [47]
		Side-stream when insects are caught in the prevention of pest outbreak [35, 53]	
	Seasonal supply [19]	Can be bred on cheap bio-waste [84], easy to massively rear [84], tremendous potential as a natural resource because of high biodiversity of insects [68]	Can be bred on cheap bio-waste [124]
Purification of chitin	Long processing times, high temperatures, and high concentration of acids and alkalis are necessary to purify [10, 19]	Lower level of inorganic material as compared to crustaceans [124]	No demineralization needed and mild extraction parameters [19]
Production of chitosan	Well-established method for industrial production of chitosan	Limited literature data; same procedure as crustaceans is mostly applied	No chemical deacetylation needed. Fermentation leads to a more reproducible product by better controlled process parameters [57]
	High cost and laborious process, harsh process parameters, high quantity of waste water [19]		
	Enzymatic deacetylation needs pretreatment [104], high cost of enzymatic deacetylation, and long reaction time [100]		
End product	High molecular weight [19]	No literature data found	Medium–low MW [19], a more narrow molecular mass distribution than the chitosan produced from seafood [13]
	Animal protein contamination with possible allergenic properties, which limits the use in biomedicine and various other sectors [19]		More controlled molecular weight DDA and distribution of deacetylated groups [9]
	Heterogeneous biomass leads to inconsistent levels of DDA and high MW [19, 125]		Physical properties of extracted chitosan notably related to the growth substrate composition [47]

is not native to animal resources, it is present as structural components in the cell wall of fungi from the Zygomycetes class, such as *Mucor*, *Absidia*, and *Rhizopus* [55]. In addition, recent observations suggested that non-Zygomycetes plant and insect pathogenic fungi also have high proportions of chitosan in the cell walls [121]. These types of fungi allow the direct production of chitosan, avoiding the need for a deacetylation step to transform chitin to chitosan. Additionally, fungal chitosan is free of allergenic shrimp protein and has been reported to have beneficial properties in terms of homogeneous polymer length and solubility over the current marine source [58]. However, the processes of chitosan production from fungi are not scaled up to industrial level [106].

As insect farms are currently being established in Europe with focus on selected insect species, insects become a potential alternative source for chitin production. Compared to crustaceans, the insect-based source (exoskeletons) is more homogeneous (mostly one species) and is not seasonally dependent. As with fungi, the mineral content is lower, which justifies reducing the demineralization step to a minimum. On the other hand, the chitin production process from insects is less studied, and mostly procedures elaborated for crustaceans are applied.

Although commercial facilities for chitin and chitosan production exist (for Crustaceans), the examples mentioned in the previous sections were mainly performed at lab scale (<1–40 g). Upscaling to pilot and full scale usually requires modifications to the protocols. Lopez et al. estimated, based on lab date, the environmental impact of chitin production processes that involve strong acids and bases at high temperature in multiple process steps [122]. The chemical extraction of chitin would require, per ton chitin, 72.7 m^3 of water, 12231 kWh, 727 kg of NaOH, 2319 kg HCl, and 354 kg NaClO, and would produce 1.8 tons of CO_2 and 21.8 tons of wastewater. Switching to enzymatic deproteintion and water recovery would generate only half of the chitin from the same biomass, but could reduce the environmental impact per ton to 0.9 m^3 of water, 516 kWh, 46 kg of NaOH, 339 kg HCl, 84 kg NaClO, and 3.7 kg of enzymes, and would produce 0.08 tons of CO_2 and 0.2 tons of wastewater. Both approaches were predicted to be profitable. Gómez-Rios (2017) also reported on profitable chitosan production from shrimp shell waste, with low volumetric yields and high water requirements as main drawbacks. Re-use of water, including enzymatic processes and co-valorization of other compounds, would increase the sustainability of the process [123].

1.6 Outlook

Chitin is a natural polysaccharide that is omnipresent in nature. Despite the diverse potential applications of chitin, the compound is underspent. Upscaling of the multistep extraction process and the production of reproducible chitin and chitosan products in a more sustainable way are challenges that need to be overcome to increase the commercial production activities of crustaceans and extend them to other source such as fungi and insects.

References

1. R. A. A. Muzzarelli, J. Boudrant, D. Meyer, N. Manno, M. Demarchis, and M. G. Paoletti, "Current views on fungal chitin/chitosan, human chitinases, food preservation, glucans, pectins and inulin: A tribute to Henri Braconnot, precursor of the carbohydrate polymers science, on the chitin bicentennial," *Carbohydr. Polym.*, vol. **87**, no. 2, pp. 995–1012, 2012.

2. A. Jardine and S. Sayed, “Challenges in the valorisation of chitinous biomass within the biorefinery concept,” *Curr. Opin. Green Sustain. Chem.*, vol. **2**, no. 2016, pp. 34–39, 2016.
3. K. Kaur, V. Dattajirao, V. Shrivastava, and U. Bhardwaj, “Isolation and characterization of chitosan-producing bacteria from beaches of Chennai, India,” *Enzyme Res*., vol. **2012**, Article ID421683, 2012.
4. H. Merzendorfer, “The cellular basis of chitin synthesis in fungi and insects: Common principles and differences,” *Eur. J. Cell Biol.*, vol. **90**, no. 9, pp. 759–769, 2011.
5. H. Ehrlich, "Chitin and collagen as universal and alternative templates in biomineralization," *Int. Geology Rev.*, vol. **52**, no. 7–8, pp. 661–699, 2010.
6. M. Zuber, K. M. Zia, and M. Barikani, “Chitin and chitosan based blends, composites and nanocomposites,” In: Thomas S., Visakh P., Mathew A. (eds) *Advances in Natural Polymers. Advanced Structured Materials*, vol. **18**, Springer, Berlin, Heidelberg, pp. 55–119, 2013.
7. M. Rinaudo, “Chitin and chitosan: Properties and applications,” *Prog. Polym. Sci.*, vol. **31**, no. 7, pp. 603–632, 2006.
8. K. Kurita, “Chitin and chitosan: Functional biopolymers from marine crustaceans,” *Mar. Biotechnol.*, vol. **8**, no. 3, pp. 203–226, 2006.
9. V. Zargar, M. Asghari, and A. Dashti, “A review on chitin and chitosan polymers: Structure, chemistry, solubility, derivatives, and applications,” *ChemBioEng Rev.*, vol. **2**, no. 3, pp. 204–226, 2015.
10. I. Younes and M. Rinaudo, “Chitin and chitosan preparation from marine sources. Structure, properties and applications,” *Mar. Drugs*, vol. **13**, no. 3, pp. 1133–1174, 2015.
11. J. Z. Knaul, S. M. Hudson, K. A. M. Creber, and N. Carolina, “Crosslinking of chitosan fibers with dialdehydes: Proposal of a new reaction mechanism,” *J. Polym. Sci. B: Polym. Phys.*, vol. **37**, pp. 1079–1094, 1999
12. M. Zuber and K. M. Zia, *Advances in natural polymers*, vol. **18**, pp. 55–119, 2013.
13. I. Aranaz et al., “Functional characterization of chitin and chitosan,” *Curr. Chem. Biol.*, vol. **3**, pp. 203–230, 2009.
14. K. Y. Zhu, H. Merzendorfer, W. Zhang, J. Zhang, and S. Muthukrishnan, “Biosynthesis, turnover, and functions of chitin in insects,” *Annu. Rev. Entomol.*, vol. **61**, no. 1, pp. 177–196, 2016.
15. Y. W. Cho, J. Jang, C. R. Park, and S. W. Ko, “Preparation and solubility in acid and water of partially deacetylated chitins,” *Biomacromolecules*, vol. **1**, no. 4, pp. 609–614, 2000.
16. K. Kurita, “Controlled functionalization of the polysaccharide chitin,” *Prog. Polym. Sci.*, vol. **26**, no. 9, pp. 1921–1971, 2001.
17. A. Einbu, *Characterisation of chitin and a study of its acid-catalysed hydrolysis*, Thesis, Norwegian University of Science and Technology, 2007.
18. L. Hyman, “The occurrence of chitin in the Lophophorate phyla,” *Biol. Discov. Woods hole*, vol. **24**, no. 5, pp. 362–369, 1958.
19. S. Kaur and G. S. Dhillon, “The versatile biopolymer chitosan: Potential sources, evaluation of extraction methods and applications,” *Crit. Rev. Microbiol.*, vol. **40**, no. 2, pp. 155–175, 2014.
20. M. Poulicek and C. Jeuniaux, “Chitin biodegradation in marine environments: An experimental approach,” *Biochem. Syst. Ecol.*, vol. **19**, no. 5, pp. 385–394, 1991.
21. S. M. Holl, J. Schaefer, W. M. Goldberg, K. J. Kramer, T. D. Morgan, and T. L. Hopkins, “Comparison of black coral skeleton and insect cuticle by a combination of carbon-13 NMR and chemical analyses,” *Arch. Biochem. Biophys.*, vol. **292**, no. 1, pp. 107–111, 1992.
22. M. Bo et al., “Isolation and identification of chitin in the black coral *Parantipathes larix* (Anthozoa: Cnidaria),” *Int. J. Biol. Macromol.*, vol. **51**, no. 1–2, pp. 129–137, 2012.
23. C. Nielsen, “Entoprocta,” *Encycl. Life Sci.*, vol. **13**, no. 1997, pp. 1–2, 2001.
24. H. Ehrlich et al., “Identification and first insights into the structure and biosynthesis of chitin from the freshwater sponge *Spongilla lacustris*,” *J. Struct. Biol.*, vol. **183**, no. 3, pp. 474–483, 2013.
25. E. S. Abdou, K. S. A. Nagy, and M. Z. Elsabee, “Extraction and characterization of chitin and chitosan from local sources,” *Bioresour. Technol.*, vol. **99**, no. 5, pp. 1359–1367, 2008.

26. F. A. A. Sagheer, M. A. Al-Sughayer, S. Muslim, and M. Z. Elsabee, "Extraction and characterization of chitin and chitosan from marine sources in Arabian Gulf," *Carbohydr. Polym.*, vol. **77**, no. 2, pp. 410–419, 2009.
27. C. Aumeier and D. Menzel, *Secretions and Exudates in Biological Systems*, vol. **12**, January. 2012.
28. M. A. Rahman and J. Halfar, "First evidence of chitin in calcified coralline algae: New insights into the calcification process of Clathromorphum compactum," *Sci. Rep*., vol. **4**, Article 6162, 2014.
29. Y. Nemcova, "Detection of cell wall structural polysaccharides by cellulase–gold and chitinase-gold complexes," *Czecg Phycol.*, vol. **3**, pp. 31–36, 2003.
30. N. Pearlmutter and C. Lembi, "Localization of chitin in algal and fungal cell walls by light and electron microscopy," *J. Histrochem. Cytochem.*, pp. 782–791, 1978.
31. I. Maier, E. Parodi, R. Westermeier, and D. G. Müller, "*Maullinia ectocarpii* gen. et sp. nov. (Plasmodiophorea), an intracellular parasite in *Ectocarpus siliculosus* (Ectocarpales, Phaeophyceae) and other filamentous brown algae," *Protist*, vol. **151**, no. 3, pp. 225–238, 2000.
32. M. Kaya, T. Baran, and M. Karaarslan, "A new method for fast chitin extraction from shells of crab, crayfish and shrimp," *Nat. Prod. Res.*, vol. **29**, no. 15, pp. 1477–1480, 2015.
33. N. H. Marei, E. A. El-Samie, T. Salah, G. R. Saad, and A. H. M. Elwahy, "Isolation and characterization of chitosan from different local insects in Egypt," *Int. J. Biol. Macromol.*, vol. **82**, pp. 871–877, 2016.
34. T. Spranghers et al., "Nutritional composition of black soldier fly (*Hermetia illucens*) prepupae reared on different organic waste substrates," *J. Sci. Food Agric.*, vol. **9**, no. 8, pp. 2594–2600, 2017.
35. M. Kaya et al., "Comparison of physicochemical properties of chitins isolated from an insect (*Melolontha melolontha*) and a crustacean species (*Oniscus asellus*)," *Zoomorphology*, vol. **133**, no. 3, pp. 285–293, 2014.
36. M. Kaya, O. Seyyar, T. Baran, S. Erdoğan, and M. Kar, "A physicochemical characterization of fully acetylated chitin structure isolated from two spider species: With new surface morphology," *Int. J. Biol. Macromol.*, vol. **65**, pp. 553–558, 2014.
37. T. Kawahara, "Invasion into Japanese water by the European barnacle *Balanus improvisus* Darwin," *Nature*, vol. **197**, no. 487, pp. 912–914, 1963.
38. A. Sombke and G. D. Edgecombe, "Morphology and evolution of Myriapoda," *Arthropod Struct. Dev.*, vol. **43**, no. 1, pp. 3–4, 2014.
39. W. Arbia, L. Arbia, L. Adour, and A. Amrane, "Chitin extraction from crustacean shells using biological methods –A review," *Food Technol. Biotechnol.*, vol. **51**, no. 1, pp. 12–25, 2013.
40. S. Kaur and G. S. Dhillon, "Recent trends in biological extraction of chitin from marine shell wastes: A review," *Crit. Rev. Biotechnol.*, vol. **35**, no. 1, pp. 44–61, 2015.
41. M. Rhazi, J. Desbrières, A. Tolaimate, A. Alagui, and P. Vottero, "Investigation of different natural sources of chitin: influence of the source and deacetylation process on the physicochemical characteristics of chitosan," *Polym. Int.*, vol. **49**, no. 4, pp. 337–344, 2000.
42. M. Kaya, S. Erdogan, A. Mol, and T. Baran, "Comparison of chitin structures isolated from seven Orthoptera species," *Int. J. Biol. Macromol.*, vol. **72**, pp. 797–805, 2015.
43. M. D. Finke, "Complete nutrient content of four species of feeder insects," *Zoo Biol.*, vol. **32**, no. 1, pp. 27–36, 2013.
44. H. Yu et al., "Effect of venom from the jellyfish *Nemopilema nomurai* on the silkworm *Bombyx mori* L," *Toxins (Basel).*, vol. **7**, no. 10, pp. 3876–3886, 2015.
45. M. Kaya, V. Baublys, I. Šatkauskiene, B. Akyuz, E. Bulut, and V. Tubelyte, "First chitin extraction from *Plumatella repens* (Bryozoa) with comparison to chitins of insect and fungal origin," *Int. J. Biol. Macromol.*, vol. **79**, pp. 126–132, 2015.
46. S. Erdogan, M. Kaya, and I. Akata, "Chitin extraction and chitosan production from cell wall of two mushroom species (Lactarius vellereus and Phyllophora ribis)," in *Proceedings of the 6th international advances in applied physics and materials science congress & exhibition (APMAS 2016)*, 2017.

47. T. Wu, S. Zivanovic, F. A. Draughon, and C. E. Sams, "Chitin and chitosan-value-added products from mushroom waste," *J. Agric. Food Chem.*, vol. **52**, no. 26, pp. 7905–7910, 2004.
48. T. Wu, S. Zivanovic, F. A. Draughon, W. S. Conway, and C. E. Sams, "Physicochemical properties and bioactivity of fungal chitin and chitosan," *J. Agric. Food Chem.*, vol. **53**, no. 10, pp. 3888–3894, 2005.
49. A. Zamani, A. Jeihanipour, L. Edebo, C. Niklasson, and M. J. Taherzadeh, "Determination of glucosamine and N-acetyl glucosamine in fungal cell walls," *J. Agric. Food Chem.*, vol. **56**, no. 18, pp. 8314–8318, 2008.
50. M. Falk, D. G. Smith, J. McLachlan, and A. G. McInnes, "Studies on chitan (β-(1-4)-linked 2-acetamido-2-deoxy-D-glucan) fibers of the diatom *Thalassiosira fluviatilis* Hustedt," *Can. J. Chem.*, vol. **44**, no. 9096, 1965.
51. R. A. A. Muzzarelli, *Chitin Nanostructures in Living Organisms*, In: Gupta N. (eds) *Chitin. Topics in Geobiology*, vol. **34**. Springer, Dordrecht, 2011.
52. M. Kaya, K. Sofi, I. Sargin, and M. Mujtaba, "Changes in physicochemical properties of chitin at developmental stages (larvae, pupa and adult) of *Vespa crabro* (wasp)," *Carbohydr. Polym.*, vol. **145**, pp. 64–70, 2016.
53. M. Kaya, T. Baran, S. Erdoɨan, A. Menteş, M. Aşan Özüsaɨlam, and Y. S. Çakmak, "Physicochemical comparison of chitin and chitosan obtained from larvae and adult Colorado potato beetle (*Leptinotarsa decemlineata*)," *Mater. Sci. Eng. C*, vol. **45**, pp. 72–81, 2014.
54. M. Rhazi, J. Desbrières, A. Tolaimate, A. Alagui, and P. Vottero, "Investigation of different natural sources of chitin: Influence of the source and deacetylation process on the physicochemical characteristics of chitosan," *Polym. Int.*, vol. **49**, no. 4, pp. 337–344, 2000.
55. J. Ruiz-Herrera, *Fungal Cell Wall: Structure, Synthesis and Assembly*, 2nd edn. CRC Press, Boca Raton, 2016.
56. B. Aguilar-Uscanga and J. M. François, "A study of the yeast cell wall composition and structure in response to growth conditions and mode of cultivation," *Lett. Appl. Microbiol.*, vol. **37**, no. 3, pp. 268–274, 2003.
57. G. S. Dhillon, S. Kaur, S. K. Brar, and M. Verma, "Green synthesis approach: Extraction of chitosan from fungus mycelia," *Crit. Rev. Biotechnol.*, vol. **33**, no. 4, pp. 379–403, 2013.
58. V. Ghormade, E. K. Pathan, and M. V. Deshpande, "Can fungi compete with marine sources for chitosan production?," *Int. J. Biol. Macromol.*, vol. **104**, pp. 1415–1421, 2017.
59. D. Kafetzopoulos, A. Martinou, and V. Bouriotis, "Bioconversion of chitin to chitosan: Purification and characterization of chitin deacetylase from *Mucor rouxii*," *Proc. Natl. Acad. Sci. USA*, vol. **90**, no. 7, pp. 2564–2568, 1993.
60. A. Percot, C. Viton, and A. Domard, "Optimization of chitin extraction from shrimp shells," *Biomacromolecules*, vol. **4**, no. 1, pp. 12–18, 2003.
61. J. A. Vázquez, I. Rodríguez-Amado, M. I. Montemayor, J. Fraguas, M. P. Del González, and M. A. Murado, "Chondroitin sulfate, hyaluronic acid and chitin/chitosan production using marine waste sources: Characteristics, applications and eco-friendly processes: A review," *Mar. Drugs*, vol. **11**, no. 3, pp. 747–774, 2013.
62. J. Li, J. Revol, and R. Marchessault, "Effect of degree of deacetylation of chitin on the properties of chitin crystallites," *J. Appl. Polym.* Vol. **65**, no. 2, pp. 373–380, 1997.
63. M. H. Mohammed, P. A. Williams, and O. Tverezovskaya, "Extraction of chitin from prawn shells and conversion to low molecular mass chitosan," *Food Hydrocoll.*, vol. **31**, no. 2, pp. 166–171, 2013.
64. M. Bajaj, A. Freiberg, J. Winter, Y. Xu, and C. Gallert, "Pilot-scale chitin extraction from shrimp shell waste by deproteination and decalcification with bacterial enrichment cultures," *Appl. Microbiol. Biotechnol.*, vol. **99**, no. 22, pp. 9835–9846, 2015.
65. A. Khanafari, R. Marandi, and S. Sanatei, "Recovery of chitin and chitosan from shrimp waste by chemical and microbial methods," *J. Environ. Heal. Sci. Eng.*, vol. **5**, no. 1, pp. 1–24, 2008.
66. K. Tokatli and A. Demirdoven, "Optimization of chitin and chitosan production from shrimp wastes and characterization." *J. Food Process. Preserv*., vol. **42**, no. 2, e13494, 2017.

67. M. Finke and R. Verde, "Estimate of chitin in raw whole insects," *Zoo Biol.*, vol. **26**, no. 2, pp. 201–214, 2007.
68. S. Liu et al., "Extraction and characterization of chitin from the beetle *Holotrichia parallela* Motschulsky," *Molecules*, vol. **17**, no. 4, pp. 4604–4611, 2012.
69. A. Tolaimate, J. Desbrières, M. Rhazi, A. Alagui, M. Vincendon, and P. Vottero, "On the influence of deacetylation process on the physicochemical characteristics of chitosan from squid chitin," *Polymer (Guildf).*, vol. **41**, no. 7, pp. 2463–2469, 2000.
70. J. Jung and Y. Zhao, "Characteristics of deacetylation and depolymerization of β-chitin from jumbo squid (*Dosidicus gigas*) pens," *Carbohydr. Res.*, vol. **346**, no. 13, pp. 1876–1884, 2011.
71. I. Younes, S. Hajji, M. Rinaudo, M. Chaabouni, K. Jellouli, and M. Nasri, "Optimization of proteins and minerals removal from shrimp shells to produce highly acetylated chitin," *Int. J. Biol. Macromol.*, vol. **84**, pp. 246–253, 2016.
72. L. Manni, O. Ghorbel-Bellaaj, K. Jellouli, I. Younes, and M. Nasri, "Extraction and characterization of chitin, chitosan, and protein hydrolysates prepared from shrimp waste by treatment with crude protease from bacillus cereus SV1," *Appl. Biochem. Biotechnol.*, vol. **162**, no. 2, pp. 345–357, 2010.
73. S. Kumari, P. Rath, A. Sri Hari Kumar, and T. N. Tiwari, "Extraction and characterization of chitin and chitosan from fishery waste by chemical method," *Environ. Technol. Innov.*, vol. **3**, pp. 77–85, 2015.
74. A. Tolaimate, J. Desbrieres, M. Rhazi, and A. Alagui, "Contribution to the preparation of chitins and chitosans with controlled physico-chemical properties," *Polymer (Guildf).*, vol. **44**, no. 26, pp. 7939–7952, 2003.
75. A. Waśko, P. Bulak, M. Polak-Berecka, K. Nowak, C. Polakowski, and A. Bieganowski, "The first report of the physicochemical structure of chitin isolated from Hermetia illucens," *Int. J. Biol. Macromol.*, vol. **92**, pp. 316–320, 2016.
76. G. Galed, B. Miralles, I. Paños, A. Santiago, and Á. Heras, "N-deacetylation and depolymerization reactions of chitin/chitosan: Influence of the source of chitin," *Carbohydr. Polym.*, vol. **62**, no. 4, pp. 316–320, 2005.
77. D. Gómez-Ríos, R. Barrera-Zapata, and R. Ríos-Estepa, "Comparison of process technologies for chitosan production from shrimp shell waste: A techno-economic approach using Aspen Plus®," *Food Bioprod. Process.*, vol. **103**, pp. 49–57, 2017.
78. K. L. B. Chang and G. Tsai, "Response surface optimization and kinetics of isolating chitin from pink shrimp (*Solenocera melantho*) shell waste," *J. Agric. Food Chem.*, vol. **45**, no. 5, pp. 1900–1904,1997.
79. J. Synowiecki and N. A. Al-khateeb, "Production, properties, and some new applications of chitin and its derivatives," *Food Chem.*, vol. **43**, no. 2, pp. 145–171, 2003.
80. K. S. Chae, C. S. Shin, and W. S. Shin, "Characteristics of cricket (*Gryllus bimaculatus*) chitosan and chitosan-based nanoparticles," *Food Sci. Biotechnol.*, vol. **27**, no. 3, pp. 1–9, 2018.
81. M. Kaya et al., "Extraction and characterization of chitin and chitosan with antimicrobial and antioxidant activities from cosmopolitan Orthoptera species (Insecta)," *Biotechnol. Bioprocess Eng.*, vol. **20**, no. 1, pp. 168–179, 2015.
82. H. Ai, F. Wang, Q. Yang, F. Zhu, and C. Lei, "Preparation and biological activities of chitosan from the larvae of housefly, *Musca domestica*," *Carbohydr. Polym.*, vol. **72**, no. 3, pp. 419–423, 2008.
83. A. T. Paulino, J. I. Simionato, J. C. Garcia, and J. Nozaki, "Characterization of chitosan and chitin produced from silkworm crysalides," *Carbohydr. Polym.*, vol. **64**, no. 1, pp. 98–103, 2006.
84. C. Song, H. Yu, M. Zhang, Y. Yang, and G. Zhang, "Physicochemical properties and antioxidant activity of chitosan from the blowfly *Chrysomya megacephala* larvae," *Int. J. Biol. Macromol.*, vol. **60**, pp. 347–354, 2013.
85. Waśko et al., "The first report of the physicochemical structure of chitin isolated from *Hermetia illucens*." *Int. J. Biol. Macromol*., vol. **92**, pp. 316–320, 2016.
86. E. B. Ibitoye, I. H. Lokman, M. N. M. Hezmee, Y. M. Goh, A. B. Z. Zuki, and A. A. Jimoh, "Extraction and physicochemical characterization of chitin and chitosan isolated from house cricket," *Biomed. Mater.*, vol. **13**, 025009, 2018.

87. F. Di Mario, P. Rapanà, U. Tomati, and E. Galli, "Chitin and chitosan from Basidiomycetes," *Int. J. Biol. Macromol.*, vol. **43**, no. 1, pp. 8–12, 2008.
88. J. Synowiecki and N. A. Al Khateeb, "Production, properties, and some new applications of chitin and its derivatives," *Crit. Rev. Food Sci. Nutr.*, vol. **43**, no. 2, pp. 145–171, 2003.
89. A. M. Abdel-Mohsen et al., "Novel chitin/chitosan-glucan wound dressing: Isolation, characterization, antibacterial activity and wound healing properties," *Int. J. Pharm.*, vol. **510**, no. 1, pp. 86–99, 2016.
90. Y. A. Skorik, A. V. Pestov, and Y. G. Yatluk, "Evaluation of various chitin-glucan derivatives from *Aspergillus niger* as transition metal adsorbents," *Bioresour. Technol.*, vol. **101**, no. 6, pp. 1769–1775, 2010.
91. M. Versali, F. Clerisse, J.-M. Bruyere, and S. Gautier, "US7556946B2.pdf," US7556946B2, 2003.
92. C. K. S. Pillai, W. Paul, and C. P. Sharma, "Chitin and chitosan polymers: Chemistry, solubility and fiber formation," *Prog. Polym. Sci.*, vol. **34**, no. 7, pp. 641–678, 2009.
93. P. Methacanon, M. Prasitsilp, T. Pothsree, and J. Pattaraarchachai, "Heterogeneous N-deacetylation of squid chitin in alkaline solution," vol. **52**, pp. 119–123, 2003.
94. M. Bajaj, J. Winter, and C. Gallert, "Effect of deproteination and deacetylation conditions on viscosity of chitin and chitosan extracted from *Crangon crangon* shrimp waste," *Biochem. Eng. J.*, vol. **56**, no. 1–2, pp. 51–62, 2011.
95. C. M. de Moura, J. M. de Moura, N. M. Soares, and L. A. de A. Pinto, "Evaluation of molar weight and deacetylation degree of chitosan during chitin deacetylation reaction: Used to produce biofilm," *Chem. Eng. Process. Process Intensif.*, vol. **50**, no. 4, pp. 351–355, 2011.
96. C. H. Chen, F. Y. Wang, and Z. P. Ou, "Deacetylation of β-chitin. I. Influence of the deacetylation conditions," *J. Appl. Polym. Sci.*, vol. **93**, no. 5, pp. 2416–2422, 2004.
97. S. V. Nemtsev, A. I. Gamzazade, S. V. Rogozhin, V. M. Bykova, and V. P. Bykov, "Deacetylation of chitin under homogenous conditions," *Prikl. Biokhimiya i Mikrobiol.*, vol. **38**, no. 6, pp. 609–615, 2002.
98. P. Broussignac, "Chitosan: A natural polymer not well known by the industry," *Chim. Ind. Genie Chim.*, vol. **99**, no. 9, p 1241–1247, 1968.
99. K. Kurita, K. Tomita, T. Tada, S. Ishii, S.-I. Nishimura, K. Shimoda. "Squid chitin as a potential alternative chitin source: Deacetylation behavior and characteristic properties," *J. Polym. Sci. A*, vol. **21**, no. 2, pp. 485–491, 1993.
100. C. Liu, G. Wang, W. Sui, L. An, and C. Si, "Preparation and characterization of chitosan by a novel deacetylation approach using glycerol as green reaction solvent," *ACS Sustain. Chem. Eng.*, vol. **5**, no. 6, pp. 4690–4698, 2017.
101. T. H. D. Ngo and D. N. Ngo, "Effects of low-frequency ultrasound on heterogenous deacetylation of chitin," *Int. J. Biol. Macromol.*, vol. **104**, no. B, pp. 1604–1610, 2016.
102. I. Younes et al., "Use of a fractional factorial design to study the effects of experimental factors on the chitin deacetylation," *Int. J. Biol. Macromol.*, vol. **70**, pp. 385–390, 2014.
103. B. Focher, P. L. Beltrame, A. Naggi, and G. Torri, "Alkaline N-deacetylation of chitin enhanced by flash treatments. Reaction kinetics and structure modifications," *Carbohydr. Polym.*, vol. **12**, no. 4, pp. 405–418, 1990.
104. Y. Zhao, R. D. Park, and R. A. A. Muzzarelli, "Chitin deacetylases: Properties and applications," *Mar. Drugs*, vol. **8**, no. 1, pp. 24–46, 2010.
105. Y. Kim, Y. Zhao, K. Oh, V. Nguyen, and R. Park, "Enzymatic deacetylation of chitin by extracellular chitin deacetylase from a newly screened Mortierella sp DY-52," *J. Microbiol. Biotechnol.*, vol. **18**, no. 4, pp. 759–766, 2008.
106. D. K. Rane and G. D. Hoover, "Production of chitosan by fungi," *Food Microbiol.*, vol. **7**, no. 1, pp. 11–33, 1993.
107. K. M. Abdel-Gawad, A. F. Hifney, M. A. Fawzy, and M. Gomaa, "Technology optimization of chitosan production from Aspergillus niger biomass and its functional activities," *Food Hydrocoll.*, vol. **63**, pp. 593–601, 2017.

108. H. Miyoshi, K. Shimura, K. Watanabe, and K. Onodera, "Characterization of some fungal chitosans," *Biosci. Biotechnol. Biochem.*, vol. **56**, no. 12, pp. 1901–1905, 1992.
109. W. P. Wang, Y. M. Du, and X. Y. Wang, "Physical properties of fungal chitosan," *World J. Microbiol. Biotechnol.*, vol. **24**, no. 11, pp. 2717–2720, 2008.
110. S. A. White, P. R. Farina, and I. Fulton, "Production and isolation of chitosan from *Mucor rouxii*," *Appl. Environ. Microbiol.*, vol. **38**, no. 2, pp. 323–328, 1979.
111. S. Arcidiacono and D. L. Kaplan, "Molecular weight distribution of chitosan isolated from *Mucor rouxii* under different culture and processing conditions," *Biotechnol. Bioeng.*, vol. **39**, pp. 281–286, 1992.
112. J. Synowiecki and N. A. A. Q. Al-Khateeb, "Mycelia of *Mucor rouxii* as a source of chitin and chitosan," *Food Chem.*, vol. **60**, no. 4, pp. 605–610, 1997.
113. S. C. Tan, T. K. Tan, S. M. Wong, and E. Khor, "The chitosan yield of Zygomycetes at their optimum harvesting time," *Carbohydr. Polym.*, vol. **30**, pp. 239–242, 1996.
114. T. Wu, S. Zivanovic, F. A. Draughon, and C. E. Sams, "Chitin and chitosan—value-added products from mushroom waste," *J. Agric. Food Chem.*, vol. **52**, no. 26, pp. 7905–7910, 2004.
115. T. Kleekayai and W. Suntornsuk, "Production and characterization of chitosan obtained from *Rhizopus oryzae* grown on potato chip processing waste," *World J. Microbiol. Biotechnol.*, vol. **27**, no. 5, pp. 1145–1154, 2011.
116. P. Pochanavanich and W. Suntornsuk, "Fungal chitosan production and its characterization," *Lett. Appl. Microbiol.*, vol. **35**, pp. 17–21, 2002.
117. A. Zamani, L. Edebo, B. Sjostrom, and M. J. Taherzadeh, "Extraction and precipitation of chitosan from fungal cell wall by dilute sulfuric acid," *Biomacromolecules*, vol. **8**, no. 12, pp. 3786–3790, 2007.
118. T. Wu, "Production and Characterization of Fungal Chitin and Chitosan," *Grad. Sch*., 2004.
119. D. Knorr, M. D. Beaumont, and Y. Pandya, Potential of acid soluble and water soluble chitosan in biotechnology. *In Chitin and chitosan*. London, New York: Elsevier Applied Science, 1989.
120. W. L. Teng, E. Khor, T. K. Tan, L. Y. Lim, and S. C. Tan, "Concurrent production of chitin from shrimp shells and fungi," *Carbohydr. Res.*, vol. **332**, no. 3, pp. 305–316, 2001.
121. P. B. Nahar, V. Ghormade, and M. V. Deshpande, "The extracellular constitutive production of chitin deacetylase in *Metarhizium anisopliae*: Possible edge to entomopathogenic fungi in the bio-control of insect pest," *J. Invertebr. Pathol.*, vol. **85**, pp. 80–88, 2004.
122. C. Lopes, L. T. Antelo, A. Franco-Uria, A. A. Alonso, and R. Pérez-Martin, "Chitin production from crustacean biomass: Sustainability assessment of chemical and enzymatic processes," *J. Clean. Prod.*, vol. **172**, pp. 4140–4151, 2018.
123. D. Gómez-Ríos, R. Barrera-Zapata, and R. Ríos-Estepa, "Comparison of process technologies for chitosan production from shrimp shell waste: A techno-economic approach using Aspen Plus®," *Food Bioprod. Process.*, vol. **103**, pp. 49–57, 2017.
124. G. L. Dotto and L. A. Pinto, *Frontiers in Biomaterials: Chitosan Based Materials and its Applications*, Bentham Science publishers, Sharjah, 2017.
125. M. Kannan, M. Nesakumari, and K. Rajarathinam, "Production and characterization of mushroom chitosan under solid-state fermentation conditions," *Adv. Biol. Res.*, vol. **4**, no. 1, pp. 10–13, 2010.